HONG KONG MEDICAL JOURNAL
香港醫學雜誌

Vol 12 No 6 December 2006
Supplement 4

Fourth International Huaxia Congress of Endocrinology
15 – 18 December 2006

Fourth International Huaxia Congress of Endocrinology
15 – 18 December 2006

Organizing Committee

Chairman
Pik-To CHEUNG (Hong Kong)

Vice-chairman
Shing-Chung SIU (Hong Kong)

Members
Fredriech KW CHAN (Hong Kong)
Cheung-Hei CHOI (Hong Kong)
Chun-Hoi CHUNG (Hong Kong)
Karen SL LAM (Hong Kong)
Jenny YY LEUNG (Hong Kong)
Kathryn CB TAN (Hong Kong)
Peter CY TONG (Hong Kong)
Man-Wo TSANG (Hong Kong)
William SB YEUNG (Hong Kong)
Yan GAO (Beijing)
Guangwei LI (Beijing)
Guang NING (Shanghai)
Low-Tone HO (Taipei)
Jen-Der LIN (Taipei)

Secretary
Gladdie FY CHU (Hong Kong)

Scientific Committee

Co-Chairmen
Karen SL LAM (Hong Kong)
Kathryn CB TAN (Hong Kong)

Secretary
Alice ST WONG (Hong Kong)

Members
Simon CL AU (Hong Kong)
Annie WC KUNG (Hong Kong)
Louis CK LOW (Hong Kong)
Ronald CW MA (Hong Kong)
Sau Cheung TIU (Hong Kong)
Vincent TF YEUNG (Hong Kong)
William SB YEUNG (Hong Kong)
Changyu PAN (Beijing)
Weiping TENG (Shenyang)
Zhengpei ZENG (Beijing)
Tien-Chun CHANG (Taipei)
Lee-Ming CHUANG (Taipei)
Wayne HH SHEU (Taichung)

Sub-Committees

Finance/Exhibition
Man-Wo TSANG (Hong Kong)

Press & Publicity
Fredriech KW CHAN (Hong Kong)

Publication
Peter CY TONG (Hong Kong)

Social
Cheung-Hei CHOI (Hong Kong)

Venue & Accommodation
Chun Hoi CHUNG (Hong Kong)

International Advisory Committee

Co-Chairmen
Jialun CHEN (Shanghai)
Annie WC KUNG (Hong Kong)

Members
Ken KY HO (Australia)
Yifan SHI (Beijing)
Peter CK LEUNG (Canada)
Norman CW WONG (Canada)
Rosie TT YOUNG (Hong Kong)
Siew-Pheng CHAN (Malaysia)
Kok-Onn LEE (Singapore)
Ah-Chuan THAI (Singapore)
Tong-Yuan TAI (Taipei)
Keh-Sung TSAI (Taipei)
Frederick CW WU (UK)
Christina WANG (USA)

* Oral Presentations O2, O17, O20, O21, and O39 have been cancelled.

PL1 Pheochromocytoma: Pathogenesis and Clinical Management

Zheng-pei ZENG, Han-zhong LI, Ai-lun LUO, Da-wei LIU, Ming LI, An-li TONG, Fang LI, Lin LU, Guo-qian LIU, Dong-mei LIU, Shi CHEN, Wei LIANG, Tong-hua LIU
Peking Union Medical College Hospital (PUMCH), Chinese Academy of Medical Sciences, Beijing 100730, China

Adrenal hypertension including pheochromocytoma and primary hyperaldosteronism is the main cause of endocrine hypertension. The prevalence of pheochromocytomas or primary hyperaldosteronism may be as high as 1.9% and 5-15% respectively, in the population with hypertension. It is very important for detection, diagnosis and treatment endocrine hypertension at early stage. Study of pathogenesis and management of adrenal hypertension must be paid more attention.

429 cases (401 patients) with pheochromocytoma were diagnosed and treated from 1939 to 2006 (4 cases in 1939-1959, 10 cases in 1960-1969, 30 cases in 1970-1979, 74 cases in 1980-1989, 119 cases in 1990-1999, and 192 cases in 2000-2006) in Peking Union Medical College Hospital (PUMCH), Beijing, China. 401 patients suffered from pheochromocytoma (222 men and 179 women), aged from 7-75 years (mean age 39.9±16.5 years). There were 34 (8.5%) children and adolescent (≤20 years). 286 cases (71.3%) of pheochromocytoma arose from adrenal medulla. There were 106 (26.4%) extra-adrenal paraganglioma including the para-aortic region (81 cases), the urinary bladder (10 cases), the cardiac (5 cases), the thorax (5 cases), the liver (1 case), the head and neck region (4 cases). 5 cases located both of adrenal and extra-adrenal, 4 cases showed adrenal medulla hyperplasia. There were 86 patients (21.4%) with malignant pheochromocytoma and 30 patients (7.5%) with familial pheochromocytoma including 24 multiple endocrine neoplasia type 2 (MEN-2), 5 VHL syndrome and 1 family pheochromocytoma. The pheochromocytoma was diagnosed by measuring catecholamine levels in plasma or 24-hour urine using biochemical methods or HPLC. Pharmacologic tests, such as regitine test, glucagon test were applied to some patients as well. CT scan, MR image, ^{131}I-MIBG and/or ^{111}In-Octreotide uptake scintiscanning were performed for localization of the tumors. Surgical removal of tumor, especially by laparoscopic surgery, was proven to be an effective treatment to pheochromocytoma with alpha-adrenergic blocking drug before operation. Otherwise, we have treated malignant pheochromocytomas with or without metastasis by ^{131}I-MIBG or ^{111}In-Octreotide.

Molecular genetic studies have revealed that approximately 50% of patients with non-syndromic familial pheochromocytomas had germline VHL gene mutations. The genetic mutations of the RET proto-oncogene on chromosome 10q for MEN 2a and 2b, on chromosome 3p for VHL, and on chromosome 17q for familial neurofibromatosis. Gene mutations of the succinate dehydrogenase family, the SDHD gene on chromosome 11q and the SDHB gene on chromosome 1p, may account for up to 12% of pheochromocytoma. Genetic testing should be considered in all patients with pheochromocytoma and paraganglioma. Otherwise, vascular peptides (endothelin, urotensin II, adrenomedullin et al) and cytokines (TNFα, TGFα, VEGF et al) may play an important role in regulation of blood pressure, cell proliferation, differentiation, apoptosis and pathogenesis of pheochromocytoma.

PL2 Update on the Management of Short Stature

Pinchas COHEN
Divison of Endocrinology and Diabetes, Department of Pediatrics, Mattel Children's Hospital at the University of California, Los Angeles, David Geffen School of Medicine at UCLA, Los Angeles CA 90095-1752, USA

Therapy for short children has continuously expanded in terms of the diagnostic criteria and now includes nearly a dozen different indications. Historically, GH treatment of short stature employed fixed, weight-based, GH dosing, however, in recent years, several alternative approaches have been proposed in order to optimize height outcome. The three general strategies that have been employed in this regard include: (1) Prediction model-based dosing, whereby baseline characteristics of patients determine the starting GH dose which remain fixed thereafter, (2) Auxology-based dosing, in which the GH dose is increased in response to poor growth velocity, and (3) IGF-based dosing, an approach that involves GH dose titration designed to achieve a desired IGF-1 SDS level. Recent randomized controlled studies have been performed to define potential improvements in GH treatment, including (A) dose response studies in GH-deficient children randomized to doses up to 100 mcg/kg/day of GH, (B) IGF-based dose titration studies in which short IGF-deficient (IGFD) children (with both GHD and ISS) were randomized to a fixed (40 mcg/kg/day) or variable doses of GH (designed to reach 0 SDS +1 SDS, or +2 SDS of IGF-1). The results of these studies clearly demonstrated that there is a dramatic variability in the growth response of both GHD and ISS children to GH therapy that is dependent on a variety of factors including the degree of GH deficiency and the degree of IGF deficiency. These studies also indicate that both the dose of GH and the serum IGF-1 levels achieved during GH therapy are critical determinants of treatment outcome. While children with severe GHD typically respond well to traditional low doses of GH, children with ISS/IGFD increase their height by an average of 0.8 SDS in the first year in response to doses of GH averaging 60 mcg/kg/day, achieved through an IGF-based dose titration protocol. In conclusion, in both GHD and non-GHD patients with short stature and IGFD, adjusting the GH dose to achieve an IGF-1 in the upper normal range results in a significant increase in growth. This new paradigm in growth hormone therapy appears to be a well-tolerated and effective treatment modality for children requiring GH, which may be combined with additional modalities for treatment optimization. Furthermore, monitoring IGF-1 levels during GH therapy is a tool for decreasing potential adverse events.

PL3 Management of Pituitary Tumors in the 21st Century

Edward R. LAWS, John A. JANE Jr.
Department of Neurosurgery, University of Virginia Health System, PO Box 800212, Charlottesville, VA 22908-0212, USA

The outcomes in a surgical series of over 4,000 pituitary tumors are presented.

Non-functioning pituitary adenomas most often present as macroadenomas and cause visual field deficits and hypopituitarism. Of patients with visual deficits, surgery improves visual loss in approximately 87%. Postoperative worsening of vision occurs in 4% of patients and in the remainder vision is unchanged. Twenty-seven percent of patients presenting with hypopituitarism experience postoperative recovery of hormone secretion. Operative mortality for these larger, and often, more invasive, tumors is higher than for the hyperfunctioning adenomas and reaches just over 1%. For similar reasons, tumor recurrence is also an issue. Ten-year recurrence/persistence rates are approximately 16%, although only 6% require reoperation. Long-term follow-up finds 83% of patients alive and well without evidence of disease.

Criteria for reporting remission from acromegaly require normalization of age-adjusted IGF-1 levels; random growth hormone less than 2.5 nanograms per milliliter (ng/ml); and nadir growth hormone during an OGTT of less than 1 ng/ml. Using these strict criteria, transsphenoidal surgery obtains remission in 88% of those with microadenomas and 55% of macroadenomas. Acromegalic symptoms are improved in 95%. Recurrence at ten years is less than 2%. Ninety-seven percent of patients have preserved normal pituitary function. Seventy-two percent of patients with greater than ten year follow-up, including those with adjunctive therapy, are alive and well without evidence of active disease.

Patients with prolactinomas who present for surgery are most often those who have failed medical management. Prolactin levels are normalized in 87% of patients with microadenomas and 56% of those with macroadenomas. The recurrence rate among those patients who are normalized after a transsphenoidal operation is 13% at ten years. Preserved pituitary function occurs in all but 3%.

Surgical management of Cushing's disease achieves a 91% remission rate for patients with microadenomas, but falls to 65% for those with macroadenomas. Although up to 12% of adults may experience recurrence after ten years, a higher percentage of children develop recurrence of Cushing's disease. Adjunctive radiosurgery has achieved remission in approximately 73% of patients whose disease either did not remit following surgery or recurred.

During this first century of transsphenoidal surgery for pituitary adenomas, significant technical refinements such as the introduction of endoscopy have occurred and have broadened the scope of this versatile approach. As we enter the next millennium, advances in medical knowledge and technology will continue in parallel and will usher in new, more effective treatments for this complex disease.

PL4 The International HapMap Project

Pak C. SHAM
Chair Professor in Psychiatric Genomics, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong

The Human Genome Project (HGP), which was initiated in 1990 with the goal of obtaining a complete sequence of all 3 billion base-pairs of the genomes of a few individuals, was completed in 2003. The International HapMap Project, a sequel to the HGP, was initiated in 2003 to characterize individual differences in DNA sequence. The recent completion of the Phase 1 of this project heralds a new era in human genomics research (The International HapMap Consortium, 2005). This collaborative Project between 6 countries (USA, Canada, UK, Japan, China, Nigeria) has created a detailed map of single-nucleotide polymorphisms (SNPs) of the human genome on four populations: Yoruba in Ibadan, Nigeria (YRI), Japanese in Tokyo (JPT), Han Chinese in Beijing (CHB), and individuals of European descent in Utah, USA (CEU). Phase 1 of the Map contains genotype information on over 1,000,000 SNPs, while Phase 2 will increase this number to 3,500,000. The Hong Kong HapMap Group, consisting of researchers from the University of Hong Kong, the Chinese University of Hong Kong, and the Hong Kong University of Science and Technology, contributed 2.5% of the genotyping of Phase 1 of the project. The group has genotyped over 20,000 SNPs on the four populations using the Sequenom Mass-Array system at the Genome Research Centre in the University of Hong Kong.

The HapMap will be a valuable resource for the search of genetic variation that influences predisposition to common diseases, or response to treatments. Central to the rationale behind the HapMap Project is the frequent occurrence of strong linkage disequilibrium (i.e. allelic association) between SNPs that are in physically proximity to each other. This means that the search for disease-related variants in a genomic region can be most efficiently done by initially screening a subset of SNPs that "capture" all the variations in the region. However, the pattern of linkage disequilibrium in the genome is highly variable and unpredictable, so that a random choice of SNPs could be both incomplete and redundant, while a preliminary study to find a set of representative SNPs would be time-consuming and costly. The availability of the Haplotype Map now allows investigators to confidently select SNPs to study the genomic region of interest. As well as providing a resource for genetic studies of human diseases, the HapMap also contains data that might throw light on phenomena such as recombination, drift, mutation and selection in the human genome.

The HapMap does have some limitations. First, the study includes only a small number of human populations, and the extent to which the maps can be generalized to other populations remains uncertain. Second, the project necessarily emphasized common variants, and it is unclear to what extent rare variants can be tagged by common variants, and how important they are for common diseases.

The International HapMap Consortium. The Haplotype Map of the Human Genome. Nature 2005;437:1299-320.

PL5 Adipokines and the Metabolic Syndrome

Lee-ming CHUANG
Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan

The metabolic syndrome (MS) is a common disorder characterized by a clustering of a variety of atherogenic risk factors, including visceral obesity, hypertriglyceridemia, low HDL-cholesterol level, high blood pressure, and high fasting blood sugar level. Different criteria of the MS appear in recent years with special emphasis on visceral obesity by IDF in 2005. Recent studies show that white adipose tissue is an endocrine organ by secreting a variety of adipokines, including leptin, adiponectin and others, in addition to the role of lipid storage. Among the factors secreted from adipocytes, adiponectin has been shown to be strongly correlated with various clinical phenotypes of metabolic syndrome, suggesting its role in pathogenesis of the metabolic syndrome. Moreover, lifestyle intervention and pharmacological intervention of the metabolic syndrome have been shown to be correlated with beneficial adiponectin-raising effect. In this presentation, I will selectively discuss the biology of adiponectin and its role in pathogenesis of MS and its potential use in clinical medicine.

PL6 Aldosterone, Cardiovascular Damage and the New Biology of Mineralocorticoid Receptors

J.W. FUNDER
Prince Henry's Institute of Medical Research, Clayton Road, Clayton 3168, Victoria, Australia

In progressive heart failure spironolactone added to standard of care produced a 30% improvement in survival, and 35% less hospitalization.[1] In heart failure post myocardial infarction, the selective mineralocorticoid receptor (MR) antagonist eplerenone similarly proved efficacious,[2] and in essential hypertension reduced cardiac hypertrophy and proteinuria.[3] Although in experimental mineralocorticoid/salt hypertension eplerenone prevented/reversed vascular inflammation and perivascular fibrosis, in the trials cited above[1-3] baseline plasma aldosterone levels are low normal and sodium status unremarkable, calling into question how MR blockade is so clinically efficacious. MR are unusual in that they have equivalent high affinity for aldosterone and glucocorticoids, which circulate at >1000-fold higher concentrations. In epithelial aldosterone target tissues (and the vascular wall) they are 'protected' by the enzyme 11β hydroxysteroid dehydrogenase, which lowers intracellular cortisol (to levels still x10 those of aldosterone) and stoichiometrically generates NADH in converting cortisol to receptor inactive cortisone. Cortisol-occupied MR are normally inactive, but are activated when intracellular redox status changes, by 11βHSD blockade or reactive oxygen species generation. Under physiologic conditions MR are thus largely (epithelia, vascular wall) or overwhelmingly occupied—but not activated—by normal circulating concentrations of glucocorticoids. Cortisol is thus a bivalent ligand for MR, and overwhelmingly likely as the candidate pro-inflammatory activator of MR in hypertension and heart failure. MR blockade may thus prove efficacious not only in those conditions, but in other inflammatory disorders such as atherosclerosis[4] or autoimmune disease.[5]

References

1. Pitt B, Zannad F, Remme WJ, et al. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. N Engl J Med 1999;341:709-17.
2. Pitt B, Remme W, Zannad F, et al. Eplerenone, a selective aldosterone blocker, in patients with left ventricular dysfunction after myocardial infarction. N Engl J Med 2003;348:1309-21.
3. Pitt B, Reichek N, Willenbrock R, et al. Effects of eplerenone, enalapril, and eplerenone/enalapril in patients with essential hypertension and left ventricular hypertrophy: the 4E-left ventricular hypertrophy study. Circulation 2003;108:1831-8.
4. Takai S, Jin D, Muramatsu M, Kirimura K, Sakonjo H, Miyazaki M. Eplerenone inhibits atherosclerosis in nonhuman primates. Hypertension 2005;46:1135-9.
5. Gottlieb B, Laurent LP. Spironolactone in the treatment of myasthemia gravis. Lancet 1961;2:528-9.

PL7 Novel Role of GnRH-II in Reproduction

Peter C.K. LEUNG
Department of Obstetrics and Gynecology, University of British Columbia, Vancouver, Canada

The classical form of GnRH (GnRH-I) is a decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2) that plays a key regulatory role in mammalian reproduction. Recent studies have identified a distinct gene encoding a second form of GnRH, in humans and other primates. This second form of human GnRH (GnRH-II), which was first identified in the chicken hypothalamus, differs from GnRH-I by three amino acid residues at positions 5, 7, and 8. As GnRH-II has been conserved throughout vertebrate evolution, it has been proposed that this form of GnRH is biologically significant. In contrast to GnRH-I, GnRH-II is expressed primarily in extra-pituitary tissues and appears to have a limited regulatory effect on gonadotropin secretion.

The receptor for GnRH-I (type-I GnRHR) belongs to the rhodopsin-like G-protein coupled receptor superfamily, members of which contain a characteristic 7-transmembrane-domain structure. The detection of two distinct forms of GnRH in human tissues would suggest that multiple forms of the GnRHR are also present. Indeed, two genes encoding a second GnRHR have been identified in the human genome. However, one of these genes encodes an antisense mRNA transcript whereas the second contains a stop codon has been identified in the nucleotide sequence of exon of this other putative GnRHR-II gene. In view of these observations, it is currently accepted that the type-I GnRHR is the mutual receptor of GnRH-I and GnRH-II.

In the human ovary, we have shown that the expression of GnRH-I, GnRH-II and GnRHR is temporally and spatially specific. Immunohistochemical staining indicated that both forms of hormones and the receptor were predominantly localized to the granulosa cells of pre-ovulatory follicles and corpus luteum. GnRH-I exerts an anti-steroidogenic effect in human granulosa cells, i.e. it inhibits gonadotropin-induced progesterone accumulation. Similarly, treatment of granulosa cells with GnRH-II attenuates gonadotropin-stimulated progesterone production. The action of GnRH-I and GnRH-II are mediated via a PKC- and Erk-dependent signal transduction mechanism. Treatment of granulosa cells with GnRH-I produces a biphasic response in its own mRNA level such that high concentrations decrease the GnRH-I mRNA level, while low concentrations have the opposite effect. In contrast, treatment with GnRH-II results in a homologous down-regulation of its mRNA level at all concentrations examined. The expression of GnRH-I and GnRH-II is regulated differentially by FSH and LH/hCG such that gonadotropins increase GnRH-II mRNA levels but decrease those of GnRH-I in granulosa cells. GnRH-I and GnRHR mRNA levels are down-regulated by 17β-estradiol, while estradiol increases GnRH-II gene expression. These observations strongly implicate an autocrine role, particularly for GnRH-II, on ovarian hormone production and follicular development.

In the human placenta, GnRH-I has been localized to the villous cytotrophoblasts, syncytial trophoblast and extravillous trophoblast (EVT) columns of the human placenta and in enriched populations of these distinct trophoblastic cell types. To date, the major function of GnRH-I in the human placenta is believed to be the autocrine/paracrine regulation of hCG production. These regulatory effects appear to be receptor-mediated as GnRH-antagonists are capable of inhibiting the stimulatory actions of both GnRH-I and its agonists in cultured placental explants. In addition, GnRH-I and its agonists have been shown to increase the secretion of prostaglandin E2 and F2a, P4, E2 and estriol by chorionic villous explants in vitro. As all of these hormones play key roles in the decidualisation of endometrial stromal cells, and both GnRH and E2 have been shown to inhibit the differentiation of villous cytotrophoblasts along the non-invasive pathway, these observations support our hypothesis that GnRH-I plays a key regulatory role in the functional maturation of both the human placenta and decidua. Recently, we have determined that GnRH-II expression is restricted to the subpopulations of mononucleate cytotrophoblasts (villous and extravillous) of first trimester chorionic villi. It appears that GnRH I and GnRH II are key regulators of the proteolytic mechanism operative in highly invasive EVTs in vitro. In particular, we have determined that GnRH-I and to a greater extent, GnRH II regulate the balance between uPA/PAI-1 and MMP-2/MMP-9/TIMP-1 in these cells. In addition, we have preliminary evidence that GnRH-I and GnRH-II potentiate the invasive capacity of these cells.

We have determined that GnRH-I and GnRH-II mRNA and protein expression levels are significantly higher in decidual tissues obtained during the first trimester of pregnancy than in secretory endometrium. Similarly, GnRH-I and GnRH-II mRNA levels were significantly higher in highly enriched populations of stromal cells isolated from the deciduas. GnRH-I and GnRH-II increased uPA mRNA and protein expression levels in decidual stromal cells isolated from first trimester decidual tissues in a dose- and time-dependent manner. In contrast, GnRH-I increased whereas GnRH-II decreased PAI-1 mRNA and protein expression levels in these primary cell cultures. Taken together, these observations strengthen our hypothesis that the direct biological actions of GnRH-I and GnRH-II on the human placenta and endometrium regulate the coordinated development of the maternal and fetal cellular compartments and that altered expression levels of these hormones result in dysynchrony and an unfavourable pregnancy.

(Supported by the Canadian Institutes for Health Research)

PL8 Relationship between Insulin Resistance and Hypertension

Guang-wei LI
China-Japan Friendship Hospital , Beijing 100029, China

Hypertension is the most important risk factor for CHD and stroke in the Chinese population. However it is not easy treating it to target level with the antihypertensive medications available. The IDF global guidline (2005) for blood pressure control in diabetics even asked people to accept the fact that in a number of people blood pressure will not reach 140/80 mm Hg after taking three to five antihypertensive drugs. Thus it is not unreasonable to find some other cause for hypertension in order to improve blood pressure control. One of the first studies supporting an association between insulin resistance and hypertension was reported by Welborn in 1966. Subsequently Ferrari et al provided evidence that genetic predisposition to essential hypertension coexists with insulin resistance in 1991.

Our study in offspring of hypertensive family showed that insulin sensitivity index 1/(FINS*FPG) in first and second degree relatives of hypertension is only 57% and 85% compared with controls, and family history of hypertension is the most strong contributor to insulin sensitivity of the fist degree relatives (account for 18% of insulin sensitivity change). Family history of hypertension may or may not inherit hypertension in the offspring because of the different subtype of G protein β3 background, however it inherits insulin resistance and related abnormalities including higher level of plasma glucose, triglyceride, and lower HDL-c. Subtype of GNB3 C825T significantly correlated to insulin sensitivity index and SBP. If fasting insulin level increased from 12 to 32 mu/l , SBP will increase from 132 to 151 mm Hg and DBP will increase from 85 to 94 mm Hg in subjects with CT and TT subtype of GNB3. In overweight and/or obese non-diabetic subjects with essential hypertension who received Rosiglitazone (8 mg/day) for four weeks, SBP reduced by 17 mm Hg and DBP reduced by 11 mm Hg (n=90). Results of this study is concordant with some other reports in Diabetes Care 2003-2005. Although these studies are not randomized, it more or less suggested improving insulin sensitivity may play an important role in the management of essential hypertension. 12 month results of the RECORD study demonstrated that adding rosiglitazone to metformin or sulphonylurea in type 2 diabetes induced clinical and statistical reduction of blood pressure. In a recent clinical trial, our study group also found that adding rosiglitazone to metformin or sulphonylureas causes significant blood pressure reduction in type 2 diabetes with hypertension (SBP reduced by 11 mm Hg, DBP reduced by 6 mm Hg).

As for insulin resistance per se or its compensated hyperinsulinaemia induces blood pressure elevation, our studies support that the offender is hyperinsulinaemia. Unpublished data of the Da-Qing Study showed it is the insulin but not insulin sensitivity at baseline predicted blood pressure changes. IGT subjects moved to the either direction of reversing to NGT or worsening to diabetes showed significant reduction of blood pressure independent of body weight change also suggests decreasing of insulin may be the cause of blood pressure reduction in the study population.

Someone had suggested in 1995: the management of hypertension in obese subjects should include lifestyle intervention, improve insulin sensitivity and standard blood pressure lowering medication.

PL9 Vascular Dysfunction in Diabetes

Kathryn C.B. TAN
Department of Medicine, University of Hong Kong, Hong Kong

The macro- and microvascular complications of diabetes are the major causes of morbidity and mortality in patients with diabetes. It has been shown that endothelial dysfunction plays an important role in the pathogenesis of both micro- and macroangiopathy in diabetes. Endothelial dysfunction has been consistently demonstrated in patients with type 1 or type 2 diabetes and pre-dates the development of vascular complications. There is endothelial cell activation, reduction of vascular nitric oxide bioavailability, impairment of flow-mediated vasodilation and increased vascular stiffness. The aetiology of endothelial dysfunction in diabetes is complex with multiple mechanisms operating in concert to induce both functional and structural abnormalities in the vessels. Hyperglycaemia leaves a long-term imprint on the vessel wall and adversely affects endothelial cell function by increasing oxidative stress, enhancing the formation of advanced glycation end products and inducing chronic subclinical inflammation. Conventional risk factors like dyslipidaemia, hypertension and smoking also significantly contribute to the pathogenesis of endothelial dysfunction in diabetes. Improving our understanding of the complex mechanisms involved in the development of endothelial dysfunction in diabetes will enable new therapeutic strategies to be developed.

PL10 Changing Epidemiology and Treatment Strategies in Osteoporosis

Keh-sung TSAI
Department of Laboratory Medicine and Internal Medicine, National Taiwan University College of Medicine, Taipei, Taiwan

The prevalence of postmenopausal osteoporosis in the aging females and senile osteoporosis in the two genders are rapidly growing in the world and much more so in the Chinese societies. Surveys in the past two decades showed substantial variations in the incidence rate of hip fractures in different Chinese populations. Most likely, these variations are a reflection of the degree of urbanization and industrialization. The incidences of hip fractures in Chinese populations in Taiwan and Hong Kong are already among the highest, and the case numbers are still increasing, mainly because of the aging trends of the populations.

Various medical regimens of osteoporosis showed strong evidences to reduce the fracture risks in the past decades. In Chinese population, these drugs seemed equally effective. Yet, because of the different level of economical establishment, somewhat different strategies should be applied in Chinese populations. Currently, a new diagnosis and management guideline based on the 10-year absolute fracture risk is under construction. With some minor modification, this guideline is supposed to be useful for all the countries. There should be an opportunity for us to develop and validate a guideline for the Chinese populations.

S1.1 Genetic and Clinical Study of Hereditary Endocrine and Metabolic Diseases

Guang NING
Shanghai Clinical Center for Endocrine and Metabolic Diseases, Department of Endocrinology and Metabolism, Rui-Jin Hospital, Shanghai Jiao-Tong University School of Medicine, Shanghai 200025, China

Objective: To investigate the genetic defects and the mechanism for hereditary endocrine diseases. **Methods:** Sequencing, in vitro expression, epigenetic approaches, computer modeling and functional analysis were adopted to study the association of genotype with phenotype. **Results:** (1) The database of tissue bank, DNA and serum samples from the patients with hereditary endocrine diseases was built. (2) 21 hereditary endocrine diseases were recruited in the database and 19 gene mutations were detected, 14 of which were novel gene defects which had not been previously reported. (3) A novel locus on chromosome 20p13 responsible for a familial autosomal dominant neurohypophyseal diabetes insipidus (ADNDI) was identified in a diabetes insipidus family. (4) A correlation between phenotype and genotype was established in a 17OHD family based on functional study and computer modeling. **Conclusion:** A translational medicine from bench to clinical work was realized based on genetic analysis platform.

S1.2 Diagnosis of Inborn Errors of Metabolism

Xiao-ping LUO

(Abstract not received at the time of printing)

S1.3 Clinical Approach to Carnitine Deficiency Disorders

Jia-woei HOU
Department of Medical Genetics, Pediatric Metabolism and Endocrinology, Chang Gung Children's Hospital, Taoyuan, Taiwan

Carnitine (β-hydroxy-γ-trimethylaminobutyric acid) plays an essential role in the transfer of long-chain fatty acids across the inner mitochondrial membrane. This transfer requires enzymes and transporters that accumulate carnitine within the cell (OCTN2 carnitine transporter), conjugate it with long chain fatty acids (carnitine palmitoyl transferase 1, CPT1), transfer the acylcarnitine across the inner plasma membrane (carnitine-acylcarnitine translocase, CACT), and conjugate the fatty acid back to coenzyme A for subsequent beta oxidation (carnitine palmitoyl transferase 2, CPT2). These carnitine-cycle defects will impair the fatty acid oxidation in mitochondria with multi-organ involvement. Secondary free-carnitine deficiency, more common seen, is often a causative factor of metabolic crises in certain organic acidemias/acidurias.

Deficiency of the OCTN2 carnitine transporter causes primary carnitine deficiency, characterized by increased losses of carnitine in the urine and decreased carnitine accumulation in tissues. Patients can present with Reye-like illness (hypoketotic hypoglycemia and hepatic encephalopathy), or with skeletal and cardiac myopathy. This disease responds to carnitine supplementation dramatically. Recurrent attacks of fasting hypoketotic hypoglycemia are the initial features of defects in the liver isoform of CPT1. These patients have elevated levels of plasma carnitine. The heart and the muscle are usually unaffected. CACT deficiency presents in most cases in the neonatal period with hypoglycemia, hyperammonemia, and cardiomyopathy with arrhythmia leading to early infant death. Plasma carnitine levels are extremely low. Deficiency of CPT2 present more frequently in adults with rhabdomyolysis triggered by prolonged exercise. More severe variants of CPT2 deficiency present in the neonatal period similarly to CACT deficiency associated or not with multiple congenital anomalies.

Diagnosis of various forms of carnitine deficiency disorders depend on the assay of the plasma or urine acylcarnitine profile by urinary/plasma/tissue acylcarnitine profiles, or the newer tandem mass spectrometry using small volumes of plasma or filter paper blood spots. Cultured skin fibroblasts and cultured lymphoblasts have been used to measure the in vitro activities of specific steps in the fatty acid-oxidation pathway. All the genetic disorders of carnitine-cycle defects are inherited in autosomal-recessive fashion. The mutations in homozygous patients and heterozygous carriers could be detected by direct DNA sequencing on related genes such as *SLC22A5* (*OCTN2*), *CPT1*, *CPT2*, and *CACT*. Treatment for these patients consists in L-carnitine supplement, a low-fat diet along with medium chain triglycerides that can be metabolized by mitochondria independently from carnitine, and avoidance of fasting and sustained exercise.

S1.4 Citrin Deficiency—an Old Adult Disease with Newly Defined Pediatric Manifestations

P.T. CHEUNG
Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Hong Kong

Type II citrullinaemia (CLTN2 OMIM 603471), first described in 1968, is predominantly identified in Japanese adults classically presenting with recurrent hyperammonaemic encephalopathy and could be successful treated by liver transplantation. The primary defect was secondary hepatic argininosuccinate synthetase deficiency resulting in the urea-cycle-defect phenotype. Studies of informative families revealed an autosomal recessive inheritance and in 1999, genetic linkage studies led to the cloning of the responsible gene SLC25A13 on chromosome 7, encoding a mitochondrial aspartate-glutamate carrier named citrin. Interestingly, the same genetic defects are found responsible for another distinct phenotype in babies, designated neonatal intrahepatic cholestasis caused by citrin deficiency (NICCD), in 2001. Subsequently an extended range of clinical phenotypes have been unraveled in children including hypergalactosaemia, hypermethioninemia, hypertyrosinemia, hypoglycaemia and failure to thrive. Likewise, specific genetic diagnosis has helped revealing variant adult citrin deficiency phenotypes including juvenile onset chronic pancreatitis, non-cirrhosis related hepatocellular carcinoma and non-alcoholic hepatic steatosis.

In Hong Kong, we diagnosed the first child with NICCD initially referred for potential liver transplantation in April 2005, caused by compound heterozygote mutations with a known mutation (1638-1660dup – the third commonest in Chinese) and a novel 22.7 kb deletion involving the 3' regions of both the SLC25A13 and flanking cytoplasmic dynein intermediate chain 1 gene (DNCI1) genes. Till now, over 12 paediatric patients have been diagnosed through prospective workup of new patients, re-investigation of old patients with compatible clinical problems, retrospective review of positive plasma amino acid results from clinical biochemistry laboratory and genetic analysis of archival liver biopsy samples. However, only 2 adult CLTN2 patients have so far been identified through literature search and review of liver transplantation record at Queen Mary Hospital.

Recent genetic mutation analysis[1] revealed that the heterozygote carrier rate is alarmingly high in South-East Asia – 1/69 in for Japanese, 1/48 for Southern Chinese (1/940 for Northern Chinese) and 1/112 for Korean. Theoretically the homozygous rate for Southern Chinese could be about 1:10000, which translates into as many as 700 affected subjects in Hong Kong and 50,000 subjects in Southern China (assuming a population of 0.5 billion). More affected subjects are thus expected in Hong Kong. It is also important for clinicians looking after patients from these regions in particular to watch out for citrin deficiency based on the classic as well as variant phenotypes.

Reference

1. Lu YB, Kobayashi K, Ushikai M, et al. Frequency and distribution in East Asia of 12 mutations identified in the *SLC25A13* gene of Japanese patients with citrin deficiency. J Hum Genet 2005;50:338-46.

S2.1 Ultrasound-guided Intraparenchymal Implantation of Plasmid-electroporated Primary Hepatocytes Function as Autologous Insulin-secreting Bioimplants: Evidence of Metabolic Correction and Delayed Secondary Complications in a Pre-clinical Porcine Model of Diabetes Mellitus

Nelson K.F. CHEN[1], Jen-san WONG[1], Irene H.C. KEE[2], Siang-hui LAI[3], Choon-hua THNG[4], Wai-har NG[1], Robert T.H. NG[2], Jaichandran SIVALINGAM[1], Jason VILLANO[2], Pierce K.H. CHOW[2], Oi-lian KON[1]
[1] Division of Medical Sciences, National Cancer Centre, Singapore
[2] Department of Experimental Surgery, Singapore General Hospital
[3] Centre for Forensic Medicine, Health Sciences Authority of Singapore
[4] Department of Oncologic Imaging, National Cancer Centre, Singapore

Gene- and cell-based approaches are emerging strategies for diabetes treatment. Directed differentiation of stem/progenitor cells into β-cells is widely regarded as promising, but the feasibility of modifying adult somatic cells has been questioned. In a pre-clinical porcine diabetes model, we electroporated autologous primary hepatocytes isolated from surgically resected liver wedges with a human insulin plasmid construct and immediately performed ultrasound-guided intraparenchymal reimplantation. This bioimplant significantly corrected hyperglycemia and glucose intolerance in a 'dose-dependent' manner for at least 8 months. Treated swine showed glucose-stimulated human C-peptide secretion without evidence of porcine β-cell regeneration. Fluctuations in ambient glucose concentration appropriately altered human insulin cDNA transcription and human C-peptide secretion within minutes. Treatment halted or delayed the onset of ocular, microvascular and macrovascular complications assessed by light and electron microscopy of retinae, kidneys and carotid arteries. Transcriptomes of aortae, retinae, kidneys and livers showed significant correction of dysregulated gene expression in treated animals.

S2.2 Islet Transplantation

Jyuhn-Huarng JUANG
Division of Endocrinology and Metabolism, Department of Internal Medicine, Chang Gung University and Memorial Hospital, Taoyuan, Taiwan

In diabetes, insulin secretion is either completely absent (type 1) or inappropriately regulated (type 2). In contrast to intensive insulin treatment, transplantation of insulin-producing tissue, an attractive alternative, offers a more physiological approach for precise restoration of glucose homeostasis, thereby may reverse the metabolic and neurovascular complications of diabetes. Compare to the whole or segmental pancreas transplantation, replacement of endocrine pancreas is more physiological and has the following advantages: it is simpler and safer for the recipient, it can be repeated several times, islets can be tested and manipulated before implantation, and islet banking can be performed by cryopreservation.

From 1990 through 2004, a total of 941 adult islet allotransplantations have been performed worldwide. In 237 pretransplant C-peptide negative patients with type 1 diabetes mellitus who received adult islet allograft between 1990 and 1999, one-year patient and graft survival (as defined by basal C-peptide ≥0.5 ng/ml) rates were 96% and 41%, respectively, and 11% of the recipients were insulin independent at one year posttransplant. The success enhanced by the Edmonton protocol has fostered phenomenal progress in the field of clinical islet transplantation in the past 5 years, with 1-year rates of insulin independence after transplantation near 80%. Long-term function of the transplanted islets, however, even under the Edmonton protocol, seems difficult to accomplish, with only 10% of patients maintaining insulin independence 5 years after transplantation. These results differ from the higher metabolic performance achieved by whole pancreas allotransplantation, and autologous islet cell transplantation, and form the basis for a limited applicability of islet allografts to selected adult patients.

Candidate problems in islet allotransplantation deal with alloimmunity, autoimmunity, and the need for larger islet cell masses. Employment of animal islets and stem cells, as alternative sources of insulin production, will be considered to face the problem of human tissue shortage. A new regenerative hypothesis envisions that the endogenous pancreas maintains the ability to resupply, at a very low pace, new insulin producing cells to compensate for the beta-cell mass lost as a consequence of autoimmune or other toxic injury. The favorable conditions and the extent of this phenomenon are still mostly unknown and require careful investigation. Whether the potential of self-healing process proofs critical to be exploited as a possible curative, novel therapy for type 1 diabetes, it is not clear but offers very exciting prospectives. In this contest, a role of islet transplantation, either as provider of possible trophic factors or to relief the diabetic individual from insulin demand during the phase of self-regeneration of the insulin producing cells, as shown in animal experiments, could still be critically important. All together, islet cell transplantation is moving forward.

S2.3 Berberine in the Treatment of Diabetes

Ming-dao CHEN, Li-bin ZHOU, Ying YANG, Xiao-ying LI, Guang NING, et al
Ruijin Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai 200025, P.R. China

Berberine is one of the major constituents of Chinese herb Rhizoma coptidis which has been used to treat diabetes mellitus for more than one thousand years. During recent years, we have explored the metabolic effects and its mechanisms of berberine *in vivo* and *in vitro*. In a randomized, placebo-controlled, multi-center clinical trail, oral administration of berberine for 3 months in newly diagnosed type 2 diabetes significantly decreased body mass index, fasting and 2h plasma glucose after glucose loading, HbA1c, triglycerides, total cholesterol and LDL-C. In high fat diet-fed insulin resistant SD rats, treatment with berberine for 6 weeks significantly reduced body weight, epididymal fat pads weight, plasma glucose, fasting and 2h serum insulin after glucose loading, plasma triglycerides and free fatty acid. However, in isolated rat islets, berberine did not stimulate insulin secretion, suggesting that glucose lowering effect of berberine seems to be through promoting glucose uptake in peripheral tissue. Subsequently, we found that berberine stimulated glucose uptake in 3T3-L1 adipocytes in a dose- and time-dependent manner with the maximal effect at 12 h. Glucose uptake was increased by berberine in 3T3-L1 preadipocytes as well as C2C12 myotubes along with increasing of glucose consumption, which was additive to that of insulin in 3T3-L1 adipocytes, even at the maximal effective concentrations of both components. Besides, the reduction ofa insulin-stimulated glucose uptake by TNF-α and palmitate was antagonized by berberine. Unlike insulin, the effect of berberine on glucose uptake was insensitive to wortmannin, an inhibitor of PI 3-kinase, and SB203580, an inhibitor of p38MAPK. Berberine activated ERK1/2, but PD50985, a MEK inhibitor, only partially decreased berberine-stimulated glucose uptake. Berberine did not induce Ser-473 phosphorylation of Akt, nor enhance its insulin-induced phosphorylation. Although the expression and cellular localization of GLUT4 were not altered by berberine, nor GLUT1 gene expression, genistein, a tyrosine kinase inhibitor, did completely block berberine-stimulated glucose uptake in 3T3-L1 cells, suggesting that its role in glucose transport via increasing GLUT1 activity. Berberine inhibited 3T3-L1 adipocytes differentiation, PPARγ expression, and isoprenal-sitmulated lipolysis while enhanced AMP-activated protein kinase (AMPK) and acetyl-CoA carboxylase (ACC) phosphorylation. All these findings demonstrate that berberine increases glucose uptake through a mechanism distinct from insulin and activated AMPK appears to be involved in the metabolic effect of berberine.

S2.4 Renin-angiotensin System Blockade as a Novel Treatment Strategy for Diabetes

Po-sing LEUNG
Department of Physiology, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China

Recent clinical trials have shown that blockade of the renin-angiotensin system (RAS) is protective against the new onset of type 2 diabetes mellitus (T2DM) in high-risk individuals, such as those with hypertension or chronic heart failure. Moreover, a recent report of a meta-analysis of these randomized clinical studies concluded that the mean weighted relative risk for T2DM development was reduced by 25% in those patients treated with angiotensin II receptor blockers and/or angiotensin-converting enzyme inhibitors. In spite of these firm clinical data, the mechanistic mechanism(s) mediating the protective activity of RAS blockade have yet to be resolved. Of particular interest is the recently identified local pancreatic RAS and, perhaps more importantly, the finding that it becomes upregulated in animal models of T2DM and islet transplantation. This putative local RAS regulates pancreatic islet blood flow, oxygen tension, and islet (pro)insulin biosynthesis. It may also have a role in NADPH oxidase-mediated reactive oxygen species generation, thereby causing oxidative stress-induced pancreatic beta-cell apoptosis and fibrosis. Moreover, findings that RAS blockade improved beta cell secretory function and beta cell mass in T2DM experimental animals indicate that inhibition of the RAS activation may play a pivotal role in protecting pancreatic islet cell function and furthermore may prevent the development of overt T2DM. Such data support the involvement of the local pancreatic RAS in islet function and islet transplantation. Thus RAS blockade could contribute to the development of a novel therapeutic strategy in the prevention and treatment of patients with diabetes.

S3.1 Optimal Gonadotrophin Regimen for Assisted Reproduction

Ernest Hung-yu NG
Department of Obstetrics and Gynaecology, The University of Hong Kong, Hong Kong SAR, China

Recruitment and development of multiple follicles in response to gonadotrophin stimulation are the key factors leading to a successful outcome of assisted reproduction treatment such as the *in vitro* fertilization (IVF). Poor ovarian response may be associated with poor pregnancy rates while exaggerated ovarian response leads to an increased risk of ovarian hyperstimulation syndrome and impaired IVF success. Optimal gonadotrophin regimen should be tailed to the need of individual patients according to their ovarian reserve.

Different gonadotrophins are also associated with comparable pregnancy rates. Gonadotrophin releasing hormone agonists and more recently antagonists are now routinely employed to prevent premature LH surges, which occur in about 20% of the IVF cycles using gonadotrophin alone. There is no difference in the live birth rate between the use of agonists and antagonists. When the agonists are used in a long protocol, the pregnancy rate is significantly higher than that used in a short protocol. The role of LH supplementation will also be discussed.

S3.2 Paracrine Regulation of Folliculogenesis

Wei GE
Department of Biology, The Chinese University of Hong Kong, Hong Kong

In vertebrates, the pituitary gonadotropins—follicle-stimulating hormone (FSH) and luteinizing hormone (LH)—play vital roles in controlling ovarian development and function. However, it is widely believed that the activities of FSH and LH are mediated or modulated by a variety of locally produced peptide or protein factors that form an intimate regulatory network within and between the ovarian follicles. As a popular vertebrate model for genetic and developmental studies, zebrafish has caught tremendous attention in the past two decades; however, its utility has quickly been extended to other areas including physiology. In the past few years, a variety of growth factors have been identified and characterized in the zebrafish ovary including activin and epidermal growth factor (EGF), and lines of evidence from our laboratory and others point to the existence of an ovarian network of communication involving these factors. We have demonstrated that activin subunits are predominantly expressed in the somatic follicle cells whereas activin receptors and its intracellular signaling proteins are abundantly expressed in the oocyte, suggesting an activin-mediated follicle cell-to-oocyte signaling mechanism in the zebrafish ovarian follicle. In contrast, EGF and EGF-like peptides such as transforming growth factor α (TGFα) and betacellulin (BTC) are predominantly expressed in the oocyte, whereas their common receptor EGFR is exclusively expressed in the follicle cells, suggesting that the members of EGF family may serve as the signaling molecules from the oocyte to control the function of somatic follicle cells. Further experiments in our laboratory have shown that EGF works on the follicle cells by regulating the expression of activin and its binding protein follistatin. Evidence has also been obtained in our laboratory for roles of activin and EGF systems in mediating pituitary gonadotropin actions in the zebrafish ovary.

S3.3 Luteinizing Hormone Receptor Mutations as a Cause of Infertility

Wai-yee CHAN
Laboratory of Clinical Genomics, National Institute of Child Health and Human Development, Bethesda, MD and Department of Pediatrics, Georgetown University, Washington, DC, USA

Objective: To understand the molecular genetics of human luteinizing hormone receptor (hLHR) mutations in infertility. **Methods:** Mutations were determined by genomic DNA sequencing. Biological and genomic effects of mutated hLHR were studied in cultured cells. **Results:** Disruption of LH signaling due to mutation of the hLHR causes infertility. There are two groups of disease-causing mutations. Inactivating mutations lead to the development of Leydig Cell Hypoplasia (LCH), an autosomal recessive disorder in males. Homozygous males are infertile. Disease phenotype varies from hypogonadism to male pseudohermaphroditism. These patients have elevated plasma levels of LH and low levels of testosterone that do not respond to hCG stimulation. Various forms of mutations of the hLHR have been identified in LCH patients. These mutations are not localized and cause variable degree of loss of receptor activity which correlates with the severity of the disease. Homozygous inactivating mutations of the hLHR in females cause hypogonadism, cystic ovaries and infertility. Activating mutations of the hLHR result in Familial Male-limited Precocious Puberty (FMPP). Abnormal germ cells had been identified in some FMPP patients. Transgenic animal studies showed that founder mice expressing LHR carrying an activating mutation homologous to that (Asp578His) identified in the hLHR of patients with testicular tumor were infertile. Expression profiling of cells expressing the different activating hLHR mutants revealed differential genomic effect of the mutations. There are also a number of LCH and FMPP patients without identifiable mutation in the hLHR gene. **Conclusions:** Inactivating mutations of the LHR cause subnormal or absent production of testosterone resulting in subnormal sexual development and infertility. Activating mutations may also result in reduced fertility.

S3.4 Nuclear Orphan Receptor, COUP-TFII, in Development and Diseases?

Sophia Y. TSAI[1], Isao KURIHARA[1], Dong-kee LEE[1], Vincent Cheng-tai YU[1], Norio TAKAMOTO[2], Francesco J. DEMAYO[1] and Ming-Jer TSAI[1]
[1] Department of Molecular and Cellular Biology and Program of Developmental Biology, Baylor College of Medicine
[2] Okayama University Medical School, Japan

COUP-TFII is a member of the orphan nuclear receptor that belongs to the nuclear receptor superfamily. Genetic ablation of COUP-TFII results in early embryonic lethality due to cardiovascular defects. COUP-TFII is highly expressed in the uterine stroma and COUP-TFII +/- females show significantly reduced fecundity, irregular estrus cycles, delayed puberty and retarded postnatal growth, likely contributed by reduced decidualization that affect embryo implantation. In addition, we showed that COUP-TFII mediates Indian hedgehog (Ihh) signaling in the uterus. Conditional ablation of COUP-TFII in the uterus phenocopies the defects exhibited by the conditional ablation of Ihh or PR, resulting in infertility due to disruption of implantation and decidualization. We also showed that the expression of estrogen receptor target genes in the epithelium is enhanced in the absence of COUP-TFII. Our results not only indicate that COUP-TFII expression in the stroma is under the control of Ihh secreted from the epithelium, it also reveals that COUP-TFII mediates hedgehog/PR signaling to inhibit estrogen receptor function and provides a window of receptivity for embryo implantation.

S4.1 Melanocortin Receptors and Obesity in Humans

Ya-xiong TAO
Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL 36849, USA

The neural melanocortin receptors, melanocortin-3 and -4 receptors (MC3R and MC4R), have been shown to regulate different aspects of energy homeostasis in rodents. Whereas MC4R regulates food intake and energy expenditure, MC3R does not regulate food intake. Though still incompletely understood mechanism, MC3R regulates energy storage. Human genetic studies showed that mutations in the MC4R gene are the most common monogenic form of obesity. More than 90 distinct mutations have been reported from various cohorts. Functional analyses of the mutant receptors revealed multiple defects, including cell surface expression, ligand binding and signaling. We have proposed a classification scheme to catalogue the ever-increasing array of MC4R mutations. Functional analysis of the four inactivating MC3R mutations will also be summarized. These results suggest that MC3R mutations might be a rare cause of monogenic obesity.

S4.2 Adiponectin Enhances GNRH Secretion in GT1-7 Cells

Xiao-ying LI, Jun YANG, Hui-jie ZHANG, Guang NING
Shanghai Clinical Center for Endocrine and Metabolic Diseases, Department of Endocrinology and Metabolism, Rui-Jin Hospital, Shanghai Jiao-Tong University School of Medicine, Shanghai 200025, China

Objective: To investigate the adipokine-brain inter-talk and its potential role in hypogonadotropin hypogonadonism, PCOS and other disorders. **Methods:** RT-PCR and immunocytochemistry was adopted to detect adiponectin receptor expression in hypothalamus and GnRH cell line, GT1-7. LHRH secretion was measured in the medium of GT1-7 cells treated with globular and full length adiponectin expressed in *E. coli*. Furthermore serum adiponectin was determined in patients with HH and PCOS. **Rusults:** Adiponectin receptor type 1 and 2 were expressed in hypothalamus and GT1-7 cells. Adiponectin enhanced GnRH secretion from GT1-7 cells by raising cAMP levels. Serum adiponectin concentration elevated in HH and reduced in PCOS patients. **Conclusion:** Brain-adiponectin inter-talk possibly involves in hypothalamus-pituitary-gonads activity.

S4.3 Adipokine and Body Fat Distribution

Pei-wen WANG[1], Yu-fan CHENG[2], Leung-chit PSANG[2], Chia-wei LIOU[3], Ching-jung HEIEH[1], Shao-wen WENG[1], Hock-liew ENG[4], Iya CHEN[1], Jung-hui LI[2]
Chang Gung Memorial Hospital-Kaohsiung Medical center, Chang Gung University College of Medicine, Kaohsiung, Taiwan
[1] Department of Internal Medicine
[2] Department of Radiology
[3] Department of Neurology
[4] Department of Pathology

Objective: All traits of metabolic syndrome (MtS) are risk factors for atherosclerosis. Although the original conceptualization of this syndrome was based on insulin resistance (IR), the pathogenesis has been unclear. Accumulating evidence suggest that deranged adipocyte metabolism and altered body fat distribution are important determinants of IR. This study is designed to examine the relationships between body fat distribution, IR and the adipokine regulation in our population. **Methods:** The 450 participants (90 with type 2 diabetes [T2DM], 44 with impaired fasting glucose [IFG], and 316 normal glucose controls) all conformed to the following requirements: age ≥35 years, no history of alcoholism, hepatitis B or C, and not under thiazolidinedione therapy. Intraperitoneal fat (IP-fat), retroperitoneal fat (RP-fat), subcutaneous fat (SC-fat) and fatty liver index were measured by computed tomography. The fatty liver index was calculated as the liver/spleen (L/S) ratio of the average Hounsfield values from five 3-mm-slices. Total body fat mass was measured by the electrical bioimpedance method. IR index was estimated with homeostasis model assessment (HOMA). Serum levels of adipokines were checked by commercially available ELISA kits. The relationships among the variables were analyzed by Pearson correlation and stepwise multiple regression analyses. The level of significance was taken as $p \leq 0.05$. **Results:** For the 450 subjects, the total body fat mass correlated with IP fat ($r=0.13$, $p=0.014$) and SC fat ($r=0.11$, $p=0.034$) values. The three components of visceral fat measurements (IP-fat, RP-fat, liver fat) correlated well with each other (all $r>0.4$, $p<0.001$), but none correlated with the SC-fat. The IR indexes of the 360 non-diabetic subjects correlated with total body fat ($r=0.15$, $p=0.007$), IP-fat ($r=0.25$, $p<0.001$), and liver fat ($r=0.23$, $p<0.001$) readings, but not with the SC-fat value ($r=0.05$, $p=0.41$). Regression analysis revealed that IP-fat and liver fat were independent predictors of IR. The prevalence of fatty liver (L/S ratio<1.0) in individuals with T2DM, IFG and controls was 57.8%, 31.8% and 20.6%, respectively. Liver fat measurement correlated with IR index and all traits (waist circumference, BMI, BP, fasting sugar, HDL-cholesterol and triglyceride) of MtS. The fatty liver indexes in subjects with 0 (N=65), 1 (N=111), 2 (N=118), 3 (N=85), and ≥4 (N=71) traits of MtS were 1.25±0.13, 1.18±0.16, 1.12±0.21, 1.05±0.25 and 0.92±0.25, respectively. The difference is statistically significant ($p<0.001$). Adipokines were checked in 100 subjects with high visceral fat and 100 age- and sex-matched controls that were not on medication for hyperlipidemia. Of all the adipokines checked (hsCRP, adiponectin, IL-6, TNF-α and leptin), adiponectin ($r= -0.23$, $p=0.004$) and hsCRP ($r=0.20$, $p=0.015$) correlated with IR in non-diabetic subjects. For these two cytokines associated with IR, their independent predictors, among the five compartments of body fat, were liver fat and total body fat. We further compared the fatty liver index to the five traits of MtS for its association with the dysregulation of adipokines. Regression analysis revealed that the fatty liver index was a better predictor of CRP and adiponectin than any of the five traits of MS. **Conclusion:** For Chinese adults over age of 35, their IR is predicted by their IP-fat and liver fat, as well as serum levels of adiponectin and hsCRP. Our data suggest that fatty liver index is a useful marker for MtS and can be a better predictor of subclinical chronic inflammation than the current recognized traits of MtS.

S4.4 Adiponectin and Cardiovascular Diseases

Wing-sun CHOW
Department of Medicine, Queen Mary Hospital, Hong Kong

The growing epidemic of cardiovascular disease worldwide is closely associated with an increased prevalence of insulin resistance and type 2 diabetes due to excess body weight and sedentary lifestyles. Obesity, especially central obesity, has been shown to predict cardiovascular morbidity and mortality in long-term epidemiological studies. Although their exact pathogenic relationship remains poorly understood, intensive research efforts are underway to elucidate the mechanisms by which excess adiposity contributes to the development of cardiovascular diseases.

Adipose tissue is now recognized to be an important endocrine organ that secretes various biologically active peptides, such as adiponectin, TNF-alpha, resistin and interleukin-6, which in turn regulate a variety of homeostatic processes. Adiponectin, a hormone secreted almost exclusively from adipocytes, is found in high abundance in the circulation, and has insulin-sensitizing, anti-inflammatory and anti-atherogenic properties. Adiponectin knockout mice exhibited insulin resistance, enhanced vascular stenosis following arterial injury, and increased myocardial infarct size following coronary artery occlusion, which could be prevented by the adenovirus-mediated supplementation of adiponectin.

In humans, circulating adiponectin level is inversely related to the degree of adiposity and positively associated with insulin sensitivity. Low adiponectin levels are found in the presence of obesity, type 2 diabetes, hypertension and coronary artery disease, and are associated with features of vascular dysfunction. Hypoadiponectinaemia has also been shown to be an independent risk factor of hypertension in a cross-sectional study, and predicts the development of type 2 diabetes, hypertension, the metabolic syndrome and myocardial infarction in prospective studies. Taken together, hypoadiponectinemia plays an important role in linking obesity with various cardiometabolic risk factors, which in turn may lead to increased cardiovascular morbidity and mortality.

S5.1 Angiotensin II and Atherogenesis: New Insights

Willa HSUEH

(Abstract not received at the time of printing)

S5.2 Therapeutic Implications of Lipids and Inflammation: Role of Insulin Resistance

Wayne H-H. SHEU
President, Taiwanese Association of Diabetes Educators (TADE); Professor of Medicine and Chief, Division of Endocrinology and Metabolism, Department of Medicine, Taichung Veterans General Hospital, Taichung, Taiwan

The metabolic syndrome (MS) is a cluster of metabolic abnormalities with insulin resistance as a major characteristic. Two of main adverse consequences of the MS is development of diabetes and cardiovascular disease (CVD). Central pathophysiologic features include: insulin resistance, atherogenic dyslipidemia, chiefly present as low HDL-C together with increases in triglycerides and small dense, low density lipoprotein particles, hypertension, a proinflammatory state, with increases in acute-phase reactants, and a prothrombotic state. Although lifestyle and overeating seem to be the triggering pathogenic factors, genetic elements are also involved in the pathogenesis of MS. When present, insulin resistance results in impaired insulin action in insulin-sensitive tissues such as muscle, fat, and liver. Insulin resistance results in abnormalities of glucose metabolism, with reduced peripheral disposal of glucose in muscle and increased hepatic glucose output in the fasting state. Elevated insulin levels themselves are atherogenic by inducing an oxidative stress and by stimulating sympathetic-nerve activity. Ectopic fat deposition, stress, proinflammatory state, and a maladaptive response of innate immunity may together concur to the development of the MS. When this condition is acknowledged, substantial modification of life style and correction of each single risk factor should be pursued without uncertainties and without hierarchical approach; this means that each risk factor should be treated and brought to target. Recent large scale clinical trials indicated that therapeutic lipid-lowering agents, in particularly statins, are effective in reducing lipids and inflammatory response. Some statins are also shown to lower LDL-cholesterol level as well as improve insulin resistant state in non-diabetic hypercholesterolemic individuals.

S5.3 Small Molecules Regulate Apolipoprotein Ai Expression

Norman C.W. WONG
Departments of Medicine and Biochemistry, University of Calgary, Calgary, AB, T2N4N1, Canada

Objective: The anti-atherogenic properties of apolipoprotein AI (apoA-I) underlie the search for drugs to enhance its expression and thus lower the risk of cardiovascular disease (CVD). Drugs that increase apoA-I expression are lacking. We studied the role of two small molecules, thyroid hormone (L-T3) and resveratrol to increase apoA-I expression. **Results:** This study showed that L-T3 increased abundance of the protein, hepatic mRNA and transcription of the gene in rats. L-T3 action in Hep G2 cells mimicked hormone action *in vivo*. Promoter analysis revealed that T3-receptor interacted with a site near -200 bp of the gene. Another small molecule resveratrol believed to have cardioprotective properties may also exert its effect by enhancing apoA-I expression. In both Hep G2 and CaCO2 cells exposed to 5-10 μM resveratrol increased apoA-I fluorescence in treated but not untreated cells and abundance of the protein increased by 2- to 4-fold measured using western blot analysis. Next, the apoA-I promoter constructs localized actions of resveratrol to a site near -190 of the gene. Neither L-T3 nor resveratrol, a stilbene can be used as drugs to enhance apoA-I expression because of cardiotoxic and absorptive problems, respectively. This prompted the synthesis of a new family of compounds that activate apoA-I in cells and animals. **Conclusion:** Both L-T3 and resveratrol enhance apoA-I gene expression that, in part, is mediated by effects at separate sites of the promoter. A new family of small molecules not related to L-T3 or resveratrol should enable us to test the hypothesis that increasing apoA-I will lower the risk of CVD.

S5.4 Evidence Based Management of Diabetic Dyslipidaemia

R.S. SCOTT
Christchurch Hospital and Christchurch School of Medicine, Christchurch, New Zealand

People with diabetes have a dyslipidaemia that is both quantitative and qualitative for lipid abnormalities; viz: elevated triglycerides and low high-density lipoprotein (HDL) cholesterol. LDL lowering with statins improves outcomes and of the many intervention studies, only ASPEN using low dose Atorvastatin 10 mg/day has not shown benefit amongst people without serious renal disease. The TNT study showed that intensive statin therapy further reduces risk. However the residual CVD risk in those with type 2 diabetes remains high.

Two intervention trials, the VA-HIT and the BIP Study, indicated that fibrates might reduce CVD event rates in diabetes. The FIELD Study was designed to conclude if fibrates were able to reduce CVD events in those with Type 2 DM. 9795 men and women, with and without prior CVD, were randomised to micronised Fenofibrate 200 mg/day or matching placebo for an average of 5 years. Although most had MS, the population was low risk for future CVD events. Fenofibrate treatment gave a non-significant 11% reduction in the primary endpoint (non-fatal myocardial infarction [MI] and CHD death) and a significant 11% reduction in pre-specified composite endpoint of CVD events including significant reductions in non-fatal MI and in revascularization procedures. Stroke rate was not reduced. Total mortality, CVD mortality and CHD mortality increased but not significantly. Total CVD events fell by 19% ($p<0.004$) in patients with no previous CVD but not in those with prior CVD. As expected, people with the metabolic syndrome (ATP III) were at higher risk than without (14.5 vs 11.3% CVD event rate) but did not receive greater benefit from Fenofibrate. The reasons for the lack of benefit from Fenofibrate in high-risk subjects will be discussed.

Statins thus remain first-line therapy for lipid lowering in type 2 diabetic patients both without and with previous CVD. The benefit of combination of statin with fibrate will be addressed by the ongoing study, ACCORD but this study may still lack the power to provide clarification on benefit of statin fibrate combination therapy.

S6.1 Oxidative Stress and Diabetic Nephropathy

Hung-chun CHEN
Department of Internal Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

Partial reduction of oxygen leads to the generation of potentially toxic reactive oxygen species (ROS) that includes superoxide anion, hydrogen peroxide, hydroxyl radical, and hypohalous acids. A large body of evidence accumulate over the last decade indicates that ROS are important mediators of ischemic, toxic, and immune-mediated tissue injury. ROS are highly reactive and unstable chemical species that have been implicated in the mediation of vascular and tissue damage associated with several diseases, including diabetic nephropathy. We have demonstrated that native and oxidized low density lipoprotein (LDL), both are commonly found to be elevated in diabetic serum, are potent stimulants of superoxide production in freshly isolated diabetic rat glomeruli. Basal production of superoxide is closely related with the production of endothelin-1 (ET-1) that is a powerful vasoconstrictor. ROS scavengers suppress ET-1 production both in vitro and in vivo, and ROS donor enhances ET-1 production. Both insulin and heparin suppress the basal and LDL-stimulated production of superoxide in diabetic glomeruli but not in normal glomeruli. Induction of heat shock protein 70 may offer protective effects on mesangial cells against oxidative injury although the methods of induction deserved further studies. Pravastatin shows an additional effect as an antioxidant besides its hypolipidemic effect, it suppresses the production of superoxide and fibronectin of glomerular mesangial cells stimulated with LDL, oxidized LDL and/or high glucose. Our results demonstrate that ROS are closely related with the pathogenesis of diabetic glomerulosclerosis, and the effective therapy against ROS may have beneficial effects in diabetic nephropathy.

S6.2 Optimal Management of Diabetic Nephropathy

Juliana C.N. CHAN
Department of Medicine & Therapeutics, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong

On a global basis, 1 million people develop end stage renal disease (ESRD) yearly, with 40-60% of them due to diabetes. Over 90% of people on dialysis reside in N America, Europe and Japan while many more are dying from ESRD in developed areas due to lack of access to dialysis.

There is now conclusive evidence showing that optimal control of blood pressure, blood glucose and inhibition of the renin angiotensin-aldosterone system (RAAS) prevent the onset and progression of nephropathy in both type 1 and type 2 diabetes. However, despite receiving the best care, many patients continue to develop ESRD. In this regard, activation of the innate inflammatory system, either in response to obesity or low grade chronic inflammation may be an important promoter of diabetic nephropathy. Other therapeutic strategies such as dual blockade of the RAAS, aggressive lipid lowering, use of PPAR γ or α agonists, anti-thrombotic or anti-platelet therapy remain to be tested despite having theoretical benefits. The effects of novel therapies targeting at signaling or enzymatic pathways such as aldose reductase or PKC inhibitors on diabetic nephropathy are also being developed and evaluated.

Chronic kidney disease (CKD) is a powerful and independent predictor for cardiovascular disease in both diabetic and non diabetic patients. Apart from alteration in metabolic milieu, anemia, oxidative stress and abnormal calcium metabolism all contribute to increased cardiovascular risk. While there are ongoing studies to examine the effect of erythropoietin therapy on cardiovascular events in patients with diabetic nephropathy, their effects on progression of renal function warrants further evaluation. On the other hand, disease management delivered by a multidisciplinary team with particular focus on periodic assessments, treatment to targets and patient empowerment has been shown to substantially reduce risk of death and ESRD in type 2 diabetic patients with moderate renal impairment.

In conclusion, given the humanistic and socioeconomic implications of diabetic kidney disease, there is a need to adopt a cohesive and multi-level strategy to use policies to change environment and health care system to make preventive care more sustainable and affordable while at the same time encouraging both scientific and health care community to develop innovative therapies and care models to fight this global epidemic of diabetes and cardio-renal complications.

S6.3 Molecular Genetics of Diabetic Microangiopathy

Sookja K. CHUNG
Department of Anatomy and Research Center of Heart, Brain, Hormone & Healthy Aging, The University of Hong Kong, Hong Kong SAR, China

Recently, diabetes mellitus was recognized as the epidemic in developing countries and as one of the major threats to human health in the 21st century due to diabetes-associated microvascular diseases, such as retinopathy, nephropathy and neuropathy, and macrovascular diseases, such as atherosclerosis, leading to ischemic tissue damages in heart, retina, brain and leg. Although tight control of blood glucose greatly reduces the incidence of these complications, a significant fraction of diabetic patients with good glycemic control still develop these diseases. Therefore, it is imperative to understand the underlying mechanisms of these diseases such that effective treatment or preventive methods should be developed to augment euglycemic control. Although the exact mechanism for the pathogenesis of these diseases is not completely understood, several major factors have been proposed for microvascular complications. They include the exaggerated flux through the polyol pathway, increased production of reactive oxygen species (ROS) by the mitochondrial respiratory chain, non-enzymatic glycation, protein kinase C (PKC) activation, and increased flux through hexosamine pathway. Among them, aldose reductase (AR) in the polyol pathway, which consists of two enzymes, AR, which reduces glucose to sorbitol with the aid of NADPH, and sorbitol dehydrogenase (SDH), which converts sorbitol to fructose by using NAD^+, received the most attention. In animal studies, there is strong evidence that an exaggerated flux through AR, the first and rate-limiting enzyme of the polyol pathway and accumulation of sorbitol leading to osmotic stress, is the major culprit in the pathogenesis of diabetic complications. Recently, we revisited AR and SDH by further understanding its gene structure, regulation, expression, and normal physiological function using AR- and SDH-deficient mice. In addition, the genetically manipulated transgenic mice with over-expression of AR in tissues prone for microvascular complications and AR- and SDH-deficient mice were generated to further understand their role in potential pathogenic mechanisms of diabetic complications and its interaction with other proposed pathways involved in diabetic complications. The new evidences for the contribution of activated AR in oxidative stress leading to diabetic microvascular complications have been presented using AR-deficient and AR inhibitor treated animals. In support of such animal studies, there are now numerous studies describing the association of AR gene markers with more susceptibility to diabetic microvascular complications in diabetic patients. These revisited efforts of understanding of role of AR in the pathogenesis of diabetic microvascular complications should warrant the continued development of better AR inhibitors for the treatment and prevention of diabetic microvascular complications.

S6.4 Periodontal Disease as a Diabetic Complication

W. Keung LEUNG
Faculty of Dentistry, The University of Hong Kong, Hong Kong

Periodontal disease is reported to be the sixth most common complication of diabetes mellitus (DM) and links between these two conditions are emerging. It has been suggested that DM predisposes individuals who have inadequate oral hygiene to periodontal disease, through a combination of mechanisms such as defective polymorphonuclear leukocyte function, altered synthesis of collagen and glycosaminoglycan by gingival fibroblasts, chronic vascular pathology and compromised wound healing. **Objective:** To study among a group of ≥41-year-old Chinese type 2 DM patients, their oral health status and association with DM complications. **Methods:** 364 type 2 DM patients and 161 age- and sex-matched DM free controls (with or without hypertension) of predominantly low-income attending a charitable hospital in Hong Kong was surveyed. DM control, diabetic medical complications and oral health status were recorded. **Results:** Most DM subjects had fair diabetic control. Both groups (control/DM) had poor oral health: DMFT=14.5/16.8, CPI 4=36%/50%, (P<0.02) while DM subjects had less DT (adjusted)=2.1/1.4 (P<0.01). 294 (81%) of the 362 successfully follow-up DM individuals had at least one medical complication. Regression analyses showed advanced periodontal attachment loss among the subjects surveyed was associated with age, male gender, smoking and DM; and MT together with DMFT were associated with age, female gender, smoking, DM and hypertension. **Conclusions:** Oral health of the predominantly low-income, middle-aged to elderly patients surveyed was poor. Type 2 DM subjects were affected more by attachment loss, MT while less DT than controls. Subjects with hypertension were also found to have higher odds for MT. Poor dental health was not associated with DM complications.

S7.1 Conn's Syndrome: Which Diagnostic Strategy?

Cheung-hei CHOI
Endocrine team, Department of Medicine, Queen Elizabeth Hospital, Hong Kong

More than 50 years has passed since Jerome W Conn described the new clinical syndrome of hypertension and hypokalaemia at the Central Society for Clinical Research in Chicago, Illinois, which he termed 'primary hyperaldosteronism' (PAL). This syndrome was considered to be an uncommon cause of hypertension in the 60's to early 90's, but recently some authorities, particularly in Australia, have shown an increasing numbers of incidence by means of active screening in all hypertensive patients. As all endocrine diseases, the strategies for diagnosis are (1) to screen the high-risk groups; (2) to confirm the diagnosis and (3) to localize the tumor.

1. **To screen the high risk groups**
   High index of suspicion is required for patients presented with hypokalaemia, young age of onset and hypertension resistance to several anti-hypertensive agents (other than aldosterone antagonist). Spot/24-hour urine potassium and transtubular potassium gradient (TTKG) are useful to confirm the renal loss of potassium and possibly hyperaldosteronism. The use of aldosterone-renin ratio (ARR) to screen for PAL is quite controversial and confusing, due to the widely different cutoff levels used in studies. Our study confirmed the superiority of ARR to either aldosterone or plasma renin activity (PRA) alone as a screening test for PAL. Positive ARR cutoff levels were dependent on the condition of testing. Using a cutoff of 650 pmol/L per ng/mL·h when patients were sampled in the morning after being seated for 30 minutes, ARR had a sensitivity and specificity of 96.8 (83.2-99.5)% and 94.1 (71.2-99.0)% respectively.

2 **To confirm the diagnosis**
   Traditionally, iv saline suppression and fludrocortisone suppression are used as confirmatory tests for patients with abnormal screening tests. The profile of these tests will be discussed.

3 **To localize the tumor**
   Three types of tests are useful in localizing the tumor and differentiating adenoma or hyperplasia. The advantages and disadvantages of biochemical test like balance test (postural change of aldosterone levels), imaging like CT or MRI scan and adrenal vein sampling will be evaluated in more details.

S7.2 Surgical Treatment of Intersex Disorders

Paul K.H. TAM
Department of Surgery, The University of Hong Kong, Hong Kong

The birth of an infant with ambiguous genitalia is one of the most difficult challenges faced by physicians who care for children. Intersex management is complicated by psychological, social, and sexual concerns, as well as by medical ones. Historically, it has been a priority to assign a gender quickly, and to proceed with surgical treatments to establish that gender, physically and functionally. In recent years, there are also alternate views on these practices. Here, an up-to-date surgical approach in the management of intersex disorder is discussed.

S7.3 PET-CT in the Management of Endocrine Diseases

Shuo GAO

(Abstract not received at the time of printing)

S7.4 Management of Multiple Endocrine Tumours: a Multidisciplinary Approach

Chung-Yau LO
Division of Endocrine Surgery, Department of Surgery, University of Hong Kong Medical Centre, Queen Mary Hospital, Hong Kong

Multiple endocrine neoplasia (MEN) is an autosomal dominant familial disorder and two major types, MEN 1 and MEN 2, are recognized. Each type is characterized by the occurrence of two or more endocrine neoplasms associated with hyperfunction and malignancy within specific endocrine organs. MEN 1 syndrome has a variable expression and a high penetrance, which is age-accumulative. The clinical manifestations are dependent on the organs affected or the hormones secreted and age specific disease manifestation has been demonstrated. Insulinoma and prolactinoma occur more commonly in younger age group while life-threatening gastrinoma and islet cell carcinoma are prevalent among those >40 years of age. The menin gene is a tumour suppressor gene localized to chromosome 11q13 and the inheritance of the MEN 1 gene is not incompatible with long-term survival. Zollinger-Ellison-Syndrome secondary to hypergastrinaemia and pancreatic islet cell carcinoma with distant metastases are the major causes of death. Clinical criteria formulated to screen patients with suspected MEN 1 identified 60% of the patient as compared to 5% by non-selected approach. The identification of the MEN 1 gene and the availability of the genetic analyses provide a means of early detection of carriers, genetic counseling, presymptomatic biochemical screening and early surgical management for this condition. On the other hand, MEN 2A, MEN 2B, and familial medullary thyroid cancer (MTC) comprise the MEN 2 syndrome. A mutation in the RET proto-oncogene on chromosome 10q11.2 can be identified by genetic testing in 99% of cases. The ability to determine gene carrier status provides an opportunity to perform early prophylactic thyroidectomy. Pheochromocytoma may be the first manifestation of MEN 2A patients and is frequently reported to manifest in patients with RET mutation at codon 634. MEN 2 patients should be screened annually for urinary catecholamine levels during follow-up and localization studies should be performed for those with documented catecholamine excess. Laparoscopic unilateral adrenalectomy, based on imaging, can be adopted for majority of patients because the development of bilateral lesions can be asynchronous in many patients. All patients with MTC or bilateral pheochromocytomas should have a careful family history taken and genetic screening for RET germline mutations. In summary, an understanding of the spectrum of disease and the manifestations of each component of MEN syndrome is crucial for accurate detection, staging, and surveillance in this diverse patient group. Biochemical evaluation, hormonal assessment, anatomic and functional imaging modalities, and genetic counseling play a vital role in the diagnosis, localization and treatment of the disease. A multidisciplinary approach involving cooperation between endocrinologists, surgeons, biochemists, radiologists and oncologists is pivotal for optimizing patient management.

S8.1 Predisposition to Ovarian Cancers: a Proteomics Approach

Alice Sze-tsai WONG
Department of Zoology, University of Hong Kong, Hong Kong

Objective: To study the pattern of protein expression associated with a predisposition to develop ovarian cancer. **Methods:** Prophylactic oophorectomy is used to prevent ovarian carcinoma in high-risk populations who have a strong family history of breast/ovarian cancer. In ovarian specimens of these women, the ovarian surface epithelium (OSE), which is tissue of origin of epithelial ovarian cancer, often shows altered morphology, growth patterns, and differentiation features that are believed to be preneoplastic. This study has used a proteomics approach, based on two-dimensional gel electrophoresis and mass spectrometry, to compare the protein profiles of OSE from women with a history of familial ovarian cancer (FH-OSE), i.e. at least two first-degree relatives with such cancer and/or testing positive for *BRCA1* mutations, to those without such history. **Results:** Of >1500 protein spots, 18 were found to be significantly altered in FH-OSE. Proteins upregulated include chaperone proteins, metabolic enzymes, and several proteins associated with actin modification and membrane trafficking. In contrast, proteins that exhibited underexpression include glycolytic enzymes, the 27-kDa heat shock protein and tropomyosin. A number of the alterations seen were accompanied with protein modifications, which have not been previously reported. **Conclusion:** Our findings define the preneoplastic changes in the OSE of women at a high risk of developing ovarian cancer. Protein alterations seen in these tissues may be potential early and sensitive markers for the evaluation of cancer risk.

S8.2 Age-related Relative Testosterone Deficiency: Pathophysiology and Interim Management Guidelines

Peter Yi-wen LIU
Department of Andrology, ANZAC Research Institute and Concord Hospital, Concord NSW, 2139, Australia

The increased longevity apparent in many communities worldwide, including China, has created a need to foster healthy aging. Devising safe and effective approaches that prolong independent, and enjoyable living is therefore a priority. Androgen replacement therapy for older men holds promise in this regard since blood testosterone fall by 1-2% each year, creating a state of relative (relative to young men) androgen deficiency. This small but significant fall in lifetime systemic testosterone exposure over 6 decades is marked jointly by: (i) high-frequency, low-amplitude and quantifiably disordered pulses of pituitary luteinizing hormone (LH); (ii) normal or heightened LH secretion following single or repeated stimulation with the hypothalamic peptide, gonadotropin-releasing hormone (GnRH); (iii) reduced testosterone secretory-burst mass; and (iv) impaired Leydig-cell testosterone production in response to stimulated endogenous LH secretion or infused recombinant human LH. These interconnected pathophysiological findings suggest that androgen deprivation in the older male arises from multisite integrative failure in the GnRH-LH-testosterone axis which, in combination with the clinical similarities between many features of aging and characteristics of organic androgen deficiency in younger men, has led to the hypothesis that older men may benefit for testosterone replacement therapy. However, such therapy in older men remains controversial in stark contrast to testosterone replacement for organic androgen deficiency in younger men which is widely regarded as safe, affordable and effective. At present, best practice interim guidelines for treatment are premised upon normative reference ranges derived from young healthy adults and suggestive clinical features.

S8.3 Androgen Deficiency in Chinese Men with Aging

J.Y. LI
Department of Endocrinology, PLA General Hospital, Beijing, China

Slow and progressive decline of serum levels of androgens in men with aging has been demonstrated by cross-sectional and longitudinal studies. A part of aging men concomitantly present clinical symptoms and response to testosterone supplementation therapy (TST). It is widely accepted that androgen deficiency in the aging male (ADAM) or late-onset hypogonadism in males (LOH) is a real clinical entity.

For examining what happens to Chinese men, a survey was conducted in Beijing, Shanghai, Xian and Chongqing. A total of 1080 healthy men aged from 20 to more than 70 years old were enrolled. It was found that except for total testosterone (TT), Chinese healthy men presented progressive declines of calculated free testosterone (cFT), testosterone secretion index (TSI, the ratio of TT to LH) and free testosterone index (FTI, the ratio of TT to SHBG). Spearman's analysis showed a significant correlation between the androgen serum levels and age. The decline rate of cFT is 3% after 40, about 20% after 50, and 33% after 70 years old. The variation of TSI with aging paralleled closely the changes of cFT. Therefore, TSI is a good surrogate for cFT, because SHBG is not a routine test in clinical practice. On the other hand, the serum levels of LH, FSH and SHBG were progressively increased in men with aging.

On the self-scale basis, every subject answered 18 questions, including physical, neuropsychological and sexual dysfunctions. The frequency and severity of these symptoms were correlated to age and androgens. And a recommended questionnaire with 12 questions for screening ADAM in Chinese men was created. The results of the preliminary clinical study revealed that patients with symptomatic ADAM had a good response to 10 weeks TST by oral Andriol® in dose of 120 mg daily.

The causes for androgen deficiency in the aging men are complicated. We examined the enzymes in the pathway of testosterone biosynthesis, and found that the expression of 17β-HSD III mRNA was significantly decreased in the aging rat.

S8.4 Androgens in Fertility Control and Replacement Theory

Christina WANG, Ronald SWERDLOFF
Division of Endocrinology, Department of Medicine, Harbor-UCLA Medical Center & Los Angeles Biomedical Research Institute, Torrance, California 90509, USA

Objective: To discuss the recent developments of androgen replacement and its utilization in male contraceptive regimens. **Results:** In recent years, there is a surge in pharmaceutical interest in the development of testosterone delivery methods as well as novel compounds for androgen substitution in men. The main indication for androgen substitution is male hypogonadism which is more common in aging men than younger men. Many of the older hypogonadal men are not currently receiving treatment. Studies are being proposed or ongoing to prove that patient-reported outcome parameters (vitality, frailty, sexual dysfunction, cognition) improved after androgen replacement in older men as shown in younger men. In the development of male hormonal contraception, androgens are an essential component of the regimen. Maintenance of androgen dependent functions is necessary when endogenous testosterone production is suppressed by exogenous hormones. At present the most promising androgen for male contraception is intramuscular testosterone undecanoate injections. **Conclusion:** Development of androgen delivery systems and novel androgen receptor modulators will change our current management of hypogonadism and future male fertility control.

S9.1 Obesity Assessment in Asians in Relation to Cardiovascular Risk

E. Shyong TAI
Consultant and Clinical Scientist, Department of Endocrinology, Singapore General Hospital, Singapore 169608, Singapore

Obesity is associated with an increase in the risk of cardiovascular disease (CVD). To a large extent, this may relate to the presence of multiple CVD risk factors associated with obesity. In Asia, it has been suggested increased levels of CVD risk factors occur at lower levels of body mass index compared to Caucasian populations. Several studies have shown that the body mass index significantly underestimates the level of obesity in several Asian populations. For this reason, the WHO has recommended "public health action points" for Asians which are lower than the WHO cut-offs for overweight and obesity. Emerging data suggests that the waist circumference may underestimate the degree of visceral obesity amongst Asians in a similar way. In line with these findings, both the International Diabetes Federation and the American Heart Association/National Heart Lung and Blood Institute incorporated ethnic specific cut-offs for waist circumference in their recommendations for the identification of individuals with the metabolic syndrome.

S9.2 The Application of the Metabolic Syndrome in Predicting Cardiovascular Events in Subjects with Type 2 Diabetes Mellitus

Peter C. TONG[1], Alice P. KONG[1,3], Wing-yee SO[1], Xilin YANG[1], Chung-shun HO[2], Ronald C. MA[1], Risa OZAKI[1], Chun-chung CHOW[1], Christopher W. LAM[2], Juliana C.N. CHAN[1], Clive S. COCKRAM[1]
The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, NT, Hong Kong
[1] Department of Medicine and Therapeutics
[2] Department of Chemical Pathology
[3] Li Ka Shing Institute of Health Sciences

Objective: To compare the predictive value for cardiovascular disease of the International Diabetes Federation (IDF) definition (with Asian criteria for central obesity) of the metabolic syndrome (MES) to existing criteria of the National Cholesterol Education Program's Adult Treatment Panel III (NCEP) in a cohort of Chinese subjects with type 2 diabetes. **Methods:** 4762 subjects with type 2 diabetes were categorized according to the criteria of IDF and NCEP. Cardiovascular endpoints were defined as cardiovascular mortality and morbidity including unstable angina, myocardial infarction, stroke, revascularization or heart failure. **Results:** The mean age of subjects was 59.2 ± 13.5 years and the median follow-up period was 38.9 (interquartile range: 21.8-54.1) months. 1886 subjects (39.6%) fulfilled the criteria of both IDF and NCEP ('IDF/NCEP'). 468 (9.8%) and 924 (19.4%) complied only with the criteria of IDF ('IDF-only') or NCEP ('NCEP-only'), respectively. Subjects fulfilling 'NCEP-only' were thinner, had a higher frequency of dyslipidemia and worse renal function than the 'IDF-only' group. Among individual cardiovascular risk factors of MES, only systolic blood pressure and HDL-Cholesterol were predictors of cardiovascular events. Compared to patients fulfilling 'IDF-only', those without MES, fulfilling 'IDF/NCEP' or 'NCEP-only' had hazard ratio (95% CI, p-value) of 1.39 (0.79-2.45, p=0.249), 2.04 (1.20-3.47, p=0.009) or 2.48 (1.44-4.26, p=0.001) of developing cardiovascular events, respectively. **Conclusions:** With established type 2 diabetes, the IDF definition of MES failed to identify a subgroup of patients who were at highest risk. Practitioners must recognize the appropriate setting for its application.

S9.3 Small Lipid-binding Proteins as Novel Biomarkers for Obesity-related Metabolic and Cardiovascular Diseases

Ai-min XU, Yu WANG, Annette TSO, Jia-liang ZHANG, Karen LAM
Department of Medicine & Research Center for Heart, Brain, Hormone and Healthy aging, University of Hong Kong, Hong Kong

Objective: To use an integrated proteomics and genome-wide interrogation approach to identify novel circulating biomarkers for obesity-related metabolic and cardiovascular diseases. **Methods:** Two-dimensional gel electrophoresis and tandem mass spectrometry-based techniques were used for systematic characterization of proteins secreted in mature adipocytes. Microarray analysis was used for identification of differentially expressed genes with secretion signals in db/db diabetic mice. Several in-house ELISA methods were used for validating clinical utilities of the identified novel biomarkers in predicting obesity-associated metabolic syndrome and diabetes. **Results:** We have identified two novel obesity-associated circulating biomakers, including adipocyte-fatty acid binding protein (A-FABP) and lipocalin-2, both of which are produced from adipocytes and possess lipid-binding activities. In humans, circulating levels of both A-FABP and lipocalin-2 are closely associated with a cluster of cardio-metabolic risk factors. In a 5-year prospective study, serum levels of A-FABP at baseline are predictive of the development of the metabolic syndrome (p=0.012). On the other hand, serum lipocalin-2 levels are independently associated with hs-CRP, a well-established marker of inflammation. In an interventional study including 32 patients with type 2 diabetes, we found that rosiglitazone-mediated decreases in lipocalin-2 concentrations correlated significantly with increases in insulin sensitivity (r=0.527, p=0.002) and decreases in hs-CRP concentrations (r=0.509, p=0.003). **Conclusions:** A-FABP and lipocalin-2 represent a novel class of circulating biomarkers for risk stratification and therapeutic monitoring of obesity-related metabolic syndrome and cardiovascular disorders.

S9.4 Bariatric Surgery—Role in Obesity Management

Simon K.H. Wong
Department of Surgery, Pamela Youde Nethersole Eastern Hospital, Hong Kong

With the increasing prosperity and westernization of people's life, obesity has become an important health topic in Hong Kong. We define "morbid obesity" as the body weight index (BMI) exceeded 35 kg/m^2, and the life expectancy of these patients will be severely affected by their obese-related disease and their mortality rate is twice of that of the healthy people. The goal of weight-reduction therapy is to improve health by modifying obesity-related disease and the risk for future obesity-related medical complications. Non-surgical intervention, like dieting and exercise plus augmented by drug therapy and behavioral modification, often provide an unsustainable and insufficient weight loss in these severely obese patients. Surgical methods provide the last resort for weight control, which usually induce a major change in life-style and eating habit. According to USNIH & IFSO Consensus, surgical therapy is the only effective long-term weight reduction treatment for morbidly obese patients with a more substantial weight loss (>50% excessive body weight). The prevalence of cardiovascular risk factors will be reduced markedly after surgery, and in most patients reverse diabetes, hypertension and pulmonary dysfunction.

Indication of obesity surgery in Caucasian countries includes: BMI >40 kg/m^2 or BMI >35 kg/m^2 and suffered from severe obese-related disease with failure in previous weight reduction therapy. Patients suffered from endocrine cause of obesity and patient had major psychiatric disease were usually excluded from surgery. In Asia countries, our risk of developing DM is higher at relative lower BMI patients than Caucasian and this issue had altered our threshold in performing bariatric procedures among Asian.

Traditional weight reduction surgery included gastric restrictive procedures (e.g. vertical-banded gastroplasty) or combining gastric restrictive with mal-absorptive procedure (e.g. Roux-en Y gastric bypass, biliopancreatic diversion). However, open surgery for severely obese patient carries significant operative morbidity, especially respiratory and wound-related problems. With the advanced development in laparoscopic surgery and the introduction of laparoscopic adjustable gastric band (LAGB), morbidity of these procedures has significantly reduced. Even though surgical treatment is the only effective method of weight reduction in morbidly obese patients, the ultimate success of this procedure still heavily dependent on the will of the patient and the collaboration among the "Obesity Management Team" (physicians, surgeons, dietitian, psychiatrist, anesthetist, psychologist and physiotherapist, etc).

S10.1 Osteoporosis: When to Start Treatment?

Tai-pang IP
Osteoporosis Centre, Department of Medicine & Rehabilitation, Tung Wah Eastern Hospital, Hong Kong

The most important clinical consequence of osteoporosis is a fracture which causes significant disability and reduction in the quality of life of the fractured patients. It also imposes a great economic burden to the community with the rising prevalence of fracture in Asia. Effective medical therapies that have been proven to reduce the risk of fracture have been developed in the past one to two decades. Clinicians are now facing the challenge of identifying the appropriate patients for treatment.

Clinically, the diagnosis of osteoporosis is made with the World Health Organisation (WHO) criteria of a bone mineral density (BMD) T-score of ≤ –2.5 measured at the lumbar spine or hip by the dual-energy X-ray absorptiometry (DXA). In patients who have not experienced a fragility fracture, the decision to start treatment has been conventionally based on the BMD criteria. Although patients with osteoporosis have an increase in relative risk of fracture, clinical studies have shown that more than half of the fractures actually occur in patients with just low bone mass (osteopenia) or even normal BMD so that BMD is a relatively insensitive predictor of fracture.

The reason is that BMD is just one of the factors that determine bone strength which is the most important single endogenous factor that determines the propensity of a bone to fracture; the other elements being the structural properties, the material properties, the microarchitecture and the degree of remodeling of the bone. At the same time, a number of clinical factors have also been found to significantly affect the risk of fracture notably the age of the patient.

To maximize the cost-effectiveness of osteoporosis treatment, the ideal strategy would be to select patients on the basis of their absolute fracture risk. In analogy for selecting patients for lipid lowering therapy basing on the 10-year coronary risk, the WHO Osteoporosis Task Force is developing a model to determine the 10-year fracture risk using clinical risk factors. Through optimizing risk assessment in individual patients, treatment can be effectively and efficiently targeted for those patients who should derive the greatest benefit.

S10.2 Bone Micro-architecture and Osteoporosis

E.Y. LIAO

(Abstract not received at the time of printing)

S10.3 Clinical Manifestations of Primary Hyperparathyroidism (with Analysis of 280 Cases)

Mei LI, Xiao-ping XING, Xun-wu MENG, Ou WANG, Wei-bo XIA, Yan JIANG, Ying-ying HU, Huai-cheng LIU
Department of Endocrinology, Peking Union Medical College Hospital, Beijing 100730, China

Objective: We analyzed the clinical characteristics of 280 cases of primary hyperparathyroidism (PHPT) admitted to the Peking Union Medical College Hospital (PUMCH) during 1958 to 2005 to: (1) summarize the characters of PHPT in PUMCH, (2) compare the clinical characters of different pathologies, (3) compare the manifestations of PHPT in PUMCH with that in USA, and (4) to investigate the distribution of calcium-sensing receptor (CASR) gene polymorphisms in healthy young women and PHPT patients of Han nationality in Beijing area to preliminarily observe the relationship between CASR genotype and clinical severity of PHPT. **Subjects and Methods:** 280 patients of PHPT admitted in PUMCH during 1958 to 2005 (88 males and 192 females) aged 41.9±15.6 years (10-78 years old) with history of 5.0±5.2 years (1 month-30 years) were retrospectively analyzed. The diagnosis was confirmed by pathology. No evidence of multiple endocrine neoplasia was found in any cases. Serum total calcium (TCa), ionized calcium (ICa), phosphorus (P), AKP, PTH, TRAP, 25(OH)D_3 and urinary Ca levels were determined. CASR gene polymorphisms were studied in 45 PHPT patients and 202 healthy young women. Whole blood genome DNA as extracted by QIAGEN DNA extraction kit. A986S and G990R genotypes were determined by mutagenically separated polymerase chain reaction (MS-PCR) and PCR-RFLP, respectively. **Results:** 1. The clinical manifestations could be classified into 4 types: bone resorption only (41.1%), bone resorption with urolithiasis (34.6%), urolithiasis only (10.0%) and simple hypercalcemia (14.3%). (1) The skeletal disorders included pain (83.9%), movement disorder (67.1%), skeletal deformity (43.9%), shorten of stature (6.7±4.7 cm) and pathologic fractures (33.6%). Radiological evidence of bone resorption, osteomalacia and osteoporosis were seen in 76.4%, 42.2% and 91.1% of patients, respectively. Patients with urolithiasis, gastrointestinal symptoms, polydipsia and polyuria accounted for 44.6%, 55.4% and 57.1%, respectively. (2) Laboratory examinations: The average levels of serum TCa, ICa, P, AKP, 24h urinary Ca were 2.96±0.37 mmol/L, 1.53±0.26 mmol/L, 0.72±0.18 mmol/l, 805±947 U/L and 9.40±5.91 mmol, respectively. Serum PTH concentrations were 14.2± 13.3 times normal. The mean serum 25(OH)D_3 level was 10.0±10.0 pg/ml. As preoperative localization, the coincidence rates of neck palpation, CT scan, B-US and ^{99m}Tc-sestamibi were 28.9%, 60.9%, 81.0% and 92.7%, respectively. The hypocalcemic syndromes after operation occurred on the operation day to the subsequent 12 days (1.8±1.8 day) and sustained 1 day to 1 year. The amelioration and complete remission of pain were obtained 2.2±2.6, 5.8±7.6 months after operation. 2. Comparison among different pathologic types of PHPT: Parathyroid adenoma, hyperplasia and carcinoma accounted for 74.3%, 18.6% and 7.1% of patients. M/F ratio was higher in carcinoma group. The rates of skeletal malformation and osteomalacia were lower in hyperplasia and carcinoma group, skeletal resorption and pathologic fracture were less frequent in hyperplasia group than in other two groups; Symptoms of gastrointestinal tract, polydipsia, polyuria and changes in urinary system were more frequent in carcinoma group. Blood TCa, ICa and 24h urinary calcium levels were significantly higher in carcinoma group and similarly between adenoma and hyperplasia group. The rate of hypercalcemic crisis was higher in carcinoma group. The sizes of involved parathyroid gland(s) were smaller in hyperplasia group than in the other two groups. The recurrence rate was higher in carcinoma group. 3. Compared with PHPT in New York (1984-1999, n=143), our patients were younger (41.9 vs. 55 years old). The ratio of carcinoma was higher (7.1% vs. 0.3%). The clinical appearance was more obvious. The serum calcium and PTH levels were higher (2.96 ±0.37 vs.2.68±0.02 mmol/l, 14.2 vs. 1.86 times normal, respectively). The average 25(OH)D_3 concentration was lower (10.0±10.0 vs. 21±1 ng/ml). The abnormal parathyroid glands were heavier (6.3±9.1 vs. 1.6±1.8 g). 4. In the 45 PHPT patients: (1) The frequencies of genotypes were as follows: no SS, AA 88.9% and AS 11.1% for A986S, RR42.2%, GR48.9% and GG8.9% for G990R. The G990R genotype distribution in PHPT patients and healthy women was significantly different ($P<0.05$). The frequency of R allele in G990R genotype was higher in PHPT group. (2) In subjects carrying AA and AS genotype, serum TCa was (3.03±0.50) vs. (2.84±0.12) mmol/L, ICa was (1.56±0.32) vs. (1.42±0.07) mmol/L, serum P was (0.71±0.18) vs. (0.69±0.07) mmol/L, serum PTH was (13.13±11.26) vs. (9.25±7.78) times normal. There were significant difference in serum TCa, ICa and PTH (P=0.046, 0.022 and 0.045, respectively). (3) In subjects carrying GG+GR and RR genotype, serum TCa was (3.00±0.46) vs. (3.04±0.52) mmol/L, ICa was (1.50±0.21) vs. (1.58 ±0.39) mmol/L, serum P was (0.71±0.16) vs. (0.71±0.18) mmol/L, serum PTH was (11.84±10.63) vs. (13.89±11.51) times normal. There were significant difference in serum TCa and ICa (P=0.013 and 0.005, respectively). **Conclusions:** PHPT was characterized by skeletal resorption and/or urolithiasis, elevation of serum calcium and PTH levels. B-US and ^{99m}Tc-MIBI scan were effective preoperative localization techniques. Patients could be treated successfully by surgery. In patients of parathyroid carcinoma, the ratio of M/F was higher, polydipsia, polyuria and changes in gastrointestinal or urinary system were more frequently, the serum and urinary calcium levels were higher, rate of hypercalcemic crisis and recurrence were much higher. Compared with PHPT in New York, our patients were characterized by earlier onset, more evident manifestations and higher serum calcium and PTH levels. The ratio of carcinoma was higher in our group. CASR gene polymorphisms might play a role in the clinical severity of PHPT.

S10.4 Bone Change in Primary Hypoparathyroidism

Fredriech Kwok-wing CHAN
Department of Medicine, Queen Elizabeth Hospital, Hong Kong

Parathyroid hormone plays an important role in the regulation of bone metabolism. It regulates calcium homeostasis by affecting intestinal calcium absorption, renal calcium excretion and the rate of bone resorption. Parathyroid hormone acts directly on the skeleton to promote calcium release from bone and on the kidney to enhance calcium reabsorption. It also acts indirectly on the intestinal tract to increase calcium absorption by facilitating the renal conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D. In the state of primary hypoparathyroidism, defective parathyroid hormone secretion leads to the hallmark of hypocalcaemia and hyperphosphataemia. There are multiple etiologies, the commonest of which is due to surgical removal of the parathyroid glands during parathyroid or thyroid surgery and particularly after radical surgery for carcinoma involving structures in the neck. Other causes can be idiopathic, autoimmune or genetic in origin. Since parathyroid hormone has dual properties on bone, both catabolic and anabolic, it is interesting to review how the bone mineral density and structure change in patients with chronic hypoparathyroidism.

As early as in 1967, Dimich et al[1] observed an increase in radiographic bone density in some patients with hypoparathyroidism. Hossain et al[2] attempted to quantify it by measuring metacarpal cortical areas with the use of radiogrammetry in patients with hypo- and hyperparathyroidism. The technique was however not sensitive enough to show difference in premenopausal women but in postmenopausal women with hypoparathyroidism, no postmenopausal bone loss was demonstrable. In early 1980s, Seeman et al[3] measured bone mineral density at the midradius and distal radius by single photon absorptiometry and at the lumbar spine by dual photon absorptiometry in 100 patients with various types of endocrine disorders. Twenty of them (16 female and 4 male) had hypoparathyroidism occurring as a complication of surgery for Ca thyroid or non-toxic goiter. In patients with postsurgical hypoparathyroidism, the mean standard deviation from the age- and sex-specific normal mean was positive and significantly greater than zero at all three scanning sites. A greater increase of BMD was also noted at the lumbar spine than the radius. A decade later, Abugassa et al[4] also used photon absorptiometry to measure the skeletal mass in 13 females with hypoparathyroidism secondary to thyroid surgery for thyroid carcinoma at 10-13 years after surgery. Bone mass in this cohort was 21-28% above that of a control group of 13 patients who had normal parathyroid function after thyroid surgery. Two years later, another study showed that hypoparathyroidism retarded the rate of postmenopausal bone loss as measured by dual-energy X-ray absorptiometry (DXA) in 33 postmenopausal females with post-thyroidectomy hypoparathyroidism.[5] By use of lateral scanning of the third lumbar vertebra with DXA, Duan et al[6] demonstrated that BMD Z score was higher at the trabecular-rich vertebral body (1.02±0.47 SD) as well as predominantly cortical posterior process (0.98±0.66 SD) in 10 postmenopausal women with postsurgical hypoparathyroidism. In the cross-sectional analysis, BMD in patients with hypoparathyroidism was higher compared with age predicted mean at the lumbar spine, proximal femur but not at the distal radius. Moreover, the BMD Z scores correlated with duration of hypoparathyroidism. During longitudinal follow-up over a period of 5 years, BMD remained unchanged in the patients with hypoparathyroidism but decreased at the femoral neck in controls. Most of these studies were confined to postmenopausal females and postsurgical hypoparathyroidism. Questions arise as to whether the same change in bone mineral density occurs in patients with idiopathic hypoparathyroidism in whom there is no prior history of thyroid function abnormalities and in whom the onset of hypoparathyroidism may be more insidious.

We measured the BMD of lumbar spine and proximal femur in 14 patients, 8 with idiopathic hypoparathyroidism and 6 with postthyroidectomy hypoparathyroidism, using DXA.[7] Their age ranged from 23-57 years old, with a mean of 42.5 years. The age of our patients was younger than that of previous studies. Eight of them were female and five were pre-menopausal. While previous studies attributed the increase in BMD to attenuation of post-menopausal bone loss by hypoparathyroidism, we could show that even in male and younger population before menopause, patients with hypoparathyroidism has a higher BMD than the normal sex- and age-matched controls. This was particularly evident at the lumbar spine, with positive Z score of 1.93 ±1.03 SD, whereas Z score at the femoral neck was 1.14±0.62 SD. Six of the eight patients with idiopathic hypoparathyroidism were male. Subgroup analysis showed that those with postthyroidectomy hypoparathyroidism had a mean lumbar spine BMD of 1.434 g/cm^2 (mean Z score 2.26) and femoral neck BMD of 1.026 g/cm^2 (mean Z score 1.31), compared with a mean BMD of 1.364 g/cm^2 (mean Z score 1.68) and 1.022 g/cm^2 (mean Z score 1.02) at spine and hip, respectively, for those with idiopathic hypoparathyroidism. Although there was a trend towards higher lumbar spine and femoral neck Z score in patients with postthyroidectomy hypoparathyroidism, statistical analysis did not reveal any significant difference in BMD, T scores, and Z scores of the lumbar spine and proximal femur between the two groups. Possible caveats are the small sample size and the difference in sex distribution between the two groups. The latter is unlikely to be significant, because areal BMD of the male skeleton is higher than that of the female because of the greater size of bones in men; yet, this study showed a trend towards higher BMD in the female-predominant postthyroidectomy hypoparathyroidism. Apparently, the transient period of high bone turnover caused by thyrotoxicosis before surgery does not jeopardize bone mass. Indeed, it may even expand the remodeling space for osteoblastic bone formation after surgery.

In conclusion, others and we have demonstrated consistently that the state of chronic hypoparathyroidism is associated with increased BMD, most notably at the spine. Those with idiopathic hypoparathyroidism have a similar degree of increase in BMD as those with postthyroidectomy hypoparathyroidism. Whether or not it can be translated to a reduction in fracture risk is still not known. We shall explore the underlying mechanism of the increased BMD from the perspectives of biochemical markers and bone histomorphometry. Recently, data are also present on the bone geometry of patients with hypoparathyroidism.

References

1. Dimich A, Bedrossian PB, Wallach S. Hypoparathyroidism. Clinical observation in 34 patients. Arch Intern Med 1967;120:449-58.
2. Hossain M, Smith DA, Nordin BE. Parathyroid activity and postmenopausal osteoporosis. Lancet 1970;1:809-11.
3. Seeman E, Wahner HW, Offord KP, Kumar R, Johnson WJ, Riggs BL. Differential effects of endocrine dysfunction on the axial and the appendicular skeleton. J Clin Invest 1982;69:1302-9.
4. Abugassa A, Nordenstrom J, Eriksson S, Sjoden G. Bone mineral density in patients with chronic hypoparathyroidism. J Clin Endocrinol Metab 1993;76:1617-21.
5. Fujiyama K, Kiriyama T, Ito M, et al. Attenuation of postmenopausal high turnover bone loss in patients with hypoparathyroidism. J Clin Endocrinol Metab 1995;80:2135-8.
6. Duan Y, De Luca V, Seeman E. Parathyroid hormone deficiency and excess: similar effects on trabecular bone but differing effects on cortical bone. J Clin Endocrinol Metab 1999;84:718-22.
7. Chan FK, Tiu SC, Choi KL, Choi CH, Kong AP, Shek CC. Increased bone mineral density in patients with chronic hypoparathyroidism. J Clin Endocrinol Metab 2003;88:3155-9.

S11.1 Acromegaly Registry in Taiwan

Jen-der LIN
Division of Endocrinology and Metabolism, Department of Internal Medicine, Chang Gung Memorial Hospitals, Chang Gung University, Taiwan, ROC

The study examines the characteristics of acromegalic patients in Taiwan and the initial results of developing a registration program for acromegalic patients. The study retrospectively examined charts of clinically diagnosed acromegalic cases at Chang Gung Hospitals between January 1977 and June 2006. The goal of this study is to elucidate the clinical features of acromegaly, as well as diagnostic modalities, treatment, complications, comorbidities and follow-up status. 207 patients were diagnosed as acromegaly in Chang Gung Hospitals, Taiwan. Inclusion criteria were clinically suspicious acromegaly with elevated or persisted serum GH level as over or equal to 5 ng/mL or oral glucose tolerance test (OGTT) with unsuppressed GH (>2 ng/mL). Typical clinical features with the past history of pituitary surgery in other hospital or empty sella were included too. Acromegalic patients including 113 cases in Linkou, 14 cases in Keelung, 49 cases in Taipei, 3 cases in Jiayi and 28 cases in Kaohsiung CGMH were enrolled. There were 106 females (51.2%) and 101 males (48.8%). Of the patients, 153 cases (73.9%) received surgical treatment. In most surgical cases, a trans-sphenoidal approached was applied to remove pituitary adenoma (trans-sphenoid: 93.5%, trans-cranial: 6.5%, trans-sphenoid & trans-cranial; operation over twice: 12.4%, total cases number=153). Number of operations was as follows: once-131 cases; twice-19 cases; and three times: 3 cases. The patients received external radiation rate was only 22.7% (n=47). In our limited information, there were 23.9% (n=138) of patients diagnosed as panhypopituitarism postoperatively. Of these cases, 12 cases received Sando LAR therapy with the fixed dose 20 mg every 4 weeks. Mean GH level and IGF-1 level decreased from 62.7±85.3 ng/mL and 917.4±264.4 ng/mL to 10.2±11.9 ng/mL and 512.0± 229.2 ng/mL, respectively. In this retrospective chart review analysis, 93.7% (n=194) in patients were still survival. To obtain the incidence and prevalence data about the cases of acromegaly, registration studies are important.

S11.2 Multiple Endocrine Neoplasia-1 – the Chinese Experience

Tjin-shing JAP
Section of Biochemistry, Veterans General Hospital- Taipei, Taiwan ROC

Patients with multiple endocrine neoplasia type 1 (MEN1) may develop parathyroid, enteropancreatic endocrine and pituitary adenomas. MEN1 is an autosomal dominant disorder resulting from loss of function of a tumor suppression gene located on the chromosome 11q. The gene comprises 10 exons that encode 610 aminoacids product- menin. One of our studies was analyze 9 patients with MEN-1 with clinical presentations and their family members and to determine the mutation in menin. Of all 20 symptomatic individuals studied, all had primary hyperparathyroidism (100%), 11 pancreatic endocrine tumors (55%) and 8 pituitary tumors (40%). Eight patients (40%) had pituitary tumors, including 6 prolactinoma, 1 growth hormone-secreting tumor and 1 non-secreting tumor. Pancreatic involvement was present in 11 affected subjects (55%), including 4 insulinomas and 7 gastrinomas. Totally, we have identified *MEN1* gene mutation of nine Han Chinese kindreds with MEN1 living in Taiwan, including five novel mutations. To sum up, only 85.7% (18/21) of affected families a causal mutation is identifiable in Chinese, including 3 insertions, 2 deletions, 1 deletion/insertion, 2 abnormal splicing, 4 nonsense mutations and 6 missense mutations. As compared with the mutations in *MEN1* gene in the southern Han Chinese with MEN1, we found no similar mutations in the Han Chinese in Taiwan.

S11.3 Expression and Molecular Regulation of the *Cyclooxygenase-2* Gene in Gastroenteropancreatic Neuroendocrine Tumors and In Vitro Growth Suppression by a Selective COX-2 Inhibitor: NS-398

Feng GAO, Lu-lu CHEN
Department of Endocrinology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China

Objective: Cyclooxygenase-2 (COX-2) is the inducible key enzyme of arachidonic acid metabolism and its expression is mainly regulated at the transcriptional level. COX-2 induction is critically involved in the pathogenesis of inflammatory and neoplastic lesions. The pathophysiological role of COX-2 in gastroenteropancreatic neuroendocrine tumors (GEP-NETs), however, is unclear. Therefore, *cox-2* expression as well as the molecular mechanisms controlling transcriptional regulation of the *cox-2* gene were investigated in this tumor entity. In addition, the growth-inhibitory properties of COX-inhibitors on GEP-NET cells were analyzed. **Materials and Methods:** Expression of the *cox-2* gene was analyzed in 48 GEP-NET tissues by immunohistochemistry. In a subset of tumors as well as in the permanent NET cell lines QGP-1, BON and LCC-18, *cox-2* expression was assessed by Western blotting and *gapdh*-controlled duplex RT-PCR. The promoter region responsible for *cox-2* gene expression in NET cells was characterized by 5' deletion analysis, element transfer experiments using a heterologous promoter system and site-directed mutagenesis employing *cox-2*-luciferase reporter gene constructs. Nuclear proteins regulating the *cox-2* gene in NET cell lines were identified by standard EMSA ("Electrophoretic-Mobility-Shift-Assays") techniques. To analyze the functional relevance of transcription factors, expression constructs encoding dominant-negative transcription factor mutants were applied. COX enzymatic activity was indirectly quantified via determining prostaglandin E_2 (PGE_2) levels by enzyme immunoassay. Anchorage-dependent and -independent growth of NET cell lines was assessed using established proliferation assays. **Results:** COX-2 was detected immunohistochemically in 42 of 48 NET tissues. Within tumor tissues, COX-2 immunoreactivity was located in epithelial tumor cells as well as in inflammatory and endothelial cells. All metastatic foci analyzed, stained strongly positive for COX-2. Moreover, *cox-2* expression was found in 5 of 6 tumors by Western blotting and in 9 of 9 tissues by *gapdh*-controlled duplex RT-PCR. All permanent NET cell lines showed *cox-2* expression, however, remarkable quantitative differences between individual lines were revealed. A proximal overlapping CRE-Ebox sequence located at base pairs -56 to -48 was identified as essential basal enhancer element controlling *cox-2* expression in GEP-NET cells. Moreover, USF1/-2 and CREB transcription factors were recognized as nuclear proteins binding to the overlapping CRE-Ebox element and thus regulating *cox-2* promoter activity. COX-2-specific as well as non-selective COX inhibitors were found to reduce NET cell proliferation, suggesting that COX-2 inhibitors can suppress NET cell growth through COX-2-dependent and -independent mechanisms. **Conclusion:** This study demonstrates that the majority of GEP-NETs express the *cox-2* gene and for the first time identifies the functional interplay of CREB and USF1/-2 transcription factors with a proximal CRE-Ebox element as a central mechanism underlying *cox-2* gene expression in GEP-NET cells. Moreover, COX-2 inhibitors are capable of suppressing NET cell growth *in vitro*. Further analysis of the molecular control and the pathogenic role of COX-2 in neuroendocrine tumors may add to a better understanding of NET pathobiology and can probably help to develop novel therapeutic and/or diagnostic approaches.

S11.4 Estrogen-dependent Breast Cancers: Pathophysiology and Management

Yi-ming MU, Wei-dong HAN
Department of Endocrinology, Chinese PLA General Hospital, Beijing 100853, China

Estrogen and its receptor α (ERα) play a key role in the development and metastasis of breast carcinoma. As a transcriptional factor, ERα regulates the expression of E2 target genes through the interaction with transcriptional co-activators after combining to DNA in direct or indirect manners, which occurs after ERα combines to E2 and changes its conformation. Transcriptional coactivators and inhibitive factors of ERα play an essential role in regulating the function and activity of E2 target genes. After combing to E2, the configuration of ERα changes, then the binding sites to the co-activators are exposed. Many multimeric protein complexes are attracted here, and the transactivation of E2 target genes are activated. In the recent 10 years, more than 30 co-activators (CBP/P300/SHARP/AIB1 et al) of ERα and several multimeric protein complexes (SWI/SNF/CARM/PRMT1 et al) have been recognized. As the E2 target genes, cyclin D, MTA_3, E_2F_1, bcl-2, cad, cathepsin, c-fos, human vitamin D_3, $IGFBP_4$ and RARα have been confirmed to be related with the proliferation of the breast carcinoma to date. And E-cadherin is the gene that has been confirmed to be associated with the metastasis of the breast cancer, however the exact molecular biological mechanism is still unclear.

LRP16 is a human gene, which was originally recognized from peripheral lymphocyte cells by our group in 2000 using restriction length genomic scanning (RLGS), and then the cDNA was isolated using the rapid amplification of cDNA end (RACE) technique. LRP16 codes for a protein of 325 amino acids, which is mainly localized in the nucleus. Previous studies have demonstrated that E2 activates the LRP16 mRNA levels and reporter gene activities in MCF-7 human breast cancer cells. Recently, a maximally responsive fragment (-101 to -14 bp) within 5'-proximal region of LRP16 to estrogen was also mapped, which conferred the estrogen response through ERα/Sp1 interaction. Ectopic expression of LRP16 in breast cancer cells stimulates cyclin E gene expression and cell proliferation. Here, we present new data supporting a role for estrogenically regulated LRP16 as an estrogen receptor α(ERα) coactivator, providing a positive feedback regulatory loop. LRP16 is shown to interact with ERα in vitro and in whole cell lysates, while suppression of LRP16 expression inhibits the expression of several ERα upregulated genes including cyclin D1 and c-Myc, but results in the increase of E-cadherin expression through ERα mediation. Chromatin immunoprecipitation (ChIP) analysis demonstrates the presence of an ERα/LRP16 complex on the pS2 promoter and LRP16 inhibition of ERα binding to the E-cadherin promoter, indicating that LRP16 can co-operate with ERα or antagonize ERα for promoter binding in a gene specific manner. In general, these data suggest that LRP16 may be essential for ERα signaling and important in breast cancer progression.

S12.1 Investigation of the Association on Subclinical Thyroid Dysfunction

Yan-ming GAO, Gui-zhi LU, Jun ZHOU, Qiu-ming JIANG, Hui-min ZHANG, Ling-ding XIE, Song DONG, Xiao-hui GUO, Yan GAO
Department of Endocrinology, Peking University First Hospital, Beijing 100034, China

Objective: To investigate (1) prevalence and incidence of the subclinical thyroid dysfunction in physical examination population in Beijing area; (2) 2-year follow-up of the patients with thyroid dysfunction and to make an analysis of its natural course; (3) the association between subclinical hypothyroidism (sub-hypo), dyslipidemia and non-alcoholic fatty liver; (4) cytologic pathology of subclinical thyroid dysfunction. **Results:** (1) Total prevalence of sub-hypo and subclinical hyperthyroidism (sub-hyper) are 3.7% and 1.9% respectively. The positive rate of TPOAb in population with sub-hypo were 47.1% and significant higher than the 18.5% of euthyroid population (p=0.000); (2) 1966 people of initial physical examination population were followed up 2 years later. Total incidence of sub-hypo and sub-hyper are 1.72%/yr and 0.25%/yr respectively. (3) In 2 years follow-up study, 13 patients (22.4%) with sub-hypo returned to normal; 44 (75.9%) remained subclinical state and 1 (1.7%) progressed to overt hypothyroidism. 22 patients (73.3%) with sub-hyper returned to normal, 2 (6.7%) remained sub-hyper, 1 (3.3%) developed overt hyperthyroidism and 5 (16.7%) turned to sub-hypo. The initial concentration of serum TSH affected the natural course of sub-hypo (>10 mIU/L) and sub-hyper (<0.15 mIU/L). (4) Subclinical hypothyroidism was not associated with serum total cholesterol level but positively associated with increasing serum triglyceride. It seems that sub-hypo does not increase the prevalence of non-alcoholic fatty liver. (5) The cytologic pathological results showed that Hashimoto's thyroiditis was the most common cause in sub-hypo (81.71%). In sub-hyper, Hashimoto's thyroiditis (41.86%), goiter (34.88%) and thyroid adenoma (13.95%) were all seen frequently. **Conclusions:** (1) The prevalence and incidence of subclinical thyroid dysfunction were higher in physical examination population of the elderly and the female. (2) Only a minor group with subclinical thyroid dysfunction has developed overt thyroid abnormity in a certain period of time. Initial concentration of serum TSH may affect progression to overt hypo- and hyperthyroidism. (3) It seems that sub-hypo does not increase the prevalence of non-alcoholic fatty liver. (4) Hashimoto's thyroiditis is the most common cause, not only in sub-hypo but also sub-hyper.

S12.2 Thyroid Function Testing in Pregnancy

Zhong-yan SHAN
Department of Endocrinology and Metabolism, Institute of Endocrinology, the First Affiliated Hospital, China Medical University, Shenyang 110001, China

Thyroid disease in pregnancy has become a hot topic in endocrinology, obstetrics and gynecology in recent years. The reason is (1) recent studies found that maternal subclinical hypothyroidism during pregnancy results in intellectual impairment of their child. However, the maternal free T_4 (FT_4) is critical for fetal brain development and maturation, regardless of the TSH, during pregnancy, especially, in the first trimester. It is not clear how subclinical hypothyroidism impairs fetal brain development. (2) The normal reference ranges for parameters of thyroid function testing in pregnancy are different from nonpregnant population due to significant changes in maternal thyroid physiology. Unfortunately, currently there are no reliable trimester-specific reference ranges for TSH, TT_4 and FT_4.

In the lecture I will be mainly focus on: (1) Why to use trimester specific reference ranges to evaluate thyroid function in pregnancy? Sensitive TSH is regarded as the preferred marker in diagnosis of thyroid disease. In pregnancy, thyroid is stimulated by human chorionic gonadotropin (HCG) due to structural homology with TSH, which results in a modest increase in serum T_4 level. HCG peaks at approximately 10 weeks of gestation and declines after 12 weeks. This results in a significant fall in serum TSH in the first trimester. The fetus begins to produce thyroid hormones at around 12 weeks of gestation. Before this time, the maturing fetal brain depends on the circulating maternal T_4. In view of the reasons trimester-specific TSH and T_4 reference ranges are needed for evaluating pregnant women. (2) How to determine the trimester specific reference ranges for TSH, FT_4 and TT_4? Establishment of reference ranges must consider iodine and thyroid autoimmune status. The number of fetus is also one of influencing factors. Our study showed that serum TSH level was significantly lower in pregnant women than that in nonpregnant women in the first trimester in adequate iodine intake areas. The median TSH concentration was 0.78 (0.09-2.96 mU/L) at 12 weeks of gestation. TSH level in pregnant women with positive thyroid autoimmune antibodies (TAA) [1.33 (0.17-3.61 mU/L)] was markedly higher than that in pregnant women without TAA. (3) Which marker should be used to diagnose thyroid diseases and monitor treatment effect in the first trimester? Serum TSH level decreases and TT_4 concentration increases significantly in the first trimester compared with nonpregnant population under untreated condition. The median TT_4 was 12.35 μg/dL, which was 1.65 fold of baseline TSH. Serum TT_4 level had no significant change in pregnant women with subclinical hypothyroidism after 1-month treatment. TT_4 level was elevated after 2-month treatment. Serum FT_4 increased significantly after 1 months of treatment. Changes of serum TSH and FT_4 level are more rapid than TT_4, while FT_3 and TT_3 levels almost have no change after L-T_4 replacement started before 8 weeks gestation.

S12.3 Integration of Western and Traditional Chinese Medicine Therapy of Graves' Disease

Jia-jun ZHAO, Xia ZHONG, Men REN, Wen-xia HAN, Ling GAO
Department of Endocrinology , Shandong Provincial Hospital, Shandong University, Jinan 250021, China

Objective: In addition to the signs and symptoms of high T3 and T4, patients with Graves' Disease (GD) often have other signs including a diffuse goiter, audible bruitand exophthalmos, Treatment with western medicine alone does not address the significant signs of a goiter and exophthalmos. We investigated the effect of the addition of Traditional Chinese Medicine (TCM) to Western medicines to treat GD with exophthalmos and goitre. **Methods:** 132 untreated-GD patients were treated with western medicine (methimazole 30 mg/day or propylthiouracil 300 mg/day, western medicine alone group), while 526 untreated-GD individuals were treated with the western medicine and TCM (30 g/day, combined-treatment group). The aim of the latter prescription is to strenthen the body's natural healthy energy to drive out the bad substances; this has the combined aim of treating both GD' originally and in addition to regulate the body's general immunity. **Results:** (1) There was a reduction in thyroid volume, in 87.1% of the 526 patients in the combined treatment group compared to 31.7% of 132 patients in the western medicine alone group ($p<0.01$). (2) In the 216 GD patients with exophthalmos in the combined-treatment group, 87.5% of the patients had a significant reduction in the severity of their exophthalmos. In fact, the exophthalmos in 139 patients completely disappeared. In contrast. only 50% of the GD patients in western treatment alone group had significant reduction in their exophthalmos. (3) The rate of relapse was 23.6% in the combined treatment group versus 47% in the western medcine alone group. **Conclusion:** Therapy with the combined-treatment of western and TCM is effective in reducing goiter size and reducing exophthalmos of Graves' patients. This integration of Western and TCM is able to achieve the effect of complementing each other's strengths as well as making up for each other's deficiencies.

S12.4 Differentiated Thyroid Cancer Genetics

Rue-tsuan LIU
Division of Metabolism, Chang Gung Memorial Hospital, Kaohsiung, Taiwan

In the past 2 decades, information on expression of oncogenes in human thyroid follicular cell is rapidly accumulating. Genetic alterations in four oncogenes, namely *RAS* point mutations, *RET* rearrangements (*RET*/PTC), *NTRK1* rearrangements (*TRK*) and *BRAF* point mutations have been identified in human papillary thyroid carcinomas. These oncogenes act along the RET/PTC(TRK)-RAS-BRAF-MEK-MAPK kinase pathway, mediating a number of cellular fates including growth, proliferation and survival in thyroid cells. *RAS* point mutations have been reported in each benign and malignant histological type of thyroid neoplasia. However, it appears to selectively occur in follicular adenomas, follicular carcinomas, poorly differentiated and anaplastic carcinomas, and in some papillary carcinomas. Somatic rearrangement of the cell-surface transmembrane tyrosine kinase receptor *RET* or *NTRK1* is restricted to papillary thyroid carcinoma. Differences in the frequency of *RET* have been described in PTCs collected from various geographical area. Using RT-PCR to amplify fusion products of *RET*/PTC1, *RET*/PTC2 and *RET*/PTC3 from frozen tissues, however, the prevalence of *RET* rearrangements varies between 0% and 20% in most series of sporadic PTCs. *NTRK1* rearrangements are less frequently found in PTCs than *RET* rearrangements. Mutations of the *BRAF* protein serine/threonine kinase gene have recently been identified in a variety of human cancers, especially in melanoma and papillary thyroid carcinomas. Among benign and malignant thyroid tumors, $BRAF^{V600E}$ mutations were reported to be restricted to papillary carcinomas and poorly differentiated and anaplastic carcinomas arising from papillary carcinomas. The prevalence of *BRAF* mutations reported from different population was quite consistent, detected in 36% to 46% of papillary carcinomas in most series. To elucidate the molecular basis for the tumorigenesis of papillary carcinoma in Taiwan, we systematically evaluated the known oncogenes in a thyroid tumor cohort to determine their individual significance in the pathogenesis of papillary carcinoma in this area. We have previously shown that, of 105 cases of PTC, 49 (47%) had heterozygous mutations T1799A in exon 15 of *BRAF* gene. Eight of 105 PTCs (8%) had *RET* rearrangements. Of these tumors, 3 involved *RET*/PTC1 and 4 involved *RET*/PTC3. We identified one tumor as having an *ELKS-RET* rearrangement. One of 105 PTCs (1%) had *NTRK1* rearrangement (*TRK-T2*). We did not find *RAS* mutations in the PTCs studied. Correlation between *BRAF* mutations and various clinicopathological parameters in this studied cohort did not reveal any association with age at diagnosis, sex, tumor size, histological variants of PTC, multicentricity, cervical lymph node metastases, extrathyroidal invasion, distant metastases and clinical stage. Our data, which show lack of overlap between *RET*/PTC, *TRK*, *RAS* or *BRAF* mutations in papillary carcinomas, confirm and extend previous evidence indicating that alteration of any component in the RET/PTC(TRK)-RAS-BRAF-MEK-MAPK signaling pathway is sufficient for the initiation of sporadic PTCs and may provide a more reliable means for further confirmation of genetic alteration along this signaling pathway in individual PTC.

Recently, a new PAX8-PPAR gamma1 gene fusion has been identified at significant frequency in follicular thyroid carcinomas and in follicular adenomas as well. In our study, PAX8-PPARγ1 mutations were detected in a significant proportion of follicular adenoma (5 of 10, 50%) and follicular carcinoma (3 of 6, 50%). *Ras* and PAX8-PPARγ1 mutations were demonstrated in the majority of follicular carcinomas (5 of 6, 83%), suggesting that these two oncogenes may play important roles in the tumorigenesis in follicular carcinoma in Taiwan.

MicroRNAs (miRNAs) are a class of small non-coding RNAs involved in a wide range of basic processes such as cell proliferation, development, apoptosis and stress response. It has recently been found that an aberrant miRNA expression profile that clearly differentiates PTCs from normal thyroid tissues. These data indicate a miRNA signature associated with PTCs, and suggest miRNA deregulation as an important event in thyroid cell transformation.

DC1.1 Role of CDE in Diabetes Care

Alison EVERT
Diabetes Care Center, University of Washington Medical Center, Seattle, Washington, USA

Diabetes has reached epidemic levels in the United States (US) and around the world. The importance of optimal blood glucose control in preventing diabetes as well as its devastating complications has been well documented. One of the biggest challenges facing people with diabetes is learning how to live with, and manage their condition on a daily basis.

A majority of people with diabetes receive their diabetes care exclusively from their physician. The role of non-physician health care professional in the treatment of diabetes has emerged over the last 25 years. Currently there are over 15,000 Certified Diabetes Educators (CDE) in the US. A variety of health care professionals can become CDEs and include some of the following professions; nurse, dietitian, pharmacist, exercise physiologist, social worker, and physician. The goal of diabetes self-management education provided by CDEs is to help individuals with diabetes to acquire the knowledge, skills, attitudes, and behaviors to optimize both their self-management of diabetes and their quality of life. The role of the CDE on the diabetes health care team will be explored in this session and will include discussion of the scope of practice from the entry level nurse or dietitian to the advanced practice CDE and the variety of practice settings where diabetes education can occur.

DC1.2 Outcome of Diabetes Nurse Clinic

Frances K.Y. WONG

(Abstract not received at the time of printing)

DC1.3 Diabetes Education — Cornerstone in Diabetes Care

Wayne H-H. SHEU
President, Taiwanese Association of Diabetes Educators (TADE); Professor of Medicine and Chief, Division of Endocrinology and Metabolism, Department of Medicine, Taichung Veterans General Hospital, Taichung, Taiwan

The importance of taking aggressive management of diabetes to prevent the devastating complications of the disease has become evident. The role of glucose control in preventing diabetes complications is supported by 2 large, prospective, randomized clinical trials: the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS). Both studies demonstrated a clear association between blood glucose control and sustained, decreased rates of macro- and micro-vascular complications. Thus, all health care providers need sufficient diabetes knowledge to provide safe, competent care to persons with or at risk for diabetes.

As management of diabetes becomes increasingly complex, it is imperative that diabetes health care professionals be well educated and appropriately credentialed. Expertise in diabetes care develops through experience, continuing education and individual study. Diabetes educators use established principles of teaching and learning theory and lifestyle counseling to help diabetes patients confidently and effectively manage the disease. Instruction is individualized for persons of all ages, incorporating cultural preferences, health beliefs, and preferred learning styles of the diabetes patients. The primary goal of diabetes education is to provide knowledge and skill training that help individuals identify barriers and to facilitate problem-solving and coping skills to achieve effective self-care behavior and behavior change.

Based on recommendation from American Association of Diabetes Educators, there are 6 standards of "practice" for diabetes educators, namely, (1) Assessment, (2) Outcome Identification, (3) Planning, (4) Implementation, (5) Evaluation, (6) Documentation. There are also 8 standards of "professional performance" for diabetes educators, namely, (1) Quality of Care, (2) Professional Performance Appraisal, (3) Professional Development, (4) Collegiality, (5) Ethics, (6) Collaboration, (7) Research, (8) Resource Use. These standards might be modified over different cultures and systems.

Taiwanese Association of Diabetes Educators (TADE) established since 1996 aiming to promote skill and booster knowledge of diabetes education in order to cope with increasing need of diabetes care. Currently, we have membership up to 10,000, composing of MD, RN, RD, Pharmacists and other diabetes care subspecialties. More than 2,500 diabetes educators (CDE) certified after certain hours of course, training and passing tests. It is our mission to provide comprehensive care, to promote better quality of life for diabetes subjects through team-cared strategies. A total of 126 diabetes care settings, composed of MD, RN, RD, certified by TADE, provide care for a certain portion of diabetes subjects in Taiwan. I would like to share our experience during my presentation.

DC1.4 Advanced Diabetes Nursing Practice in Mainland China

Qing-qing LOU
Diabetes Nurse Specialist, Sir Run Run Shaw Hospital, Medical College of Zhejiang University, China

Diabetes is already a health crisis of enormous proportions. The burden on patients and society of late diabetes complications is overwhelming. The projections indicate that by 2025, 6.3% of the global adult population will have diabetes (334 million) with an even greatest burden falling in the developing countries. At that time, China will have over 100 million diabetes patients. The battle against diabetes will not be won without team work of multidisciplinary professionals. In the developed countries and areas, nurses are playing very important roles in diabetes care, while in Mainland China, nurses have not been involved in diabetes education until late 1990s.

In this presentation, we will discuss the background of diabetes in Mainland China, and the factors that keep us from growing professionally. Also we will reflect on experiences and examples of advanced diabetes nursing practice in Mainland China.

DC2.1 Why Do We Need Diabetes Team Care?

S.T. TSAI

(Abstract not received at the time of printing)

DC2.2 Psychological Challenges in Diabetes

Peter W.H. LEE
Clinical Health Psychology Programme, Department of Psychiatry, The University of Hong Kong, Hong Kong

Kierkegaard (1959) noted rightly that "If you really want to help somebody, first of all you must find him where he is and start there. This is the secret of caring….Helping somebody implies your understanding more than he does, but first of all you must understand what he understands….All true caring starts with humiliation". Experienced clinicians have long realized that management of chronic illnesses like diabetes cannot rely on a one sided, authoritative or paternalistic approach from the doctor or specialist nurse dictating what patients should or should not do. To effectively manage the challenges imposed by diabetes, the prerequisite task is to establish a safe and trusting therapeutic rapport with the patient before moving on to strive to contain the harm of diabetes with collaborative and synergistic efforts between the clinician and the patient. "Compliance" or adherence to medical advice, whether it be the medication regimen, habit and lifestyle changes, blood glucose monitoring or behavioural preventive measures, is not automatic. There is a danger that metabolic goals are vigorously pursued at the psychological expense of miserable patients so much so that they eventually choose to abandon the agreed quest for metabolic perfection when face with unacceptable psychological costs. Compliance is not automatic. A patient's motivation is complex and frequently goes beyond simple health concerns. Important patient related variables had been highlighted in the literature on health belief. Patients' perceptions of their vulnerability, the severity of the illness, sense of control, effectiveness of the treatment, related costs and barriers to treatment are potent variables affecting adherence. On the other hand, Ley (1997) reminded us that the consultation related variable which is consistently associated with compliance is "the patient's satisfaction with the consultation". The psychological challenges of diabetes extend beyond blood glucose control and prevention of long-term complications. In particular, the clinician has to manage the high prevalence of dysphoric emotions (with an estimated ten percent of the diabetic population suffering from a major depressive disorder and thirty percent having depressive symptomatology), help patients maintain an optimal and satisfying quality of life despite the illness, promote effective stress management, satisfactory weight control, and deal with idiosyncratic concerns like sexual dysfunctions. In managing diabetes, the clinician also has to work against the adverse influence of the life long nature of the illness. A life-long affliction creates almost certain periodic fatigue, demoralization, and resentment in patients, particularly during periods of high stresses. The clinicians' effectiveness in managing diabetes rests largely in their success in empowering patients to do what is best for themselves, in the most willing and informed manner. The skills required are multifaceted. Good management starts with effective psychoeducation, clear communication, successful formation of trusting rapport and a long lasting therapeutic relationship. The challenge in managing diabetes is to be able to cut through ignorance to knowledge, from knowledge to realistic attitudes and beliefs, and finally from attitudes/beliefs to effective actions. The effective clinician is one who can balance and deliver informational as well as emotional care. A good clinician strives to be one whom patients can trust and work with; one who understands, respects and is respected in return, one who listens and is willing to answer questions; and one who is comfortable to be with.

DC2.3 Role of Diabetes Nurse in Complication Screening

Elaine LEUNG
Nurse Specialist, Diabetes Care, Queen Mary Hospital, Hong Kong

The total number of diabetes patients under the care of Hospital Authority of Hong Kong in 2004/2005 was about 200,000. It not only represented nearly 90% of known diabetes population in Hong Kong, but also accounted for over 0.8 million SOPC attendance and over 1 million bed days as in-patient, with major diagnosis being chronic complications for diabetes. To face with this huge burden on medical expenditure, prompt diagnosis and treatment may be viewed as a key element to avoid the need for more complicated and expensive treatment regimens.

International guidelines recommend that diabetic patients should be periodically screened for early evidence of the complications so that timely intervention can be initiated appropriately to improve clinical outcomes. Although these guidelines are not difficult to follow and implement, owing to constrain in resource, many health care providers still not yet incorporated guidelines into their practice. It leads to not every diabetic patient in Hong Kong can receive regular complication assessment. Every effort should be made to facilitate health service providers' adherence to these guidelines. It is commonly accepted that a structured program of complication assessment is an effective strategy to facilitate adherence, as well as a conductive way in achieving the goal of comorbidities reduction associated with delayed diagnosis of diabetic complications.

Diabetes nurse has a long history in Hong Kong. Apart from promoting diabetes self-management as an integral part of diabetes care, we also strike for the collaboration of multidisciplinary task force in providing quality health services. When we first initiated complication screening in the 90s, the role of diabetes nurse was mainly concentrated on the program implementation. As the demand was increased tremendously after its recognition among both patients and health service providers, support from other health professionals, such as podiatrist and optometrist, was indicated. Our role was then extended to the coordination among different health workers to facilitate seamless service provision.

In 2002, when the Hospital Authority took over the management of the first 5 GOPCs, complication screening was also rolled out from secondary health care level to the primary one. To improve chronic disease management among the primary health care, diabetes nurses were invited to design and conduct the first structured diabetes education course for nurses in GOPC. As variation in practice across different clusters has been identified, representatives from Diabetes Educators' Group worked for a user manual to enhance consistency and quality of results collection during the regular complication screening program. The role of diabetes nurse in this perspective became professional training.

Facing the rapid changing health service environment, the role of diabetes nurse should be adjusted according to newly arose demands. Our role in complication screening should not be limited to solely addressing the screening and preventive services, but should also extend to education, counseling, and supporting clients in adopting and maintaining healthy lifestyles. Being an explorer for a new model of health care provision, as well as facilitating other health care service providers in providing quality services to our clients, may be our direction for future development.

DC2.4 New Approaches in Diabetes Dietary Management

Flavia U
Hong Kong SAR

Medical Nutrition Therapy (MNT) is important in preventing diabetes, managing existing diabetes, and preventing the rate of development of diabetes complications. Yet, many misconceptions exist concerning nutrition and diabetes. The goal of nutrition recommendations is to make people with diabetes and health care providers aware of beneficial nutrition interventions. This requires the use of the best available scientific evidence while taking into account treatment goals, strategies to attain such goals and changes individuals with diabetes are willing and able to make. It is important that all the team members, including physicians and nurses, be knowledgeable about MNT and support its implementation.

MNT involves a nutrition assessment to evaluate the patient's food intake, metabolic status, lifestyle and readiness to make changes, goal setting, dietary instruction, and evaluation. To facilitate the adherence, the plan should be individualized and take into account cultural, lifestyle, and financial considerations. Monitoring of glucose and A1C, lipids, blood pressure, and renal status is essential to evaluate nutrition-related outcomes. If goals are not met, changes must be made in the overall diabetes care and management plan.

Both the amount as well as the type of carbohydrate in a food influence blood glucose level. The total amount of carbohydrate consumed is a strong predictor of glycemic response, and thus, monitoring total grams of carbohydrate, whether by use of exchanges or carbohydrate counting, remains a key strategy in achieving glycemic control. The use of glycemic index (a measure of the effect of type of carbohydrate) can provide additional benefit on glycemic control.

Overweight and obesity are strongly linked to the development of type 2 diabetes and can complicate its management. Obesity is also an independent risk factor for hypertension and dyslipidemia as well as cardio-vascular diseases, which is the major cause of death in those with diabetes. Moderate weight loss improves glycemic control, reduce CVD risk and can prevent the development of type 2 diabetes in those with pre-diabetes.

We will discuss carbohydrate counting, glycemic index, lipid lowering diet, meal spacing and current recommendations.

SC1.1 推行優質糖尿病護理的障礙

Eva C.Y. KAN 簡靜兒

在1922年以前，糖尿病是不治之症。直至Banting醫生和BEST醫生發現胰島素之後，糖尿病治療開始展示一線生機。醫護人員為協助糖尿病人延長生命，開始教導他們注射胰島素，從此時開始便確知糖尿病人教育的重要性。直至1972年，糖尿病人教育才正式納入為糖尿治理的重要一環。如今，糖尿教育已成為優質糖尿病治理不可或缺的單元。

糖尿護理的精髓是透過教育糖尿病人，幫助他們正面積極地面對糖尿病。香港有系統的糖尿護理和病人教育始自1980年代中期，由四位註冊護士將他們在澳洲所學所見，實踐在本地的糖尿護理，自此糖尿護理發展更一日千里，本地護理的模式及準則更成為西太平洋區糖尿病人教育及護理典範。

儘管如此，糖尿病護理在發展過程中，其實也經歷不少障礙和挑戰，講者很榮幸有機會與在座各位分享這個題目，推行優質糖尿病護理的障礙。首先，講者會重溫糖尿護理的發展與現時服務病人的護理準則，然後會透過「優質改進圈」的五個重要單元：「人」,「機」，「物」，「法」和「環」去審視實踐優質糖尿病護理時遇到的障礙，那些是可以克服的，那些是尚需要我們繼續努力去解決。護士和病人在糖尿病治理過程中關係密切，是對等的合作伙伴，希望藉此論述，護士與病人間增進更透切的瞭解，令糖尿病者的自我照顧和管理更加提升。

SC1.2 Experience of a Key Member of the Diabetes Team

W.K. LEUNG

(Abstract not received at the time of printing)

SC1.3 Dietetic Management of Diabetes and Dyslipidaemia

Selina KHOR
State Registered Dietitian, UK

Food is an essential element in life. Physiologically, it is a source of energy; a source of health. It can be a source of enjoyment and gratification. For people with diabetes and dyslipidaemia, it can be a source of anxiety as diet is an important element in the management of the conditions. The role of dietitian is to help them to eat healthily while keeping an optimal control of the conditions. The sequence of dietetic care can be done in three steps: to translate the scientific message to down-to-earth information; to impart knowledge according to their ability to comprehend and accept; to help them to cope with the change and live with the condition while enjoying food as healthy persons. The presentation will be conducted in Cantonese with an introduction of the latest dietary guidelines for diabetes and dyslipidaemia. It will be followed by the application of the guidelines such as healthy food choices, shopping tips, menu planning, cooking and eating out tips etc. Other aspects such as the common food beliefs and food fads will also be discussed. At the end of the presentation, the audiences are expected to be able to adopt a healthful diet suitable for diabetes and dyslipidaemia.

SC1.4 Do We Need Dental Care in Diabetic Patients?

C.S. CHU

(Abstract not received at the time of printing)

SC2.1 實現良好糖尿病控制的要訣

M.W. TSANG 曾文和

醫患携手A B C …

糖尿病的併發症除了眼，腎、週邊神經外，還有心、腦、週邊血管栓塞等。治療糖尿病的目的:
* 減輕、消除高血糖引起的徵象;
* 減少糖尿病的併發症;
* 減少糖尿病對生命歷程的影響。

糖尿病控制及併發症研究(DCCT)，英國糖尿病前瞻性研究(UKPDS)證明平均血糖（糖化血紅素 HbA1c）與血管併發症有直接相關。因此，各地糖尿學會一致推薦HbAlc <6.5%為良好糖尿病控制的指標。與此同時，全面的糖尿病控制更不可忽視高血壓、高血脂等對糖尿病人的大血管併發症的防冶。現就達到良有糖尿病控制提供以下要訣，以供參考。

醫護		病患者
Anti-atherosclerosis 血管硬化冶療	A	HbA1c 糖化血紅素
Blood pressure 高血壓冶療	B	Blood glucose monitoring 自我血糖監測
Cholesterol 高血脂冶療	C	Compliance 藥物，飲食依從性
Diabesity 糖尿與肥胖冶療	D	Dietetic management 營養飲食控制
acEi* 糖尿腎的冶療	E	Exercise 運動控制
Intensive management 加强的冶療方案	I	Insulin resistance/secretion 胰島素抵抗與分泌的認知
anti-Smoking 禁菸	S	Screening of complication 併發症篩檢

*ACEI=血管緊張素轉化酶抑製劑

以上建議基於循證醫學，病人教育，專科護理，及營養冶療學等範籌，本着醫患本一家的精神，共同面對日益嚴重的糖尿病相關問題。

SC2.2 Exercise Therapy in Diabetes and Obesity

Clare C.W. YU
Institute of Human Performance, University of Hong Kong, Hong Kong

Exercise is one of the important components in the management of diabetes and obesity. However, the adherence to exercise programs in many patients has been poor. How can it be improved? The talk will provide update information regarding the effects of different modes of exercise on glucose metabolism, risk factors for complication, and weight management in patients with type 2 diabetes mellitus. In addition, exercise recommendations and practical issues in this population will also be discussed.

SC2.3 Abdominal Obesity, a Cardiovascular Risk of DM Patients

S.C. SIU
DM Centre, Tung Wah Eastern Hospital, Hong Kong SAR

Abdominal obesity is common in people of Hong Kong, especially in the middle aged and elderly. Simply, it is the preferential accumulation of fat within the abdominal cavity. People with abdominal obesity have a higher chance of developing diabetes mellitus, hypertension and cardiovascular diseases. The risk of cardiovascular diseases is even greater for people with diabetes. Though the detection of abdominal obesity is straightforward and easy, it is regrettably very often missed, ignored or denied by both patients and health carers. Abdominal obesity is in fact frequently considered 'normal' or of little clinical significance by all. Attention to and active management of abdominal obesity will greatly improve glucose control and reduce the risk of developing major cardiovascular diseases like myocardial infarction and stroke.

01 Ala45Thr Variation in NeuroD1 Gene is Associated with Early-onset Type 2 Diabetes with or without Diabetic Pedigree in Chinese

Li-mei LIU, Kun-san XIANG, Tai-shan ZHENG, Ming LI, Wei-ping JIA, Hui-juan LU
Department of Endocrinology and Metabolism, Shanghai Jiaotong University Affiliated No.6 People's Hospital, Shanghai Clinical Center for Diabetes, Shanghai Diabetes Institute, Shanghai 200233, P.R. China

Objective: Based on onset-age stratified analysis may be useful to determine the association of NeuroD1-Ala45Thr variation with susceptibility to genetic heterogeneous type 2 diabetes mellitus (T2DM), we investigated the Ala45Thr variation in unrelated early-onset and late-onset T2DM with or without diabetic pedigree and unrelated non-diabetic control subjects in Chinese. **Methods:** 175 early-onset and 194 late-onset type 2 diabetic patients were further divided into two subgroups according to with or without diabetic pedigree respectively. This NeuroD1-Ala45Thr variation were screened by PCR-direct sequencing in above 369 type 2 diabetic patients and 87 unrelated non-diabetic control subjects. We then compared the distribution of the Ala45Thr variation among the groups, searching for the predictive trends. **Results:** Frequencies of the variant (AA+GA genotype) in early-onset T2DM are obviously elevated, especially among diabetic pedigree subjects when compared to non-diabetic controls (p=0.003) and late-onset T2DM subjects (p=0.014). However, no significant differences were observed between late-onset T2DM with or without diabetic pedigree and non-diabetic control subjects. **Conclusions:** Our results suggest that (1) the NeuroDl-Ala45Thr variation may itself have an important role in susceptibility to or be in disequilibrium with early-onset T2DM in Chinese; (2) the Ala45Thr may affect the onset pattern of T2DM, i.e., early-onset but not late-onset T2DM in Chinese; and (3) onset-age stratified analysis may be useful to determine the association of NeuroD1-Ala45Thr variation with susceptibility to genetic heterogeneous T2DM in Chinese.

03 The NCEP Criteria for Metabolic Syndrome Identifies Type 2 DM Patients with Increased Risk for CKD Compared with the IDF Criteria

R.C. MA, A. LUK, W.Y. SO, A.P. KONG, V. NG, C.C. CHOW, C.S. COCKRAM, J.C. CHAN, P.C. TONG
Department of Medicine and Therapeutics, Prince of Wales Hospital, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China

Objective: Metabolic syndrome has been found to be associated with chronic kidney disease (CKD). We compared the predictive value for CKD of the new International Diabetes Federation (IDF) definition of the metabolic syndrome (MES) in a cohort of Chinese subjects with type 2 diabetes to existing criteria of the National Cholesterol Education Program's Adult Treatment Panel III (NCEP). **Methods:** Subjects with type 2 diabetes who underwent assessment for complications between 1995 and 2000 were included and categorized according to different definitions of MES. Glomerular filtration rate (GFR) was calculated using the abbreviated equation developed by the Modification of Diet in Renal Disease (MDRD) study. CKD was considered present if eGFR was <60 mL/min per 1.73 m^2. **Results:** Of 5134 subjects (median age 59.1±13.5), 1996 (38.9%) fulfilled the criteria of both IDF and NCEP ('IDF/NCEP'). For the remaining patients with MES, 506 (9.9%) and 966 (18.8%) complied only with the criteria of IDF ('IDF-only') or NCEP ('NCEP-only'), respectively. Subjects fulfilling 'NCEP-only' were thinner, had a more adverse lipid profile, worse renal function and a higher prevalence of albuminuria than the 'IDF-only' group. Subjects fulfilling 'IDF-only' did not have increased risk of CKD. Subjects fulfilling 'NCEP-only' had increased risk of CKD, OR 2.03 (95% CI 1.53-2.70). The risk of CKD increased with increasing components of MES, with OR 1.71 (95% CI 1.05-2.81), 2.74 (95% CI 1.70-4.42) and 3.85 (95% CI 2.30-6.45) for subjects fulfilling 3, 4 or 5 components of NCEP-MES respectively. **Conclusions:** With established type 2 diabetes, the NCEP definition of MES identifies a subgroup of patients at highest risk of CKD. The IDF criteria fail to identify such subjects. Given the association of CKD and cardiovascular events, the NCEP criteria remains valuable among subjects with established type 2 diabetes.

04 The Adherence of Medical Nutrition Therapy in Diabetes—Based on Diabetes Case Management Program 2001, Taiwan

M.M.T. FUH[1], C.C. LIN[2]
[1] Medicine, China Medical University Hospital, Taichung 404, Taiwan
[2] Family Medicine, China Medical University Hospital, Taichung 404, Taiwan

Objective: In order to evaluate the opportunity for the long-term MNT intervention and ensuing demonstration of overall quality of diabetes care, a nationally standardized healthcare program—DCMP 2001 was implementing in a medical center, Mid-Taiwan. **Methods:** From 2003 to June 2004, 5404 diabetes beneficiaries were randomly recruited in DCMP 2001 via monthly outpatient visits. All the participants were divided up into 5 groups with duration of diabetes, less than 1 year, 1 to <6 years, 6 to <11 years, 11 to <16 years and over 16 years. Accordingly, the diabetes should tri-monthly be able to have dietary consultation after seeing physician. The nutritional assessment and MNT intervention were achieved after adequate record of dietary history. The comparisons of actual and recommendatory total daily caloric intakes and percentages of macronutrients were performed. Comparisons between groups on the amount of daily calorie intakes were performed using one-way ANOVA. Chi-Square tests were used to compare if subjects in groups with different durations of diabetes were improving the adherence. **Results:** The distributions of difference (A-R) between actual (A) and recommendatory (R) daily caloric intakes and the percentage distributions of macronutrients in these 5 groups indicated patients' adherence of eating habits to MNT in different time intervals of having diabetes. Statistically, neither the A-R distributions nor the percentage distributions of macronutrient consumptions were significantly different in these 5 groups of diabetes. **Conclusion:** The results clearly indicated that a considerable opportunity for the long-term MNT intervention has constantly existed whenever the time we start. Furthermore, the ensuing improvement of the overall dietician's quality of dietary care in diabetes via the implementation of DCMP 2001 would become possible.

05 Study of the Role of AMPK in the Regulation of Insulin Secretion

Xiao WANG, Yan-yun GU, Li-bin ZHOU, Ji-ping LI, Tian-hong LUO, Guo LI, Min LUO
Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai Clinical Center for Endocrine and Metabolic Diseases, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Objective: To investigate the role of AMPK in the regulation of insulin secretion. **Methods:** Rat and mouse islets were isolated by perfusing the pancreas with collagenase via the pancreatic duct. Islets were incubated in RPMI 1640 with 0.5% BSA overnight, then incubated in culture medium with different concentration of glucose, glutamate, palmitate and metformin for different time. **Results:** Our findings showed that elevations in glucose concentration from 2.8 mmol/L to 16.7 mmol/L markedly reduced the phosphorylation of AMPK at Thr-172 in islets, which were associated with a progressive increase in insulin secretion. Thr-172 phosphorylation is very important for the activity of AMPK. AMPK activity was negatively related to insulin secretion. 10 mmol/L glutamine inhibited AMPK phosphorylation and increased GSIS. Metformin activated AMPK, but decreased insulin secretion. 0.4 mmol/L Palmitate inhibited GSIS and increased islets AMPK phosphorylation at 16.7 mmol/L glucose concentration. **Conclusion:** These findings suggested that AMPK was involved in the regulation of GSIS. Inhibition of AMPK activity is important for GSIS, while palmitate increased AMPK activity, thus AMPK might be a possible target for palmitate to intervene GSIS.

O6 Study of the Effect on Islets Cultured In Vitro and STZ-induced Diabetic Rats In Vivo of the EGF-like Domain of Betacellulin

Hong LI[1,2], Guo-ting WU[1], Feng-ying LI[2], Wei-bin ZHOU[2], Yan-yun GU[2], Tian-hong LUO[2], Guo LI[2], Min LUO[2]
[1] The Tenth People's Hospital Affiliated to Tongji University, 200072, China
[2] Shanghai Institute of Endocrine and Metabolic Disease, Ruijin Hospital of Shanghai Jiaotong University, 200025, China

Objective: To study the effect of the EGF-like domain of betacellulin (BTCe) on rat islets cultured in vitro and STZ-induced diabetic rats. **Methods:** Abundant BTCe protein was obtained by prokaryotic expression and identified by SDS-page, Western blot and mass spectrometry (MS). Rat islets were cultured in vitro with 1 nmol/l BTC or BTCe. The level of glucose-stimulated insulin secretion (GSIS) was measured on day 1, 3, 5, 7, 10, 15, 20. STZ-induced diabetic rats were transfected with pcDNA-BTCe by a single injection into muscles. **Results:** During a 20-day culture in vitro, the GSIS of islets was much improved in the presence of 1 nmol/l BTC or BTCe though there was no change in gene expressions of insulin, glucagon, PDX-1 and Glut-2. Fifteen to twenty days after plasmid transfection into muscle, the blood glucose of diabetic rats was markedly reduced to nearly 10 mmol/l from over 20 mmol/l. There appeared lots of PDX-1 positive duct cells and insulin positive cells in pancreas from diabetic rats by immunofluorescence. **Conclusion:** BTCe can effectively improve the level of GSIS when islets were cultured in vitro. It was not possible that BTCe executed the protective effect on rat primate islets in vitro through up-regulating the expressions of the four key genes. BTCe can ameliorate the hyperglycemia of diabetic rats perhaps by promoting the regeneration of PDX-1 positive duct cells and differentiation of some precursor beta cells.

O7 Bone Marrow Harbors Islet-like Cells in Hyperglycemic Rats

Xiao-hong WU, Jian ZHU, Jing-jing JIANG, Yu XU, Cui-ping LIU, Kuan-feng XU, Xiao-dong MAO, Chao LIU
Department of Endocrinology, First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China

Objective: To observe whether rat bone marrow harbor islet-like cells in hyperglycemic state. **Methods:** Prolonged and transitory hyperglycemic state was set up in SD rats induced by i.p. streptozotocin or 50% glucose solution. Age-matched normal SD rats were used as controls. Rat bone marrow cells were isolated and cultured in low glucose DMEM supplemented with 10% FBS. Cell morphology was observed under converted microscopy. Islet hormones expressions were observed by immunofluorescence under laser con-focal microscopy. Genes related to islet cells development and functions were detected by RT-PCR. The positive cells of islet hormones and CO-expressed rates of insulin and CD45/CD90 were detected by flow cytometry. Insulin release after glucose challenge was tested with ELISA. **Results:** Primary cultured bone marrow cells were round, polygonal or spindle-like cells adherent to the dishes that were single scattered or formed several cell clones. The gene and protein of islet hormones could not be detected. However, bone marrow cells from transitory hyperglycemic and diabetic rats could form cell clusters which expressed the proteins and genes of islet hormones, as well as the glucose transport-2 (GLUT-2), glucokinase (GK), glucagon like peptide-1 receptor (GLP-lR), PDX-1, Ngn3, NeuroD1, Pax-6, NKX2.2 genes. But two weeks after transitory hyperglycemia, the above positive genes disappeared in bone marrow cells. Flow cytometry showed that the insulin-positive cells from diabetic rats bone marrow accounted for 6.9±2.9%, C-peptide 6.6±1.3%, glucagon 8.2±0.4%, somatostatin 2.3±1.1%, islet amyloid polypeptide 2.3±0.7%, insulin co-expressed with CD90/CD45 were 5.9% and 1.9%. However, glucose stimulated insulin release of these cells was not detected. **Conclusion:** Rat bone marrow harbored islet-like cells in prolonged or transitory hyperglycemic state, which might be derived from adult stem cells of bone marrow.

O8 Differences in Genes Expression for Pancreatic Islet Development and Differentiation Related Genes between Wild Type and MEN1 Knockout Embryonic Stem Cells

Guo LI, Hong-li ZHANG, Tian-hong LUO, Ling FENG, Yu ZHAO, Min LUO
Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai Clinical Center for Endocrine and Metabolic Diseases, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China

Objective: The MEN-1 syndrome has the strongest association with the development of pancreatic endocrine tumors. It is generally accepted that abnormal development and differentiation could lead to tumorigenesis. Thus men1 gene may be related to development and differentiation of the pancreatic islet. In this study, we want to investigate the physiological function of menin in the pancreatic islet development and differentiation. **Methods:** We used embryoid bodies (EBs) formed from wild-type (Men1+/+) and mutant (Men1 -/-) ES cells as a model system to examine the expression profile of three different categories of genes which include genes relevant to pancreatic islet development and differentiation, such as PDX-1, HNF6, IGF-1, Nkx6.1, Nkx2.2, HNF4α, FGF2, genes involved in the endoderm development: sox17, GSC, Foxa2, by RT-PCR and Real-time quantitative PCR. **Results:** Among these genes, a difference of the time of gene expression between wild-type and mutant EB was observed for Sox17 and Foxa2. Sox17 expression in mutant EBs was detected on the 3rd day, while its expression in wild-type EBs was not observed until the 5th day. The expression pattern of Foxa2 was very similar to that of SOX17, with its expression detected on the 5th day in wild-type EBs and 7th in the mutant EBs. The expression level of GSC, PDX-1, IGF-1, FGF2, IGF1, NKX6.1, NKX2.2 and HNF6 were different between the two EBs during the cultivation, with the most prominent difference appearing at day 3-5, after that, the expression tend to be in the same level, except for NKx2.2 which was downregulated at day 10 in mutant EB. **Conclusion:** Sox l7 and Foxa2 are putative markers of definitive endoderm development. The definitive endoderm is derived from the epiblast during gastrulation, and, at the early organogenesis stage, forms the primitive gut tube, which gives rise to the digestive tract, liver, pancreas and associated visceral organs. It suggests that men1 may be involved in endoderm development, influencing the development of pancreatic islet, possibly through its interaction with soxl7 or Foxa2. Nkx2.2 plays a unique role in endocrine pancreas development. Menl gene may act through downregulation of nkx2.2 gene involved in the pancreatic islet differentiation which further leads to the tumorogenesis of islet. But the conclusion still needs further investigation.

O9 Expression of Human Insulin Gene in Gastrointestinal Tracts

Li NIU, Yan-cheng XU, Jia-zhong SUN
Department of Endocrinology, Zhongnan Hospital of Wuhan University, Wuhan 430071, China

Objective: To study the expression of human insulin genes in gastrointestinal tracts of rats with type 1 diabetes. **Methods:** Plasmids isolated with a large-scale alkaline lysis procedure were purified. Chitosan-DNA nanoparticles were transfected to diabetes rats by lavage and coloclysis respectively. 2 control groups were treated with chitosan and normal sodium respectively. Fasting blood glucose and plasma insulin were measured. RT-PCR analysis was performed to confirm the expression of human insulin gene. **Results:** Fasting blood glucose levels of the lavage group and coloclysis group decreased significantly compared with control groups ($P<0.01$). And plasma insulin levels of the lavage group and coloclysis group were much higher than those of the control groups accordingly ($P<0.01$). The human insulin gene mRNA was only detected in the lavage group and the coloclysis group. **Conclusion:** Human insulin gene embedded with chitosan nanoparticle can be transfected to rats successfully by gastrointestinal tracts, indicating that chitosan is a promising non-viral vector. Compared with other non-viral vectors, chitosan has some merits as follows: (1) It has good biocompatibility, without any toxicity, and can be obtained economically. (2) We can regulate the transfection efficiency of chitosan and intaking of cells by changing molecular weight of chitosan, plasmid concentration, and the ratio of chitosan and plasmid. (3) After the plasmid is embeded in chitosan, it can resist degradation of nucleas.

O10 Relation of Serum Vitamin B12 to Bone Mineral Density of Postmenopausal Women

Li YOU[1], Zheng-yan SHENG[1], Li-meng ZHANG[2], Jin-yu CHEN[1]
[1] Osteoporotic Department, Affiliated First People Hospital, Shanghai Jiaotong University, Shanghai 200080, China
[2] Nuclear Medicine Department, Affiliated First People Hospital, Shanghai Jiaotong University, Shanghai 200080, China

Objectives: To study whether in postmenopausal women levels of serum vitamin B12 are related to bone mineral density (BMD). **Methods:** (1) Experimental subjects: 93 voluntary women, who had been postmenopausal for at least 12 months and were recruited from those (487 women) of our first osteoporotic outpatients (age 65.62±11.49) between August 2005 and December 2005. All subjects gave their informed consent to participate into the study. All subjects had no use of multivitamin B and vitamin D supplements, no gastric surgery, no liver and renal abnormality, no use of hormone in 6 months, no diabetes mellitus, hyperthyroidism and hyperparathyroidism or other metabolic bone diseases, no excessive smoking and alcohol. (2) Groups: osteoporosis (n=29), osteopenia (n=50) and normal (n=13). (3) Serum sample: blood sample from fasting subjects were collected between 8am-10am, and serum was stored at -20° C, within 3 days, samples were measured. (4) Biochemical assays: [1] serum vitamin 12 levels were measured using chemiluminescence (DPC corporation), the sensitivity of this assay is 50 pg/mL, normal range 174-878 pg/mL, [2] serum BGP and serum PTH levels were measured by chemiluminescence (equipment is Roche E170). [3] serum ALP, serum Ca and serum P were measured by automated biochemistry techniques. [4] BMD was measured using dual energy X-ray analysis (GE PRODIGY Inc.) at the lumbar spine (L1-l4) and at the left hip (total hip, trochanter, Ward's area and femoral neck). (5) Statistical analyses: BMD values, biochemical parameters were expressed as mean±SD. Multiple regression analysis was used to assess the relationship between BMD values and serum vitamin B12 levels. Comparisons between two groups were made with *t*-tests. P values less than 0.05 were considered significant. All statistical analysis were performed by SPSS11.5 system. **Results:** (1) Osteoporotic women had lower values of serum vitamin B12 (osteoporosis group 512.55±209.85 pg/ml, osteopenia group 551.29±237.71 pg/ml and normal group 565.71±189.03 pg/ml), but no significance. (2) Low vitamin B12 level and low BMD value at each of the hip sites and for total hip are positive correlation (total hip r=0.25, P<0.01, trochanter r=0.239, P<0.05, shaft of femur r=0.257, P<0.01 and femoral neck r=0.212, P<0.05). (3) Low vitamin B12 level and low BMD value at lumbar spine (L1-L4) are no correlation (r=0.141, P>0.05). **Conclusions:** Serum vitamin B12 deficiency may be an important risk factor for osteoporosis.

O11 IGF-1 Maybe the Earliest Marker Detecting Osteopenia/Osteoporosis in Females

Jian-min LIU, Hong-yan ZHAO, Guang NING, Yong-ju ZHAO, Ying CHEN, Lian-zhen ZHANG, Man-yin XU, Jia-lun CHEN
Department of Endocrine and Metabolic Diseases, Rui-jin Hospital, Shanghai Second Medical University; Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai 200025, China

Objectives: To investigate the changing patterns of serum insulin like growth factor-1 (IGF-1), osteoprotegerin (OPG), leptin, osteoclacin (OC) and urinary N-terminal crosslinking telopeptide of type I collagen (NTx), and bone mineral densities at lumbar spine and femoral neck along with age, and find out the one that changes earliest, and explore its (their) role(s) in detecting osteopenia/osteoporosis patients in female. **Methods:** (1) BMDs of lumbar spine and femoral neck were assessed using dual-energy X-ray absorptiometry (DXA) (Lunar Expert 1313) in 504 pre- and postmenopausal women aged between 20-75. They were divided into normal, osteopenic and osteoporotic groups according to WHO criteria. (2) Serum concentrations of IGF-1, OPG, leptin, OC and urinary levels of NTx were measured. The participants were classified into 6 groups according to every 10 years of age: 20-29, 30-39, 40-49, 50-59, 60-69 and 70-79. The nonparametric tests were used to compare the differences of above parameters and BMDs among the groups in order to find out the marker(s) which changes earliest along with age. (3) The role of the selected marker which changes earliest with age in diagnosing osteopenia/osteoporosis was analyzed with receiver operating characteristic (ROC). The area under the curve (AUC), sensitivity and specificity parameters were obtained from the ROC analysis. T score was also calculated for each tested values. **Results:** (1) All these markers showed a descending or ascending changes with age (P<0.0001). Serum levels of leptin and IGF-1 changed earliest at 30. Serum levels of OC and OPG markedly decreased or elevated after 40; while urinary NTx significantly increased after 50; and BMDs at L2-4 and femoral neck dropped markedly after 50. (2) Leptin and IGF-1 were chosen as the earliest markers which change with age. The AUCs of these two makers in diagnosing osteopenia/osteoporosis in ROC analysis were: 0.527±0.029 (P=0.345) and 0.776±0.022 (P<0.0001), respectively. If the serum level of IGF-1 at 159.1 ug/ml, eg T score -1.5 were adopted as a cut-point, it can find out the patients with osteopenia/osteoporosis with the sensitivity of 73% and specificity of 67%. **Conclusion:** Serum IGF-1 and leptin are the earliest markers changing with age. However, serum IGF-1 is more useful in detecting osteopenia/osteoporosis. A routing check of this marker in young women maybe helpful in finding out the potential patients at an early stage.

O12 Impact of Multiple Candidate Genes on Bone Mineral Density in Chinese Women

Hong-yan ZHAO, Jian-min LIU, Guang NING, Yong-ju ZHAO, Yin CHEN, Li-hao SUN, Lian-zhen ZHANG, Man-yin XU, Jia-lun CHEN
Department of Endocrine and Metabolic Diseases, Rui-jin Hospital, Shanghai Jiaotong University Medical School; Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai 200025, China

Objectives: Osteoporosis is a multifactorial and polygenic disease caused by the combined effects of genetic and environmental factors. Genetic factors have been estimated by twin and family studies to be responsible for 75%-80% of the variance in bone mineral density (BMD). So, the purpose of this study was to assess the contribution of osteoprotegerin (OPG), parathyroid hormone (PTH), calcitonin receptor (CTR), osteocalcin (BGP), and leptin receptor (LEPR) gene polymorphisms to the variation of BMD in Chinese women. **Methods:** Bone mineral density at lumbar spine and femoral neck were measured by dual-energy X-ray absorptiometry (DEXA) in 504 Chinese women (282 pre- and 222 postmenopausal). Using polymerase chain reaction (PCR) and direct sequencing technique to identify OPG gene polymorphism. PTH, CTR, BGP and LEPR genes were evaluated through PCR and restriction fragment length polymorphism. The differences of BMD at the lumbar spine (L_{2-4}) and femoral neck (FN) across each genotypes (OPG Lys3Asn, PTH 3244 G/A, CTR 1377 C/T, BGP 298 C/T, LEPR Gln223Arg) were tested by analysis of covariance (ANCOVA) adjusted for age and BMI. **Results:** In postmenopausal women, we found that individuals with Asn-Asn genotype of the OPG gene have significantly higher BMD at the lumbar spine (L_{2-4} BMD) compared to those with Lys-Lys genotype (p=0.007), while women with bb genotype of the PTH gene have higher L_{2-4} BMD compared to those with BB genotype (p=0.002). No significant association was observed between BMDs and CTR, BGP and LEPR polymorphisms. Multiple regression analysis revealed that BMI and age accounted for the variance of BMD in premenopausal women. As for the postmenopausal women, age, BMI, OPG Lys3Asn genotype and PTH 3244G/A genotype accounted for the variance of BMD. Logistic regression analysis showed that OPG Lys3Asn (p=0.008) and PTH 3244G/A (p=0.03) polymorphisms were independent risk factors of osteopenia/osteoporosis in postmenopausal women. **Conclusions:** In conclusion, genetic variation in the OPG and PTH gene is associated with BMD in Chinese postmenopausal women.

O13 The Effect of Treatment Discontinuation on the BMD of Osteoporotic Patients Who Received Alendronate for 4 Years

Yu-cho WOO, Jenny Y.Y. LEUNG, Man-choi WAN, Chun-por WONG
Department of Integrated Medical Service, Ruttonjee and Tang Shiu Kin Hospitals, Hong Kong SAR, China

Objective: To study the effect of treatment withdrawal on the bone mineral density (BMD) of osteoporotic patients who had received 4 years of Alendronate (ALN). **Methods:** We followed 37 Chinese female patients with osteoporosis who had completed 4 years of ALN 10 mg daily with calcium carbonate 1000 mg/day. BMD of the lumbar spine and hip was measured by dual-energy X-ray absorptiometry at baseline, after 4 years of ALN and upon drug discontinuation for 1.5 year. **Results:** 37 patients were included in the analysis. BMD was measured at baseline (0 month), at time of treatment withdrawal (0.5±7.5 months) and 20.6±9.5 months after ALN withdrawal. After completion of 4 years of ALN, BMD at spine (L1-4) increased by 16.7% ($p<0.05$) while total hip BMD increased by 7% ($p<0.05$). Upon discontinuation of ALN for about 21 months, there was no significant decrease in BMD at spine (L1-4) (0.88 g/cm^2 vs 0.88 g/cm^2, p=0.736). However, the total hip BMD decreased from 0.68±0.09 g/cm^2 to 0.67±0.09 g/cm^2 (p=0.014) within same period. **Conclusion:** After ALN withdrawal, spine BMD could be maintained. However, a small but significant decrease in BMD at total hip region was noted.

O14 A Study on Vitamin D Status of Referrals to a Local Osteoporosis Centre

Winnie Zee-man WAT, Jenny Yin-yan LEUNG, Annie Wai-chee KUNG
Department of Medicine, Pamela Youde Nethersole Eastern Hospital, Chai Wan, Hong Kong SAR
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong SAR

Objectives: This cross-sectional study aims to (1) determine the vitamin D status in subjects at high risk of osteoporosis in Hong Kong and (2) to identify risk factors for vitamin D insufficiency in this cohort. **Methods:** Subjects who were assessed at the Osteoporosis Centre of Queen Mary Hospital were recruited. Those who were taking drugs or having diseases that would affect the vitamin D status, serum calcium level and bone mineral density were excluded. Subjects were assessed using a standardized questionnaire on their basic demographic data and detailed medical, dietary, social and drug history. Blood was taken for serum 25-hydroxy-vitamin D which was measured by radioimmunoassay. **Results:** 244 Chinese, 87 male (35.7%) and 157 female (64.3%), with mean age of 71.1±8.7 (mean±SD) years were included for data analysis. The mean 25-hydroxy-vitamin D (25OHD) was 30.6±11.02 ng/ml. 39 (16%) subjects had serum 25OHD level below 20 ng/ml, while 116 (51.7%) subjects had levels below 30 ng/ml. Using multiple logistic regression, the independent predictive factors for vitamin D insufficiency (defined as serum 25OHD <20 ng/ml) were identified as male sex (p=0.002, OR 3.31, 95% CI 2.54-4.09), daily outdoor time less than 60 minutes (p=0.022, OR 2.54, 95% CI 1.74-3.34) and winter season (p=0.040, OR 1.63, 95% CI 1.16-2.10). **Conclusions:** Vitamin D insufficiency is common in subjects at high risk of osteoporosis in Hong Kong. Male sex, daily outdoor time less than 60 minutes and winter season were independent predictive factors for vitamin D insufficiency.

O15 Clinical Parameters Analysis of 160 Cases with Primary Hyperparathyroidism in Ruijin Hospital

Xiao-yan HE, Qing HAN, Jian-ming LIU, Guang NING
Department of Endocrine and Metabolic Diseases, Ruijin Hospital, SJTU, Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrine and Metabolic Diseases, 197 Shanghai Rui-jin Er Road, Shanghai 200025, China

Purpose: To analyze the clinical characteristics of primary hyperparathyroidism (PHPT) patients diagnosed in Ruijin Hospital from 1956 to 2005, and compared them with the contemporary PHPT patients in western world and developing area. **Methods:** Retrospective analysis was applied in studying the clinical features of 160 PHPT patients admitted to Ruijin Hospital during 1956 to 2004. Hyperparathyroidism was diagnosed histologically and biochemically. Many online literatures were reviewed to find out the western and developing area PHPT characteristics. Comparison was made between ours and theirs. **Results:** Totally 160 cases of PHPT were collected. 130 cases were done operations. The pathological examinations indicated that parathyroid adenoma was the main cause of the disease, accounting for 86.1.0%, followed by parathyroid proliferation (5.9%) and cancer (7.7%). There were 6 cases of ectopic hyperthyroidism, 4 in thymus, 1 in thyroid and 1 on sternoclavicular joint. However, our cohort still manifested "classical" presentations, which were in sharp contrast with the "modern" asymptomatic Western features. The patients in our cohort were: (1) Younger: the age of onset (ours vs West): 39.07±15.82 vs 55±12 yr; (2) With obvious symptoms: the most common one was urinary symptoms (82.1%), with renal stone 39.3%, and renal failure 28.6%; bone disease and gastrointestinal tract symptoms occurred in 48.2% and 46.4% patients, which was similar with western patients before 1985 (bone lesion 50-60%, and renal stone 50-80%), while in sharp difference with them in recent 20 years (bone lesion 10-20%, and renal stone 5-20%). (3) With higher biochemical markers: serum calcium: 3.08±0.49 mmol/L vs 2.10±0.15 mg/dL; serum PTH 976.27±839.52 vs 128±96 ng/mL; urinary calcium 320±160 vs 249±122 mg/dL; serum AKP 422.21 ±681.02 vs 110±60 IU/L. (4) With low Vitamin D: 25-OHVitaminD 18.03±16.13 ng/mL vs 21 ng/ml. **Conclusion:** The PHPT in our cohort 1956-2005 had a younger age of onset and more severe clinical manifestations compared with modern western patients. The clinical presentations of PHPT in China are still "classical", obviously VitD deficiency and/or genetic difference may be responsible for this discrepancy.

O16 Use of Intra-operative Parathyroid Hormone Assay Improves Cure Rates in Primary Hyperparathyroidism—Experience in a Single Centre

Wai-keung CHICK[1], Ronald Pak-kin HO[1], Philip Lap-fai TANG[1], Angel On-kei CHAN[2], Anthony Chi-chung SHEK[2], Fredriech Kwok-wing CHAN[3], Sau-cheung TIU[3]
[1] Department of Surgery, Queen Elizabeth Hospital, Hong Kong SAR
[2] Department of Pathology, Queen Elizabeth Hospital, Hong Kong SAR
[3] Department of Medicine, Queen Elizabeth Hospital, Hong Kong SAR

Objective: Intra-operative parathyroid hormone assay (IOPTH) has been widely used for confirmation of surgical success in parathyroidectomy in the recent years. It is still controversial whether IOPTH can improve the cure rates in primary hyperparathyroidism. In this study, we focused on whether there was a change in cure rates after the use of IOPTH in a single centre. **Methods:** 46 consecutive patients, who received minimally invasive parathyroidectomy (MIP) with intra-operative rapid PTH assay for primary hyperparathyroidism from March 2002 to March 2005, were compared to a historical cohort of 36 consecutive patients with conventional parathyroidectomy (bilateral exploration without IOPTH assay) performed between January 1987 and July 1996. **Results:** The frequency of parathyroidectomies increased markedly from 3.4 cases per year to 15 cases per year. For the historical group, 1 patient had persistent hypercalcemia and 1 had recurrence of hypercalcemia, making the cure rate being 94.4%. For the MIP group, 41 cases had resection of a single adenoma, followed by a >50% drop in IOPTH level, and were cured. 5 patients required conversion into bilateral exploration due to difficult anatomy in 3 patients with adenomas, intra-thyroidal adenoma in 1 patient and hyperplasia in 1. All patients in the MIP group were cured. The cure rate in this group was 100%. **Conclusion:** Our result indicates that the use of IOPTH improves cure rates in primary hyperparathyroidism. IOPTH can allow recognition of missed abnormal glands not discovered during localization procedures or operations. However, other factors like improvement in localization procedures may also contribute to the better result and further investigation is necessary.

O18 The Genetic Analysis to Pseudohypoparathyroidism

Li-hao SUN, Jian-min LIU, Hong-yan ZHAO, Guang NING
Shanghai Clinical Center for Endocrine and Metabolic Diseases, Department of Endocrine and Metabolic Diseases, Ruijin Hospital, China

Objective: Pseudohypoparathyroidism (PHP) refers to a heterogeneous group of rare metabolic disorders characterized by hypocalcemia and hyperphosphatemia due to PTH resistance. In our study, we evaluated whether molecular diagnosis is a useful tool to characterize AHO and PHP. **Methods:** We collected clinical, biochemical data of 8 patients characterized by PTH resistance from unrelated families. Genomic DNA was extracted from peripheral blood leukocytes of these patients. The 13 exons of GNAS1 gene were screened for mutations by PCR and direct sequencing of the amplified products. **Results:** We found PHP Ia patients are characterized by the classic laboratory findings and features of Albright's hereditary osteodystrophy (AHO), including short stature, developmental delay, brachydactyly, and heterotopic calcifications. Heterozygous mutations in the GNAS1 gene encoding the $G_s\alpha$ protein by direct DNA sequencing have been found in PHP Ia patients. **Conclusions:** GNAS1 gene is associated with several human endocrine disorders, including PHP. Clinical data combined with genetics analysis are propitious to the diagnosis of PHP.

O19 Muscular Biopsies on the Patients with Periodic Hypokalemic Paralysis of Hyperthyroidism of Graves Disease

Ming-cai QIU, Hong-yan WEI, Ji-yuan FAN
Division of Endocrinology, Tianjin Medical University Hospital, 300052, China

Objectives: Periodic hypokalemic paralysis of hyperthyroidism of Graves disease is the disease commonly seen in our endocrine clinic while its pathogenesis still remains unclear. To investigate if there are autoimmune injuries to the muscular cells, such as immunoglobulin and complex depositions. **Methods:** Muscular biopsies were carried out to study the autoimmune injuries on 15 patients with Hypokelamic Periodic Paralysis in Hyperthyroidism of Graves Disease (HPPHGD), 5 patients with Hyperthyroidism of Graves Disease without Hypokelamic Periodic Paralysis (HGDHPP), 7 patients with Familial Hypokalaemic Periodic Paralysis (FPP) and 17 normal subjects. **Results:** IgG, IgA, IgM, C3, C1q and FRA were detected in the HPPHGD, HGDHPP and FPP groups while only a little found in the normal subjects. The data suggest that there were a plenty of immunoglobulins and complex deposited on the muscular cells which may block the channels for ions exchange between the intracellular and extracellular fluids, leading to the hypokalemic paralysis. The light density study showed that the value of light density of the all immunoglobulins and complex in HGDHPP was the highest and higher than the other three groups, such as IgG, IgA and C3 ($P<0.05$) while no significant difference was found among the HGDHPP, FPP and normal subjects I in IgG, IgA and C3 ($P>0.05$). The IgM expression in HPPHGD group was more significant than both FPP and normal groups while no significant difference in comparison with normal group ($P>0.05$). There was no significant difference among the 4 groups in both C1q and FRA ($P>0.05$). **Conclusion:** There was a plenty of IgG, IgA, IgM, C3, C1q and FRA deposited on the surface of skeletal muscular cells in HPPHGD group while more significant expression of IgG, IgA and C3 was detected on the skeletal muscular cells in HPPHGD patients than HGDHPP, FPP and normal subjects, suggesting that the hypokalemic paralysis might be due to the deposition of a huge amount of immunoglobulins and supplements on the surface of muscular cells, leading to the hypokalemic paralysis.

O22 Reference and Optimal Intervals of Thyrotropin, and the Influence of Iodine on the Reference Range: Results of a 5-Year Follow-up Study in Areas with Different Iodine Intakes

Hai-xia GUAN, Zhong-yan SHAN, Yu-shu LI, Xiao-chun TENG, Wei-ping TENG
Department of Endocrinology & Institute of Endocrinology, First Affiliated Hospital of China Medical University, Shenyang, Liaoning Province 110001, P.R. China

Objective: To establish a reference interval and optimal interval for serum TSH, and to assess the factors (especially iodine) that might influence the reference interval for TSH in reference group. **Methods:** We selected a reference population from 3761 adults to establish a reference interval for TSH. 2727 (80.02% of 3408, 656 men and 2071 women) with normal TSH levels at the original survey were followed up 5 years later. Serum TSH, TPOAb, TgAb and urinary iodine were measured. **Results:** A group of 2237 (59.48% of the whole population, 630 males and 1607 females) participants fulfilled the criteria for establishing a reference range for TSH. The reference range for TSH was dropped to 0.30-4.79 mU/L. In reference population, both the median TSH levels and TSH reference intervals showed an iodine-related increase. The median levels of TSH in Panshan, Zhangwu and Huanghua were 1.22 mU/L, 1.41 mU/L and 1.99 mU/L, respectively ($P=0.000$). The intervals in Panshan, Zhangwu and Huanghua were 0.33-3.50 mU/L, 0.20-4.62 mU/L and 0.56-6.00 mU/L, respectively. TSH concentrations obtained in the follow-up study showed a good correlation to those in the original survey ($r=0.576$, $P=0.000$). A rise in baseline serum TSH above 1.9 mU/L was associated with an increased incidence of development of supranormal TSH; and a descent in baseline serum TSH below 1.0 mU/L, subnormal TSH. **Conclusion:** We calculated a TSH reference interval of 0.30-4.79 mU/L for Chinese population considering the wide variation of iodine nutrition in populations. Iodine nutrition is an important factor associated with TSH concentration even in the rigorously selected reference population. Baseline TSH of 1.0-1.9 mU/L is a relatively safe interval with the lowest incidence of abnormal TSH in five years.

O23 Screening for Pregnant Women with Abnormal Thyrotropin in Early Pregnancy in Iodine-adequate Area

Xiao-hui YU, Zhong-yan SHAN, Yan-yan CHEN, Jia LI, Chen-ling FAN, Rui GUO, Hong WANG, Wei-ping TENG
Department of Endocrinology and Metabolism, Institute of Endocrinology, First Affiliated Hospital, China Medical University, Shenyang 110001, China

Objective: To acquire the prevalence of abnormal thyrotropin (TSH) in early pregnancy in iodine-adequate area and to explore the factors causing increased TSH level. **Methods:** 2108 pregnant women whose gestation ages were less than 8 weeks (G8) and 162 12-week pregnant women (G12) were enrolled. Serum TSH, free thyroxine (FT_4), thyroid peroxidase antibody (TPOAb) and urine iodine excretion were detected. **Results:** (1) Median urine iodine of G8 and G12 were 185.5 μg/L and 183.5 μg/L, respectively. (2) According to nonpregnant reference interval (0.3-4.8 mIU/L), decreased TSH prevalence were 5.26% in G8, 14.82% in G12 and 5.78% in controls. The prevalence in G12 was significantly higher than that in G8 and controls (both P=0.000). And increased TSH prevalence were 3.37%, 0.62% and 2.78%, respectively. The prevalence in G12 was slightly lower than that in G8 and controls. (3) TSH reference intervals for G8 and G12 established in euthyroid pregnant women were 0.25-4.66 mIU/L and 0.09-2.96 mIU/L, respectively. (4) According to new reference, decreased TSH prevalence were 4.18% in G8 and 3.09% in G12. And increased TSH prevalence were 3.61% and 3.71%, respectively. In G12, the two decreased TSH prevalence were different (P=0.000) and increased TSH prevalence was higher according to new reference. So if to evaluate TSH level of G12 according to nonpregnant reference, 11.73% of G12 would be misdiagnosed as decreased TSH and 3.09% of increased TSH would be missed diagnosed. (5) In early pregnancy, serum TSH level was correlated with serum FT_4 level (r= –0.205, P=0.000). Serum TSH level in positive TPOAb subjects was higher than that in negative ones (P=0.000). (6) Logistic analysis showed that increased TSH level in early pregnancy was associated with positive TPOAb and serum FT_4 level. **Conclusion:** It is important to establish and monitor appropriate reference intervals for proper interpretation of TSH measurements in early pregnancy. The status of thyroid autoimmunity and serum FT_4 level can affect serum TSH level in early pregnancy.

O24 The Target Antigens of Antineutrophil Cytoplasmic Antibodies (ANCA) Induced by Propylthiouracil

Ying GAO[1], Ming-hui ZHAO[2], Xiao-hui GUO[1]
[1] Department of Endocrinology, Peking University First Hospital, Beijing 10003, China
[2] Department of Nephrology, Peking University First Hospital, Beijing 10003, China

Objective: Antineutrophil cytoplasmic antibody (ANCA) has been well documented in association with propylthiouracil (PTU), and some patients with PTU-induced ANCA also develop clinical vasculitis. The aim of the current study was to detect ANCA specificities in sera from patients with PTU-induced ANCA with and without clinical vasculitis. **Methods:** Sera from 65 patients with PTU-induced ANCA were collected, and 27 of these patients were diagnosed with PTU-induced ANCA associated systemic vasculitis (AASV). Indirect immunofluorescence assay and antigen-specific ELISAs were used to detect ANCA and their antigen specificities. The seven known target antigens included myeloperoxidase (MPO), proteinase 3, human leukocyte elastase, lactoferrin, cathepsin G, azurocidin and bactericidal/permeability-increasing protein (BPI). **Results:** In IIF assay, P-ANCA was found in 58/65 (89.2%) sera, C-ANCA in two, both P-ANCA and C-ANCA in five, respectively. MPO (60%) and lactoferrin (63.1%) were the two most common target antigens detected in sera from all the patients. 25/27 sera from patients with PTU-induced AASV recognized multiple target antigens, which was significantly higher than those (13/38) from patients without (P<0.001). Except anti-BPI antibodies, the prevalence of antibodies against the other six target antigens was significantly higher in patients with clinical vasculitis than that in patients without (P<0.05, respectively). **Conclusion:** Antibodies against multiple ANCA specific antigens, especially the antigens rather than MPO and PR3, might be the characteristic of PTU-induced ANCA. Patients with antibodies against more ANCA specific antigens might be at increased risk of developing overt clinical vasculitis. The mechanism of ANCA production in PTU-induced cases was different from that in primary AASV.

O25 Successfully Established an Animal Model of Graves' Disease

Bing-yin SHI, Li-ping WU, Chun-rong CHEN, Li-ying GUO, Li XU, Ling LAN, Juan LIU, Jin-an ZHANG, Li-ru XUN
Department of Endocrinology, First Hospital Affiliated to Medial College of Xi'an Jiaotong University, Xi'an 710061, P.R. China

Objective: To induce an animal model of Graves' disease by immunizing inbed BALB/c mice with recombinant adenovirus expressing the thyrotropin receptor A-subunit (Ad-TSHR289). **Methods:** Ad-TSHR289 was constructed by the method of AdMax. Female BALB/c mice (age, 6-8 wks) were divided randomly into experimental and control group, and injected intramuscularly with Ad-TSHR289 and control adenovirus (Ad-null) respectively three times at 3-week intervals. Blood samples were obtained 8 wks after the third injection by extirpating eyeball for anti-TSHR autoantibodies (TRAb), total thyroxine (TT_4) and thyrotropin (TSH) assay. Their thyroids were removed and fixed with 10% Formalin, the tissues embedded in paraffin and stained with HE for histological examination. **Results:** 50% of immunized mice developed the higher level of TRAb, 21.4% showed obviously hyperthyroidism with positive TRAb and elevated TT_4 with suppressed TSH levels. The mice with higher TT_4 showed weight loss in varied degree. The thyroid glands from hyperthyroid mice displayed diffuse enlargement with hypertrophy and hypercellularity of follicular epithelial and papillary poles protruding into the follicular lumen, thyroid epithelial cells are cuboidal or columnar with occasional intracellular vacuolation, decreased amounts of colloid were also observed. Findings consistent with high secretary activity, contrast with flattened thyroid epithelium in euthyroid animals. There was no inflammatory cell infiltration. **Conclusion:** It is shown from present study that, as a primary target antigen of GD, TSHR A subunit play an important role in the development and progression of the disease. Our results demonstrate that we have successfully established an animal model of resembling some characteristics of human Graves' disease by immunization with recombinant adenovirus expressing the thyroid stimulating hormone receptor A-subunit.

O26 Estrogen Regulates Cell Proliferation and Apoptosis, its Role in Thyroid Cancer Development

George G. CHEN, Qiang ZENG, Alexander C. VLANTIS, Andrew C. VAN HASSELT
Department of Surgery, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, New Territories, Hong Kong SAR, China

Objective: Apoptosis plays an important role in the pathogenesis of thyroid disorders including thyroid cancer. There is strong evidence suggesting the development of thyroid cancer is associated with female sex hormones, particularly the estrogen. The incidence of thyroid cancer is roughly three times more frequent in women than in men. Moreover, the incidence decreases after menopause in females. The aim of the project was to explore how estrogen contributed to the development of thyroid cancer. **Results:** Our lab has examined various thyroid cancer cells and explored how estrogen affects the proliferation and growth of thyrocytes. The function of estrogen is mediated through two related but distinct estrogen receptors (ERs), ERα and ERβ, both of which express in thyroid cancers. Estrogen can promote cell survival through an ERα signaling pathway, and cell apoptosis through an ERβ signaling pathway in thyroid cancer cells. Both of these pathways appear to be non-genomic instead of classical/genomic one. ER-mediated cell survival or death is regulated by various kinases and other growth factors which are related to various apoptotic molecules. **Conclusion:** The estrogen can have opposite effects on cell proliferation, growth and apoptosis, depending on the balance between ERα and ERβ in cells. Functionally, the pathway of ERα is much stronger than that of ERβ in thyroid cancer cells.

027 Lithium Stimulates Sodium/Iodide Symporter Expression But Not Radioiodide Uptake in Human Follicular Thyroid Cancer Cell Line Via Cyclic AMP-Independent Pathway

Kam-tsun TANG, Chin-chang CHEN, Chen-hsen LEE
Department of Medical Research & Education; and Department of Surgery, Taipei Veterans General Hospital, Taipei, Taiwan

Objective: Decrease/increase in sodium/iodide symporter (NIS) expression have been reported in well-differentiated thyroid cancers, suggested that defects in NIS expression/trafficking may be the cause of poor iodide uptake in thyroid cancers. Lithium has been used as an adjuvant to enhance radioiodide uptake (RAIU) in thyroid cancer therapy. This study aims to investigate the effects of lithium on NIS in thyroid cancer. **Methods:** Follicular thyroid cancer cell lines (CGTH W1) were incubated with lithium, TSH, cAMP and a protein kinase A inhibitor H89 alone or in combination. After incubation, cells were harvested for NIS expression, translocation, RAIU and signal transduction analyses. **Results:** There was no basal NIS gene expression in CGTH W1 cells. Either lithium or TSH stimulated NIS gene and protein expressions in a dose response manner with the peak at 2 mM or 1 mU/mL, respectively. The maximum increase in NIS gene and protein expressions were observed at 4h and 24h, respectively. TSH-induced NIS was detected on the membrane and stimulated RAIU. However, lithium-induced increase in NIS was limited in the cytosol and no significant increase in RAIU was observed. The addition of TSH facilitated the lithium-induced NIS targeting on the membrane resulted in an increase in RAIU, which were partially blocked by H89. cAMP had similar effect as TSH on lithium-induced NIS and RAIU, and could be completely blocked by H89. H89 could not block the lithium-induced NIS gene and protein expressions. **Conclusion:** Lithium induced NIS gene and protein expression, but had no effect on NIS translocation or RAIU in CGTH W1 cells. The effect of lithium on NIS expression is not cAMP-dependent, but NIS trafficking and RAIU are cAMP dependent. Multiple signal transduction pathways are involved in the TSH-induced NIS expression and iodide uptake.

028 An Evaluation of the International Diabetes Federation Definition of Metabolic Syndrome in the Chinese Patients Older Than 30 Years and Diagnosed with Type 2 Diabetes Mellitus

B. LU, Y.H. YANG, X.Y. SONG, X.H. DONG, Z.Y. ZHANG, L.N. ZHOU, Y.M. LI, N.Q. ZHAO, X.X. ZHU, R.M. HU
Department of Endocrinology, Huashan Hospital, Institute of Endocrinology and Diabetology, Fu Dan University, 200040, China

Objective: The purpose of this study was to determine the most accurate metabolic syndrome (MS) definition among the definitions proposed by the International Diabetes Federation (IDF), the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III [ATP III]) and the World Health Organization (WHO) by making a comparison of the three definitions and evaluation of cardiovascular disease risks. **Methods:** 1039 Chinese patients diagnosed with type 2 diabetes aged over 30 were investigated by randomized cluster sampling in the Shanghai downtown and 1008 patients analyzed in this study. Body mass measurements including height, weight, waist circumference and hip circumference, resting blood pressure, fasting blood measures and carotid atherosclerotic measurements including common carotid artery intima-media thickness (IMT) and plaque of carotid artery were investigated. The IDF definition was compared with the other two definitions and the carotid atherosclerosis was evaluated among the patients defined by these definitions. **Results:** (1) The MS prevalence was 50.0%, 55.7% and 70.0% under the IDF, ATPIII and WHO definitions respectively. The prevalence of central obesity defined by the ATPIII definition was 4.1% in men and 30.2% in women. (2) The male patients had greater waist circumference, waist hip ratio and the female patients had greater body mass index. (3) MS patients defined by the IDF definition had greater waist circumference and BMI. (4) The percentage of all the participants categorized as either having or not having the MS was 69.9% (under the IDF and ATPIII definitions) and 70.2% (under the IDF and WHO definitions). (5) Common carotid artery IMT of MS patients defined by the IDF definition was thicker than those defined by the WHO and ATPIII definitions (1.04 mm vs 0.99 mm; 1.04 mm vs 0.96 mm respectively; $P<0.01$) and the percentage of carotid plaque of MS patients defined by IDF definition was greater than those defined by WHO and ATPIII definitions (74.8% vs 62.7%; 74.8% vs 59.6% respectively; $P<0.01$). **Conclusion:** The prevalence of MS was 50.0%, 55.7% and 70.0% under the IDF, ATPIII and WHO definitions respectively. The preferable IDF definition might be a better predictor of cardiovascular disease risk in the Chinese patients diagnosed with type 2 diabetes compared with the ATPIII and WHO definitions.

O29 Epidemiological Study of Metabolic Syndrome in Schoolchildren: Relationship with Adipocytokines, Obesity and Insulin Resistance

Ming LI[1], Jie MI[2], Hong CHENG[2], Kui ZHANG[1], Dong-qing HOU[2], Xiao-yuan ZHAO[2], Cong-yuan WU[1]
[1] Department of Endocrinology, Peking Union Medical College Hospital, Beijing 100730, China

Objective: To investigate the effects of obesity on the prevalence of the metabolic syndrome (MS) and its relation to insulin resistance and levels of adipocytokines in a large population-based study of schoolchildren in Beijing area. **Methods:** A total of 3515 schoolchildren (1155 health and others with at least one MS component, male/female: 1792/1723) were selected from the Beijing Child and Adolescent Metabolic Syndrome Study, a representative sample of 19 593 children aged 6-18 y in 2004. Examination included anthropometry, body composition by BIA; pubertal development, and levels of fasting lipid profile, insulin, leptin, resistin and adiponectin. The overweight status was defined according to International Obesity Task Force (IOTF) BMI cut-offs and the MS was diagnosed by a modified IDF definition in 2005. **Results:** In general, the prevalence of MS increased with the severity of overweight and reached about one fifth in overweight and obese children (male/female: 19.8% vs 17.4%). The prevalence of the MS increased significantly with increasing insulin resistance (HOMA-IR) after adjustment of obesity (BMI by age and Z-score). The level of leptin increased and adiponectin decreased with the increasing of obesity and numbers of MS component, even after adjustment for gender and pubertal development, but no similar trend was observed in resistin levels. **Conclusions:** The prevalence of the MS was high among overweight and obese schoolchildren in Beijing and it increased with worsening of obesity. Biomarkers of the increased risk of adverse cardiovascular outcomes, such as hyperleptinaemia and hypoadiponectinaemia, are already present in these children.

O30 Is Central Obesity–based Metabolic Syndrome More Strongly Associated with Stroke in Chinese Adult Population? Report from the Technical Working Group of China National Nutrition and Health Survey, 2002

Guang-wei LI[1], Chong-hua YAO, Yi-song HU, Feng-ying ZHAI, Ling-zhi KONG, The Technical Working Group of China National Nutrition and Health Survey
[1] Dept. of Endocrinology, China-Japan Friendship Hospital, Beijing 100029, China

Background: Metabolic syndrome (MS) has been recognized as a constellation of risk factors for cardiovascular disease and stroke. However the importance of central obesity must be the prerequisite component of the syndrome remains unclear. **Objectives:** To clarify whether the central obesity–based MS is more strongly associated with stroke in Chinese adult population. **Methods:** 71,971 Subjects from 31 provinces, autonomous regions, and municipalities in China were enrolled in a randomized survey on malnutrition, hypertension and diabetes by multi-steps cluster sampling method from Aug-Oct 2002 to Sep-Dec 2003. Height, weight, waist, blood pressure and the laboratory parameters including fasting plasma glucose, total cholesterol, triglyceride, HDL-C and LDL-C were measured in 43,469 subjects with age from 20 to 75 years old participants. Physicians and neurologists diagnosed stroke were recorded during the survey. Metabolic syndrome was defined by 2004 IDF criteria. All the subjects were stratified by the number of cardiovascular risk factors, the central obesity (CO), hypertension (HT), hypertriglyceridemia (HTG), hyperglycemia (HGLY) and low-HDL. Prevalence of stroke in different groups was compared. Logistic regression analysis was performed to evaluate the importance of central obesity–based MS on stroke in Chinese population. **Results:** Distribution of stroke patients were 6.8% (26/382) in group without any risk factor, 51% (194/382) in group with one or two risk factors, and 37.7% (145/382) in IDF defined MS group, while as only 4.5% (17/382) in the non-obese MS group. Prevalence of stroke in the (HT+HGLY+LOW-HDL) group is 4.23%, which ranks the top of all cardiovascular risk factor specified groups, but it only account for 2.09% of the whole strokes. Although the prevalence (2.45%) in IDF_MS group ranks No. 2, it accounts for as much as 37.7% of the total strokes. Those with prevalence rank No. 3 to No. 5 were: 2.07% in (CO+HGLY) group, 1.93% in (CO+HT) group, 1.37% in any CO-free three or more risk factor combination group. Other groups with prevalence greater than 1% were: HGLY+low-HDL group (1.23%), HT group (1.21%), HT+HGLY group (1.05%), HT+HTG group (1.01%). Taken together, stroke in the central obesity–related groups, MS, CO+HGLY and CO+HT group, account for 50% of the total. Strokes in the hypertension-related groups (HT only, HT with one or more than one other risk factor except central obesity–based MS) account for 40% of the total, which even more than that in MS group. Logistic regression analysis demonstrated that IDF defined MS and group with HT+HGLY+LOW-HDL have the greatest risk to stroke compared with the risk factor–free group (OR=8.33, OR=11.24). CO+HGLY and CO+HT groups followed (OR=6.42, OR=4.85) after the adjustment of age, sex, smoke and LDL-C. **Conclusion:** (1) Central obesity–based MS mostly contributed to the development of stroke, however it only accounts for 38% of the total stroke; (2) hypertension only, hypertension combined with obesity, hypertension combined with both hyperglycemia and low-HDL, and obesity with hyperglycemia also play very important roles to induce stroke. (3) In order to prevent stroke in Chinese adult population we should pay much attention to obesity-based MS and also should not forget the importance of some other groups related to hypertension and hyperglycemia, although they do not meet the criteria of IDF_MS because of being not central obese or only having less than three risk factors.

O31 Low Circulating Adiponectin Level Predicts the Development of Hypertension in Chinese

L.H.Y. ONG, W.S. CHOW, A.W.K. TSO, A. XU, C.H.Y. FONG, K.S.L. LAM
Division of Endocrinology, Department of Medicine, Queen Mary Hospital, Hong Kong SAR

Objective: To assess whether low adiponectin level is associated with development of hypertension in human. **Methods:** A nested-case control study with normotensive subjects (BP<130/85 mm Hg) recruited from the Hong Kong Cardiovascular Risk Factors Prevalence Study and were followed up prospectively for 5 years. At 5-year follow-up, 75 recruited subjects (cases) developed hypertension (BP≥130/85 mm Hg or on regular anti-hypertensive treatment). Controls consisted of age and sex-matched subjects (n=150, matched to cases at a ratio of 2:1) who remained normotensive at 5 years. Blood pressure was measured as the mean of two readings taken after sitting for at least 10 minutes. Adiponectin level was measured with an in-house ELISA assay. **Results:** Hypertensive subjects had more adverse risk factors, including higher BMI, waist circumference and waist hip ratio, mean arterial pressure (MAP) at baseline, compared to controls. Baseline adiponectin levels were lower in hypertensive subjects (1.75±0.48 μg/ml vs 1.93±0.38 μg/ml, hypertensive vs control, p=0.003). Logistic regression analysis showed that baseline sex-adjusted adiponectin level (OR 0.32, 95% CI 0.13-0.82, P=0.018) was an independent negative predictor of hypertension development at 5 years. **Conclusion:** Low circulating adiponectin level independently predicts development of hypertension.

O32 Association between Melanocortin 4 Receptor Variants and Obesity

Cong-rong WANG, Qi-chen FANG, Rong ZJANG, Cheng HU, Xiao-jing MA, Wei-ping JIA
Department of Endocrinology and Metabolism, Shanghai Jiaotong University Affiliated No.6 People's Hospital, Shanghai Clinical Center for Diabetes, Shanghai Diabetes Institute, Shanghai 200233, P.R. China

Objective: To investigate the association between variants of melanocortin-4 receptor (MC4R) gene and obesity. **Methods:** (1) The genotypes of three variants, nt-216C/T, nt-178A/C and Val103I1e, were determined through DNA sequencing in 563 Chinese from Shanghai, including 258 individuals with body mass index (BMI) over 30 kg/m^2 and 305 individuals with BMI less than 23 kg/m^2. (2) Phenotypes measured were height, weight; waist, hip and femoral circumference; blood pressure; plasma glucose level of blood obtained at 0 and 120 minutes during 75 g oral glucose tolerance test; serum lipid levels including total cholesterol, triglyceride, high-density and low-density lipoprotein cholesterol and percentage of body fat. (3) |D'| and r^2 values were calculated. **Results:** (1) The frequencies of nt-216C/T, nt-178A/C and Val103I1e were 1.6%, 2.5% and 3.2% respectively in the Chinese; (2) The three variants were in low linkage disequilibrium; (3) Logistic regression showed that the Val103I1e variant was an independent risk factor for obesity (OR=0.414, P=0.040). The frequency of lie was less in the obese individuals compared with the controls. (4) No association between the Val103I1e variant and clinical parameters was detected in subjects with both normal weight and obesity. **Conclusion:** Val103I1e variant of MC4R gene was associated with obesity in Chinese.

O33 Resistin Production from Adipose Tissue is Decreased in db/db Obese Mice, and is Reversed by Rosiglitazone

Hong-ying YE[1 2 3], Ai-min XU[1 2], Ruby L.C. HOO[1 2], Herbert ZHANG[1 2], Rachel WONG[1 2], Karen S.L. LAM[1 2]
[1] Department of Medicine, University of Hong Kong, Hong Kong SAR, China
[2] Research Centre of Heart, Brain, Hormone, and Healthy Aging, University of Hong Kong, Hong Kong SAR, China
[3] Department of Endocrinology, Huashan Hospital, Fudan University, Shanghai 200040, China

Objective: Resistin has been proposed to play a role in linking obesity and insulin resistance. This study was designed to (1) investigate the expression profiles of resistin in db/db obese/diabetic mice and its association with metabolic parameters; and (2) to evaluate the effects of Rosiglitazone on production of resistin. **Methods:** db/db (-/-) obese/diabetic mice and their lean litter mates were used for this study. Epididymal fat pads were excised from mice of different age (from 5 to 12 weeks) for ex vivo incubation. Resistin levels in serum and conditioned culture medium of fat pads were measured using an in-house ELISA assay. The gene expression of resistin was determined by real-time PCR. Rosiglitazone (20 mg/kg/day) or the vehicle (PBS) was administered into db/db mice by daily intra-gastric gavage. Differentiated 3T3-L1 adipocytes were used for in vitro evaluation. **Results:** At each time point, the secretion of resistin from the fat pads in db/db mice was significantly lower than that in lean mice ($P<0.01$). Real time PCR showed that mRNA expression of the resistin gene in fat tissue of db/db mice was decreased by 60.5% compared to their lean controls ($p<0.05$). Serum levels of resistin were comparable between the obese and lean group, perhaps due to the increased total fat mass in db/db mice. Correlation analysis showed that serum resistin levels were negatively correlated to the body weight ($r= –0.515$, $P=0.000$) and fasting glucose level ($r= –0.357$, $P=0.002$). Notably, treatment of db/db mice with Rosiglitazone increased the serum resistin levels by 66.4%. In 3T3-L1 adipocytes, Rosiglitazone (10 μM) markedly enhanced the secretion of resistin by 120% ($P<0.01$) and its gene expression by 78.1% ($P<0.01$). **Conclusion:** Both resistin gene expression and its secretion from the epididymal adipose tissue are decreased in db/db obese/diabetic mice, while the insulin-sensitizing drug Rosiglitazone increases resistin production. Our results do not support the role of resistin as an etiological link between obesity and diabetes.

O34 Emodin Promotes Preadipocytes Differentiation, Increases Adipocytes Glucose Uptake and Inhibits Lipolysis in 3T3-L1 Cells

Ying YANG, Wen-bin SHANG, Li-bin ZHOU, Bo-ren JIANG, Hua JIN, Jin-feng TANG, Feng-ying LI, Ming-dao CHEN
Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai Clinical Center for Endocrine and Metabolic Diseases, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China

Objective: To investigate the effect of emodin on preadipocytes differentiation, glucose uptake and lipolysis in 3T3-L1 cells. **Methods:** (1) Adipocytes differentiation was estimated by Oil Red O staining of intracellular lipids. (2) 2-deoxy-[3H]-D-glucose uptake was performed in differentiated adipocytes. (3) The altering of Glut1 and Glut4 was observed by Northern blot and Western blot. (4) The lipolytic effect was determined on the base of the glycerol released from the cells. **Results:** (1) Oil red O staining and measurement of glycerol-3-phosphate dehydrogenase (GPDH) activity indicated that emodin promoted the differentiation of 3T3-L1 preadipocytes to adipocytes. (2) Glucose transport rate significantly increased after the incubation with 50 μM emodin for 6 hours. (3) 6.25 um emodin showed a significant increase in glucose uptake in the presence of 100 nM insulin, however, 50 um emodin impaired insulin-stimulated glucose transport. (4) Emodin induced glucose uptake was partly abolished by wortmannin, a specific inhibitor of phosphatidylinositol 3'-kinase (PI3K), and independed on MEK inhibitor or activation of Akt or PKC. (5) Emodin increased glucose transport in 3T3-L1 adipocytes through enhanced Glut1 and Glut4 expression as well as Glut4 translocation. (6) The isoproterenol-induced lipolysis in the cells was reversed by treatment with 50 um emodin. **Conclusion:** These results supported evidence that emodin is effective in regulating the glucose and lipid metabolism of 3T3-11 cells.

O35 Molecular Mechanism Underlying Berberine Improving Glucose Metabolism

Li-bin ZHOU, Ming-dao CHEN, Ying YANG, Wen-bin SHANG, Xiao WANG, Feng-ying LI, Jin-feng TANG, Shang-quan LIU, Guo-yao YUAN
Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai Clinical Center for Endocrine and Metabolic Diseases, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China

Objective: The aim of this study was to characterize the effect of berberine on glucose metabolism in *in vitro* and *in vivo*, and to investigate whether insulin signaling pathway was involved in the action. **Methods:** Basal and insulin-stimulated 2-deoxy-D-[3H]glucose uptake were measured in the presence and absence of berberine or specific inhibitors of insulin signaling pathway. Insulin resistance animal model was induced by SD rats fed with high fat diet for fourteen weeks and then treated with berberine for six weeks. Insulin sensitivity was estimated by oral glucose tolerance test and insulin tolerance test. Insulin signal and AMP-activated protein kinase (AMPK) activity were detected by western blot. **Results:** Berberine stimulated glucose uptake in 3T3-L1 adipocytes in a dose- and time-dependent manner with the maximal effect at 12 h. Glucose uptake was also increased by berberine in 3T3-L1 preadipocytes and C2C12 myobutes. Berberine-stimulated glucose uptake was additive to that of insulin in 3T3-L1 adipocytcs, even at the maximal effective concentrations of both components. Tumor necrosis factor-α (TNF-α) reduced insulin-stimulated glucose uptake, which was antagonized by berberine. Unlike insulin, the effect of berberine on glucose uptake was insensitive to wortmannin, an inhibitor of phosphoinositol 3-kinase, and SB203580, an inhibitor of p38MAPK. Berberine activated ERK1/2, but PD50985, a MEK inhibitior, only decreased berberine-stimulated glucose uptake by 32%. Berberine did not induce Ser-473 phosphorylation of Akt, nor enhance insulin-induced phosphorylation of Akt. Meanwhile, the expression and cellular localization of GLUT4 were not altered by berberine. However, genistein, a tyrosine kinase inhibitor, did completely block berberine-stimulated glucose uptake in 3T3-L1 adipocytes and preadipocytes. In *in vivo* experiment, the body weight, liver weight and epididymal fat pads weight of high fat fed rats (HF) were significantly higher than those of berberine treatment group (HF+B) and normal control group (NC). Fast plasma glucose, insulin and 2h plasma glucose, insulin after taking glucose in HF+B were significantly lower than those in HF (all $P<0.01$). Plasma glucose and insulin at all time points in HF were significantly higher than those in NC. Homa-IR of HF was markedly higher than that of HF+B ($P<0.01$). The glucose-lowering effects after the administration of insulin at all time points in HF+B were stronger than those in HF with 23% and 7% reduction in 15 min, respectively. Berberine, decreased plasma triglycerides in high fat fed rats as well. AMPK activity was increased by berberine in 3T3-L1 adipocytes and muscle in high fat fed rats. **Conclusions:** Berberine increases basal and insulin-stimulated glucose uptake through a mechanism distinct from insulin and may improve insulin resistance in part via stimulation of AMPK activity.

O36 A Study for Insulin Sensitivity and First-phase Insulin Secretion and Their Inflammatory Factors in Obesity with Hyperglycemia in 30 Min and/or 60 Min during OGTT

Jie HONG, Wei-qiong GU, Yi-fei ZHANG, et al
Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai Second Medical University, Shanghai 20025, China

Objective: To investigate insulin sensitivity and first-phase insulin secretion in obesity with hyperglycemia in 30 min and/or 60 min during OGTT (≥11.1 mmol/L, post-loading hyperglycemia, PLH). Meanwhile to evaluate their inflammatory factors (including IL-18, adiponectin and hs-CRP). **Methods:** A total of 165 subjects including lean controls 38, simple obesity 49, obesity with PLH 22, obesity with IGT 56 underwent 75 g oral glucose tolerance test (OGTT) and insulin-modified reduced sample number (n=12) of Bergman's minimal model method with frequently sampled intravenous glucose tolerance test (FSIGTT). Insulin resistance was determined by the HOMA-IR from homeostasis assessment and by the insulin sensitivity index (S_I) from the FSIGTT. Insulin secretion was determined by the $(I_{30min}-I_{0min})/(G_{30min}-G_{0min})$ from OGTT and by the acute insulin response to glucose (AIRg) during FSIGTT. The disposition index (DI), the product of AIRg and S_I was used to determine whether AIRg was adequate to compensate for insulin resistance. **Results:** (1) Compared with normal controls, the value of HOMA-IR was significantly increased and S_I was significantly decreased in those three groups ($P<0.01$) and with no significant difference between each other. (2) The AIRg value in simple obesity group is significantly increased and greater than that of normal control, PLH and IGT group ($P<0.01$, $P<0.05$ and $P<0.01$), and there was no significant difference among the latter three groups. The value of $(I_{30min}-I_{0min})/(G_{30min}-G_{0min})$ is significantly increased in simple obesity group, and higher than normal controls, PLH and IGT group ($P<0.01$), This index in PLH is significantly higher than that in normal controls ($P<0.01$), and there was no significant difference between PLH and IGT group and between normal controls and IGT group. (3) Compared with normal group, the DI value is significantly decreased in those three groups ($P<0.01$), and this index in simple obesity group is significantly higher than those in PLH and IGT group ($P<0.01$), and there was no significant difference between the latter two groups. (4) IL-18, hs-CRP was increased in IGT and PLH group and adiponectin was decreased in both groups without significant difference. **Conclusions:** Obese patients with hyperglycemia in 30 min and/or 60 min during OGTT in Shanghai area have a similar degree of insulin resistance and dysfunction of β cell. Also they have atherosclerosis risks. Compared with NGT, the PLH subjects also have hyperlipidemia, hyperuricemia and hypertension. So we should pay close attention to this kind of population because they have high risk factor for T2DM theoretically. We suggest doing OGTT in high-risk subjects for DM to screen PLH.

O37 Evaluation of Insulin Secretion and Insulin Resistance and the Selection of Hypoglycemic Drugs—a Multicentre Clinical Study

Yu-qian BAO, Wei-ping JIA[1], Xin GAO[2], Wei LIU[3], Hui-li XING[4], Zhi-min LIU[5], Zheng-yan SHENG[6], Ren-ming HU[7], Guang NING[8], Da-jing ZOU[9], Bo FENG[10], Jun-xi LU[1], Jian ZHOU[1]

[1] Department of Endocrinology and Metabolism, Shanghai Jiaotong University Affiliated No. 6 People's Hospital, Shanghai Clinical Center for Diabetes, Shanghai Diabetes Institute, Shanghai 200233, P.R. China
[2] Zhongshan Hospital affiliated to Fudan University
[3] Renji Hospital affiliated to Shanghai Jiaotong University
[4] Xinhua Hospital affiliated to Shanghai Jiaotong University
[5] Changzheng Hospital affiliated to Second Military Medical University
[6] No.1 People's Hospital affiliated to Shanghai Jiaotong University
[7] Huashan Hospital affiliated to Fudan University
[8] Ruijin Hospital affiliated to Shanghai Jiaotong University
[9] Changhai Hospital affiliated to Second Military Medical University
[10] Shanghai East Hospital affiliated to Shanghai Tongji University

Objective: To study the evaluation of pathophysiological function and its effect on the treatment in newly diagnosed type 2 diabetes individuals. **Methods:** Totally 322 newly diagnosed type 2 diabetes individuals were included, which were divided into normal and impaired islet function groups by arginine stimulation test. The former was distributed to repaglinide (Novo Norm), rosiglitasone (Avandia) and metformin subgroups and the latter to repaglinide, rosiglitasone and glipizide subgroups randomly. Homeostasis model assessment insulin resistance index (HOMA-IR) was applied to estimate insulin resistance. **Results:** (1) Compared with baseline fasting plasma glucose (FPG), post prandial plasma glucose (2hPG) and hemoglobin Alc (HbAlc) were significantly decreased in every subgroup after 3 months and 6 months of treatment periods ($P<0.01$). The percentage of HbAlc well controlled was 63.5%. (2) In normal islet function group compared with baseline the area under the curve (AUC) of arginine stimulation test was increased and proinsulin (PI) was reduced in rosiglitasone subgroup ($P<0.01$) and HOMA-IR was decreased in metformin subgroup ($P<0.05$). (3) In impaired islet function group in comparison with baseline, the increment of true insulin (ΔTI), ΔTI/PG, AUC and true insulin (TI) were elevated in both repaglinide and glipizide subgroups ($P<0.05$-0.01). In rosiglitasone subgroup ΔTI/PG and AUC were increased ($P<0.01$) and HOMA-IR and PI were declined ($P<0.01$) compared with pre-treatment. **Conclusions:** (1) In newly diagnosed type 2 diabetes patients the level of blood glucose was well controlled by oral hypoglycemic agents according to the evaluation of pathophysiological function. (2) Acute insulin release and TI could be increased in newly diagnosed type 2 diabetes patients treated with repaglinide and glipizide. (3) Thiozolidinediones not only improved β cell function by increased insulin sensitivity and decreased PI but also increased acute insulin release in patients with decreased early insulin secretion. (4) In newly diagnosed type 2 diabetes individuals the effectiveness of blood glucose control was related to increase in insulin secretion and insulin sensitivity.

O38 Changes in Serum Inflammation Markers and Nuclear Factor κB Activity in Peripheral Blood Mononuclear Cell and the Effects of Different Hypoglycemic Agents in Newly Diagnosed Type 2 Diabetics

Xiu-lin SHI, Fang-ping LI, Feng LI, Meng-yin CAI, Li YAN, Yan LI, Hua CHENG
Department of Endocrinology, The Second Affiliated Hospital, Sun Yat-sen University, Guangzhou 510120, China

Objective: To investigate the change of serum inflammation marker levels including hs-CRP, TNF-α and IL-6 and NF-κB activity in peripheral blood mononuclear cell (PBMNC) and the effects of different hypoglycaemic agents treatment in patients with newly diagnosed type 2 diabetes. **Methods:** 68 normal individuals and 101 newly diagnosed type 2 diabetics were selected. The diabetic patients were divided into group A (with 7.0 mmol/L<FPG<11.1 mmol/L) and group B (with FPG≥11.1 mmol/L). The patients of group A were randomised to group A_1 (insulin [novolin]) and group A_2 (oral hypoglycemic agents). Group A_2 were further subdivided into group A_{2a} (25 kg/m^2<BMI≤35 kg/m^2, metformin ([glucophage]) and group A_{2b} (20 kg/m^2<BMI≤25 kg/m^2, gliclazide [diamicron]). Serum hs-CRP, TNF-α, IL-6 were assayed by enzyme linked immunosorbent assay. Phosphorylation status of NF-κB p65 of PBMNC was determined by immunoblotting with phosphor-specific antibodies to NF-κB p65(Ser^{536}). Inflammation markers repeated at 0 weeks, 2 weeks and 12 weeks after glycemic control. **Results:** Levels of serum hs-CRP, TNF-α and IL-6 were higher in diabetic patients than that in control subjects ($P<0.01$). The levels of phosphorylation NF-κB P65 in PBMNC was increased newly diagnosed type 2 diabetic patients compared with control subjects (0.73±0.15 vs 0.47±0.13, $P<0.05$). In insulin group, the serum levels of hs-CRP, IL-6 and the levels of phosphorylation NF-κB P65 were decreased, TNF-α level did not change significantly after glycemic control comparing with baseline levels. In Metformin group, the serum levels of hs-CRP, IL-6, TNF-α and the levels of phosphorylation NF-κB P65 were decreased after glycemic control comparing with baseline levels. In Gliclazide group, there were no significant changes of the serum levels of hs-CRP, IL-6, TNF-α and levels of phosphorylation NF-κB. **Conclusions:** The levels of serum hs-CRP, IL-6 and TNF-α and the levels of phosphorylation NF-κB P65 in PBMNC were increased in newly diagnosed T2DM patients compared with that in control subjects, suggesting that newly diagnosed T2DM patients were in the state of chronic non-specific inflammation. In patients with type 2 diabetes treated with insulin, metformin treatment was associated with improvement of low-grade inflammation.

O40 Diabetic Retinopathy: Relationship with APOE and Oxidative Stress

Shuk-woon MA, Iris BENZIE, Vincent YEUNG
Department of Health Technology & Informatics, The Hong Kong Polytechnic University, Hong Kong SAR

Objective: To investigate whether diabetic retinopathy (DR) is related to *APOE* by comparing Type 2DM subjects with (+DR) and without (–DR) retinopathy. **Methods:** 406 consenting Chinese Type 2DM subjects (mean [SD] age: 59.3 [10.3] years) were recruited. Fasting venous blood was sampled for *APOE* genotyping, plasma glucose (FPG), allantoin/urate (as an index of oxidative stress) at entry. Duration of DM was recorded. Patients were monitored for development of complications for up to 3 years. ANOVA was used to compare results across genotypes. Unpaired *t* test was used to compare data of +DR and –DR groups for each genotype. **Results:** Genotypic frequencies were ε2/3 (13.8%), ε3/3 (69.7%), ε3/4 (12.6%). The ε3/4(+DR) (n=15) subjects had significantly higher FPG (9.8 [2.4] vs 7.5 [1.7] mM) and longer DM duration (13.4 [5.5] vs 8.2 [5.3] years) than ε3/3(+DR) (n=96) subjects ($p<0.05$). ε3/4(+DR) and ε2/3(+DR) (n=16) had significantly higher allantoin/urate ratios than their –DR counterparts: 28 [51] vs. 11 [8] and 27 [23] vs. 11 [7] respectively ($p<0.05$). **Conclusion:** DR developed in ε3/3 subjects who had lower FPG and shorter DM history compared with ε3/4 subjects, and in those ε2/3 and ε3/4 subjects with higher oxidative stress at baseline. The role of *APOE* genotype and oxidative stress in DR deserves further study as this may help identify subjects at increased risk of complications and facilitate early intervention.

O41 Prevalence and Characteristics of Undiagnosed Peripheral Vascular Disease (PVD) in High-risk Type 2 Diabetic Patients of a Regional Diabetes Centre in Hong Kong

Ho-ching LEUNG, Shing-chung SIU, Ka-wai WONG
Department of Medicine and Rehabilitation, Tung Wah Eastern Hospital, Hong Kong SAR

Objectives: To investigate the prevalence of undiagnosed PVD by using ankle-brachial index (ABI) measurement, and the characteristics of those patients in a regional diabetic centre in Hong Kong. **Methods:** 142 male type 2 diabetic patients with age ≥55 without history of PVD were recruited and ABI measured. Background information including BMI, waist circumference, BP, glycaemic and lipid profile, DM-related complication and medication history were collected. **Results:** The prevalence of undiagnosed PVD in our cohort was 12%. Those who have PVD were older ($p<0.01$), with higher HbA1c ($p=0.02$) and SBP ($p=0.03$). More proportion of PVD subjects were having retinopathy ($p=0.02$). In multiple logistic regression, age, HbA1c and presence of retinopathy were independent predictors of the presence of PVD with adjusted odds ratio of 1.2, 1.4 and 5.7 respectively. **Conclusions:** PVD is underdiagnosed in diabetic patients. Screening with ABI measurement is recommended in all diabetic patients, especially in high-risk group.

O42 Function Study of BNIP3: a Novel Gene Related with Diabetic Cardiomyopathy

Yan-yun GU, Jian YANG, Di ZHANG, Wen-zhong ZHOU, Feng-ying LI, Wei-bin ZHOU, Tian-hong LUO, Guo LI, Min LUO
Shanghai Institute of Endocrine and Metabolic Diseases. Shanghai Clinical Center of Endocrine and Metabolic Diseases, Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200025, China

Objective: In our previous study, bnip3 was in first time found to be the most obvious upregulated gene in cardiac muscle in DD-PCR comparison between diabetic rat and normal control. With this indication, we dig into how this gene participates in diabetic complication and what results it will bring in along with its high expression level in hyperglycemia. **Methods:** Construct BNIP3-EGFP and PGEX-4T2-BNIP3. Transfecting H9C2 cell line (ATCC CRL1446) with BNIP3-EGFP and BL21 strain with PGEX-4T2-BNIP3. Chemical siRNA targeted to bnip3 is applied to intervene the BNIP3-EGFP positive H9c2 cell with lipofectime 2000 to knock down bnip3. Different glucose levels 5.8, 17.6, 25, 33 mmol/l have been applied on different kinds of cell culture conditions. Northern blotting, realtime PCR, western blotting have been used to testify the expression level of binp3 and other genes. FCM is taken in testing apoptosis level (Annexin V) and mitochondria potential (JC-l). **Results:** Only high glucose level (33 mmol/l) can raise up the apoptosis level, BNIP3 will raise up the apoptosis level especially under high glucose, probably though injure the mitochondial potential. Bnip3 also can cause expression level of diabetic cardiomyopathy related gene pln & ctgf changing in the similar way as the high glucose does. **Conclusion:** BNIP3 expression level can be effected by high glucose 1evel, which can subsequently causes mitochondrial induced cell apoptosis and other diabetic cardiomyopathy related pathways. ctgf is related to cardiomyocyte fibrosis and pln is related to intracellular calcium concentration regulation. So bnip3 may play a key role in diabetic cardiomyopathy pathogenesis.

O43 Effect of High Glucose and SB203580 on the Expression of P38MAPK and PEDF in Cultured Rat Glomerular Mesangial Cells

Yong XU, et al
The Hospital of Luzhou medical college, Luzhou, Sichuan 646000, China

Objective: Diabetic nephropathy is one of important diabetic microvascular complications. Recent studies have emphasized the important role of P38MAPK signal transduction pathways in the pathophysiological processes. The effect of PEDF which is identified as an inhibitor of neovascularization in the pathogenesis of diabetic nephropathy and the association between PEDF and P38MAPK signal transduction pathways remain largely unknown. In order to explore the mechanism of P38MAPK and PEDF in diabetic nephropathy and the relation between P38MAPK and PEDF, we investigated the effect of high glucose and inhibitor of P38MAPK-SB203580 on the expression of P38MAPK and PEDF in cultured rat glomerular mesangial cells and discuss the mechanism of P38MAPK signal transduction pathways in diabetic nephropathy and the relation between P38MAPK and PEDF. We have found new evidence for prevention and cure of diabetic nephropathy. **Methods:** Cultured HBZY-l rat glomerular mesangial cells were divided into 7 groups: Normal Glucose-treated group, High Glucose-treated group (including different concentration glucose), mannitol-treated group (as osmotic controls), SE3203580 and High Glucose-treated group, DMSO and High Glucose-treated group. The expression of phospho-P38MAPK was measured by indirect indirect-immunofluorescence. We also measured the expression of P38MAPK and PEDF by Real time Quantitative PCR. **Results:** (l) Normal Glucose-treated group has expression of phospho-P38MAPK, P38MAPK and PEDF. (2) The expression of phospho-P38MAPK and P38MAPK were increased in high glucose-treated group and mannitol-treated group. (3) The expression of phospho-P38MAPK in high glucose-treated groups were decreased by adding SB203580. (4) The expression of PEDF was decreased in high glucose-treated group, the expression of PEDF was negatively correlated with concentration of glucose. (5) SB2003580 can prevent the decreased expression of PEDF induced by high glucose. **Conclusions:** (l) High glucose can increase significantly the expression of phospho-P38MAPK and P38MAPK in cultured mesangial cells. High glucose can decrease significantly the expression of PEDF in cultured mesangial cells, which is independent of the osmotic. (2) SB2003580 can inhibit the downregulated expression of PEDF induced by high glucose, the mechanism was correlated with P38MAPK signal transduction pathways.

O44 Hepatocyte Growth Factor Protects Human Endothelial Cells against Advanced Glycation End Products-induced Apoptosis and Its Mechanism

Yi-jun ZHOU[1], Jin ZHANG[2]
[1] Department of Endocrinology and Metabolism, Fourth Affiliated Hospital, China Medical University, Shenyang 110032, China
[2] Department of Endocrinology and Metabolism, First Affiliated Hospital, China Medical University, Shenyang 110001, China

Objective: To investigate inhibiting effect of hepatocyte growth factor (HGF) on endothelial cells apoptosis induced by advanced glycation end products (AGEs) and its possible mechanism. **Methods:** Human umbilical vein endothelial cells (HUVECs) were cultured *in vitro* and were treated with AGEs in the presence or absence of HGF. The cell viability was measured by methyl thiazolyl tetrazolizm (MTT) assay, the earlier apoptosis was detected by flow cytometry with Annexin-FITC/PI double staining, morphology of cell apoptosis was observed by Wright's-Giemsa staining, Acridine Orange and Hoechst 33258 fluorescence staining, and the expression of apoptosis-associated genes Bax, Bcl-2 and NF-κB were detected by western blotting. The activities of caspase-3 and caspase-9 were detected by enzyme-linked immunosorbent assay. **Results:** Treatment of HUVECs with AGEs changed cell morphology and decreased cell viability, leading to apoptosis. Apoptosis was induced by AGEs in a dose- and time-dependent fashion. AGEs markedly elevated Bax and decreased NF-κB, but not Bcl-2 expression. Additionally, AGEs significantly inhibited cell growth through a pro-apoptotic action involving caspase-3 and -9 activations in HUVECs. Pretreatment with HGF protected against AGEs-induced cytotoxicity in the endothelial cells. HGF significantly promoted the expression of Bcl-2 and NF-κB, while decreased the activities of caspase-3 and -9 without affecting Bax level. **Conclusion:** AGEs can induce the apoptosis of endothelial cells *in vitro*. HGF effectively attenuate AGEs-induced endothelial cells apoptosis through upregulating Bcl-2 and NF-κB gene expression and inhibiting caspase-3 and -9 activations.

O45 Etiology Distribution of 1007 Cases with Chief Complaint of Short Stature

Hui PAN, Hui-juan ZHU, Xin LI, Feng-ying GONG, Ming-ming HU, Feng GU, Xue-yan WU, Jie-ying DENG, Zi-men JIN, Yi-fan SHI
Department Endocrinology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy Science, Beijing 100730, China

Objective: To study the etiology distribution of short stature patients. **Methods:** The clinical data of 1007 patients with chief complaint of short stature treated in Peking Union Medical College Hospital between 1990-2006, were analyzed for etiology distribution retrospectively. **Results:** 1007 patients included 336 females and 671 males, the first visit age was 12.6±4.1 years. 1007 cases consisted of 550 cases (54.6%) with growth hormone deficiency, 106 cases (10.5%) with Turner syndrome, 106 cases (10.5%) with idiopathic short stature, 45 cases (4.5%) small for gestational age, 26 cases (2.8%) with central diabetes insipidus, 13 cases (1.4%) with central diabetes insipidus and growth hormone deficiency, 20 cases (2.0%) with hypothyroidism, 11 cases (1.1%) with precocious puberty, 1 case (0.6%) with congenital skull-clavicle agenesis. The common etiology of male cases were growth hormone deficiency, idiopathic short stature, central diabetes insipidus and small for gestational age, those of female patients were Turner syndrome, growth hormone deficiency and idiopathic short stature. **Conclusion:** Most common etiology of these cases with chief complaint of short stature are growth hormone deficiency, Turner syndrome, idiopathic short stature, small for gestational age, central diabetes insipidus and hypothyroidism.

O46 The Changes of Body Composition in Adolescence with Idiopathic Growth Hormone Deficiency After 1-Year Treatment of Recombinant Human Growth Hormone

Qin-yong WU, Hui-juan ZHU, Xin LI, Hui PAN, Dian-xi ZHANG, Feng-ying GONG, Jie-ying DENG, Yi-fan SHI
Department of Endocrinology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing 100730, China

Objective: To assess the changes of body composition in idiopathic growth hormone deficiency (IGHD) after 1 year treatment of recombinant human growth hormone (rhGH). **Methods:** 17 IGHD cases (male 14, female 3), chronological aged (CA) (14.6±4.5 years), bone age (BA) (12.1±0.8 years) and growth velocity (2.9±0.7 cm/year) before treatment were injected subcutaneously with rhGH (0.5 u/kg) daily before sleep for one year. Height, weight, fat percentage (F%), free fat mass (FFM), grips of both hands, serum insulin-like growth factor (IGF)-1 and insulin-like growth factor binding protein (IGFBP)-3 were measured before and after 1, 3, 6, 9, 12 months of treatment. A total of 177 normal northern Chinese children (boy 95, girl 82, CA 6-16 years) were used as control group. **Results:** The height, weight, FFM, serum IGF-1 and IGFBP-3 of hGHD cases were significantly lower, F% was significantly higher, but body mass index (BMI kg/m^2) value was not significantly different from that of the control group. From one month to end of treatment of rhGH, F% of patients was decreased significantly ($P<0.05$), and was not significantly different from that of control group. Body height, weight, growth velocity, FFM, serum IGF-1 and IGFBP-3 were significantly increased ($P<0.05$), and were negatively correlated with decreased F% ($P<0.01$) after treatment of 6 months. At the end of treatment, the grips value of both hands of patients were significantly increased than those at the beginning of treatment. **Conclusions:** Increased height, FFM and grip, decreased F% of IGHD cases after treatment of rhGH in IGHD patients results indicated that they have significant changes in body composition.

O47 The Clinical Features and Outcomes in Acromegaly

Feng GU, Zi-meng JIN, Jie-ying DENG, Xue-yan WU, Hui-jian ZHU, Hui PAN, Nai-shi LI, Ming-ming HU, Qin-yong WU, Yi-fan SHI
Department of Endocrinology, Peking Union Medical College Hospital, Beijing 100730, China

Objective: To analyse the clinical features and outcomes in acromegalic patients which were diagnosed in Peking Union Medical College Hospital from 1978 to 2006. **Methods:** We retrospectively analyzed the clinical data of 778 acromegalic patients diagnosed and followed up in the department of endocrinology, compared their different clinical features and the outcomes with different treatments (include surgery, radiation and medication). **Results:** In 778 patients (male 361, female 397), the average onset age was 30.4±4.5 yrs, and the average follow-up duration was 14.0 ±8.7 yrs. The pathologic diagnosis in these patients included 680 cases of GH-secreting tumors, 89 cases of multi-hormone secreting tumors (secreting GH, PRL, FSH, LH and TSH), 7 cases of MEN-1 and 2 cases of McCune-Albright syndrome, in which macroadenoma accounted for tip to 90%. They had different clinical manifestations, with acral and soft-tissue enlargement (100%), deadlims (92%), OSAS (90%), headache (89%), hypogonadism (56%), pituitary apoplexy (25%) and vision damaged (24%). Hypertension occurred in 35% patients, abnormal glucose metabolism occurred in 70% patients (in which 25% was DM, 47% was IGT and 28% was IFG). Malignancy occurred in 4.6% patients. These patients were treated with different methods, which were surgery (94%) (53% with surgery, 41% surgery plus radiation, 4% with medicine plus surgery and/or radiation, etc), radiation (5.2%) and medication (0.8%). After treatment, the rate of the chemical relief of acromegalic symptoms in these patients were 70% in surgery group, 84% in surgery plus radiation group, 97% in medication plus surgery and/or radiation group and 87% in radiation group. Hypertension improved in 81% patients, abnormal glucose metabolism improved to normal glucose level in 37% DM patients and from DM to IGT in 24% patients after treatment. Occurrence of pituitary hypofunction after treatment were 27%, 68% and 40% in surgery, surgery plus radiation and radiation groups respectively. **Conclusion:** Acromegaly is usually occurred in the prime of one's life. Most of the patients at first visit had macroadenoma combined with different extent of reversible hypertension and abnormal glucose metabolism. Malignancy prevalence is much higher in acromegaly. Surgery therapy is still the leading way for acromegaly. The prevalence of hypofunction in pituitary is highest with radiation therapy.

O48 Classical Pituitary Apoplexy—a Retrospective Analysis in a Tertiary Hospital in Hong Kong

H.K. PANG, Ronald C.W. MA, Jenny Y.Y. LEUNG, Francis C.C. CHOW
Department of Medicine, Pamela Youde Nethersole Eastern Hospital, Hong Kong SAR

Objectives: To review the clinical features, neuro-ophthalmological presentation and pituitary hormonal dysfunction of patients with classical pituitary apoplexy (CPA), and to study the visual and pituitary hormonal outcome of these patients. Comparison of visual and pituitary hormonal outcome will be made between the surgically treated group and the conservative treated group. **Methods:** Retrospective analysis of case records of 22 patients with CPA managed at the Prince of Wales Hospital from 1994- mid 2005. **Results:** The most frequent presenting symptoms were impaired visual acuity (VA) (73% of patients) and headache (68%). At time of acute CPA, 85% had hypogonadotrophin hypogonadism, 63% had hypocortisolism and 41% had central hypothyroidism. 14 patients (64%) underwent surgical decompression, while 8 (36%) were managed conservatively. There was no mortality in both groups. Up to 1 year, 92% in the surgically treated group had improvement in VA comparing with 66% in the conservative treatment group (P=0.371). Improvement of visual field and ophthalmoplegia reached 55% and 80% respectively in the surgical group. Up to 1 year, hypocortisolism, hypothyroidism and hypogonadism were present in 71%, 64% and 72% respectively in the surgical treatment group. In comparison, hypocortisolism, hypothyroidism and hypogonadism were found to be 75%, 36%, and 66% respectively in the conservative treatment group (P=1.0, P=0.378, P=1.0 respectively). **Conclusions:** The clinical features of CPA were similar to other studies in literature. For those patients presented with VA impairment, 87% of them had improvement in VA up to 1 year after CPA. Surgical decompression may result in better outcome in VA. Permanent hypocortisolism, hypothyroidism and hypogonadism could be frequently found in 73%, 55% and 71% of the patients respectively up to 1 year after CPA.

O49 Clinical and Basic Research of MEN1

Xiao-hua JIANG, Xiao-ying LI, Guang NING, et al
Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai Second Medical University, Shanghai 20025, China

MEN1 is an inherited tumor syndrome characterized by the development of tumors of parathyroid, pancreatic islets and anterior pituitary. Heterozygous germline mutations of the MEN1 gene are responsible for the onset of MEN1. In the present study, we investigated the probands and their relatives from nine unrelated Chinese families with MEN1. Direct sequencing analysis identified two novel mutations 373_374insl8 and 822delT, and six previously reported mutations 357_360delCTGT, 427_428delTA and 432C>T of the MEN1 gene, etc. Moreover, loss of heterozygosity (LOH) at Chromosome 11q13 was detected in gastrinoma, insulinoma and parathyroid adenoma from the probands of Family A and B, but not in parathyroid adenoma from the proband of Family D, which was identified with a heterozygous mutation 822delT. RT-PCR showed that mutant MEN1 transcripts remained in the MEN1 related endocrine tumors from the probands in Family B and D, whereas menin was not detected in these tumors by immunohistochemistry and western blot. In conclusion, our study has further demonstrated that the development of MEN1 related endocrine tumors results from complete menin expression defects due to MEN1 mutation and LOH. In addition, some unknown mechanisms may be also responsible for MEN1.

O50 The Clinical Analysis and the Research of RET Proto-oncogene in Twenty MEN2 Pedigrees

Yu-lin ZHOU, Yong-ju ZHAO, Bin CUI, Li-qun GU, Jie HONG, Yi-fei ZHANG, Xiao-ying MA, Shao-xing ZHU, Zhao-li YU, Min YIN, Tie-yun ZHAO, Jing-bo LIU, Chang-you CHENG, Jian-jun LI, Zhi-qiang YIN, Li-ming WANG, Bu-yun XIAO, Guang NING
Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrine and Metabolic Diseases, Department of Endocrinology and Metabolism, Ruijin Hospital, Medical School of Shanghai Jiaotong University, China

Objective: To detect the phenotype and the mutations of RET proto-oncogene in fifteen MEN2A and five MEN2B probands and their family members. **Methods:** Totally 119 family members of fifteen MEN2A pedigrees and 5 patients with typical phenotype of MEN2B and 23 corresponding family members were recruited. Total genomic DNA was extracted from peripheral blood for PCR. PCR products of exon 8, 10, 11, 13, 14, 15 and exon l6 of the RET proto-oncogene were purified and direct gene sequencing was performed. **Results:** (1) The germline mutations of RET proto-oncogene were detected in 49 patients of the sixteen MEN2A pedigrees. From them, 37 patients had MEN2A phenotype and the others were gene carriers The mean age when the former was diagnosed of MEN2A is significantly later than the latter's (43.0±13.9 vs 9.8±7.4 yrs, P<0.01). The incidences of MTC, PCC and HPT in 37 MEN2A patients were 91.9%, 56.8% and 10.8%. The mean age when they occurred was 33.6±12.4 yrs, 35.4±12.3 yrs and 38.5±15.2 yrs differently. Five germline mutations which all located in codon 634 of exon11 in RET proto-oncogene were detected in fifteen MEN2A pedigrees. They were C634W (13.3%), C634Y (46.7%), C634R (26.7%), C634F (6.7%) and C634S (6.7%). Through the analysis of the genotype-phenotype of 37 MEN2A patients, no statistic significance has been found in the diagnosed age of MEN2A, the incidence of PCC, the percentage of cervical lymph node metastasis and the five different genotypes. The significant analysis of HPT has not been done for it has only been found in patients with C634R and C634Y. (2) The phenotype and gene mutation of MEN2B only occurred in five MEN2B patients and none of their family members. The incidences of MTC, marfanoid habitus, neuroma of tongue and lips, eyes' disorders, intestinal disorders and PCC when they were diagnosed of MEN2B were 100%, 100%, 100%, 80%, 60% and 60% differently. The age when their MTC and PCC occurred was 20.0±8.l yrs and 28.3±2.5 yrs. Only M918T of exon16 in RET proto-oncogene was found. **Conclusion:** (l) The mean age which 37 patients of fifteen MEN2A pedigrees were diagnosed is 43.0±13.9 yrs. It's older than correlative report overseas which is probably related to the lack of cognition of MEN2A in our country. The occurrence of MTC in MEN2A patients is earlier than PCC and HPT. The incidence of MTC and PCC is basically consistent with the oversea archive, while the incidence of HPT is lower than the corresponding report. The detected mutations of RET proto-oncogene in MEN2A patients were all located in codon 634 of exon11. This is relatively simple compared with foreign reports. The highest incidence of live germline mutations is C634Y followed by C634R, which is a little different from oversea reports that the incidence of C634R is the highest. According to the reports abroad, no statistic significance has been found between the incidence of PCC and different genotypes of codon 634 in MEN2A patients whereas the incidence of HPT is closely related to C634R and C634Y. (2) The mean age when they get MTC and PCC is later than the related report abroad while PCC occurs after MTC. The incidences of other MEN2B clinical symptoms are higher than the reports abroad. They are considered to be related to the late diagnose of MEN2B. The percentage of pure proband getting the RET germline mutation (100%) was obviously higher than that of abroad (50%). What's more, only a singe germline mutation of RET proto-oncogene (M918T) was found. (3) The mean age of MEN2A patients when MTC and PCC develop is separately later than that in MEN2B. The degree of these diseases and their aggressiveness is also lower in MEN2B patients.

O51 Genetic Analysis for 11 Patients with 17alpha-hydroxylase/17,20 Lyase Deficiency

Min NIE, Zhao-lin LU, Mei-li SUN, Kui ZHANG
Department of Endocrinology, Peking Union Medical College Hospital, 100730, China

Objective: To analyze the genetic characteristics of 11 patients with 17α-hydroxylase /17,20 lyase deficiency (17OHD) or isolated 17,20 lyase deficiency (ILD) in north China. **Methods:** The subjects were 10 17OHD patients and 1 ILD patient who were diagnosed in the Peking Union Medical College Hospital from 2003 to 2006. We amplified all eight exons of the CYP17 gene, including the exon-intron boundaries, by the polymerase chain reaction and determined their nucleotide sequences. **Results:** Nine mutations, including 4 missense mutations, 2 deletion mutations, 2 splice site mutations and 1 compound mutation were identified, three of which are novel mutations, and the other six have been described previously. The 3 undescribed mutations were R449C; a GT to GC mutation at g.2261 in the splice donor site of intron 1; an AG to AA mutation at g.3927 in the splice acceptor site of intron 1 and the other six mutations were Y329K,418X; I259H,274X; R496C; R362C; Y398V; Del D487_F489. The mutation Y329K, 418X in exon 6 was the most common, accounting for half of the mutant alleles, including 4 homozygotes and 4 compound heterozygotes. The mutation R449C may be selectively disrupt 17,20 lyase activity. **Conclusion:** We identified novel mutations of the CYP17 gene in 11 Chinese 17OHD patients. The information gained provides a new insight into the molecular pathogenesis of the disorder.

O52 Age-related Changes in Adrenomedullin Expression and Hypoxia-inducible Factor 1 Activity in the Rat Lung and Their Responses to Hypoxia

Isabel S.S. HWANG, M.L. FUNG, E.C. LIONG, G.L. TIPOE, F. TANG
Departments of Physiology and Anatomy, and the Research Centre of Heart, Brain, Hormone and Healthy Aging, Faculty of Medicine, the University of Hong Kong, Hong Kong SAR, China

Objective: To investigate the effects of hypoxia on adrenomedullin (AM) gene expression during aging. **Methods:** Male rats aged 3 months, 12 months and 20 months were subjected to breathing 8% of oxygen for 6 hours. AM levels in the lung were measured by RIA while mRNA levels of preproAM, its receptor component proteins and hypoxia-inducible factor-1α (HIF-1α) were determined by RNase protection assay and/or RT-PCR. HIF binding to DNA was measured by electrophoretic mobility shift. **Results:** In the lung, there was an age-related increase in basal levels of preproAM mRNA and AM and of the binding of hypoxia-inducible factor-1α (HIF-1α) to DNA. Upon hypoxic stimulation, HIF binding to DNA increased in the young and middle-aged rats, but not in the old rats. AM gene expression increased in response to hypoxia in rats of all ages but the increase was much less in the old rats. AM peptide levels in the lung decreased with age in hypoxia. In male rats aged 3 months and 20 months subjected to hypoxia, preproAM, CRLR (calcitonin receptor-like receptor), RAMP (receptor activity modifying proteins) mRNA and HIF-1 mRNA levels showed an increase in basal levels but a diminished response to hypoxia in the old rats. Polysome profiling demonstrated decreases in the percentages of translatable preproAM mRNA in response to hypoxia, with a greater decrease in the old than the young rats. **Conclusion:** The age-dependent decrease in the hypoxic response of the AM system in the lung was associated with high basal levels of HIF activity and AM expression in the old rats, but less translatable preproAM mRNA in the old rats in response to hypoxia. Thus the HIF-AM pathway may be impaired in the aged lung and other mechanisms may be present to maintain an AM response to hypoxia.

(This study was supported by a CRCG grant from the University of Hong Kong).

O53 Cyclooxygenase-2 is an Essential Mediator of Gonadotropins-induced Apoptosis of Human Ovarian Surface Epithelial Cells

Yuen-lam PON, Alice S.T. WONG
Department of Zoology, University of Hong Kong, Pokfulam Road, Hong Kong SAR

Objective: To investigate the signaling mechanism underlying gonadotropins-mediated apoptosis of human ovarian surface epithelial (OSE) cells. **Methods:** OSE cells were treated with surge concentrations of gonadotropins (FSH and LH/hCG), membrane, cytosolic, and nuclear fractions were prepared by differential centrifugation. Expression of N-cadherin, β-catenin, and cyclooxygnease-2 (COX-2) were examined by immunoblot analysis. β-catenin/T-cell factor (TCF) transcriptional activity was measured in a luiferase reporter gene assay. **Results:** We demonstrated that disruption of N-cadherin-mediated cell-cell adhesion by gonadotropins is an important molecular event in the apoptosis in human OSE. This downregulation of N-cadherin was correlated with a redistribution of β-catenin from the plasma membrane to the nucleus, resulting in the activation of β-catenin/T Cell Factor (TCF) transcription. These events were mediated via a phosphatidylinositol 3-kinase (PI3K)-dependent phosphorylation of the inhibitory serine 9 residue of glycogen synthase kinase-3β (GSK3-β). In addition, we showed that COX-2, a target of β-catenin/TCF, is an important mediator of the gonadotropins-induced apoptosis of OSE. **Conclusion:** These findings shed new light on the molecular mechanism underlying OSE survival and death, a process that is anticipated to have important implication in the ovulation and ovarian cancer risk.

P1 Diabetes Mellitus and Impaired Glucose Tolerance in Dalian, China: an Epidemiological Survey

Meng YU[1], Gui-xian YU[2], Xiao-ping LIANG[2], Li-xin ZU[2], Lei LUO[2], Shu-min MU[2], Hui WANG[2], Lin-jing WANG[2], Li-jun XU[2], Wei XU[3], Chang-chen LI[3]
[1] Dalian University Zhongshan Hospital, Dalian, Liaoning 116001, China
[2] Dalian Port Hospital, Dalian, Liaoning 116001, China
[3] Dalian Medical University, Dalian, Liaoning 116001, China

Objective: The WHO has termed the increased prevalence of diabetes and obesity as a "21st Century epidemic". China is also thought to be severely affected by diabetes. However, reliable, standardized data on prevalence and characteristics of diabetes and glucose intolerance in Chinese populations remain sparse. **Methods:** A total of 5169 randomly selected adults aged 20 years and older were examined at Dalian, a city with a population of about 6 millions. The diagnosis of diabetes (DM) and impaired glucose tolerance (IGT) was made following the WHO 1999 criteria for DM diagnosis. **Results:** Prevalence of diabetes was 9.2% (2.87% in the group aged 20 to 29; 3.13% in the group aged 30-39; 9.6% in the group aged 40 to 49; and 12.3% in the group aged more than 50 years). In addition, the prevalence of IGT was 12.5% (1.19% in the group aged 20 to 29; 6.8% in the group aged 30-39; 12.9% in the group aged 40 to 49; and 15.8% in the group aged more than 50 years). About 57.9% of all cases of diabetes were undiagnosed before the survey, among which the prevalence of hypertension, obesity, dyslipidemia and metabolic syndrome were 44.2%, 45.3%, 43.4% and 28.9%, respectively. **Conclusion:** Diabetes and IGT are not uncommon in the city of Dalian. About one fifth of all adults in the chosen community had DM and IGT. Since China is a developing country and the lifestyle is fairly traditional by international standards, these findings are unexpected.

P2 Study the Prevalence of Type 2 Diabetes Mellitus in Adolescents of Xi'an Urban Areas in 2001

Yu HUA, Zhu-fang TIAN, Jin-hui ZHANG, Yang-mei LV, Min-wen ZHU, Heng LI, Fang WANG, Xiang WANG, Ning LI, Xin JIA, Ying JIANG, Xi-ling XIAO, Ping LIU
Department of Endocrinology, Xi'an Center Hospital, Xi'an Shannxi 710003, China

Objective: The aim of the study was to determine the prevalence of type 2 diabetes mellitus (T2DM) in adolescents of Xi'an urban areas and to investigate associated risk factors. **Methods:** A survey was conducted among 3000 students aged 9 to 18 years in 2001, who were selected by cluster sampling. Investigate content (1) questionnaire: inquiring the weight of birth, occupation of parents, income of the family, family history of T2DM, hours of activity, diet, smoking and drinking and so on. (2) Physical examination: height, weight, circumference of waist and hip. (3) Blood glucose measurement: take orally 75 g glucose fasting, capillary blood glucose (CBG) were measured respectively after 2 hrs. Subjects with CBG≥6.7 mmol/L received a standard oral glucose tolerance test (OGTT), impaired fasting glycaemia (IFG) were diagnosed by fasting plasma glucose (FPG) ≥5.6 mmol/L. **Results:** 3 Female subjects were diagnosed as T2DM among 2956 and received treatment; 4 were diagnosed as IFG, two were female; 2 were impaired glucose tolerance (IGT), one was female; 1 was IFG companioning IGT, female. The standard prevalence is 0.952%, 1.001%, 0.569%, 0.192% respectively. Prevalence of gender has no significance. All positive subjects have no significance in weight of birth, family history of T2DM and income of family compared with normal subjects. Some BMI and WHR of positive subjects were not acquired and was not statistics. All subjects denied drinking and smoking. **Conclusion:** The prevalence of T2DM, IFG and IGT in adolescents of Xi'an urban areas is high and National prevention programs are needed. Our study could not find the relationship between associated risk factors and T2DM, IFG and IGT because our sample was not enough and some data could not be acquired.

P3 Cost Analysis of Diabetes from One Chinese Hospital between the Years 2000-2004

Zu-qian LU, Chen-wei HU, Zhang-rong XU
Diabetic center, the 306th Hospital of PLA, Beijing, China

Background: Diabetes mellitus have already become one chronic non-communicable disease that affects the health of China people and the Chinese health care system at many levels. According to Chinese Diabetes Association, Chinese people have diabetes mellitus. Diabetes and its chronic complications bear heavy burden to society and family in China. The prevalence of diabetes is rapidly increasing in this country as the economic develops. Therefore, it is very important to analysis the in-hospital expenditure of diabetic patients using the medical economics method scientifically. This is of significantly importance to highly use limited medical resources to properly manage the diabetic patients. **Objective:** The goal of this analysis was to evaluate the medical costs of diabetes patients from one Chinese hospital between the years 2000-2004. **Methods:** All subjects are from diabetic center in the 306th hospital of PLA in China between the years 2000-2005. All data were obtained from the cover page of database of in-patient case record. Student *t* test was used to compare to two group data. **Results:** 2794 patients were admitted to hospital between the years 2000-2004, with man 2085 and female 1934, with age from 14-98 years old (66.1 ±11.9). 42 patients had type 1 diabetes, and 1146 patients had type 2 diabetes. The other 1606 patients are not marked with types of diabetes. The order of the first diagnosis for in-patient is as follows: type 2 diabetes (4019 RMB Yuan [28.51%]), cerebral infarction (2794 RMB Yuan [7.37%]); hypertension (2065 RMB Yuan [2.79%]); coronary heart disease (1934 RMB Yuan [2.39%]); others (1087 RMB Yuan [27.05%]). There is no difference among the ages of in-patients between the years. The average total medical costs of in-patients per year is 6557 RMB Yuan, 6887 RMB Yuan, 8235 RMB Yuan, 9633 RMB Yuan, and 11785 RMB Yuan from 2000 to 2004, respectively. If based on the cost of the year 2000, the ratio is increase 5%, 26%, 47%, and 80% for the average total medical cost per year from 2001 to 2004, respectively; 17%, 19%, 47%, and 96% for drugs cost; 5%, 29%, 56%, and 92% for examinations. When the diabetic patients had cerebral infarction, the average total medical, drugs, and examination costs of in-patients per year increased 1.01, 1.14 and 1.10 times, respectively; when the diabetic patients had hypertension, those costs above increased 1.16, 1.37 and 1.12 times, respectively; when diabetes had cholecystitis and or gallstones, those costs above increased 1.24, 1.11 and 1.18 times, respectively; when the diabetes mellitus had influ, those costs above increased 1.46, 1.44 and 1.28 times, respectively. The in-hospital days for patients are significantly delayed as the patient had the above diseases, and the average total medical cost also increased significantly. **Conclusions:** The above data indicate that the average total medical, drugs and examination cost in this society is dramatically increased yearly, and this will give heavy burden to this society. When the diabetes complicated with other disease including cerebral infarction, hypertension, and coronary heart disease, and so on, the costs are increased rapidly. It is suggested that the behavior intervention may be an effective way to decrease the diabetes-related costs in this society.

P4 An Investigation of Chronic Diseases for Elderly People in the Community of Shenyang

Jin ZHANG
Department of Endocrinology, The First Affiliated Hospital of China Medical University, Shenyang 110001, China

Objective: To study the prevalence of elderly chronic disease and emphasize the importance for community health, and then strengthen the strategy of the prevention and cure in community. **Methods:** The measurement of blood glucose, blood pressure, weight, and WHR were performed for 2853 elderly people. **Results:** More than 60 years elderly people are 87%; the level of glucose more than normal (FBG>5.6 mmol/L and PBG>7.8 mmol/L) are 14.4%. The prevalence of hypertension was 57.5%. The rate of overweight (BMI>27 kg/m^2) was 72.2%. **Conclusion:** The prevalence of diabetes, hypertension and obesity in the elderly people are highest in the investigation of community. Blood glucose should be measured in plan every year for the elderly people, then diabetes can be diagnosed and treated early. It should be recognized that overweight and obesity are important risk factors for diabetes, hypertension and cardiovascular diseases. It is suggested that the prevention and cure of chronic diseases in elderly people should be emphasized.

P5 Clinical Analysis of Disturbance of Carbohydrate Metabolism in 35 Patients with Epidemic Hemorrhagic Fever

Fang XU, Yan LIU, Peng GAO
Department of Endocrine, The First Hospital of JiLin University, Chang Chun 130021, China

Objective: Epidemic hemorrhagic fever can cause dysfunction of many organs including pancrease, but we do not clearly know the relationship of EHF, pancrease and hyperglycemia. So we discuss the clinical data of carbohydrate catabolism in patients with epidemic hemorrhagic fever to find out the clinical characters of them. **Methods:** We analyse the characters of history, clinical manifestations, blood glucose and complications of 35 patients in our hospital. **Results:** We find that their blood glucose has come to the diagnosis standards of diabetes mellitus and the decline of blood glucose after treatment compared with the first days in hospital is statistically overt. There are 6 patients with diabetic ketoacidosis (17.17%) compared with 3 patients with nonketotic hyperosmolar diabetic coma. Most have dysfunction of liver and kidney. **Conclusions:** Patients with epidemic hemorrhagic fever can have abnormal metabolism of carbohydrate, elevetion of blood glucose and acute complications of diabetes mellitus. The causes of hyperglycemia may be (1) hemorrhagic fever virus can infect pancrease and cause its dysfunction; (2) because of stress state, many anti-insulin hormones are secreted; (3) the sensitivity of liver to insulin is damaged by its dysfunction. Most of them can have better control of blood glucose after dietetic therapey, insulin (intravenous or intramuscular), monitoring of blood glucose, fluid replacement and treatment of epidemic hemorrhagic fever.

P6 Ault Nesidioblastosis: a Case Report

Ming-chen HSIEH, Duan WU, Shih-ming HUANG
Division of Endocrinology and Metabolism, Department of Internal Medicine, Buddhist Tzu- Chi General Hospital, Hualien, Taiwan

Hypoglycemia is a common cause of hospitalization in patients with type 2 diabetes usually caused by diabetic insulin overdose, a missed meal or unexpected exercise. But other disease states may also influence hypoglycemia too. Nesidioblastosis is an uncommon but clinically important cause of hypoglycemia in the adult population. It characterizes a condition with diffuse proliferation of disordered islet cells from pancreatic ductal cells. Patients with nesidioblastosis usually present from birth to age 18 months, with most cases diagnosed shortly after birth. We report a 70-year-old woman with recurrent hypoglyceamia and hyperinsulinaemia. The selective arterial stimulation venous sampling (ASVS) showed insulin values were elevated at dorsal pancreatic artery and proximal splenic artery. Resected pancreas proved a focal nesidioblastosis. The rarity of pancreatic tumours other than nesidioblastosis in adult is such that the experience of any one surgeon or institution is small.

P7 Clinical Characteristics and Classification of Diabetic Patients with Ketosis-onset

Fang FANG[1,2], Pei-li GU[1], Yong-ju ZHAO[1]
[1] Shanghai Institute of Endocrine and Metabolic Diseases, Ruijin Hospital, Shanghai Jiatong University School of Medicine
[2] Department of Endocrinology, Diabetic Laboratory, Shanghai Jiaotong University Affiliated First People's Hospital, Shanghai 200080, China

Objective: To identify a helpful way to classify the ketosis-onset diabetes patients at onset. Help the clinicians evaluate these patients easily and quickly. **Methods:** We performed a retrospective analysis together with prospective follow-up of satisfied patients admitted to the Shanghai Clinical Center for Endocrine and Metabolic Diseases in Ruijin Hospital. Patients diagnosed as diabetes ketosis or ketoacidosis were entered into the analysis, and the duration of hyperglycemic symptoms were restricted within 6 months. The clinical characteristics at onset and after six months' follow-up were registered carefully. According to presence or absence of markers of B-cell autoimmunity (A+ or A-), and quantitative differences in β-cell function (β+ or β-), four groups are identified among the ketosis-onset diabetes: 17 in A+β-, 26 in A-β-, 10 in A+β+ and 46 patients in A-β+. Using statistical analysis, significant differences are found between the 4 groups, with regard to both the clinical characteristics and HOMA2 index at the onset of disease or the prognostics after 6 months following up. **Results:** Patients in A+β- have the earliest onset ages, the highest DKA recurrence rate (29.4%) and the lowest insulin discontinued rate (0%), without any other metabolic dysfunction. Patients in A-β- group are numerously similar to those in A+β-, besides a higher rate of first-degree relatives with diabetes and negative autoimmunity. Patients in A-β+ are described as adult-onset diabetes with apparent metabolic dysfunction. After six months' follow-up, HbA_{1C} decreases most significantly while fasting C-peptide arises most significantly in this group. Patients in A+β+ group are numerously similar to those in A-β+, without positive autoimmunity. **Conclusion:** It is suggested that patients with an initial onset of ketosis or ketoacidosis are diverse. The four groups in this study possibly represent the autoimmune type 1 diabetes (A+β-), idiopathic diabetes (A-β-), latent autoimmune diabetes in adults (A+β+) and type 2 diabetes (A-β+), respectively. Although the classification method in our work is not the best approach, it is a helpful and simple tool for clinician to evaluate and classify diabetes without genotypic analysis.

P8 The Blood Beta-hydroxybutyrate Levels Vary in Different Types of Diabetes Patients

Ming LI, Song-hua WU, Yi SHI, Xiao-jing MA, Wei-ping JIA
Department of Endocrinology and Metabolism, Shanghai Jiaotong University Affiliated No.6 People's Hospital, Shanghai Clinical Center for Diabetes, Shanghai Diabetes Institute, Shanghai 200233, P.R. China

Objective: To improve the clinical acknowledgement and treatment of early stage of keto-metabolism mal-function, this study focuses on discussion of the real existed keto-metabolic mal-functions in different types of diabetes, and its relationship with FPG. **Methods:** 101 cases as control (45 males, 56 females, ages between 55.85±15.86), 42 cases of type 1 diabetic patients (20 males, 25 females, ages between 40.78±17.73), 101 cases of type 2 diabetic patients (48 males, 53 females, ages between 63.92±11.75) and 12 cases of MDM (6 males, 6 females, ages between 42.92±7.69). All patients' fasting blood glucose and blood HBA level were tested. The relationship between fasting blood glucose and blood HBA level has also been studied in TlMD and T2MD. **Results:** The blood HBA level of diabetic groups is remarkably higher than normal group ($p<0.0l$). The HBA level of type 1 diabetic patients is the highest ($p<0.001$), in compare with T2DM ($P<0.0001$). The HBA level of type 2 diabetic patients has no relationship with FPG ($r=0.15$, $p=0.14$), but the HBA level of type 1 diabetic patients was obviously related with FPG ($r=0.68$, $p<0.0001$). In type 1 diabetic group, the HBA level of patients with FPG≥11.1 mmol/l is higher than those with FPG<11.1 mmol/l ($p<0.0001$). **Conclusions:** Both T1DM and T2DM patients have shown abnormity of keto-metabolism with mild elevation of blood glucose level. T1DM patients are more likely to suffer from diabetic ketosis and ketoacidosis with elevated blood glucose level. Blood glucose level should be controlled strictly to prevent keto-acidosis.

P9 Diabetes Ketoacidosis in Atypical Diabetes Mellitus

Grace Pui-sze HUI, Man-wo TSANG
Division of Endocrinology, Department of Medicine and Geriatrics, United Christian Hospital, HKSAR, China

Objective: To compare the clinical and biochemical features of type 1, type 2 and atypical diabetes. **Methods:** We reviewed 119 admissions of patients with discharge diagnosis of Diabetes ketoacidosis (DKA) between January 2004 and April 2006 admitted to the Medical Unit of United Christian Hospital. Patients were verified to have DKA according to the diagnostic criteria of American Diabetic Association (plasma glucose >15 mmol/L, pH<7.3, serum bicarbonate <18 mmol/L and positive urine ketone). Patients were classified as having type 1, type 2 or atypical diabetes based on previous history, present treatment after 3 to 30 months of follow-up or C-peptide result on glucagon stimulation test. **Results:** Of 119 admissions, 91 admissions are confirmed to be DKA. Seventy-one patients were involved, one lady had 7 admissions for DKA within the period of study. Among the 91 admissions, there was 5 mortality (5.5%). The mean age of the patients was 48.5±17.7 years (M 31, F 40). Of the 71 patients admitted for DKA, the age of onset of diabetes was significantly lower in the type 1 group (30.1±16.5 years) when compared with the type 2 (49.8±12.4 years) and the atypical group (48.9±13.0 years) (P<0.001 and P=0.003 respectively). The age of DKA was also lower in the type 1 group (35.1±17.1 years), with P<0.001 when compared with type 2 diabetes (59.7±10.3 years), and P=0.027 when compared with the atypical group (48.9±13.0 years). The age at presentation of DKA was younger in the atypical group when compared with the type 2 diabetes (P=0.009). Duration of diabetes and the body mass index were not significantly different between the 3 groups. Regarding the biochemical characteristics of the three groups. All these groups had similar degree of hyperglycaemia on arrival to the Emergency Department. Serum bicarbonate and pH level in the type 1 group (pH 7.1±0.1, HCO3 6.7±4.2 mmol/L) were lower than in the type 2 (pH 7.2±0.2, HCO3 10.5±4.9 mmol/L) and the atypical group (pH 7.3±0.1, HCO3 10.3±5.0 mmol/L) (P<0.05). The HbA1c in the atypical group (14.1±2.3%) was higher than both type 1 and type 2 diabetes (10.6±3.7% and 12.0±2.6% respectively) (P<0.05). **Conclusion:** DKA occurs in patients with type 1 and also type 2 diabetes. In this study, a third group was identified, these patients were first presented as diabetes with DKA at an older age compared with the group of type 1 diabetes, but comparable with type 2. Their ketosis was less severe compared with type 1 group but comparable with type 2. However, they have higher HbA1c at the time of diagnosis. This group of patient requires further follow-up and monitoring to assess their long-term insulin requirement.

P10 Relationship between the Hepatocyte Nuclear Factor-1α Gene Variants and Diabetes-related Traits in the Chinese

Rong ZHANG, Cong-rong WANG, Qi-chen FANG, Cheng HU, Xiao-jing MA, Wei-ping JIA
Department of Endocrinology and Metabolism, Shanghai Jiaotong University Affiliated No.6 People's Hospital, Shanghai Clinical Center for Diabetes, Shanghai Diabetes Institute, Shanghai 200233, P.R. China

Objectives: In the previous study, we had found that a common haplotype (GCGC, formed by four tagging SNPs, rs1169294, rs1169301, rs3751156, rs2259820) of the hepatocyte nuclear factor-1α gene (HNF-1α) was found to be associated with decreased risk of type 2 diabetes the haplotype decreased the risk for type 2 diabetes in the Chinese subjects. The aim of this study was to analyze the association between nine variants of the HNF-1α gene and diabetes-related traits. **Methods:** In 152 probands from early-onset and/or multiplex type 2 diabetes pedigrees and 93 unrelated subjects with normal glucose tolerance, nine SNPs (rs1169289, re1169288, rs1169294, rs3751l56, rs1169301, rs2259820, rs2464196, rs2464195, rs735396) were identified and genotyped. Statistical analyses were performed to investigate whether these SNPs were associated with diabetes-related traits in our samples. **Results:** In the control group, three SNPs (rs1169294, rs3751156, rs2259820) in the HNF-1α gene were associated with BMI (P=0.0049, 0.0514, 0.0514, respectively). In the haplotype analysis, the common haplotype GCGC carriers had a significantly lower BMI level (P=0.0065). **Conclusion:** The variants of the HNF-1α gene may play a role in the total body fat in the Chinese.

P11 Activating Transcription Factor 6 Ala145Pro Variant is Associated with Glucose and Lipid Metabolism in the Chinese

Cheng HU, Rong ZHANG, Hui WAN, Xiao-jing MA, Cong-rong WANG, Qi-chen FANG, Wei-ping JIA
Department of Endocrinology and Metabolism, Shanghai Jiaotong University Affiliated No.6 People's Hospital, Shanghai Clinical Center for Diabetes, Shanghai Diabetes Institute, Shanghai 200233, P.R. China

Objective: To investigate the relationship between activating transcription factor 6 (ATF6) gene Ala145Pro (GCG>CCG) variant and glucose and lipid metabolism in the Chinese. **Methods:** The genotypes were determined through PCR-RFLP in 689 Chinese in Shanghai. Among them, 361 were subjects with normal glucose regulation, 250 were newly diagnosed diabetic patients without taking any drug and 78 were probands of early-onset type 2 diabetes pedigrees. Phenotypes related to glucose and lipid metabolism were measured, including BMI waist-to-hip ratio, blood pressure, levels of glucose and insulin during OGTT, fasting serum lipid levels, body fat percentage and distribution. **Results:** (1) C allele was the rare allele of this polymorphism. Minor allele frequencies were 0.306, 0.278 and 0.224 in subjects with normal glucose regulation, newly diagnosed type 2 diabetes patients and probands of early-onset type 2 diabetes pedigrees respectively. (2) The frequency of C allele is significantly lower in probands from early-onset type 2 diabetes patients compared with subjects with normal glucose regulation (P=0.0350). (3) In subjects with normal glucose regulation, the C allele carriers had a significantly lower level of high-density lipoprotein cholesterol (P=0.0140). (4) In type 2 diabetic patients, the C allele carriers had a significantly higher level of low-density lipoprotein cholesterol (P=0.0409). (5) After gender and age adjusted, C allele carriers had a significantly lower level of high-density lipoprotein cholesterol (P=0.0274) in the subjects with normal glucose regulation. **Conclusion:** These findings suggested that variant of ATF6 played a role in glucose and lipid metabolism in the Chinese.

P12 The SNPS Analysis of PGC-1A Gene and Its Interaction with MEF2C on the Development of Type 2 Diabetes

Wen-sheng LU, Hua CHENG, Qin HUANG, Li YAN, Chao-gang CHEN, Mu-chao WU, Dan LIU, Fen LI, Yi-qin QI
The Endocrinology of The Second Affiliated Hospital of Sun Yat-sen University, Guangzhou 510120, P.R. China

Objective: This study aimed to screen all exon region of PGC-1α gene for single nucleotide polymorphisms (SNPs) in Han Chinese type 2 diabetic patients and to investigate the relationship between SNPs of PGC-1α gene and the susceptibility of type 2 diabetes mellitus in Han Chinese population and to predict the secondary and tertiary structure of PGC-1α domain, to analyze functional parameter and to probe the impact of gene mutation on structure by a series of bioinformatics softwares. **Methods:** The all exon region of PGC-1α gene was analyzed by means of PCR-SSCP and direct sequencing technologies in 50 Han Chinese type 2 diabetic patients. The identification of gene types was made by PCR-RFLP method. The secondary structure and three-dimensional crystal structure of PGC-1α protein were predicted by a series of bioinformatics softwares. **Results:** There are at least four variations of PGC-1α gene exons in Han Chinese diabetic population that were Thr394Thr (G→A), Gly482Ser (G→A), Thr528Thr (G→A) and Thr612Met (C→T) variations. The frequencies of G and A alleles of Gly482Ser polymorphism of PGC-1α gene were 59% and 40.1% in diabetic group and 70.7% and 29.3% in nondiabetic group respectively (P=0.0002). The Gly482 mutation might increase hydrophilicity and decrease a hydrogen bond which is very critical to the tertiary structure and its function. **Conclusion:** Gly482Ser (G→A) variation might decrease the bonding force between PGC-1α and MEF2C to increase the risk of type 2 diabetes in Han Chinese population.

P13 Association Between Single Nucleotide Polymorphisms in the PGC-1β Gene and Type 2 Diabetes Mellitus and Related Metabolic Disease

Rong LI, Su-hua ZHANG, Li-lin GONG, Wen-yu ZHANG, Xiao-su BAI, Mao-sheng YANG
Department of Endocrinology, The First Affiliated Hospital, Chongqing University of Medical Sciences, Chongqing 400016, China

Objective: To study the association of single nucleotide polymorphisms (SNPs) in peroxisome proliferators-activated receptor γ coactivator-1β (PGC-1β) with T2DM and with related metabolic disease. **Methods:** (1) All of 12 exons in PGC-1β gene, including exon-intron boundaries and promoter region were amplificated, and the PCR products were examined by denaturing high performance liquid chromatography (DHPLC) followed by sequencing to search SNPs, then pairwise linkage disequilibrium (LD) test and haplotype were examined. (2) The missense variants were genotyped in 474 T2DM patients and in 313 controls in Chongqqing area to investigate their genetic association with T2DM and obesity. **Results:** A total of 9 SNPs were identified in PGC-1β gene, including four missense variants (*Ala203Pro, Arg265Gln,Val279Ile, Arg292Ser*) in exon 5, one silent variant (*Leu42Leu*), two SNPs in promoter region (*-1263G>A, -985C>T*) and two SNPs in intron (*IVS2-132 G>A, IVS9 -31G>C*). The four missense variants were in LD and in a haplotype block. There was no association between *Ala203Pro*, *Arg265Gln, rg292Ser* and T2DM (P>0.05). The SNP of *Arg265Gln* showed an increased risk with obesity. In male, subjects with *Arg265Gln* GA/AA genotype had higher BMI, waist and WHR than those with GG genotype (P<0.05). Among four missense SNPs genotyped, three common haplotypes (frequency >0.05) accounted for 95.3% of the observed haplotypes. There were no association between the three haplotypes and T2DM (P>0.05). The frequency of haplotype H3 was higher in obesity group than in controls (16.6% *vs* 12.8%, P=0.039). **Conclusion:** There is no association between PGC-1β SNPs and T2DM in Chongqing area. *Arg265Gln* and a common haplotypes in PGC-1β gene may contribute to the pathogenesis of obesity, especially in male.

P14 Study on the Association of Polymorphism in the TCF7L2 Gene with Risk of Type 2 Diabetes in Chinese Population

Wen-yi LI, Tian-hong LUO, Hong-li ZHANG, Jia XU, Qin ZHANG, Xian-ling ZHANG, Li-hong XU, Yu ZHAO, Guo LI, Min LUO
Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai Clinical Center for Endocrine and Metabolic Diseases, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China

Objective: Recent studies revealed that a common microsatellite (DG10S478) within intron 3 of the transcription factor 7–like 2 *(TCF7L2)* is associated with type 2 diabetes in an Icelandic case-control sample and two additional case-control cohorts in white patients drew the similar conclusion. In this study, we explored the relationship between microsatellite DG10S478 with type 2 diabetes in Chinese subjects. **Methods:** 466 diabetic patients and 450 well-matched healthy controls were genotyped for DG10S478 by ABI3700 sequencer, and the genotype were used along with their phenotype, for association analyses with T2DM. **Results:** 5 alleles of DG10S478 were found in this study population: -8, 0, 4, 8, and 12. The major allele is 0 with a mean frequency of 95.6%. Nonezero allele has a frequency of 3.9% and 4.8% in control individuals and type 2 diabetes patients respectively. 6 different genotypes were found: -8/-8, -8/0, 0/0, 0/4, 0/8, 0/12, with a frequency of 0, 5.8%, 92.2%, 1.3%, 0.7% and 0 respectively in normal subjects, and 0.2%, 6.9%, 90.5%, 1.3%, 0.6% and 0.4% respectively in diabetic patients. No evidence of association of DG10S478 with type 2 diabetes has been found, by X2 test (P=0.315), in our study population. However, when genotypes were compared between normal controls and diabetic patients, the genotype -8/-8 and 0/12 were only found in diabetic subjects, and genotype -8/0 showed a tendency of increased frequency in diabetic patients (6.9% vs 5.8%). **Conclusion:** Our data suggest that the genetic variation D10S478 in the TCF7L2 gene do not have a remarkable effect on type 2 diabetes in Chinese population. But considering the low frequency of the polymorphisms of TCF7L2 in Chinese population, the sample number in this study is still relative small to totally exclude the minor effect of the TCF7L2 on the susceptibility of T2DM in Chinese population. Association studies with larger sample number as well as functional research of the variant will help us on the understanding of the role TCF7L2 played in the pathogenesis of T2DM.

P15 Association of SNPs in the Proximal Promoter Region of the APM1 Gene with Serum Adiponectin Level and Type 2 Diabetes in Shaanxi Chinese

Lan HE, Ping LIU, Feng YE, Jian-ning LI, Chun-ping DONG
Department of Endocrinology, The First Affiliated Hospital of XI'an Jiaotong University, Xi'an 710061, China

Objective: To study the correlation between SNPs -11377C/G and serum adiponectin level in type 2 diabetes. **Methods:** The PCR-RFLP techniques were adopted to test SNPs in the proximal promoter region of the APM1 gene in 101 non-diabetes control group and 203 diabetes group. 203 diabetes patients were underwent 75 g glucose OGTTs with measurement of serum adiponectin, glucose, insulin, lipids. Insulin secretion index and insulin resistance index were caculated. **Results:** Serum adiponectin level in the diabetes group were statistically lower than non-diabetes control group (12.8±6.5 mg/L, 15.0±6.9 mg/L, $P<0.05$). The distribution of APM1 gene polymorphism in non-diabetes group and diabetes group was C/C53 (52.5%), C/G38 (37.6%), G/G10 (9.9%) and C/C107 (54.0%), C/G74 (37.4%), G/G17 (8.6%). There is not statistical significance. In non-diabetes control group serum adiponectin levels of the GG genotype at position -11377 were lower than that of the CC genotype, but the TG levels of the GG genotype was much higher than that of the CC genotype ($P<0.05$). **Conclusions:** SNP-11377C/G modulates adipocyte-secreted adiponectin hormone level and may contribute to the genetic risk for T2DM in Shaanxi Chinese.

P16 The Correlativity Study of Peroxisome Proliferator Activated Receptor γ_2 Gene Polymorphism with Susceptibility of Diabetic Nephropathy in Type 2 Diabetes Mellitus

Yu-zhi YANG, Wei ZHU
Heilongjiang Province Hospital, 150036, China

Objective: To study the relationship between peroxisome proliferator activated receptor γ_2 gene polymorphism and susceptibility of diabetic nephropathy in type 2 diabetes mellitus. **Methods:** Polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) was used to examine the polymorphism of peroxisome proliferator-activated receptor γ_2 gene in 78 patients with type 2 diabetes mellitus (41 cases with diabetic nephropathy [DN group]; 37 cases without diabetic nephropathy [non-DN group]) and 38 controls. The serum TNF-α was measured by ELISA. **Results:** (1) The frequency of Pro allele and Ala allele are 0.957 and 0.043 in Han people of North China. (2) There was no significant difference in the frequency of genotypes (PP, PA, AA) among DN group, non-DN group and control group ($X^2=1.13$, $P>0.05$). (3) There was no significant difference in allele freguency (Pro, Ala) among DN group, non-DN group and control group ($X^2=1.08$, $P>0.05$). (4) Compared with non-DN group patients and controls, serum TNF-α level of DN group patients was significantly higher ($P<0.001$). Serum TNF-α level of diabetic patients of non-DN group was significantly higher than that of controls ($P<0.01$). **Conclusions:** There was no significant association between PPARγ_2 gene polymorphism and susceptibility of diabetic nephropathy in type 2 diabetes mellitus in Han people of North China. But serum TNF-α level of patients with diabetic nephropathy was significantly higher than those of controls and patients without diabetic nephropathy. Serum TNF-α level of diabetic patients was higher than that of controls.

P17 The Relationship Between the Polymorphism in PPAR-γ_2 Gene and Coronary Heart Disease in Type 2 Diabetes Mellitus and Diabetic Nephropathy with the Han Nationality in Baotou

Feng WEI, Jin-jing WANG, Xiao-jing HUO, et al
Department of Endocrinology, The First Affiliated Hospital of Innermongolia Science & Technology University, Baotou 014010, China

Objective: To investigate the relationship between the Pro12Ala polymorphism of PPAR-γ_2 and coronary heart disease (CHD) in T2DM, diabetic nephropathy (DN) in Baotou. **Methods:** We selected 268 subjects with the Han nationality in type 2 diabetes (87 diagonsised as CHD in type 2 diabetes, 82 diagonsised as DN and 99 diagonsised as T2DM without complications). 120 non-diabetes subjects as control. Genomic DNA was extracted from peripheral blood leucocytes of the participants. The Pro12Ala polymorphism was screened by means of polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) in all subjects. **Results:** (1) The frequency of the Pro12Ala mutation is significantly lower in the subjects of T2DM than the controls. X/A genetype is associated with a decreased risk for the development of T2DM. (2) The frequency of the Pro12Ala mutation are significantly higher in T2DM and CHD than in T2DM. X/A genetype is associated with a increased risk for the development of T2DM and CHD. (3) The frequency of the Pro12Ala mutation has no significantly relationship to DN. Compared with the subjects with PP genetype Ua/c are significantly lower than the subjects with X/A genetype in the DN group ($P<0.05$). **Conclusion:** The frequency of the Pro12Ala mutation are independent protect factor for the development of the T2DM. X/A genetype is associated with a decreased risk for the development of T2DM. X/A genetype is associated with an increased risk for the development of T2DM and CHD. And X/A genetype has no relationship to the development of obesity and the development of DN.

P18 Serous Protein Fingerprint Profile and Decision Tree Diagnostic Pattern of Type 2 Diabetic Nephropathy

Ye-hong YANG[1], Shuo ZHANG[1], Jie-feng CUI[2], Bin LU[1], Xue-hong DONG[1], Xiao-yan SONG[1], Ying-kun LIU[2], Xi-xing ZHU[1], Ren-ming HU[1]
[1] Institute of Endocrinology and Diabetology, Department of Endocrinology, Huashan hospital, Fudan University, Shanghai 200040, China
[2] Proteome research section, Liver Cancer Institute, Zhongshan Hospital, Fudan University, 136 Yi Xue Yuan Road, Shanghai 200032, China

Objective: We aimed to use SELDI and bioinformatics to define and validate a DN-specific proteomic pattern of serum. **Methods:** We used SELDI to obtain protein or polypeptide patterns from serum samples of 65 patients with DN and 65 non-DN subjects. From signatures of protein or polypeptide mass, we established a model for diagnosing the presence of DN. We estimated the proportion of correct classifications from the model by applying it to a masked group containing 22 patients with DN, 15 healthy individuals, and 13 diabetic patients with normal urinary albumin. **Results:** The intensities of 22 peaks detected appeared upregulated, while 24 peaks downregulated, in DN group as compared to non-DN groups more than 2 folds ($P<0.01$). The algorithm identified a diagnostic DN pattern of 6 protein/polypeptide masses. On masked assessment, prediction models based on these protein/polypeptides obtained a sensitivity of 90.91% and specificity of 89.29%. **Conclusion:** These observations suggest that DN patients have a unique cluster of molecular components in sera, which are present in their SELDI profile. Identification and characterization of these molecular components help in the understanding of the pathogenesis of DN. Serous protein signature combined with a tree analysis pattern can provide a novel clinical diagnostic approach of DN.

P19 Role of rAAV2/GAD65 in Prevention of Autoimmune Diabetes in NOD Mice

Yan-hui WU, Qiang LI
Department of Endocrinology, The Second Affiliated Hospital of Harbin Medical University, Harbin 150086, China

Objective: To investigate whether treatment with recombinant adeno-associated virus serotype 2 expressing glutamic acid decarboxylase (rAAV2/GAD65) could prevent the development of diabetes in NOD mice. **Methods:** Seven-week-old female NOD mice were injected i.p. with various doses of rAAV2/GAD65 or rAAV or PBS as a control. The blood glucose was measured twice a week. We then examined the incidence of diabetes, insulitis, T-cell proliferative response to GAD, amounts of anti-GAD antibodies and cytokine production. **Results:** (1) Administration of rAAV2/GAD65 to NOD mice prevented diabetes and insulitis in a dose-dependent manner. (2) Splenic T cells from rAAV2/GAD65-treated mice did not proliferate in response to GAD65. (3) The amount of antibodies to GAD was increased. (4) The production of interleukin-4, interleukin-10 and transforming growth factor-β increased, whereas the production of interferon-γ and interleukin-2 decreased in rAAV2/GAD65-treated mice after stimulation with GAD. **Conclusion:** Immunogene therapy using rAAV2/GAD65 results in the prevention of autoimmune diabetes in NOD mice. The therapeutic effect was due to the induction of immunological tolerance through active suppression of effector T cells by inducing a Th2 response and inhibiting the Thl-mediated β cell destruction in NOD mice. This treatment provides a new method and might have therapeutic value for the prevention of type I diabetes.

P21 Experimental Study of the Effects of Adipocytes on Insulin Postreceptor Signal Transduction Pathway in Insulin Target Cells Using a Co-culture System

Xiao-ying DING[1], Yong-de PENG[1], Wei-ping DONG[1], Yu-fei WANG[1], Xiao-jie PAN[1], Shang-quan LIU[2], Ying YANG[2]
[1] Department of Endocrinology, Diabetic Laboratory, Shanghai Jiaotong University Affiliated First People's Hospital, Shanghai 200080 ,China
[2] Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai Jiaotong University Affiliated Ruijin Hospital, Shanghai 200025, China

Objective: A co-culture system of adipocytes and insulin target cells (islet cell, myocyte, hepatocyte) were established to observe the effects of adipocytes factors on insulin target cells ultramicro-structure and insulin post-receptor signal transduction pathway, by which to investigate the cross-talk coupling mechanism between adipose tissue and insulin resistance tissue. **Methods:** 3T3-L1 cells were cultured in normal culture medium and co-culture system where 3T3-L1 cells were differentiated and co-cultured with hepatocyte, myocytes and islet cells. Adipocytes were cultured in Millicell apparatus (4 cm in diameter) seeded into the other bigger culture plates (10 cm in diameter) which contain different insulin target cells at different concentration to glucose and insulin. Ultramicro-structure of islet cell, myocyte, hepatocyte in different media were observed by transmission electronic microscope. Western blot was employed to measure the expression cytoplasmic level and phosphorylations level of IRS-1, IKK and mTOR in hepatocytes, myocytes and islet cells. The insulin levels in culture supernatant were determined by radioimmunoassay. **Results:** The observation of transmission electronic microscope showed that lipid accumulation were observed in the cytoplasm of hepatocytes accompanying partially organelle lost and the impairment of insulin-induced increase in glucose uptake were observed in myocytes accompanying with glycogen granule accumulation as well as mitochondria swelling in the co-culture system. The ability in secreting insulin of islet cell was degraded in adipocyte/islet cell co-culture system. There was an up-regulated serine phosphorylation and decreased tyrosine phosphorylation of IKK, IRS-1 in co-culture system groups. **Conclusion:** Adipocyte a kind of active endocrine cell also inflammatory cell. The abnormality in serum level and function of diverse adipokines may be involved in interfering with the multiple step phosphorylations and the expression of insulin post-receptor signal transduction in insulin target cells.

P22 1α,25-Dihydroxyvitamin D_3 Protects Islet Cells from Apoptosis Induced by Proinflammatory Cytokines

Z. ZHOU, Y. ZHANG, X. LI
Diabetes Center, Central South University, 410011, China

Background and Aims: Epidemiology researches have demonstrated that vitamin D supplement is associated with a decreased risk of type 1 diabetes, and studies on type 1 diabetes animal model—NOD mice have also shown that treatment with 1α,25-Dihydroxyvitamin D_3 ($1,25D_3$) arrests the progression of type 1 diabetes. They found the protective roles of $1,25D_3$ on islet β cells mainly through down-regulation of Th1 cells that was dominated in insulitis. In this study, we will focus on whether $1,25D_3$ have direct influences on β cells, and investigate the protective effects of $1,25D_3$ on islet cells apoptosis induced by proinflammatory cytokines. **Materials and Methods:** The expression of vitamin D receptor (VDR) protein was identified by indirect immunofluorescent and western-blotting assay. An insulitis model in vitro was established by NIT-1 cells treated with IL-β (100 IU/ml)+IFN-γ(500 IU/ml). Four different treatment groups were designed as (IL-β+IFN-γ), ($1,25D_3$+IL-β+IFN-γ), $1,25D_3$ alone and vehicle control. The rates of cells apoptosis were analyzed using Annexin V/PI staining by Flow Cytometry. The enzymatic activity of the caspases-3 class of NIT-1 cells was determined by colorimetric assay. **Results:** (1) VDR protein was present in NIT-1 cells' nuclei. (2) The apoptosis rate of NIT-1 cells treated for 48 h with (IL-1β+IFN-γ) was 25.35%±4.5%, relative (to vehicle) activity of Caspase 3 was 3.21±0.78. In vehicle control, there was 1.74%±0.79%, 1, respectively. Comparing the two parameters between (IL-1β+IFN-γ) group and vehicle control, there revealed a clear increase of apoptosis and activity of caspase 3 in cytokine-induced cells (both $P<0.01$); whereas treatment with physiological concentration of $1,25D_3$ (10^{-8} mol/L) alone, apoptosis rate was 1.4%±0.58%, activity of Caspase 3 was 1.12±0.46. Compared with control group, no significant difference was found (both $P>0.05$); and there was 7.7.%±2.5%, 1.65±0.68 respectively in ($1,25D_3$+IL-1β+IFN-γ) treatment group. Compared to (IL-1β+IFN-γ) group or vehicle control, there was significantly difference between them (both $P<0.05$). **Conclusion:** (1) VDR protein is expressed in NIT-1 cells. (2) Treatment combing IL-1β with IFN-γ can induce cells apoptosis of NIT-1, whereas cotreatment with $1,25D_3$ could protect from apoptosis of NIT-1 cells, implying that $1,25D_3$ may directly counteract insulitis induced by proinflammatory cytokines. The protective effect of $1,25D_3$ is correlated with down-regulation of caspase 3 activity.

P23 Effect of Survivin Overexpression on Cytokine-induced Apoptosis in NIT-cells

Ming-tong XU, Hua CHENG, Feng LI, Sheng-neng XUE, Li YAN
Department of Endocrinology, The Second Affiliated Hospital of Sun Yat-sen University, Guangzhou 510120, China

Objectives: To evaluate the effect of survivin overexpression on cytokine-induced apoptosis in NIT-1 cells and its influence on glucose-stimulated insulin secretion. **Methods:** NIT-1 cells were divided into control group, group transfected with pcDNA 3.0-GFP and group transfected with pcDNA 3.0-GFP-Sur. Each group was divided into non-cytokine intervention sub-groups (C, GFP and SUR group) and cytokine intervention sub-groups (CC, GFP-C and SUR-C group). Apoptotic NIT-1 cells were quantified by Hoechst 33342 stain, TUNEL and hypodiploid nucle analyzed by flow cytometry. Insulin secretion was evaluated in all groups. **Results:** There were more apoptotic cells in cytokine intervention sub-groups compared with that in the non-cytokine intervention sub-groups in the same group ($P<0.01$). Among cytokine intervention groups, the percentage of apoptotic cells in the SUR-C group was lower than that in the CC group (Hoechst 33342: 5.78±0.81% vs 10.69±1.35%, $P<0.01$; TUNEL: 6.31±0.77% vs 16.76±3.02%, $P<0.01$; hypodiploidy: 9.67±2.36% vs 16.56±0.9%, $P<0.01$), and was also lower than that in the GFP-C group ($P<0.01$), whereas was higher than that in the C group ($P<0.01$). In each group, the concentration of insulin was lower in the sub-group with cytokine intervention than that in the sub-group without cytokine intervention ($P<0.01$). In the sub-groups with cytokine intervention, the SUR-C group had a trend to increase insulin secretion compared with the CC group and GFP-C group, but did not reach statistical significance. **Conclusions:** Overexpression of survivin may partly protect the NIT-1 cells from cytokine-induced apoptosis.

P24 Islet-brain 1 Protein Inhibits Cytokine-induced Apoptosis of β Cells

Qi SUN, Luo-lan XIANG, Yu-xiu LI, Heng WANG
Department of Endocrinology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, 100730, China

Objective: To investigate the mechanism of Islet-Brain-1 (IB1) inhibited apoptosis of islet cells induced by cytokines. **Methods:** The total RNA was extracted from human insulinoma. IB1 gene was amplified by PCR from human IB1 cDNA library. The eukaryotic expression vector encoding IB1 was constructed by inserting the IB1 cDNA in the *EcoR I*/*Kpn I* sites of the pEGFP-N1 vector with the green fluorescent. The construct was transfected into RIN 5mF cell line and screened by G418 and obtained the stably transfeced cell line. For induction of apoptosis, IL-1β, TNF-α and IFN-γ was used at a different concentration for 48 h. Apoptosis cells evaluate using Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling (TUNEL). **Results:** The RT-PCR products for IB1 (AA1-280) generated from human insulinoma was 840kb bp long, and sequence analysis showed that it contained the same sequence as the one published in Gen-Bank. Two bands showed that pEGFP-N1 vector encoding IB1 digested by *EcoR I* or *Kpn I*. It showed IB1 gene constructed successfully. After transfected, RIN5mF cells can express IB1 it proved by inverted fluorescence microscope, flow cytometer and Western Blot. The TUNEL method identifies apoptotic cells after cytokine-induced. The results show that IFN-γ or TNF-α alone cannot induce apoptosis of RIN5mF cells. IL-1β, either alone or in combination with IFN-γ or TNF-α resulted in apoptosis of wild RIN5mF, but not transfected cells. **Conclusions:** IB1 protein can inhibit cytokine-induced apoptosis of β cells.

P25 Effects of Pentoxifylline on Apoptosis of β-Cell in NOD Mice

Li-ping GU, Yi-jie WU, Yu-fei WANG, Nian-wei SHI, Wei-ping DONG
Department of Endocrinology, Diabetic Laboratory, Shanghai Jiaotong University Affiliated First People's Hospital, Shanghai 200080, China

Objective: In China, the incidence of type 1 diabetes is about 10%, if LADA is included, of all diabetes. How to prevent type 1 diabetes is focused in most current research. In this study, NOD mice as type 1 diabetes animal models, are administered Pentoxifylline (PTX) before diabetes onset to identify the drugs whether can safely and efficiently intervene type 1 diabetes as well as explore its possible mechanism. **Methods:** Female NOD mice, aged eight-week-old, divided into two groups randomly. All of the mice received cyclophosphamide (CP) at a dose of 200 mg/kg on days 1 and 14. Thirty-two NOD mice were treated with PTX by intraperitoneal injection at a dose of 80 mg/kg three times a day from days 0 to 3 and from days 13 to 16, or two times a day from days 4 to 12 and from days 17 to 26. As control, thirty-three NOD mice were administered normal saline solution with the same volume and same time like Group PTX. Diabetes was defined as the presence of that random glucose lever was equaled or exceeded 16 mmol/L for two consecutive days. The mice sacrificed when diabetes was found or were killed until the end of the experiment. The apoptosis of β-cell was detected by TUNEL, the expressions of caspase-3 in the islet cells were detected by immunohistochemistry and the expressions of Fas, FasL and caspase-8 both in pancreas and spleen were detected by semiquantitative reverse transcriptase (RT)-PCR. **Results:** At last, number of diabetic mice were 23 in control and 13 in group PTX (69.70% vs 40.63%, $P<0.05$). The apoptosis index was much lower in group PTX (4.80±0.60%, $P<0.01$), comparing with group control (9.04±1.02%). The expression of caspase-3 in islet of the mice in PTX group (22.67± 10.07%) were much lower than that of control group (37.33±14.37%). The expression of Fas in pancreas in group PTX was decreased comparing with group control, but there was not any significant difference ($P>0.05$). The expressions of FasL and caspase-8 in pancreas in group PTX were much lower than those in group control (FasL, $P<0.05$, caspase-8, $P<0.01$). We also found in PTX group, the expressions of caspase-8, Fas, FasL mRNA in spleen were much higher than those in group control. **Conclusions:** Our results suggest that PTX would delay or prevent the development of diabetes in NOD mice. The medicine can downregulate the expressions of Fas, FasL, caspase-8 and caspase-3 in pancreas, and then decrease the apoptosis of islet β-cells. Further study needs to focus whether there is a relationship between the upregulation of Fas, FasL, caspase-8 expressions in spleen and protection NOD mice from diabetes.

P26 Effects of Tripterygium Wilfordii Ploylycosidium on Apoptosis of β-cell in NOD Mice

Li-ping GU, Yi-jie WU, Yu-fei WANG, Nian-wei SHI, Wei-ping DONG, Jian LIN
Department of Endocrinology, Diabetes Research laboratory, Shanghai Jiaotong University Affiliated First People's Hospital, China

Objective: Type 1 diabetes is an autoimmune disease involved T-cell mediated destruction of islet β-cells of the pancreas. Apoptosis plays an important role in the decrease of the number of β-cells. In this study, NOD mice as type 1 diabetes animal models, are administered tripterygium wlfordii ploylycosidium (TWP) before diabetes onset to identify the drugs whether can safely and efficiently intervene type 1 diabetes as well as explore its possible mechanism. **Methods:** Female NOD mice, aged eight-week-old, divided into two groups randomly. All of the mice received cyclophosphamide (CP) at a dose of 200 mg/kg on days 1 and 14. Thirty NOD mice were treated with TWP by intraperitoneal injection at a dose of 5 mg/kg two times a day from days 0 to 3 and from days 13 to 16, or one time a day from days 4 to 12 and from days 17 to 26. As control, thirty-three NOD mice were administered normal saline solution with the same volume and same time like Group TWP. Diabetes was defined as the presence of that random glucose lever was equaled or exceeded 16 mmol/L for two consecutive days. The mice sacrificed when diabetes was found or were killed until the end of the experiment. The apoptosis of β-cell was detected by TUNEL, the expressions of caspase-3 in the islet cells were detected by immunohistochemistry and the expressions of Fas, FasL and caspase-8 in pancreas were detected by semiquantitative reverse transcriptase (RT)-PCR. **Results:** The incidence of diabetes in TWP group was 43.33%, which was obviously lower than 69.70% in the control group ($P<0.05$). Compare to the control group (9.04%), the apoptosis index of beta-cell was decreased in TWP group (5.06%, $P<0.01$). The expression of caspase-3 in islet of the mice in TWP group (24.89±10.12%) were much lower than that of control group (37.33± 14.37%), and the expression of caspase-8 mRNA in pancreas in TWP group (0.84±0.11) were also much lower than that of control group (1.22±0.13, $P<0.01$). Expression of Fas in pancreas in group TWP was decreased, compared to control group, but no significant difference could be found ($P>0.05$). The expressions of FasL in pancreas in group TWP (0.80 ±0.11) were also much lower than those in group control (1.33±0.17, $P<0.01$). **Conclusions:** Our results suggest that TWP would delay or prevent the development of diabetes in NOD mice. The medicine can downregulate the expressions of Fas, FasL, caspase-8 and caspase-3 in pancreas, and then decrease the apoptosis of islet β-cells.

P27 The Role and Mechanism of Angiotensin II on Beta Cells

Feng-ying LI, Jing-yan TIAN, Qiang-su GUO, Ming-dao CHEN
Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai Clinical Center of Endocrine and Metabolic Diseases, Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200025, China

Background: There were some evidences that ACE inhibition or Angiotensin II receptor blockade protected from the prevalence of cardiovascular diseases and the development of type 2 diabetes in hypertensive patients. The existence of an intrinsic pancreatic RAS in islets may have pathophysiological roles in secretion, proliferation and apoptosis. The aim of the study is to explore the effect and mechanism of Angiotensin II on beta cells. **Materials and methods:** Western blotting was used to determine AT1 protein expression on beta cells. We measured changes in intracellular calcium by microfluorimetry using Fura 3-loaded beta cells. Insulin secretory responses were determined by RIA. The cell cytotoxicity assay was measured by cell counting kit-8. **Results:** Mouse beta cells expressed AT1. Beta cells exposed to 20-l00 nM Angiotensin II for 8 hours showed an increased basal and glucose-stimulated insulin secretion ($P<0.05$). The cell viability was increased to 1.7 fold. The apoptosis of beta cells was increased and Losartan had the protective effects against Angiotensin II on beta cells by functionally increasing in intracellular calcium at 16.7 mmol/l glucose, but Angiotensin II1 had no effect on it. **Conclusions:** These data demonstrate that the AT1 is expressed on beta cells and that angiotensin II had effects on mouse beta cells to promote its proliferation and insulin secretion, which is inhibited by Losartan, most probably through AT1.

P28 The Role and Mechanism of AT1 on Islets

Jing-yan TIAN, Feng-ying LI, Yan-yun GU, Su-yuan JIANG, Cheng-lin SUN, Lei QIAN, Jin-feng TANG, Hong-li ZHANG, Xiao WANG, Tian-hong LUO, Guo LI, Min LUO
Shanghai Institute of Endocrinology and Metabolism, Shanghai Clinical Center of Endocrine and Metabolic Diseases, Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200025, China

Background: There were some evidences that ACE inhibition or Angiotensin II receptor blockade protected from the prevalence of cardiovascular diseases and the development of type 2 diabetes in hypertensive patients. The existence of an intrinsic pancreatic RAS in islets may have pathophysiological roles in secretion, proliferation, apoptosis and fibrosis. The aim of this study is to explore the effect and mechanism of AT1 on islets. **Materials and methods:** Pancreatic islets were isolated from the pancreata of male Sprague-Dawley rats by the collagenase digestion and by the dextran gradient centrifugation method. Western blotting, real-time polymerase chain reaction (PCR) and immunofluorescence was used to determine AT1 protein expression. The insulin secretion was measured by radioimmunoassay. We measured changes in intracellular calcium by microfluorimetry using Fura 3-loaded islets. The cell cytotoxicity assay was measured by cell counting kit-8. We adopted RNAi aimed to decrease the AT1 express to study the effect and mechanism of Angiotensin II on islets. **Results:** Rats islets expressed AT1. After decreased AT1 express by the ways of RNAi, the secretion of insulin and glucagon of Angiotensin II on islets were different from the control. The cell viability was decreased. Some changes in intracellular calcium by microfluorimetry using Fura 3-loaded islets had happened. **Conclusions:** These data demonstrate that the AT1 is expressed in islets and that Angiotensin II effects on islets to promote proliferation and secretion most probably through AT1. This may be the mechanism of ACE inhibition or Angiotensin II receptor blockade decrease the prevalence of type 2 diabetes.

P29 Telmisartan: a New Perspective Therapeutic Option for Obese Type 2 Diabetes? The Effects of Telmisartan Compared with Pioglitazone on PARγ Activation

Xiao-na YU, Jun HE
Department of Biopharmaceutics, Beijing University of Chinese Medicine, Beijing 100102, China

Objective: To observe the different effects of angiotensin receptor blockers (ARBs) telmisartan and thiazolidinediones (TZDs) pioglitazone on PPARγ activation, to provide a new therapeutic option for metabolic diseases and to initiate the development of new drugs. **Methods:** The C57BL/6J mice were fed a high-fat diet followed by l0 weeks' treatment with telmisartan and pioglitazone. Body weight was determined frequently during treatment. Oral glucose tolerance tests and ITT were performed at the end of the treatment period. Total body fat content (in grams) was measured by nuclear magnetic resonance. **Results:** The fasting plasma glucose and insulin levels in telmisartan group are higher than the vehicle group (separately $P<0.05$, $P<0.01$), and in pioglitazone group are significantly higher than the vehicle group (both $P<0.05$). The body fat content is significantly decreased in telmisartan group ($P<0.01$), whereas significantly higher in pioglitazone group ($P<0.01$). **Conclusion:** Compared with pioglitazone, telmisartan improved moderately insulin sensitivity and glucose tolerance in diet-induced obese mice in the absence of weight gain. This may provide a new therapeutic option for better management in metabolic diseases and may initiate the development of new classes of antidiabetic drugs.

P30 Improving Effects of Renin-angiotensin System Blockade on Pancreatic Islets Function in Type 2 Diabetes Rats via Downregulation of iNOS

Li YUAN, Xin LI, Guo-ling XU, Cui-juan QI, Bei ZHANG, Min ZHOU
Department of Endocrinology, Union Hospital, Tongji Medical College, Huazhong University of Science & Technology, Wuhan 430022, China

Objectives: To study the impact of perindopril and valsartan on the morphology and function of pancreatic islet cell in type 2 diabetes rats, and to investigate the protective mechanisms of the renin-angiotensin system blockade on the pancreatic islet cells in type 2 diabetes rats. **Methods:** Type 2 diabetes rats models were created by high fat high caloric laboratory chow plus intraperitoneal injection of a small dose of streptozotocin. Rats were divided into normal group (n=10), diabetic group (n=8), perindopril treated group (n=10) and valsartan treated group (n=10). The last two groups were given perindopril or valsartan treatment for eight weeks, respectively. To measure the islet function by intraveneous glucose tolerance test, islet morphology and intraislet insulin expression by immunohistochemistry, expression of transforming growth factors-β1 (TGF-β1) as a symbol of severity of fibrosis, expression of induced nitric oxide synthase (iNOS) as a symbol of oxidation stress. To detect the apoptosis of islet β-cell by TUNEL, angiotensin (AGT) and proinsunlin mRNA by RT-PCR. **Results:** Compared to normal group, pancreatic islet function in diabetes group was lost, and first-phase insulin secretion, expressed as the incremental area under the insulin curve in the first 10 min, was reduced by 67% ($P<0.01$). The intraislet β-cell relative volume was decreased by 68% ($P<0.01$), and β-cell nuclear density was decreased by 48% ($P<0.01$). Insulin relative concentration dropped obviously. The apoptosis of islet cell and the expression of local iNOS were increased. TGF-β1 relative concentration was increased significantly, and the relative volume of interstitial cell expressing TGF-β1 was enhanced by 1.52 fold. Local AGTmRNA was increased, and proinsulin mRNA was decreased by 31% ($P<0.01$). Compared to diabetes group, first-phase insulin secretions were increased by 41% ($P<0.01$) and 33% ($P<0.01$), respectively, and the intraislet β-cell relative volume was increased by 84% ($P<0.01$) and 78% ($P<0.05$) in perindopril treated group and valsartan treated group. After perindopril treated and valsartan treated, Islet-cell apoptosis per islet area and the intraislet iNOS expression was decreased significantly. The relative volume of interstitial cell expressing TGF-β1 was decreased by 44% ($P<0.01$) and 36% ($P<0.01$), respectively. Local AGT mRNA was decreased, and proinsulin mRNA was increased by 23% ($P<0.01$) and 22% ($P<0.01$), respectively. **Conclusions:** The activation of pancreatic local RAS played an important role in the development of type 2 diabetes mellitus through promoting apoptosis of islet cells and fibrosis via increasing oxidation stress. The renin-angiotensin system blockade could improve the islet morphology and function in type 2 diabetes rats, and possible mechanism was decreasing damage of oxidation stress on islet via downregulation of local iNOS.

P31 Reversal of Streptozotocin-induced Diabetic Rat Using Islet-like Cells Generated In Vitro from Bone Marrow Mesenchymal Stem Cells

Xiao-hong WU, Cui-ping LIU, Kuan-feng XU, Xiao-dong MAO, Jian ZHU, Jing-jing JIANG, Dai CUI, Mei ZHANG, Yu XU, Chao LIU
Department of Endocrinology, First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China

Objective: To observe the effect of islet-like cells generated in vitro from bone marrow mesenchymal stem cells (BM-MSCs) in the treatment of streptozotocin-induced diabetic rat. **Methods:** BM-MSCs were isolated from SD rats and induced to differentiate into islet-like cells under defined conditions. Differentiation was evaluated using electron microscopy, RT-PCR, immunofluorescence and flow cytometry. Insulin release after glucose challenge was tested with ELISA. Then allogeneic islet-like cells were transplanted into diabetic rat via portal vein. Blood glucose levels were monitored and islet hormones were detected in the liver and pancreas of recipients by immunohistochemistry. **Results:** BM-MSCs were spheroid adherent monolayers with high CD90, CD29 and very low CD45 expression. Typical islet-like cells clusters were formed after induction. Electron microscopy revealed that secretory granules densely packed within the cytoplasm of the differentiated cells. The spheroid cells expressed islet related genes and hormones. The insulin-positive cells accounted for 19.8% and mean fluorescence intensity increased by 2.6 folds after induction. The cells could secret a small amount of insulin that was increased by 1.5 folds after glucose challenge. After transplantation, islet-like cells could locate in the liver expressing islet hormones and lower the glucose levels of diabetic rats during day 6 to day 20. **Conclusion:** Islet-like cells differentiated from BM-MSCs in vitro could alleviate the hyperglycemia of diabetic rats.

P32 Inducing ICCS from CK7 Marked Mouse Pancreatic Ductal Epithelia Cells

Jian YANG, Yan-yun GU, Di ZHANG, Wen-zhong ZHOU, Feng-ying LI, Wei-bin ZHOU, Tian-hong LUO, Guo LI, Min LUO
Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai Clinical Center of Endocrine and Metabolic Diseases, Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200025, China

Background: Since Edmenton method was first used in clinic iselt tansplantation, it has been believed to be a physical and ideal remedy for type 1 or some late type 2 diabetes. While the lack of human islet resources and immunosuppression treatment after transplantation have become the problems which impede the application of this method. Alternative islet sources have been the heated research target during this years. Pancreatic ductal epithelial cell seems to be a more reliable and available resources. **Objectives:** Using laser microdissection to purify pancreatic ductal epithelial or classic mixture culture to induce functional ICSS. ICSS are found in the second day on inducing. **Methods:** Using FGF-7, EGF, nicotine and BSA to induce ICSS in vitro. Immnunochemistry, realtime PCR, and RIA insulin detection are applied to compare the expression level of insulin, glucagon, PDX-1, NK2.2, NK6.1 in ICSS. T test, Anova is applied in statistic issues. **Results:** Marker of ductal epithelial CK7 was found co-expression with PDX-1 either in vivo or in vitro. Both methods could induce ICSS. Insulin expression level increased along with the elongation of inducement ($p<0.05$) with either method, but secretion function did not develope to ideal degree. Further study testified that the secreting related gene such as NFAT or MAP2 was still in low expression level in ICCS. **Conclusion:** Mouse pancreatic ductal epithelial has the potential to induce ICCS, though the ICCS induced by them could express considerable insulin and glucagon, they are short of secretion function to let hormone out. It seemed that endocrine secretion mechanism will be another focus for studies on pancreatic islet in vitro induction. There is a long but hopeful way from ICCS to Islet.

P33 Experimental Study on Isolation, Culture and Differentiation of Human Pancreatic Ductal Endothelial Cell In Vitro

Yu-fei WANG, Wei-ping DONG, Jian-bo WANG, Yong-de PENG
Department of Endocrinology, Diabetes Research laboratory, Shanghai Jiaotong University Affiliated First People's Hospital, Shanghai 200080, China

Objective: To explore the method of isolation, identification and in vitro differentiation of human pancreatic ductal endothelial cell. **Methods:** Islets were isolated using digestion by semi-automatic method. The duct were picked up for digestion by using 0.25% trypsin and 2 mg/ml dispase, the digested cells were cultured with 0.5% serum DME medium (bFGF, EGF, HGF and NAA). The expressions of nestin and CK-19 were tested by immunocytochemistry, while the gene expressions of insulin, glucagon and PDX-1 were tested by RT-PCR methods. **Resu1ts:** The nestin and the CK-19 positive cells can be found within 12 wks. After adding bFGF, EGF, using HGF and NAA, the cells proliferated fast and formed new islet-like cell clusters (ICCs). RT-PCR showed that there is a high level of expression on insulin, glucagon and PDX-1. **Conclusion:** The nestin and the CK-19 positive cells can be isolated from human pancreatic ductal endothelial cell and can be differentiated in islet-like cell clusters.

P34 The Functional Maturation of Insulin Secretion during Pancreatic Development in Rat

Chao LIU, Qing-xin YUAN, Li-ping TENG, Wei DE, Cui-ping LIU, Kuan-feng XU, Xiao-dong MAO
Department of Endocrinology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China

Objective: To define key genes relevant to insulin synthesis and exocytosis and investigate the improved process of insulin secretion. **Methods:** Pancreata of SD rat at embryonic day 12.5 (E12.5), E15.5, E18.5 and newborn, 21 days after birth, adult rats were dissected respectively. Genechips from Affymetrix company were applied to explore gene expression profiles. Some genes related to insulin synthesis and secretion were verified by realtime PCR. Intraperitoneal glucose tolerance tests (IPGTT) and ELISA was done to detect the function of pancreatic islet. **Results:** Among genes related to insulin synthesis and release, insulin and relevant transcription factors such as PDX-1 began to be expressed from E12.5; Munc13-l, syntaxin-1a, Rab3a, Vamp-2, Glut-2 began to be expressed from E15.5, while GCK was expressed at late stage of embryonic day. The result of IPGTT revealed that newborn rats before a week after birth were significantly glucose intolerant by showing an increased excursion in blood glucose in response to a glucose challenge. Basic blood insulin level was detected at E18.5 and increased rapidly thereafter. However, in response to a glucose load, only rats older than a week after birth began to show normal insulin secretory response gradually. **Conclusion:** Potential ability of insulin synthesis and release in rat occurs from early and middle stage of pancreatic development. But the normal insulin secretion response to glucose challenge starts later.

P35 Isolation and Purification of Islets of Langerhans from Human Adult Pancreas

Yu-fei WANG, Wei-ping DONG, Jian-bo WANG, Yong-de PENG
Department of Endocrinology, Diabetes Research laboratory, Shanghai Jiaotong University Affiliated First People's Hospital, Shanghai 200080 ,China

Objective: To establish a method for isolation and purification of Langerhans islets from human adult pancreas. **Methods:** 30 patients of PTDM during January 2000 and July 2006 from both outpatient and inpatient department of our hospital were retrospectively reviewed. General condition, blood glucose, glycosylated hemoglobin (HbA_{1C}), blood concentration of cyclosporine (CSA), islet function and islet antibodies were detected and compared with those of type 2 diabetes mellitus (T2DM) patients of the same DM duration. **Results:** 12 pancreas were isolated, for each pancreas, yield was 282,000±149,000 islet equivalents (IEQ), corresponding 1295±515 IEQ for per gram of pancreas, purity of islet was 89.3%±4.3% and the viability of islet was 96.2%±2.4% Insulin release for low glucose and high glucose without cultured and cultured for 24 hours were 31.4±2.9 vs 81.5±9.4 and 24.2±4.5 vs 41.9±4.5 respectively. The stimulate index (SI) were 2.61±0.27 and 1.82±0.46 respectively. **Conclusion:** The purified and active islet was obtained via this method.

P36 Adjustment of the Cultured Period Improves Islet Transplantation Outcome

Mei ZHANG, Chao LIU, Shu-hang XU, Cui-ping LIU
Department of Endocrinology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China

Objective: To evaluate the effect of transplantation of islets that had undergone different periods of in *vitro* culture. **Methods:** Islets were digested by pancreatic ductal injection of collagenase and purified by Histopaque 1077 density centrifugation method. Then, the islets were cultured in RPMI 1640 with L-glutamine, 1% HEPES, 10% fetal calf serum, at 37°C in a humidified atmosphere of 95% air, 5% CO2 for different period. Glucose stimulated insulin secretion test was performed to evaluate the function of isolated islets in vitro. Cultured islet with different time periods was allogeneically transplanted under the kidney capsule of streptozotocin-induced diabetic BALB/cByJ mice to evaluate the function of isolated islets in vivo. **Results:** Mice showed an islet yield of 166.5±23.47 *per* pancreas. Islet viabilities were more than 95%. Glucose stimulated insulin secretion test in isolated islets after cultured different period were good. There was no difference between the two groups that cultured for 2-4 h and 12-24 h in glucose stimulated insulin secretion. Diabetic mice after transplantation of cultured 2-4 h islets survived for 6.25±0.96 days. However, the survival of cultured 12-24 h islets were 10.6±1.82 days. The cultured 12-24 h islets also showed better blood glucose levels during 1-3 day after islet transplantation than that cultured for 2-4 h. **Conclusions:** These results demonstrate that transplantation of cultured islet allografts can promote the success of islet transplantation to reverse diabetes in the mouse model.

P37 The Relationship between Diabetes Mellitus and Pancreatic Cancer

Yu-fan WANG, Li ZHAO, Ming-yu GU, Li-li YAO, Shuai YAN, Yong-de PENG
Department of Endocrinology, Diabetic Laboratory, Shanghai Jiao Tong University Affiliated First People's Hospital, Shanghai 200080, China

Objective: In the early nineteenth century, the relationship between diabetes mellitus (DM) and pancreatic cancer (PC) was recognized, but till now it still remains a matter of controversy whether DM is the cause or the consequence of PC. The study was to explore the relationship between DM and PC, and improve the early diagnosis of PC. **Methods:** The 83 cases of PC with and without DM admitted to our hospital during Dec, 2003-Feb, 2005 were retrospectively reviewed. Among the diabetics, 13 patients of PC who were newly diagnosed DM or deterioration of DM were compared with another 20 type 2 diabetics (T2DM) in age, BMI, diabetic family history and lab data, with matched DM duration between two groups. **Results:** (1) Among 83 patients with PC, 35 (42.17%) accompanied by DM. DM duration was less than 2 years in 24 (68.57%) patients, with more than 2 years in 8 (22.86%) patients and with unknown DM duration in 3 patients. DM and PC were diagnosed simultaneously in 14 (40%) patients. The hypertention prevalence was higher in DM group than in non-DM group (45.71% vs 25%, $P<0.05$). (2) The prevalence of abdominal pain ($\chi^2=5.332$, $P<0.01$) and bad appetite ($\chi^2=3.867$, $P<0.01$) was lower in DM group. (3) And there was less elevated direct bilirubin (DB) ($\chi^2=23.46$, $P<0.01$) in DM group. (4) There was no significant difference in tumor location, metastasis, operation and life span between two groups. (5) 13 cases of PC with DM were compared with 20 cases of T2DM. The patients of PC with DM were older (68.93±10.62 vs 55.6±11.14 y, $P<0.01$) and leaner (BMI 21.51±1.98 vs 23.87±3.04 kg/m^2, $P<0.05$). **Conclusion:** Clinically, PC is a progressive cancer. DM occurs frequently in patients with PC and does not influence its clinical feature and prognosis. PC should be screened in recent-onset and deteriorative diabetics with advanced age and remarkable weight loss.

P38 Expression of munc13-l in Rats During Normal and Intrauterine Growth Retardation Pancreatic Development and Its Effect on Insulin Secretion

Qing-xin YUAN, Chao LIU, Li-ping TENG, Wei DE, Cui-ping LIU, Kuan-feng XU, Xiao-dong MAO
Department of Endocrinology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China

Objective: To investigate the expression of munc13-l in rats during normal and intrauterine growth retardation (IUGR) pancreatic development and determine its effect on insulin secretion. **Methods:** Pancreata of rat E12.5, E15.5, E18.5, new-born, 21 after birth and adult rats were dissected under microscope. The rat models of IUGR were made by 50% calorie restriction in pregnant rats from gestational day 15 until term. After extraction of total RNA and protein, the techniques of RT-PCR, real-time PCR and Western blot were used to study the expression of munc13-l and insulin secretion. Immunohistochemistry and immunofluorescence were used to define the location of munc13-l. Blood insulin level was detected in normal and IUGR rats. **Resu1ts:** Munc13-l was located at islet along with insulin. The genes of insulin and munc13-l began to be expressed from E12.5 and E15.5 respectively and their expressions were increased as the fetus grew. The result of Western blot showed that the expression of munc13-l was low at E15.5 and E18.5 and increased later. This was coincident with the higher insulin level after E18.5. The blood insulin level and the expression of munc13-l were reduced simultaneously in IUGR models compared with normal newborn rats. **Conclusions:** Munc13-1 plays an essential role in insulin exocytosis.

P39 宫内发育迟缓大鼠胰岛素释放功能的研究

刘超，袁庆新，刘翠萍，徐宽枫，茅晓东
南京医科大学第一附属医院内分泌科，210029

目的：胚胎胰腺发育和成年糖尿病有着密切的联系。宫内发育迟缓（IUGR）是孕期常见的并发症，也是围产期及日后多种疾病尤其是糖耐量异常和成年糖尿病的重要危险因素。本研究观察宫内发育迟缓新生大鼠血糖、血胰岛素释放功能的改变，并探讨造成这种异常的机制。**方法：**SD孕鼠被随机分为两组：正常对照组和宫内营养不良组。自孕15天开始给予宫内营养不良组孕鼠50%卡路里限制直至新生鼠出生，造成宫内发育迟缓动物模型。宫内发育迟缓新生大鼠出生后，部分给予正常哺乳，部分继续给与半量饮食，观察其体重和胰腺重量的变化。分离提取正常对照组和宫内发育迟缓组新生大鼠的胰腺组织，提取上述胰腺组织的总RNA及蛋白质，运用RT-PCR技术检测胰岛素合成和胞吐相关基因的表达，Western-blot、免疫组化等技术确定相关蛋白的表达情况。鼻尖采血收集新生1天、3天、7天、10天、12天鼠血，测定血糖水平，颈静脉采血以ELISA法测定血胰岛素的浓度，腹腔葡萄糖耐量试验（IPGTT）确定胰岛功能。**结果：**宫内发育迟缓新生大鼠出生时体重和胰腺重量比对照组明显减轻，给与正常饮食后，体重和胰腺重量增加，接近正常对照组；虽然两组新生大鼠胰岛素基因和Pdx-1基因的表达无明显变化，但宫内发育迟缓组新生鼠胰岛素胞吐相关基因munc13-1, syntaxin1a等的表达显著减少。与正常新生鼠相比，宫内发育迟缓组新生鼠基础胰岛素水平较低，葡萄糖负荷后胰岛素释放反应亦明显减弱，但出生后给与正常饮食后，减弱的葡萄糖耐量能逐渐恢复。**结论：**宫内发育迟缓可导致大鼠胰岛功能完善过程的异常，新生大鼠胰岛功能减低，血胰岛素和葡萄糖水平改变。造成这种异常可能主要是由于胰岛素释放(胞吐)功能减弱所致；给与正常饮食后，这种改变能逐渐恢复。

P40 Isolation and Characterization of Adipose-derived Mesenchymal Stem Cells in Rats

Hong QIAO, Qiang LI, Jin-chao ZHANG, Yu-qian SUN
Department of Endocrinology, the Second Affiliated Hospital of Harbin Medical University, Harbin 150086, China

Objective: Adipose tissue presents a source of multipotent mesenchymal stem cells. They are abundant, readily accessible and proliferate fast. They are able to differentiate into different cell types. But the stroma-vascular fraction (SVF) of adipose tissue has recently been described to be composed of endothelial cells, preadipocytes and vessel smooth muscularis cells. In this study, we have detected the isolation approach and the phenotype of the adipose tissue-derived mesenchymal stem cells: CD105, CD31, Vimentin and α-SMA. **Methods:** *Cell isolation methodology:* from Wistar rat epididymal fat pad, miced the tissue into small fragments, then digested with type I collagenase for 1 hour at 37°C. The dispersed tissue was filtered through an 80 um nylon mesh and centrifuged for 5 min at 800 rpm/m. The stromal vascular fraction could be separated by centrifugal force from the floating primary adipocytes, and cultured them in DMEM with 10% fetal bovine serum. Passage 3 or 4 is to be used. *Immunofluorescence staining:* First, cultured cells were fixed with paraformaldehyde. Second, the cells were incubated at the appropriate Ab1, then stainned with the FITC or PE-labelled antibody. Samples were incubated at 37°C for 60 min and washed twice in PBS. The labeled samples were analyzed with a FACScan. **Results:** The cells stainned with the FITC-labelled anti-CD105 or anti-Vimentin antibody were all green fluorescence; the cells stainned with the TRITC-labelled anti-CD31 or SMA antibody were not red fluorescence. **Conclusion:** This isolation approach is easy and possible; the cells express CD105 and Vimentin, but don't express CD31 and SMA.

P41 Detection and Study for the New Protein My027 of Human Adipocytes

Xiao-hua LI[1,2], Sheng ZHENG[1], You-ping LIU[1], Hua-feng LI[1], Tian-hong LUO[1], Guo LI[1], Min LUO[1]
[1] Shanghai Institute of Endocrine and Metabolic Disease, Department of Endocrinology and Metabolism Disease, Shanghai Jiaotong University affiliated Ruijin Hospital
[2] Department of Endocrinology, Diabetic Laboratory, Shanghai Jiaotong University Affiliated First People's Hospital, Shanghai 200080, China

Objective: To identify novel proteins by proteomic study, and study their structure and function. **Methods:** The expressed proteins of human adipocytes were identified by 2-D and mass spectrometry. The new protein identified was firstly confirmed by RT-PCR, DNA sequencing, and prokaryotic expression. Then its structure and function were predicated via bioinformatics. And the activity of the fusion protein GST-My027 was also assayed in vitro. **Results:** My027 is a novel protein of the human adipocytes identified by 2-D and mass spectrometry. It was confirmed by RT-PCR, sequencing, and prokaryotic expression. And the expression of My027 mRNA was identified in many tissues, meaning it may be an important functional protein. It was suggested via bioinformatics that My027 protein might be located in cytoplasm, had highly conversed structure, and shared 95.7% similarity with glyoxalase I, which imply it might be highly homologous to glyoxalase superfamily. The data of the activity analysis in vitro also showed that protein My027 could catalyse the methyglyoxal into S-lactoglutathione, just the same as the glyoxalase I. **Conclusion:** A novel protein My027 was identified and confirmed; and it might have the same function as the glyoxalase I. The work we have done made it possible for further studying the function of My027.

P42 Study on Differentially Expressed Genes Associated with Signal Transduction in Adipose Tissues of Insulin Resistant Type 2 Diabetes Mellitus by cDNA Microarray Analysis

Jiao-yang ZHENG, Bin LU, Zhim LIU
Department of Endocrinology, Changzheng Hospital, Second Military Medical University, Shanghai 200003, China

Objective: To elucidate the mechanism of IR of T2DM and provide a new therapeutic method, the gene expression profiles associated with signal transduction in IR were compared with those of normal omental adipose tissue utilizing cDNA microarray analysis. **Methods:** Both mRNA from IR and the normal omental adipose tissue were reversely transcribed to cDNAs with the incorporation of fluorescent dUTP (cy25 or cy23) for preparation of hybridization probes. The mixed probes were hybridized to cDNA microarray, which was scanned for the fluorescent signals and showed differences between the two adipose tissues. **Results:** We identified 82 differently expressed genes between the two tissues, 19 differently expressed genes were associated with signal transduction. In IR 12 genes were upregulated while 7 were downregulated. **Conclusion:** The genes with altered expression and involved in cell proliferation, immune response and metabolism etc, and are correlated with signal transduction. The cDNA microarray provides a powerful approach to elucidate the mechanism of signal transduction of IR.

P43 Study of Molecular Mechanism of T2DM Caused by ADRP Gene Mutation

Tian-hong LUO, Yu ZHAO, Hong-li ZHANG, Wen-yi LI, Jia XU, Li-hong XU, Qin ZHANG, Xian-ling ZHANG, Guo LI, Min LUO
Medical Faculty of Shanghai Jiaotong University, Ruijin Hospital, Shanghai Research Institute of Endocrine and Metabolic Diseases, Shanghai Clinical Center for Endocrine and Metabolic Diseases, 200025, China

Objective: We have previously reported that D9S171 and D9S175 microsatellite are linked with T2DM in East Chinese population, and a further polymorphism and mutation screening of genes near D9S171 showed that a missense mutation in exon 8 of adipocyte differentiation related protein (ADRP) gene (Thr394-Ala) cosegregated with a T2DM pedigree, indicating a possible relationship of ADRP gene with T2DM. In this study, we used an expression vector to study the effect of the mutation on cellular lipid droplet formation. **Methods:** Full length ADRP cDNA was amplified by RT-PCR using a pair of ADRP specific primer and total RNA isolated from mouse adipose tissue. A a/g mutation was then inserted into position 1258 of ADRP mRNA by site specific point mutation technique. Wild-type and mutated ADRP cDNA were then inserted into the right place of the expression vector pEGFPC3, leading to the expression of wild-type and mutated ADRP-GFP fusion protein. The vector was then transfected to the NIH3T3 cell with lipofectamine 2000, and the distribution of the fusion protein as well as the formation of small lipid droplet was examined under fluorescence microscope. **Results:** The sequence of full length mouse ADRP cDNA, amplified by RT-PCR, as well as that of the mutated ADRP cDNA (at 1258 a/g, was confirmed by direct sequencing. The cDNA was then inserted into pEGFPC3 vector, and the vector carrying wild-type and mutated ADRP gene was transfected into the NIH3T3 cell respectively. 24 after transfection, fluorescence of NIH3T3 cell was examined under fluorescence microscope, which showed a diffuse distribution, inconsistent with the distribution of small lipid droplet, for mutated ADRP-GFP fusion protein compared to the point-like distribution, consistent with the distribution of small lipid droplet, for wild-type ADRP-GFP fusion protein. After coloration with Oil-red, mean number of small lipid droplet in mutated ADRP transfected cells was 36% less than that in wild-type ADRP transfected cells. **Conclusion:** Thr394-Ala mutation caused a functional deficiency of ADRP gene, leading to the difficulty of ADRP protein locating to the surface of small lipid droplet, which results in the defect of lipid droplet formation as well as the cellular lipid metabolism. But the relationship of the defect with pathogenesis of T2DM still needs to be elucidated by in vivo study.

P44 Adipose Tissue Specific CETP Expression in Mice: Impact on Glucose Metabolism and Adipocytes

Hong-wen ZHOU[1,2], David JANG[1], Chien-ping LIANG[3], Chao LIU[2], Xian-cheng JIANG[1]
[1] Department of Anatomy and Cell Biology, SUNY Downstate Medical Center, 450 Clarkson Ave. Box 5, Brooklyn, NY 11203, USA
[2] Department of Endocrinology, the first affiliated hospital of Nanjing Medical University, 300 Guangzhou Road, Nanjing 210029, China
[3] Division of Molecular Medicine, Department of Medicine, Columbia University, New York, NY 10032, USA

Objective: To investigate the impact of adipose CETP (Cholesteryl ester transfer protein) expression on glucose metabolism and adipocyte function, we established adipose tissue specific CETP transgenic (CETPTg) mice, using aP2 promoter. **Methods:** We constructed aP2-CETP transgenic gene and established CETPTg mice. Northern Blot Analysis was used for the CETP mRNA tissue distribution investigation. Lipid extraction and assays were done using epididymal fat pads and adipocyte size was investigated, insulin levels were determined using an enzyme-linked immunosorbent assay kit, glucose and insulin tolerance tests were performed among 14-week-old male littermates. **Results:** CETP mRNA is predominantly expressed in adipose tissue in transgenic mice. CETPTg adipocytes are smaller with poor lipid than those in WT mice. We found that aP2-CETPTg mice had higher fasting glucose levels (98±10 vs 75±11, $p<0.05$) and showed glucose intolerance compared to WT mice. However, insulin tolerance tests revealed that the insulin sensitivity of aP2-CETPTg mice was increased. We then measured fasting and 30 min glucose-induced insulin levels. We found that aP2-CETPTg mice had significantly lower insulin concentration than that of WT ones. All these results indicated a defect of insulin secretion in aP2-CETPTg mice. **Conclusion:** We established adipose tissue specific CETP transgenic mice. Adipose tissue CETP can be secreted into the circulation and makes a contribution to plasma lipoprotein metabolism. Thus, CETP joins the growing list of molecules secreted by adipose tissue which have more widespread systemic effects. Surprisingly, CETP expression in adipose affected lipid contents and size of adipocytes, and the CETP transgenic mice have impaired glucose intolerance but with higher insulin sensitivity. These changes could be associated with reduced insulin secretion.

P45 Effects of Berberine on the Proliferation and Differentiation and Adipocytokine Expression in Human Preadipocytes

Jing YANG, Jin-hua YIN
Department of Endocrinology, The First Hospital of Shanxi Medical University, 030001, China

Objective: To characterize the effects of berberine on the proliferation and differentiation and adipocytokine expression in human preadipocytes. **Methods:** Human omental preadipocytes were cultured. Its proliferation and differentiation were detected by MTT and oil red O staining methods after adding different concentrations of berbering in the culture. The mRNA expression level of peroxisome proliferation activated receptorγ2 (PPARγ2), the key protein on controlling preadipocytes differentiation, was detected by reverse transcriptase polymerase chain reaction (RT-PCR). The adiponectin and leptin mRNA and protein were detected by RT-PCR and enzyme-linked immunosorbent assay (ELISA). **Results:** MTT staining showed an absorbance of berbering group with different concentrations (0.1 μmol/l, 1 μmol/l, 10 μmol/l), significantly higher than that of control group ($P<0.05$). During the differentiation period of preadipocytes, there were less and smaller lipid droplets in the adipocytes treated with berberine of different concentrations (1 μmol/l, 10 μmol/l) as compared with the untreated control cells. The absorbance of berbering group was also significantly lower than that of control group in oil red O staining ($P<0.05$). RT-PCR showed that berberine inhibited the expression of PPARγ2, leptin and adiponectin mRNA. ELISA showed that berberine of the concentration of 10 μmol/l significantly reduced the secretion of leptin and adiponectin both of differentiating and differentiated preadipocytes ($P<0.05$). **Conclusion:** Berberine promotes the proliferation of human preadipocytes. It inhibits differentiation of the cells and the expression of leptin and adiponectin mRNA and protein, which may be associated with its effect on decreasing the expression of PPARγ2 Mrna.

P46 Molecular Mechanism of Improved Insulin Resistance by Glucagon-like Peptide-1 in 3T3-L1 Adipocytes

Hong G, Yi-sheng Y, Jun Y, Zhi-guo Z, Xin-jun W, Xiao-ying L, Guang N
Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrine and Metabolic Diseases, Department of Endocrinology and Metabolism, Ruijin Hospital, Medical School of Shanghai Jiaotong University, China

Objective: To explore the effect of GLP-1 on glucose transport and mechanism of improved TNF alpha-induced insulin resistance by glucagon-like peptide-1 in 3T3-L1 adipocytes. **Materials and methods:** 3T3-L1 fibroblasts were grown and differentiated in culture plates. Cells were incubated in serum-free DMEM containing 0.2% BSA overnight then incubated in indicated concentrations of insulin, GLP-1 and TNF alpha prior to performing the glucose uptake assay. The protein expression and phosphorylation of Akt, GSK-3β were detected by western blot. The translocation and expression of GLUT-4 were detected by subcellular fractionation. **Results:** (1) GLP-1 increased glucose transport in basal and after insulin stimulation by activating PI-3 kinase. (2) Glucose transport, Akt and GSK3β activation were induced by insulin and were inhibited by TNF alpha in both expression and activation. GLUT-4 expression and translocation are also decreased by TNF alpha. Treatment of the cells 30 minutes with GLP-1 reverted the inhibition in phosphorylations and translocation partly, but have no effect on expression of kinases. 24 hours treatment of the cells with GLP-1 reverted the decrease of protein expression by TNF alpha. **Conclusions:** GLP-1 can protect 3T3-L1 adipocytes from insulin resistance induced by TNF alpha by increasing expression and phosphorylation of Akt, GSK-3β and GLUT-4 translocation.

P48 Ginsenoside Rb1 Promotes Adipogenesis and Inhibits Lipolysis in 3T3-L1 Adipocytes

Wen-bin SHANG, Ying YANG, Bo-ren JIANG, Hua JIN, Li-bin ZHOU, Shang-quan LIU, Ming-dao CHEN
Shanghai Institute of Endocrine and Metabolic Diseases, Ruijin Hospital, School of Medicine, Shanghai Jiaotong University, 197 Ruijin Road II, Shanghai 200025, China

Objective: Evidence has been accumulated that ginseng and its main active constituents, gensenosides, possess antidiabetic and insulin-sensitizing property which may be partly realized by regulating adipocyte development and functions. In the present study, we explored the effect of ginsenoside Rb1, the most abundant ginsenoside in ginseng root, on adipogenesis and lipolysis of 3T3-L1 cells. **Methods:** 3T3-L1 fibroblasts were induced under standard differentiation process in the presence of 0. l μM, l μM, 10 μM, 100 μM ginsenoside Rb1 for 6 days. Oil red O staining, measurement of triglyceride contents and glucose uptake assay were performed. The expressions of mRNA and protein of PPARγ2, C/EBPα, ap2, GLUT1, and GLUT4 were analysed with quantitative real time-PCR and immunoblot. MTT was used to observe the effect of 48h treatment with Rb1 on preconfluent 3T3-L1 preadipocytes. Lipolysis of adipocytes was examined by the measurement of glycerol released from adipocytes treated with Rb1 for 1h. **Results:** Ginsenoside Rb1 facilitated adipogenesis of 3T3-L1 preadipocytes in a dose-dependent manner, 10 μM Rb1 increased lipid accumulation by about 56%. Treatment of differentiating adipocytes with 10 μM Rb1 increased the expressions of mRNA and protein of PPARγ2 and C/EBPα, as well as mRNA of ap2, one of their target genes. After treatment of differentiating adipocytes with Rb1, basal and insulin mediated glucose transport augmented significantly accompanied by up-regulation of mRNA and protein level of GLUT4, but not of GLUT1. In addition, Ginsenoside Rb1 inhibited the proliferation of preconfluent 3T3-L1 preadipocytes. Ginsenoside Rb1 inhibited basal lipolysis in adipocytes in a dose-dependent manner. However, it did not affect isoproterenol stimulated lipolysis. **Conclusion:** Ginsenoside Rb1 promotes adipogenesis, inhibits lipolysis and increases basal and insulin mediated glucose transport in cultured adipocytes. Therefore, anti-diabetic and insulin-sensitizing activity of ginsenosides is, at least in part, involved in the enhancing effect on PPARγ2 and C/EBPα expression to promote adipogenesis and glucose uptake, and the inhibiting effect on lipolysis in adipocytes to reduce lipotoxicity.

P49 Cloning and Expression of Visfatin, and Measurement of the Biological Activity In Vitro

Xin-jun WANG, Xiao-ying LI, Lei YE, Hong GAO, Zhi-guo ZHANG, Yi-sheng YANG, Guang NING
Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrine and Metabolic Diseases, Department of Endocrinology and Metabolism, Ruijin Hospital, Medical School of Shanghai Jiaotong University, China

Objective: Visfatin was recently identified as a protein highly expressed a in adipose tissue with insulin-mimetic effect and is a candidate cytokine to help explain the association between adipose tissue expansion, insulin resistance and type 2 diabetes. The present study was aimed to obtain recombinant visfatin expressed in *Escherichia coli* (*E. coli*) and observe the biological activity in vitro. **Methods:** For cloning of mouse visfatin gene, a pair of specific primers was designed based on the published sequence of this gene. A 1474 bp specific DNA fragment corresponding to the encoding region of visfatin was obtained by RT-PCR from the total RNA of mouse liver. The amplified cDNA was inserted into the IPTG-inducible expression plasmid pET-32c and its sequence was confirmed by DNA sequence analysis. The recombinant plasmid was transformed to the competent cells of *E. coli* BL21 (DE3). Visfatin was expressed after IPTG induction. The fusion protein was digested by enterokinase and purified by NTA resin. The biological activity was observed by glucose transportation in 3T3-L1 adipocytes. **Results:** The visfatin gene was successfully cloned, expressed and purified. The purified visfatin could facilitate glucose transportation in 3T3-L1 adipocytes. **Conclusion:** All these results suggested that the recombinant protein had biological activity, and provided a useful tool in further studies.

P50 Relationship between the Expression of Visfatin in Visceral Fat Tissue and Insulin Resistance in SD Rats Induced by High Fat Diet

Xiao-yi ZHOU, Yan-cheng XU
Department of Endocrinology, Zhongnan Hospital of Wuhan University, 430071, China

Objective: To explore effects of IR induced by high fat diet (HFD) on expressions of visfatin in visceral tissues of SD rats. **Methods:** 24 SD rats were randomly divided into 2 groups, HFD group and control group. After one week adaptive feeding, fasting blood glucose (FBG) and plasma insulin (PINS) were measured, then rats were fed with regular diet and self-made HFD respectively to induce IR models. Weights of rats were measured weekly. FBG, PINS, and HOMA-IR were measured after 12 weeks feeding. Then, all rats were killed and fat of epididymidis, mesentery, folded peritoneum and pad of kidney were isolated as visceral fat and weighed. Expression levels of visfatin mRNA were measured by RT-PCR separately, and analysis of correlation between HOMA-IR, visceral fat mass and visfatin/B-actin Reinhoit Zahl were carried out correspondingly. **Results:** IR was successfully induced; high fat group had a great increase in visceral fat mass ($p<0.01$) and displayed obvious visceral obesity. Their FBG increased slightly ($p>0.05$); PINS and HOMA-IR increased evidently ($p<0.05$), visfatin expressions of visceral fat tissues in HFD group were significantly higher ($p<0.05$) compared with control group. HOMA-IR and visceral fat mass had a positive correlation with visfatin/B-actin Reinhoit Zahl respectively. **Conclusion:** Rats with IR that induced by HFD have significantly higher levels of visfatin mRNA in visceral tissues, and visfatin mRNA expression levels had a positive correlation with HOMA-IR and visceral fat mass, which suggests the possible role of visfatin performed in occurrence and development of IR.

P52 Receptor an Postreceptor Signalling Pathways Underlying the Endothelial Action of Adiponectin, a Fat-derived Anti-Anterogenic Hormone

K.Y. CHENG, A. XU, K.S.L. LAM
Department of Medicine and Research Centre of Heart, Brain, Hormone & Healthy Aging, University of Hong Kong, Hong Kong

Background and objective: Adiponectin is a fat-derived hormone with potent anti-atherogenic activity. This hormone can protect vascular endothelium through enhancing vascular reactivity by increasing nitric oxide (NO) production in endothelial cells (1). However, the intracellular signalling pathways that mediate the actions of adiponectin in endothelial cells remain largely elusive. The major objective of this study is to investigate the role of two putative adiponectin receptors (adipoR1 and adipoR2) (3) and the adaptor protein containing PH domain, PTB domain, and leucine zipper motif (APPL1) (2) in mediating the endothelial actions of adiponectin. **Results:** Adiponectin stimulated NO production through activating AMPK, Akt, eNOS phosphorylation in a time-dependent manner. Simultaneous knock-down of both adipoR1 and adipoR2, or APPL1 expression, largely attenuated adiponectin-induced AMPK, Akt, eNOS phosphorylation and NO production in endothelial cells. Our affinity pull down experiments demonstrated that the cytoplasmic tails of both adipoR1 and adipoR2 interacted with APPL1. Co-immunoprecipitation experiments showed that APPL1 was associated with Akt2, eNOS and HSP90 in endothelial cells. **Conclusion:** These results suggest that adipoR1 and adipoR2 are functionally complementary in mediating adiponectin's endothelial actions, and that APPL-1 may serve as a proximal adaptor signaling molecules that links adiponectin receptors with Akt2, HSP90 and eNOS activation.

P53 Effect of Simvastatin on Adiponectin and NF-kappa B Inhibitor Kinase mRNA Expression in Insulin-resistant Rats Adipose

Jian DU, Li-juan CUI, Yu-yan ZHAO, Xiao-juan ZHAO
Department of Endocrinology, No.1 Hospital, China Medical University, Shenyang 110001, China

Objective: To study the effect of simvastatin on adiponectin and NF-kappa B inhibitor kinase (IKK) mRNA expression in insulin-resistant rats adipose. **Methods:** Insulin-resistant rat model was induced by high fat diet feeding, then assessed by euglycemic-hyperinsulinemia clamp technique. Insulin-resistant rat was fed with simvastatin 10 $mg \cdot kg^{-1} \cdot d^{-1}$ in gavage. The adiponectin and IKK mRNA expressions were tested with quantitative RT-PCR. **Results:** The glucose infusion rate (GIR) in high-fat diet group decreased significantly compared with normal control group fed with basic chow ($GIR_{60\sim120}$ [0.76±0.28 *vs* 4.26±0.70] $mg \cdot kg^{-1} \cdot min^{-1}$, $P<0.05$). There were no obvious difference on adiponectin mRNA expression between simvastatin treated group and high-fat diet group not treated with simvastatin (A $value_{adiponectin/\beta\text{-actin}}$ 0.25±0.12 *vs* 0.29±0.11, $P>0.05$); but they decreased significantly compared with normal control group, respectively (A $value_{adiponectin/\beta\text{-actin}}$ 0.25±0.12 *vs* 1.18±0.12, 0.29±0.11 *vs* 1.18±0.12, $P<0.05$). The IKK mRNA expression of adipose in simvastatin-treated group decreased significantly compared with high-fat diet group not treated with simvastatin (A $value_{IKK/\beta\text{-actin}}$ 0.15 ±0.03 *vs* 1.21±0.03, $P<0.05$), though was not obviously different from normal control group (A $value_{IKK/\beta\text{-actin}}$ 0.15±0.03 *vs* 0.15±0.03, $P>0.05$). **Conclusion:** Simvastatin may restore the IKK mRNA expression in insulin-resistant rats adipose induced by high fat diet feeding, which may be benefit from the anti-inflammatory effect beyond the lipid-lowering effect.

P54 The Effect of Telmisartan on Body Weight, Adipokines Expression and Insulin Resistance in SD Rats with High-fat Diet

Yan JIANG, Yan LI
Department of Endocrinology, The 2nd Affiliated Hospital of Sun Yat-sen University, Guangzhou 510080, China

Objective: To investigate whether RAS plays a role in the development of obesity induced with high-fat diet and its possible mechanism. **Methods:** 40 SD rats were randomly divided into four groups (n=10 per group) as follows: the 1st was fed with standard diet; the 2nd was fed with telmisartan and standard diet; the 3rd was fed with high-fat diet; the 4th was fed with telmisartan and high-fat diet. Animals were treated for 10 weeks. Body weight was measured at the beginning and every week of the experiment. At 10th week, fasting levels of glucose, lipids, FFA and insulin were also measured. HOMA-IR was calculated according to fasting serum concentrations of insulin and glucose. The expression of adipokines (TNF-α, IL-6 adiponectin, MCP-1 and CD68) in adipose tissue was determined by semi-quantitative RT-PCR. **Results:** There were significant differences between four groups in the trend of body weight changing with the time: the body weight of high-fat diet group was significantly higher than control treated group and high-fat diet treated group. Telmisartan had no significant effect on the expression levels of TNF-α, IL-6 adiponectin, MCP-1 and CD68 mRNA. **Conclusions:** Telmisartan treatment for 10 weeks inhibited the increase of body weight induced by high-fat diet fed suggested that AngII is involved in the development of obesity.

P55 Development of Monoclonal Antibody-based ELISA for Human Adiponectin and Evaluation of Applications

Ming LI[1], Jin-hua YIN, Jie MI, Shan GAO, Kui ZHANG[1], Cong-yuan WU[1]
Department of Endocrinology, Peking Union Medical College Hospital, Beijing 100730, China

Objective: Adiponectin (APN), proposed by IDF as one of the 'platinum standard' for research on metabolic syndrome (MS), exists in human plasma as a trimer, a hexamer and high-molecular-weight multimer; to further investigate its clinical significance, we use the newly produced monoclonal antibodies (mAb) to establish ELISA methods for measurement, and evaluate their application. **Methods:** Using hybridoma technique, we produced mouse anti-human APN mAbs. Based on those mAbs, sandwich avidin-biotin amplified ELISAs were developed. Furthermore, serum total APN levels, as well as levels of true insulin, proinsuluin and leptin in 410 adult pedigrees of type 2 diabetes and 965 schoolchildren were all measured by our in-house ELISAs and compared relationship with insulin resistance and MS components. **Results:** mAbs against human APN with different specificity were prepared. Sandwich ELISAs were developed with different specificity and sensitivity for polymer and for total APN. Comparison with commercially available method, this ELISA for total APN showed good properties. Preliminary clinic use showed that decreased total APN levels associated with the fundamental components of the MS i.e., obesity, insulin resistance, hyperglycemia, hypertension and dyslipidemia, both in adults and in children. **Conclusion:** Our novel ELISA for APN, combination with our early-developed in-house ELISAs for insulin, proinsulin and leptin, may construct an important platform for research and clinic use in MS, type 2 DM and other obesity-related diseases.

P56 Effects of gAd on the ROS Induced by Hyperglycemia

Jia ZHOU, Chuan YANG, Ming-tong XU, Li YAN, Hua CHENG, Zu-zhi FU
Department of Endocrinology, Second Affiliated Hospital of Sun Yat-Sen University, Guangzhou 510120, China

Objective: To observe effects of gAd on the ROS induced by hyperglycemia in NIT-1 cells. **Methods:** NIT-1 cells were divided into six groups: normal glucose concentration (5.6 mmol/L) and high glucose concentration (30 mmol/L) without or with gAd, DPI (NG, HG, NG+gAd, HG+gAd, NG+DPI, HG+DPI). In vitro the cells were cultured in the different medium as mentioned above for 72 hours. Then the cells were used for 2',7'-dichlorodihydrofluorescein diacetate (H_2DCF-DA) staining to detect the level of ROS and evaluate the expression of AdipoR1 mRNA by RT-PCR. **Results:** The levels of ROS in NG, HG, NG+gAd, HG+gAd groups have statistical significance (F=16.797, P=0.000). And the levels of ROS in NG, HG, NG+DPI, HG+DPI groups (0.94±0.26, P<0.05) also have statistical significance (F=5.653, P=0.025). The interaction among the glucose concentration, gAd and DPI is significant (P<0.05). The expression of AdipoR1 in NG, HG, NG+gAd, HG+gAd groups does not have statistical significance (F=0.005, P=0.945). And the mRNA of AdipoR1 in NG, HG, NG+DPI, HG+DPI groups also does not have statistical significance. The interaction among the glucose concentration, gAd and DPI is not significant (P>0.05). **Conclusions:** The hyperglycemia could induce the increase of ROS in NIT-1 cells. The gAd or inhibitor of NAD(P)H oxidase(DPI) could inhibit the increase of ROS caused by hyperglycemia, possibly through an NAD(P)H oxidase-linked mechanism.

P57 Effects of Globular Adiponectin, Glucose and Free Fatty Acid on AMPK and ACC Phosphorylation in INS-1 Cells

Yu TONG[1], Dan-shan HUANG[2], Michael BRYER-ASH[2]
[1] Department of Endocrinology, First Hospital, Peking University 100034, China
[2] Division of Endocrinology, Diabetes and Hypertension, David Geffen School of Medicine, University of California, Los Angeles 90095, USA

Objective: To investigate effects of glucose and free fatty acid of different concentrations on phosphorylation of AMPK and ACC in INS-1 cells, and globular adiponectin effects on phosphorylation of AMPK and ACC. **Methods:** INS-1 cells were cultured and treated with 5 mmol/L glucose or 0.25 mmol/L free fatty acids, and time courses and dose responses of different dosage of glucose and fatty acid on phosphorylation of AMPK and ACC were measured. We measured the effects of the pharmacological AMPK activator AICAR and globular adiponectin on phosphorylation of AMPK and ACC. **Results:** Glucose and fatty acid of different concentrations inhibited the phosphorylation of AMPK and ACC at 60 mins, but AICAR increased the phosphorylation of AMPK and ACC significantly, while 2.5 mg/L globular adiponectin increased the phosphorylation of AMPK and ACC by 23% (p<0.05) and 50% (p<0.05) respectively, at baseline. In the presence of 5 mmol/L glucose, globular adiponectin increased AMPK and ACC phosphorylation by 1.4 fold (p<0.05) and 3 fold (p<0.01), respectively. In the presence of 0.25 mmol/L free fatty acid, globular adiponectin increased AMPK and ACC phosphorylation 3 fold (p<0.05) and 5 fold (p<0.01) respectively. **Conclusion:** In cultured islet cells, glucose and free fatty acid of various concentrations inhibits the phosphorylation of AMPK and ACC, but AICAR and globular adiponectin 2.5 mg/L increases the phosphorylation level. This may constitute a mechanism to increase fatty acid oxidation and decrease triglyceride accumulation in islet β-cells.

P58 Correlation of Serum Adiponectin, Leptin and its Ratio with Insulin Resistance in First-Degree Relatives of Type 2 Diabetic Patients

Shan GAO[1], Ming LI, Ju-ming LU, Xiu-juan ZHANG[1], Xu REN[1], Hai-hui LI[1], Rong-zhou ZHANG[1]
[1] Department of Endocrinology, JingXi Branch, Chao Yang Hospital, Capital University of Medical Sciences, Beijing 100043, China

Objectives: To study the association of serum leptin and adiponectin with insulin resistance in first-degree relatives of type 2 diabetes mellitus (T2DM), and to investigate the role of leptin and adiponectin in development of T2DM. **Methods:** Serum adiponectin and leptin levels in 71 patients with newly diagnosed of T2DM, 55 subjects with IGT/IF and 174 NGT from first-degree relatives of T2DM, and 114 subjects of NGT without T2DM family history as control group (NC), were measured by ELISA. Insulin resistance was evaluated by HOMA-IR. **Results:** From NC group to NGT, to IGT/IFG and to T2DM group, the serum levels of leptin were increased progressively, which positively correlated with HOMA-IR ($r=0.35$, $p<0.001$). The serum levels of adiponectin were significantly decreased from NC group to NGT group, to IGT/IFG and to T2DM progressively, and negatively correlated with HOMA-IR ($r= –0.41$, $p<0.001$). The adiponectin/leptin ratio was reduced significantly in groups of diabetic pedigree from NGT to IFG/IGT and T2DM, and correlated with HOMA-IR significantly ($r= –0.53$, $p<0.001$). **Conclusions:** Decreased serum adiponectin levels and increased leptin levels, in parallel with increased insulin resistance, were already present in NGT pedigrees of T2DM, and worsening with the proceeding of glucose tolerance. We speculate the change of adiponectin/leptin ratio may be a reflection of genetic deficiency in T2DM pedigrees, and play an important role in the pathogenesis of insulin resistance and T2DM; thus the adiponectin/leptin ratio is potent to be an early predicator of T2DM.

P59 Resistin/Adiponectin Ratio as a New Atherosclerotic Index in Type 2 Diabetic Patients: Relationship of Index to Carotid Intima-media Thickness

Ming LI[1], Cui-ping LIU, Bin LI, Xiao-yan CHEN, Li-mei CUI, Jing TAO, Xiao-min ZHEN
[1] Department of Endocrinology, Peking Union Medical College Hospital, Beijing 100730, China

Objective: To explore the relationship of serum resistin, adiponectin, and leptin concentrations to carotid intima-media thickness (CIMT) in patients with type 2 diabetes mellitius (T2DM). **Methods:** 121 patients with T2DM were included in this cross-sectional study. Fasting levels of resistin, adiponectin, leptin, true insulin and proinsulin were measured by ELISAs. CIMT was measured using high-resolution B-mode ultrasound. The patients were subdivided into control group (CIMT <1 mm) and observe group (CIMT ≥1 mm). **Results:** Fasting levels of CRP, resistin (16.3±15.5 vs 19.0±13.8 ng/ml, $P<0.01$) and ratio of resistin/adiponectin (1.16±1.25 vs 1.66±1.43, $P=0.005$) were significantly increased in observe group. Fasting proinsulin and proinsulin/true insulin ratio in observe group were significantly higher than those in control group. Serum levels of adiponectin (22.4±20.3 vs 16.4±13.5 ug/ml, $P=0.05$) were significantly lower than those in the control group ($P<0.05$). Correlation analysis showed that resistin /adiponectin ratio ($r=0.316$, $P<0.001$), rather than resistin ($r=0.229$, $P<0.01$) and adiponectin alone, was more strongly associated with CIMT. **Conclusion:** Given the opposite effects of resistin and adiponectin on the inflammatory process, we speculate that relative proportion of resistin-to-adiponectin might potentially influence cardiometabolic risk. Our study suggests that the resistin/adiponectin ratio can serve as a new atherosclerotic index (a clinical marker of atherosclerosis) in patients with T2DM.

P60 The Relationship between Resistin with Obesity and Type 2 Diabetes Mellitus

Mei-hua LIANG, Qiang LI, Jin-chao ZHANG
The 2nd affiliated hospital of Harbin Medical University, Harbin 150086, China

Objective: To research the relationship between resistin with obesity and type 2 diabetes mellitus. **Methods:** 80 healthy person, 67 obesity, 77 type 2 diabetes and 102 type 2 diabetes with obesity were chosen. ELISA was used to test resistin, true insulin, proinsulin. RIA was used to test glucagon. And we test fasting glucose, lipid and blood pressure of these subjects. Height, weight and other index were recorded. SPSS13.0 was used to analyze. **Results:** The resistin level was not significantly different among these groups. No relationship was found between resistin and sensitivity of insulin. Resistin was associated with TC in all of obesity and diabetes. Resistin was related to WHR in all of obesity. In pure obesity resistin was positively related to PI. **Conclusions:** These findings suggest that resistin is not associated with type 2 diabetes directly, but in obesity and diabetes it may influence lipid metabolism and in obesity it may influence the formation of insulin resistance.

P61 Serum CRP Levels are Equally Elevated in Newly Diagnosed Type 2 Diabetes and Impaired Glucose Tolerance and Related to Adiponectin Levels and Insulin Sensitivity

Guo-yue YUAN[1], Li-bin ZHOU[2], Jin-feng TANG[2], Ying YANG[2], Wei-qiong GU[1], Feng-ying LI[2], Jie HONG[1], Yan-yun GU[2], Xiao-ying LI[1], Guang NING[1], Ming-dao CHEN[1 2]
[1] Ruijin Hospital affiliated to Shanghai Second Medical University, Shanghai, China
[2] Shanghai Institute of Endocrine And Metabolic Diseases, China

Objective: To measure the serum highly sensitive C-reactive protein (hs-CRP) and adiponectin levels and assess the SI and AIR in normal control (NC) subjects, patients with impaired glucose tolerance (IGT) and newly diagnosed type 2 diabetes mellitus (DM), and to explore the possible correlation between hs-CRP and blood glucose, insulin, lipids, insulin sensitivity index (SI), acute insulin response (AIR), and adiponectin. **Methods:** Age- and sex-matched 28 normal subjects, 31 patients with IGT, and 31 patients with newly diagnosed type 2 DM were included in the study. SI and AIR were assessed by the reduced sample number of Bergman's minimal model method with intravenous glucose tolerance test in subjects of each group. **Results:** Compared with NC group, serum hs-CRP was significantly increased in IGT and type 2 DM groups ($p<0.001$), although there was no significant difference between the latter groups. Hs-CRP was negatively correlated with high density lipoprotein cholesterol (HDL-C), SI and adiponectin levels ($p<0.05$ - $p<0.001$), and positively correlated with systolic blood pressure (SBP), fasting blood glucose (FBG), BMI, waist-to-hip ratio (WHR), postprandial blood glucose (PBG), fasting serum insulin (FINS) and postprandial serum insulin (PSI) ($p<0.05$ - $p<0.001$). In general multivariate regression, only adiponectin was the significantly independent determinant for serum hs-CRP ($p<0.0001$). **Conclusions:** Elevated serum hs-CRP may play a role in the development of metabolic syndrome-related manifestations, type 2 DM and atherosclerosis. This elevation is accompanied by the opposite changes of adiponectin.

P62 Effects of Rosiglitazone on Adipocytokines and Insulin Resistance in Newly Diagnostic Type 2 Diabetic Patients

Jing YANG[1], Jin-hua YIN[1], Ming LI
[1] Department of Endocrinology, First Affiliated Hospital, Shanxi University of Medical, Taiyuan 030001, China

Objective: To investigate the effects of rosiglitazone (ROS) on serum adipocytokines and insulin resistance (IR) in the newly diagnostic patients with type 2 diabetes mellitus (T2DM). **Methods:** 38 T2DM patients were given ROS 4 mg/d for 12 weeks. The changes of weight, blood pressure, fasting and postprandial 2h plasma glucose (FPG, P2BG), true insulin (TI) and proinsulin (PI), serum adiponectin (APN), leptin (LEP), resistin (RSN) and retinol binding protein 4(RBP-4), and serum lipid were examined in 24 normal control subjects and patients at the baseline and after treatment. **Results:** Compared with the controls, fasting PI, PI/TI ratio, HOMA-IR and LEP, RSN, RBP-4 were significantly higher, but APN was lower in T2DM before treatment. BMI, hepatic and renal function, serum lipids did not significantly change, while plasma glucose, PI, RSN, LEP and HOMA-IR were significantly reduced after 12-week treatment. The APN was significantly increased (by 2 fold) after treatment (12.6 vs. 27.8 μg/ml, $P<0.01$). Partial analysis showed LEP was positively correlated with sex, BMI, fasting TI, PI and HOMA-IR. RSN was positively correlated with PI/TI ratio, but negatively correlated with fasting TI. APN was correlated with age, BMI, RBP-4, TI, PI and HOMA-IR. Serum RBP-4 was correlated with BMI, APN and HOMA-IR. Multivariate regressive analysis revealed the most important factors affecting the HOMA-IR were LEP, APN and BMI ($R^2=0.655$, $P<0.01$). **Conclusion:** Increased serum LEP, RSN, RBP-4, and decreased serum APN, correlating closely with IR, were found in the newly diagnostic patients with T2DM. ROS treatment can decrease plasma glucose levels; improve insulin sensitivity and β-cell function at least partly through improving the profile of adipocytokines.

P63 Long-term Intensive Glycemic Control, Lipid Control and Blood Pressure Control Ameliorate the Decreased Level of Plasma Adiponectin in Type 2 Diabetes

Jian-mei YANG, Xiao-hui GUO, Hong WANG, et al
The First Affiliated Hospital, Beijing University, Beijing 10034, China

Objectives: To study the level of plasma adiponectin in the patients with type 2 diabetics (T2DM) and the relationship of adiponectin with the levels of glucose, blood pressure and blood lipids in T2DM. To investigate whether long-term intensive glycemic control, lipid control and blood pressure control would ameliorate the decreased level of plasma adiponectin in patients with T2DM. **Methods:** We measured plasma adiponectin in 106 cases of T2DM diagnosed by criteria of WHO (1999), 49 cases of normal subjects matched to the patients of T2DM in sex and age. The routine clinical data were also checked up in all subjects. Of all patients 68 cases were given long-term intensive glycemic, lipid and blood pressure control. After 1 year plasma adiponectin was determined in those patients again. **Results:** (1) The level of plasma adiponectin was decreased significantly in patients with type 2 diabetes compared with normal control. (2) The level of plasma adiponectin was correlated with sex (positively with male, negatively with female), also correlated negatively with waist circumference, waist to hip ratio (WHR), diastolic blood pressure, fasting plasma glucose (FPG), postprandial 2h plasma glucose (2hPG) and triglycerides (TG), and also correlated positively with HDL. In multivariate regression, FPG and HDL-C were significantly independent determinants for plasma adiponectin concentration. (3) After 1 year intensive glycemic, lipid and blood pressure control the levels of TG, LDLC were significantly reduced and the level of HDL was significantly increased, FPG, 2hPG and GHbA1c were significantly reduced, and simultaneously the decreased level of plasma adiponectin was significantly increased. **Conclusions:** The level of plasma adiponectin is decreased in patients with type 2 diabetes. Adiponectin appears to be closely related to glycemic and lipid disorder. Long-term intensive glycemic control, lipid control and blood pressure control could ameliorate the decreased levels of plasma adiponectin in patients with T2DM.

P65 Serum Visfatin Concentration among Normal and Diabetic Subjects Type 2 Diabetic Patients and Normal Subjects in a Chinese Population

Xin-jun WANG, Ji-guang WANG, Min XU, Peng-fei DU, Yan WEN, Gu-liang WANG, Xue-mei LUO, Yi-sheng YANG, Jun YANG, Jian-min LIU, Wei-qing WANG, Guang NING, Xiao-ying LI
Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrine and Metabolic Diseases, Department of Endocrinology and Metabolism, Ruijin Hospital, Medical School of Shanghai Jiaotong University, China

Objective: To investigate the relationship between serum visfatin concentrations and anthropometric or metabolic measurements in diabetic patients or normal subjects. **Methods:** A cross-sectional study was performed in 58 type 2 diabetic patients and 79 normal subjects from the Shanghai Qingpu Community. Serum visfatin was measured by EIA. **Results:** (1) No significant difference of visfatin concentrations was found between diabetic group and normal control group. (2) Serum visfatin was negatively correlated with body weight, body mass index (BMI), waist circumference and hip circumference in normal subjects, while in diabetic subjects, all the relationships disappeared. (3) Multiple regression analysis showed that waist circumference was the independent relative factor for influencing serum visfatin concentration. **Conclusion:** Serum concentrations of visfatin displayed negative relation with adiposity index in Chinese healthy subjects. Visfatin might play a role in glucose homeogenisis on physiological concentrations.

P66 The Visfatin Level Increases in Metabolic Syndrome Patients and Its Correlation with the Components of MS

Fang LIU, Xin WANG, Jun-xi LU, Hui-juan LU, Jun XIE, Wei-ping JIA
Department of Endocrinology and Metabolism, Shanghai Jiaotong University Affiliated No.6 People's Hospital, Shanghai Clinical Center for Diabetes, Shanghai Diabetes Institute, Shanghai 200233, P.R. China

Objective: Visfatin is a new adipose cytokine, and it is not clear whether this hormone changes in patients with metabolic syndrome. To investigate the change of adiponectin and visfatin levels in patients with metabolic syndrome (MS), and their relation with the components of MS. **Methods:** Based on WHO 2000 standards, 42 MS patients were chosen, and 30 healthy subjects as control group. After measuring the height, weight and waist circumference, the fasting blood of MS group and control group were collected and the fasting levels of glucose, insulin, adiponectin and visfatin were determined. The adiponectin levels was measured with radioimmunoassy (RIA), and the visfatin with enzyme-linked immunoassay (EIA). The HOMA-IR was calculated based on FPG and PINS, HOMA-IR=FPG*FINS/22.5. The statistical data were analyzed with SPSS10.0 software. **Results:** The visfatin levels of MS group increased significantly than control group (97.57±40.69 ng/ml vs 50.54±19.14 ng/ml, $p<0.05$), and the adiponectin levels were lower significantly than control subjects (7.98±3.62 ug/ml vs 13.55±5.72 ug/ml, $p<0.01$). The visfatin levels were positively correlated to waist circumference ($r=0.415$, $p<0.01$) of MS patients, but there was no correlation between the visfatin level and other metabolic indexes, as adiponectin, FINS, BMI, HOMA-IR, or triglycerol. There was positive correlation between adiponectin level and HDL-C ($r=0.579$, $p<0.01$), but it is negatively correlated with HOMA-IR ($r= –0.601$, $p<0.01$) and waist-hip ratio (WHR) ($r= –0.531$, $p<0.01$) in these MS patients. **Conclusions:** The visfatin level increased significantly in MS patients, but the adiponectin levels decreased markedly. Visfatin may involve in the development of visceral obesity, and the adiponectin may resist the insulin resistance and dyslipidemia. The visfatin levels may have no obvious relation with the adiponectin in patients with MS.

P67 Fasting Serum Ghrelin and Visfatin Levels and Their Relationships with Insulin Resistance in Type 2 Diabetes

Wen-hui WANG, Ning LOU, Ying CUI, Hui MA
Endocrinologic Department of Jinan Central Hospital, Clinical College of Shandong University, Shandong 250013, China

Objective: To measure levels of fasting serum ghrelin and visfatin in obese and non-obese patients with T2DM, and to explore the relationships of these with age, BMI, WHR, BF%, blood glucose, lipids, FINS, HOMA-IAI, HOMA-IR and between themselves. **Methods:** 58 type 2 diabetes patients were selected and divided into obese group of 30 and non-obese group of 28 due to BMI≥25 or <25. 30 non-diabetic controls were selected. Fasting ghrelin and insulin were measured with RIA, and visfatin with ESLIA. BMI, WHR, BF% were calculated with stature, weight, waistline and age of subjects. **Results:** In the diabetes, the fasting serum ghrelin was significantly lower, while the visfatin was higher than that of the control (p=0.005 and 0.002). The fasting serum ghrelin was negatively correlated to BMI, WHR, BF%, FINS and HOMA-IR while the visfatin was positively correlated to WHR, FINS and HOMA-IR in T2DM. No relationship of ghrelin and visfatin was observed. After multiple regression analysis, the BMI and the WHR seemed to be the best variable in predicting ghrelin and visfatin levels respectively in T2DM. **Conclusion:** The fasting serum ghrelin was reduced while the visfatin was elevated inT2DM. Fat and insulin resistance may contribute to the change of these cytokines.

P68 The Serum Adiponectin Levels and Correlations with Markers of Inflammation in Patients with Coronary Artery Disease

Hong TAO, Shu-hua MI, Rui CHEN, Xiao-mei YANG
Department of Endocrinology, Beijing Anzhen Hospital, Capital University of Medical Sciences, Beijing 100029, China

Objective: To investigate the association of serum adiponectin concentrations with markers of inflammation and other risk of coronary artery disease (CAD) in patients with CAD. **Methods:** From Aug 2004 to July 2005, 105 cases aged 30-70 with angiographically confirmed CAD and 42 age- , BMI-, and gender-matched controls were included in the study. The serum adiponectin, TNF-α, IL-6 were determined by ELISA. And the high sensitive C-reactive protein (hs-CRP) determinations were done by an immunoturbidimetric assay. **Results:** Serum adiponectin level was 41.4±21.4 ng/ml in the patients with CAD, a value significantly less than that of 74.9±68.4 ng/ml in the control subjects (P<0.01). Next, we subgrouped the CAD patients according to the presence of DM. It was further reduced to 36.6±18.2 ng/ml in the subjects having both CAD and DM (n=61), a value significantly less than that of 45.0±22.9 ng/ml in the absent of diabetes (n=40, P <0.05). As compared with the group of control, patients with CAD also had higher levels of TNF-α, IL-6, hs-CRP and HOMA-IR (P<0.01). After careful adjustment for age and gender, a significant negative correlation between adiponectin and the level of BMI, waist circumference, HOMA-IR, hs-CRP, IL-6, TNF-α, TG was found from Pearson correlation analysis (P<0.01), whereas the value of HDL-c was positive correlated with adiponectin (P<0.01). Multiple step regression analysis showed that IL-6, TG and HDL-c were independently correlated with the level of adiponectin. **Conclusion:** Adiponectin is close correlated with inflammation factors related to atherogenesis, and it may have potential pathophysiological role in atherosclerosis.

P69 Relationship between the Level of Resistin and Insulin Resistance in Patients with Hyperthyroidism

Zheng-fang LI, Shu-yun TONG, Chang LIN, et al
Department of Endocrinology, The Second Affiliated Hospital of Kunming Medical College, Kunming 650101, China

Objective: To investigate the relationship of serum resistin concentration with insulin resistance in patients with hyperthyroidism. **Methods:** The fasting serum resistin conentrations were measured with enzyme immunoassay in 32 patients with hyperthyroidism and 40 healthy controls, and fasting plasma glucose, insulin, height, body weigth were measured to calculate body mass index (BMI) and insulin resistance index (HOMA-IR). Correlated factors of serum resistin were analyzed. **Results:** The fasting serum resistin (10.97±6.37 ng/ml), insulin (7.76±2.92 mU/L), glucose (5.38 ±0.95 mmol/L) concentrations and HOMA-IR (2.2±1.03) were significantly higher than those in the healthy controls (7.09±4.36, 6.37±2.67, 4.79±0.48, 1.43±1.12), all $p<0.05$. Resistin showed a significant correlation with FT3 ($r=0.448$, $p=0.011$), FT4 ($r=0.405$, $p=0.023$) and HOMA-IR ($r=0.392$, $p=0.036$) in patients with hyperthyroidism and negatively correlated with sTSH ($r= –0.361$, $p=0.045$). After adjustment for age and BMI, partial correlation analysis showed that serum resistin concentration in patients with hyperthyroidism were correlated with FT3, FT4, HOMA-IR and sTSH. **Conclusion:** Resistin might play important roles in the mechanisms of glycometabolic disorder and insulin resistance in patients with hyperthyroidism.

P70 Persistence of High Prevalence of IDD in a District in North India Despite 2 Decades of Universal Salt Iodization Program: an Urgent Need for Intervention

Sushil Kumar GUPTA[1], Subhash YADAV[1], Manoj JAIN[2], Anand MISHRA[3], Ashutosh SRIVASTAVA[1], Praveen VP[1], Raman BOUDALA[1], Ashwani TANDON[1], Manish HORA[4], Amit RAWAT[2], Manoj SHUKLA[1], Satish BABU[1], P.K. AWASTHI[1], Madan M. GODBOLE[1]
Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India 226 014
[1] Department of Endocrinology
[2] Department of Pathology
[3] Department of Endocrine Surgery
[4] Department of Nuclear Medicine
[5] Department of Biostatistics

Keeping in view the estimated figures of 330 millions at risk, 150 millions with goiter, and 2.2 millions cretins, India has implemented universal salt iodization (USI) program since year 1985. Effectiveness of USI has rarely been objectively studied with a statistically designed population sample size. District Gonda in sub-Himalayan belt in North India had an estimated pre-iodination goiter prevalence rate of 69%. An objective assessment of IDD was carried out in 2800 subjects residing in 12 blocks of using 30 cluster design of WHO representing the size proportionate to population. Goiter prevalence, urinary iodine levels, thyroid function tests and salt iodine contents at house hold level were studied. There is a significant overall reduction in goiter prevalence from 69% to 27.2% but it brings out a very disturbing findings namely (1) Goiter prevalence rate is still way above the internationally accepted norm of 10%, (2) Six blocks of Gonda district still suffer from severe iodine deficiency (30% and above), (3) More than 30% population of these 6 blocks show urinary iodine excretion less than 50 ug/dl, and (4) IDD associated primary hypothyroidism (as judged by serum TSH >5.0 mIU/L and FT4 <10 pmol/L) was observed in approximately 10% of the study subjects. 20% and 50% of subjects still receive negligible iodine or suboptimal iodine respectively from salt consumption. The household washing of "*Bargada*" salt is a standard practice in 90% of families and is probably the major cause of persistent severe iodine deficiency. These data demonstrate persistently high prevalence of IDD in rural population possibly due to lack of proper monitoring, lack of emphasis in hot spots of IDD and lack of public awareness regarding IDD.

P72 CTLA-4 Gene A-G Polymorphism in Graves' Disease

Grace Pui-sze HUI, Man-wo TSANG
Division of Endocrinology, Department of Medicine and Geriatrics, United Christian Hospital, HKSAR, China

Objective: To see if CTLA-4 exon 1 polymorphism (49 A/G) is associated with Graves' disease (GD) in Hong Kong Chinese adult patient. **Methods:** Two hundred and twelve patients (154 females and 58 males) with GD were recruited in the out-patient bases, compared with 226 racially matched control subjects with no history of thyroid disease or any other autoimmune disease. Genomic DNA was extracted from the blood samples. The polymorphism at position 49 A/G was analyzed by polymerase chain reaction, restriction fragment length polymorphism and DNA sequencing. **Results:** There was no difference between the genotype distribution and the allele frequency when comparing the disease group with the control group. Though there was slight increase in frequency of G allele in male patient when compared with the control, but it was not statistically significant. The clinical characteristics of the GD patient with genotype A/A, A/G and G/G were also of no difference. **Conclusion:** GD is a condition with both genetic and environmental contribution. CTLA-4 gene polymorphism in the exon 1 position 49 has failed to show an association with GD in this study. Further study is suggested to delineate the association of CTLA-4 gene polymorphism and other non-genetic cause with GD in this locality.

P73 Study on the Change of Cytokines Levels in the Patients with Graves Disease

Ze-lin LIU[1], Yu-lin WANG[1], Lu LI[1], Ren ZHOU[1], Hui QI[2], Yan WU[1]
[1] Department of Endocrinology, Shenzhen People's Hospital, The Second Affiliated Hospital of Jinan University, Shenzhen 518020, China
[2] Clinical study center

Objective: To investigate the role of the IL-6, IFN-γ, TNF-α in the pathogenesis of Graves disease (GD) and try to find the index of diagnosing GD. **Methods:** Serum concentrations of IL-6, IFN-γ, TNF-α in 68 patients with Graves disease and 29 healthy subjects were measured by RIA and double-antibodies ELISA. Meanwhile the levels of TSH, FT3, FT4 also were measured in them. **Results:** IL-6, IFN-γ, TNF-α levels in GD patients were significantly higher than those in control subjects ($P<0.05$). There were no correlations between the serum levels of FT3, FT4 and IL-6, IFN-γ, TNF-α ($P>0.05$). **Conclusion:** IL-6, IFN-γ, TNF-α has taken part in the GD pathogenesis and may play some important roles in the immunopathogenesis of GD and could act as referance indexes to diagnose GD.

P74 The Investigation on the Change of Graves Disease Patients' Serum IL-10, IL-18 and Hemo-lymphocyte Subset

Hui HUANG
The Second People's Hospital of Shenzhen, China

Objective: To study the change and correlation of serum's IL-10, IL-18 and hemo-lymphocyte subset at different stages of treatment in Graves disease, reveal the immune pathogenesis and guide the clinical therapy of Graves disease. **Methods:** After collecting 60 Graves disease patients and 20 controls, propylthiouracil (PTU) was administrated to the patients, the change of serum FT3, FT4 and TSH were checked after one or two months of treatment. When the pathogenetic conditions were relieved, the PTU dosage for patients was lessened. 50 mg/day PTU was administrated to the patients for maintenance 3-5 months later. Rechecking the serum FT3, FT4 and TSH per 2-3 months and collecting the peripheral blood and serum sample at the each stage of therapy. The percentage of peripheral blood T lymphocyte subset $CD3^+CD4^+$, $CD3^+CD8^+$, $CD19^+$ and natural killer (NK) cells were measured by cytometry, and the serum IL-10, IL-18 were analyzed by enzyme-linked immunosorbent assay (ELISA). **Results:** Compared with the normal controls, the percentage of $CD3^+CD4^+$ lymphocytes and natural killer cells ($P<0.05$) in Graves disease patients were much lower obviously, however, the percentage of $CD3^+CD8^+$ lymphocytes and serum concentration of IL-10 and IL-18 in Graves disease patients were higher than the normal controls. After 2 to 3 months of treatment, the percentage of NK cells increased obviously and $CD3^+CD8^+$ cells began to reduce ($P<0.05$). The serum concentration of IL-10 and IL-18 were also reduced dramatically ($P<0.01$). At the stage of maintained therapy, the serum concentration of IL-10 and IL-18 continued reduced, but were still higher than that of the normal control ($P<0.01$). The percentage of peripheral blood T lymphocyte subset $CD3^+CD4^+$ and NK ($P<0.05$) were higher than that of untreated. 21 patients were relapsed after half to one year of drug withdrawal. The percentage of T lymphocyte subset $CD3^+CD4^+$, $CD3^+CD8^+$ and NK cells is similar to that of untreated. 39 Graves disease patients were not relapsed after two years of drug withdrawal, and their serum IL-10 and IL-18 as well as the percentage of T lymphocyte subset were as normal as that of the controls. There existed linear positive correlation between the percentage of T lymphocyte subset $CD3^+CD4^+$, $CD3^+CD8^+$, CD4+/CD8+ and NK cells and serum IL-10 concentration. **Conclusions:** There exists significantly immunologic derangement in the patients with Graves disease. The antithyroid drugs can repair this kind of immunologic derangement step by step. During this course, the changes of peripheral blood T lymphocyte subset and serum IL-10 or IL-18 levels were not coincidence with that of FT3, FT4 and TSH levels, the maintenane therapy is necessary for the Graves disease.

P75 Evaluated the Insulin Secretion and Insulin Resistance in Patients of Graves Disease and IGT by Hyperglycemic Clamp

Zhen LIANG, Guo-chun LUO, Qin-hong HU, et al
Department of Endocrinology, The Shenzhen Affiliated Hospital of Southern Medical University, Shenzhen 518035, China

Objective: To investigate the state of insulin secretion and insulin resistance in patients of Graves disease and IGT by hyperglycemic clamp. **Methods:** Six patients of Graves disease with impair glucose tolerance were selected as GD+IGT group, ten healthy volunteers as normal control group (NC group). All subjects were required to fast for 12 hours and then underwent hyperglycemic clamp to assay insulin secretion and insulin sensitivity. **Results:** All subjects appear two-phase insulin release, first peak of insulin secretion appeared in 4-6 minutes after injection 20% glucose. Then the insulin secretion returned the lowest value during 10-30 minutes. After the lowest value, the insulin concentration increased slowly and reached a stable top level at 120-150 minutes. Insulin secretion of GD+IGT group was significantly higher than that in NC group, 1^{st} phase insulin secretion was 636.31±105.54 mIU/L vs 233.56±21.33 mIU/L, P=0.001. 2^{nd} phase insulin secretion was 146.68±25.0 mIU/L vs 67.06±6.23 mIU/L, P=0.03. The maximal insulin secretion during 120-150 minutes was 195.05±32.94 mIU/L vs 87.64±9.78 mIU/L, P=0.04. The hyperglycemic clamp insulin sensitivity index (dividing the average glucose metabolic rate by $INS_{120\text{-}150}$ during 120-150 minutes) significantly lower in GD+IGT group than that in NC group (11.52±1.90 vs 21.72±3.25, P= 0.04). **Conclusions:** Patients of Graves disease with IGT appeared significant insulin resistance and compensated elevated insulin secretion.

P76 Changes of High-sensitivity C-reactive Protein and Tumor Necrosis Factor-α in Graves' disease

Li-zhen LIAO, Guo-wen LONG, Fei-qi LIU, Hui-ling HU, Guo-hua LI, Ni FENG
Central Hospital of Xiangtan City, Hunan Province 411100, China

Objective: To investigate the changes of high-sensitivity C-reactive protein (hsCRP) and tumor necrosis factor-α (TNF-α) in Graves' disease (GD). To explore the evidence of inflammatory in Graves' disease. **Methods:** One hundred and forty patients with Graves' disease were selected as GD group (which excluded other thyroid gland diseases and factors influencing hsCRP and TNF-α) and 200 healthy volunteers who matched GD patients in age and sex as control group were selected. We used a high-sensitive immunological assay to determine the levels of serum C-reactive protein and a radioimmunoassay to TNF-α. The difference between the two groups was compared and the relationship of these indexes to thyroid hormones was analyzed. **Results:** The values of hsCRP (mg/L) in healthy controls were 0.512± 0.338 (0.563±0.355 for men, 0.462±0.315 for women). The values of hsCRP of women were significantly lower than those of men (p=0.035). The values of TNF-α (μg/L) in healthy controls were 0.530±0.564. The difference of TNF-α in both sexes has no statistical significance (p>0.05). The levels of hsCRP (1.889±4.005) and TNF-α (1.247±2.206) for GD group were significantly higher than those of healthy controls (p<0.001). The values of hsCRP and TNF-α had no significant correlation with free triiodothyronine (FT3) and free thyronine (FT4) (p>0.05). **Conclusion:** The levels of hsCRP and TNF-α of patients with GD are significantly higher than those of healthy controls. The inflammatory factors such as hsCRP and TNF-α play important roles in the pathogenesis of Graves' disease.

P77 Levels of Serum High-sensitivity C-reactive Protein in Patients with Graves' Disease with Different Complications

Li-zhen LIAO, Guo-wen LONG, Fei-qi LIU, Hui-ling HU, Ni FENG, Guo-hua LI
Central Hospital of Xiangtan City, Hunan Province 411100, China

Objective: To study the relationships between high-sensitivity C-reactive protein (hsCRP), an inflammatory marker, and the different complications of Graves' disease (GD). **Methods:** The GD group were divided into seven sub-groups: GD without complications as group 1, thyrotoxic crisis as group 2, thyrotoxic heart disease as group 3, Graves' ophthalmopathy as group 4, thyrotoxic periodic paralysis as group 5, leucocytes decrease as group 6, and liver damage as group 7. The levels of serum hsCW of GD and healthy controls and those of the different sub-groups of GD were compared. **Results:** The values of serum hsCRP (mg/L) of 200 healthy controls were 0.512±0.338 and those of the different sub-groups of GD were as follows: group 1 (n=97) 1.527±1.660, group 2 (n=2) 47.115, group 3 (n=26) 3.737± 6.803, group 4 (n=24) 3.060±4.264, group 5 (n=6) 1.245±1.120, group 6 (n=21) 1.044±1.494 and group 7 (n=23) 1.311± 1.706. There were no significant difference (p>0.05) between the age, course of disease, free triiodothyronine (FT3), free thyronine (FT4) and thyrotropin receptor antibody (TRAb) in GD groups. The values of serum hsCRP in patients with GD were higher than those of healthy controls. The values of hsCRP in the group 1, 3, 4, and 7 were significantly higher than those of healthy controls (p, respectively, <0.001, 0.023, 0.008, 0.035), while, there were no statistical significance (p>0.05) between group 5, 6 and healthy controls. The values of hsCRP in two cases for group 2 were 64.68 and 29.55, respectively. The levels of hsCRP in more than grade 4 Graves' ophthalmopathy were higher than those of less than grade 3 (p<0.05). The levels of hsCRP in patients with more than II° heart failure were higher than those of patients without heart failure (p=0.006). **Conclusion:** The levels of serum hsCRP in patients with GD were higher than those of healthy controls. The patients with critical complications have higher levels of serum hsCRP. According to the average of hsCRP, the sub-groups of GD are arranged as follows: group 2, group 3, group 4, group 1, group 7, group 5 and group 6.

P78 Variance of CD4+CD25+T Regulatory Cells in Graves Disease

Shan DANG, Bing-yin SHI
Department of Endocrinoloy, First Hospital Affiliated to Xi'an Medical College of Jiaotong University, Xi'an 710061, China

Objective: To investigate the levels of CD4+CD25+T regulatory (Tr) cells in the peripheral blood of the patients with Graves disease (GD). **Methods:** Thirty-seven GD patients (including 21 newly diagnosed GDs pre-treatment and 16 relieved patients after more than 6 months' regular treatment with methimazol) and 13 healthy individuals were included in this study. Levels of CD4+CD25+Tr cells in the peripheral blood were measured by flow cytometry. **Results:** The level of CD4+CD25+Tr cells in GD patients before treatment were significantly lower than normal controls ($p<0.05$), CD4+T cells are on the contrary. No significant correlation was found between levels of CD4+CD25+T regulatory cells and other throid function parameters including TT_3, TT_4, TGAb and TMAb. **Conclusion:** These data suggest that CD4+CD25+Tr cells might play an essential role in the pathogenesis of Graves disease.

P79 Allogeneic Antigen-specific $CD4^+CD25^+$ Regulatory T Cells as Suppressor Cells In Vitro and In Vivo

Chao LIU, Mei ZHANG, Shu-hang XU, Cui-ping LIU, Kuan-feng XU, Xiao-dong MAO
Department of Endocrinology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China

Objective: Induction of antigen-specific unresponsiveness to grafts is the ultimate goal for organ transplantation. We induced allogeneic antigen-specific $CD4^+CD25^+$ regulatory T cells in vitro and examined whether these cells generated in vitro have the suppressive function. **Methods:** Antigen-specific $CD4^+CD25^+$ regulatory T cells were generated by the addition of multiple intravenous injections of ICR mice splenocytes in vivo. Spleen cells were then pooled and enriched for $CD4^+CD25^+$ T cells by negative and positive selection by MACS in vitro. Characterizations of donor antigen-specific $CD4^+CD25^+$ regulatory T cells were tested by mixed lymphocyte reaction (MLR) consisting of BALB/cByJ splenocytes as responder and MMC-treated ICR splenocytes as stimulator. The abilities of these cells were tested on islets transplantation model in vivo. **Results:** In the MLR, donor antigen-specific T cells displayed little proliferation with allogeneic-antigen existence, although they proliferated in response to concanavalin A. The anergic state was reversed by the addition of exogenous IL-2. Furthermore, these cells inhibited the proliferative responses and IFN-γ production of naïve $CD4^+CD25^-$ T cells to alloantigens in vitro. And they prolong islet graft survival and normoglycemia in transplanted allogeneic diabetic mice in vivo. **Conclusions:** Donor-specific splenocytes transfusion-induced $CD4^+CD25^+$ T cells were allogeneic antigen-specific. They were anergic and retained their ability to suppress antigen-driven responses of $CD4^+CD25^-$ T cells in vitro and in vivo. This procedure might be clinically useful for promoting allograft survival.

P80 Study on the Association of Thyrotropin Receptor Antibodies with the Sensitivity to Glucocorticoid Therapy in Graves's Ophthalmopathy Patients

Ke-hui LIU, Qing LIU, Wei-ying GUO, Shu-gang XI
Department of Endocrinology, The First Hospital, Jilin University, Changchun 130021, China

Objective: Graves's ophthalmopathy (GO) is a kind of organ-specific autoallergic disease. Thyrotropin receptor antibodies (TRAb) are concerned with the onset and activity of GO. We observed changes of serum TRAb level in GO patients pre- and post-glucocorticoid treatment and investigated the association of TRAb level with the sensitivity to glucocorticoid therapy. **Methods:** Total 37 subjects with active GO were treated with oral prednisone, while treated with antithyroid drugs to rectify hyperthyroidism and retain pituitary-thyroid axis' function normal. We observed ocular symptoms and optic nerve function, judged ophthalmopathy activity with Mouritis' clinical activity scores. Patients were grouped into sensitive (S) group and non-sensitive (NS) group, according to the relieve degree and clinical activity of their ophthalmopathy after steroid treatment. TRAb positive rate and titer of the two groups were compared. **Results:** In S group (n=28, account for 75.7%), the positive rate and titre of TRAb prior treatment were 76.53% and 12.54± 5.62 U/L. The positive rate and titre of TRAb post-treatment were 22.18% and 7.82±4.91 U/L, were significant lower than data prior treatment (P<0.05). In NS group (n=9, account for 24.3%), the positive rate and titre of TRAb prior treatment were 67.24% and 11.07±4.63 U/L. The positive rate and titre of TRAb post-treatment were 59.74% and 10.81 ±5.96 U/L, had no significant difference with data prior treatment. Prior treatment, there was no significant difference of TRAb titres between two groups. Post-treatment, TRAb titres in NS group were significant higher than in S group (P<0.05). **Conclusion:** It suggested that TRAb's variations of GO patients were closely correlated with the sensitivity to glucocorticoid therapy. Serum TRAb level may be used as a major index to evaluate therapeutic sensitivity to glucocorticoid therapy in GO.

P81 Valuable Features for Predicting the Remission of Graves Hyperthyroidism After Antithyroid Drug Treatment

Xing-jun LIU, Bing-yin SHI, Hao LI
Department of Endocrinology, First Hospital Affiliated to Medical College of Xi'an Jiaotong University, Xi'an 710061, P.R. China

Objective: Antithyroid drug treatment (ATDT) is effective in achieving euthyroidism in Graves' hyperthyroidism, but with a potential problem of a high relapse rate after an apparently successful treatment course. We investigated the outcome of Graves' hyperthyroidism after ATDT and valuable factors predicting the remission. **Methods:** 133 patients mainly with mild and moderate goiter in our hospital from Feb 2000 to Feb 2005 were included. All patients received methimazole for a total course of 25.2±11.6 months and were followed for over one year. Lasting remission was defined as a clinical and laboratory picture of euthyroidism for at least one year after stopping antithyroid drug. **Results:** 118 of 133 patients (88.7%) still remained in remission after up to 1 years of follow-up while 15 patients (11.3%) relapsed. Of the various clinical and laboratory features studied, past GD history, continuous obvious goiter, positive TSAb and unstable TSH status during therapy are related to the subsequent risk of relapse in stepwise logistic regression analysis (OR value is separately 4.98, 20.54, 9.99 and 5.07), but other clinical parameters including age, sex, initial goiter, ophthalmopathy, thyroxine and triiodothyronine level, TGA and TMA level do not reach significance. Also, it is found that patients with a minimal MMI maintenance dosage of 2.5 mg or 1.25 mg daily have lower relapse rate than those with 5 mg daily (6/85 vs. 9/48, p<0.05). **Conclusion:** (a) Regular and individualized ATDT can achieve 88.7% remission rate in selected Graves hyperthyroidism patients, and the valuable features predicting remission include small goiter, TSAb negative, and small dosage of MMI before stopping treatment. (b) Past GD history, continuous obvious goiter, positive TSAb and unstable TSH status during therapy were associated with relapse.

P82 The Clinical Analysis of 75 Patients of Thyrotoxic Heart Disease

Hong-hua WU, Xiao-hui GUO, Yan-ming GAO
Department of Endocriology, Peking University First Hospital, Beijing, China

Objective: To analyse the clinical information of 75 patients of hyperthyrotoxic heart disease retrospectively. **Clinical information:** 75 patients of hyperthyrotoxic heart disease were included, male 30, female 45, mean age 54.3±14.4 years (17-84 years), mean course 7.5±7.6 years (l month-30 years). Heart failure was present in 47 patients (62.7%), 20 (42.6%) were left heart failure, 5 (10.6%) were right heart failure, 22 (46.8%) were both heart failure; 47 (62.7%) patients occurred atrial fibrillation, 2 paroxysmal supraventricular tachycardia, 4 frequent ventricular premature, 2 sick sinus syndrome, 28 nonspecificity ST-T change; 48 (65.8%) patients occurred left atrial enlargement, 31 (42.5%) left ventricular enlargement, 30 (41.l%) right atrial enlargement, 35 (48%) right ventricular enlargement, 18 (24.7%) both heart enlargement—3 present as dilated cardiomyopathy. 45 patients occurred mitral valve regurgitation, 45 tricuspid regurgitation, 34 aortic valve regurgitation, 3 pulmonary valve regurgitation, 6 mitral valve calcified, and 2 prolapse, l3 aortic valve calcified. 33 patients occurred pulmonary artery hypertension, 43.86±6.7 mm Hg; LVEF 60.38±18.65%. **Conclusion:** Hyperthyrotoxic heart disease is a common heart disease but easy to be made misdiagnosis and missed diagnosis. The clinical manifestation is multiformity that left, right or both heart enlargement could be involved; tachy- or bradyarrhythmia can occur in hyperthyrotoxic heart disease patients. Atrioventricular valve regurgitation was very common. Hyperthyroidism can exist in various heart disease and aggravate it. Thyroid function should be investigated to exclude hyperthyrotoxic heart disease when routine treatment was ineffective in heart disease. The key point of treatment in hyperthyrotoxic heart disease was to treat hyperthyroidism, and the prognosis was good if the treatment is prompt.

P83 The Treatment Investigation of 50 Patients of Thyrotoxic Heart Disease with Heart Failure

Hong-hua WU, Xiao-hui GUO, Yan-ming GAO
Department of Endocriology, Peking University First Hospital, Beijing, China

Objective: To investigate the treatment of 50 patients of thyrotoxic heart disease with heart failure retrospectively. **General information:** 50 patients of thyrotoxic heart disease with heart failure were included, male 18, female 32, mean age 54 years (17-84 years), course l month-30 years; 20 (40%) were left heart failure, 8 (16%) were right heart failure, 22 (44%) were both heart failure; 33 (66%) patients occurred atrial fibrillation, 36 (80%) patients occurred left atrial enlargement, 17 (38%) were left ventricular enlargement, 19 (42%) were right atrial enlargement, 22 (49%) were right ventricular enlargement, 14 (31%) both heart enlargement. LVEF 60.38±18.65%, 15 patients LVEF <50%. **Treatment:** About hyperthyroidism: 37 (74%) patients used antithyroid drugs, 11 (22%) patients used ^{131}I, 3 (6%) undertook operation. About heart failure: 38 (76%) patients used digitaloid drugs, 43 (86%) used β adrenergic receptor blocker, 42 (84%) used vasodilator agent, 41 (82%) used diuretic agent, 17 (34%) accepted antibiotics. **Therapeutic efficacy:** The heart failure symptoms significantly improved in 43 (86%) patients, 7 (14%) died. **Conclusion:** The key point of treatment in thyrotoxic heart disease with heart failure was to treat hyperthyroidism. These patients had poor response to digitaloid drugs, and may induce digitalic toxicosis in advanced stage. It also had poor response to vein vasodilator agent, but had curative effect to diuretic and β adrenergic receptor blocker. Atrial fibrillation with hyperthyroidism should enhance anticoagulation and antiplatelet treatment to prevent thromboembolism complication.

P84 A Retrospective Review on the Management of Thyroid Nodules

Chi-pun CHAN, Ip-tim LAU
Department of Medicine, Tseung Kwan O Hospital, HKSAR, Hong Kong

Objective: To evaluate the diagnostic values of fine-needle aspiration cytology (FNA) of a palpable thyroid nodule and ultrasound-guided fine-needle aspiration cytology. **Methods:** Patients who had thyroidectomy performed during the period from Jan 2000 to Dec 2005 were reviewed. Cytology was categorized as non-diagnostic, benign, indeterminate, or malignant. They were grouped into two groups according to the method of FNA: either guided by US (US-FNA) or by manual palpation (palpation-FNA). **Results:** 196 patients had thyroidectomy. 25.8% of the patients harbored malignancy. There were only 16 US-FNA. All US-FNA were performed using the 'dominant' nodule approach i.e. FNA done on the largest one if there were multiple nodules. Overall, the rates of non-diagnostic and indeterminate results that turned out to be malignant were 50% and 23.1%, respectively. 71.4% and 12.5% of benign results by US-FNA and palpation-FNA respectively subsequently turned out to be malignant ($p<0.01$). 37.8% and 21.4% of indeterminate results by US-FNA and palpation-FNA, respectively, turned out to be malignant; however, the difference was not statistically significant. The rate of non-diagnostic cytology was 2.2% in palpation-FNA group. There was no non-diagnostic result in US-FNA group. **Conclusion:** A 'dominant' nodule approach for US-FNA was inferior to palpation-FNA in accuracy. A prospective study of US-FNA guided by other sonographic features, rather than size alone, can be done to evaluate its diagnostic value. These features include hypoechogenecity, irregular margins, presence of microcalcification and intranodular hypervascularity.

P85 Study of Thyroid Nodule in 271 Cases by FNAC

Gui-zhi LU, Song DONG, Yan-ming GAO, Xiao-hui GUO
Department of Endocrinology, Peking University First Hospital, Beijing 100034, China

Objective: To investigate the clinical diagnostic value of the thyroid fine needle aspiration cytologic (FNAC) in the thyroid nodules. **Methods:** 271 thyroid FNAC results of thyroid nodules were analyzed in Peking University First Hospital from June 1994 to December 2005, as well as were contrasted with the serum levels of Tg, thyroid US and histopathologic diagnosis. **Results:** (1) The 271 FNAC results (male 37, female 274, from 15 to 75 years old) showed the incidence of malignant lesions and suspected malignant lesions were 1.48% and 5.90% respectively, the rate of benign lesions was 78.6%, goiter (29.15%), Hashimoto's Thyroiditis (26.57%) and thyroid adenoma (15.13%) were more common in benign, while unsatisfactory smear was in 14.02% of all cases. (2) Compared FNAC results of thyroid nodule with the positive rate of Tg we can see that 34 of the total 108 cases (31.48%) were Tg positive, 24 of 95 benign lesions (25.26%) were Tg positive, 3 cases were Tg positive in 4 malignant lesions (75.00%) and in suspected malignant lesions 7 of the total 9 cases (77.78%) were Tg positive. The positive rate of Tg in malignancy and in suspected malignancy was relatively higher than that in benign lesions ($P=0.004$). (3) When contrasting FNAC results of thyroid nodule to thyroid US we showed all 96 unique nodules cytological diagnosis included 86 benign (89.58%), 3 malignancy (3.12%), 7 suspected malignancy (7.29%). The rate of thyroid adenoma, goiter and Hashimoto's Thyroiditis were 31.25%, 23.96% and 21.88% relatively in benign nodules. Of 137 multiple nodules cytological diagnosis included 127 benign (92.70%), 1 malignancy (0.73%), 9 suspected malignancy (6.57%). The rate of goiter and Hashimoto's Thyroiditis were 40.87% and 37.23% relatively in benign. (4) When comparing 24 cytological and histological diagnoses, all 14 cases with a cytological diagnosis of benign diseases were confirmed to benign nodules on histological results, three of four patients with a cytological diagnosis of malignant lesions had thyroid carcinoma, five of six cases with a cytological diagnosis of suspicious lesions were confirmed to benign diseases on histological results, only one was malignant lesions. The diagnostic accuracy of FNAC was 75.00%, as well as the rates of false positive and false negative were 25.00% and 0.00% relatively. **Conclusion:** FNAC is a reliable method to define the benign or malignant nature of thyroid nodules with a high diagnostic accuracy. FNAC should be carried out actively to increase the preoperative warning rate of thyroid carcinoma.

P86 The Mouse Gonad Transcriptome Correlation Map Associated with Murine Embryonic Gonad Development

Tin-lap LEE[1], Diana ALBA[1], Vanessa BAXENDALE[1], Owen M. RENNERT, Wai-yee CHAN[1,2]
[1] Laboratory of Clinical Genomics, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD
[2] Departments of Pediatrics and Biochemistry & Molecular Biology, Georgetown University School of Medicine, Washington, DC, USA

Objective: To reveal the global genomic changes during the development of male embryonic gonads from E10.5 to E17.5 in terms of chromosomal mapping. **Methods:** To understand the mechanisms that regulate such developmental transition, we performed Serial Analysis of Gene Expression (SAGE) to profile the genes and novel transcripts expressed in male embryonic gonads at E10.5, E11.5, E12.5, E13.5, E15.5 and E17.5 with an average of 152K coverage for each of the SAGE libraries. We developed algorithms to assign the SAGE tags to the corresponding chromosomal position based on the Unigene assignments. Chromosomal "hotspots" with significant changes at any time point will be highlighted based on co-localization of gene expression, the biological implication of significant chromosomal clusters will be extracted and further analyzed by linking to different mutation and functional annotation databases. **Results:** Altogether, we have identified more than 70 000 genes in the six gonad libraries, far beyond the coverage that current Affymetrix mouse microarray platform can provide. Importantly, we observed a prominently increase in global genomic activity as the gonad develops from E10.5 to E17.5, and identified several important chromosomal regions related to the developmental processes and validated by established mouse models with gonad and developmental disorders. **Conclusion:** The study provides a new way of visualizing transcriptome data and allows rapid identification of chromosomal segments important in male embryonic gonad development. This candidate regions represent possible treatment targets and as markers in early diagnosis for male gonadal hormone disorders.

P87 Functional Study of a Novel Acrosome-specific Gene (AEP1/VAD1.3) Using Conditional Gene Knock Out Approach

Yan ZUO, Yuen-tsung TAM, William S.B. YEUNG, Kai-fai LEE
Department of Obstetrics and Gynaecology, The University of Hong Kong, Hong Kong SAR, China

Objective: *AEP1*/VAD1.3, a novel acrosome gene, was first identified from a retinol treated Vitamin A deficiency (VAD) rat model with synchronized spermatogenesis. AEP1 was expressed specifically in the testes from Day 25 when spermatids form. AEP1 immunoreactivity was localized at the acrosome region of the rat, human, monkey and porcine spermatids and spermatozoa, suggesting that AEP1 may play a significant role on acrosome formation. We hypothesized that *AEP1* deficiency may cause acrosomal defect in mouse sperm leading to infertility. **Methods:** To elucidate the role of AEP1 during spermatogenesis, a Cre-LoxP conditional gene knock out approach will be used to generate *AEP1* deficient mice for functional studies. **Results:** Conditional target vector was constructed by inserting the LoxP sites flanking the exons 2 and 3 of the AEP1 gene. A neo cassette flanked by Frt sites and a splice-receptor conjugated EGFP gene for in vitro selection of recombinant embryonic stem (ES) cells and Loxed AEP1 allele in sperm, respectively, were inserted downstream of the exons 3. In vitro recombinant occurred when the targeting vector was transformed into *E. coli* harboring Cre or Flp recombinases targeting for LoxP and Frt sites of the targeting vector, respectively. The conditional targeting construct was used to electrophorate 129/SvEv embryonic stem (ES) cells to generate conditional KO mice. **Conclusion:** The conditional knockout targeting vector for *AEP1* gene was generated and characterized. Generation of AEP1 conditional knockout animal may allow us to understand the role of AEP1 on acrosome formation and infertility in human.

(This work was supported in part by a RGC grant HKU7537/05M to KFL.)

P88 Characterization of a Novel Acrosome-expressing Protein 2 (AEP2/VAD1.2) during Spermatogenesis

Yuen-tsung TAM, Yan ZUO, John M.C. LUK, William S.B. YEUNG, Kai-fai LEE
Department of Obstetrics and Gynaecology, The University of Hong Kong, Hong Kong SAR, China

Spermatogenesis is a complex process in which undifferentiated male germ cells undergo mitotic and meiotic divisions, followed by a dramatic morphological reorganization to generate spermatozoa that are capable of fertilizing an oocyte. Maturation and differentiation of male germ cells take place within seminiferous tubules and the process occurs in a cyclic manner. Recently, we used vitamin A-deficiency (VAD) rat model to synchronize spermatogenesis in rat testis and mRNA differential display to profile the gene expression patterns in retinol re-initiated (PVA-VAD) spermatogenesis. Twelve cDNA fragments including VAD1.2 and VAD1.3 that are differentially expressed in testis were isolated. **Objectives:** To characterize the acrosome-expressing protein 2 (AEP2/VAD1.2). **Methods and Results:** AEP2 was expressed in mouse on postnatal Day 25 coincided with the formation of spermatids. The gene coding for AEP2 is located on chromosome 11E1 containing 5 exons. RT-PCR and Northern blot analysis suggested that AEP2 was highly expressed in the germ cell of mouse testis at stages X-XII. Immunohistochemistry and immunofluorescence colocalization studies revealed an acrosome-specific expression pattern in developing spermatids of rat, mouse, human, pig and monkey. Western blot analysis confirmed a protein band of size 40-kDa in both rat and mouse testis lysates. **Conclusions:** The specific temporal and spatial expression of AEP2 suggested that it may play important roles in the maturation and differentiation of spermatids during spermiogenesis.

(This work is supported in part by a RCG grant HKU7357/05M to KFL.)

P89 Regulation of Olfactomedin Isoform Expressions in Human Endometrium

Pak-yiu NG, Yunao LIU, Ernest H.Y. NG, Pak-chung HO, William S.B. YEUNG, Kai-fai LEE
Department of Obstetrics and Gynaecology, The University of Hong Kong, Hong Kong SAR, China

Objective: Previous microarray studies showed that olfactomedin 1 (OLFM1) gene is down-regulated in the receptive window (LH+7) of human endometrium. In this study, the expression of OLFM mRNA isoforms in IVF patients was studied, and correlated with the lower pregnancy rate in excessive responders (serum E_2 ≥20 000 pmol/L at hCG day) of the stimulated group. **Methods:** Quantitative PCR was carried out using TaqMan Assay probes to detect OLFM isoforms in endometria of IVF patients. Immunohistochemistry was carried out to study the localization of OLFM. Human endometrial stromal and epithelial primary cultures were isolated and treated with estrogen (E) and/or progesterone (P) to study the hormonal regulation of OLFMs. **Results:** Endometrial OLFM-1/-2 mRNA levels decrease significantly from proliferative to secretory phases of the menstruation cycle. OLFM protein was found to be highly expressed in glandular and luminal epithelium of human endometrium. Patients in natural cycles at LH+7 (n=15) had a higher expression of OLFM-1/-2 mRNA than those from stimulated cycles (15 moderate and 17 excessive responders). Yet, no significant difference was found between the stimulated groups. A significant reduction in OLFM-2 mRNA was detected under E or P treatment in cultured epithelial cells. **Conclusion:** The expression of OLFM-2 mRNA is down-regulated by hormones while the decreased expression of OLFM-1/-2 in the stimulated groups may not be the cause of lower pregnancy rate in the excessive-responders of IVF patients.

(This work is supported in part by a CRCG grant to KFL and RGC grant HKU 7514/05M to PCH.)

P90 Aberrant Angiopoietins 1 to 2 and Angiopoietin 2 to VEGF Ratio in Endometrium of Excessive Ovarian Responders during In-vitro Fertilization Treatment

Yin-lau LEE, Yunao LIU, Kai-fai LEE, Ernest H.Y. NG, William S.B. YEUNG
Department of Obstetrics and Gynaecology, The University of Hong Kong, Hong Kong, China

Objectives: Angiogenesis is a process regulated by various angiogenic and anti-angioenic factors. To study the regulation of angiogenesis in human endometrium during the implantation window, we compared the expression of Angiopoietin 1 (Ang-1), Angiopoietin 2 (Ang-2) and VEGF mRNA and protein expression in the peri-implantation endometrium of patients between natural and stimulated cycles in assisted reproduction cycles. **Methods:** Human endometrial biopsies were collected 7 days after human chorionic gonadotrophins injection in stimulated cycles or after leutinizing hormone surge in natural cycles. Ang-1, Ang-2 and VEGF mRNA and protein expression were compared in natural cycles (N), stimulated cycles with peak serum E2 <20,000 pmol/L (moderate responders, M) and ≥20 000 pmol/L (excessive responders, H) using real time PCR and Western blot analysis. **Results:** The expression of Ang-1 mRNA in endometrial tissue from Groups M and H was significantly lower ($P<0.001$) than that of Group N. However, Group H had significantly higher Ang-2 ($P=0.002$) and VEGF ($P<0.05$) mRNA expressions than that in Group M and N. Ang-1 to Ang-2 ratio was significantly lower in Groups M and H when compared to Group N, while Ang-2 to VEGF ratio was significantly higher in Group H than in Groups M and N ($P=0.006$). Negative correlation was found between Ang-1 to Ang-2 mRNA ratio and serum estrogen level ($P=0.001$). Using Western blot analysis, Ang-1 was down-regulated while Ang-2 was up-regulated in Group H. No significant difference was found in VEGF protein expression among the three groups. **Conclusion:** The decreased Ang-1 to Ang-2 ratio and increased Ang-2 to VEGF ratio in excessive responder suggested dys-regulation of angiogenesis during perimplantation period may affect normal endometrial development leading to lower implantation and pregnancy rate in these patients.

P91 Effects of Cyclic vs Sustained Estrogen Administration on Peripheral Immune Functions in Ovariectomized Mice

Jing LI, Robert W. MCMURRAY
Department of Endocrinology, Institute of Endocrinology, the First Affiliated Hospital, China Medical University, Shenyang 110001, P.R. China

Objective: To compare the effects of cyclic *vs* sustained estrogen treatments at the same total dose on peripheral immune functions in ovariectomized (OVX) adult mice. **Methods:** Six-week-old Balb/c mice were randomly assigned to three groups after OVX surgery. (1) Cyclic estradiol (E2) group: implanted with placebo pellet and *s.c.* injected with 17β-E2 in corn oil once every 4 days; (2) sustained E2 group: implanted with E2 pellet and injected with corn oil once every 4 days; (3) OVX control: implanted with placebo pellet and injected with corn oil as above. After 6 weeks of treatment, all the mice were immunized with DNP-KLH. Ten days later, they were sacrificed. A series of peripheral immune functions were determined. **Results:** Total E2 doses received from the two ways of E2 administration were almost same (cyclic E2 group 44.8 μg; sustained E2 group 44.2 μg). There was no significant difference in uterine weight gain between the two E2 groups. Both cyclic and sustained E2 treatments significantly reduced the percentage of splenic $B220^+sIgM^+$ cells, enhanced IFN-γ production and suppressed IL-6 production from Con A-stimulated splenocytes; no significant differences were found in these effects between the two ways of E2 administration. Either E2 treatment had no influence on splenic weight or the proportion of splenic $CD4^+$ or $CD8^+$ T cells, Con A-stimulated IL-2 or IL-4 secretion or proliferative response. Serum anti-DNP IgG1, IgG2b, and IgG3 levels were significantly increased in both cyclic and sustained E2 groups compared to OVX control; however, there were no significant differences in these anti-DNP levels between the two E2 groups. **Conclusion:** The two ways of estrogen administration (cyclic *vs* sustained) at the same total dose have no differential effects on peripheral immune functions in OVX mice.

P92 Preparation of Protein and Polyclonal Antibody of AD-004 and Preliminary Study on Its Function in the Adrenal and Testis

Jie QIAO, Xia CHEN, Sheng-xian LI, Xue-song LI, Qin-yun MA, Huai-dong SONG, Ming-dao CHEN
Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai Clinical Center of Endocrine and Metabolic Diseases, Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200025, China

Objective: AD-004 was first identified from human adrenal by our group, which acted as a novel adenylate kinases through crystal structure analysis. However, the physiological function of the AD-004 gene is remained unknown. To prepare rabbit antibody against mouse AD-004 by expressing AD-004 in the prokaryotic expression system and identify its distribution in the testis and adrena. **Methods:** The full-length cDNA of mouse AD-004 was cloned into PET28 plasmid and the protein was induced in *E. coli* BL21 bacteria by adding IPTG and then purified by Ni2+-NTA column. The purified protein was used as an immunogen to prepare polyclonal antibody (pAb) of AD-004. The specificity of the antibody was detected by Western blotting. Using this pAb, we carried out immunohistochemical assay in the mouse adrenal and testis. **Results:** 6His fused AD-004 could be expressed efficiently in the prokaryotic system. Western blot analysis showed that the polyclonal antibody could bind to purified AD-004 with high specificity and sensitivity. AD-004 was abundantly identified in the adrenal medulla and mainly expressed in the Leydig cells of testis interstitial. **Conclusion:** The mouse protein of AD-004 was obtained from the prokaryotic expression system. The rabbit anti-AD004 antibody has been prepared successfully. The fact that AD-004 protein was specifically localized in the interstitial of testis, suggested that AD-004 may play a role in the synthesis of sex-steroid hormone.

P93 Study on Cytogenetics of 23 Cases of Testicular Deficiency

Wan-chun CAI, Zheng ZHANG
Dept. of Endocrinology, Fifth Hosp. of PLA, China

Objectives: In order to study cytogenetics analyses of 23 cases of testicular deficiency. **Methods:** The volume of testis ≤8 ml was inspected at single side or both sides in the patients. Two main clinical types are classified according to the serum level of gonadotrophin: high gonadotrophin type (17 cases) and the low one (6 cases). **Results:** The karyotypes of chromosome of 23 patients were distributed as follows: Klinefelter's Syndrome 11 cases, 47,XXY (9/11) and 46,XY/47, XXY (2/11); Sertoli cell only Syndrome 2 cases, 46,XY: male Turner's Syndrome 1 case, 45,XO/46,XY; Testicular Feminization Syndrome 2 cases, 46,XY; real Hermaphrodism 1 case, 46,XX/46,XY. Above 17 cases belonged to Hypergonadotrophic Hypogon adism. The rest 6 cases (pituitary dwarfism 3 cases; Frohlich's Syndrome 2 cases; Kallman Syndrome 1 case) belonged to Hypogonadotrophic Hypogonadism and the karyotypes of chromosome were 46,XY. **Conclusion:** The inspection of cytogenetics was clinically important to the patients with testicular deficiency.

P94 Studies of Caveolin Expression and Caveolar Function in the Rodent Testes and Testicular Cell Lines

Chak-leung AU, Sin-man KWOK, Lam YEUNG, Lai-fong CHAN
Department of Physiology, The Chinese University of Hong Kong, Shatin, Hong Kong SAR

Objective and Methods: In view of the importance of caveolae in regulating many aspects of cellular functions such as signal transduction, cholesterol trafficking, oncogenic transformation, transcytosis, etc, the aims of the present study were: (a) to examine the caveolin mRNA and protein expression in the rodent testes and testicular cell lines using immunohistochemisty, RT-PCR and Western blot, (b) to identify proteins associated with the caveolar fraction using cold triton extraction and sucrose density gradient separation, and (c) to examine the effect of caveolar disruption on LH signalling and androgen production by a mouse Leydig cell line (MLTC-1). **Results:** Cav-1 immunoreactivity was localized in Leydig cells with variable staining found in Sertoli cells and spermatocytes depending on the stages of the spermatogenic cycle. These cellular localizations were confirmed using RT-PCR and Western blot on cDNA and protein prepared from adult rat testes, primary testicular cells or cell lines. Cav-1α/β, phosphoCav-1 and Cav-2α/β were shown to be present. In the caveolar membrane fraction, the following molecules were identified: LH receptor, Gsα protein, ERK 1/2, c-src tyrosine kinase, insulin receptor, connexin 43, calcium sensing receptor and inducible nitric oxidase synthase. In MLTC-1 cells, prior treatment with methyl-β-cylcodextrin (MBCD, 5 mM for 3 h) to disrupt caveolae led to reduction in hCG-induced steroidogenic acute regulatory (StAR) protein mRNA expression at 1-6 h after treatment. Total androgen production by MBCD-treated cells recovered when replaced with 22R-hydroxycholesterol (5 μg/ml), while StAR mRNA expression remained suppressed. **Conclusion:** The present data demonstrated an important role of caveolae in LH/hCG signaling in Leydig cells.

P95 Effect of Recombinant Human Growth Hormone Therapy in Children with Idiopathic Short Stature of Middle or Later Period Puberty

Rong XIANG
Department of Pediatrics, The Nan Kai Hospital of Tianjin 300100, China

Objective: To assess the efficacy of recombinant human growth hormone (rhGH) in treatment of idiopathic short stature (ISS) children with the middle or later period of puberty. **Methods:** 19 children with ISS middle or later period of puberty who were divided into 3 groups according to their BA. A group consisted of 10 cases BA was 13.0-13.9 years, 7 boys and 3 girls; B group was composed of 6 cases BA was 14.0-14.9 years, 4 boys and 2 girls; C group was composed of 3 cases BA was 15.0-16.0 years, 2 boys and a girl. The enrolled children were treated with subcutaneous injection of rhGH (0.18-0.2 IU/kg) daily before sleep for six months. **Results:** The mean height of 3 groups increased from 138.4± 1.2 cm, 144.2±1.8 cm, 52.8±4.4 cm to 144.4±1.6 cm, 148.7±1.2 cm, 155.3±6.5 cm respectively. The growth velocity of 3 groups increased strikingly during first months, but decreased during 4-6 months in B and C groups, The change of the growth velocity among 3 groups is statistically $P<0.05$. There were no change in mean body weight and BA during the whole course of rhGH therapy. Serum thyroxine, glucose and blood, urine Rt levels remained normal in all ISS children. **Conclusion:** rhGH is an effective therapy for promoting growth in children with ISS middle or later period of puberty, but must be observed closely.

P96 Analysis of a Long-term Follow-up in 30 Cases of Adolescent Pituitary Hyperplasia

Hui PAN[1], Hui-juan ZHU[1], Xin LI[1], Ming-ming HU[1], Feng GU[1], Xiao-chuan PING, Xue-yan WU[1], Tao ZHANG[2], Zi-meng JIN[1], Yi-fan SHI[1]
[1] Department of Endocrinology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing 100730, China
[2] Department of Radiology, Peking Union Medical College Hospital, Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing 100730, China

Objective: Pituitary hyperplasia is common in puberty children. To analyse the causes of misdiagnosis and mistreatment in adolescent pituitary hyperplasia by the summary of clinical character and the imaging changed in 30 cases which were classified as pituitary microadenoma because of the enlargement of pituitary. **Methods:** We had a prospective study on 30 cases visited our hospital from 1996 to 2006. The staging of pubic hairs and breast were according to Tanner stage, and measuring volume of testicles was performed using Prader Orchidometer. All the hormones tested and imaging examination were normal at first visit in this group, then followed up to evaluate the same exams every 6 to 12 month. **Results:** The mean age of the 30 patients was 14.3±2.5 (ranged from 12 to 19), and the mean body height was 1.61±0.07 m, the mean weight was 52.5±10.2 kg. All of them have begun puberty onset. In 18 female patients, 8 cases of them visited for routine medical examinations, 6 of them visited for short stature, 4 of them visited for menoxenia. In 12 male patients, 6 cases of them visited for fat and small genitalia, 4 of them visited for routine medical examinations, 1 of them was classified as pituitary microadenoma by MRI of saddle area in our hospital, the other one had a MRI exam because his mother was diagnosed as prolactinoma. Each of the 30 patients had more than twice MRI exams in our hospital, and on MR image, 24 of them showed convax in the superior margin of pituitary, 8 of them showed dissymmetry of pituitary and bias of stalk hypophysial, 7 of them showed the left low signal in T1 and high signal in T2 but the diameters were less than 3 mm while the enhancement showed homogeneous. In 18 female patients, the coronal height of the pituitary gland was 8.8±2.3 mm, the coronal width was 14.7±2.0 mm, and the anteriorposterior diameter was 9.3±2.1 mm. In 12 male patients, the coronal height of the pituitary gland was 8.1±2.3 mm, the coronal width was 14.0±2.1 mm, and the anteriorposterior diameter was 8.8±1.4 mm. In 20 of the patients (4 male and 16 female), the coronal height of the pituitary gland was more than 9 mm, while it was more than 10 mm in 12 cases (2 male and 10 female). 28 patients of the group have had one more MRI exam after 1 or 2 years, and on MR image, 8 of them showed no changed in the saddle area images and the diameters, the other 20 patients (4 male and 16 female) showed the coronal height of the pituitary gland was 6.1 ±2.0 mm, the coronal width was11.0±1.6 mm, and the anteriorposterior diameter was 6.5±1.1 mm, which were smaller than previous result. The PRL levels in two patients were higher than normal (one was 2.89 pmol/L, and the other was 1.91 pmol/L, the normal level was less than 1.20 pmol/L). One of the two had taken Metoclopramide because of nausea, and the level of PRL became 0.62 pmol/L after stop using the drug for one month. During the follow-up from 6 months to 2 years, no abnormality was found in the physical examination and hormone test results. 28 cases were misdiagnosed as pituitary microadenoma by local hospital, and 2 of them were treated with resection of pituitary adenoma with transsphenoidal but the pathological findings revealed the samples were normal. And 8 of the 28 cases were classified as pituitary microadenoma and considered radiation therapy, then visited our hospital, and one of the 8 patients appeared a severe anxiety disorders which need antianxiety treatment after classified as pituitary microadenoma. **Conclusions:** The most of pituitary enlargement patients (the coronal height of the pituitary gland is more than 9 mm in prepuberty adolescents) without any symptoms in clinic are physiological pituitary hyperplasia. It could be considered physiological pituitary hyperplasia when the MRI and hormone examinations are normal. To avoid misdiagnosing and mistreating such as pituitary operation, biopsy or Gamma knife treatment, we need careful MRI exam and enhancement, complete hormone tests, and long-term follow-up when it's necessary.

P97 Energy Expenditure in Institutionalized Developmentally Delayed Children Using Indirect Calorimetry

Peggy Siu-pik LEE[1], Chung-hung KO[2], Ka-ming CHEUNG[2], Alex Kwok-hing CHAN[2], Wan-ting TSE[2], Heung-chin CHUI[3]
[1] Dietetic Department, United Christian Hospital, 130 Hip Wu Street, Kwun Tong, Kowloon, Hong Kong SAR
[2] Pediatric Department, Caritas Medical Centre, 111 Wing Hong Street, Shamshuipo, Hong Kong SAR
[3] Geriatric and Medical Department, Caritas Medical Centre, Hong Kong SAR

Objective: To compare measured energy expenditure (MEE) using indirect calorimetry and three published equations including the Recommended Daily Allowance (RDA), a formula specific for estimating energy needs in children with cerebral palsy (CP) and energy needs calculated by using body surface area (BSA). **Methods:** A total of 8 patients residing at the Pediatrics Developmentally Delayed Unit at Caritas Medical Center, Hong Kong were recruited. Energy expenditure was obtained using the three predicted equations and direct measurement by indirect calorimetry. **Results:** Energy expenditure of six subjects were obtained from each method including: MEE 541 kcal (±63 kcal), BSA 1073 kcal (±95.7 kcal), RDA 1882 kcal (±134 kcal) and CP 1740 kcal (±197 kcal). Friedman test showed that the three predicted equations were significantly different from the predicted ones (p=0.001). Intraclass correlation coefficient (ICC) =0.4072 (p=0.1984). The energy expenditure measured by indirect calorimetry was only 50.4% of the BSA, 28.7% of the RDA and 36.8% of the CP equations. In addition, the pre- and post-test vital signs variations including body temperature (p=0.302), pulse rate (p=0.088) and blood pressure (p=0.075) were found not to be statistically significant with the introduction of sedatives. **Conclusion:** The preliminary results showed that measured energy expenditure varied significantly from predicted equations and interrater reliability was very poor between the 4 methods. Pre- and post-test vital signs were not significantly affected by the use of sedatives and thus ensure the accuracy of the measured result. Nonetheless, a larger sample size would be prudent to enhance power of the study.

P98 The Growth and Biochemical Change in an Adult Patient with Untreated Congenital Hypothyroidism

Kit-yu LAU, Lap-ming WONG
Department of Paediatrics and Adolescent Medicine, Tuen Mun Hospital, Hong Kong

With the implementation of neonatal screening, untreated congenital hypothyroidism is rare nowadays. An 18-year-old gentleman, who was a new immigrant, presented to us with extreme short stature (114 cm, i.e. 35 cm below 3% centile), truncal obesity, delayed development and accidental finding of hypercholesterolemia. Investigations revealed primary hypothyroidism (TSH 882 mIU/L), hyperprolactinemia (prolactin 1332 mIU/L) and dyslipidemia (total cholesterol 13 mmol/L). Further imaging revealed ectopic thyroid gland, pericardial effusion and bilateral basal ganglion calcifications. L-T4 replacement was started, resulting in gradual normalization in thyroid function, prolactin level and lipid profile. There was a significant improvement in his obesity and generalized edema. A 1 cm height gain was noted 2 months after the initiation of treatment. **Conclusion:** There is an overall improvement in physical, biochemical and psychosocial parameters after L-T4 treatment in patients with long standing untreated hypothyroidism.

P99 An Adolescent Girl with Delayed Puberty Owing to Macroprolactinoma

Lap-ming WONG, Kam-fuk FOK, Yee-ling KAN
Department of Paediatrics and Adolescent Medicine, Tuen Mun Hospital, Tuen Mun, Hong Kong SAR, China

A 14-year-old girl was presented with delayed thelarche, premature adrenarche and recurrent headache for 2 years. She had no other systemic symptoms and past health was good. Physical examination, including visual perimetry and fundi, revealed no significant abnormality and her pubertal stage was Breast 1, Pubic 3 and Axilla 1 according to Tanner staging. Full blood count, ESR and thyroid function test were unremarkable. Gonadotropin-releasing hormone stimulation test showed pubertal response. Prolactin level was markedly raised at 154 350 miu/L. Imaging of brain showed a 6.3 cm tumor in sellar and para-sellar region, which compressed left frontal lobe and extended to sphenoid sinuses and left infra-temporal fossa. Bromocriptine was started and prolactin level returned to near normal 3 months later. The drug was well tolerated with no complication of tumor or treatment noted. A repeat MRI brain demonstrated a shrinkage of tumor. **Conlcusion:** Macroprolactinoma is a rare cause of pubertal delay in girl and bromocriptine treatment is effective.

P100 A 12-year-old Girl with Virilisation and Right Ovarian Mass

Lap-ming WONG, Wing-lai MAK, Sau-har LUK, Yin-fong KWOK
Department of Paediatrics and Adolescent Medicine, Tuen Mun Hospital, Tuen Mun, Hong Kong SAR, China

A 12-year-old Chinese girl was referred for acne, deepening of voice, prominent "Adam apple" and hirsutism involving face and four limbs for 2 years. Secondary amenorrhoea occurred at 3 months before presentation. Past health was unremarkable. Body weight and height corresponded to mid-parental height percentile. Physical examination confirmed the features in history. The pubertal stage was B1P5A3M1, according to Tanner Staging. Pubic hair extended upwards along linea alba. Clitoromegaly was noted at 2.8 cm long and 1 cm in diameter while the rest of genitalia was normal. No abdominal mass was palpable. Blood pressure was 126/66 mm Hg. Serum oestradiol and progesterone levels were at 126 pmol/L and 18.26 nmol/L respectively. Serum FSH, LH and prolactin were at 7.6 iu/L, 3.4 iu/L and 367 miu/L respectively. Serum α feto-protein, β-HCG, CEA and CA 125 were not raised. The karyotype was 46XX. However, serum testosterone and DHEAs were raised at 7.93 nmol/L (ref 0.1-2.46) and 12.1 μmol/L (ref <8) respectively. Short Synacthen test using Tetracosactide 250 mcg intravenously showed that both the baseline and the peak 17-hydroxyprogesterone levels were more than 60 nmol/L, while the baseline and the peak cortisol levels were at 113 nmol/L and 450 nmol/L respectively. Bone age was at 15.3 years (Tanner and Whitehouse). CT scan of abdomen and pelvis showed that both adrenal glands were normal. A 57x30x40 mm soft tissue mass with small calcified foci were found at the right ovary. Urine for steroid profile revealed increased excretion of androgen metabolites. Venous sampling procedure at various sites was carried out. Testosterone level was markedly raised from right ovarian vein. At laparotomy, a firmed mass of right ovary was identified and removed. Histology confirmed fibrothecoma. Serum testosterone and DHEAs returned to normal 2 days after the operation. The patient then resumed thelarche and had menarche 2 months later. At the latest follow-up, which was 5 years after the operation, she had full pubertal development and regular monthly menstrual cycles. Deepening of voice and laryngomegaly remained same, though clitoromegaly became less prominent.

P101 Cyp21 Gene Point Mutations Study in Twenty-three 21-Hydroxylase Deficiency Patients

Hui-jie ZHANG, Jun YANG, Shou-yue SUN, Li-hao SUN, Ting-wei SU, Yu-hong CHEN, Jie HONG, Jian-min LIU, Xiao-ying LI, Guang NING
Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrine and Metabolic Diseases, Department of Endocrinology and Metabolism, Ruijin Hospital, Medical School of Shanghai Jiaotong University, China

Objective: The major cause of congenital adrenal hyperplasia (CAH) is 21-hydroxylase deficiency, which accounts for 90%-95% of all cases in most population. Our study was conducted to characterize the molecular basis of the 21-hydroxylase deficiency and to obtain the spectrum of the CYP21 gene mutations in a group of Chinese patients, and analyze the relationship of genotype and phenotype. **Methods:** To detect the distribution of gene mutations in Chinese population samples from 23 patients with 21-hydroxylase deficiency, including their parents or brothers/sisters. Blood samples were obtained for extraction of peripheral blood lymphocytes. The primary PCR amplified two overlapping specific DNA fragments of CYP21 gene. Then, the DNA fragments are used to sequence directly. Furthermore, the promoter region of CYP21 gene is amplified and sequenced when the coding regions had no mutations. Heterozygosis was confirmed by subcloning. **Conclusion:** Through analysis of 23 patients with 21-hydroxylase deficiency, the most common mutations was I2g and I172N which present on 34% affected alleles, then followed by 7DELL, S494N, R103K, E3A8nt, Q319X, R357W, accounting for 32%, 31%, 4%, 4%, 4% of all identified mutations respectively. 15 kinds of mutations were found in 23 patients with 21-hydroxylase deficiency, including 4 kinds of mutations that was never reported in Chinese population. Compoud mutations were identified in 21 patients, accounting 91%. Totally, 52% of alleles could be detected to present more than two mutations. Our study provide important information for diagnosis, treatment and genetic counseling.

P102 Clinical and Molecular Genetic Study of Three Kindreds with Five Patients Suffering from P450c17 Deficiency

Jie QIAO, Yan-yun GU, Xia CHEN, Jing GONG, Ying-li LU, Yu-guo YANG, Wan-ling WU, Huai-dong Song, Ming-dao CHEN, Jia-lun CHEN
Department of Endocrinology, the Ninth People Hospital, Shanghai Jiao Tong University, Shanghai 200011, Shanghai Institute of Endocrinology, Ruijin Hospital, Shanghai Jiao Tong University, Shanghai 200025, China

Objective: To study clinical and molecular genetic features of three kindreds with five patients suffering from P450c17α deficiency. **Methods:** Serum ACTH and steroid hormones of the family members were measured by radioimmunassay, enzyme-immunoassay. A short ACTH test was performed in heterozygotes without apparent symptoms. Eight exons of CYP17 gene were screened by polymerase chain reaction (PCR) and autosequencing. **Results:** The five patients with karyotype 46,XX presented with hypertension, hypokalemia and hypogonadism although they had different onset of age. One of the kindred contained three sisters suffering from the disease. All the five patients had abnormally high levels of ACTH, low levels of cortisol and sex steroids, low levels of 17-hydroxyprogesterone. In short ACTH test subtle metabolic abnormality appeared in some of the family members. 5 patients were detected to be complex heterozygotes with Asp^{487}-Ser^{488}-Phe^{489} deletion in one allele of CYP17 gene. Other mutations were founded in the exons 6 of CYP17 gene. **Conclusion:** The five patients with a classical presentation were diagnosed as P450c17 deficiency. The activity of 17α-hydroxylase and 17,20-lysase were severly abolished. Short ACTH test represent useful markers for identification of heterozygotes. Asp^{487}-Ser^{488}-Phe^{489} deletion was the most frequent mutation in the CYP17 gene in China. It is of great value to establish measures screening heterozygotes with 9bp deletion.

P103 17-Hydroxysteroid Dehydrogenase 3 Deficiency—One Case Report

Jun YANG, Guang NING, Jia-lun CHEN, Man-yin XU, Jie HONG, Li-hao SUN, Xiao-ying LI
Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrine and Metabolic Diseases, Department of Endocrinology and Metabolism, Ruijin Hospital, Medical School of Shanghai Jiaotong University, China

Objective: 17-hydroxysteroid dehydrogenase 3 (17HSD3) deficiency is one of the rare cause of male pseudohermaphroditism. We reported 1 case here and reviewed the literature in order to improve the knowledge of the disease. **Materials and method:** The clinical data of the patient were collected and analysed, and using PCR sequencing and subcloning, the genetic diagnosis was performed. **Results:** Clinical data showed the characteristics of male pseudohermaphroditism. Hormone assay showed the precursors of testosterone were accumulated and s HCG stimulation test and confirm the diagnosis. **Conclusions:** The diagnosis of 17β HSD3 deficiency should be considered in those who was male pseudohermaphroditism without abnormality in adrenal steroid biosynthesis, and absent mullerian ducts and normal wolffian duct, and virilize at puberty with gynecomastia. HCG test provides the evidence in the clinical and gene detection will confirm the diagnosis.

P104 Exploring the Issue about Surgical Intervention in Intersex of Children

Su YAN[1], Wei-jue XU[1], En XU[1], Li-ping SHENG[1], Jian-wen CHEN[1], Dai-xian GONG[1], Hong-da ZHU[2], Xiao-ying LI[2], Guang NING[2]
[1] Department of Pediatric Surgery, Ruijin Hospital, Shanghai Jiaotong University Medical School 200025, China
[2] Department of Endocrine, Ruijin Hospital, Shanghai Jiaotong University Medical School 200025, China

Objective: To explore the issue about the surgical intervention in the intersex of children. **Methods:** Analyzed 26 patients suffering from intersex in our department during 1993-2006 years, 4 patients were FPH with 46 XX karyotype, 5 were TH with 46XX in 3 cases and 46XX/46XY in 2 cases, 16 patients were MPH with 46XX in 2 cases and 46XY in 14 cases, another one case was partial gonadal dysgenesis with persistence of Mullerian duct syndrome and had a 46XX/46XY. **Results:** 5 patients with FPH were performed reduction clitoroplasty or were done flap vaginoplasty simultaneously, and treated with hydrocortisone after operation, the prognosis is good. In 5 TH cases, 2 cases were removed ovotestis and changed the rearing gander from female to male, 1 kept the female gender and 2 kept the male gender. In 16 FPH cases, 3 cases kept female gender and 7 changed female to male gender, 1 patient was done the orchidopexy and will keeping female gender until the puberty, most of the patients who changed their gender were younger than 4 years old. All patients who choose the male gender should be done cosmetic operation several times. The girls have male gender identity and boys have female gender identity in most of MPH. One testis was malignant in the patient with partial gonadal dysgenesis, 3 patients who were MPH had the mammary development. **Conclusion:** Advancing of social, the gender choice of intersex will be diversity, and individuality, surgical intervention should be considered carefully.

P105 Evaluation of Efficacy of Repaglinide Versus Glibenclamide in Newly Diagnosed Type 2 Diabetic Patients Using Continuous Glucose Monitoring System

Ju-ming LU, Xian-ling WANG, Chang-yu PAN, Yi-ming MU, Jing-tao DOU, Jian-ming BA
Department of Endocrinology, Chinese PLA General Hospital, Beijing 100853, China

Objective: To evaluate the efficacy of repaglinide on daily blood glucose (BG) profiles versus glibenclamide in newly diagnosed type 2 diabetic (T2DM) patients using continuous glucose monitoring system (CGMS). **Methods:** This was an open, randomized, controlled trial with 20 newly diagnosed T2DM patients assigned to treatment with either repaglinide (0.5 mg tid) or glibenclamide (2.5 mg bid) for 4 weeks. Daily BG monitoring by CGMS for 3 days was performed at week 0 and week 4. Fasting and 2-hour post breakfast plasma glucose (PG) and insulin as well as serum fructosamine were also assessed in both groups at week 0 and week 4. **Results:** A total of 20 newly diagnosed T2 DM patients were enrolled, 10 subjects (6 males and 4 females, 45±5 years old) were randomized to the repaglinide group and 10 patients (5 males and 5 females, 47±4 years old) to the glibenclamide group. Using CGMS data collected during the 3-day monitoring, compared to week 0, there were significant reductions in BG at all time points after 4 weeks of treatment in both groups except for post-lunch BG in the glibenclamide group. Between group comparisons show greater reductions in mean BG levels (11.5±1.4 to 8.3±0.7 mmol/L vs 11.0±1.0 to 9.2±0.8 mmol/L, p=0.003), BG excursion as shown by standard deviation (1.8±0.3 to 1.2±0.3 vs 1.8±0.2 to 1.7±0.3 mmol/L, p<0.001), 2h post prandial (12.2±1.3 to 8.5±0.6 vs. 12.3±1.1 to 10.4±0.7 mmol/L, p=0.002) and peak postprandial BG levels (14.4±1.1 to 9.7±0.8 vs. 14.0± 1.3 to 11.8±1.0 mmol/L, p=0.000) in the repaglinide than the glibenclamide group. Reduction in BG levels at 3 am was less in the repaglinide than the glibenclamide group (8.4±0.9 to 7.5±0.5 vs. 8.2±0.6 to 6.4±0.3 mmol/L, p=0.017). There were also greater reductions in fructosamine levels in the replaginide than the glibenclamide group. Two-hour plasma insulin levels increased significantly only in the glibenclamide group. **Conclusions:** Using CGMS, 4-week treatment with repaglinide was associated with greater and smoother reduction in BG levels than glibenclamide.

P106 The Observation of Therapeutic Efficacy of NoVoMix30 for Patients with Type 2 Diabetes after CSII Therapy

Yue-hong LIU, Dong-hong WU, Dian-xin LIU, et al
Department of Endocrinology, The First Hospital of Harbin, 150010, China

Objective: To investigate the efficacy, safety and patients satisfaction of NoVoMix30 therapy for patients with type 2 diabetes after CSII therapy. **Methods:** A total of 40 type 2 diabetic patients (FBG <7.0 mmol/L, 2h BG <10.0 mmol/L with CSII therapy) were randomly assigned (1:1) to NoVoMix30 group (twice daily) or control group (bolus human regular insulin and basal NPH insulin) in a 12-week study. Efficacy was assessed with HbA1c and seven-point blood glucose. Safety was assessed with the incidence of hypoglycemia. Treatment satisfaction was determined with a self-administered questionnaire. **Results:** HbA1c values were not significantly different between the two groups (6.94± 0.18% vs 6.83±0.21%, *P*>0.05). Safety assessments were comparable for both treatment groups. A total of 90% of NoVoMix30-treated subjects satisfied NoVoMix30 (twice daily) therapy for reasons of convenience, flexibility, ease of use. **Conclusion:** NoVoMix30 therapy provided efficacy, safety, convenience and flexibility for type 2 diabetes after CSII therapy.

P107 Evaluation of the Superiority of Insulin Glargine as Basal Insulin Replacement by Continuous Glucose Monitoring System

Ju-ming LU, Xian-ling WANG, Chang-yu PAN, Yi-ming MU, Jing-tao DOU, Jian-ming BA
Department of Endocrinology, Chinese PLA General Hospital, Beijing 100853, China

Objective: To compare the effects of glargine with NPH on 24-h blood glucose profiles and hypoglycemia events in type 2 diabetic patients, whose blood glucose were not well controlled with sulphanylureas, by continuous glucose monitoring system (CGMS), and to evaluate the superiority of glargine as basal insulin replacement in type 2 diabetic patients. **Methods:** 24 cases with T2DM, whose blood glucose was not well controlled with sulphanylureas, were enrolled. At first, they were treated with extended-release glipizide (glucotrol XL) 5 mg before breakfast daily for 2 weeks, then randomized to glargine combined with glucotrol XL group (16 cases) or NPH combined with glucotrol XL group (8 cases) and treated for 12 weeks. CGMS were carried at the 2nd week after treatment with glucotrol XL, and at 12th week after randomization. The differences of blood glucose profiles and nocturnal hypoglycemia events between two groups were compared. **Results:** (1) The HbA1c levels were significantly reduced in both groups with glargine or NPH treatment compared with patients treated only with glucotrol XL (from 8.77±1.18% to 7.62±0.98% in glargine group, and from 8.75±1.24% to 7.43±0.73% in NPH group) (P<0.05). (2) When FPG were well controlled in both groups (glargine group vs NPH group: 6.0±1.0 vs 5.8±1.3 mmol/L), the blood glucose level at pre-supper (6.0±0.7 vs 7.1 ±1.0 mmol/L) and bedtime (7.8±1.2 vs 9.2±2.0 mmol/L) were lower (P<0.05) respectively, the blood glucose at 3:00am (5.1±0.9 vs 4.2±0.8 mmol/L) were higher (P<0.05), the rate of nocturnal hypoglycemia (1/16 vs 4/8) were less (P=0.028), $T_{PG\leq 3.0mmol/L}$ at night were lower (2.56±1.79% vs 5.88±1.96%) in glargine group than those in NPH group. (3) CGMS showed that the blood glucose profile in glargine group was in a more smoother level during night, and the excursion was also smaller during daytime than that in NPH. **Conclusion:** T2DM patients whose blood glucose levels were not well controlled with sulphanylureas alone, after combined treatment with glargine at bedtime, had better controlled blood glucose at pre-supper and bedtime, and less nocturnal hypoglycemia compared with NPH when FPG levels were well controlled in both groups. So glargine may be a more ideal basal insulin replacement than NPH in type 2 diabetic patients with poor glucose controlled with sulphanylureas only.

P108 The Clinical Value Evaluation of 24 Continuous Glucose Minitoring and Utilizing Value to Insulin Bump Treatment

Dong-hong WU, Bin-hua XU, Huan-yu ZHAO, Yue-hong LIU, Yan SU, Yao CHENG, Dian-xin LIU, Jie LIU, et al
Harbin First Hospital, Harbin 150001, China

Objectives: To study the clinical value evaluation of continuous glucose minitoring (CGMS) and continuous subcutaneous insulin infusion (CSII). **Methods:** The 62 study subjects were wore CGMS and observe the 24 hours blood glucose, meanwhile 30 of them were utilizing CSII therapy, compared with 42 CSII control with using SMBG. The 62 study subjects were wore CGMS for 3-5 days (4-7 capillary glucose measurements were taken each day). **Results:** (1) The sensor showed close agreement to capillary blood glucose meter readings, with a median daily correlation of 0.92; (2) high and low pattern identification was greater with the CGMS (191 patterns) than with logbook records (42 patterns) for 62 patients. (3) In subjects of HbA_1C (6.2±0.8%), experienced a total of 98 hypoglycemic events, and 9 of these patients experienced 54 events where the glucose levels were <40 mg/dL, but none of them experienced that the glucose levels were <40 mg/dL with SMBG. (4) FBG and PBG in CSII with CGMS and in CSII with SMBG were all significantly low, but not statistically significant in two groups. But the time of glycemic control targets in CSII with CGMS were significantly lower than that in CSII with SMBG. The rate of hypoglycemia in CSII with CGMS were significantly lower than that in CSII with SMBG. **Conclusion:** The CGMS provides a comprehensive, reliable picture of an individual patient's blood glucose levels throughout the monitoring period. The information that was obtained could be used to alter the diabetes regimen and impact glycemic outcome.

P110 Predictors for the Long-term Remission of Type 2 Diabetes Induced by Short-term Intensive Insulin Treatment

Bo ZHANG, Ya-li AN, Qiu-hong GONG, Ying SHUAI, Shi BU, Yan-yan CHEN, Jin-ping ZHANG, Xue-li LIU, Wen-ying YANG, Guang-wei LI
Department of Endocrinology, China-Japan Friendship Hospital, Beijing 100029, China

Objective: To investigate the predictors for the long-term remission of type 2 diabetes induced by short-term intensive insulin treatment. **Methods:** 54 cases of diabetic patients with high level of hyperglycemia and duration less than 5 years received the two-week long intensive insulin treatment. The standard meal test and the IVGTT were done at the baseline and 24 hr after treatment respectively. Remission meant that the diabetic patients should maintain the desired glycaemic control without any hypoglycaemic agents within at least one year after the short-term intensive insulin treatment. **Results:** The duration and fasting plasma glucose (FPG) level 24 hr after treatment were lower in remission group (n=31) than those in non-remission group (n=23) by ANOVA analysis, while the TNF-α at baseline, AIR (peak value of insulin level in IVGTT) and $\Delta I_{30}/\Delta G_{30}$ levels after treatment were higher in remission group, $P<0.05$. Logistic regressive analysis showed that only duration (Log) was independently related with the remission, $\beta= -1.291$, $P<0.01$. The FPG after treatment ($\beta= -1.509$, $P<0.01$) and ΔTNF-α(Log) ($\beta=2.856$, $P<0.05$) were independent predictors for the remission if the duration was not taken into account. **Conclusion:** The duration is the most important predictor for the long-term remission event. The contribution of TNF-α deserves a further investigation.

P111 Effect of Insulin Therapy on Testosterone Deficiency in Sexual Dysfunctional Men with Early Type 2 Diabetes

Chun-sheng HAN, Xiu-fang DING, Xiang-ping LUAN, Wen-hua LI
Department of Endocrinology, the Third Affiliated Hospital, Qiqihaer Medical University, Heilongjian Province 161000, China

Objective: Sexual dysfunction that is often found in the male patients with type 2 diabetes results from testosterone deficiency associated with insulin deficiency. We have therefore studied the effect of insulin treatments on testosterone deficiency in sexual dysfunctional men with type 2 diabetes. **Methods:** The randomized male-outpatients with type 2 diabetes treated with oral hypoglycemic agents complained of sexual disorder within previous 1 year. The patients were further characterized by measurement of serum testosterone concentration under the lowest limit (normal range, 260-1320 ng/dl), and treated with insulin replacing oral hypoglycemic agents to attempt to reach a set standard (fasting plasma glucose <7.0 mmol/L, postprandial glucose <7.8 mmol/L; hemoglobin A_{1C} <6.5%). The differences between pre and post treatments after three months of insulin therapy were evaluated by serum testosterone concentration and hemoglobin A_{1C} levels. **Results:** In the present study from seventeen male patients, the mean age were 46.5±5.83 y, mean body mass index 25.7±2.2 kg/m^2 and mean duration of diabetes 8.2±4.6 months. There was a significant difference in serum testosterone concentration measured in all patients between pre and post treatments with insulin injection (155.5±74.8 ng/dl vs 344.0±198.9 ng/dl, $p<0.05$). Also was showed another significant difference by contrast between 11 patients (11/17, 64%) with the serum testosterone concentration returned to normal range (410.5±22.3 ng/dl) and 6 patients (6/17, 36%) still under normal range (215.7±29.6, $p<0.05$), but no significant difference with hemoglobin A_{1C} between both of the 11 and 6 patients (6.4±0.9% vs 6.8±0.3%, $p<0.05$). **Conclusion:** The results suggest that insulin therapy in sexual dysfunctional men with early type 2 diabetes can increase serum testosterone concentration to normal range and contribute to the recovery of sexual function.

P113 Effects of Treatment of Hyperhomocysteinemia on the Levels of TGF-β_1 and MAlb/cr in Type 2 Diabetes Mellitus

Jie LIU, Hong YUE, Jin-kang SHAO, et al
Department of Endocrinology, Shanxi Provincial People's Hospital, Shanxi, China

Objective: To clarify the effects of supplementation of folate, methylcobalamin and vitamin-B_6 on the hyperhomocysteinemia and the levels of plasma transforming growth factor β_1 (TGF-β_1) and ruinous microalbumin/creatinine (MAlb/cr). **Methods:** Ninty-six cases of type 2 diabetes mellitus concomitant hyperhomocysteinemia were divided into four groups: folate group (take folate 5 mg evergy day), methylcobalamin group (take methylcobalamin 0.5 mg evergy day), 3B group (take folate 5 mg, methylcobalamin 0.5 mg and vitmanin-B_6 10 mg evergy day) and control group (not to take every Vitamin B medicines). Plasma homocysteine (Hcy), folate, VitaminB_{12}, HbA_{1c}, fasting blood glucose, post-prandial blood glucose, cholesterol, triglyceride, TGF-β_1, and ruinous MAlb/cr levels were measured. The mutations of the C677T transition of methylenetetra-hydrofolate (MTHFR) and the T27796C transition of cystathionine β-synthase (CBS) were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFCP). Plasma Hcy, TGF-β_1 levels and ruinous MAlb/cr levels were measured after four weeks of treatment. **Results:** Before treatment there was no difference on the levels of Hcy, TGF-β_1, MAlb/cr, general and biochemical data, but after treatment the levels on Hcy, TGF-β_1, MAlb/cr were significantly decreased among three treatment groups. The decreased ranges of Hcy levels on control group was 1.5±1.2, methylcobalamin group was 7.2±5.0, the folate group was 7.0±7.0, while the 3B group was 16.8±16.8, the 3B group had highly decreased ranges of Hcy levels among four groups. There were no apparent changes on the Hcy levels between methylcobalamin group and folate group after treatment. The decreased ranges of TGF-β_1 levels on control group was 18.1±6.9, methylcobalamin group was 32.8±11.4, the folate group was 26.2±13.7, while the 3B group was 33.9±11.5, the 3B group had highly decreased ranges of TGF-β_1 levels among four groups. The methylcobalamin group had lower levels of TGF-β_1 than folate group after treatment. The decreased ranges of MAlb/cr levels on control group was 12.5±10.0, methylcobalamin group was 16.2±20.0, the folate group was 27.9±49.3, while the 3B group was 33.5±16.8, the 3B group had highly decreased ranges of MAlb/cr levels among four groups. **Conclusion:** Supplementation of folate, methylcobalamin and vitamin-B_6 may reduce the plasma Hcy, TGF-β_1 levels and ruinous MAlb/cr levels.

P114 A Fifteen Years' Commitment of the Diabetes Educators Group in Developing Diabetes Education in Hong Kong

Winnie M.W. CHENG[1], Eva C.Y. KAN[2], JoJo Y.M. KWAN[3]
Diabetes Center
[1] Queen Elizabeth Hospital, Hong Kong SAR, China
[2] Alice Ho Miu Ling Nethersole Hospital, Hong Kong SAR, China
[3] Our Lady of Maryknoll Hospital, Hong Kong SAR, China

Objective: To establish diabetes patient education in HK, so as to empower diabetic patients to achieve ideal metabolic control, prevent complications and maintain the ultimate quality of life. **Methods:** The Diabetes Educators Group (DEG) was formed by a group of nurses in 1989, aiming to promote local diabetes education. In collaboration with a local nursing Institute, the DEG trains local diabetes nurses (DMN) since 1991. And every DMN joins DEG. Through peer support and work projects for the communities, the group increases its cohesiveness. With continuous professional and graduate education, the group equips themselves with the ability of service development for both hospital and community based care. The unfailing support from endocrinologists has provided the group with autonomy and backup for developing effective client-centered services. **Results:** Diabetes patient education is well accepted by both health professionals and patients in HK today. DMN becomes a crucial member in the Diabetic Care Team. Driven by evidence and protocols, Diabetes Nurse Clinic has been set up across 15 local diabetes centers. **Conclusions:** Fifteen years concerted effort of the group has just laid the foundation of local patient education. DEG will continue to work together to advance educational practice, continuously evaluate and improve service effectiveness in future.

P115 Association of Health Literacy, Complication Awareness and Diabetic Control in Patients with Type 2 Diabetes

Holly Ying-ho TANG[1], Samantha Mei-che PANG[2], Moon-fai CHAN[2], Grace Sau-ping YEUNG[1], Vincent Tok-fai YEUNG[1]
[1] Department of Medicine and Geriatric, Our Lady of Maryknoll Hospital, Hong Kong SAR, China
[2] School of Nursing, The Hong Kong Polytechnic University, Hong Kong SAR, China

Objective: To determine the relationship between health literacy, complication awareness and diabetic control among patients with type 2 diabetes. **Background:** Studies on patients' complication awareness and relationship with diabetic control have been conducted in the West but rarely in the East. Moreover, to our knowledge measurement of health literacy has not been reported among Chinese diabetic patients. **Methods:** 149 Chinese diabetic subjects (mean=59.8 years, range: 27-90 years) who attended DM complication assessment in the Diabetes Centre of a local hospital from September 2005 to February 2006 were recruited. Data were collected by in-person survey using a structured questionnaire. The questionnaire comprised three parts: (1) demographics, (2) a self-developed patient awareness score, and a modified Chinese version of Summary of Diabetes Self-Care Activities (C-SDSCA) measure, and (3) the Chinese version of the Short Test of Functional Health Literacy in Adults (C-S-TOFHLA). Diabetic control was assessed by the most recent HbA1c level. **Results:** An adjusted multiple regression models indicated that health literacy ($p<0.001$), management of treatment in the C-SDSCA measure ($p=0.030$), patient awareness score ($p=0.035$), gender ($p=0.023$), and duration of DM ($p<0.001$) were related to patients' diabetic control. **Conclusions:** In order to develop effective patient education and improve patients' diabetic control and awareness of complications, educational strategies need to take into consideration patients' health literacy level and self-care skills. Tailor-made strategies should be pursued to cater for patients with low health literacy level so as to enhance treatment adherence and improve diabetic control.

P116 Correlates of Psychological Distress in Chinese Elderly Patients with Type 2 Diabetes Mellitus

Yuk-ling TSANG, Doris Sau-fung YU
4/F, School of Public Health, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR

Objective: To identify the correlates of psychological distress among the Chinese elderly patients with type 2 diabetes mellitus (DM). **Methods:** This was a cross-sectional correlational study with data obtained from a consecutive sample of community-dwelling Chinese elderly patients with type 2 DM (n=106) with measures of psychological distress (Hospital Anxiety and Depression Scale), functional status (Barthel Index), and perceived social support (Medical Outcomes Study Social Support Survey). Clinical and demographic data were also collected. Stepwise multiple regressions were used to identify the significant correlates of psychological distress among demographic, clinical and psychosocial factors. **Results:** Four variables, including gender, presence of stroke, nephropathy and size of social network in total explained 52% of the variance in psychological distress. Among these significant correlates, the presence of stroke was the most strongly related to psychological distress, with standardized coefficient as 0.639 ($p<0.0001$). **Conclusion:** The findings suggest the heightened risk of Chinese elderly type 2 diabetic patients, who are female, with comorbidity of stroke and complication of nephropathy, in developing psychological distress. Improving psychological well-being of this group of patients entails improving their social network.

P117 Improvement on Glycaemic Control at Diabetes Nurse Clinic

Amy Shun-wah YEE, Moon-fai CHAN, Elaine Lai-yee LEUNG
Department of Medicine, Diabetes Centre, Queen Mary Hospital, Hong Kong SAR, China

Objective: To investigate the effectiveness of a diabetes nurse clinic intervention in controlling the poor glycaemia of older patients with type 2 diabetes. **Methods:** A quasi-experimental design with pre- and follow-up tests is used. The study was conducted in a regional acute hospital in Hong Kong. A total of 150 (75 controls and 75 cases) poor glycaemic control older patients with diabetes were recruited in the study. The biomedical data were collected at pre- and follow-up period and compared between groups. **Results:** The study results show an effective intervention of diabetes nurse clinic in giving consultation and education to the type 2 diabetic patients. Subjects in the nurse follow-up group showed an improvement in the HbA1c and reduction on healthcare utilization. **Conclusion:** The study provided evidence to support the diabetes nurse clinic in treating the older patients with diabetes.

P118 Need of Insulin Treatment in Relation to BMI and Duration of Diabetes in a Cohort of 4120 Chinese Type 2 Diabetes Patients

Wing-bun CHAN, Wing-yee SO, Ronald Ching-wan MA, Osaki RIZA, Chun-chung CHOW, Juliana Chung-ngor CHAN
Department of Medicine and Therapeutics, Prince of Wales Hospital, Chinese University of Hong Kong, Hong Kong SAR

Objective: To investigate factors which predict insulin requirement in type 2 diabetes patients. **Methods:** 4119 Type 2 diabetes subjects who were not treated with insulin were identified and recruited through complication screening from 1994 to 2001. All subjects underwent complication screening with documentation of metabolic control, anthropometric parameters, diabetes related complications and drug treatment at time of recruitment. They were subsequently followed up till end of 2001 with median duration follow up of 4.06 years. Insulin requirement were identified through prescription record. **Results:** At baseline, the 4119 subjects had a mean age of 58.6±13.5 years and disease duration of 6.2±5.7 years. Their baseline HbA1c was 7.5±1.7%. On subsequent follow-up, 12.9% of the subjects require insulin treatment. Those require insulin treatment has longer duration of diabetes (8.5 vs 5.9 years), younger onset of disease (51.3 vs 52.8 years), lower BMI (24.4 vs 25.2 kg/m^2), higher HbA1c (9.0 vs 7.3%), worse lipid profile and higher albuminuria. There was significant interaction between BMI and disease duration on need of insulin ($P<0.001$). A lower BMI was associated with need of insulin with short disease duration while the impact of BMI on insulin requirement gradually became U shape with long disease duration. **Conclusion:** Type 2 diabetes patients with lower BMI, longer disease duration and worse metabolic control were associated with future insulin treatment. With longer disease duration, obese subjects were also more likely to require insulin.

P119 Insulin Resistance and Its Relative Hormones of Gestational Diabetes Mellitus

Chun-fang SHEN, Jie HONG, Wei-qiong GU, Yi-fei ZHANG, Duan-duan LAI, Qing SHI, Min XU, Xiao-ying LI, Guang NING
Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrine and Metabolic Diseases, Ruijin Hospital, Medical School of Shanghai Jiaotong University, 197 Ruijin Er Road, Shanghai 200025, China

Objective: To evaluate the correlation among placental hormones, high sensitivity C-reactive protein (hsCRP), interleukin-18 (IL-18), testosterone and insulin resistant, β-cell function in gestational diabetes mellitus and nondiabetic pregnant women. **Methods:** We collected 50 patients with gestational diabetes mellitus and 50 control pregnant women without diabetes. All subjects were measured for serum placental hormones, high sensitivity C-reactive protein, interleukin-18, testosterone, glucose and insulin. **Results:** The serum hsCRP, IL-18, testosterone levels in gestational diabetes mellitus were significantly higher than those of controls. Insulinogenic index of β-cell ($\Delta I_{30}/\Delta G_{30}$) in gestational diabetes mellitus was significantly lower than that of controls. CRP, IL-18, testosterone were positively correlated with insulin resistance index (HOMA-IR) ($P<0.01$). Further analysis revealed that BMI, IL-18 and testosterone contributed to HOMA-IR ($r^2=0.466$, $P<0.001$). CRP was positively correlated with pre-pregnant weight, pre-pregnant BMI, fasting insulin, HOMA-IR and IL-18, but was inversely correlated with $\Delta I_{30}/\Delta G_{30}$ ($r= -0.509$, $P<0.001$). **Conclusion:** CRP, IL-18, testosterone were positively correlated with insulin resistance index (HOMA-IR). High levels of testosterone and IL-18 were independently with the risk of GDM. Increased testosterone and inflammation were associated with insulin resistance during pregnancy.

P120 The Differences of Insulin Action and Secretion in the Morning and Afternoon Oral Glucose Tolerance Test

Dee PEI, Chang-hsun HSIEH, Ko-lin KUO, Jiunn-diann LIN, Chung-ze WU, Yi-jen HUNG, Jer-chuan LI, Tso-fu WANG, Chien-hsing LEE, Shi-wen KUO
Department of Internal Medicine, Buddhist Tzu Chi General Hospital, Xindian, Hualien, Tri-Service General Hospital, Taipei, Taiwan

Objective: The relationships between the plasma glucose levels during an oral glucose tolerance test in the morning and after 8 hours fasting in the afternoon are interesting and had been studied in several studies. However, it is still interesting to know the differences between the insulin action and secretion in these two conditions. To better understand their relationships, the parameters for insulin action and secretion derived from oral glucose tolerance test (OGTT) were compared to those derived from the frequent sample intravenous glucose tolerance test (FSIGT) done in the morning in this study. **Methods:** Twenty-two subjects with various glucose tolerance with mean age of 52.4±2.4 years old were enrolled this study. Each subject was assigned for 3 different tests, which included OGTT in the morning and afternoon (M-OGTT, A-OGTT respectively) and FSIGT. The surrogates of insulin resistance (IR_{OGTT}) or insulin sensitivity (SI_{OGTT}) were compared with the insulin sensitivity derived from FSIGT (SI_{FSIGT}). In the same time, the insulin secretion ($ISEC_{OGTT}$) calculated from the OGTT was compared with acute insulin response to glucose load (AIRg) derived from FSIGT. **Results:** The IR_{OGTT} and SI_{OGTT} of the M-OGTT were unanimously more insulin resistance than the A-OGTT. They were also significantly better correlated with the SI_{FSIGT} (mean r=0.62 vs. 0.47, p<0.01). At the same time, higher $ISEC_{OGTT}$ levels in M-OGTT were also noted and, again, the correlation was better in the morning with AIRg (mean r=0.41 and 0.20, p=0.021). **Conclusions:** From this study, we concluded that compared to A-OGTT, higher insulin resistance and insulin secretion could be noted in the morning. In the meanwhile, both the insulin action and secretion of the M-OGTT are better correlated with the SI_{FISGT} and AIRg respectively. There are significant differences in the glucose metabolism during morning and afternoon. Thus data derived in the afternoon could not be used to evaluate the insulin action and secretion or to diagnose diabetes.

P121 The Longitudinal Study of Different Glucose Tolerance in First-degree Relative of Type 2 Diabetes

Jing LI, Li YAN, Li-hong CHEN
Department of Endocrinology, The Second Affiliated Hospital, SUN Yat-sen University, Guangzhou 510120, China

Objective: To investigate the role of insulin secretion as well as insulin sensitivity in the onset and development of type 2 diabetic patients in different glucose tolerance of first-degree relatives with type 2 diabetes. **Methods:** 131 NGT and 35 IGT people without family history of diabetes (named NGT_{NF} and IGT_{NF} respectively), 24 NGT and 29 IGT patients who were first-degree relatives of type 2 diabetes (named NGT_F and IGT_F respectively). Measurements of β cell function and insulin sensitivity including OGTT and IVGTT. AIR_{3-5} reflected first-phase insulin secretion function. $\Delta I_{30}/\Delta G_{30}$ reflected early-phase insulin secretion function. S_{IM} reflected insulin sensitivity. Plasma glucose was measured by the automatic biochemical meter using glucose dehydrogenase technique. Serum specific insulin was measured by the resolved time fluorescence immunoassay technique in the Auto-DELFIA analyzer. **Results:** AIR_{3-5} between groups of NGT_F and NGT_{NF} was significantly different at baseline (P=0.02) and this time (=0.03). AIR_{3-5} between the two groups of IGT_F and IGT_{NF} was not significantly different at baseline, but the result of this time is significantly different (P=0.01). $\Delta I_{30}/\Delta G_{30}$ between NGT_F and NGT_{NF} was not significantly different at baseline and this time. But the change of $\Delta I_{30}/\Delta G_{30}$ between the 2 groups of IGT_F and IGT_{NF} was significantly different. At this time the result of the two groups is different (P=0.01). In IGT_F, S_{IM} was significantly decreased. In IGT_{NF}, S_{IM} did not change. S_{IM} was significantly different between the two groups this time. **Conclusion:** The dysfunction of islet β cells shows in the different glucose tolerance of first-degree relative with family history of diabetes more earlier and more remarkable than that in the groups without family history of diabetes.

P122 The Role of Insulin Secretion and Insulin Sensitivity in the Progress from NGT to IGT or from IGT To NGT

Jing LI, Li YAN, Li-hong CHEN
Department of Endocrinology, The Second Affiliated Hospital, SUN Yat-sen University, Guangzhou 510120, China

Objective: To investigate the role of insulin secretion as well as insulin sensitivity in the onset of type 2 diabetic patients in China. **Methods:** 70 individuals with NGT and IGT are studied. Measurements of β cell function and insulin sensitivity include OGTT and IVGTT. $AIR_{3\text{-}5}$ reflected first-phase insulin secretion function. $\Delta I_{30}/\Delta G_{30}$ reflected early-phase insulin secretion function. S_{IM} reflected insulin sensitivity. Plasma glucose was measured by the automatic biochemical meter using glucose dehydrogenase technique. Serum specific insulin was measured by the resolved time fluorescence immunoassay technique in the Auto-DELFIA analyzer. **Results:** In progressors (IGT), $AIR_{3\text{-}5}$ decreased by -13.68 mu/l, compared with the baseline of which is significantly different (P=0.024). In nonprogressors (IGT), $AIR_{3\text{-}5}$ increased +28.43 mu/l, compared with the baseline of which was also significantly different. The change in $AIR_{3\text{-}5}$ differed significantly between the 2 groups (*P*=0.007). $\Delta I_{30}/\Delta G_{30}$:$\Delta I_{30}/\Delta G_{30}$ decreased in progressors (IGT) and $\Delta I_{30}/\Delta G_{30}$ increased in nonprogressors (IGT), the change in $\Delta I_{30}/\Delta G_{30}$ differed significantly between the 2 groups (*P*<0.036). S_{IM}:S_{IM} decreased by -11.38 L^2/mol*mU in progressors (NGT), compared with the baseline of which was significantly different. The change in S_{IM} differed significantly between the 2 groups (*P*<0.035). S_{IM} increased by +15.28 L^2/mmol*mU in the group from IGT to NGT, compared with the baseline of which was significantly different (*P*=0.001). In logistic regression, low S_{IM} was significantly contributed to the transition from NGT to IGT. **Conclusion:** During the progression from NGT to IGT, insulin resistance plays a major role.

P123 Study on Insulin Resistance and Function of β-cells in Individuals with IFG and IGT

Bing HUANG[1], Xue XIE[1], Wen-qun HAN[1], Yu-qin ZHAO[2], Yu-qing CHEN[2]
[1] Endocrine Department of Chengdu No.2 People's Hospita, 610017, China
[2] Nuclear Medicine Department of Chengdu No.2 People's Hospital, 610017, China

Objective: To compare insulin resistance and β-cells function in individuals with IFG and IGT for the purpose of providing evidences for appropriate medical interventions. **Methods:** We have studied 220 subjects through health examination and out-patient recruiting between 2003 and 2005, and among them 47 with IFG, 132 with IGT and 41 with IGR. After OGTT, the individuals with fasting blood sugar ≥6.1 mmol/L or random blood sugar ≥7.8 mmol/L were included and those with diagnosed diabetes mellitus, obesity or hyperlipemia were excluded. We detected such indexes as blood sugar, insulin and blood fat are detected, then, HOMA-β, HOMA-IR, $\Delta I_{ns30}/\Delta G_{30}$, area under blood sugar time curve, area under insulin time curve were calculated and compared. The difference between groups was compared using SNK method of ANOVA. **Results:** The fasting insulin in IFG and IGT group are 17.0±8.6 mU/L and 19.5±9.4 mU/L respectively. Two-hour insulin in IFG and IGT group are 61.2±16.3 mU/L and 78.3±29.8 mU/L respectively. HOMA-IR in IFG and IGT group are 4.2±1.2 and 3.4±1.6 respectively. HOMA-β in IFG and IGT group are 8.2±1.0 and 15.3±1.4 respectively. $\Delta I_{ns30}/\Delta G_{30}$ in IFG and IGT group are 4.1±0.9 and 6.3±0.4 respectively. Insulin resistance and β-cells failure are observed in both IFG and IGT groups. Insulin resistance and β-cells failure in IFG group are more obvious than that of IGT group. **Conclusions:** There is insulin resistance and β-cells failure both in patients with IFG and in those with IGT, but it's more obvious in the former. The primary reason of IFG is insufficient insulin excretion and/or insulin resistance. The latter happens in liver where gluconeogenesis during fasting leads to increasing of fasting blood sugar. The insulin resistance while IGT happens in peripheral tissues, such as fat tissue and skeletal muscles. IGT is manifested with insufficient insulin excretion after sugar load, increasing postprandial blood sugar and minor impaired β-cells function. IFG and IGT, the pre-diabetes, have same pathology but different pathogenesis. Accordingly, it's necessary to adopt different measures while preventing intervention of diabetes mellitus.

P124 The Comparison of Insulin Sensitivity, Glucose Sensitivity and First Phase Insulin Secretion in Patients Treated with Repaglinide or Gliclazide

Jiunn-diann LIN, Chung-ze WU, Ko-lin KUO, Yi-jen HUNG, Jer-chuan LI, Tso-fu WANG, Chien-hsing LEE, Shi-wen KUO, Chun-chun YANG, Dee PEI
Department of Internal Medicine, Buddhist Tzu Chi General Hospital, Hualien and Taipei Branch, Tri-Service General Hospital, Taipei, Taiwan

Objective: Traditional sulfonylureas with long half-life, such as gliclazide, have sustained stimulation on the insulin secretion than the short acting insulin secretagogue-repaglinide. In this hospital cohort, randomized, cross-over study, we used the frequent sampled intravenous glucose tolerance test (FSIGT) to evaluate the insulin sensitivity (SI), glucose sensitivity (SG) and acute insulin response after glucose load (AIRg) after 4 months' treatment with either gliclazide or repaglinide. **Methods:** We enrolled 20 fresh type 2 diabetes with mean age of 49.3±2.2 years old and BMI 25.4± 0.9 kg/m^2. After titration and maintain dose stage, all cases were randomized to the repaglinide or gliclazide treatment for 4 months. At the end of each treatment period, a FSIGT was done. **Results:** There were no significant differences between fasting plasma glucose, insulin, BMI, blood pressure, glycated hemoglobin and lipids. The SI was higher, but SG and AIRg were lower in the repaglinide treatment group. No significance was noted in these comparisons. The DI is the product of SI and AIRg and there was also no difference noted. **Conclusion:** In conclusion, non-significantly higher AIRg, but lower SI and SG could be noted after 4 months of repaglinide treatment compared with the gliclazide. However, since the DI was also non-significantly higher in the repaglinide group, the repaglinide might be superior in the treatment of type 2 diabetes.

P125 Evaluation of First Phase of Insulin Secretion in Newly Diagnosed Type 2 Diabetics Using Nateglinide-intravenous Glucose Insulin Release Test

Guo-chun LUO, Zhen LIANG, Qing-hong HU, et al
Department of Endocrinology, Shenzhen Second People's Hospital, Shenzhen 518035, China

Objective: To evaluate the function of first phase of insulin secretion of pancreatic B cells in newly diagnosed type 2 diabetics using nateglinide-intravenous glucose insulin release test (NG-IVGIRT). **Methods:** NG-IVGIRT and IVGIRT were done in 8 patients with newly diagnosed type 2 diabetes mellitus and NG-IVGIRT was done in 8 normal people. Insulin and glucose of blood were determined at -15, 0, 2, 4, 6, 8 and 10 min in NG-IVGIRT or IVGIRT. **Results:** Comparison between NG-IVGIRT and IVGIRT in the diabetics: Comparison in the results of NG-IVGIRT between the diabetics and the normals: The median of insulin in NG-IVGIRT was 12.65 mU/l at -15 min, 15.65 mU/l at 0 min, 30.60 mU/l at 2 min, 39.65 mU/l at 4 min, 31.45 mU/l at 6 min, 23.75 mU/l at 8 min and 24.75 mU/l at 10 min, and that in IVGIRT 12.40 mU/l, 12.55 mU/l, 12.45 mU/l, 12.85 mU/l, 12.75 mU/l, 11.90 mU/l and 12.10 mU/l, respectively. The responses of 0-10 min insulin to NG-IVGIRT were significantly higher than those to IVGIRT in the diabetics (P all<0.01). The AUC of insulin was 191.10 (46-251.6) mU/l in NG-IVGIRT and 74.60 (29.4-166.6) mU/l (P<0.01). The AUC of plasma glucose was 109.48±2.96 mmol/l in NG-IVGIRT and 121.25±3.17 mmol/l (P<0.01). Comparison in the results of NG-IVGIRT between the diabetics and the normals: The median of insulin in the normals was 14.69 mU/l at -15min, 28.90 mU/l at 0 min,158.6 mU/l at 2 min, 220.6 mU/l at 4 min, 218.8 at 6 min, 220.10 mU/l at 8 min and 212.35 mU/l at 10 min, and that in the diabetics was 12.65 mU/l, 15.65 mU/l, 30.60 mU/l, 39.65 mU/l, 31.45 mU/l, 23.75 mU/l and 24.75 mU/l, respectively. The response of insulin to NG-IVGIRT in the normals was much higher than that in the diabetics (P all<0.01). The AUC of insulin to NG-IVGIRT was apparently elevated and the AUC of glucose to NG-IVGIRT reduced in the normals compared with those in the diabetics. **Conclusion:** The results indicated that the reserve of first phase insulin secretion of pancreatic B cells in newly diagnosed type 2 diabetics could be provoked in some degree by NG-IVGIRT and there was a big difference in the reserve of the first phase of insulin secretion provoked by NG-IVGIRT between newly diagnosed type 2 diabetics and normal people.

P126 Hyperglycemic Glucose Clamp Technique Evaluates the Effects of Short-term Intensive Insulin Therapy on the Functions of Pancreatic Islets Beta Cells in Newly Diagnosed Type 2 Diabetics

Guo-chun LUO, Zhen LIANG, Qing-hong HU, et al
Department of Endocrinology, Shenzhen 2nd People's Hospital, Shenzhen 518035, China

Objective: Hyperglycemic glucose clamp technique (HGCT) was performed to evaluate the effect of short-term intensive insulin therapy on the first-phase insulin secretion (1PH), second-phase insulin secretion (2PH) and maximum insulin secretion (MIS) in newly diagnosed type 2 diabetics. **Methods:** Twelve volunteers with normal glucose tolerance (NC group) and six newly diagnosed type 2 diabetics (DM group) were included in this study. HGCT was performed to assess the function of pancreatic islets beta cells in normal group and DM group and then repeated in the 6 patients following two-week intensive insulin therapy. **Results:** 1PH, 2PH and MIS were 256.93±36.21 mIU/l, 63.45±4.60 mIU/l and 80.13±5.35 mIU/l in NC group respectively, and were 94.85±19.41 mIU/l, 33.79±9.18 mIU/l and 39.27±12.30 mIU/l in DM group respectively. There was significant difference (P all<0.01) in 1PH, 2PH and MIS between NC group and DM group. 1PH was significantly improved in the diabetics following 2-week insulin intensive treatment compared with that before the treatment (135.25±26.65 mIU/l vs 94.85±19.41 mIU/l, P=0.01), as well as 2PH and MIS were slightly increased (39.85±8.6 mIU/l vs 33.79±9.18 mIU/l, P=0.09, 46.22±11.03 mIU/l vs 39.27±12.30 mIU/l, P=0.08, respectively). There was significant difference in 1PH, but no apparent difference in 2PH and MIS between 6 patients with diabetes before and after the insulin intensive treatment. **Conclusions:** Short-term intensive insulin therapy can significantly improve the 1PH and slightly improve the 2PH and MIS in newly diagnosed type 2 diabetics by eliminating the glucose toxicity.

P127 Study on Relationship between Type 2 Diabetes with Insulin Resistance and Traditional Chinese Medicine Syndrome Type

Hong-fang LIU, Lian-jie WANG, Zhen-hua CHAO
Dong Zhemen Chinese Hospital, Beijing 100700, China

Objective: To observe the relationship between traditional Chinese medicine XU-SHI syndrome type and type 2 diabetes with insulin resistance. **Methods:** 87 cases of type 2 diabetes patients were divided into insulin resistance group and no-insulin resistance group according to serum insulin level. The traditional Chinese medicine syndrome type were judged. The blood glucose and serum insulin in fasting and after 75 g glucose, serum cholesterol and triglyceride were detected. **Results:** Compared with the no-insulin resistance group, the fasting blood glucose and serum insulin after 75 g glucose, IBM and serum triglyceride were increased and insulin resistance index decreased in insulin resistance group. The mostly appeared syndrome type in insulin resistance group is excess superficiality syndrome type, in no-insulin resistance group is deficiency syndrome type with excess superficiality syndrome type. **Conclusion:** The insulin resistance of type 2 diabetes has the relationship with the excess superficiality syndrome type.

P128 SelS mRNA Expression in Omental Adipose Tissue is Associated with Homa-IR and Serum SAA in Patients with Type 2 Diabetes Mellitus

Qian XING, Bo LV, Jian-ling DU, Li-li MEN
Department of Endocrinology, First Affiliated Hospital of Dalian Medical University, 116011, China

Objective: To research the expression of SelS/Tanis mRNA in omental adipose tissues in patients of T2DM and the relationship of SelS mRNA with Homa-IR and serum SAA level. **Methods:** To extract total RNA from human omental adipose tissues. Semi-quantitative RT-PCR was conducted to compare the expression of SelS gene in omental adipose tissues of ten type 2 diabetic subjects with twelve non-diabetic individuals. The subjects were blood sampled. To calculate insulin resistance index (Homa-IR) and to detect SAA level by ELISA. The relationship of SelS mRNA expression in omental adipose tissues with Homa-IR and serum SAA level in all subjects was analysed. **Results:** It suggests that the total RNA extracted from human omental adipose tissues was complete. Omental adipose tissues SelS mRNA expression, Homa-IR and SAA level in diabetic subjects were higher than those in control subjects. SelS mRNA expression was positively correlated with Homa-IR and serum SAA level in two groups. **Conclusions:** SelS gene segment was cloned successfully from human omental adipose tissues by RT-PCR. SelS may have the role of insulin resistance in Chinese with T2DM of Dalian. SelS may act as the receptor of serum SAA and play an important role in the development of T2DM and atherosclerosis.

P129 Correlation between Serum Level of IL-18 and Insulin Resistance in PCOS Patients

Yi-fei ZHANG, Yi-sheng YANG, Jie HONG, Wei-qiong GU, Chun-fang SHEN, Min XU, Peng-fei DU, Xiao-ying LI, Guang NING
Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrine and Metabolic Diseases, Ruijin Hospital, Medical School of Shanghai Jiaotong University, 197 Ruijin Er Road, Shanghai 200025, China

Objectives: Overproduction of proinflammatory factors is associated with obesity and diabetes. Interleukin (1L)-18 as a member of IL-1 cytokine family is increased in obese, in diabetic, and even in polycystic ovary syndrome (PCOS) patients. In the present study we evaluated the association of serum IL-18 levels with insulin resistance in PCOS women. **Methods:** Forty-two PCOS women and 38 control subjects were enrolled in this study and matched with respect to age and body mass index (BMI). Serum IL-18 levels and hormones were measured for all subjects. Furthermore, euglycemic hyperinsulinemic clamp test was performed in selected 30 PCOS women and 11 control subjects. **Results:** Serum IL-18 levels were elevated in PCOS women compared with the control (p=0.033). IL-18 levels were positively correlated with homeostasis model assessment index (HOMA) β index, which assesses β cell function (p=0.035), but were inversely correlated with clamp indices, which best represent insulin resistance status: M, Clamp ISI*100, and MCRg values (p=0.006, 0.010, and 0.009 respectively). No correlation was found between IL-18 and age, BMI, waist-to-hip ratio (WHR), lipid profile, dehydroepiandrosterone-sulfate (DHEAS), sex hormone-binding globulin (SHBG), or fasting insulin levels. **Conclusion:** In the present study, serum IL-18 levels were significantly increased in PCOS women and firmly associated with insulin resistance displayed by euglycemic hyperinsulinemic clamp test. It indicates that IL-18 may be a contributing factor linking inflammation and insulin resistance in PCOS women.

P130 CSII Improving HOMA-IR in Type 2 Diabetes. A Complex Compound of Glucose NEFA and Insulin Blocked Themselves and Induced Insulin Resistance?

Xiao-ge DENG, Xin SU, Guo-liang SUI, Hong ZHANG, Yi-qun PENG, Wei-li TANG, Ru-chun DAI, Xiang-hang LUO, Ji-ping MAO
Department of Endocrinology, Second Xiang-Ya Hospital of Central South University, 139# Renmin-Zhong Road, Changsha Hunan 410011, P.R. China

Objective: Evidences showed that insulin resistance (IR) of the type 2 diebetes patients (T2DP) could be released after effective therapy. We believed that was related to a complex compound (CC) in the blood, and it should come from insufficient insulin effects and should be overcome if enough insulin was added. For this reason, we try to see whether it was true and how. **Methods:** A self-controlled trial was performed at 77 T2DP, who had hyperglycemia and indications to receive insulin therapy. Many indices related to the effects of insulin were measured before and after the continuous subcutaneous insulin injection (CSII). **Results:** Paired-samples *t* test showed that after CSII, (1) blood glucose, HOMA-IR, NEFA, C-peptide, osmolarity, BUN, proteins, creatinine were decreased significantly ($p<0.05$-$p<0.0001$); (2) insulin, sodium, chlorine, CO2CP were increased significantly ($p<0.05$-$p<0.0001$); (3) no significant differences at the potassium, super sensitive C reactive protein, HbA1C, HOMA-β-cell, and hepatic enzymes ($p>0.05$); and (4) excepting hyperglycemia, hyperinsulinemia and high HbA1C, other indices were inside normal ranges. **Conclusion:** The total effect of CSII was balancing the ratios among glucose, NEFA, proteins and others in the blood to normal. The hyperinsulinemia with obviously decreased blood glucose after CSII, supported that some thing had prolonged the half life of insulin in the blood, because it had to understand that IR at the target organs were able to prolonged the half life of insulin in the blood. Further studies about the CC will bring us a novel concept about the IR of T2DP.

P131 Expression of Presenilins-associated Rhomboid-like Protein (PSARL) in the Skeleton Muscles of Insulin Resistance and Diabetes Rats

Hui-qin TANG, Yan-cheng XU
Department of Endocrinology, Zhongnan Hospital of Wuhan University, China

Objective: To investigate the different expression of presenilins-associated rhomboid-like protein (PSARL) in rats with insulin resistance, rats with type 2 diabetes and normal rats. **Methods:** Rat models of type 2 DM were established with high fat diet and low dose streptozotocin. Rat models of IR were established with high fat diet. Sprague-Dawley rats used in the research were randomized into 3 groups: normal control rats (group NC); insulin resistence rats (group IR) and T2DM rats (group T2DM). After 12 hours fasting, weight of body, FBG and FINS were recorded for all rats. Gene expressions of PSARL in gastrocnemius muscles were detected by RT-PCR. **Results:** Compared with NC group, gene expressions of PSARL in insulin resistance and type 2 DM groups were significantly decreased, what's more, gene expressions of PSARL in type 2 DM was less than that of insulin resistance group. The mitochondria shapes were badly destroyed in gastrocnemius muscle cells of insulin resistance and type 2 diabetes rats. **Conclusions:** Gene expressions of PSARL would decrease in rats with insulin resistance and rats with type 2 diabetes. PSARL might play an important role in the development of insulin resistance and type 2 diabetes by affecting mitochondrial functions.

P132 The Gene Expression of InsR and GLUT4 of Different Tissues in KKAy Mice with Insulin Resistance

Ju-min SONG, Xiao-mei LIU, Wei WANG, Ji-ling YAN, Jun MA
Shanghai university of TCM, Shanghai 201203, China

Objective: To study the gene expression of InsR and GLUT4 of different tissues in KKAy mice with insulin resistance and the effects of TCM on them. **Methods:** Healthy male C57BL mice were set up as normal control group fed with normal diet. KKAy mice with spontaneous insulin resistance were selected as KKAy group and TCM group fed with high fat diet. The TCM group was treated with Fufang Huanglian Jiangtangpian for two weeks. The numbers of InsR in liver was measured by radioactive labeling-ligand method. The mRNA expression of InsR, GLUT4 were tested with RT-PCR analysis. **Results:** The levels of FBG, insulin and blood fat of the KKAy mice were increased. The InsR numbers were decreased. The mRNA expression of InsR and GLUT4 in different tissues were significantly decreased. TCM can decrease the FBG, increase the numbers of InsR in liver and the mRNA expression of InsR and GLUT4. **Conclusion:** The insulin resistance of KKAy mice was relevant to InsR and GLUT4 of the insulin signal transduction path, which fits the feature of type 2 diabetes mellitus. TCM can improve these conditions.

P133 To Study the Effect of Sulindac on Glucose Metabolism Insulin Sensitivity in Rat of Insulin Resistance Induced by High Fat Diet

Xiao-ying Ding, Yong-de PENG, Wei-ping DONG, Yu-fei WANG, Xiao-jie PAN
Department of Endocrinology, First People's Hospital of Shanghai Jiaotong University, Shanghai, 200080 Institute of Diabetes, China

Objective: To observe the effect of sulindac on insulin resistance induced by high fat diet in rat changes of insulin sensitivity in rat models with insulin resistance induced by high fat diet. To investigate the effects of sulindac on rats with insulin resistance. **Methods:** Normal male SD rats were divided into two groups, taking either normal chow or high fat diet, 20 weeks, HF rats were divided into two groups taking either normal chow or high fat diet. 340 male SD rat of 20 weeks old were randomized into two groups, normal feeding group and high feeding in group; rats was investigated by hyperinsulinemic-euglycemic clamp. 20 weeks after feeding, rat models with IR were evaluated with glucose ring clap technique, level of serum FFA was assayed with chromatometry, levels of serum triglyceride, total cholesterol were measured by using biochemical enzymic technique. Changes of body mass, glucose infusion rate and serum FFA in rats were compared between the groups. In rats with 20-week high-fat feeding, euglycemic-hyperinsulinemia clamp technique was performed in order to estimate their insulin in sensitivity; high fat diet induced insulin resistance rats were selected as the subject to evaluate the effect of sulindac on insulin sensitivity. Euglycemic insulin clamp technique is the main method, meanwhile, the level was measured. After 20 weeks of feeding rats with high fat foods, glucose tolerance fasting glucose, serum fasting insulin, triglyceride, free fatty acid levels, glucose infusion rate were tested when the experiment finished. Treating rats with sulindac as a treatment group and with saline as a control group for 4 weeks, continued to take high fat diet, another ten rats took additional sulindac gavage, four weeks later, oral glucose tolerance test and insulin tolerance test were performed for estimating insulin sensitivity. **Results:** The results showed that glucose infusion rate in rats with high-fat feeding was decreased as compared with the control rats, moreover, high-fat feeding can decrease insulin in sensitivity index and increase fasting plasma insulin of the rats. The results suggested that high-fat feeding the high fat diet induced rats without treatment had lower GIR level. There was an obvious increase in GIR of group treated by sulindac, meanwhile, the treatment group showed much lower TG than control group. Compared with rats in place group, there was significant increasd serum fasting insulin, triglyceride and free fatty acid levels, impaired glucose tolerance and decreased glucose infusion rate, the treatment of sulindac reduced the differences between control and treatment group. Comparison of blood lipid and FFA after 20 weeks feeding, levels of TG and FFA in the high fat feeding group were significantly higher than those in the normal feeding group. Comparison of body mass, blood lipid, level of insulin and glucose infusion rate, level of body mass and serum insulin in rats of high fat feeding group were significantly higher than those in the normal feeding group, and the glucose infusion rate was obviously lower than that in the normal feeding group. The body weight of HF group was significantly higher than those of HF+sulindac group and Nc group. Fasting plasm glucose insulin and plasm glucose, insulin 2h after taking glucose in HF rats were significantly higher than those in NC rats. Sulindac inhibits serine phosphorylation of insulin receptor substrate. **Conclusion:** Long-term high fat diet resulted in insulin resistance, sulindac was able to reverse insulin resistance through promoting targeting serine kinases, regulates IRS-1 serine 307 phosphorylation. Glucose infusion rate in rats fed with high fat diet decreased obviously which indicates that high fat diet can induce severe insulin resistance. Sulindac could improve insulin resistance, has a treatment effect to rats with insulin resistance, probably through increasing sensivity of rats to insulin by raising the amount of signal transduction. This effect may be partly achieved by deducing TG in liver euglycemic-hyperinsulinemic clamp technique. Furthermore, high doses of sulindac improved insulin sensitivity in rats.

P134 Effects of Ecdysterone on Insulin Sensitivity and Glucose Metabolism in Insulin-resistant Cell Model

Qiu CHEN[1], Yong-peng XIA, Zong-yin QIU
[1] Department of Endocrinology, Affiliated Hospital of Luzhou Medical College, Luzhou City, Sichuan Province 646000, China

Objective: To investigate the effects of ecdysterone on insulin sensitivity and glucose metabolism in the insulin resistant HepG2 cell model induced by high concentration of insulin. **Methods:** The insulin-stimulated glucose incorporation rate was determined with ^{3}H-D-glucose uptake test which was used to estimate insulin sensitivity of HepG2 cell, and glucose consumption, the amounts of glucose disappeared from the culture medium of HepG2 cells within 24 hours were determined. **Results:** The incubation of insulin resistance cells with ecdysterone $1x10^{-6}$-10^{-4} M could significantly increase the glucose uptake and glucose consumption of the cells compared with that of control cells ($P<0.01$). The glucose uptake and glucose consumption of insulin resistance cells were not of difference in the insulin resistance cells with ecdysterone and pioglitazone ($P>0.05$). **Conclusion:** Ecdysterone could improve the insulin sensitivity in the cell model and might attenuate the aggression of insulin resistance. The effect to improve the insulin sensitivity with ecdysterone was similar to that with pioglitazone.

P135 Erythropoietin Inhibits Insulin-stimulated Glycogen Synthesis in Primary Cultured Hepatocytes

Jian ZHU, Xiao-hong WU, Cui-ping LIU, Chao LIU
Department of Endocrinology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China

Background/Objective: Long-term EPO treatment could ameliorate insulin resistance in anemia patients with diabetes. However, little is known about the directly metabolic effects of EPO in the liver, which is target tissue of insulin. Studies were performed to examine whether EPO influences carbohydrate metabolism, which changes play an essential role in insulin resistance. **Methods:** (1) Primary cultured mice hepatocytes were treated with glucoregulatory hormones such as insulin, glucagons in the presence or absence of EPO. Cellular glycogen deposition and degradation were compared. In addition, insulin signaling was subsequently analyzed in hepatocytes which treated with or without EPO. (2) The insulin tolerance test was performed to evaluate insulin sensitivity in mice treating with or without EPO. **Results:** EPO inhibited insulin-stimulated glycogen deposition, but had no effect on glycogen degradation stimulated by glucagons. Compared with the control group, the level of blood glucose in mice with EPO subcutaneous injection is higher. In addition, experiments with immunoprecipitation revealed that EPO had no effect on the upstream of insulin signaling including an activation of its receptor and phosphorylation of PKB (AKT), indicating that EPO presumably affects further downstream of these events. **Conclusions:** These results demonstrate that EPO interacts with insulin on glucose metabolism in hepatocytes. EPO may be involved in glucose regulation in addition to stimulates the proliferation and differentiation of colony-forming unit erythroid.

P136 The Effects of Dexamethasone and Glucagon on Expression FoxO1 on Hepatic Cells (HepG2)

Yu-xiu LI, Xiao-jun ZHU, Qi SUN, Qing-rong PAN, Ping ZENG, Jing-bo ZENG, Heng WANG
Department of Endocrinology, Peking Union Medical College Hospital, Bejing 100730, China

Objective: To investigate the effects of glucagon and dexamethasone on the expression of FoxO1 in HepG2 and relationship with expression of PCKl and PGC-lα. **Methods:** FoxO1, PCKl and PGC-lα mRNA level and protein levels were observed by real time RT-PCR technology and western blot technology on hepatic cells (HepG2) culture with glucagon and dexamethasone. **Results:** Dexmethasone significantly increased the mRNA levels of FoxO1 in HepG2 cells, which reached the maximum by one hour. Dexmethasone increased the protein level of FoxO1 paralleling with the mRNA levels. However, glucagon did not have any significant effect on the expression of FoxO1 in the mRNA or protein levels in HepG2 cells. The peak time of FoxO1, PCKl and PGC-lα mRNA expression with stimuli of dexamethasone were the same at 1 hour, means there was no sequence relationship among them. **Conclusion:** Dexmethasone promotes the expression of FoxO1 in HepG2 but not glucagons.

P137 An Evaluation of the International Diabetes Federation Definition of Metabolic Syndrome in the Chinese Patients Older Than 30 years and Diagnosed with Type 2 Diabetes Mellitus

B. LU, Y.H. YANG, X.Y. SONG, X.H. DONG, Z.Y. ZHANG, L.N. ZHOU, Y.M. LI, N.Q. ZHAO, X.X. ZHU, R.M. HU
Department of Endocrinology, Huashan Hospital, Institute of Endocrinology and Diabetology, Fu Dan University, 200040, China

Objective: The purpose of this study was to determine the most accurate metabolic syndrome (MS) definition among the definitions proposed by the International Diabetes Federation (IDF), the Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III [ATP III]) and the World Health Organization (WHO) by making a comparison of the three definitions and evaluation of cardiovascular disease risks. **Methods:** 1039 Chinese patients diagnosed with type 2 diabetes aged over 30 were investigated by randomized cluster sampling in the Shanghai downtown and 1008 patients analyzed in this study. Body mass measurements including height, weight, waist circumference and hip circumference, resting blood pressure, fasting blood measures and carotid atherosclerotic measurements including common carotid artery intima-media thickness (IMT) and plaque of carotid artery were investigated. The IDF definition was compared with the other two definitions and the carotid atherosclerosis was evaluated among the patients defined by these definitions. **Results:** (1) The MS prevalence was 50.0%, 55.7% and 70.0% under the IDF, ATPIII and WHO definitions respectively. The prevalence of central obesity defined by the ATPIII definition was 4.1% in men and 30.2% in women. (2) The male patients had greater waist circumference, waist hip ratio and the female patients had greater body mass index. (3) MS patients defined by the IDF definition had greater waist circumference and BMI. (4) The percentage of all the participants categorized as either having or not having the MS was 69.9% (under the IDF and ATPIII definitions) and 70.2% (under the IDF and WHO definitions). (5) Common carotid artery IMT of MS patients defined by the IDF definition was thicker than those defined by the WHO and ATPIII definitions (1.04 mm vs 0.99 mm; 1.04 mm vs 0.96 mm respectively; $P<0.01$) and the percentage of carotid plaque of MS patients defined by IDF definition was greater than those defined by WHO and ATPIII definitions (74.8% vs 62.7%; 74.8% vs 59.6% respectively; $P<0.01$). **Conclusions:** The prevalence of MS was 50.0%, 55.7% and 70.0% under the IDF, ATPIII and WHO definitions respectively. The preferable IDF definition might be a better predictor of cardiovascular disease risk in the Chinese patients diagnosed with type 2 diabetes compared with the ATPIII and WHO definitions.

P139 Metabolic Syndrome and Its Effect on Glucose Regulation in High-risk Population for Diabetes in Beijing

Guang-ran YANG, Shen-yuan YUAN, et al
Department of Endocrinology, Tongren Hospital Affiliated to Capital University of Medical Sciences, Beijing 100730, China

Objective: To investigate the prevalence and characteristics of metabolic syndrome in Beijing and the relationship between metabolic syndrome and the transformation of glucose regulation. **Methods:** In 1995-1996, 20 682 residents, randomly selected and over 25 years of age, were screened in Beijing area. Those whose capillary glucose concentration was above 120 mg/dl two hours after 100 g steam-bread were subjected to 75 g oral glucose tolerance test. ECG examination was also carried out in these people together with the measurement of blood pressure and determination of plasma cholesterol, triglycerides, insulin and urine albumin excretion. 1302 people were selected. **Results:** (1) 518 people had metabolic syndrome, 39.8% in 1302 people by CDS diagnostic criteria higher than that in 1995, $p<0.01$. The prevalence of hyperglycemia was much more higher in 2000 than that in 1995. (2) The prevalence of MS in Daxing was 47.25%. There was statistically significance among the parameters of each region. (3) According to the result of oral glucose tolerance test (OGTT) in 1995, 1302 people were divided into three groups: normal glucose tolerance (NGT) 482, impaired glucose regulation (IGR) 416, and diabetes mellitus (DM) 404. After 5 years only 366 persons were NGT. The prevalence of hyperglycemia in NGT with MS was significantly higher than that in NGT without MS. There was statistically significance in age, BMI, blood pressure, postprandial plasma glucose, triglycerides, HOMA index between two groups. (4) Moreover compared to IGR without MS, the prevalence of DM in IGR with MS was much higher (38.4% vs 19.7%). And its age, BMI, blood pressure, postprandial plasma glucose, plasma cholesterol, triglycerides, HOMA index were statistically higher than those in IGR without MS, respectively. (5) In diabetes group, diabetic patients who had MS had much higher prevalence diabetic nephropathy, coronary heart disease and cerebral infarction than those without MS. **Conclusion:** MS in Beijing had regional characteristics. The prevalence of MS in counties was higher than that in towns. Hyperglycemia was the important factor of MS increase. MS may affect the change of different glucose metabolism of people. Insulin resistance may be the major reason.

P140 Epidemiological Study of Metabolic Syndrome and Its Related Diseases in Jiangsu Province

Yu FENG, Wei TANG, Yu DUAN, Xiao-dong MAO, Shu-hang XU, Shang-yong FENG, You-wen QIN, Cui-ping LIU, Jun-jian CHEN, Wei LI, Rui-fang BO, Chao LIU
Department of Endocrinoiogy, The First Affiliated Hospital of Nanjing Medical University, Jiangsu Province People Hospital, Nanjing 210029, China

Objective: To investigate the prevalence and epidemic of metabolic syndrome (MS) and its related diseases. **Methods:** (1) 6758 residents from 6 districts of Jiangsu Province, who had lived for at least five years and aged over 20, were enlisted in our research. (2) Fasting plasma glucose, triglycerides (TG), high-density lipoprotein-C (HDL-C) were measured. (3) The diagnosis of MS was based on the definition of IDF 2005. (4) Statistical analyses were completed using SPSS software. **Results:** (1) The crude prevalence of central obesity, hypertension (≥140/90 mm Hg), IFG, hypertriglyceridemia and hypoalphalipoproteinemia-C were 37.3%, 36.2%, 19.8%, 24.2%, 35.1% and the standardized rates were 30.1%, 27.7%. 15.2%, 20.1%, 33.1% respectively. (2) The crude prevalence of MS were 21.1%, 15.5%, 24.6% in total population, male and female while the standardized rate were 15.9%, 13.1%, 17.9% accordingly. The incidence of hypertension (>130/85 mm Hg), hypertriglyceridemia, IFG, hypoalphalipoproteinemia-C were 87.4%, 59.0%, 44.0%, 67.2% respectively among MS individuals, of which 54% incorporated only 2 metabolic abnormities, 34.4% incorporated 3 metabolic abnormities and 11.6% had 4 metabolic abnormities. (3) Multiple logistic regression analysis showed that the risk factors of MS were female, age and MS family history while smoking was protective factor. **Conclusions:** (1) There is a high prevalence of MS and its related diseases in Jiangsu Province. (2) Aged people and female were at higher risk. Hypertension was the most frequent metabolic abnormity among MS individuals. Most MS individuals incorporated only 2 metabolic abnormities. (3) Female, age and MS family history were risk factors of MS while smoking exerts protective effect.

P141 The Mean High-density Lipoprotein (HDL-C) Level in Shanghai Community

Qing LI, Wei-ping JIA, Jun-xi LU, Yuan-ming WU, Su-ying JIANG, Kun-san XIANG
Department of Endocrinology and Metabolism, Shanghai Jiaotong University Affiliated No.6 People's Hospital, Shanghai Clinical Center for Diabetes, Shanghai Diabetes Institute, Shanghai 200233, P.R. China

Objective: To determine the mean high-density lipoprotein (HDL-C) level in Shanghai community. **Methods:** A total of 5852 Shanghai Chinese (men 2430, women 3198) aged over 20 years were included. Body Mass Index (BMI), blood pressure, fasting plasma glucose, lipid profile and plasma insulin concentrations were measured in all subjects. **Results:** (l) The mean high-density lipoprotein (HDL-C) level was 50.69±0.15 mg/dl in our two communities. The women had a significantly higher mean HDL-C level than the men (52.00±0.20 mg/dl vs 48.97±0.22 mg/dl, P<0.01). (2) According to CHINA criteria, 7.73% of our community people had low HDL-C. According to ATPIII criteria, 15.16% of our community people had low HDL-C. (3) The proportion of diabetes mellitus (DM), impaired glucose regulation (IGR), hypertension (HTN), hypertriglycermia, obesity is 22.27%, 12.27%, 47.73%, 60.45% and 57.27% respectively in the population whose high-density lipoprotein cholesterol (HDL-C) were less than 35 mg/dl. The proportion of DM, IGR, HTN, hypertriglycermia and obesity is 12.29%, 12.97%, 45.05%, 62.46% and 52.56% respectively in the population whose HDL-C were between 35 mg/dl and 40 mg/dl. The proportion of DM, IGR, HTN, hypertriglycermia and obesity is 10.34%, 9.35%, 36.26%, 43.63% and 31.3% respectively in the population whose HDL-C were equal to or larger than 60 mg/dl. (4) The multiple stepwise regression analysis in which HDL-C as dependent variable and age, sex, BMI, WHR, FPG, SP, DP, TG, HOMA-IR as independent variables showed that sex, HOMA-IR, BMI, age, TG, FPG and WHR were influencing factors of HDL-C. **Conclusions:** (l) The concentration of HDL-C is different between men and women and among races. (2) That improving the condition of hypo-HDL-C and its concomitant metabolic diseases is significantly important for decreasing the incidence rate and mortality rate of cardiovascular and cerebrovascular diseases.

P142 The Characteristics of Hypertensive Subjects with Metabolic Syndrome and its Components in Communities

Jun-xi LU, Yu-qian BAO, Wei-ping JIA
Department of Endocrinology and Metabolism, Shanghai Jiaotong University Affiliated No. 6 People's Hospital, Shanghai Clinical Center for Diabetes, Shanghai Diabetes Institute, Shanghai 200233, P. R. China

Objective: To study the clinical characteristics of hypertensive subjects with metabolic syndrome and its components in communities. **Methods:** Totally 5628 subjects aged over 20 years were included. According to 1999 WHO definition of metabolic syndrome these individuals were divided into 4 groups as follows: non-metabolic disorder group, isolated hypertension group, hypertension with one component of metabolic syndrome group, hypertension with two components of metabolic syndrome group, hypertension with three components of metabolic syndrome group. **Results:** (1) Among subjects with hypertension, 15.37% are patients with isolated hypertension, 32.40% are patients with one component of metabolic syndrome, 33.36% are patients with two components of metabolic syndrome, 18.87% are patients with three components of metabolic syndrome. (2) Body mass index (BMI), waist (W), waist:hip ratio (WHR), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-c), fasting plasma glucose (FPG), 2h postprandial plasma glucose (2hPG), fasting serum insulin (FIN), 2h postprandial serum insulin (2hIN) and insulin resistant index (HOMA-IR) in three groups of hypertension with one component of metabolic syndrome, hypertension with two components of metabolic syndrome and hypertension with three components of metabolic syndrome were significantly increased than those of isolated hypertension group (P<0.01). (3) The hypertension patients showed a higher insulin resistance, despite the other status of metabolic disorder. And the hypertension patients with more components of metabolic syndrome showed a higher chance to get insulin resistance. (4) In a multiple stepwise regression analysis, all of FPG, 2hPG, FIN, 2hIN, BMI, SBP and TC were risk factors of HOMA-IR. **Conclusions:** (1) In community based population, isolated hypertension patients were rare. About 85% were with more than one metabolic disorder, more than half were metabolic syndrome. (2) The hypertension patients showed a higher percentage of total body fat, abdominal fat and levels of total cholesterol, triglyceride, low-density lipoprotein cholesterol and serum insulin, despite the other status of metabolic disorder. (3) Individuals with hypertension showed severe insulin resistance status, the more components that the individuals carried, the severer the insulin resistance status were. (4) The percentage of total body fat, levels of plasma glucose, serum insulin, total cholesterol and systolic blood pressure were independent risk factors of insulin resistance.

P143 Do Different Combinations of Metabolic Syndrome Components Have Equal Contribution to the Development of Stroke in Chinese Adult Population? — Report from The Technical Working Group of China National Nutrition and Health Survey, 2002-2003

Guang-wei LI[1], Chong-hua YAO, Yi-song HU, Feng-ying ZHAI, Ling-zhi KONG, and The Technical Working Group of China National Nutrition and Health Survey
[1] Dept. of Endocrinology, China-Japan Friendship Hospital, Beijing 100029, China

Background: Stroke is one of the leading causes of death worldwide. Metabolic syndrome greatly increases the risk of cardiovascular disease and stroke. It is not clear as to whether the different combinations of metabolic syndrome components have equal effect on the development of stroke. **Objectives:** To investigate the difference of different combinations of metabolic syndrome components have equal effect on the development of stroke in Chinese population. **Methods:** 71,971 Subjects from 31 provinces, autonomous regions, and municipalities in China were enrolled in a randomized survey on malnutrition, hypertension and diabetes by multi-steps cluster sampling method from Aug-Oct 2002. Height, weight, waist, blood pressure and the laboratory parameters including fasting plasma glucose, total cholesterol, triglyceride, HDL-C and LDL-C were measured in 43,469 subjects with age from 20 to 75 years old participants. Physicians and neurologists diagnosed stroke based on ICD 9 (Code 430, 431, 434, and 436) were recorded during the survey. Metabolic syndrome was defined by 2004 IDF criteria. All the subjects were stratified by the number of cardiovascular risk factors, the central obesity (CO), hypertension (HT), hypertriglyceridemia (HTG), hyperglycemia (HGLY) and low-HDL. Prevalence of stroke in different groups were compared. Logistic regression analysis was performed to evaluate the contribution of different combination of MS components on stroke after the adjustment of age, sex, smoke and LDL-C in Chinese population. **Results:** Prevalence of stroke was 0.2% (26/13195) in the group without any risk factor, however it was greater than 1% in any type of combination of MS. The highest stroke-risk existed in MS groups with the combination of (CB+HT+HGLY+L-HDL) or with the combination of (CB+HT+HGLY), of which prevalence of stroke was 4.51% and 4.31% respectively. Groups with prevalence of stroke greater than 2% were those with the combination of (CB+HT+HGLY+L-HDL+HTG) or (CB+HT+HTG) or (CB+HT+L-HDL+HTG) or (CB+HT+HGLY+HTG) or (CB+HT+L-HDL), of which prevalence of stroke was 2.62%, 2.62%, 2.58%, 2.49% and 2.06%. The MS group with the lowest stroke-risk was group with (CB+L-HDL+HTG), its prevalence was 0.52%. After adjustment of age, sex, smoke and LDL-C, multivariable logistic regression analysis showed that the combination of (CB+HT+HGLY+L-HDL) remain ranks top of the stroke-risk (OR=). Groups with combination of (CB+HT+HTG+L-HDL), (CB+HT+HGLY), (CB+HT+HGLY+L-HDL+HTG), and (CB+HGLY+L-HDL) followed, of which OR were 12.55, 10, 8.9, 8.3 and 8.1 respectively. Group with the lowest stroke-risk was that group with (CB+L-HDL+HTG), its OR was 3.3. **Conclusion:** Different combinations of metabolic syndrome components are not equally contributed to the development of stroke in Chinese adult population, of which group with (CB+HT+HGLY+L-HDL) or (CB+HT+HGLY) ranks the top risk, while as group with (CB+HTG+L-HDL) ranks the lowest risk.

P144 Does Metabolic Syndrome with Hyperglycemia More Strongly Contribute to the Development of Stroke in Chinese Adult Population? — Report from The Technical Working Group of China National Nutrition and Health Survey, 2002-2003

Guang-wei LI[1], Chong-hua YAO, Yi-song HU, Feng-ying ZHAI, Ling-zhi KONG, and The Technical Working Group of China National Nutrition and Health Survey
[1] Dept. of Endocrinology, China-Japan Friendship Hospital, Beijing 100029, China

Background: Both diabetes and metabolic syndrome greatly increases the risk of cardiovascular disease and stroke. However, it remains unclear as to the difference of the impact of metabolic syndrome with or without hyperglycemia on the development of stroke. **Objectives:** To investigate if metabolic syndrome with or without hyperglycemia differently contributed to the development of stroke in Chinese population. **Methods:** 71,971 Subjects from 31 provinces, autonomous regions, and municipalities in China were enrolled in a randomized survey on malnutrition, hypertension and diabetes by multi-steps cluster sampling method from Aug-Oct 2002. Height, weight, waist, blood pressure and the laboratory parameters including fasting plasma glucose, total cholesterol, triglyceride, HDL-C and LDL-C were measured in 43,469 subjects with age from 20 to 75 years old participants. Physicians and neurologists diagnosed stroke based on ICD 9 (Code 430, 431, 434, and 436) were recorded during the survey. Metabolic syndrome was defined by 2004 IDF criteria. Prevalence of stroke in the metabolic syndrome groups with or without hyperglycemia was compared. Logistic regression analysis was performed to evaluate the contribution of different combination of MS components on stroke after the adjustment of age, sex, smoke and LDL-c. **Results:** Prevalence of metabolic syndrome was significantly influenced by plasma glucose levels in both males and females: in age <35, ~45, ~55 and ≥55 females with FPG <5.6 mmol/l and FPG ≥5.6 mmol/l were 2% vs 21.7%, 5.3% vs 41.4%, 11.1% vs 56%, 15% vs 67.2%, in males were 4.7% vs 45.7%, 7.45% vs 57.4%, 7.7% vs 53.8% and 7.7% vs 56.3% respectively. Prevalence of stroke in subgroups with 0, 1, 2 risk factors were 0.19%, 0.63% and 1.45%, whereas in metabolic syndrome subgroups with FPG <5.6 mmol/l, ~7.0 mmol/l and ≥7 mmol/l were increased step by step, the prevalence of stroke were 2.18%, 3.16% and 3.62% respectively. After the adjustment of age and sex, multivariable logistic regression analysis showed that both metabolic syndrome with or without hyperglycemia were more strongly associated with the risk of stroke: OR in metabolic syndrome subgroups with FPG <5.6 mmol/l, ~7.0 mmol/l and ≥7 mmol/l were 7.47, 8.51 and 9.0 compared with the subgroup that have no risk factors. It was much higher than the subgroups with 1 (OR=2.16) or 2 risk factors (OR=4.45). lf further adjusted LDL-c and smoke, the contribution of hyperglycemia on the development of stroke seems attenuated (OR in metabolic syndrome subgroups with FPG <5.6 mmol/l, ~7.0 mmol/l and ≥7 mmol/l were 7.23, 8.04 and 8.25). **Conclusion:** Hyperglycemia greatly increases prevalence of metabolic syndrome in both male and female Chinese. Metabolic syndrome with hyperglycemia more strongly contributed to the development of stroke in Chinese population, meanwhile LDL-c may play important roles independently.

P145 Relationship between Hyperuricemia and Metabolic Syndrome in a Part of Population Undergone Health Check-up in Wuhan Area

Pei-wen LIU, Lu-lu CHEN, Hui SUN, Rui ZHOU, Jie MA, Hong-mei ZHANG, Yue-wei ZHOU, Yan TAO, Lan-lan YI, Wang-dong WANG, Qing-ling ZENG
Xinhua Hospital, Wuhan 430015, China

Objective: To evaluate possible relationship between hyperuricemia (HUA) and metabolic syndrome (MS). **Methods:** Totally 1278 persons (male 919, female 359) who underwent health check-up were enrolled in this study. The prevalence rate of HUA and MS were calculated and analyzed. Correlation analysis was performed among HUA and obesity, waist-hip circumference ratio (WHCR), impaired fasting glucose, hypertension, hypertriglyceridemia, hypercholesterolemia and other signs of MS. Multivariate regression analysis was carried out on the blood uric acid and homeostasis model assessment insulin resistance index (HOMA-IR). **Results:** (1) The prevalence of obesity, impaired fasting glucose, hypercholesterolaemia, hypertriglyceridemia, hypertension in HUA group was signficantly higher than that in the normal blood uric acid group (P<0.05). (2) The standardized prevalence of HUA in high WHCR group was significantly higher than that in normal WHCR group (P<0.001). (3) The standardized prevalence of HUA in MS group was significantly higher than that in the normal group (P<0.001). (4) The standardized prevalence of HUA in the overweight and obese groups was significantly higher than that in the normal weight and lean groups (P<0.001). (5) A positive correlation was found between UA and log HOMA-IR on multivariate regression analysis (P=0.002). **Conclusion:** The blood uric acid was significantly positively correlated to HOMA-IR. Hyperuricemia is associated with obesity, high WHCR and dyslipidemia, and therefore it may be an important factor in occurrence and development of MS.

P146 Changes of Insulin Sensitivity and Plasma Resistin Levels in Subjects with Metabolic Syndrome

Ai-sheng WEI, Jian-hong YE, Ping CHEN, Fu-neng WANG, Fa-sheng CHEN, Jiang-ming LANG
Department of Endocrinology, Affiliated Foshan Hospital of Traditional Chinese Medicine, Guangzhou Traditional Chinese Medicine University, Foshan 528000, China

Objective: To investigate changes of insulin sensitivity and plasma resistin levels in subjects with metabolic syndrome (MS). To investigate the relationships between insulin sensitivity and plasma resistin levels in subjects with MS. **Methods:** 30 subjects aged from 36 to 60 years with MS were selected as MS groups. 20 subjects with normal glucose tolerance, normal serum lipids, normal blood pressure, normal liver function and renal function were selected as control group. The age and sex were matched between the two groups. Insulin sensitivity was evaluated by way of hyperinsulinemic euglycemia clamp technique and plasma resistin concentrations were detected by enzyme linked immunosorbent assay (ELISA). **Results:** (1) During the steady state (last 30 mins) in the hyperinsulinemic euglycemia clamp, M in MS group was significantly lower than that in controls (4.13±1.34 $mg \cdot kg^{-1} \cdot min^{-1}$ *vs* 8.33±1.59 $mg \cdot kg^{-1} \cdot min^{-1}$, respectively, *P*<0.01). M was inversely associated with BMI, WC, TG, FINS and leptin (r= -0.457, -0.497, -0.580, -0.581, -0.580, *P*=0.011, 0.005, 0.001, 0.001, 0.001, respectively), positively associated with HDL-C and adiponectin (r=0.448, 0.560, *P*=0.013, 0.001, respectively). (2) Plasma resistin levels in MS group was not significantly different from that in controls (10.91±4.02 vs 11.35±4.04 ng/mL, respectively, *P*>0.05 for all correlations). No significant association of resistin with BMI, WC, FPG, 2h PG, FINS, TG, HDL-C and M was found (r=0.156, 0.115, 0.138, -0.057, 0.103, 0.175, -0.149, -0.122, respectively, *P*>0.05 for all correlations). **Conclusions:** Our finding showed insulin sensitivity of subjects with MS in this study was significantly decreased. Plasma resistin levels were not significantly different between MS and control group and no significant association of resistin with M.

P147 The Relationship between Metabolic Syndrome and Nonalcoholic Fatty Liver Disease in Non-obese Impaired Glucose Tolerant Subjects

Ying LU, Yan-xa HUANG, Xiao-yi SONG
Department of Medicine, Hospital of Guangzhou Economic and Technological Development District, Guangzhou 510730, China

Objective: To investigate the relationship between metabolic syndrome (MS) and nonalcoholic fatty liver disease (NAFLD) in non-obese impaired glucose tolerant (IGT) subjects. **Methods:** 126 non-obese IGT subjects were divided into 64 IGT with NAFLD and 62 IGT without NAFLD by abdominal ultrasonography examined. Body mass index (BMI), blood pressure, fast and OGGT 2 hour plasma glucose, fast and OGGT 2 hour plasma insulin, plasma lipids and aminotransferase were measured in the two groups. Insulin resistance (IR) was estimated from plasma insulin and glucose as the homeostasis model assessment (HOMA) index. Multivariate logistic regression analysis was used to identify the variables independently associated with NAFLD. **Results:** The group with NAFLD had significantly higher BMI (25.7±1.5 vs 24.8±1.7, P=0.01), fast plasma glucose (6.5±0.4 vs 6.3±0.4 P=0.018), triglycerides (2.6±1.2 vs 2.0±0.8, P=0.002), fast insulin (16.2±5.5 vs 12.5±4.8 P<0.001), 2 hour insulin (58.6±24.8 vs 47.8±20.9, P=0.01), HOMA-IR (4.7 ±1.8 vs 3.5±1.5, P<0.001), alanine aminotransferase (ALT) (23.0±8.8 vs 17.2±8.4, P<0.001), aspartate aminotransferase (AST) (20.3±6.4 vs 16.8±5.4, P=0.001) and MS prevalence rate (86% vs 58%, P<0.001) than the group without NAFLD. Multivariate logistic regression analysis (forward LR) showed HOMA-IR (OR 1.492, 95%CI 1.141-1.951; P=0.003) and MS (OR 3.322, 95%CI 1.221-9.040; P=0.019) were independently associated with NAFLD. **Conclusion:** Non-obese IGT subjects with NAFLD have significantly higher insulin resistance, MS prevalence rate and liver injury than those without NAFLD, and NAFLD is independently associated with MS in non-obese IGT subjects. MS plays a role not only in occurrence of NAFLD but also in liver injury.

P148 Complications of Overweight and Obesity in Children and Adolescents Children and Adolescents

Hui-juan ZHU, Yi-fan SHI, Hui PAN, Ming-ming HU
Department of Endocrinology, Peking Union Medical College Hospital, Beijing 100730, China

Objective: Obesity is now well known as a medical problem among children. The prevalence of overweight status has tripled worldwide in the last 2 to 3 decades including China. Outcomes associated with obesity in adults are now affecting children. This study is to compare the complications of overweight and obesity in children and adolescents with those of obese adults. **Methods:** Blood pressure, oral glucose tolerance status, uric acid, the prevalence of nonalcoholic steatohepatitis (NASH) were compared between 142 children or adolescents and 198 adults with simple obesity of outpatient. Patients were divided into three subgroups in accordance with age as less than 18 years old, 19-40 and 41-60 years old. SPSS 11.0 was used in statistical analysis. **Results:** The prevalence of hypertension in obese children was 18.2%, BMI and waist circumference (WC) were the risk factors of hypertension, the blood pressure had no significant difference between groups adjusted with age. 10.9% children were diagnosed with type 2 diabetes, 29.1% children with IGT. The prevalence of abnormal glucose metabolism of children and adolescents was higher than that of adults aged 19-40 years old (IGT 13.9%, DM 5.6%) (p=0.0097) .The level of blood uric acid were higher than normal in 35.3% obese children. It had no significant difference between groups (27.7% and 25%, respectively). 83.3% children were diagnosed with NASH, there was no significant difference between subgroups (85.0% and 92.4%, respectively). The levels of amino transferase were related to BMI, WC, level of fasting blood glucose and insulin. 49.5% obese children had the symptom of snore which was less than the prevalence of the other elder subgroups. 19.3% girls has the history of irregular menses or amenorrhea. **Conclusion:** The prevalence of complications related to obesity of children and adolescents was not less than that of adults. The screening and treatment of these outcomes should be taken into account among overweight and obese children.

P149 Insulin Resistance in Obese Accompanying Hypertriacylglycemia Patients

Ding-yu CHEN, Ling HE, Zheng-hua XIAO
Department of Endocrinology, Guangzhou 1st Hospital, 510180, China

Objective: To investigate insulin resistance level in obese accompanying hypertriacylglycemia patients. **Methods:** Ninety out-patient clinic subjects receiving health examination in 2004 with body mass index (BMI) over 25 kg/m^2 were enrolled. Blood pressure, height, weight, waistline, hip circumference, body lipid proportion, body lipid mass, blood lipid, blood uric acid, glycosylated hemoglobin (HbA1c), blood glucose and insulin level at fasting and 2 hours after taking 75 g glucose were measured and homeostasis model assessment (HOMA) were chosen to evaluate insulin resistance level (HOMA-IR). **Results:** (1) The prevalence of hypertriacylglycemia, hypercholesterolemia and ortholiposis in this obese group were 36.6%, 37.8% and 25.6%, respectively. (2) Levels of HOMA-IR and WHR were higher in patients with hypertriacylglycemia than in normal triglyceride ones (0.91 vs 0.87, P=0.008; 4.62 vs 3.36, P=0.012). (2) Blood uric acid, body lipid mass, FBS and Fins were higher in insulin resistance group than those without insulin resistance (440.9 vs 369.0, P=0.043; 24.92 vs 21.24, P=0.005; 5.38 vs 4.46, P=0.01; 20.03 vs 10.48, P=0.000). (3) HOMA-IR index was positively correlated with body lipid mass and TG level, respectively (r=0.306, P=0.000, N=65; r=0.288, P=0.000, N=81). **Conclusions:** Insulin resistance exists in these obese accompanying hypertriacylglycemia subjects and screening for these disorders is necessary in obese patients.

P150 Serum Profile of Proinflammatory Factors (CRP, IL-18 and adiponectin) in Obesity with Glucose Intolerance

Wei-qiong GU, Jie HONG, Yi-fei ZHANG, et al
Medical Faculty of Shanghai JiaoTong University, Ruijin Hospital, Shanghai Research Institute of Endocrine and Metabolic Diseases, Shanghai Clinical Center for Endocrine and Metabolic Diseases, 200025, China

Objectives: To explore the serum profile of proinflammatory factors, represented by CRP, IL-18 and adiponectin in obesity with glucose intolerance. **Methods:** 167 obese subjects (BMI >25 kg/m^2) were divided into NGT, IGT and DM groups according to the results from OGTT. The SI and AIRg were estimated by FSIGT combined with Bergman's minimal model. Fasting serum was drawn so as to test hs-CRP, IL-18 and adiponectin by ELISA and RIA respectively. **Results:** Insulin resistance resisted in all obese subjects. Serum CRP and IL-18 levels increased in IGT and DM groups, while adiponectin decreased compared to NGT group, the difference among groups was significant. BMI, serum glucose and lipid level effected the changing of these three factors in varying extent. Insulin sensitive index was only negtively correlated to adiponectin. No relationship was found among CRP, IL-18 and adiponectin. **Conclusion:** In obese NGT, IGT and DM groups, serum CRP and IL-18 show an increase tendency while adiponectin dcreases, which indicate the different role of these proinfammatory factors played in the development of diabetes mellitus.

P151 Genetic Variant of Adipocyte–fatty acid–binding protein (A-FABP) with a Favourable Phenotype against Obesity and Metabolic Syndrome

D.C.Y. YEUNG, A. XU, P.C. SHAM, X.M. ZHANG, J.Y. XU, K.S.L. LAM
Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, HKSAR, China

Objective: Adipocyte-fatty acid-binding protein (A-FABP) is an adipokine regulating systemic insulin sensitivity, lipid and glucose metabolism. We have previously shown its release from adipocytes and it is present in significant concentrations in the circulation. In humans serum A-FABP levels correlate significantly with features of the metabolic syndrome, a closely related cluster of cardiovascular risk factors which includes central obesity, dyslipidemia, hyperglycaemia and hypertension. This study aims at identifying polymorphisms in the human A-FABP gene, which may be used as genetic markers for predicting the development of the metabolic syndrome. **Methods:** About 1000 bp of the promoter region and four exons of A-FABP gene of 48 subjects with BMI <25 and 52 subjects with BMI≥25 were directly sequenced. The sequences of the A-FABP promoter region of a further 126 subjects were also elucidated. Statistical analysis was computed using SPSS14.0, while linkage analysis was done by Haploview. **Results:** Four polymorphisms, rs16909225, rs3834363, rs16909220 and Ins+4200C, were identified, of which rs16909225, rs16909220 and Ins+4200C were completely linked ($r^2 = 1$). Age- and sex-adjusted individuals who were homozygous for the variant rs3834363 showed significantly lower serum A-FABP levels (p=0.001), BMI (p<0.001), waist circumference (p=0.002), fasting insulin (p=0.001), HOMA insulin resistance index (p=0.003) and triglycerides (p=0.003). **Conclusion:** The DNA sequence harbouring rs3834363 contains a potential C/EBP-alpha binding site, and the significant decrease in A-FABP levels in rs3834363 homozygotes may be due to the loss of transcription factor binding. The question of whether rs3834363 is a functional polymorphism of A-FABP is being examined. To summarize, individuals homozygous for the A-FABP rs3834363 genetic variant possess a favourable phenotype against obesity and the metabolic syndrome.

P152 Screening and Identification on Differential Proteins of Human Visceral and Subcutaneous Adiposities

Xiao-hua LI[1 2], Sheng ZHENG[1], You-ping LIU[1], Hua-feng LI[1], Tian-hong LUO[1], Guo LI[1], Min LUO[1]
[1] Shanghai Institute of Endocrine and Metabolic Disease, Department of Endocrinology and Metabolism Disease, Shanghai Jiaotong University affiliated Ruijin Hospital
[2] Department of Endocrinology, Diabetic Laboratory, Shanghai Jiaotong University Affiliated First People's Hospital, Shanghai 200080, China

Objective: To screen the differential proteins of visceral and subcutaneous adipocytes and analyze their function. **Methods:** The total proteins of visceral and subcutaneous adipocytes were separated by two-dimensional gel electrophoresis (2-DE). After silver staining, the differentially expressed proteins were analyzed by using imaging analysis software PDQuest 7.2 and 10 differentially expressed spots were identified by MALDI-TOF-MS. **Results:** The average matching rate between the two teams is 0.71 (r=0.71). And the average differential spots is 82. After identified by mass spectrum, we found six differential proteins, including Kruppel-like factor 15, Adipsin, Ribosomal protein s6 kinase, Ras-related protein Ral-A, Rho-kinase and a new protein My027. They involved in the cell differentiation, apoptosis, signal transduction of insulin, and energy metabolism, which involved in signal transduction of insulin, lipid metabolism, and cell differentiation. **Conclusion:** We screened and identified the differential protein profiles between the visceral and subcutaneous adipocytes. The differentially expressed proteins we reported might play an important role in the pathogenesis of the visceral obesity-related metabolic disorders, and provide a basis for further work on why visceral fat accumulation can be dangerous.

P153 Study on the Differentially Expressed Genes in Visceral Adipose Tissue in Obese and T2DM Patients as well as its Relationship with Adipocyte Differentiation

Yu ZHAO, Tian-hong LUO, Hong-li ZHANG, Wen-yi LI, Li-hong XU, Qin ZHANG, Shen ZHENG, Guo LI, Min LUO
Medical Faculty of Shanghai JiaoTong University, Ruijin Hospital, Shanghai Research Institute of Endocrine and Metabolic Diseases, Shanghai Clinical Center for Endocrine and Metabolic Diseases, 200025, China

Objective: To identify genes that are differentially expressed in omental fat in normal subjects, obese subjects and obese diabetic patients, and to study the expression pattern of these differentially expressed genes during preadipocyte differentiation, in order to clarify the relationship of preadipocyte differentiation with pathogenesis of obesity and T2DM. **Methods:** Gene expression profile of omental fat from normal weight subjects, obese subjects and obese diabetic patients were compared using high-density cDNA microarray, to identify adipose-specific genes associated with obesity and T2DM. Then genes with the most prominent difference of expression in obese and dibetic patients were selected and their expression patterns during preadipocyte differentiation were examined. **Results:** 119 and 46 genes were up- and down-regulated respectively in obese patients, while 256 and 58 genes were up- and down-regulated respectively in obese diabetic patients. Among them, 77 and 8 genes were up- and down-regulated in both obese and obese diabetic patients, these genes include immunoregulating, signal transduction and energy metabolism, especially lipid synthesis related genes as well as some functionally undetermined genes. We then selected some genes with the most prominent difference between normal subjects, obese subjects and obese diabetic patients and examine their expression pattern during 3T3L1 preadipocyte differenciation. The results showed that the expression level of RGS2, ADAMTS1 and KLF4 increased 15.2, 8.3 and 3.1 times respectively after preadipocyte was induced into mature adipocyte, indicating a close relationship of these genes with differentiation and maturation of preadipocyte. **Conclusions:** Our study demonstrated that there is similarity between gene expression profile of omental adipose tissue from obese subjects and obese diabetic subjects. The differentially expressed genes include signal transduction, immunoregulating and energy metabolism, as well as preadipocyte differentiation related genes. A further functional analysis of these genes will probably shed light on the pathogenesis of obesity and T2DM, as well as provide new therapeutic target.

P154 The Relationship between Expression of Prepeo-orexin and Orexin Receptors Gene and Hypothalamic-pituitary-adrenal Axis in Dietary Obesity Rats

Li-bin LIU, Li-ping LIAO, Zhou CHEN
Department of Endocrinology, Union Hospital, Fujian Medical University, Fujian Institute of Endocrinology, China

Objective: To investigate the relationship between expression of prepeo-orexin and orexin receptors gene and hypothalamic-pituitary-adrenal (HPA) axis in dietary obesity rats. **Methods:** SD rats were fed with high-fat diet to induce obesity. Expression of prepro-orexin, orexin receptors 1 and 2 mRNA in the hypothalamus and adrenal, and expression of corticotrophin releasing hormone (CRH) mRNA in the hypothalamus were detected by RT-PCR. The concentration of serum corticosterone and 24 hours urine corticosterone were assayed by ELISA. Analyze the relationship between orexin and HPA axis by correlation analysis. **Results:** Expression of prepro-orexin mRNA in the hypothalamus was decreased in obese rats ($P<0.05$). Expression of orexin receptor and CRH mRNA in the hypothalamus show no obvious difference between obese rats and controls. Expression of orexin receptor-2 mRNA in adrenal was increased in obese rats. Serum corticosterone and 24 hours urine corticosterone were reduced in obese rats. In obese rats, expression of prepro-orexin mRNA in the hypothalamus was inversely correlated to that of orexin receptor-2 in adrenal, and expression of orexin receptor-2 in adrenal was positively correlated to that of CRH. Serum corticosterone concentration was inversely correlated to expression of CRH and orexin receptor-2 gene in adrenal. And there was no correlation between corticosterone level and weight or Lee's index. **Conclusions:** Expression of prepro-orexin in the hypothalamus may be decreased in dietary obese rats. Expression of orexin receptor-2 in adrenal was correlated to expression of CRH mRNA, and corticosterone concentration.

P155 Rosiglitazone Decreases Alzheimer-like tau Phosphorylation in Hippocampus in Obese Rats

Yan YANG, Shu-hong HU, Mu-xun ZHANG
Department of Endocrinology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

Aim: To study phosphorylation of tau protein in the hippocampus of obese rats during insulin resistance and the effect of rosiglitazone administration on tau phosphorylation. **Methods:** Wistar rats were randomized into 3 groups. The control group (CTL) was fed with normal food. The other two groups were fed with high sugar, high fat and high protein diet for 3 months (OB group), followed by plus rosiglitazone at a dose of 3 $mg \cdot kg^{-1} \cdot d^{-1}$ (TZD group) for 4 weeks. The plasma insulin level was measured by RIA method, and the plasma glucose by glucose-oxidase method. The indexes of insulin resistance were calculated by HOMA-IR. The expression of Tau[$pS^{396/404}$] was observed by immunohistochemical assay, as well as total tau level and the phosphorylation level of tau at individual phosphorylation sites (Ser199, Thr212, Ser214, Ser396 and Ser422) were analyzed by Western blots. **Results:** We found no difference of plasma glucose levels among OB, TZD and CTL groups. Plasma insulin was significantly higher in OB group than in CTL group, and TZD treatment reduced it to the control level. Insulin resistance, which was calculated by HOMA-IR, was significantly higher in OB than CTL group, and TZD treatment restored insulin sensitivity to nearly the control level. The expression of phosphorylated tau modestly increased in CA3 sector of hippocampus in OB group compared with TZD group and CTL group. Neither obesity-induced insulin resistance nor TZD treatment changed the total level of tau protein in the hippocami of rats. However, Tau was found to be hyperphosphorylated at several AD-related phosphorylation sites (Ser199, Thr212, Ser214, Ser396 and Ser422) in OB group. TZD treatment reduced obesity-induced hyperphosphorylation of Ser199, Thr212, Ser396 and Ser422 of tau significantly and of Ser214 of tau to the control level. **Conclusions:** These findings suggest (1) that insulin resistance leads to hyperphosphorylation of tau protein, and rosiglitazone can partially reverse it, and (2) insulin resistance induced by obesity causes a downregulation of insulin signal transduction and the consequent upregulation of GSK-3β activity, which leads to hyperphosphorylation of tau protein.

P156 Preventing Dyslipidemia and Altering Insulin Sensitivity of Chickpea and Its Extracts in Rodents Fed with a High-fat Diet

Jin-feng TANG, Ying YANG, Yi-bo ZHANG, Li-bin ZHOU, Feng-ying LI, Ming-dao CHEN
Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai Clinical Center of Endocrine and Metabolic Diseases, Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200025, China

Objective: This study evaluated the effects of chickpea and its extracts on preventing dyslipidemia and altering insulin sensitivity in obese rats and mice feeding with high fat diet. Methods: (1) Male rats were fed normal fat diet (NFD), high fat diet (HFD) or HFD+chickpea (HFD+CP) for 6 months. (2) Male ICR mice received NFD (n=10) and HFD (n=50) for 100 days, the HFD animals were randomly divided into five groups of 10 mice each, which were administered distilled water, chickpea aqueous extract (CPA 0.2 g/kg/day, 0.4 g/kg/day), and ethanolic extract (CPE 0.2 g/kg/day, 0.4 g/kg/day). The NFD group was also administered distilled water. **Results:** (1) Dietary chickpea significantly suppressed the HFD-induced increase in body weight gain and epididymal fat pad weight, caused favorable plasma lipid levels reflecting decreased TG, LDL-C, and LDL-C/HDL-C ($p<0.05$). (2) Rats fed the HFD had higher TG concentration in muscle and liver, whereas the addition of chickpea to the HFD drastically lowered TG concentrations in muscle (39%) and (23%) liver, further research observed by transmission electron microscopy (TEM) showed that dietary chickpea normalized the increased intramyocellular fat in soleus muscle. (3) The activities of lipoprotein lipase (LPL) in epididymal fat and hepatic triglyceride lipase (HTGL) in liver recorded a 40% and 23% increase in HFD rats compared with NFD rats respectively, dietary chickpea completely normalized the levels. (4) Insulin tolerance test (ITT), oral glucose tolerance test (OGTT) as well as insulin releasing test (IRT) showed that the administration of chickpea significantly improved insulin resistance, prevented postprandial hyperglycemia and hyperinsulinemia induced by long-term high fat diet. (5) Further research in HFD induced mice showed that the ethanolic extract rather than the aqueous extract was responsible for these beneficial effects from chickpea. **Conclusion:** Our findings provide a biochemical and nutritional basis for the use of chickpea as a functional food factor, which may have important implications for the reduction of body fat accumulation and for the prevention of diabetes.

P157 The Effect of Short-term and Long-term Fasting on the Expression and Secretion of Ghrelin in Normal, Obese and Obese after Weight Reduction Rats

Huai-dong SONG, Li SHAO, Rong-ying LI, Xue-song LI, Shuang-xia ZHAO, Jie QIAO, Ming-dao CHEN
Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai Clinical Center of Endocrine and Metabolic Diseases, Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200025, China

Objective: Ghrelin, secreted from stomach, plays an important role in appetite regulation and energy imbalance, while its expression and secretion is regulated by feeding status. In the present study, we aimed at investigating the effect of short-term and long-term fasting on the expression and secretion of ghrelin in normal, obese and obese after weight reduction rats, further to know the factors implicating in regulating ghrelin in response to fasting. **Methods:** (1) Establishing obese model with high fat diet and then reducing the weight of some obese rats with very low caloric diet. (2) Normal, obese and obese after weight reduction rats were fasted for different periods, then stomach and serum were selected for analysis. The expression and secretion of ghrelin was studied using northern blot and RIA. (3) The secretion pattern of leptin and ratio of epididymal fat to weight were further analyzed. **Results:** (1) The expression and secretion of ghrelin was increased first, then began to decrease in response to different periods of fasting, with higher level of secretion after fasting for 24h, while its expression at 72h. (2) There was no significant change of ghrelin expression and secretion in obese rat in response to different periods of fasting. Consistent with other reports, we also found that both expression and secretion of ghrelin were decreased in obese rats in feeding status. (3) The patterns of ghrelin expression and secretion in obese rats after weight reduction were similar to normal rats in response to different periods of fasting. (4) The secretion of letpin in rats was decreased with fasting in all these models. In other words, the secretion pattern of leptin was same in different models in response to fasting. After fasting, the ratio of epididymal fat to weight is higher in obese rats and returned to similar level after losing weight, when compared with normal rats. **Conclusion:** Body fat may play an important role in the regulation of expression and secretion of ghrelin in different models after various periods of fasting and ghrelin seems to be a key factor in energy metabolism during long-term life.

P158 Effects of Short-term and Long-term Fasting on Transcriptional Regulation of Genes Involved in Glucose and Lipid Metabolism in Rat Tissues

Rong-ying LI, Shuang-xia ZHAO, Jie QIAO, Li SHAO, Huai-dong SONG, Ming-dao CHEN
Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai Clinical Center of Endocrine and Metabolic Diseases, Ruijin Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200025, China

Objective: Many genes involved in metabolism were identified showing differential expression in fasting rat adipose tissue with cDNA chip. Then we aimed at studying the expression patterns of these genes in rat tissues after different periods of fasting, to evaluate at transcriptional level the role of these genes in the process of transition from short-term to long-term fasting. **Methods:** cDNA chip was used to study genes profile of normal and fasting rat adipose tissue, both Northern blot analysis and real time PCR were performed to investigate the expression of genes involved in metabolism in rat tissues in response to different periods of fasting. **Results:** 98 genes/ESTS were identified to show different expression. Among these genes, genes implicated in glucose metabolism and fatty acid or lipid metabolism displayed the greatest transcriptional changes with fasting. Northern blot analysis and real time PCR were performed to investigate the expression patterns of these genes in rat tissues after short- and long-term starvations. The results of the increased expression of the pyruvate dehydrogenase kinase4 (PDK4) gene and decreased pyruvate dehydrogenase (PDH) in rat muscle together with decreased fatty acid synthase (FAS) in rat adipose tissue after one day of fasting (F1) suggested from transcriptional level that glucose aerobic oxidation was down-regulated in rat muscle and synthesis of saturated fatty acids was inhibited in rat adipose tissue after short-term fasting. It was noted that the transcriptions of genes involved in the fatty acid oxidation, such as LCAH, ACO, CPT-I and CAT were greatly increased in F1 rat liver, then began to decrease in F3 and 5-day fasting (F5) rat liver. **Conclusion:** These data suggest that inhibition of the oxidation of lipid may play an important role in the phase II-phase III of fasting transition in the long-term fasting rats. The present study provides a broad perspective of the molecular events occurring physiologically in white adipose tissue in response to starvation.

P159 Effects of Intermittent High Glucose on Gene Expression Profiles in Human Umbilical Vein Endothelial Cells—a Preliminary Study

Hong-da ZHU, Huai-dong SONG, Ming-dao CHEN
Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai Clinical Center for Endocrine and Metabolic Diseases, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China

Objective: Vascular complications are the most important cause of the morbidity and mortality in diabetic patients. However, the mechanism of the endothelial cell dysfunction under hyperglycemia has not been clearly elucidated so far. To explore the gene expression profiles of human umbilical vein endothelial cells under intermittent high glucose circumference. **Methods:** For mimicking the glucose excursion in the physiological states, a second group with intermittent high glucose levels (5 and 25 mmol/L) was added in addition to a continuous high glucose level group (25 mmol/L). Besides, the cells under 5 mmol/L of glucose level served as control. The duration of intervention lasted 7 days. Affymetrix U133 plus 2 chips were used for detecting the gene expression profiles of the 3 groups. **Results:** It revealed that more genes up- or down-regulated in intermittent high glucose group than in continuous high glucose group. The discrepancy at the number of the down-regulated genes was even bigger. Both of the high glucose groups rendered the expression of the apoptosis-related gene, BAX, up-regulated, however, different effects on the other genes within apoptosis pathway were found. In the intermittent high glucose group, a great number of the genes related to the oxidative phosphorylation up-regulated by the similar folds, most of them were enzymes. But the gene expression profile of the continuous high glucose group was more likely as the control group than the intermittent ones. **Conclusion:** These results suggest that high level of glucose makes the endothelial cells tend to apoptosis. The mechanisms of intervention by continuous high glucose and fluctuation of glucose levels group are different. The intermittent high glucose levels may do more harm to the endothelial cells than that in continuous high glucose environment.

P160 The Effect of Increase of Mitochondrial Biogenesis on Hyperglycemia-induced Reactive Oxygen Species Generation

Xin-yu WANG, Guo-chun LUO, Ling-bo LV, Huai-xin YU
Department of Endocrinology, Shen Zhen Second People's Hospital, 518035, China

Objective: Excessive reactive oxygen species (ROS) accumulation induced by hyperglycemia is the leading cause of diabetic macro and micro vascular complications. Much data from cell and animal studies indicate that excessive ROS in endothelial cells decrease the mitochondrial number, function and mitochondrial biogenesis. We postulated that increasing mitochondrial biogenesis could decrease intracellular ROS level. To verify the notion above, peroxisome proliferator activated receptor coactivator 1α (PGC-1α), a major regulator of oxidative metabolism and mitochondrial biogenesis will be over expressed in endothelial cells, aimed to increase mitochondrial biogenesis, meanwhile, ROS level will be observed. **Methods:** Human Umbilical Vein Endothelial Cells (HUVEC) were isolated from umbilical cord veins and cultured in 5 mM glucose or 30 mM glucose medium respectively. Confluent HUVEC cells were exposed to retroviral vectors, pLenti6/V5 TOTO PGC-1α at an moi of 50 for 12 h. The expression of PGC-1α and Cytochrome C reflecting mitochondrial biogenesis were analyzed by Western blotting with chemiluminescence detection. ROS was measured by flow cytometry. **Results:** ROS level in 30 mM glucose medium HUVEC cells was siganificantly higher than that in 5 mM glucose medium HUVEC cells ($p<0.01$); Compared with control (HUVEC cells infected by non insert retrovirus), Cyt C expression was obviously increased in HUVEC cells infected by pLenti6/V5 TOTO PGC-1α; ROS level decreased dramatically in HUVEC cells infected by pLenti6/V5 TOTO PGC-1α both in 5 mM glucose and 30 mM glucose medium, but reduced extent of ROS in 30 mM glucose HUVEC cells was more obvious than that in 5 mM glucose. **Conclusion:** PGC-1α increases mitochondrial biogenesis that reduced accumulation of ROS in endothelial cells. The data suggest that PGC-1α could play a crucial protective role in vascular complications of diabetes. It needs to further investigate the pathway how PGC-1α regulate mitochondrial biogenesis and mitochondrial metabolism.

P161 Effects of Advanced Glycation End Products on Caveolin-1 and Endothelial Nitric Oxide Synthase in Endothelial Cells

Sammy SHIU, Kathryn TAN
Department of Medicine, University of Hong Kong, Hong Kong SAR, China

Objective: Recent studies have suggested that advanced glycation end products (AGEs) may be one of the causes of endothelial dysfunction in diabetes mellitus. However, the underlying mechanism(s) are not fully understood. Since AGEs bind to the receptor for advanced glycation end products (RAGE) in caveolae, this study was performed to investigate whether AGEs influence endothelial nitric oxide synthase (eNOS) activity by affecting caveolin-1 which is involved in the post-translational regulation of eNOS. **Methods:** Caveolin-1 enriched membrane fractions were isolated from human aortic endothelial cells using a modified detergent-free extraction procedure. Isolated caveolae were subjected to Western blotting analyses with anti-caveolin-1, eNOS and RAGE antibodies. Nitric oxide (NO) production in response to AGEs was measured by colorimetric assay. **Results:** AGEs reduced NO production by endothelial cells in a dose-dependent manner despite no significant change in eNOS mRNA and protein in total cell lysate. Treatment with 200 μg/mL AGEs significantly increased protein levels of caveolin-1 (24.4%±3.7 vs. 13.5±5.6 in untreated control, p=0.03) and RAGE (30.0%±6.9 vs. 12.6±2.1, p=0.04) in caveolae compartment which was associated with a reduction in eNOS activity and NO production. This resulted in increased expression of adhesion molecules like vascular cell adhesion molecule-1 in the endothelial cells. **Conclusion:** Our results suggest that AGEs induce endothelial dysfunction by increasing caveolin-1 expression in endothelial cells and reduce eNOS activity and NO production. Whether the up-regulation of caveolin-1 is mediated by the interaction of AGEs with RAGE warrants further investigations.

P162 Effect of Advanced Glycation End Products on Expression of Hepatocyte Growth Factor in Human Umbilical Vein Endothelial Cells

Jin ZHANG[1], Yi-jun ZHOU[2]
[1] Department of Endocrinology and Metabolism, First Affiliated Hospital, Shenyang 110001, China
[2] Department of Endocrinology and Metabolism, Fourth Affiliated Hospital, China Medical University, Shenyang 110032, China

Objective: To investigate the effects of advanced glycation end products (AGEs) on protein and mRNA expression of hepatocyte growth factor (HGF) in cultured human umbilical vein endothelial cells (HUVECs). **Methods:** HUVECs were cultured *in vitro* and treated with AGEs at different concentrations for 24 h and at a concentration of 400 mg/L for different time. The HGF mRNA was determined by reverse transcriptase-polymerase chain reaction (RT-PCR) and HGF protein was analyzed by immunocytochemistry. **Results:** Immunocytochemical staining showed that after treated with AGEs at different concentrations (100 mg/L, 200 mg/L, 400 mg/L) for 24 h, integrated optical density (OD) of HGF in HUVECs was significantly higher than that of control group ($P<0.05$). After treated with AGEs at a concentration of 400 mg/L for 6 h, 12 h and 24 h, the OD of HGF in HUVECs were strikingly higher than that of 0 h. After 48 h, the OD of HGF was decreased. RT-PCR showed that treated with AGEs at different concentrations for 24 h, expression of HGF mRNA were increased in HUVECs compared with control group ($P<0.05$). The expression of HGF mRNA was elevated after treated with 400 mg/L AGEs for 6 h and reached the peak at 24 h. After 48 h, the expression of HGF mRNA was decreased. **Conclusion:** Advanced glycation end products may induce the expression of HGF mRNA and protein in the early stage then caused a gradient decrease of HGF expression in HUVECs.

P163 Resistin Inhibits Production of Nitric Oxide through AMP-activated Protein Kinase Signaling in Vascular Endothelial Cells

Zhi-zhen LI, Fang-ping LI, Li YAN, Jian-hong YE, Jia ZHOU, Hua CHENG, Zu-zhi FU
Department of Endocrinology, The Second Affiliated Hospital, SUN Yat-sen University, Guangzhou 510120, China

Objective: To investigate the effects and mechanisms of resistin on HUVECs NO production and the role of resistin in the pathogenesis of atherosclerotic disease. **Methods:** HUVECs were cultured for experiments. Passage 3-5 cells were treated with different concentration of resistin (15, 50, 100 ng/mL) for 24 h. NO production was measured by DAF-2DA fluorescence. eNOS and AMPK phosphorylation were measured by western-blot. **Results:** The NO production of resistin treated groups were significantly lower than that of control group ($p<0.05$). With the decrease of NO production, the phosphorylation of eNOS and AMPK were decreased significantly ($p<0.05$ vs control). However, when AICAR was used for activating AMPK in 50 ng/mL resistin treated group, the phosphorylation of eNOS and AMPK were significantly increased compared with group treated with 50 ng/mL resistin but not treated with AICAR. At the same time, AICAR treatment increased NO production for 1.5-2 folds. These results suggest AMPK was involved in the cell-signaling of resistin-inhibited endothelial cell NO production. **Conclusions:** Resistin inhibits NO production in HUVECs. The main point of the mechanisms of resistin regulated endothelial NO production may be the regulation of eNOS phosphorylation through AMPK signaling. Our findings suggest that resistin-mediated inhibition of endothelial cell NO production may contribute to cardiovascular disease.

P164 The Relationship between Plasma Osteoprotegerin and Endothelium-dependent Arterial Dilation in Type 2 Diabetes

Guang-da XIANG, Hui-ling SUN, Lin-shuang ZHAO, et al
Department of Endocrinology, Wuhan General Hospital of Guangzhou Command, Wuluo Road 627, Wuhan 430070, Hubei Province, China

Objective: To investigate the relationship between the plasma osteoprotegerin (OPG) levels and endothelium-dependent arterial dilation in type 2 diabetic patients. **Methods:** The subjects included 40 newly diagnosed type 2 diabetic patients and 46 healthy subjects. All patients were then given insulin therapy for 6 months. Plasma OPG concentration was measured in duplicate by a sandwich ELISA method, and high-resolution ultrasound was used to measure brachial artery diameter at rest, after reactive hyperemia and after sublingual glyceryltrinitrate (GTN). **Results:** Plasma OPG level in patients before treatment was 3.36±0.32 ng/L, which was significantly higher than that in control (2.38±0.25 ng/L) ($p<0.01$). After 6 months treatment, OPG levels decreased markedly (2.83±0.34 ng/L) ($p<0.01$). The flow-mediated endothelium-dependent arterial dilation in patients before treatment was 3.21±0.52%, which was significantly lower than that in control (4.46±0.56%) ($p<0.01$), and improved markedly after 6 months treatment (4.03±0.49%) ($p<0.01$). In multivariate analysis, OPG was significantly associated with endothelium-dependent arterial dilation, fasting blood glucose (FBG), hemoglobin A1c (HbA1c) and ultra sensitive C-reactive protein (CRP) at baseline ($p<0.01$). The absolute changes in OPG were significantly correlated with the changes in endothelium-dependent arterial dilation, FBG, HbA1c, and CRP in diabetic patients during the course of treatment ($p<0.01$). **Conclusion:** This study shows that plasma OPG levels are elevated in newly diagnosed diabetic patients, and that plasma OPG levels are significantly associated with vascular endothelial function.

P165 Pro-opiomelanocortin Overexpression Alters the Endothelin-1 Homeostasis and Angiogenic Functions in Human Endothelial Cells

Hing-chung LAM, Shiao-mei KUO, Peyru LIN, Guei-sheung LIU, Ming-hong TAI
Department of Medical Education and Research, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan 81346, R.O.C.

Objective: To investigate the effect of pro-opiomelanocortin (POMC) overexpression on the endothelin-1 (ET-1) homeostasis and angiogenic functions in human EA.hy926 endothelial cells. **Methods:** Adenovirus vectors encoding POMC (Ad-POMC) and green fluorescent protein (GFP; Ad-GFP) were generated and were employed in the present study. ET-1 was measured by the quantitative enzyme immunoassay technique. Cell proliferation, migration, as well as tube formation assays were performed as previously described (Atherosclerosis 2006;186:448-57). **Results:** POMC gene delivery significantly decreased the ET-1 release ($p<0.001$) without affecting the ET-1 messenger RNA level. It also significantly inhibited the migration ($p<0.01$) and tube-forming capability ($p<0.01$) of endothelial cells. Exogenous ET-1 could partially reverse the POMC-induced inhibition of tube formation ($p<0.05$). **Conclusion:** It suggests that the interaction between POMC and ET-1 may contribute to the pathogenesis of endothelial dysfunction.

P166 Effect of Homocysteine on Expression of ICAM-1 of Human Vessel Endothelial Cells in High Glucose

Zhuo-wei ZHANG, Jie LIU
Department of Endocrinology, Shanxi Province People's Hospital, Shanxi Taiyuan, 030012, China

Objective: To investigate the effect of Homocysteine (Hcy) on the production of reactive oxygen specine (ROS) and the expression of intercellular adhesion molecule-1 (ICAM-1) in human vessel endothelial cells cultured in high glucose. **Methods:** In vitro raises the human vessel endothelial cells, the experimental simulation diabetes high Hcy condition, stochastically divides into four groups: control group; high glucose group; Hcy group; high glucose with Hcy, the activities of SOD and the level of MDA in the supernatant were measured by spectrophotometry and the expression of ICAM-1 mRNA in the cells were measured by RT-PCR. **Results:** Compared with the control group, high glucose and Hcy groups SOD obviously reduces ($P<0.01$), but the MDA obviously elevates ($P<0.01$), and high glucose or Hcy stimulated the expression of ICAM-1 mRNA ($P<0.01$). Also this kind of change is more obvious in the high glucose with Hcy group ($P<0.05$), and lengthens as necessary has the obvious increase the tendency. **Conclusion:** Hcy might up-regulate the expression of ICAM-1 mRNA through oxidative stress mechanism, thus damage the vessel endothelial cell, aggravates the diabetes vessel complications. Therefore studies the role and the partial mechanisms which Hcy to prevents and controls the diabetes complication to have the vital significance, and intervenes the hyperhomocysteinemia for the clinical early time to provide the new theory basis.

P167 The Relation of Adipose Tissue in Abdominal Body Composition of Dual-energy X-ray Absorptiometry (DEXA) and Insulin Resistance of Type 2 Diabetes Mellitus

Ling JIANG, Ning SU
Department of Endocrinology, Qilu Hospital of Shandong University, Jinan 250012, China

Objective: To study the relationship between abdominal body composition, especially fat tissue, of dual-energy X-ray absorptiometry (DEXA or DXA) and insulin resistance of type 2 diabetes mellitus (T2DM). Meanwhile, to prompt a new custom DEXA region of interest (ROI). **Methods:** Thirty-nine T2DM patients of the outpatient department and endocrinology department of QILU Hospital, Shangdong University, whose insulin sensitivity has not been interfered by extrinsic insulin or thiazolidinedione etc, and twenty-two healthy objects are included. Their age, height, waist circumstance etc, were recorded. Body composition analyses, especially in a new ROI by DEXA were performed on them. This ROI is defined as rectangle area between the two horizontal lines passing the intervertebral space of L3-L4 and the clearance between L5 and sacrum. The other borderlines distinguish the abdominal tissue from the other exactly. The objects' fast insulin (FINS) was measured by time-resolved fluoroimmunoassay (Tr-FIA) and fast plasma glucose (FPG) by the way of glucose oxidase (GOD). Homa model IR index was used to indicate IR. Statistical management is carried out in SPSS 11.0 software. **Results:** (1) The mean Log Homa IR of T2DM group was higher than that of control group (0.33±0.20 vs 0.08±0.11, P<0.01). (2) In T2DM group, ROI soft tissue adipose percentage (FAT100), fat mass (Fat), body mass index (BMI), waist circumference (Waist), waist to hip ratio (WHR) are all correlated significantly with LogHomaIR (P<0.01), especially FAT100 (R=0.591, P<0.001). In control group, ROI, FAT100, Fat, total body average fat percentage (AVGFAT100) are all correlated with LogHomaIR (P<0.01). FAT100 is still the most significant parameter (R=0.796, P<0.001) by Pearson correlation analysis. (3) Multifactor linear regression analyses suggest that in T2DM group, FAT100 is a strong predictor of LogHomaIR and in control group FAT100. WHR are the strongest. The effect of FAT100 in the latter (group) is stronger than that in the former. **Conclusions:** (1) The adipose tissue mass of abdominal body composition is an important influencing factor on insulin resistance. (2) The fat tissue mass in the region between the two horizontal lines passing the intervertebral space between L3-L4 and the clearance between L5-sacrum is a strong predictor of insulin resistance, which is a better predictor than simple indexs BMI, Waist and WHR.

P168 Antioxidative Molecular Mechanism of Traditional Chinese Medicine Qinghuoyihao against Detrimental Effects of High Level Glucose on Endothelial Cells

Xin GAO, Bin ZHANG
Department of Endocrinology & Metabolism, Zhongshan Hospital, Fudan University, Shanghai 200032, China

Objective: Our previous study has demonstrated that a prescribed traditional Chinese medicine preparation—Qinghuoyihao (QHYH) can decrease urinary microalbumin excretion in type 2 diabetic patients and improve microvascular endothelial cells growth in high glucose medium with similar effects to a natural antioxidant N-acetylcysteine (NAC). This study was to explore the antioxidative molecular mechanism of QHYH against the noxious effects of high glucose level on endothelial cells. **Methods:** Electron paramagnetic resonance (EPR) together with spin trap technique was applied to determine the antioxidative capacity of QHYH and its each constitutional material in vitro. And effects of QHYH on cellular superoxide anion production was observed when endothelial cells were cultured in a high glucose medium. The effects of QHYH on endothelial cells cultured in high glucose media were investigated with serum pharmacological approaches. Expression of uncoupling proteins (UCPs) mRNA in endothelial cells cultured in media with 5.56 mM glucose (NG group), 35 mM glucose (HG group), 35 mM glucose and 1:100 QHYH (QHYH group), and 35 mM glucose plus 10 mM NAC (NAC group) were analyzed by real time quantitative RT-PCR, and the expression of UCP molecules in the endothelial cells were studied by Western blot analysis. **Results:** EPR determination revealed that QHYH could extinguish 82.2% of the radicals generated in a hydroxyl free radical model system and the scavenging capacity of single component medicine ranged from 23.1% to 46.2%. Like NAC, serum from SD rats which were administered with QHYH could improve endothelial cells' vitality in high glucose milieu. Cellular superoxide anion production increased when endothelial cells were cultured in media with 35 mM glucose and QHYH could potently scavenge the superoxide anions with a similar effect to superoxide dismutase. No obvious expression of UCP-1 or UCP-3 mRNA was observed in endothelial cells cultured in media with 5.56 mM or 35 mM glucose while distinct expression of UCP-2 mRNA was detected in the endothelial cells of NG, HG, QHYH, and NAC groups. Expression of UCP-2 mRNA in HG group was higher than that in NG group (p=0.009) and UCP-2 mRNA was further upregulated in cells of QHYH and NAC groups (p=0.009, 0.036 respectively). In Western blot analysis, expression of UCP-2 molecule were detected in cells of QHYH group and a blear band of UCP-2 molecule appeared in NAC group while no apparent UCP-2 molecule band occurred in NG or HG groups. **Conclusions:** It suggests that the prescribed traditional Chinese medicine preparation—Qinghuoyihao and its each constitutional material can scavenge oxygen free radicals in vitro with Qinghuoyihao compound recipe being more potent than any of its single component remedium. Serum materia medica of Qinghuoyihao recipe, like NAC, can protect endothelial cells against the noxious effects of high level glucose in culture media. Cellular superoxide anion production increases when endothelial cells are exposed to high level glucose and the Qinghuoyihao preparation is effective in extinguishing superoxide anions produced by the endothelial cells. In addition to its direct effects of scavenging oxygen free radicals, the traditional Chinese medicine compound recipe can upregulate expression of UCP-2 molecule in cells exposed to high glucose milieu resulting in decrease of mitochondrial superoxide anion production and this may also play an important role in its antioxidative and protective effects on endothelial cells in high glucose surroundings.

P169 Expression and Roles of SelS in Human Umbilical Vein Endothelial Cells

Jian-ling DU, Li-li MEN, Xiu-juan ZHANG, Chang-chen LI
Department of Endocrinology, First affiliated Hospital of Dalian Medical University, Dalian 116011, China

Objective: To investigate the expression and roles of SelS in human umbilica vein endothelial cells. **Methods:** Total RNAs were extracted with TRIzol from adipose issues of human. Amplify the 1102bp fragment of SelS by RT-PCR. SelS was cloned into pMD18-T vector and digested with ClaI and XhoI before insertion into the corresponding sites of pLNCX2. After transient transfection with the pLNCX2-SelS or pLNCX2 alone, cultured human umbilical vein endothelial cells (HUVECs) was treated with hydrogen peroxide (100, 200, 300, 400 μM) for 24 h, cell viability was assayed by MTT method. **Results:** A 1102bp SelS gene fragment was successfully inserted into pLNCX2. The expression of recombinant SelS in the HUVECs was 1.76-fold as higher as the endogenous level and the cells over-expressing SelS was resistant to H_2O_2. **Conclusion:** It suggests that endogenous SelS may exert beneficial cytoprotection roles on endothelial cells in systemic vascular disorders.

P170 Impaired Serum Cholesterol Efflux Potential is Associated with Endothelial Dysfunction in Type 2 Diabetic Patients

Hua-li ZHOU, Sammy SHIU, Ying WONG, Kathryn TAN
Department of Medicine, University of Hong Kong, Hong Kong SAR, China

Objective: Cellular cholesterol efflux to serum is the first step in reverse cholesterol transport and plays an important role in reducing the accumulation of excess cholesterol in arterial wall and prevents atherosclerosis. This study was performed to investigate whether abnormalities in serum cholesterol efflux potential were related to endothelial dysfunction in type 2 diabetic patients. **Methods:** 95 type 2 diabetic patients and 19 age-matched healthy controls were recruited. Endothelium-dependent vasodilation (EDVD) and endothelium-independent vasodilation (EIDVD) of the brachial artery were measured by high-resolution vascular ultrasound. Serum cholesterol efflux potential was determined by measuring the transfer of [^{3}H] cholesterol from SR-BI rich Fu5AH cells to the medium induced by the test serum. **Results:** Type 2 diabetic patients had significantly lower HDL than controls. Serum cholesterol efflux potential was reduced in diabetic patients (15.0±2.0 vs. 18.5±2.6%, P<0.001), and both EDVD (5.2±2.5 vs. 7.4±3.3%, P=0.001) and EIDVD (13.4±4.5 vs. 15.7±4.2%, P=0.045) were also impaired compared with controls. Serum cholesterol efflux potential correlated with both EDVD (r=0.23, P=0.02) and EIDVD (r=0.24, P=0.02) in diabetic patients. On multiple regression analysis of all subjects (including age, gender, the presence of diabetes, smoking status, body mass index, waist, blood pressure, triglyceride, LDL and serum cholesterol efflux potential), only serum cholesterol efflux potential remained a significant independent determinant of EDVD (r^2=0.15, P<0.001). Serum cholesterol efflux potential was not an independent determinant of EIDVD. **Conclusion:** Impaired serum cholesterol efflux potential is related to endothelial dysfunction independent of other cardiovascular risk factors.

P171 Study of the Relation between Thyroid Autoimmunity and Hepatitis C Virus Infection

Rong YANG, Zhong-yan SHAN, Chen-ling FAN, Yu-shu LI, Hai-xia GUAN, Wei-ping TENG
Department of Endocrinology & Institute of Endocrinology, Ist Affiliated Hospital of China Medical University, Shenyang, Liaoning Province 110001, China

Objective: To explore the association of autoimmune thyroid diseases as well as thyroid autoantibodies with hepatitis C virus infection. **Methods:** Four hundred and sixty-two samples with positive thyroid peroxidase antibody (TPOAb) and/or thyroglobulin antibody (TgAb) from rural areas and Shenyang were collected. Three hundred and eighty subjects with negative TPOAb and TgAb who had similar age and gender distribution and without personal and family history of thyroid diseases were selected as controls from the areas mentioned above. The anti-HCV antibody was examined in all the cases using the third-generation ELISA, HCV RNA qualitative examination was examined further in those who had positive anti-HCV antibody. Meanwhile, 39 subjects with hepatitis C, 30 healthy subjects, and 30 subjects with hepatitis B were sampled. Thyrotropin, FT_3, FT_4, TPOAb and TgAb were examined in these subjects. **Results:** There was no obvious difference in the HCV infection rate between the group with positive thyroid autoantibodies and the group with negative thyroid autoantibodies. The anti-HCV titer in the group with positive thyroid autoantibodies was obviously higher than that in the group with negative thyroid autoantibodies ($p<0.05$). The correlation between thyroid autoantibodies and serum anti-HCV titer was analyzed in the group with positive thyroid autoantibodies, and showed that there was a positive correlation between TPOAb level and anti-HCV titer ($r=0.615$, $p<0.05$). 30.77% of subjects with hepatitis C were TPOAb positive, 30.77% were TgAb positive, and there was significant difference when compared with healthy subjects and subjects with hepatitis B ($p<0.05$). **Conclusion:** In subjects with hepatitis C, positive thyroid autoantibodies was increased, which indicated that thyroid autoimmunity and hepatitis C virus infection are correlative.

P172 A Two-year Follow-up Study of 58 Patients Diagnosed as Postpartum Thyroiditis

Chen-yang LI[1], Chen-ling FAN[2], Yu-hong OUYANG[1], Ying TENG[1], Wei-ping TENG[2]
[1] Department of Obstetrics & Gynecology, Shenyang No.5 People's Hospital, Shenyang, Liaoning Province 110001, P.R. China
[2] Department of Endocrinology & Institute of Endocrinology, the First Affiliated Hospital to China Medical University, Shenyang, Liaoning Province 110001, P.R. China

Objective: To investigate the cumulative incidence of persistent hypothyroidism (PH) in patients who diagnosed as postpartum thyroiditis (PPT) and determine the factors associated with the development of PH in those patients. **Methods:** 58 patients who were diagnosed as PPT (35 overt PPT and 23 subclinical PPT) at 6th month postpartum (pp) were followed up at 12th month pp, and then every 6 months until 24th month pp. Patients were taken fasting blood samples for testing serum TSH, thyroid peroxidase antibody (TPOAb), thyroglobulin antibody (TgAb). Free T_3 (FT_3), free T_4 (FT_4) and TRAb were detected if TSH was abnormal. **Results:** Of the total 58 PPT patients, 91.4% (n=53) were successfully followed. 5 patients with overt PPT and 6 patients with subclinical PPT developed PH, that was, the cumulative incidence of PH in the studied PPT patients was 20.8%. Among 15 PPT patients who had a classical biphasic course (a thyrotoxic phase followed by a hypothyroid phase), PH was seen in 26.7% (n=4). Among 11 PPT patients with hypothyroidism only, PH was seen in 63.6% (n=7). On the contrary, none of the patients with thyrotoxicosis only had PH. All of the patients who developed PH had a higher TSH levels than 4.8 mU/L at 6th month pp. Before delivery, TSH levels of the patients developed PH were significantly higher than those of the patients with transient hypothyroidism, and the similar thing happened at the 12th month pp (all $P<0.01$). PPT patients maintained a relatively higher rate of thyroid autoantibodies. The positive rate of TPOAb at the 12th, 18th and 24th month pp was 56.6%, 50.9% and 52.8%, respectively; and the positive rate of TgAb, 35.8%, 30.2% and 30.2%, respectively. Both the positive rate and titer of antibodies in patients with PH were similar as those with transient hypothyroidism ($P>0.05$). **Conclusions:** We followed up 58 patients with PPT for 2 years pp, their cumulative incidence of PH was 20.8%. Whether a patient with PPT would develop PH depends on their manifestation during the course of disease and their TSH levels.

P173 The Dynamic Changes of IgG Subclasses of Thyroid Peroxidase Antibodies in Patients with Postpartum Thyroiditis

Yu-shu LI, Chen-yang LI, Chen-ling FAN, Hai-xia GUAN, Zhong-yan SHAN, Wei-ping TENG
Department of Endocrinology & Institute of Endocrinology, the First Affiliated Hospital, China Medical University, Shengyang 110001, China

Objective: To evaluate the dynamic changes of IgG subclasses of thyroid peroxidase antibodies (TPOAb) in patients with postpartum thyroiditis (PPT) during the first postpartum (pp) year. **Methods:** 35 cases of clinical PPT, 23 cases of subclinical PPT and 6 cases of TPOAb positive pp women without PPT were from a large survey for the prevalence of PPT. Levels of TPOAb and its IgG subclasses in sera collected just before delivery (0), at 3, 6, 9 and 12 months pp were measured by ELISA. **Results:** (1) In PPT patients, levels of TPOAb and its subclasses were lowest before delivery, and increased significantly at 3-12 months pp ($P<0.01$). The levels of IgG1 and IgG4 had no difference among 3-12 months pp. IgG2 levels increased steadily at pp months with the peak at 9 months pp. IgG3 levels reached the peak at 6 months pp, and kept high levels after that. (2) The ratio of IgG1/IgG4 in PPT patients was relatively low before delivery, and increased at pp months, reached its peak at 6 months pp ($P<0.05$, compared with 0 months), while it decreased at 9-12 month pp. (3) In TPOAb positive non PPT pp women, levels of TPOAb and its subclasses were lower before delivery and increased at 3-12 pp months, while the ratio of IgG1/IgG4 did not change significantly among 0-12 months pp. (4) The levels of all TPOAb subclasses and the ratio of IgG1/IgG4 in hypothyroid phase of PPT patients were higher than those in hyperthyroid and euthyroid phases ($P<0.05$). (5) At 6 months pp, TSH levels were positively correlated with the levels of TPOAb and all its subclasses, FT4 levels were negatively correlated with IgG1, IgG3 and IgG4 levels (all $P<0.05$). At 3-6 months pp, TSH levels were positively correlated with the ratio of IgG1/IgG4. **Conclusion:** Levels of TPOAb and all its subclasses were associated with thyroid dysfunction. The higher ratio of IgG1/IgG4 was associated with the occurrence of hypothyroid function in PPT patients.

P174 The Shifts Toward to Different Directions of Th1/Th2 Associated Cytokine Gene Expression in Autoimmune Thyroid Disease

Xin-yu WANG, Guo-chun LUO, Ling-bo LV, Huai-xin YU
Department of Endocrinology, Shen Zhen Second People's Hospital, 518035, China

Objective: To investigate the initial pathogenesis of autoimmune thyroid disease (AITD) and determine Th1/Th2 cytokine profiles in AITD. **Methods:** Newly dignosed Graves' disease (GD) or Hashimoto' disease (HD) patients were selected. The mRNA of IFN-γ, TNF-a, IL-2, IL-4, IL-6 and IL-10 were detected by means of reverse transcription polymerase chain reaction (RT-PCR). **Results:** Compared with the control, the detection rates of all 6 cytokines were significantly elevated in GD and HD. In GD, the detection rates of Th2-like cytokines (IL-4, IL-6) were much higher than those of Th1-like cytokines (IFN-γ, TNF-a, IL-2), but no change after being treated with Methimazole. In HT, the detection rates of Th1-like cytoines (IFN-γ, TNF-a, IL-2) were higher than those of Th2-like cytokines (IL-4, IL-6, IL-10). **Conclusion:** The predominant expression of Th2-like cytokines mainly mediating humoral immunity was found in GD, and the cytokine profile was unchanged after being treated with Methimazole that means anti-thyroid drug can not correct the immunity nature of GD, while there was a Th1 cytokine profile found in HT that induce cell immune response.

P175 The Avidity of PTU-induced Anti-myeloperoxidase Antibodies was Associated with Clinical Vasculitis

Ying GAO[1], Ha YE[1], Ming-hui ZHAO[2], Xiao-hui GUO[1]
[1] Department of Endocrinology, Peking University First Hospital, Beijing 100034, China
[2] Department of Nephrology, Peking University First Hospital, Beijing 100034, China

Objective: Propylthiouracil (PTU) could induce MPO-ANCA positive vasculitis. However, high titer MPO-ANCA in remission had been observed in a substantial number of patients. Therefore, whether ANCA can be used as a guide to therapy in patients with PTU-induced vasculitis remains disputable. Our previous studies indicated that patients with PTU-induced vasculitis tended to have higher avidity of anti-MPO antibodies than those with PTU-induced anti-MPO antibodies but without clinical vasculitis. We undertook the current study to detect the avidity of anti-MPO antibodies in sequential sera from patients in both active phase and remission in order to further investigate the role of avidity of MPO-ANCA on the pathogenesis of PTU-induced AASV. **Methods:** Six patients with PTU-induced vasculitis were enrolled in the current study. Serial sera in both active phase and remission were collected. MPO-ANCA avidity was assessed by antigen-inhibition ELISAs, and avidity constant (aK) was determined as the reciprocal value of MPO molar concentration in the liquid phase resulting in 50% inhibition of anti-MPO antibodies binding to MPO in solid phase ELISA. Titers of MPO-ANCA were determined by a serial dilution of sera in MPO-ELISA. **Results:** After cessation of PTU and immunosuppressive therapy, the avidity of anti-MPO antibodies decreased significantly during follow-up in sera from all the patients, and it seemed decreased more quickly than titers of MPO-ANCA. **Conclusion:** Our study indicated that avidity of anti-MPO antibodies might play an important role in the pathogenesis of PTU-induced AASV and it might be more closely associated with clinical vasculitis than the titer. PTU might be involved in the process of ANCA production.

P176 The Clinical Significance of Erythrocyte Carbonic Anhydrase Concentration in Different Thyroid Disease

Chun-xia PENG, Yan-ming GAO, Ying GAO, Wei WANG, Shu-feng WANG
Department of Endocrinology, Peking University First Hospital, Beijing 100034, China

Objective: Thyroid hormone and erythrocyte carbonic anhydrase (CA) concentration were measured in 38 patients with different thyroid disease, and the relationship between them was researched. **Methods:** 38 cases patients were divided into 3 groups: Graves disease 20, hypothyroidism 10 and hashitoxicosis 8, healthy adults 28 served as control group. The level of serum T_3, T_4, TSH and erythrocyte CA were measured once every 4 weeks in 16 weeks in all subjects. **Results:** (1) The concentration of CA in the Graves disease were significantly lower compared with the control group in 0, 4, 8 weeks after treatment ($p<0.001$), but in the 12th week after treatment, the concentration of CA was in the normal range, there was no significant difference ($p=0.103$) compared with control group. There was no difference on the concentration of CA between the hashitoxicosis group and hypothyroidism and their corresponding control group. (2) In 0 week, there was no significant difference on the level of the T_3 and T_4 between the Graves disease and the hashitoxicosis group. The CA concentration in the Graves disease was significantly lower than the hashitoxicosis group ($p=0.001$). **Conclusion:** (1) The erythrocyte concentration of CA can be used in the differentiation between the Graves disease and hashitoxicosis. And it could be used to guide therapy in different causes of thyrotoxicosis. (2) The erythrocyte concentration of CA reflects the mean level of the thyroid hormone measured 8 weeks earlier in the Graves disease.

P177 TRβ Gene Mutation Study in a Thyroid Hormone Resistance Syndrome Family

Jing-fang LIU, Bing-yin SHI
Department of Endocrinology, First Hospital Affiliated to Medical College of Xi'an Jiaotong University, Xi'an 710061, P.R. China

Objective: To study the pathogenesis and thyroid hormone receptor (TR) β gene mutation in a pedigree with thyroid horrmone resistance syndrome. **Methods:** The genomic DNA was extracted from peripheral blood leukocytes of 15 family members and 100 healthy subjects. Exon 7-10 of the TRβ gene were amplified by polymerse chain reaction (PCR) .The product of PCR were purifed and sequenced directly to detect gene mutation. **Results:** Five persons of this family had C→G transition mutation at nucleotide 1642 within exon 10 of TRβ gene, which nonsense mutation caused substitution of Proline to Alanine (P453A); C→T transition mutation at nucleotide 1020 within exon 7 of TRβ gene was also found and this synonymous mutation did not cause the change of amino acid at this position (F245F). These two mutations were heretozygote. No mutation in the exon 7-10 of TRβ gene was identified in other family members. PCR products of exon 10 were digested with Fau I. The result confirmed the presence of the mutation of the five patients and no mutation and polymorphism were found in this point of 100 healthy subjects. **Conclusion:** We first found a family with thyroid hormone resistance syndrome having successive four generation and five patients in China. P453A mutation was located in T_3-binding domain of TR. The mutant receptor not only had reduced affinity for T_3 and decreased transcriptional activity, but also impaired the activity of the wild-type receptor by the dominant negative inhibition, which was major mechanism leading to thyroid hormone resistance. F245F mutation probably was a genetic molecular marker and associated with hereditary susceptibility to some environmental factor.

P178 Case Report: Thyroid Hormone Resistance Syndrome in a Chinese Family in Hong Kong

Ho-ching LEUNG[1], Ching-wan LAM[2]
[1] Department of Medicine and Rehabilitation, Tung Wah Eastern Hospital, HKSAR
[2] Department of Medicine and Therapeutics, Prince of Wales Hospital, HKSAR

We report a familial case of thyroid hormone resistance syndrome with R320C mutation in thyroid hormone receptor beta gene. Mr Ng had incidental finding of abnormal thyroid function test (TFT) with elevated free T3, normal free T4 and elevated TSH level. He has a history of thyrotoxicosis presented with diffuse goiter and mild thyroid toxic symptom underwent subtotal thyroidectomy in 1989 and put on thyroxine supplement. Mr Ng claimed to have good thyroxine compliance. Clinically he was euthyroid. Other pituitary hormone profile was normal. TSH alpha-subunit was normal. MRI pituitary showed two tiny hypo-enhancing foci (each 2 mm in size) in pituitary gland. TRH test showed exaggerated peak response with a 6.6 fold rise in TSH after TRH injection. A followed-by T3 suppression test showed partially suppressed TSH. Gene analysis showed a R320C mutation in thyroid hormone receptor beta gene, which was a well-known disease-causing mutation in thyroid hormone resistance syndrome. Blood test screening on family members spotted out his 11-year-old son shares the same mutation. His son was clinically euthyroid with normal growth and mental development.

P179 Gene Therapy to Graves' Disease Mice by Administrating Recombinant Plasmid pcDNA3.1/hFasL In Vivo

Hua SUI[1], Chun LIU[1], Hui ZHANG[2], Xiu-juan LI[1]
[1] Department of Endocrinology, The First Affiliated Hospital, Chongqing University of Medical Sciences
[2] Department of Pathology, Chongqing University of Medical Sciences, Chongqing 400016, China

Objective: To observe gene therapeutic effects of recombinant plasmid pcDNA3.1/hFasL to Graves' disease mice. **Methods:** Graves' disease mice were established by being immunized with recombinant plasmid pcDNA3.1/hTSHR, and then were treated by administrating recombinant plasmid pcDNA3.1/hFasL in vivo as experimental objects. **Results:** (1) Serum TT4 was lower in Treated group than in Model group ([24.71±14.34 vs. 59.72±32.76] ng/ml, P<0.05), and serum TSH was higher in Treated group than in Model group ([1.27±0.40 vs 0.97±0.39] uIU/ml, P<0.05). Whereas there was no significant difference between serum TT4 and TSH in Treated group and in Model group (TT4: [24.71±14.34 vs. 19.39±7.45] ng/ml, P>0.05; TSH: [1.27±0.40 vs. l.34±0.27] uIU/ml, P>0.05). (2) The pathologic changes rate of hyperthyroid was lower in Treated group than in Model group (15.8% vs. 47.4%, χ^2=6.78, P<0.05), and there were a few ulterstructure changes such as heterochromatin margination in thyroids epithelia of Treated group mice. **Conclusion:** Gene therapy by administration of plasmid pcDNA3.1/hFasL is an effective approach for Graves' disease mice.

P180 To Investigate the Expression of Melatonin Receptor in Human Thyroid Follicular Cell Cancer Tissue

Jun-jie ZOU[1], Lan LI[1], Ye RUAN[1], Jin HE[2], Zhi-min LIU[1]
[1] Endocrinology Department
[2] Pathology Department
Changzheng Hospital, Second Military Medical University, Shanghai 200003, China

Objective: To investigate the different expression of melatonin receptor in adult thyroid follicular cell cancer and its adjacent normal thyroid tissue. **Methods:** MR binding sites were measured by radioligand binding assay. We extracted the total RNA of thyroid tissue, synthesized the primer of mt1, MT, and analyzed the mRNA of melatonin receptor by RT-PCR method. **Results:** By Scatchard analysis, Bmax of 125I Mel specific binding of thyroid follicular cell cancer tissue is 0.63±0.07 *m*B/fmol·mg^{-1}, Kd is 45.3±9.3 *c*B/pmol·L^{-1}; Bmax of 125I Mel specific binding of thyroid normal tissue is 0 58±0.04 *m*B/fmol·mg^{-1}, Kd is 43.5±8.2 *c*B/pmol·L^{-1}. MT2 and mt1 cDNA fragment of the expected size were determined by RT-PCR. MT2 in follicular cell cancer had a higher quantity than that in normal thyroid tissue (P<0.05). The expression of mtl in follicular cell cancer tissue and MT2 subtype were not significantly higher than that in normal thyroid tissue. **Conclusion:** Melatonin mt1 and MT2 subtype expressed in human thyroid follicular cell cancer tissue, MT2 subtype may be associated with the development of human thyroid follicular cell cancer.

P181 A Dose and Time Dependent Relationship in Iodine-induced Autoimmune Thyroiditis in NOD.H-2^{h4} Mice

Xiao-chun TENG, Zhong-yan SHAN, Yu-shu LI, Cheng-ling FAN, Hong WANG, Rui GUO, Wei-ping TENG
Department of Endocrinology & Institute of Endocrinology, 1st Affiliated Hospital of China Medical University, Shenyang 110001, China

Objective: To explore the relationship between iodine dose-time and autoimmune thyroiditis or serum thyroid autoantibodies (TgAb) in NOD.H-2^{h4} mice. **Methods:** One hundred twenty-eight five-week-old NOD.H-2^{h4} mice were randomly divided into five groups: normal iodine, 5-fold, 10-fold, 100-fold, and 1000-fold iodine excess. At the time point of 4, 8, 16 and 24 weeks receiving iodine water, mice were anesthetized by diethyl ether, bleed from eye socket vein, and their thyroids were collected. Indirect Ellisa method was used to measure the levels of serum thyroglobulin autoantibodies. BX51FL+DP70 photomicrograph and Image-Pro Plus 5.1 software analysis system was used to observe the pathological characteristics. **Results:** (1) With the time and dose of iodine supplementation increased, the incidence rate of autoimmune thyroiditis, as well as infiltration degree of lymph cells increased, there was a positive correlation between iodine dose and incidence rate of autoimmune thyroiditis (r=0.87-0.98, $P<0.05$) at the time of 8, 16, and 24 weeks after iodization, as well as between iodine dose and the infiltration degree of lymph cells (r=0.57, $P=0.04$) at the time of 24 weeks. (2) With the time and dose of iodine supplementation increased, the incidence rate of serum TgAb, as well as its values increased, there was a positive correlation between iodine dose and incidence rate of serum TgAb (r=0.84-0.96, $P<0.05$), as well as between iodine dose and the values of serum TgAb (r=0.40-0.62, $P<0.05$) at the time of 8, 16, and 24 weeks. **Conclusion:** (1) Iodine excess may initiate and accelerate the development of autoimmune thyroiditis, as well as serum TgAb in a dose and time dependent way. (2) Titers of serum TgAb correlated positively with the degree of thyroid lymphocytes infiltration.

P182 Iodine-induced Autoimmune Thyroiditis in NOD.H-2^{h4} Mice

Xiao-chun TENG, Zhong-yan SHAN, Jing LI, Cheng-ling FAN, Hong WANG, Rui GUO, Wei-ping TENG
Department of Endocrinology & Institute of Endocrinology, 1st Affiliated Hospital of China Medical University, Shenyang 110001, China

Objective: To investigate the kinetics of the development of iodine-induced autoimmune thyroiditis and thyroid autoantibodies in NOD.H-2^{h4} mice. **Methods:** Either one hundred and twelve five-week-old NOD.H-2^{h4} mice or Kunming mice were randomly divided into two groups, and received plain water or 0.05% iodine water. At the time point of 1, 2, 4, 8, 12, 16 and 24 week receiving iodine water, mice were anesthetized by diethyl ether, bleed from eye socket vein, and their thyroids were collected. Indirect Ellisa method was used to measure the levels of serum thyroglobulin autoantibodies. BX51FL+DP70 photomicrograph and Image-Pro Plus 5.1 software analysis system was used to observe the pathological characteristics. **Results:** (1) In iodine treated NOD.H-2^{h4} or Kunming mice, the epithelia height was decreased, the follicular lumina were enlarged and contained lots of colloid. (2) One of the control NOD.H-2^{h4} mice had autoimmune thyroiditis at the age of thirteen weeks old, but in the iodine treated group, autoimmune thyroiditis were observed as early as 1 week after they began receiving iodine water. The incidence rate of autoimmune thyroiditis was 62.5% at 8 weeks, and generally reached maximal rate of 87.5% at 12 weeks, and then remained until 24 weeks. (3) Thyroid lymphocytes infiltration appeared after receiving iodine water in NOD.H-2^{h4} mice, and generally maximal at 12 weeks and remained relatively unchanged until 24 weeks. (4) Serum thyroglobulin autoantibodies values increased after 4 weeks of iodine ingestion in NOD.H-2^{h4} mice, then increased steadily throughout the 24 weeks of experiment. On the contrary, serum thyroglobulin autoantibodies values were not detected in the control group and Kunming mice. **Conclusion:** Iodine may induce and exacerbate infiltration of the thyroid by lymphocytes in genetically susceptible NOD.H-2^{h4} mice but has no effect on Kunming mice.

P183 Effects of Iodine Supplementation on 5'Deiodinase Activity in Wistar Rats with Iodine Deficient Background

Ya-jie TONG, Zhong-yan SHAN, Wei-ping TENG
Institute of Endocrinology, Department of Endocrinology, the First Affiliated Hospital, China Medical University, Shenyang 110001, China

Objective: To investigate effects of iodine supplementation (IS) on 5'deiodinase activity and thyroid function of rats with iodine deficient background. **Methods:** Wistar rats fed with iodine deficient diet for 3 months were divided randomly into 3 groups: iodine deficient control (ID), 1-fold-physiological-need iodine supplement group (IS1) and 3-fold-physiological-need iodine supplement group (IS3), fed with distilled water, potassium iodate solutions with iodine concentrations as 100 μg/L and 330 μg/L respectively. Wistar rats fed with iodine sufficient diet continuously served as normal control (NC). Rats were killed after 1, 2, 4, 8, 12 and 24 weeks of IS. Urinary iodine, thyroid function, thyroid hormone contents and 5'deiodinase activity in tissues of thyroid, pituitary, liver and kidney were examined. **Results:** Median urinary iodine excretion was 139.43 μg/L, 14.55 μg/L, 85.62 μg/L and 409.07 μg/L in NC, ID, IS1 and IS3, respectively. Increased relative weight of thyroid in ID could be reduced by IS, but still higher than that in NC. Thyroid type I 5'deiodinase (5'D1) activity and pituitary type II 5'deiodinase (5'D2) activity in ID were significantly higher than those in NC and could be reversed by IS. 5'D1 activities of liver and kidney in ID, IS1 and IS3 were significantly lower than those in NC. Extremely low thyroid T_3, T_4 and rT_3 contents in ID could be restored to normal levels by 24w IS except thyroid T_4 content in IS3 which exceeded the normal level. In ID, T_3 content in serum, pituitary, liver and kidney remained, but T_4 content in serum, liver and kidney decreased and serum TSH increased, which could be reversed by IS. In IS3, T_4 contents in serum, liver and kidney even exceeded the normal levels after 24w IS. **Conclusion:** 1-fold IS was enough for rats with iodine-deficient background, but long-term 3-fold IS would increase T_4 levels in serum, thyroid and peripheral tissues. But T_3 contents in serum and peripheral tissues remained stable since 5'deiodinase activities in thyroid and peripheral tissues were inhibited.

P184 Effects of Chronic Iodine Excess on 5'deiodinase Activity in Wistar Rats with Iodine Sufficient Background

Ya-jie TONG, Zhong-yan SHAN, Wei-ping TENG
Institute of Endocrinology, Department of Endocrinology, the First Affiliated Hospital, China Medical University, Shenyang 110001, China

Objective: To investigate the effects of chronic iodine excess on 5'deiodinase activity and thyroid function indexes of Wistar rats. **Methods:** Wistar rats fed with iodine sufficient diet for 3 months were divided randomly into 3 groups: normal control (NC), 3-fold high iodine intake group (HI3) and 6-fold high iodine intake group (HI6), fed with distilled water, potassium iodate solutions with iodine concentrations as 300 and 660 μg/L respectively. The rats were killed after 1, 2, 4, 8, 12 and 24 weeks of high iodine intake. Urinary iodine and thyroid function were tested. Thyroid hormone contents and 5'deiodinase activity in tissues of thyroid, pituitary, liver and kidney were examined. **Results:** Median urinary iodine excretion was 139.43 μg/L, 442.07 μg/L and 887.35 μg/L in NC, HI3 and HI6 group, respectively. Type I 5'deiodinase (5'D1) activity in thyroid tissue began to decrease after 1-week high iodine intake and kept in the lower stage throughout 24-week observation. After 24-week high iodine intake type II 5'deiodinase (5'D2) activity in pituitary and 5'D1 activity in liver and kidney decreased significantly, but pituitary 5'D1 activity did not change. With iodine supplementation prolong, T_3, T_4 and rT_3 content in thyroid tissue increased significantly with a decreased T_3/T_4 ratio. After 24-week high iodine intake, T_3 content of pituitary and liver tissue in HI3 and HI6 groups showed decreasing tendency compared with that in NC group, but the difference was not significant. After 24-week high iodine intake, serum TT_4, rT_3 and TSH levels increased and serum TT_3 level decreased, but serum FT_4 and FT_3 levels did not change significantly. **Conclusion:** Long-term 3-6–fold iodine excess could inhibit 5'deiodinase activity in thyroid, liver, kidney and pituitary tissues and resulted in elevated serum T_4 and rT_3 contents. But T_3 contents in serum and peripheral tissues decreased, which indicated that the biological effects of thyroid hormone in peripheral tissues might have decreased.

P185 Alzheimer-like Hyperphosphorylation of Tau in Cerebral Cortex of Rats with Hypothyroidism

Yan YANG, Shu-hong HU, Mu-xun ZHANG
Department of Endocrinology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

Objective: Abnormal hyperphosphorylation of tau plays a critical role in the pathogenesis of Alzheimer disease (AD). We therefore studied the roles of hyperthyroidism and hypothyroidism in hyperphosphorylation of tau in cerebral cortex in rats. **Methods:** Wistar female rats were randomized into 3 groups, hyperthyroidism (group A), hypothyroidism (group B) and control (C) group. The rats in group A were plus thyroid at a dose of 0.8 $mg \cdot kg^{-1} \cdot d^{-1}$ for 4 weeks, and in group B were plus tapazole at a dose of 0.6 $mg \cdot kg^{-1} \cdot d^{-1}$ for 4 weeks. The temperatures of rats were collected in rectal. The plasma FT3, FT4 and TSH levels were measured by RIA method, and the blood glucose by glucose-oxidase method. Total tau level and the phosphorylation level of tau at individual phosphorylation sites (Ser199, Thr205, Thr212, Ser214, Ser396 and Ser422) were analyzed by Western blots. As well as the expression of Tau[pS^{214}] was observed by immunohistochemical assay. **Results:** We found that it is significantly higher in glucose and rectal temperature in group A than in group C. But in group B, these two targets were significantly lower than in group C. Neither hyperthyroid nor hypothyroid changed the total level of tau protein in the cortex of rats. However, Tau was found to be hyperphosphorylated at several AD-related phosphorylation sites (Serl99, Thr212, Ser214, Ser396 and Ser422) in group B. The expression of phosphorylated tau modestly increased in CA3 sector of hippocampus in group B compared with group C and group A. **Conclusions:** These findings suggest (1) that tau protein in cortex was hyperphosphorylated in hypothyroid rats, (2) tau hyperphosphorylation in hypothyroid status may be caused by low metabolism, and (3) high temperature and glucose metabolism in hyperthyroid status may not change the phosphorylation of tau protein.

P186 Studies on the Interactive Protein of Insulin-like Growth Factor Binding Protein-1 (IGFBP-1)

Ping WEI, Yin-zhang WANG, Dong-gang XU, Jia-xi WANG
Department of Endocrinology, Southwest Hospital, the Third Military Medical University of PLA, Chongqing 400038, China

Objective: Insulin-like growth factor binding protein-1 (IGFBP-1) is one of the important endocrine hormones. We studied on the interactive protein of IGFBP-1 with the technique of yeast two-hybrid, in order to investigate the mechanism of IGFBP-1. **Methods:** (1) The human IGFBP-1 cDNA was isolated by PCR from fetal hepatic cDNA library and confirmed by sequence analysis. (2) Yeast two hybrid system was used on studying the biological activities and mechanism of IGFBP-1. The results showed that IGFBP-1 has transcriptional activation. The relationship of the structure and function of IGFBP-1 was analysed by bioinformatics. A mutant was designed by deleting 27 amino acid of the carboxyl end of IGFBP-1. The transcriptional activation was deleted by constructing the mutant. **Results:** Seven genes, which encode proteins interacting with the mutant of the IGFBP-1, were found by screening the library using the Yeast two-hybrid system. By homology comparing, these genes identified as human metallothionein-Ie, hemoglobin, autonomously replicating sequence, hepatocellular carcinoma-associated antigen 112, fibrinogen, haptoglobin and serine (or cysteine) proteinase inhibitor. **Conclusion:** The protein interacting with IGFBP-1 may be new cues for investigating new biological activity and mechanism of IGFBP-1. FBP-1 may be new cues for investigating new biological activity and mechanism of IGFBP-1.

P187 The Effects of Interleukin-1β and Interleukin-6 on Growth Hormone Gene Promoter Activity in Rat MtT/S Cells

Feng-ying GONG, Jie-ying DENG, Yi-fan SHI
Department of Endocrinology, Peking Union Medical College Hospital, Beijing 100730, China

Objectives: To investigate the effect(s) of interleukin-1β (IL-1β) and interleukin-6 (IL-6) on the activity of human growth hormone (hGH) gene promoter in rat pituitary MtT/S cells and the molecular mechanism underlying the effect(s). **Methods:** The stable transfection method of the hGH gene promoter fused to a luciferase reporter gene was used. **Results:** IL-1β (10^3 U/ml) and IL-6 (10^3 U/ml) increased GH secretion and synthesis, and promoted the luciferase expression in stable MtT/S cells, with an action of 1.38 and 1.69 times respectively over that of controls. Among the inhibitors of intracellular signaling transduction pathways, mitogen-activated protein kinases (MAPK) inhibitor PD98059 (40 μM) and p38 MAPK inhibitor SB203580 (5 μM) blocked completely the stimulatory effect of IL-1β and IL-6, and phosphoinositide 3-kinase (PI3-K) inhibitor LY294002 (10 μM) blocked partly the induction of IL-1β. Western blot analysis demonstrated that IL-1β and IL-6 indeed increased the activation of phosphorylated MEK and p38 MAPK in MtT/S cells. Neither overexpression of Pit-1 nor inhibiting Pit-1 expression affected IL-1β and IL-6 inductions of hGH promoter activity. The stimulatory effects of both IL-1β and IL-6 were abolished following deletion of the -196 to -132bp fragment of hGH gene promoter. **Conclusions:** IL-1β and IL-6 increase the activities of hGH gene promoter in rat pituitary MtT/S cells. These stimulatory effects of IL-1β and IL-6 appear to require the intracellular MAPK, p38 MAPK and PI3-K dependent signaling pathways and a fragment of promoter sequence that spans the -196 to -132bp fragment of the gene, but is unrelated to Pit-1 protein.

P188 The Identification of Growth Hormone Releasing Hormone in Goldfish and Zebrafish and the Implications

Ivy Tik-yan LAU, Leo Tsz-on LEE, Billy Kwok-chong CHOW
Department of Medicine, The University of Hone Kong, Hong Kong SAR, China

Objective: To show that the previously identified fish GHRH-like peptides are homologs of the mammalian PACAP-related peptides (PRP) and, similar to mammals, these PRPs are also encoded in the same transcript with PACAP. **Methods:** The full-length cDNA sequences of GHRH and GHRHR from goldfish (*Carassius auratus*) and zebrafish (*Danio rerio*) were cloned. Phylogenetic analysis, sequence alignment of GHRHR vs. PRPR and GHRH vs. PRP were performed. Real-time PCR and cAMP assay were used to study their tissue distribution and their functional properties. **Results:** The newly identified cDNAs were highly conserved with their mammalian's counterparts. Goldfish GHRH was expressed exclusively in the brain while the GHRH-R was found mostly in brain and pituitary. GHRHR were responsive to GHRH with a significant and dose-dependent increase in intracellular cAMP but not the PRP. **Conclusion:** Evidences showed that the previously identified GHRH in fish is only a related peptide and so give rise to a novel evolutionary scheme of GHRH and its receptor from fish to mammals.

P189 Genetical Diagnosis of Multiple Affected Tissues in a Patient with McCune-Albright Syndrome

Ji ZHOU, Li-hao SUN, Jian-min LIU, Bin CUI, Huai-dong SONG, Xiao-ying LI, Guang NING
Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrine and Metabolic Disease, Ruijin Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai 200025, China

Objective: McCune-Albright syndrome (MAS) is a sporadic disorder characterized by the classic triad of polyostotic fibrous dysplasia, cafe-au-lait skin pigmentation, and hyperfunctional endocrinopathy. This syndrome is rare, and less than 100 cases associated with acromegaly have been described in literature. It is caused by embryonic somatic mutations leading to the substitution of His or Cys for Arg at amino acid 201 of the alpha-subunit of the signal transduction protein Gs (Gsα). We here presented a case of MAS with acromegaly, and studied the gene mutation of Gsα gene in multiple affected tissues of the patient. **Methods:** A 32-year-old man was diagnosed as McCune-Albright syndrome with the following findings: polyostotic fibrous dysplasia, cafe-au-lait spots and acromegaly. He was found associated with left pleural effusion in an ultrasonic inspection. The peripheral blood, bone tissue, skin lesion and pleura samples of the patient were collected. Genomic DNAs were isolated from these samples by using phenol-chloroform methodology. Then PCR and direct sequencing were performed. **Results:** The peripheral blood and bone tissue of the patient showed Arg201Cys (R201C) in Gsα gene, a missense point mutation (CGT→TGT) leading to the substitution of Cys for Arg at amino acid 201. But no mutation was detected in the skin and pleura samples of the patient. **Conclusion:** The gene diagnosis confirms the patient has a R201C mutation in Gsα gene, and multiple tissues are affected. The mutation occurred early in embryogenesis, and his features can be clinically different. Genetical diagnosis may help to define a more complete clinical spectrum of MAS.

P190 Basic and Clinical Research on Melatonin and Its Receptors

Zhi-min LIU[1], Ying ZHAO[2], Xiang-fang CHEN[1], Yong-quan SHI[1], Jun-jie ZOU[1], Zhu-qian LU[1], Hui ZHANG[1], Li-lin ZHANG[1], Ying LIU[1], Lan LI[1]
[1] Department of Endocrinology, Changzheng Hospital, Second Military Medical University, Shanghai 200003, China
[2] Department of Neurology

Objective: To investigate the distribution and biological characteristic of melatonin receptors in human embryo and some adult tissues. **Methods:** Melatonin receptors (MR) were measured by radio ligand assay, immunohistochemistry, RT-PCR, and situ hybridization. The effect of GTP γS and electrolyte on 125I-indomelatonin specific binding. The change of MR in physiological and pathological station by these above methods. **Results:** Melatonin specific binding sites were approved in these embryonic tissues (cerebral cortex, frontal lobe, parietal lobe, occipital lobe, temporal lobe, hippocampus, cinguium gyrus, retina, optic chiasm, olfactory ball, thalamus, hypothalamus, corpus callusum, pons, mesencephalon, cerebellum, medulla, cervical enlargement and thoracic cord, lumbar and sacral cord, spinal cord, heart, liver, lung, kidney, bladder, stomach, intestine, colon, thymus, spleen, thyroid, adrenal gland, testis, prostate, uterus, ovary), and by radioligand binding assay, meanwhile, this specific binding could be inhibited by GTP γS supporting the theory that melatonin receptor coupled to inhibitory G-protein system. Specificity analysis: 2-Indomelatonin>Melatonin>6-Chloromelatonin>6-Hydroxymelatonin>N-Acetylserotonin>5-Hydroxytryptamine >Tryptamine, 3-Acetylindole, L-Tryptophan> Norepinephrine, Epinephrine, Acetylcholine. Subcellular Distribution: Nuclear>Mitochondrial>Mocrosomal>Cytosolic fraction. GTP γS including two different concentration could reduce the content of binding sites, which were of dose-dependent. Monovalent and divalent cation could depress the specific binding of 125I-indomelatonin, it was also found the level of MR affected by divalent cation was lower than that of MR affected by monovalent. We applied the immunohistochemistry to identify and localize the mt1 and MT2 receptor subtype protein expression in human embryos tissues, furthermore, evaluated the precise distribution of mt1 and MT2 receptor subtype in specific cells. It was identified and localized the mt1 and MT2 receptor subtype mRNA and protein expression in human embryos tissues by the RT-PCR and in situ hybridization. Furthermore, the change of MR in physiological and pathological station by these above methods were approved. **Conclusion:** MR were detected in many human embryo tissues, these results provide experimental data for elucidating the mechanism of the melatonin.

P191 Study on Immuno-related Genes Expression Changes of Melatonin-treated T Lymphocyte Line

Xiang-fang CHEN[1], Zhi-min LIU[1], Ying ZHAO[2], An-mei DENG[3], Ye RUAN[1], S.F. PANG[4]
[1] Department of Endocrinology, Changzheng Hospital, Second Military Medical University, Shanghai 200003, China
[2] Department of Neurology, Changzheng Hospital, Second Military Medical University, Shanghai 200003, China
[3] Department of Clinical Experimental Diagnosis, Changzheng Hospital, Second Military Medical University, Shanghai 200003, China
[4] Department of Physiology, Medical College, University of Hong Kong

Objective: To investigate the distribution and biological characteristic of melatonin receptors in T-lymphocytes cells (Hut78), further to study the effects of melatonin on Hut78 by genomic-scale gene expression analysis of immunity. **Methods:** The reverse transcription-polymerase chain reaction (RT-PCR) was applied to examine mt1 mRNA of Hut78. The Envision method was applied to immunohistochemistry. Two cDNA probes were from mRNA of melatonin treated and untreated Hut78 cells. These two groups of cells with Cy3 or Cy5 fluorescence dyes individually, hybridized with cDNA microarray, and were scanned for fluorescent intensity. The altered gene expression was screened through the analysis of difference in gene expression profile. **Results:** RT-PCR analysis identified the existence of mt1 mRNA in the Hut78 cells investigated. Electrophoresis shows positive production of RT-PCR with the mt1 receptor subtype primer in above cells. Sequencing of RT-PCR production showed that the DNA sequence of production coincided with the sequence from Genebank. The mt1 were observed in Hut78. These buffy positive granules are scattered with some areas stronger than the others, and primarily located to cytoplasm and membrane, with rare location in nucleus. The results of mRNA profiles analysis indicated that 35 of immuno-related genes were upregulated by Mel while 7 were downregulated. **Conclusion:** The mt1 mRNA is identified by RT-PCR in the Hut78 cells investigated, furthermore, the protein molecular of the mt1 was observed in the same cells. It was indicated that melatonin have directly immune-regulative effects on Hut78. cDNA microarray technique is effective in screening the differentially expressed genes between these two groups. It was proved that Mel could activate the T lymphocyte line by regulation of some genes, further analysis of these obtained genes will be helpful to understand the molecular mechanism that Mel regulated the immunity.

P192 The Dynamics of CRH and AVP Gene Transcription within the Paraventricular of the Hypothalamus after the Injection of Interleukin-1β (IL-1β) into the Vein of the Rats

Ya-qiu JIANG, Zhong-yan SHAN, Wei-ping TENG
Department of Endocrinology and Metabolism and the Institute of Endocrinology, First Affiliated Hospital, China Medical University, 110001, China

Objective: To examine the effects of cytokine IL-1β on CRH and AVP gene transcription in the paraventricular nucleus of the hypothalamus. **Methods:** Under anesthesia the catheter were implanted into the right atria of rats. 24 hours after the surgery IL-1β (1.4 μg/kg) or saline (300 μl) was injected into the vein of conscious rats, rats were decapitated 15, 120 min after IL-1β injection, brains were rapidly removed and frozen, then sectioned (10 μm slice) using a cryostat. Plasma ACTH and AVP were measured by RIA. The expression of CRH and AVP heteronuclear RNA (hnRNA) and messenger RNA (mRNA) was detected by in situ hybridization and autoradiograph. Semiquantitative analysis was performed using NIH Image software. **Results:** Plasma ACTH and AVP reached a peak ($P<0.01$) at 15 min after IL-1β injection, then returned to the basal level by 120 min. Expression of CRH hnRNA increased markedly 15 min after IL-1β injection ($P<0.01$), then returned by 120 min. Expression of AVP hnRNA in the parvocellular division increased 15 min after IL-1β injection ($P<0.0l$), and furthermore this increase continued until to 120 min after IL-1β injection. After IL-1β injection, expression of CRH mRNA and AVP mRNA in the parvocellular division increased at 120 min ($P<0.01$). After the injection, expression of AVP hnRNA in the magnocellular division increased at 120 min ($P<0.01$), but expression of AVP mRNA in the same division was not significantly different from the basal value at any time. **Conclusion:** These results show the dynamics of CRH and AVP gene transcription in the paraventricular after the IL-1β injection into the vein, suggest that regulation of CRH and AVP gene transcription in the parvocellular division is different, and that regulation of AVP gene transcription in the magnocellular division is different from in the parvocellular division too.

P193 Neuroendocrine, Endocrine and Paracrine Regulation of Zebrafish FSHβ, LHβ and GH Expression *In Vitro*

Sze-wah LIN, Wei GE
Department of Biology, The Chinese University of Hong Kong, HKSAR

Gonadotropins (GTH)—follicle stimulating hormone (FSH) and luteinizing hormone (LH)—and growth hormone (GH) are endocrine hormones from the pituitary that play important roles in regulating vertebrate reproduction. Their expressions are finely regulated by a complex interaction of neuroendocrine, endocrine and paracrine factors, which act at both hypothalamus and pituitary levels. In order to study the effects and action mechanisms of these factors at the pituitary level, an *in vitro* pituitary cell culture system has been established in the zebrafish. In the present study, the direct effects of different hormones and peptides including gonadotropin-releasing hormone (GnRH), sex steroids (estradiol and testosterone), activin, follistatin, insulin-like growth factor I (IGF-I), epidermal growth factor (EGF), and pituitary adenylate cyclase activating polypeptide (PACAP) on the expression of zebrafish *fshb, lhb* and *gh* were determined using real-time RT-PCR. GnRH positively regulated *lhb* but not *fshb* expression. Estradiol and testosterone, on the other hand, increased the mRNA levels of both *fshb* and *lhb*. Activin significantly stimulated *fshb* while inhibited *lhb* expression; on the contrary, treatment with activin-binding protein, follistatin, dose-dependently decreased and increased *fshb* and *lhb* expression, respectively. Interestingly, IGF-I had differential effects on the expression of *fshb*, *lhb* and *gh*. The expression of *fshb* and *gh* was positively and negatively regulated by IGF-1, respectively, whereas *lhb* showed no response to IGF-I treatment. No effects of EGF and PACAP on GTH expression were observed. In summary, the present study serves as the first step towards further understanding the involvement and possible interactions of various regulatory factors in controlling reproduction of zebrafish, an increasingly popular model in vertebrate developmental biology and physiology.

P194 The Molecular Mechanism of Ectopic ACTH Syndrome

Lei YE, Xiao-ying LI, Rui-xin LIU, Na LI, Guang NING
Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrine and Metabolic Diseases, Department of Endocrinology and Metabolism, Ruijin Hospital, Medical School of Shanghai Jiaotong University, China

Objectives: To investigate the molecular mechanism for abnormal ACTH secretion and tumoriogenesis of ectopic ACTH tumors. **Methods:** Bisulphite sequencing was adopted to determine the methylation pattern of *POMC* promoter in ectopic ACTH tumors. Meanwhile expression profiles of transcription factors regulating *POMC* expression were examined by RT-PCR. Furthermore PAK3 (p21 activated kinase 3), which was overexpressed in ectopic ACTH tumors was investigated for its role in tumorigenesis and the possible signaling pathway. **Results:** The degree of hypomethylation correlated well with *POMC* overexpression. Additionally T-box binding factor (Tpit) and NeuroD1 were required for ectopic *POMC* expression. PAK3 transfection could increase the mobility of NIH3T3 cell and the JNK-MAPK pathway. Inhibition of JNK activation by SP600125 can reduce the mobility of the cells. **Conclusions:** The *POMC* promoter hypomethylation and acquired expression of some pituitary transcription factors caused *POMC* gene activation in ectopic ACTH tumors. PAK3 overexpression can activate the JNK-MAPK pathway, which may play a critical role in the tumoriogenesis and development of ectopic ACTH tumors.

P195 Clinical Retrospective Study of Adrenal Incidentalomas Via Operations of 59 Cases

Ru-pan GAO, Zhi-qiang LU, Xin GAO
Department of Endocrinology and Metabolism, Zhongshan Hospital Fudan University, Shanghai 200032, China

Objective: To improve the diagnosis and treatment of adrenal incidentalomas by analyzing clinical features of the cases confirmed by operations. **Methods:** Analyzed retrospectively the operation-confirmed adrenal incidentaloma cases admitted in our hospital in the past 3 years. Among the total 162 adrenal tumor cases via operations, 59 were adrenal incidentalomas cases (37 men, 22 women, mean age 53.00±12.70, 22-75 years). Among the 59 cases, 42 were asymptomatic and were found in routine health examinations, 6 suffered from discomfort in back and 2 from cholecystitis. Other causes include hepatic cysts, renal cyst, urinary tract infection, varicose of the leg, palpitation, lacunar infarction, coronary heart disease, adnexauteri space occupying lesion and lumbar disc herniation. **Results:** 48 cases were detected by B ultrasound and 34 were confirmed by operation (coincidence rate with preoperational diagnosis is 70.83%); 11 cases were detected by CT scan and 10 were confirmed by surgery (coincidence rate is 90.9%). Pathological results showed 17 cases of adrenal cortical adenoma, 15 cases of pheochromocytoma, 11 cases of adrenal myelolipoma, 9 cases of secondary carcinomas, 3 cases of adrenocortical carcinoma and 4 cases of ganglioneuroma. 32 samples were smaller than 4 cm, 5 of them were malignant tumors, accounting for 15.6%; 19 samples were measured from 4 to 8 cm and 5 were malignant tumors, accounting for 26.3%; 8 samples were larger than 8 cm and 5 were malignant tumors, accounting for 25.0%. No data proved significant relationship between tumor malignance and sex, age or tumor size ($p>0.05$). Obvious blood pressure fluctuation occurred in 15 cases and 9 were pheochromocytoma (8 cases have imagine proofs). Blood pressure fluctuation in the operations has significant relationship with pheochromocytoma ($p<0.05$) while no significant relation with tumor size or malignance ($p>0.05$). **Conclusion:** Special attention should be paid to adrenal incidentaloma patients because not a few of the tumors are malignant. Tumor size has no significant relationship with tumor malignance. Proper preoperational assessment can facilitate selection of ways of operation, thus avoid additional radical ones. Our results suggest preoperational function assessment is necessary, especially to patients with pheochromocytoma. In order to prevent blood pressure fluctuation, unidentified tumor cases should also be administrated with α1-adrenoceptor antagonist, similar to the treatment of pheochromocytoma.

P199 The Diagnostic Performance of Plasma Metanephrine and Normetanephrine for Pheochromocytoma/Paragaglioma in Patients with Hypertension and Retroperitoneal Mass

Ting-wei SU, Lei JIANG, Jun-ni ZHANG, Yu-xuan WU, Fu-kang SUN, Yu ZHU, Wei-qing WANG, Guang NING
Department of Endocrine and Metabolic Disease, Ruijin Hospital, Medical School of Shanghai Jiaotong University, Shanghai Clinical Center for Endocrine and Metabolic Disease; Shanghai Institute of Endocrine and Metabolic Disease, China

Objective: The purpose of this study is to evaluate the diagnostic performance of plasma metanephrine (MN) and normetanephrine (NMN) in patients with hypertension and retroperitoneal mass. **Methods:** 115 patients with hypertension and retroperitoneal mass were recruited in the study. All underwent measurement of plasma MN and NMN before operation. The diagnosis of pheochromocytoma/paragaglioma was confirmed by clinical characteristics and pathology. All data were analyzed by SPSS 11.5. **Results:** The plasma MN and NMN level were significantly different between the patients with and without pheochromocytoma/paragaglioma. The normal range of MN was 14-90 ng/L and NMN was 19-121 ng/L in our center. When the cut-off point derived from ROC curve was set at 90.1 ng/L for MN and 132.3 ng/L for NMN, the sensitivity of MN and NMN were 63.8% and 82.8%, respectively. The specificity of MN and NMN were 98.2% and 98.2%, respectively. **Conclusion:** The plasma MN and NMN levels present a high specificity in the diagnosis of pheochromocytoma/paragaglioma in the hypertension patient accompanied with retroperitoneal mass and the NMN level had higher sensitivity than MN level.

P200 DNA-based Diagnosis of a Patient with von Hipple-Lindau Syndrome Presenting with Bilateral Papillary Cystadenoma of the Epididymis

Wai-kwan SIU, Ching-wan LAM, Yan-wo CHAN
Department of Pathology, Princess Margaret Hospital, Hong Kong SAR, China

Objective: To establish a molecular diagnosis of a patient with von Hippel-Lindau syndrome (VHL), who initially presented with bilateral papillary cystadenoma of the epididymis. **Methods:** Genomic DNA was extracted from peripheral blood. All the exons and flanking introns of the *VHL* gene were amplified by PCR and directly sequenced. **Results:** A known disease-causing mutation c.694C→T (p.R161X) was identified. **Conclusion:** VHL is a dominantly inherited familial cancer syndrome caused by germline mutation of the *VHL* gene. Affected patients are predisposed to various neoplasms in the central nervous system, kidneys, adrenal glands, pancreas and reproductive adnexal organs. The presence of bilateral epididymal papillary cystadenoma is highly suggestive of VHL. DNA-based test provides a specific and reliable basis of diagnosis. It also allows for presymptomatic screening of family members and prenatal diagnosis. Close surveillance for tumours could be started in early or presymptomatic stage of the disease. Early diagnosis and treatment of the tumours in VHL improves prognosis and reduces morbidity and mortality. The underlying *VHL* mutation is also useful in counseling for prognosis, especially in estimating the risk of phaeochromocytoma.

P201 Study on Succinate Dehydrogenase Gene in Pheochromocytoma

Ya-ru ZHOU, Zheng-pei ZENG, Jing ZHANG, Yuan-jia CHEN, Han-zhong LI, An-li TONG, Lin LU, Shi CHEN, Wei LIANG, Da-chun ZHAO, Tong-hua LIU
Department of Endocrinology, Peking Union Medical College Hospital, Beijing 100730, China

Objective: To detect mutations and loss of heterozygosity (LOH) of succinate dehydrogenase B and D (SDHB and SDHD) gene in pheochromocytoma patients. **Methods:** (1) 63 patients (15 cases of familial, 48 cases of sporadic pheochromocytoma), 22 relatives of familial pheochromocytoma and 20 normal controls were screened for mutations of SDHB and SDHD gene. (2) We spanned 1p35-p36 (SDHB locus) and 11q23 (SDHD locus) LOH in paired samples of DNA extracted from tumor tissues and peripheral blood leukocytes of 40 pheochromocytoma patients. **Results:** (1) Among 63 cases, 2 had mutations of SDHB, the frequency of mutations in SDHB gene was 3.2%, both of them were sporadic extra-adrenal paraganglioma. No SDHD gene mutations were identified. (2) Among 40 cases, 15 (42.9%) had 1p35-p36 LOH, 1 patient had 1p35-p36 microsatellite instability (MSI). 1p35-p36 LOH was significantly associated with extra-adrenal tumors. Among 40 cases, 2 cases had 11q23 LOH and 2 cases had 11q23 MSI. 11q23 LOH/MSI was significantly associated with malignant extra-adrenal tumors. **Conclusions:** (1) Detect SDHB gene mutation may have significance in diagnosis of extra-adrenal paraganglioma. (2) 1p35-p36 LOH and 11q23 LOH/MSI can be used as potential genetic markers to detect malignant, extra-adrenal paraganglioma at early stage.

P202 Effects of Urotensin II on the Proliferation of Pheochromocytoma Cells

Guo-qiang LIU, Zheng-pei ZENG, Dong-mei LIU, An-li TONG
Department of Endocrinology, PUMC Hospital, CAMS&PUMC, Beijing 100730, China

Objective: Urotensin II (UII) is reported to be the most potent vasoconstrictor identified to date. To investigate the effects of UII on the proliferation of rat pheochromocytoma cell line (PC12) and human pheochromocytoma cells primary cultured in vitro. **Methods:** We observed the effects of UII at different concentrations (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} M) on the proliferation of rat PC12 cells with MTT method, and then, PC12 cells were stimulated in the presence of UII at 10^{-7} M, whose effects on the proliferation of PC12 cells was observed at 12, 24, 36, 48, 60, 72 hr. The human pheochromocytoma tissue was digested and freed tumor cells were separated for primary culture. MTT was applied to observe the effects of UII at different concentrations (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} M) on the proliferation of human pheochromocytoma cells. **Results:** UII at different concentrations had no obvious effect on the proliferation of rat PC12 cells. Neither did UII at 10^{-7} M have any effect on the proliferation at different times of culture (12-72 hr). UII (at 10^{-7} and 10^{-6} M) could promote the proliferation of human pheochromocytoma cells in the primary culture in vitro. **Conclusions:** Our study suggests that UII had no effects on the proliferation of PC12 cells, and the results also showed that UII could promote the proliferation of human pheochromocytoma cells in primary culture and it probably plays a role in the pathogenesis of pheochromocytoma.

P203 Ageing and Adrenomedullin in the Male Reproductive System of the Rat

Fai TANG, Yuk-yin LI, Wai-sum O
Departments of Physiology and Anatomy, and The Centre of Heart, Brain, Hormone and Healthy Ageing, Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China

Objective: To study the age-related changes in the gene expression of adrenomedullin (AM), its receptor (CRLR, calcitonin receptor-like receptor) and its receptor activity modifying proteins (RAMPs) in the rat testis, ventral prostate and seminal vesicle in male S.D. rats aged 3 months (young), 12 months (middle-aged) and 20 months (old). **Methods:** AM levels in the plasma, the testis, the ventral prostate and the seminal vesicles were measured by RIA. PreproAM mRNA levels in the tissues were determined by RT-PCR. **Results:** Plasma AM levels were higher in the middle-aged and old rats than in young rats. Testicular AM concentrations as well as the mRNA levels of preproAM and RAMP1 and RAMP3 increased with age. However, the AM concentrations in both ventral prostate and seminal vesicle decreased with age. In the ventral prostate, the mRNA levels of preproAM and RAMP1 declined with age while in the seminal vesicle, the mRNA levels of preproAM, RAMP2, RAMP3 and CRLR were reduced with age. **Conclusion:** These changes may be related to the decline in serum testosterone levels. It is concluded that ageing has different effects on the levels of AM and its receptors in the testis and the accessory sex glands. AM may regulate the functions of these organs and its relative importance may change during ageing (supported by a CRCG grant from the University of Hong Kong).

P204 Ageing and Adrenomedullin in the Female Reproductive System of the Rat

Yuk-yin LI, Wai-sum O, Fai TANG
Departments of Physiology and Anatomy, and The Centre of Heart, Brain, hormone and Healthy Ageing, Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China

Objective: To investigate the age-related changes in the gene expression of adrenomedullin (AM), its receptor (CRLR, calcitonin receptor-like receptor) and its receptor activity modifying proteins (RAMPs) and coupling protein (RCP) in the ovary, oviduct and uterus of female rats aged 3 months (young) and 20 months (old) at oestrus and dioestrus. **Methods:** The stages of the oestrus cycle were determined by vaginal smear. AM levels in the plasma, the ovary, the oviduct and the uterus were assayed by RIA while the mRNA levels were measured by RT-PCR. **Results:** The young rats were cycling while the old rats were in either constant oestrus or constant dioestrus. In the young rats, the plasma AM levels were higher at dioestrus than at oestrus. In the ovary of the young rat, the preproAM mRNA and AM levels were higher at dioestrus than at oestrus. Plasma AM levels were lower in the old rats than in the young rats. There were age-related decreases in AM levels at both dioestrus and oestrus in the oviduct, but only at dioestrus in the ovary and the uterus. Both preproAM mRNA and CRLR mRNA levels decreased in the ovary, oviduct and uterus of the old rats at oestrus as well as at dioestrus. In the ovary of the old rats, RAMP2 mRNA decreased at both stages. In the oviduct, mRNA levels of all RAMPs decreased at both stages. In the uterus, RAMP3 decreased at both stages while RAMP1 and RAMP2 mRNA levels decreased only at oestrus. The mRNA levels of RCP decreased in the old rats in all three tissues at both stages. **Conclusion:** These results demonstrate that ageing has similar effects on the levels of AM and its receptor component proteins in the ovary, the oviduct and the uterus. The decrease in AM actions in the female reproductive systems may contribute to the decline of reproductive function in the ageing female rat.

P205 Effect of MEN1 on Development of Embryonic Development In Vitro and Differences in Gene Expression between men1+/+ and men1 -/-l Knockout Embryonic Stem Cells

Hong-li ZHANG, Tian-hong LUO, Ling FENG, Yu ZHAO, Guo LI, Min LUO
Shanghai Institute of Endocrine and Metabolic Diseases, Shanghai Clinical Center for Endocrine and Metabolic Diseases, Ruijin Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200025, China

The MEN1 gene has been identified as the gene responsible for MEN1, a hereditary syndrome transmitted with an autosomal dominant trait. In the other's study generation of mice with a null-mutation in the men1 gene revealed a crucial role in embryonic development. In this study, we used embryoid bodies (EBs) formed from men1+/+ and men1 -/- ES cells as a model system to investigate effect of on the embryo development, which recapitulated some features of early embryogenesis in vitro. Morphological analysis showed that EBs formed by the men1 -/-l ES cells were much similar in size and number to those formed by men1+/+ ES cells during the 10 days of suspension culture. We propose proliferation capacity of ES cells seem not to be impaired in the absence of menin. We also studied on differences in gene expression between men1+/+ and men1 -/-l knockout embryonic stem cells utilizing Affymetrix chips. 115 of these genes were increased and 565 were decreased by at least 2-fold in the men-/- ES relative to men+/+ including genes involved in TGFβ Signaling Pathway (e.g. smad3, smad1, Thbs1, Zfhx1b, Runx2, Inhba), Wnt signaling Pathway (e.g. Wnt5a), G Protein Signaling Pathway (e.g. Gna13, Akap9) and genes involved in Apoptosis (e.g. Traf3, Dffb, Jun, Mdm2, Irf2). We have identified a number of putative men1 target gene such as: Thbs1, Runx2, Wnt5a, etc. More experiments are needed.

P206 Qualitative Diagnosis and Localization of Insulinoma Before Surgery—Review of 99 Cases of PUMC Hospital

Xin YUE, Feng GU, Wei-bo XIA, Xin-hua XIAO, Mei LI, Xiao-ping XING, Zheng-pei ZENG, Hong-ding XIANG, Xiao-lan LIAN, Xue-yan WU
Department of Endocrinology, Peking Union Medical College Hospital, Beijing 100730, China

Objective: To evaluate the value of different methods of hormone measurement and image study before surgery. **Methods:** We retrospectively reviewed 99 patients with insulinoma confirmed by pathological report after surgery in the PUMC Hospital from December 1989 to August 2005. **Results:** The main clinical manifestations are hypoglycemia coma, weight gain, perspiration, disorientation, hypomnesis, and dizziness. The sensitivity of diagnosis of insulinoma with fasting blood-glucose less than 50 mg/dl is 67%. The ratio of serum insulin to blood-glucose greater than 0.3 when hypoglycemia attacks is 90% sensitive. Fasting test and OGTT are not necessary for typical case. The sensitivity of ultrasound, CT, perfusion CT, endoscopic ultrasonography, DSA and ASVS is 17%, 58%, 100%, 84%, 87%, 89% respectively. **Conclusion:** Typical "Whipple's triad" and the ratio of serum insulin to blood-glucose greater than 0.3 when hypoglycemia attacks are best for the qualitative diagnosis of insulinoma. Perfusion CT has the best sensitivity and specificity among image studies currently used. DSA is more useful for the localization of smaller insulinoma, and ASVS is not better than DSA. Endoscopic ultrasonography may be replaced by some other noninvasive diagnostic methods or more accurate invasive methods. Ultrasound and CT can be used as screening methods.

P207 Pancreatic Tumor Metastatic to the Pituitary Gland: a Case Report

Yu PEI, Huo-ming CHENG, Bao-zhong ZHANG, Suo-zhu SUN, Zhi-wei ZHAN
Department of Endocrinology, PLA Second Artillery Hospital, Beijing 100088, China

Pituitary carcinomas are rare adenohypophysial neoplasms, the definition, diagnosis, therapy, and prognosis of which are controversial. It includes primary pituitary carcinomas and tumor metastasis to pituitary. Tumors metastasis to the pituitary gland are an unusual complication of systemic cancer typically seen in elderly patients with diffuse malignant disease. Breast and lung are the commonest sites of the primary tumor, whereas diabetes insipidus is the most frequent symptom at presentation. Up to now, only few cases of pancreatic carcinoma metastasis to pituitary were reported. We report a case of pancreatic carcinoma metastasis to pituitary in this paper. A 47-year-old man, was admitted to hospital with "dizziness, nausea and vomit over 1 month". He had not any history of malignancy, presented with headaches, dizziness, nausea and vomit in one month before hospitalization. Blood electrolytes disorders with hyponatremia (121 mmol/L) and hypochloraemia (76 mmol/L) were found. A mild abdominal pain was presented. Progressive bilateral deterioration of visual acuity occurred in a few days. Magnetic resonance imaging revealed a large sellar mass compressing the optic chiasm. Some endocrine examination showed hypopituitarism as below: FT_3 0.14 pg/ml (2.07-5.97), FT_4 1.43 pg/ml (7.14-19.9), T_3 0.28 ng/ml (0.8-2.2), T_4 25.62 ng/ml (50-126), TSH 0.1 uIU (0.3-5.0); E2 11.22 pg/ml (0-70), LH 0.10 mIU/ml (1.5-9.2), FSH 0.10 mIU/ml (l-14) and T 2.0 ng/ml (5-15). Abdominal CT scan showed large mass in the head of pancreas and diagnosed as pancreatic carcinoma. Decompression of the sellar region was attempted, and pathology disclosed a metastatic adenocarcinoma. Tissue from the tumor displayed positive in Ca199, and negative in ACTH, TSH, GH, FSH, LH and PRL stain by immunohistochemistry analysis. The patient was diagnosed as pancreatic tumor metastatic to the pituitary gland. He died because pancreatic carcinoma widespread metastasis. Because few cases of patients with metastasis to the pituitary were found in relevant literature, we report this unusual case.

P208 Estrogen-dependent Breast Cancers: Pathophysiology and Management

Yi-ming MU, Wei-dong HAN
Department of Endocrinology, Chinese PLA General Hospital, Beijing 100853, China

Estrogen and its receptor a (ERa) play a key role in the development and metastasis of breast carcinoma. As a transcriptional factor, ERa regulates the expression of E2 target genes through the interaction with transcriptional co-activators after combining to DNA in direct or indirect manners, which occurs after ERa combines to E2 and changes its conformation. Transcriptional coactivators and inhibitive factors of ERa play an essential role in regulating the function and activity of E2 target genes. After combing to E2, the configuration of ERa changes, then the binding sites to the co-activators are exposed. Many multimeric protein complexes are attracted here, and the transactivation of E2 target genes are activated. In the recent 10 years, more than 30 co-activators (CBP/P300/SHARP/AIB1 et al.) of ERa and several multimeric protein complexes (SWI/SNF/CARM/PRMT1 et al.) have been recognized. As the E2 target genes, cyclin D, MTA_3, E_2F_1, bcl-2, cad, cathepsin, c-fos, human vitamin D_3, $IGFBP_4$ and RARa have been confirmed to be related with the proliferation of the breast carcinoma to date. And E-cadherin is the gene that has been confirmed to be associated with the metastasis of the breast cancer, however the exact molecular biological mechanism is still unclear.

LRP16 is a human gene, which was originally recognized from peripheral lymphocyte cells by our group in 2000 using restriction length genomic scanning (RLGS), and then the cDNA was isolated using the rapid amplification of cDNA end (RACE) technique. LRP16 codes for a protein of 325 amino acids, which is mainly localized in the nucleus. Previous studies have demonstrated that E2 activates the LRP16 mRNA levels and reporter gene activities in MCF-7 human breast cancer cells. Recently, a maximally responsive fragment (-101 to -14 bp) within 5'-proximal region of LRP16 to estrogen was also mapped, which conferred the estrogen response through ERa/Sp1 interaction. Ectopic expression of LRP16 in breast cancer cells stimulates cyclin E gene expression and cell proliferation. Here, we present new data supporting a role for estrogenically regulated LRP16 as an estrogen receptor a (ERa) coactivator, providing a positive feedback regulatory loop. LRP16 is shown to interact with ERa in vitro and in whole cell lysates, while suppression of LRP16 expression inhibits the expression of several ERa upregulated genes including cyclin D1 and c-Myc, but results in the increase of E-cadherin expression through ERa mediation. Chromatin immunoprecipitation (ChIP) analysis demonstrates the presence of an ERa/LRP16 complex on the pS2 promoter and LRP16 inhibition of ERa binding to the E-cadherin promoter, indicating that LRP16 can co-operate with ERa or antagonize ERa for promoter binding in a gene specific manner. In general, these data suggest that LRP16 may be essential for ERa signaling and important in breast cancer progression.

P209 The Association among Lipids, Leptin and Leptin Receptor Polymorphisms with Risk of Breast Cancer

Cun-zhi HAN, Jing SHI, Li-li DU, Xiu-ying LIU, Jie-xian JING, Xian-wen ZHAO, Bao-guo TIAN, Fu-guo TIAN
Department of Etiology, Shanxi Cancer Institute, Taiyuan 030013, China

Objective: To evaluate the association between serum levels of leptin, lipids, and leptin receptor (LEPR) gene polymorphism and breast cancer. **Methods:** LEPR Gln223Arg polymorphism was detected by PCR-RFLP in 240 patients with breast cancer and 500 healthy controls, and assayed the levels of leptin and blood lipids by ELISA and automatic biochemistry analyzer. **Results:** In univariate regression analyses, we found serum levels of leptin, lipids (free Cholesterol, Triglyceride, Apolipoprotein A1) were significantly higher than those in the control ($P<0.05$-$P<0.001$, respectively), Apolipoprotein B was significantly lower than that of the control ($P<0.001$). x^2 statistics were used to evaluate Gln223Arg genotype AA, AG, and AA+AG distribution in breast cancer cases and the controls, significant difference were observed in both groups ($x^2=11.16$, $P=0.004$). In multivariable analyses, we found strong association among those with the high leptin level (OR 1.53, 95%CI 1.13-2.07, P=0.006), LEPR Gin223Arg polymorphism (OR 4.87, 95%CI 1.30-18.22, P=0.019), the lipids of serum, included high level of the free Cholesterol (OR 15.19, 95% CI 4.11-56.19, P=0.0001), Triglyceride (OR 5.54, 95%CI 2.33-9.89, P=0.016), Apolipoprotein A1 (OR=12.73, 95%CI 2.44-66.31, P=0.003), WHR (OR=3.68, 95%CI 1.34-10.11, P=0.011), low level of Apolipoprotein B (OR=0.05, 95%CI 0.003-0.63, P=0.021), and breast cancer risk. **Conclusion:** The study suggested that LEPR Gln233Agr polymorphism, the elevated WHR and serum level of leptin, the disorder of lipids metabolized may be correlated with increased risk of breast cancer.

P210 The Role of Glucose Regulated Protein (GRP)-78 in Regulating Cell Growth and Apoptosis in Human Breast and Ovarian Cancer Cells

Wai-yin KWAN, Bonnie Ho-yee YEUNG, Alice Sze-tsai WONG
Department of Zoology, The University of Hong Kong, Hong Kong SAR, China

Objective: To investigate the role of GRP78 in regulating cell growth and apoptosis in human breast and ovarian cancer cells and examine whether overexpression of GRP78 protects cells from endoplasmic reticulum (ER) stress and chemotherapeutic drug induced apoptosis. **Methods:** GRP78 expression was measured by Western blot and immunofluorescent microscopy. Cell cycle analyses and cell proliferation were examined by flow cytometry and MTT assay, respectively. Apoptosis was determined by DAPI and TUNEL staining. **Results:** Overexpression of GRP78 in both breast and ovarian cancer cells led to increases in cell growth, which was due to decreased apoptosis. Treatment with tunicamycin and 2-deoxyglucose induced an increase in GRP78 expression, suggesting that GRP78 overexpression may alleviate unfolded protein stress in the ER. We also showed that overexpression of GRP78 protected both breast and ovarian cancer cells against apoptosis induced by chemotherapeutic drugs such as paclitaxel. **Conclusions:** Up-regulation of GRP78 is one of the mechanisms which may enhance survival and drug resistance of breast and ovarian cancer cells.

P211 GnRH Enhances Matrix Metalloproteinases-dependent Invasion of Human Ovarian Cancer Cells

Lydia Wai-ting CHEUNG, Peter C.K. LEUNG, Alice Sze-tsai WONG
Department of Zoology, University of Hong Kong, Hong Kong

Objectives: To investigate the contribution of GnRH in the invasive behavior of human ovarian cancer cells and to unveil the mechanism underlying this process. **Methods:** *In vitro* migration and cell invasion assays were performed with two human ovarian cancer cell lines CaOV-3 and OVCAR-3 in the presence of GnRH agonist. RT-PCR, Western blot, and gelatin zymography were used to investigate the effect of GnRH on metastasis-related proteinases, matrix metalloproteinase (MMP)-2 and MMP-9. The signaling pathway involved was identified by using specific small molecule inhibitors and dominant negative mutants. **Results:** The *in vitro* assays revealed a biphasic nature of GnRH; low concentrations of GnRH agonist increased cell motility and invasiveness of CaOV-3 and OVCAR-3, but the stimulatory effect was insignificant at higher concentrations. Moreover, we demonstrated that expression and activation of MMP-2 and MMP-9 were functionally related to GnRH-mediated invasion, and this was through the c-Jun N-terminal kinase signaling pathway. **Conclusion:** These results suggest a novel role of GnRH signaling cascade in the invasive phenotype and motility of human ovarian cancer cells.

P212 Antiapoptotic Effects of Mitochondrial Manganese Superoxide Dismutase in Ovarian Cancer Cells

Kwan-yeung WONG, Bonnie Ho-yee YEUNG, Alice Sze-tsai WONG
Department of Zoology, The University of Hong Kong, Hong Kong SAR, China

Objective: To investigate the protein expression levels of manganese superoxide dismutase (MnSOD) in ovarian cancer cell lines and ovarian surface epithelial (OSE) cells and the possible correlation between MnSOD expression and resistance to stress-induced apoptosis. **Methods:** Protein expression levels were measured by immunoblot analysis. Expression of MnSOD was manipulated by overexpression or small interfering RNA. Cell proliferation was examined by cell growth, colony formation, and MTT assays, whereas cell death was measured by DAPI and TUNEL assays. **Results:** MnSOD expression was significantly higher in most ovarian cancer cell lines than OSE. Overexpression of MnSOD in ovarian cancer cells conferred a strong decrease in cell proliferation and an increase of apoptosis, whereas targeted knockdown of endogenous MnSOD reduced apoptosis. Furthermore, stimulation of mitochondrial superoxide induced an increase of MnSOD expression, suggesting that MnSOD may alleviate the reactive oxygen species stress in these cells. We also showed that overexpression of MnSOD protected ovarian cancer cells against apoptosis induced by treatment of rotenone, hydrogen peroxide, or hypoxia-mimicking agents cobalt chloride and deferoxamine. **Conclusion:** Upregulation of MnSOD is one of the mechanisms which may increase resistance to oxidative stress and apoptosis in ovarian cancer cells.

P213 Expression of Sodium-iodine Symporter in Gastric Carcinoma with Different Histological Types

Ya-xin LAI, Zhong-yan SHAN, Chen-ling FAN, Wei-ping TENG
Department of Endocrinology & Institute of Endocrinology, 1st Affiliated Hospital of China Medical University, Shenyang 110001, China

Objective: To study the expression of sodium-iodine symporter (NIS) in gastric carcinoma with different histological types. **Methods:** One hundred and eighty samples of gastric carcinoma were collected and divided into six groups according to their histological classification, each group had thirty samples. The paracancerous tissue of the above 180 samples and thirty samples of chronic gastritis were also collected as controls. The paraffin-embedded sections were stained with SP method of immunohistochemistry. **Results:** In cancerous tissue, paracancerous tissue and chronic gastritis tissue, the positive rate of NIS expression was 10.6%, 28.3% and 90% respectively, and significant differences were found among the three (all $p<0.01$). In papillary, tubular, low-differentiated, mucous adenocarcinoma, signet-cell carcinoma and undifferentiated carcinoma, the positive rate of NIS expression was 30%, 10%, 13.3%, 6.7%, 3.3% and 0 respectively, and significant differences were found between papillary adenocarcinoma and the latter three types of carcinoma ($p<0.05$). In chronic gastritis tissue and paracancerous tissue, NIS expressed on the cell membrane, while in cancerous tissue, NIS expressed not only on the cell membrane, but also in the cytoplasma. **Conclusion:** (1) In cancerous tissue, paracancerous tissue and chronic gastritis tissue, the positive rate of NIS expression increased one by one. (2) In the gastric carcinoma of different histological types involved in this study, with the malignancy of carcinoma increasing, NIS expression showed decreasing tendency. (3) In cancerous tissue, NIS expressed not only on the cell membrane, but also in the cytoplasma, while in chronic gastritis tissue and paracancerous tissue, NIS only expressed on the cell membrane.

P214 Suppression of Met Expression by RNA Interference Inhibits the Metastatic Potential of Nasopharyngeal Carcinoma Cells

Hong-yan ZHOU, Kai-fung WAN, Chris Kong-chu WONG, Alice Sze-tsai WONG
Department of Zoology, University of Hong Kong, Pokfulam Road, Hong Kong SAR, China

Objective: To investigate the effects of Met overexpression on the invasive phenotype of nasopharyngeal carcinoma (NPC), and to assess downstream signaling pathways mediated by hepatocyte growth factor (HGF). **Methods:** The NPC cell line CNE-2 was used as an *in vitro* model to examine the influence of Met overexpression in regulating cell invasion and migration. Met expression was knockdown by using Met specific siRNA, cell invasion was examined by a Matrigel invasion assay and *in vitro* migration was measured by the transwell migration assay. **Results:** Met downregulation by siRNA reduced the invasion-motile response to HGF. Treatment of the cells with specific inhibitors against Akt (LY294002) and JNK (SP600125) or expression of their dominant negative forms abolished HGF-stimulated cell invasion, associated with downregulation of matrix metalloproteinases-9. Conversely, the inhibition by ERK1/2 and p38 MAPK had no effect. **Conclusions:** Met overexpression may play an important role in the progression and metastasis of NPC by activating the Akt and JNK pathways, and raise the possibility of their application for cancer therapy.

P215 Mitogenic Effect of Estrogen in Human Ovarian Surface Epithelial Cells (OSE) Involves Downregulation of Pigment Epithelium-derived Factor (PEDF)

Lydia Wai-ting CHEUNG, Simon Chak-leung AU, Annie Nga-yin CHEUNG, Hextan Yuen-sheung NGAN, Alice Sze-tsai WONG
Department of Zoology, University of Hong Kong, Hong Kong SAR

Objectives: To characterize the regulation of PEDF expression in OSE and its role in estrogen-dependent development of ovarian cancer derived from OSE. **Methods:** PEDF expression was examined in OSE cell lines and ovarian tumor tissues using immunohistochemistry, real-time PCR and Western blot. Cell proliferation and apoptosis were determined by MTT assay, TUNEL and Hoechst staining. **Results:** PEDF immunoreactivity was lower in a majority of ovarian tumors when compared with normal tissues. In cultured human OSE, exogenous PEDF caused significant growth inhibition and apoptosis. In line with the estrogen dependency of OSE-derived ovarian tumors, we showed for the first time that estrogen-induced OSE proliferation was accompanied by a specific inhibition of PEDF expression, which could be reversed by an estrogen receptor antagonist (ICI 182,780). Addition of exogenous PEDF antagonized the growth stimulatory effect of E_2 on these cells, suggesting a negative role of PEDF in estrogen-induced OSE cell proliferation. **Conclusion:** This study is the first to identify PEDF as an ER-regulated target gene, and apart from its known function as a potent anti-angiogenic factor, is also implicated as a suppressor of estrogen-dependent ovarian tumor growth.

P216 Value of the Clinic Autoantibodies Against AT_1 Receptor with the Hormone of Nerve and Endocrine on the Diabetic with Myocardial Disease

Lin-shuang ZHAO[1], Yu-hua LIAO[2], Min WANG[2], et al
[1] Department of Endocrinology Wuhan General Hospital of Guang Zhou Military Region Wuhan 430070, China
[2] Institute of Cardiology, Union Hospital, Tongji Medical College, Huazhong University of Science Technology, Wuhan 430022, China

Objective: To explore the role of autoantibodies against AT_1-adrenergic receptor (AT_1-receptor) in the development of diabetic with myocardial disease. **Methods:** The epitopes of the second extracellular loop of AT_1 receptor (165-191) were synthesized and used respectively to screen serum autoantibodies from patients: diabetic with myocardial disease (n=49), hypertension (n=58) and from healthy blood donors (n=41, control) by ELISA, the hormone of nerve and endocrine by RIA. **Results:** In diabetic with myocardial disease, diabetes mellitus with the positive rates of autoantibodies against AT_1 -receptor were 53.1%. The positive rates were both higher than those of patients with hypertension (12.1%). They were also higher than those of healthy donors (12.2%) ($P<0.01$). The hormone of nerve and endocrine (PRA and AgII) and in patients diabetic with myocardial disease were higher than those of patients with hypertension without myocardial disease ($P<0.05$, $P<0.01$), and they were also higher than those of healthy donors. **Conclusion:** The findings suggest that these autoantibodies against AT_1-receptor, these autoantibodies may play important roles, and activate accordingly the hormone of nerve and endocrine system in the pathogenesis of diabetic with myocardial disease.

P217 The Prevalence and Related Factors of Coronary Heart Disease in High-risk Population for Diabetes in Beijing

Guang-ran YANG, Shen-yuan YUAN, et al
Department of endocrinology, Tongren hospital affiliated to Capital University of Medical Sciences, Beijing 100730, China

Objective: To investigate the prevalence and characteristics of Coronary Heart Disease in high-risk people for diabetes in Beijing and the relationship between metabolic syndrome and the occurrence of Coronary Heart Disease. **Methods:** In 1995-1996, 20 682 residents, randomly selected and over 25 years of age, were screened in Beijing area. Those whose capillary glucose concentration was above 120 mg/dl two hours after 100 g steam-bread were subjected to 75 g oral glucose tolerance test. ECG examination was also carried out in these people together with the measurement of blood pressure and determination of plasma cholesterol, triglycerides, insulin. 1302 people were selected. ECG was analyzed by Minesta code. In 1995 according to Minesta code, 231 people were diagnosed Coronary Heart Disease and 1071 persons were non-Coronary Heart Disease. In 2000 these 1071 people were followed up. **Results:** (1) In 2000, 287 were Coronary Heart Disease patients, the prevalence of Coronary Heart Disease in 1071 was 26.8%. (2) There was statistically significance in coronary heart disease between male and female (23.6% vs 29.4%). (3) The prevalence of Coronary Heart Disease in MS group (23.7%) was significantly higher than that in those without MS (33.4%). The number of Coronary Heart Disease was highest in people who had diabetes and metabolic syndrome. (4) Logistic analysis suggested that Coronary Heart Disease was correlated to MS. **Conclusion:** MS may be the predictive factor of Coronary Heart Disease. Combined with DM, MS people have higher risk of Coronary Heart Disease.

P218 Study of Carotic Intima-media Thickness and Risk Factors in Patients with Type 2 Diabetes Mellitus

Li-li YAO, Yong-de PENG, Fang FANG
Department of Endocrinology, Shanghai Jiaotong University Affiliated First People's Hospital, Shanghai 200080, China

Objective: To investigate carotid artery intima-media thickness (IMT) and relation of risk factors in patients with type 2 diabetes mellitus (DM). **Methods:** A retrospective analysis was conducted in 99 patients with type 2 diabetes to analyze the relationship between carotic intima-media thickness and risk factors. **Results:** Statistical significant association was found between IMT and age, SBP, high LDL level and HbAlc. **Conclusion:** It suggests measurement of IMT is a way to detect early in patients of high risk factors with heart diseases.

P219 Study on Serum Leptin and Its Relationship with the Predisposing Factors of Vasculopathy in Type 2 Diabetes with Coronary Heart Diseases

Jing SUI, Fu-qin CHEN, Chun-mei ZHAO
Department of Endocrinology, QiLu Hospital of Shangdong University, Jinan 250012, China

Objective: To investigate serum leptin and its relationship with the predisposing factors of vasculopathy in type 2 diabetes with coronary heart diseases. **Methods:** Serum leptin, CRP, TG, TC, LDL-C, HDL-C, FBG, HbA1C were measured in type 2 diabetic with and without CHD, and nondiabetic subjects. Insulin resistance was estimated by the insulin resistance index of homeostasis model assessment. The intima-media thickness of the common carotid artery was examined by a high resolution color doppler ultrasonography. **Results:** (1) Serum leptin concentrations were significantly higher in diabetic with CHD than those in diabetic without CHD and nondiabetic subjects. (2) Serum leptin concentrations were significantly higher in female than in male subjects. (3) Correlation analysis showed that there was independent relationship with leptin to WHR and FINS. Stepwise regression analysis showed gender and FINS entered the regression equation in the final phase. **Conclusion:** Leptin is highly correlated with the predisposing factors of vasculopathy in type 2 diabetes and diabetes with coronary heart diseases. These results suggested that the increased serum leptin concentrations in diabetes should be a risky factor of coronary heart disease.

P221 Clinical Evaluation and Risk Factors Analysis for Cardiovascular Autonomic Neuropathy in Patients with Type 2 Diabetes

Dan-yun LING[1 2], Zheng-yi TANG[1], Wei ZHANG[1], Xiao-ying LI[1], Wei-qing WANG[1], Guang NING[1]
[1] Shanghai Institute of Endocrine and Metabolic Disease, Ruijin Hospital, Shanghai Jiaotong University School of Medicine
[2] Department of Endocrinology, Diabetic Laboratory, Shanghai Jiaotong University Affiliated First People's Hospital, Shanghai 200080, China

Objective: Risk factors for diabetic cardiovascular autonomic neuropathy (DCAN) in patients with type 2 diabetes were analyzed to establish regression model for evaluating the diagnosis of DCAN. **Methods:** 325 patients with type 2 diabetes were divided into four groups (no DCAN, early DCAN, definitive DCAN and severe DCAN) according to the results of four standard function tests (30:15 ratio, HRV during deep breathing, Valsalva maneuver and SBP decrease during standing). Clinical data including disease history, EKG, nerve conduction velocity, retinoscope and Doppler of carotid and lower limb arteries, and results of serum and urine test were collected to evaluate clinical characteristics, metabolism condition, chronic diabetic complications and other diseases. SPSS (Ver 12.0) software was applied for statistic analysis. Ordinal regression was performed to identify risk factors and establish a diagnostic model. χ^2 test was used for comparison of multi-rates and calculation of exact probability was launched for rates comparison in small samples. **Results:** 64% patients had abnormal autonomic function, 30.2% with definite DCAN. There was significant difference between groups in age, average HbAlc, SBP (Systolic Blood Pressure), 24h urine albumin excretion and heart rate at rest, duration of diabetes and duration of diabetic neuropathy ($P<0.05$). However, there was no significant difference in sex, BMI, DBP (Diastolic Blood Pressure), cholesterol, triglyceride, HDL and LDL among groups. The incidence of diabetic microvascular complications, hypertension, artery mass, history of coronary heart disease, stroke history, insulin usage history increased with deterioration of DCAN ($P<0.05$). The Ordinal regression model showed that age, average HbAlc, hypertension, peripheral neuropathy, retinopathy, tachycardia at rest and duration of peripheral neuropathy were significant related factors for DCAN. Coefficients of all risk factors displayed that age which is the factor out of control had much effect on the severity of DCAN ($\beta_1\chi_2>1.512$). Apart from age, HbA1c (HbA1c>6.4%, then $\beta_3\chi_3>1.510$) was the most effective factor for prognosis when duration of peripheral neuropathy <12 years ($\beta_2\chi_2<1.428$). Influence of peripheral neuropathy was second to HbA1c ($\beta_6\chi_6=0.917$). Symptom of tachycardia at rest ($\beta_5\chi_5=0.852$), retinopathy ($\beta_7\chi_7=0.745$), hypertension ($\beta_4\chi_4=0.665$) had effects on severity of DCAN in turn after factors mentioned above. **Conclusion:** Age, hyperglycemia, hypertension, neuropathy and retinopathy and tachycardia at rest were found as risk factors for DCAN. Except age and hypertension, above risk factors were worsened by hyperglycemia. These further suggest that control of hyperglycemia is the primary task to prevent diabetic complications.

P223 Investigation on the Relationship between C-Peptide and Chronic Complications in Type 2 Diabetic Patients

Ming-yu GU, Li ZHAO, Yong-de PENG
Department of Endocrinology, Shanghai Jiaotong University Affiliated First People's I kispital, Shanghai 200080, China

Objective: C-peptide is a cleavage peptide from proinsulin to insulin. Recent studies have indicated that C-peptide may exert biological effects when administered to patients with diabetes. It can improve vessel function and autonomic nervous function. This study was done to explain the relationship of chronic compliation with C-peptide in type 2 diabetic patients. **Methods:** A retrospective analysis was conducted in 220 patients with type 2 diabetes to analyze chronic complications with C-peptide values. **Results:** Statistical significant association was found between C-peptide and diabetic retinopathy, nephropathy and neuropathy ($P<0.05$ or <0.01). **Conclusion:** It suggests that C-peptide may reduce the diabetic microangiopathy and neuropathy. It can be used as an effective tool for treatment in diabetic complications.

P224 The Association Study of C-reactive Protein and Type 2 Diabetes Mellitus With or Without Coronary Arteriosclerosis

Jing WANG
The First Hospital, Jilin University, Changchun 130021, China

Objective: To study the association between C-reactive protein (CRP) and the pathogenesis of type 2 diabetes mellitus with or without coronary arteriosclerosis. **Methods:** The determination of serum high sensitivity C-reactive protein (hsCRP) is based on an immune turbidimetric assay. Compared the differences of the common conditions, blood lipid, FPG, CRP, type of CHD and so on in four groups. **Result:** CRP level of B, C and D group were higher than those of normal controls ($P<0.01$) and CRP level of C and D group were higher than those of B group ($P<0.01$). But there was no significant difference in CRP levels between C and D group ($P>0.05$). Compared with B group, there was a long history of T2DM in C group ($P=0.05$). Compared with B group, the level of serum CRP was higher in C group ($P<0.01$). There was no significant difference in FPG and HbA_{1C} between B and C group ($P>0.05$). The patients with unstable angina had higher CRP level than those with stable angina ($P<0.01$). Serum CRP level of the patients with acute myocardial infarction was higher than that of the patients with stable angina in D group ($P<0.05$). Serum CRP concentration was positively correlated with BMI and HbA_{1C} ($P<0.05$). **Conclusion:** (1) CRP may predict the development of T2DM and play an important role in the initiation and progression of T2DM and coronary arteriosclerosis. (2) As for coronary arteriosclerosis, CRP is a common risk factor of general population, although CRP is not a special risk factor of T2DM. (3) Serum CRP level is closely associated with stability of coronary lesion. (4) Serum CRP concentration was positively correlated with BMI and HbA_{1C}.

P225 Tongmaikang Affecting Serum P-selectin, C-Reactive Protein, Anti-cardiophospholipid Antibody Levels in Patients with Non-insulin Dependent Diabetes Mellitus

Chun-lan LIU, Feng-qin JIANG, Yong LIU, Qing LIU, Wei QU, Kai-shen WANG
Department of Internal Medicine, The 456th Hospital of PLA, Jinan 250031, China

Objective: To find the possible effects of Tongmaikang on serum P-selectin, C-reactive protein, anti-cardiophospholipid antibody levels in patients with non-insulin dependent diabetes mellitus, and to see if it can alter platelet activation and pre-micro emboli condition or block the progress of diabetes mellitus. **Methods:** Sixty-eight patients with non-insulin dependent diabetes mellitus were randomized into Tongmaikang (38 patients) and Chuanxiongqin (30 patients) groups. They stopped all anti-coagulant management for at least 1 week before involved in this study. Their serum P-selectin, C-reactive protein, anti-cardiophospholipid antibody levels were detected at the beginning and 30 days after management. ELISA assayed P-selectin and anti-cardiophospholipid antibody. CRP was detected by immunity velocity scattering deep and thick method. **Results:** Serum P-selectin, C-reactive protein, anti-cardiophospholipid antibody levels were significantly decreased in both groups. However, all these parameters were even more pronouncedly decreased in Tongmaikang group compared with the control. **Conclusions:** Tongmaikang was effective in patients with diabetes mellitus with hyper coagulation and it could reduce the incidence of cardiovascular events.

P226 Effect of Fluvastatin on Expression of Connective Tissue Growth Factor in the Myocardium of Diabetic Rats

Peng-bin LAI[1], Li-yong YANG[2]
[1] Department of Endocrinology, Zhangzhou Municipal Hospital Fujian Province, Zhangzhou 363000, China
[2] Department of Endocrinology, The First Affiliated Hospital, Fujian Medical University, Fuzhou 350005, China

Objective: To investigate the effects of fluvastatin on expression of connective tissue growth factor (CTGF) in the myocardium of diabetic rats. **Methods:** Thirty-two Sprague-Dawley rats were randomized into four groups: normal control rats, untreated diabetic rats induced by streptozotocin, and diabetic rats treated with both low-dose fluvastatin and high-dose fluvastatin. After 12 weeks' intervention, body weight as well as the weights of both whole heart and its left ventricle were measured to calculate the ratio of heart weight to body weight (H/B) and the left ventricle mass index (LVMI). The mRNA expression of CTGF, transforming growth factor β_1 (TGF-β_1), fibronectin (FN), collagen type III (Col III) in the myocardium of each groups were detected by reverse transcription-polymerase chain reaction (RT-PCR). **Results:** Compared with the normal control group, H/B and LVMI were significantly increased, as was the mRNA expression of CTGF, TGF-β_1, FN, and Col III in the myocardium of untreated diabetic group. In addition to the effective regulation of lipid metabolism, fluvastatin treatment could obviously reduce the increment of H/B and LVMI ($P<0.05$-0.01), and suppress the mRNA expression of CTGF, TGF-β_1, FN, and Col III in the myocardium of diabetic rats ($P<0.01$). The down-regulation of CTGF was more significant than that of TGF-β_1. All these effects were in dose-dependent way. **Conclusions:** Fluvastatin inhibits extracellular matrix (ECM) accumulation in the myocardium of diabetic rat. Down-regulating the overexpression of CTGF in diabetic is an underlying mechanism of fluvastatin in the cardiac protection.

P227 Cloning, Protein Expression and Function Studying of 4TM Gene from Human Aorta

F.L. CHEN, X.C. WANG, Yu LIU, L.X. LI, Z.H. YANG, W.B. ZHOU, R.M. HU
Department of Endocrinology, Huashan Hospital, Institute of Endocrinology and Diabetology, Fu Dan University, 200040, China

Objective: We have prepared the diabetic monkey model by the methods of high calorie diet followed with injection of low dose streptozotocin and found 216 differential expression genes in diabetic aorta with cDNA array technique. Among those genes (ESTs), one EST was down regulated by 4 folds. We cloned the full length cDNA by bioinformatics technique and found that it contained 4 transmembrane domains. We name this gene as 4TM gene. Thus we started functional study on this full length cDNA. **Methods:** (1) Subclone and bioinformatics technique was used to clone full length 4TM cDNA. (2) To construct prokaryotic expression vector and invert into *Bacillus coli* to express 4TM protein then purify 4TM protein from *Bacillus coli* and immune rabbit to produce polyclone antibody. (3) Northern blot technique was used to establish 4TM gene expression profile in human tissues. (4) Construct pEGFP-C3-4TM vector and transfect it to 293 cell line to observe the GFP-4TM fusion protein intracellular location. (5) Realtime PCR technique was used to investigate the effect of glucose and AcLDL to 4TM gene expression in human aorta smooth muscle cell. (6) Western blot technique was used to establish 4TM protein expression profile in human tissues. (7) Immunohistochemical method was used to observe 4TM protein location in human aorta. **Results:** (1) The full length 4TM gene cDNA which contained a 1035bp ORF was cloned from human aorta. (2) The 4TM polyclone antibody was made by immune rabbit with 4TM protein. (3) It was found that 4TM gene and protein was widely expressed in human tissue. (4) 4TM protein was expressed at cell membrane. (5) 4TM gene expression was downregulated in human aorta smooth muscle cell by glucose. (6) 4TM gene expression was decreased in aorta of type 2 diabetic patients and diabetic monkey. **Conclusion:** We cloned a novel gene (4TM gene) which might be a membrane protein and widely expressed in human tissues. 4TM gene expression was decreased in aorta of type 2 diabetic patients and diabetic monkey and in human aorta smooth muscle cell by glucose. 4TM gene might be related to the etiology of diabetic macroangiopathy.

P228 Prevalence and Predictors of Microalbuminuria in Shanghai Population with Various Glucose Tolerance Levels

Li HUO[1,2], Min XU [1], Hong-er HUANG[3], Rui LI[4], Meng DAI [1], Ji-guang WANG[5], Guang NING[1], Xiao-ying LI[1]
[1] Department of Endocrinology, Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai Institute of Endocrine and Metabolic Diseases, Ruijin Hospital, Shanghai Jiaotong University School of Medicine
[2] Department of Endocrinology, Diabetic Laboratory, Shanghai Jiaotong University Affiliated First People's Hospital, Shanghai 200080, China
[3] The Center for Disease Prevent and Control Baoshan, Shanghai
[4] Shanghai Municipal Center for Disease Control and Prevention
[5] Centre for Epidemiological Studies and Clinical Trials, Ruijin Hospital, Shanghai Jiaotong University School of Medicine

Objective: To investigate the prevalence of microalbuminuria in subjects with various glucose tolerance levels and the risk factors for the development of microalbuminuria. **Methods:** 1779 subjects, including 752 with normal glucose tolerance (NGT), 505 impaired glucose tolerance (IGR), and 522 type 2 diabetes mellitus (T2DM) diagnosed according to a 75 g oral glucose tolerance test (OGTT), were recruited in this study. Urinary albumin-to-creatinine ratio (ACR) was measured for an early morning urine sample by rate-nephelometry method. **Results:** (1) ACR levels were significantly elevated in IGR and DM groups (P<0.001), the prevalence of microalbuminuria is 4.7% in NGT group, 6.1% in IGR group and 11.3% in T2DM group. (2) Logistic regression showed that microalbuminuria was significantly correlated with 2-h PG, systolic blood pressure and triglyceride. **Conclusion:** ACR levels became elevated in IGR subjects. The prevalence of microalbuminuria was higher in Chinese diabetic patients. 2-h PG, systolic blood pressure and triglyceride independently contributed to microalbuminuria.

P229 The Efficacy of Rosiglitazone Combined with Insulin and Only Insulin in Treating Diabetic Nephropathy

Cui-hua LIU, Li ZHAO
Department of Endocrinology, Donganshan Hospital, Anshan Iron and Steel Company, Anshan Liaoning 114041, China

Objective: To compare the therapeutic effect of rosiglitazone combined with insulin and only insulin in treating early stage diabetic nephropathy (DN). **Methods:** 62 patients with DN in whom microalbuminuria (MAU) were in 20-200 μg/min, body mass index (BMI) were average 27 kg/m^2, were randomly divided into two groups. First group: used rosiglitazone maleate tablets (trade name: Avandia) 4-8 mg daily, combined with small dose insulin. Second group: used short acting plus intermediate acting insulin. Two groups were used both for 6 months. **Results:** Fasting blood sugar, MAU, glycosylate hemoglobin (HbA1c) in two groups were decreased both lower than that before the treatment (P<0.01). The comparison of two groups were P>0.05. Postprandial blood sugar levels in two groups were both decreased lower than that before the treatment (P<0.01), but second group was better than first group (P<0.01). On BMI first group was better than second group (P<0.01). **Conclusions:** The efficacy of rosigitazone combined with insulin and only insulin in treating early stage diabtic nephropathy are good with to delay deterioration of DN. Rosiglitazone in complying is good, and body weight is not increased in DN with obesity, hypoglycemia is seldom. Transaminase in liver function elevates only a few, but it recovers to normal after drug withdrawal.

P230 Clinical Investigation on Type 2 Diabetics with Pretibial Black Spots by Both Skin and Kidney Biopsies

M.C. QIU, Zh.H. GAO, M. ZHU, S. LIN, H.L. HAN, J.Y. FAN, B.SH. SHEN, ZH.SH. MA, W.L. SU, W. LIU, H. GAO, SH.F. TANG
Division of Endocrinology, Tianjin Medical University Hospital, Tianjin 300052, China

Objective: To investigate the pathological abnormalities of both kidney and skin in T2 diabetes with pretibial black spots (PBS) and the correlation between the two. Also, serum electrophoresis was performed to look for abnormal proteins on the gel. **Methods:** To survey the epidemiology of T2 diabetes with pretibial black spots. By both kidney and skin biopsies, pathological and immunohistochemical abnomalities were investigated in 16 T2 diabetics with pretibial black spots while serum electrophoresis was carried out. **Results:** Among 573 DM patients, 24.78% patients had the pretibial black spots. Various pathological abnormalities were demonstrated in the 16 diabetics and same immunocomplexes were detected on both kidney and skin. An extra band was found on the electrophoresis gel with a large molecular weight. **Conclusion:** The data suggest that the same pathogenesis occur at least on the two organs detected by biopsies. The retinopathy and beta cells failure in the pancreas might be related to the same pathogenesis while hyperglycemia may be just a part of the whole disease.

P231 Effects of Diabetic Nephropathy on Plasma Brain Natriuretic Peptide Levels

Hua SUI, Chun LIU, Xiu-juan LI
Department of Endocrinology, The First Affliated Hospital, Chongqing University of Medical Sciences, Chongqing 400016, China

Objectives: To study effects of diabetic nephropathy on plasma brain natriuretic peptide levels in type 2 diabetic patients. **Methods:** Serum BNP levels and suspected influential factors (including age, sex, blood pressure, body mass index, HbA1c, blood lipid, renal function) were investigated in 77 patients with type 2 diabetes mellitus. **Results:** Serum BNP was significantly different (6.7±8.5 vs.13.2±13.1 vs. 62.7±71.6 pg/ml, $P<0.05$) among renal normal-function group (Ccr≥90 ml/min), renal mildly abnormal-function group (90 ml/min>Ccr≥60 ml/min), renal markedly abnormal-function group (Ccr<60 ml/min). Serum BNP was also different (110.6±87.1 vs. 15.3±14.2 vs. 10.8±14.0 pg/ml, $P<0.05$) among macroalbuminuric group (UAER ≥300 mg/d), microalbuminuric group (300 mg/d>UAER≥30 mg/d), macroalbuminuric group (UAER<30 mg/d). **Conclusion:** Renal dysfunction can raise serum BNP levels in type 2 diabetic patients.

P232 The Study of Clinical Features of Post-renal Transplantation Diabetes Mellitus

Yu-fan WANG, Shuai YAN, Yin ZHANG, Li ZHAO, Yong-de PENG
Department of Endocrinology, Diabetic Laboratory, Shanghai Jiao Tong University Affiliated First People's Hospital, Shanghai 200080, China

Objective: Post-renal transplantation diabetes mellitus (PTDM) was studied to explore its clinical features and improve its diagnosis and therapy. **Methods:** 30 patients of PTDM during January 2000 and July 2006 from both outpatient and inpatient department of our hospital were retrospectively reviewed. General condition, blood glucose, glycosylated hemoglobin (HbA_{1c}), blood concentration of cyclosporine (CSA), islet function and islet antibodies were detected and compared with those of type 2 diabetes mellitus (T2DM) patients of the same DM duration. **Results:** (1) 30 cases of PTDM (male/female 17/13), their ages, PTDM history, medium of DM history and medium of interval from transplantation to onset of PTDM were 51.93±7.04 (38-65) years old, 5.52±4.73 (0.08-15) years, 0.8 (0.005-7) years and 2.3 (0-12.42) years respectively. 11 cases (36.7%) had the diabetic symptom while 15 cases (50%) had DM family history. (2) Compared with T2DM of matched diabetic duration, patients of PTDM were younger (50.24±7.53 vs 59.27 ±13.4 years old, $P<0.05$). Postprandial C-peptide was lower (1394.96±835.81 vs 2406.19±1541.58 pmol/L, $P<0.05$). More patients of PTDM received insulin therapy (67.9% vs 39.3%, $\chi^2=4.595$, $P<0.05$). Difference was not statistically significant for body mass index (BMI), DM family history and fasting C-peptide. (3) Positive islet antibodies were found in 2 PTDM patients. (4) There was no correlation between blood CSA concentration and fasting blood glucose and HbA_{1c}. **Conclusion:** PTDM is a serious complication secondary to immunosuppression which has an impact on life quality and patient survival. Compared with T2DM, patients of PTDM were at earlier onset and islet release function was impaired. Furthermore PTDM is always asymptomatic. Blood glucose should be carefully followed up after kidney transplantation operation. Insulin and proper antidiabetic drugs need to be used for glucose disorder when PTDM is diagnosed.

P233 The Therapeutic Efficacy of Prostglandin E1 in Diabetic Nephropathy

Lin LIAO[1], Ming YANG[1], Stanley C.F. LIEW[2], Yong QU[1], K.O. LEE[2], Jia-jun ZHAO[1]
[1] Department of Endocrinology, Shandong Provincial Hospital, 250021, China
[2] Department of Endocrinology, The National University Hospital, Singapore

Objective: To investigate the efficacy of prostglandin E1 (PGE1) in diabetic patients with microalbuminuria or proteinuria. **Methods:** Sixty patients, 30 with microalbuminuria (30 to 300 mg/24 hrs) and 30 with clinical nephropathy (>300 mg/24 hrs) were studied. The patients had a mean (SD) age of 59 (11.1) yrs (range: 28-77 yrs), mean duration of diabetes of 10 (5.2) yrs (range: 2-20 yrs) and all had acceptable HbA1C of <7.0%, and BP <135/85 mm Hg. The 30 patients with microalbuminuria were randomly assigned to two treatment groups, microalbuminuria-Lotensin group, treated with Lotensin (benazpril/hydrochlorothiazide) 10 mg qd for 12 months, and microalbuminuria-PGE1 group who were treated with Lotensin 10 mg qd for 12 months AND had intravenous lipo-prostaglandin E1 (alprostadil injection) daily for 15 days. Similarly, the 30 patients with clinical nephropathy were also randomly assigned to nephropathy-Lotensin group and nephropathy-PGE1 group. **Results:** Urine albuminuria was significantly decreased in all the patients at the end of the one-year study period ($P<0.01$). The decrease in the PGE1-treated groups was significantly more compared with the Lotensin only groups ($P<0.01$). Urine albuminuria of some patients decreased to normal range (<30 mg/day) immediately after 15 days' PGE1 treatment and remained normal for the 12 months. At the end of 12 mths, all the patients who had received PGE1 treatment had significantly less proteinuria compared to Lotensin only patients. **Conclusion:** PGE1 is effective and rapid in decreasing albuminuria. The combination of PGE1 plus ACEI is an effective combination in decreasing proteinuria and maintaining the outcome, and is significantly more effective than therapy of Losentin alone.

P235 Effect of Rosiglitazone on the Expression of Fractalkine in Renal Cortex of Type 2 Diabetes Rats

Zi-lin SUN, Qiong WEI, Yu-yan SUN, Lin ZHANG, Yong ZHANG, Gui-ju SUN, Bi-cheng LIU, De-zai DAI, Nai-feng LIU
Department of Endocrinology, Zhongda Hospital, Southeast University, Nanjing 210009, China

Objective: To investigate the effect of Rosiglitazone on the expression of fractalkine in renal cortex of type 2 diabetic rats. **Methods:** Type 2 diabetic rats were induced by an intraperitoneal injection of low doses of streptozotocin combined with high fat diet, and were randomized to receive rosiglitazone or vehicle treatment. Serum advanced glycosylation end products (AGE-P) was measured by using flow injection assay. The mRNA and protein level of fractalkine were detected by semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemical staining, respectively. **Results:** The serum AGE-P, the level of fractalkine mRNA and protein in renal cortex of diabetic rats was significantly higher than those of normal control. However those of rats treated with Rosiglitazone were obviously lower compared to untreated group. **Conclusion:** Rosiglitazone may play its renoprotective effects via inhibition of fractalkine expression in the renal cortex.

P236 Effects of Pioglitazone on Receptor of the Advanced Glycation End Products in Renal Cortex of Streptozotocin-induced Diabetic Rats

Jian-guo SHEN, Jing-xia KONG, Yuan RUAN, Zhong-hang TONG
Department of Endocrinology, First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, China

Objective: To explore the effects of piogilitazone on mRNA expression of AGE-specfic cellular receptor (RAGE) in renal cortex of diabetic rats induced by Streptozotocin. **Methods:** We induced diabetic rats by an intraperitoneal injection of STZ in SD rats. The criterion of diagnosis of diabetes was the consecutive blood glucose level ≥16.7 mmol/L. 28 male SD rats were randomly divided into 3 groups: diabetes adding pioglitazone group (n=9, intra gastric administration pioglitazone 20 mg/kg·d) and diabetes adding BBS group (n=9) and normal control group (n=10). The body weight and blood glucose were measured every two weeks. 8 weeks later, all rats were killed and the expression of RAGE mRNA was quantified in renal cortex by reverse transcription-polymerase chain reaction (RT-PCR) respectively. **Results:** The RAGE mRNA expression was increased in renal cortex of diabetic rats. RAGE/β-actin ratio of diabetic group was significantly higher than that of the control ($P<0.01$). After treatment with pioglitazon, RAGE mRNA expression decreased significantly and RAGE/β-actin ratio was obviously lower than that of the control. **Conclusion:** The high expression of RAGE mRNA may participate in the renal damage of diabetic rats. Pioglitazone can protect the renal tissue from the impairment of hyperglycemia and advanced glycation end product.

P237 Experimental Study of Relationship between Simvastatin, P38 Mitogen-activated Protein Kinase and Diabetic Nephropathy

Yan-bo LI[1], Jun-yong HAN[2], Chi-ying AN[1]
[1] Department of Endocrinology, The First Affiliated Hospital of Harbin Medical University, Harbin 150001, China
[2] Department of Cardiology, The Fifth Affiliated Hospital of Zhongshan Medical University, Zhuhai 519000, China

Aim: To study the relationship between P38 mitogen-activated protein kinase (P38MAPK) and type IV collagen (Col IV) and fibronectin (FN); in order to explore the effect of P38MAPK in diabetic nephropathy and mechanism of preventing from diabetic nephropathy of simvastatin. **Methods:** We mimicked diabetic state *in vitro*. Rat mesangial cells (RMC) were incubated with high glucose, advanced glycosylation end products (AGE) or H_2O_2 with or without pre-treatment with SB203580 (P38MAPK specific inhibitor) or simvastatin. The expression of phospho-P38MAPK and Col IV and FN in RMC was detected by Western-blot or RT-PCR. **Results:** High glucose, AGE or H_2O_2 can activate P38MAPK and increase the expression of Col IV and FN in RMC. The expression of Col IV and FN was inhibited by SB203580. Simvastatin inhibited the activation of P38MAPK and reduced the expression of Col IV and FN. **Conclusion:** P38MAPK is an upstream signaling molecule of Col IV and FN. P38MAPK may be one of the initiating signals of diabetic nephropathy. Simvastatin can inhibit the expression of Col IV and FN by repressing P38MAPK signaling pathway and therefore prevent the development of diabetic nephropathy.

P238 Effect of Simvastatin on the Early Renal Injury of Experimental Diabetic Rats

Xin WANG, Chao LIU
Department of Endocrinology, First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China

Objective: To observe the renal protective effect of simvastatin by *in vitro* and *in vivo* experiments. **Methods:** The rat glomerular mesangial cells were treated with 5.6 mmol/L glucose or 30 mmol/L glucose with or without different concentration of simvastatin. The cell proliferation was determined by tetrazolium salt method. In the *in vivo* study, the S-D rats were randomly assigned into normal control group (N), diabetic control group (D) and simvastatin treated group (S). Eight weeks after the onset of hyperglycemia and treatment of simvastatin, the ratio of whole kidney to the body weight and the structural damage of kidney were observed, the urinary transforming growth factor-β (TGF-β) excretion and the 24-h urinary protein were measured by ELISA and the expression of the connective tissue growth factor (CTGF) of the renal cortex was also determined by semi-quantitative RT-PCR. All the data was analyzed by one way-ANOVA. **Results:** (1) The over proliferation of glomerula mesangial cells induced by high glucose was suppressed by simvastain as a dose dependent manner. (2) Simvastatin prevented the renal injury of the diabetic rats, decreased the urine TGF-β and total protein excretion rate, inhibited the cortex CTGF over expression and the enlargement of the kidney. **Conclusion:** Simvastatin prevents diabetic nephropathy by inhibiting over proliferation of mesangial cells and over expression of CTGF and TGF-β.

P239 The Effect of Resveratrol on Expression of NF-kB and MCP-1 on Renal Cortex in Experimental Diabetic Rats

Xu-lei TANG, Song-bo FU
Department of Endocrinology, The First Hospital of Lanzhou University, Lanzhou 730000, China

Objective: To investigate the effect of resveratrol (Res) on expression of NF-kB and MCP-1 on renal cortex and renal function in experimental diabetic rats. **Methods:** Diabetic model of rats was induced by streptozocin. Three groups of male Wistar rats were allotted: normal control (NC) group, diabetic control (DC) group and Res (10 $mg \cdot kg^{-1} \cdot d^{-1}$) treated group. All rats were sacrificed at the end of 10th week after models were established. (1) The serum urea nitrogen (BUN), creatinine (Cr), amount of 24 hours urine protein and renal index were measured. (2) Mean glomerular volume (MGV) and glumerular basement membrane thickness (GBMT) were observed and analysed using light microcopy and electron microscopy. (3) The level of expression of nuclear factor-κB (NF-κB) and monocytechenoattractant protein-1 (MCP-1) in renal cortex was detected using immunohistochemical methods. **Results:** The blood glucoses in NC group, DC group and Res group were respectively 4.80±1.14, 25.90±3.49 and 25.36±2.97 mmol/L. The urine protein (57.5±11.8 $mg \cdot d^{-1}$) and renal index (8.07±1.22) in DC group were significantly higher than that in NC group (13.5±3.9 $mg \cdot d^{-1}$, 3.97±0.24, $p<0.01$). Both BUN (9.7±2.9 mmol/L) and Cr (60.8±9.5 μmol/L) in DC group were remarkably higher than that in NC (6.0±1.7 mmol/L, 24.0±9.1 μmol/L, $p<0.01$); MGV ($11.7\pm2.4\times10^5$ μm^3) and GBMT (226.4±30.1 nm) in DC group were obviously increased than that in NC (5.1±1.8, 124.1±7.8, $p<0.01$). The levels of expression of NF-κB (0.162±0.034) and MCP-1 (0.730±0.145) on renal cortex were distinctly increased in DC group than that in NC (0.073±0.019 and 0.206± 0.071, $p<0.01$) group. The urine protein (20.8±8.4 $mg \cdot d^{-1}$), renal index (6.34±0.34), BUN (7.2±2.3 mmol/L), Cr (48.8±9.7 μmol/L), MGV ($7.4\pm2.2\times10^5$ μm^3) and GBMT (147.6±12.7 nm) were all remarkably reduced in Res group than those in NC group ($p<0.01$). The levels of expression of NF-κB (0.079±0.020) and MCP-1 (0.268±0.082) on renal cortex in Res group were significantly decreased than those in NC group ($p<0.01$). **Conclusion:** Resveratrol can effectively inhibit the expression of NF-κB and MCP-1 on renal cortex and improve renal function, and reduce urine protein in diabetic rats. It also can alleviate renal glomerulus hypertrophy and glumerular basement membrane thickness. Resveratrol may protect diabetic rats from nephritic damage.

P240 A Study of TGF-β1 and CTGF in the Pathogenesis of Diabetic Nephropathy and the Therapeutic Effect of MMP in Rats

Ji-xiang DONG, Zhi-hua LIU, Lu DING, Ying XIE, Ji HU, Zhi-min MA
The Second Hospital of Lanzhou University, Lanzhou 215004, China

Objective: To investigate the effect of MMP on the expression of transforming growth factor-β1 and connective tissue growth factor in the kidney of diabetic nephropathy rat. **Methods:** SD rats were randomly made to be normal controls (group A), diabetic nephropathy controls (group B) and MMF treatment group (group C). Rats of group C were given MMF 10 mg/kg/d while group B were given the same amount of NS. Observe the change of blood glucose, UAER. Sacrificed the rats after 2 mo, and observed the change of histology and expression of TGF-β1 and CTGF. Count the amount of tinct cells in glomerular and tubulointerstitium. **Results:** Blood glucose, kidney weight/body weight (KW/BW) and 24 hours urinary protein increased significantly in DN model group and MMF treatment group, compared with control group. By immunohistochemistry increased levels of TGF-β1 both in glomerular and tubulointerstitium were observed in groups B and C compared with in group A. There is a significant decrease of MMF treatment group compared with the DN group. Increased levels of CTGF both in glomerular and tubulointerstitium were shown in groups B and C compared with in group A. There is a significant decrease of MMF treatment group after 8 weeks compared with the DN group. Levels of the factors expression had positive correlations with UAER and KW/BW. Within the two factors there were also positive correlations. **Conclusion:** (1) MMF suppress the overproduction of TGF-β1 and CTGF in the kidney of diabetic nephropathy rat. (2) MMF can prevent the progression of diabetic nephropathy, reduce KW/BW and 24 hours urinary protein count, perhaps partly by suppressing the overproduction of TGF-β1 and CTGF.

P242 Change of Interstitial Cells of Cajal and Gap Junction Protein43 in the Stomach of Diabetic Rats

Han-ni WU, Xi-ting ZHANG, Han TIAN
Department of Endocrinology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China

Objective: To explore the pathogenesis of diabetic gastroparesis by examining and comparing the distribution of interstitial cells of cajal (ICC) and gap junction protein43 (connexin43, Cx43) in the stomach of normal and Streptozotocin (STZ)-induced diabetic rats by immunohistochemical study. **Methods:** 20 male Wistar rats were randomly divided into normal control group and diabetic group. The latter group was induced by STZ via celiac injection (50 mg/kg). Diabetes was defined as a blood glucose concentration of 16.7 mmol/L or high. All rats were bred 8 weeks with free access to water and food. At the end of the experiment, the phenol red solution was given orally into the stomach. After 30 minutes, the animal was decapitated, and the stomachs were removed after clamping the esophagus at the cardia and the pylorus. Gastric emptying rate was evaluated by Phenol Red test. The gastroparesis models of diabetic rats were defined by the delay of gastric liquid emptying. Immunohistochemical study was used to examine and compare the distribution of ICC and Cx43 in the stomach of normal and diabetic rats. **Results:** Compared with the control rats: (1) gastric emptying in the diabetic rats was significantly delayed (52.00±5.40 vs 74.38±3.62, $P<0.01$); (2) Immunohistochemical staining showed that ICC were densely distributed in the circular and longitudinal muscle layers of both corpus and antrum in the control rats, but greatly reduced in the stomach of diabetic group ($P<0.01$); (3) Strong Cx43 immunoreactivity was detected thoughtout the circular muscle layers, was densely homogeneous as c-kit immunopositivity in the corpus and antrum of the control rats. In the diabetic group, expression of Cx43 was obviously weak ($P<0.01$). **Conclusion:** The quantity of ICC and expressions of Cx43 are reduced. The loss of ICC and impaired intercellular communication between ICCs and smooth muscle cells may play a key role in the pathogenesis of diabetic gastroparesis.

P243 The Study on the Expression of Insulin-like Growth Factor-1 in Taste Bud Cell of Diabetic Rat

Li-hong ZHOU, Xiao-min LIU, Xiao-hong FENG
Department of Endocrinology, The First Affiliated Hospital of Haerbin Medical University, Haerbin 15000l, China

Objective: To observe the expression of the of insulin-like growth factor-1 (IGF-1) in rat taste bud cell, as well as the expression difference in taste bud cell between diabetes and control rat. **Methods:** Twenty male Wistar rats were randomly divided into the control group (10), and diabetes group (10), diabetes group were fed with high fat and calorie for 4 weeks, and then intraperitoneally injected with a low dose of STZ (25 mg/kg), after 4 weeks, rats were killed and excision of circumvallate papillae, excised papillae were immersion fixed in 4% paraformaldehyde, paraffin imbedding, the expression of IGF-1 in rats taste bud cell were examined with immunohistochemistry (S-P). **Results:** The expression of IGF-1 in rat taste bud cell was found, compared with the normal rat, the expression of VIP in diabetic rat taste bud cell is stronger than that in normal controls. **Conclusion:** IGF-1 belongs to insulin family, it can be synthesized and secreted with paracrine and/or autocrine in a variety of tissues. It can promote growth and differentiation, as well as participate in glucose and protein and fat metabolism, and have neuroprotective effect. IGF-1 may be correlated to gustatory signal communication among the cells of the taste bud and transmission to central nervous system. The significance of expression in taste bud and expression up-regulation in diabetes should be further investigated.

P244 Expression of Vasoactive Intestinal Peptide in Taste Bud Cell of Diabetic Rat

Xiao-hong FENG, Xiao-min LIU, Li-hong ZHOU
Department of Endocrinology, The First Affiliated Hospital of Haerbin Medical University, Haerbin 150001, China

Objective: To observe the expression of the vasoactive intestinal peptide (VIP) in rat taste bud cell, as well as the expression difference in taste bud cell between diabetes and control rat. **Methods:** Twenty male Wistar rats were randomly divided into the control group (10), and diabetes group (10), diabetes group were fed with high fat and calorie for 4 weeks, and then intraperitoneally injected with a low dose of STZ (25 mg/kg), after 4 weeks, rats were killed and excision of circumvallate papillae and foliate papillae, excised papillae were immersion fixed in 4% paraformaldehyde, the expression of vasoactive intestinal peptide in rat taste bud cell were examined with immunohistochemistry (S-P). **Results:** VIP is positive expression in rat taste bud cell, compared with the normal rat, the positive rate of VIP in diabetic rat taste bud cell is higher than that in normal controls. **Conclusion:** The expression of VIP in taste bud suggested that neuropeptide could play previously unrecognized functional roles in the peripheral processing of taste information. It may be participated in cell to cell communication among the cells of the taste bud, as signaling agents that likely act in concert with transduction mechanisms. The possible reason of VIP positive expression up-regulation is a compensation response.

P245 Specificity and Sensitivity of Clinical Examinations to Diagnose Diabetic Neuropathy

Qun YUAN, Shi-bai WU, Da ZHANG
Department of Endocrinology, General Air Force Hospital of P.L.A., Beijing 100036, China

Objective: (1) To discuss the correlation factors to develop diabetic neuropathy. (2) To study the association of clinical examinations between diabetic peripheral and autonomic neuropathy. (3) To identify the specificity and sensitivity of clinical examinations to diagnose diabetic neuropathy. **Methods:** 469 patients suffered from type 2 DM were involved. The clinical examinations included atherogenic index/motor nerve conduction velocity and amplitude of vibration/sensory nerves conduction velocity and F wave/quantity of vibration sensation/rising test/variance of heart rate after deep breath/Valsalva test/Baroreflex Sensitivity of vascular pressure receptor. **Results:** (1) The factors to develop diabetic peripheral neuropathy correlate successively with age, course, BMI, fasting blood glucose, HbAlc, history of diabetic retinopathy and macroangiopathy. (2) The factors to develop diabetic autonomic neuropathy correlate successively with age, gender, course, history of diabetic retinopathy. (3) There is no correlation between severity of peripheral neuropathy and autonomic neuropathy. (4) Clinical examinations with high specificity and sensitivity for diabetic peripheral neuropathy are: MCV and SCV of median nerve, SCV of peroneal nerve. (5) Rising test, Baroreflex Sensitivity of vascular pressure receptor and variance of heart rate after deep breath are specific and sensitive for diabetic autonomic neuropathy among all the clinical examinations. **Conclusion:** (1) There is no causal relation between peripheral and autonomic neuropathy. Autonomic neuropathy does not occur secondary to peripheral neuropathy. They are independent or parallel. (2) Among all the clinical examinations, the tests with high specificity and sensitivity for peripheral neuropathy are: MCV and SCV of median nerve, SCV of peroneal nerve; for autonomic neuropathy are: rising test, Baroreflex Sensitivity of vascular pressure receptor and variance of heart rate after deep breath. (3) The clinical examinations to test peripheral neuropathy or autonomic neuropathy cannot be replaced by another ones.

P246 Study on Relation Dyslipidemia to Diabetic Macular

Ling PAN, Zheng-yan SHENG, Xun XU, Jian-feng ZHU
Osteoporotic Department, Shanghai First People Hospital Affiliated to Shanghai Jiaotong University, 200080, China

Objective: Diabetic macular edema (DME), which involves retinal thickening in the macular area, is the leading cause of blindness and vision lost. Impairment in visual acuity of diabetic patients with macular edema can undeniably be decreased with systemic and ocular therapeutic intervention at early stages, as shown by numerous controlled studies. We investigated dyslipidemia to access the control of diabetes-associated metabolic abnormalities which may slow the progression of DME. Our study may assist the physician and ophthalmologist in providing patients with the optimal diabetic care available. **Methods:** The community based study was done in Beixinjing District, Shanghai. 128 patients at study entry, from a representative data underwent an epidemiologic study of a routine ocular examination between 2003-2004, were chosen to take further interview and laboratory investigations. We evaluated macular thickness among individuals by Optical Coherence Tomography (OCT), known as an effective technique, extremely sensitive for detecting early DME. **Results:** (1) In 128 DM patients 248 eyes, the median macular thickness of foveal was 164 μm (53.50 μm). (2) Multiple factors analysis on progression of DME: the macular thickness has significant correlation with age, duration, TG, LP(a), HDL, TC. (3) In model controlling for age, sex, plasma glucose and DR, the macular thickness was related with LP(a), TG, HDL. (4) LP(a), TG and duration entered multiple stepwise regression and discriminant analysis of DME. **Conclusions:** Dyslipidemia were confirmed as an important risk factor associated with DME progression. Significant independent risk factors of DME were LP(a), triglyceride, longer duration, which could predict development and progression of DME. With elevation of LP(a) and TG, the incidence of DME increased, and the severity aggravated. A renewed emphasis on preventing early DME is the effective treatments of dyslipidemia, which may offer the potential for further reduction of visual loss caused by DME.

P247 Risk Evaluation of Erectile Dysfunction Using Structured Questionnaire in Chinese Type 2 Diabetic Men

L.W.L. YU, A.P.S. KONG, Claudia TAM, P.C.Y. TONG, W.Y. SO, Norman N. CHAN, C.S. HO, C.W.K. LAM, W.B. CHAN, C.C. CHOW, C.S. COCKRAM, J.C.N. CHAN
Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, N.T., Hong Kong SAR

Objective: To explore the frequency and severity of erectile dysfunction (ED) in Chinese type 2 diabetic men using a structured questionnaire, and the associations between ED and diabetes related complications and metabolic indices. **Methods:** A consecutive cohort of 114 Chinese type 2 diabetic men aged between 25 and 76 years old attending a diabetic centre were recruited between July and September 2003. Detailed assessments, using the European DiabCare protocol and the 5-item version of the International Index of Erectile Function (IIEF-5) questionnaire, were performed. **Results:** Of the study population, the frequency of ED was 49.0% according to the National Institute of Health (NIH) Consensus Conference criteria, compared to 86.8% (40.0% having severe ED) as diagnosed by IIEF-5. ED as defined by NIH criterion was associated with age, duration of diabetes, glycosylated haemoglobin (HbA_{1c}), serum creatinine, estimated glomerular filtration rate, urine albumin-creatinine ratio (ACR), retinopathy, microvascular complications, insulin therapy and use of anti-hypertensive drugs (p=0.003, 0.001, 0.009, 0.007, 0.007, 0.013, 0.008, 0.002, 0.036 and 0.033 respectively). Using the IIEF-5 criteria, severe ED was also significantly associated with these factors together with low education level (p=0.02), hypertension (p=0.044), chronic kidney disease (p=0.002) and macrovascular complications (p=0.014). After adjusted for confounding factors by logistic regression, ED defined by NIH criterion was associated with urine ACR only (OR=1.98 [95% CI 0.98-4.00], p=0.026), whereas severe ED defined by IIEF-5 was associated with age, HbA_{1c} and urine ACR, compared to those with no or mild to moderate ED (OR=1.81 [95% CI 1.25-2.60], p=0.002; 18.03 [1.74-187.02], p=0.016; 2.50 [1.04-6.01], p=0.041 respectively). **Conclusions:** ED is highly prevalent in Chinese type 2 diabetic men and is associated with multiple risk factors and complications. Age, glycaemic control and albuminuria are predictors of severe ED. Structured questionnaire is a useful and objective tool to detect early ED which should prompt comprehensive risk assessment in these subjects.

P250 Age-related Differences in Plantar Pressure Parameters and Its Distributions in Normal Chinese

Si-liang ZHANG, Li YAN
Department of Endocrinology, The 2nd Affiliated Hospital of Sun Yat-sen University, GuangZhou 510080, China

Objective: A number of studies were performed to investigate the relationship between plantar foot pressures, neuropathy and foot ulceration in foreign country. People came to know that abnormal plantar pressure is one of the most important factors for diabetic foot ulceration. This study tried to find out normal plantar pressure parameters and its distribution in Chinese population of different age. **Methods:** There were 952 non-diabetes healthy adult volunteers participated in this study. Their gait patterns are normal and their feet are with no callosities, no physical deformity, no pain, no surgical history and intermittent claudication or symptoms and peripheral neuropathy. They were divided into five groups according to their age, and were barefoot during examination and their feet were scanned using Novel's EMED-AT force-plate gait analysis system (Germany). 'First-step' method was used. The whole foot was divided into four "mask" in gross, including the heel, midfoot, forefoot, total toes, and divided into ten "mask" in detail. Peak pressure, maximum force, contact area, contact time, force-time integrals and pressure-time integrals were measured. **Results:** We find that age was less related with MPP. However, the distribution of peak pressure in different age groups are slightly different and level of peak pressure at forefoot and heel decreased, while the level of the same parameters at midfoot and toes increased as age increased.

P251 The Influencing Factors of Plantar Pressure in Normal Chinese

Si-liang ZHANG, Li YAN
Department of Endocrinology, The 2nd Affiliated Hospital of Sun Yat-sen University, Guangzhou 510080, China

Objective: A number of studies were performed to investigate the relationship between plantar foot pressures, neuropathy and foot ulceration in foreign countries. Our study tried to find out the influencing factors of its parameters among normal Chinese. **Methods:** There were 952 non-diabetes healthy adult volunteers participated in this study who present with no callus. They were divided into two gender groups, three BMI groups, and four shoes-worn groups. We also detected another 70 volunteers who presented with callus. All volunteers were barefoot during examination and their feet were scanned using Novel's EMED-AT force-plate gait analysis system (Germany). "First-step" method was used. Peak pressure, maximum force, contact area, contact time, force-time integrals and pressure-time integrals were measured. **Results:** We find that gender affects MPP with that of female higher than that of male. Their pressure loading patterns were also different. BMI were less related with MPP, however, increased the level of peak pressure at lateral outer forefoot, midfoot, toes were found. Plantar callus would increase plantar pressure. **Conclusions:** Level of barefoot plantar pressure parameters in subjects of different shoes worn was of no diversity in our study.

P252 Related Factors in Diabetes Mellitus Patients with Diabetic History Over Ten Years Happen Diabetes Foot Gangrene

Ao TONG, Hong TANG, Na ZHAO, Hong-xia ZHANG, Xiao-yan MA, Qun YUAN, Shu-hua DONG, Xiao-qing SUN
Department of Endocrinology, General Air Force Hospital of P.L.A., Beijing 100036, China

Objective: It is a dangerous factor in diabetes patients with diabetic history for over ten years, as it may be easier to result in diabetic foot gangrene. But not all of them will get this. To discuss the relativity between the occurrence of diabetic foot gangrene and the patients' self-management, making them be used to conforming doctor's advices from the first, and reducing the diabetic foot gangrene. **Methods:** We used the form of diabetics' daily self-management ability which was designed by ourselves, and chose patients with diabetic history for over ten years, having or not having had gangrene apiece 50, investigated by person special. It contains diet control, exercise habit, insisting on taking drug, blood sugar control, actively learning about diabetic knowledge, foot health, blood sugar inspection, the level of blood glucose control, the situation about abstaining from tobacco. Each aspect owns three choices: good, middle and bad. Then analysed the result. **Results:** (1) The Group of not having diabetes foot gangrene (Non-DFG) behaves better than the diabetes foot gangrene (DFG) group at large, especially in the following aspects: insisting on drug taking (Non-DFG occupies 76%, DFG Group occupies 14%), blood sugar inspection (Non-DFG Group takes up 38%, DFG Group takes up 0%), actively diet control (Non-DFG Group accounts for 42%. the DFG Group accounts for 4%), well control of blood sugar (Non-DFG Group accounts for 70%, DFG Group accounts for 34%). (2) Patients who get high education always appear better in the aspect of actively learning about diabetic knowledge. But the number of this part is still account for small. Some of the patients do not know the serious consequences of DFG actually, and some of them never heard of DFG. **Conclusion:** (1) The recognizant extent on the diabetes mellitus and the subsequent symptom which diabetes patients know about is not tally with the trend of diabetes come on. Therefore, it is necessary to give them an extensive and constant education to improve the consciousness and self-prevention capability. (2) It is necessary for doctors and nurses to guide patients to enhance self-management ability and compliance.

P253 Recombinant Human Acidic Fibroblast Growth Factor Accelerates the Healing of Dermal Chronic Ulcers in Diabetic Rats

Qing LIU, Wei-ying GUO, Ke-hui LIU, Xiao-na XIE
Department of Endocrinology, The First Hospital, Jilin University, Changchun 130021, China

Objective: Diabetic patients' skin present wounds healing handicaps, even occur ischemic ulcer and neurotrophic ulcer. Acidic fibroblast growth factor (aFGF) is a drastic mitogen, which prominently promote wound healing. In the previous study, we successfully overexpressed human recombinant aFGF (rhaFGF) in *Escherichia coli*. We topically applied rhaFGF on dermal chronic ulcers in diabetic rats to study the healing effect of rhaFGF. **Methods:** We prepared diabetic models with 10 male Wistar rats by intraperitoneal injection with tetraoxypyrimidine, then created dermal chronic ulcers area of 2.54 cm by full-thickness ablation on both sides of the rats' backs. The wounds were sprinkled with rhaFGF (90 U/cm^2) and physiological saline, respectively. We recorded wound area by raw surface photograp and fenestrate membrane tracings, calculated wound healing ratio, measured wound cavity volume by affusion, and observed wound healing time. We also observed granulation tissue growth and epithelization in wound edge tissue, and evaluated wound healing status. **Results:** Compared with control group, the wound area and wound cavity volume at different time of rhaFGF group were significantly diminuted (p<0.05), the healing ratio was predominantly elevated (p<0.05). The healing time of rhaFGF group was significantly shortened (p<0.05), 5.4 days shorter in average. RhaFGF treatment resulted in a remarkable enhancement of granulation tissue growth and epithelization in wound. **Conclusion:** It suggested that rhaFGF topical application can significantly accelerate healing of dermal chronic ulcers in diabetic rats. The results provide potent evidence for clinical treatment diabetic dermal chronic ulcers by topical application rhaFGF, including ischemic ulcer and neurotrophic ulcer.

P254 Dynamic Changes of MMP-9 and TIMP-1 During Wound Healing of Diabetic Rats

Ping ZHU, Li YAN, Chuan YANG, Guo-juan LAO
Department of Endocrinology, The 2nd Affiliated Hospital of Sun Yat-sen University, Guangzhou 510080, China

Objective: To establish the model of diabetic wounding rats and the criteria of histological scores of wound healing. We investigated the dynamic changes of MMP-9, TIMP-1 and the ratio of MMP-9/TIMP-1 in the wounds of diabetic and non-diabetic rats. **Methods:** Diabetic rats were induced with streptozotocin. All rats were maintained for 6 weeks. A full-thickness excisional wound was created. The parameters measured were epidermal and dermal regeneration, granulation tissue thickness, angiogenesis, matrix density, and infiltrated cells with Haematoxylin-eosin, Masson's trichrome staining and immunohistochemistry. **Results:** The wound healing rate of normal rats was faster than diabetic rats. Six weeks after STZ treatment, the epidermis and dermis layer were thinner in diabetic rats than those in control rats. At 3rd day, there were a lot of fibroblast and macrophage in normal skin, while few such cells are observed in diabetic skin. The other histological scores in normal skin were higher than those in diabetic rats at 7th and 14th day. The relative values of expression of MMP-9 mRNA and protein in diabetic group were higher than those in normal group in different time points. However, the values of TIMP-1 mRNA and protein in diabetic group were significantly lower than those in control group. Significant difference was seen between two groups with the ratio of MMP-9/TIMP-1 in diabetic group higher than those in normal group. **Conclusions:** The changes of the cutaneous histology, pathophysiology and levels of MMP-9/TIMP-l in diabetic rats exist before skin wound, which may give rise to ulceration in diabetic skin. The balance of between MMP-9 and its inhibitors is abnormally in diabetic skin tissue after injury, which seems to support a delayed wound healing and lead to a failure of wound healing.

P255 Bone Mineral Content Measurement in 255 Old Patients with Diabetes Mellitus

Wan-chun CAI, Fan ZHANG, Xiao-wei LI
Dept. of Endocrinology, Fifth Hospital of PLA, Yimchuan 750004, China

Objective: To study the incidence of osteoporosis and influential factors of bone mineral content (BMC) in old diabetic patients. **Methods:** BMC was measured in 255 old diabetic patients, using model SD-1000 single photoabsorptiometer. **Results:** (1) BMC was significantly higher in males than in females and decreased with increasing ages in males and females. (2) BMC decreased with lengthening medical history. (3) In male diabetic patients BMC correlated positively with the body mass indices (BMI), but there was no significant difference between the group with BMI 20-25 kg/m^2 and the group with BMI over 25 kg/m^2 in female patients. (4) The incidence of osteoporosis in this group was 38.04% (32.45% for male : 46.15% for female). **Conclusion:** Combined osteoporosis is a common chronic complication in old patients with diabetes mellitus.

P256 Clinical Use of Quantitative Ultrasound in Type 2 Diabetes Postmenopausal Women

Bei TAO, Jian-min LIU, Hong-yan ZHAO, Li-hao SUN, Xiao-ying LI, Wei-qing WANG, Guang NING
Department of Endocrine and Metabolic Diseases, Shanghai Rui-jin Hospital, affiliated to Jiaotong University, School of Medicine, 197 Ruijin Er Road, Shanghai 200025, China; Shanghai Clinical Center for Endocrine and Metabolism Diseases; Shanghai Institute of Endocrinology and Metabolism; Endocrine and Metabolic Division, E-Institutes of Shanghai Universities (EISU)

Objective: This cross-sectional study aims to analyze the change of BMD by dual-energy X-ray absorptiometry (DXA), and SOS by quantitative ultrasound (QUS) to consider factors that might account for low bone density in type 2 diabetes. **Methods:** Seventy-six postmenopausal women with type 2 diabetes and eighty-six healthy postmenopausal women participated. SOS at the radius, the phalanx and the tibia were assessed by using the Omnisense prototype (Sunlight Ltd. Israel) in all subjects. We measured BMD of L_{2-4}, femoral neck and total hip by DXA, as well as the HbA1c, serum level of osteocalcin (OC), bone-specific alkaline phosphatase (BAP), and the urinary concentration of the N-terminal telopeptides of type I collagen (NTX/Cr) in type 2 diabetes women. DXA was also performed in the 86 healthy postmenopausal women. **Results:** In multiple regression analysis, the course of diabetes and the serum osteocalcin showed relationships between the radial SOS (β= –0.365, β=0.410, P<0.05, R^2=0.305). And the course of diabetes also showed negative relationship with the phalangeal SOS (β= –0.404, P<0.05, R^2=0.325). BMD evaluation resulted in a higher values that measured by DXA at L_{2-4}, femoral neck and total hip (1.029±0.188 vs 0.896±0.231 g/cm^2, 0.798±0.131 vs 0.744±0.118 g/cm^2, 0.868±0.139 vs 0.802±0.133 g/cm^2, P<0.05), while multi-site SOS values by QUS were lower in type 2 diabetes postmenopausal women than that of healthy postmenopausal women (4050±178 vs 4129±182 m/s, 3908±207 vs 3999±214 m/s, P<0.05), presented with the declined relationships between the L_{2-4}, femoral neck and total hip BMD and the phalangeal SOS (r=0.51, r=0.75, r=0.61 and r=0.41, r=0.50, r=0.46 in diabetes women and healthy women, respectively, differences with statistical significance, P<0.05). Even with the diminished relationship between BMD and the radial, tibial SOS. **Conclusion:** The non-paralleled change of the BMD by QUS and DXA in diabetic subjects suggested that QUS might be superior to DXA in screening low bone density in type 2 diabetes postmenopausal women, and to carrying out the primary prevention of diabetic osteoporosis and osteoporotic fracture.

P257 An Assessment of the Use of Quantitative Ultrasound and the Osteoporosis Self-assessment Tool for Asians in Determining the Risk of Non-vertebral Fracture in Postmenopausal Chinese Women

Bei TAO[1], Jian-min LIU[1], Xiao-ying LI[1], Ji-guang WANG[2], Wei-qing WANG[1], Guang NING[1]
[1] Department of Endocrine and Metabolic Diseases, Shanghai Rui-jin Hospital, affiliated to Jiaotong University, School of Medicine, 197 Ruijin Er Road, Shanghai 200025, China
[1] Shanghai Clinical Center for Endocrine and Metabolism Diseases
[1] Shanghai Institute of Endocrinology and Metabolism
[1] Endocrine and Metabolic Division, E-Institutes of Shanghai Universities (EISU)
[2] Shanghai Insitute of Hypertension

Objective: This cross-sectional study aims to assess the effectiveness of a simple, noninvasive scoring system, the Osteoporosis Self-assessment Tool for Asians (OSTA), and quantitative bone ultrasound (QUS) in assessing non-vertebral fracture risk in Chinese postmenopausal women. **Methods:** A group of 815 community-dwelling individuals participated in this study, among which 513 females were included in the analysis. Speed of sound (SOS m/s) at the radius, phalanx and tibia were assessed by using the Omnisense prototype (Sunlight Ltd. Israel). Body height and weight were measured, and body mass index (BMI) and OSTA indices were calculated. Self-reported fractures were identified using a structured questionnaire. **Results:** SOS at the radius was significantly lower among women with non-vertebral fracture than those without (3995 m/s vs 4053 m/s, P=0.044). SOS at the phalanx was significantly lower among women with a history of postmenopausal non-vertebral fracture than those without (3755 m/s vs 3841 m/s, P=0.003). SOS at the radius, phalanx and tibia showed a positive correlation with OSTA index (r=0.376-0.401, P<0.001). The prevalence of non-vertebral fractures also increased significantly with the decreasing order of OSTA index (χ^2=6.370, P=0.041). The OSTA values of ≤-1 and phalanx QUS T score of -1.95 can differentiate postmenopausal non-vertebral fracture with sensitivity 75% and 81%, respectively, and specificity 48% and 40%, respectively. Combining OSTA and phalanx QUS yielded a sensitivity of 83% and a specificity of 84% to detect postmenopausal non-vertebral fracture. **Conclusion:** We conclude that OSTA and phalanx QUS are simple and effective clinical tools for identifying postmenopausal women at increased risk of non-vertebral fractures, and can thereby facilitate the appropriate and more cost-effective use of bone densitometry to prevent osteoporotic fractures in developing countries.

P258 Relation of Serum Folate to Bone Mineral Density of Postmenopausal Women

Zheng-yan SHENG[1], Li YOU[1], Li-meng ZHANG[2], Jin-yu CHEN[1]
[1] Osteoporotic Department, Affiliated First People Hospital, Shanghai Jiaotong University, Shanghai 200080, China
[2] Nuclear Medicine Department, Affiliated First People Hospital, Shanghai Jiaotong University, Shanghai 200080, China

Objectives: To study whether in postmenopausal women levels of serum folate are related to bone mineral density (BMD). **Methods:** (1) Experimental subjects: 93 volunteer women who had been postmenopausal for at least 12 months and were recruited from those (487 women) of our first osteoporotic outpatients (age, 65.62±11.49) between August 2005 and December 2005. (2) Groups: osteoporosis (n=29), osteopenia (n=50) and normal (n=l3). (3) Serum sample biochemical assays: (a) serum folate levels were measured using chemiluminescence, (b) serum BGP and serum PTH levels were measured by chemiluminescence, (c) serum ALP, serum Ca and serum P were measured by automated biochemistry techniques. (4) BMD was measured using dual energy X-ray analysis at the lumbar spine (L1-14) and at the left hip (total hip, trochanter, Ward's area and femoral neck). (5) Statistical analyses: BMD values, biochemical parameters were expressed as mean±SD. Multiple regression analysis was used to assess the relationship between BMD values and serum folate levels. Comparisons between two groups were made with t-tests. All statistical analyses were performed by SPSS11.5 system. **Results:** (1) Osteoporotic women had lower values of serum folate (osteoporosis group [11.27±6.04 pg/ml], osteopenia group [13.18±6.14 pg/ml] and normal group [11.9±3.73 pg/ml]), no statistical significance. (2) Low folate levels and low BMD value at each of the hip sites and for total hip are no correlation (total hip r=0.005, P>0.05, trochanter r= –0.021, P>0.05, shaft of femur r=0.017, P>0.05 and femoral neck r=0.021, P>0.05). (3) Low folate levels and low BMD value at lumbar spine (L1-L4) have no correlation (r=0.078, P>0.05). **Conclusions:** Serum folate levels are not an important risk factor for osteoporosis.

P259 Relationships between Insulin-like Growth Factor-1 (IGF-1) and OPG, RANKL, Bone Mineral Density in Healthy Women

Hong-yan ZHAO, Jian-min LIU, Guang NING, Yong-ju ZHAO, Yin CHEN, Li-hao SUN, Lian-zhen ZHANG, Man-yin XU, Jia-lun CHEN
Department of Endocrine and Metabolic Diseases, Rui-jin Hospital, Shanghai Jiaotong University Medical School; Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai 200025, China

Objective: Insulin-like growth factor-1 (IGF-1) is a ubiquitous polypeptide that stimulates osteoblast activity, subsequently leading to bone matrix formation and inhibition of bone collagen degradation. IGF-1 also stimulates osteoclast formation and action, although these effects are less clear. So, the purpose of this study was to investigate the relationships between IGF-I and OPG, RANKL, and bone mineral density in healthy women. **Methods:** Lumbar spine and femoral neck BMDs in 504 healthy women (282 pre- and 222 postmenopausal) were measured by dual energy X-ray absorptiometry (DEXA). Serum levels of IGF-I were measured by RIA. Serum levels of OPG and RANKL were measured by ELISA. **Results:** Age was negatively correlated with serum levels of IGF-1 in healthy women (r= –0.702, p<0.01). On the other hand, IGF-I was negatively correlated with OPG, OPG/RANKL ratio, whereas positively correlated with RANKL. No association was found between IGF-1 and BMDs after adjusted for age. In postmenpausal women, IGF-I was lower in osteoporotic women than those in normal women (p=0.056, though no significantly so), but no differences were found among OPG, RANKL and OPG/RANKL ratio. Serum levels of OPG in the highest quintile of IGF-1 were significantly lower than those in the lowest quintile of IGF-1, while no difference was found in RANKL. In a multiple regression analysis model, menopause and serum levels of IGF-1 were the main determinants of the bone mass in healthy women. **Conclusion:** The relationship between age-related decreasing IGF-I and BMDs in healthy women was under the influence of age, whereas the effect of IGF-I on bone remodeling (bone resorption) may be mediated by OPG-RANKL system.

P260 Serum Containing Experiential Recipe-GSB Enhances Proliferation and Differentiation of Rat Marrow Mysenchymal Stem Cells In Vitro

De-wen YAN, Hai-yan LI, Lin FU, Qing-ping WU, Guo-chun LUO, Yao-ting GUI, Jian-de XIAO
Department of Endocrinology, Shenzhen Second People Hospital, Guangdong, Shenzhen, China

Objectives: To investigate the effect of serum containing GSB-extract (GSB serum) on proliferation and differentiation of rat mesenchymal stem cells (rMSCs) and study the tonifying kidney and benefiting essence of life by the traditional Chinese medicine. **Methods:** rMSCs were incubated in culture medium with different concentrations of GSB serum (5%, 10% and 20%). Cell proliferation was detected with MTT method to confirm appropriate serum concentration. The mRNA level of osteopontin, PPARγ2 and type II collage (col2α1) were assessed by RT-PCR and β-actin expression was as control. **Results:** Proliferation curve showed as S shape in groups with different concentrations of GSB serum, like as in control group. Absorbency was increased with GSB serum in a dose-dependent manner. The mRNA expression of osteopontin was detected, and the expression of PPARγ2 and col2α1 was not measured in GSB serum group, while all expressions were not observed in controls when cells were incubated for 14 days. **Conclusions:** GSB serum may promote proliferation and osteogenic differentiation of rMSCs. Experiential recipe-GSB possesses to manufacture the essence.

P261 The Effects of Puerarin and Resveratrol on Prevention of Osteoporosis and Regulation of Serum Lipids Metabolism in Ovariectomized Rats

Quan TIAN, Xu-lei TANG, Yan WANG, Meng-hai BAI
Department of Endocrinology, The Fist Hospital of Lanzhou University, Lanzhou 730000, China

Objective: To investigate the effects of puerarin and resveratrol on prevention of osteoporosis and regulation of serum lipids metabolism in ovariectomized rats. **Methods:** A total of 96 female SD rats were divided randomly into sham operated (SHAM), ovariectomized (OVX), 17β-estradiol treated, low-dose puerarin, middle-dose puerarin, high-dose pueraringroup, low-dose resveratrol middle-dose resveratrol and high-dose resveratrol group. Treatments started from one week after ovariectomized and lasted 7 weeks. The rats were sacrificed at the end of 8th week after operated. BMD was measured by dual-energy X-ray absorptiometry, and biomechanical were performed by bending and compressive test. **Results:** (1) The BMD in OVX group was much lower than that in SHAM group ($P<0.01$). The femur BMD in O+Ph group increased by 7% ($P<0.05$). (2) In O+Pm, O+Ph groups, the ELASTIC, STIFFNESS of lumbar vertebrae and M-STRESS of femur increased when compared with in OVX group ($P<0.05$). (3) In O+Pm and O+Ph groups, the levels of HDL-C increased and LDL-C decreased than in OVX group ($P<0.01$). (4) In O+Rm and O+Rh groups M-STRESS are higher than in OVX group ($P<0.05$). (5) In resveratrol group HDL-C increased than in OVX group ($P<0.01$), TG decreased than in OVX group ($P<0.01$). **Conclusion:** Puerarin and resveratrol can increase bone strength and BMD of OVX rats. They have favorable effects on serum lipids. They may be effective in prevention of osteoporosis and atherosclerosis.

P262 Vitamin D Receptor Suppresses Rat Cholesterol 7α-hydroxylase Expression through Inhibition of LXRα Signaling

Wei JIANG[1], Takahide MIYAMOTO[2], Tomoko KAKIZAWA[2], Takahiro SAKUMA[2], Ako OIWA[2], Kiyoshi HASHIZUME[2]
[1] Department of Endocrinology, Peking University First Hospital, Beijing 100034, China
[2] Department of Aging Medicine and Geriatrics, Shinshu University Graduate School, Matsumoto 390-8621, Japan

Objective: Cholesterol 7α-hydroxylase (CYP7α) is the rate-limiting enzyme in the catabolism of cholesterol to bile acid. Liver X receptor α (LXRα) mediates feed-forward induction of CYP7α and bile acid receptors negatively regulate its expression. Recently, vitamin D receptor (VDR) is known to function as a receptor responsive to the secondary bile acid. In the current study, effect of VDR on the LXR-CYP7α pathway was investigated. **Methods:** A real-time quantitative PCR (SYBR Green) was applied to determine the mRNA expression by using ABI 7000 Sequence Detection system. Cells were grown in DMEM and plasmids DNA were introduced into cells using the FuGENE 6 transfection reagent. **Results:** We demonstrated that 1,25-$(OH)_2$D3 blunted the LXRα-mediated induction of CYP7α mRNA in H4IIE rat hepatoma cells. In co-transfection experiments in HepG2 cells, VDR repressed the activity of rat CYP7α promoter in a ligand-dependent manner through inhibition of the LXRα signaling. We also confirmed the ability of VDR to repress the LXRα transcriptional activation by using a synthetic LXRα responsive reporter. Deletion analyses revealed that the ligand-binding domain of VDR was required for the suppression and the DNA-binding domain was dispensable. **Conclusions:** Given the fact that VDR can be activated by the secondary bile acid as well as 1, 25-$(OH)_2$D3, the cross-talk between LXRα and VDR signaling in regulation of bile acid metabolism might provide a novel pathway for protection against bile acid toxicity, and highlight a physiological function of VDR beyond calcium homeostasis in the body.

P264 A Chinese Family with Familial Paraganglioma Syndrome due to Succinate Dehydrogenase Deficiency

R.C.W. MA, C.W. LAM, W.B. CHAN, W.Y. SO, S.F. TONG, C.C. CHOW, C.S. COCKRAM
Department of Medicine and Therapeutics, Prince of Wales Hospital, Chinese University of Hong Kong, Hong Kong

Objective: To report the genetic characterization of a family with familial paraganglioma syndrome. **Methods:** The index case was diagnosed to have carcinoid tumour of bronchus at the age of 30 and was subsequently diagnosed to have bilateral phaeochromocytoma. The sister of the index case had bilateral carotid body tumours. Mutational analysis of succinate dehydrogenase (SDH)B and SDHD were performed. **Results:** Mutational analysis revealed the subject was heterozygous for the M1I mutation of the SDHD gene. Genetic analysis revealed that the sister also suffers from SDH deficiency with the same mutation. Presymptomatic testing confirmed the genetic diagnosis, and led to the clinical diagnosis in an otherwise asymptomatic sibling. Comparison with other known cases of M1I mutation suggests that this is a founder mutation in the Chinese population. **Conclusions:** Genetic analysis of the SDH genes can provide a specific diagnosis and allow for genetic screening of individuals with or at risk of paragangliomas.

P265 Usefulness of Non-invasive Vascular Measurements for Evaluation of Peripheral Vascular Disease in Asian Type 2 DM Patients

Kin-chap YU, Ka-fai LEE
Department of Medicine, Kwong Wah Hospital, 25 Waterloo Road, Hong Kong SAR, China

Objective: To investigate the prevalence of Peripheral Arterial Disease (PAD) in Asian DM subjects by ankle brachial index (ABI) and toe brachial index (TBI) and to explore the usefulness of brachial ankle pulse wave velocity (BaPWV) as a surrogate marker for DM complications. **Methods:** Cross-sectional study of 253 consecutive type 2 DM patients in a diabetes centre. ABI and BaPWV were measured by a volume-plethymographic apparatus while TBI was measured by a photoplethysmographic-based sensor. **Results:** Prevalence of PAD is 4.4% (ABI<0.9). Additional use of TBI in cases of suspected tunica media calcification (ABI>1.3 and TBI<0.64) did not significantly improve the sensitivity. DM patients with PAD were older, had a longer duration of DM, had a higher SBP and were more likely to have nephropathy. BaPWV was found to correlate with traditional vascular risk factors, DM macrovascular and microvascular complications. **Conclusion:** The prevalence of PAD in our population is low. BaPWV may be recommended as a cardiovascular marker if its usefulness is further confirmed in longitudinal studies.

P266 Structured Care Model in Managing Adult Type 2 Diabetic Patients with Nephropathy

G. KAM, M. NG, G. HUI, C.S. HUNG, E. CHEUNG, M.W. LAM, M. MOK, M.W. TSANG, J. CHAN
Division of Endocrinology, Department of Medicine & Geriatrics, United Christian Hospital, Hong Kong SAR, China

Objective: A multi-centre randomised prospective study to compare Structured Care (SC) and Usual Care (UC) models in managing type 2 diabetic patients with nephropathy was initiated in mid 2004 in HK. This is a report of the preliminary data we have collected in our local centre examining 15 patients who have so far completed the study. **Methods:** Adult type 2 diabetic patients aged 35-75 with plasma creatinine 150-350 μmol/L were randomised to receive 2 years of either (1) SC—patients were followed up at the Diabetes Ambulatory Care Centre 12-16 weekly by Diabetologist, with intensive reviews in between by Diabetes Nurse Specialist. Metabolic parameters were monitored at 2-3 months' intervals. Patients were treated to metabolic targets of BP <130/80 mm Hg, Hba1c <7.5%, LDL-C <2.6 nmol/L. ACEI/AIIA use was encouraged unless contraindicated, or (2) UC—patients continued their usual follow-ups at Medical/Family Medicine clinics. **Results:** 7 patients in SC and 8 patients in UC groups completed the study by September 2006. 57% of SC and 63% of UC participants were male. Mean age were 70.3±7.3 and 64.9±9.1 for SC and UC groups respectively. Patients in SC model achieved lower mean BP, fasting glucose, Hba1c, and LDL-C, while patients in UC gained more weight and had worsened Tg during follow-up. There were more AED attendance (10 vs 6) and hospital admissions (26 vs 6) in UC compared to SC model. 2 patients in UC group were started on CAPD, 1 developed IHD, and another had retinopathy; whereas 1 patient in SC model developed unilateral sensory neuropathy over lower limb during the study period (Table). **Conclusion:** Our preliminary data suggested that structured care with treat-to-target approach and utilization of ACEI/AIIA can improve all metabolic risk profiles. Whether this can improve renal outcome in diabetic patients with nephropathy awaits further analysis from the full study.

	SC baseline → 2 years	UC baseline → 2 years
BW (kg)	62.6 → 63.4	62.7 → 65.4
BP (mm Hg)	157/79 → 128/68	155/79 → 152/80
Anti HT agents	3.3 → 4	2.3 → 2.4
% on ACEI/AIIA	86 → 100	75 → 63
FG (mmol/L)	8.5±1.6 → 6.5±1.3	7.5±1.5 → 7.3±1.8
Hba1c (%)	9.3±1.9 → 7.5±0.9	8.2±2.3 → 8.5±0.8
LDL-C (mmol/L)	2.8±0.9 → 1.7±0.9	2.7±1 → 2.8±1
Triglyceride (mmol/L)	2.4±2.8 → 2.3±3.3	2.3±1.3 → 2.9±1.8

P267 Associations of Remission Status and Lanreotide Treatment with Quality of Life in Patients with Treated Acromegaly

Shih-che HUA, Yuan-horng YAN, Tien-chun CHANG
Division of Endocrinology and Metabolism, Department of Internal Medicine, Chia-Yi Christian Hospital, Chiayi 60002, Taiwan

Objective: To investigate the associations of remission status and lanreotide treatment with quality of life in treated acromegaly, by the Acromegaly Quality of Life Questionnaire (ACROQOL). **Methods:** 52 patients with treated acromegaly were recruited to complete the Chinese version of ACROQOL. The patients were divided into controlled and uncontrolled group based on the latest remission criteria and further subdivided into 4 groups based on current treatment with lanreotide or not. Comparisons between groups were analyzed. **Results:** There was no difference between controlled and uncontrolled group in the ACROQOL scores of total score, both scales and subscales. However, in the controlled group, current treatment with lanreotide, had worse ACROQOL scores in total score (p=0.021), psychological scale (p=0.011), psychological subscale "appearance" (p=0.032) and "personal relations" (p=0.010). **Conclusions:** The lanreotide treatment has negative association with quality of life in biochemically controlled acromegalic patients, especially in the psychological aspect.

P268 Effect of Anti-hypertensive Medications on Aldosterone to Renin Ratio in Screening for Primary Aldosteronism

Ace Yee LEE, Shing-chung SIU
Department of Medicine and Rehabilitation, Tung Wah Eastern Hospital, Hong Kong

Objective: To investigate the effect of anti-hypertensive medications on accuracy of aldosterone to renin ratio (ARR) in screening for primary aldosteronism. **Methods:** ARR were checked in 93 hypertensive subjects while continued their usual anti-hypertensive medications. Cut-off values for positive ARR were selected as either ARR>600 or ARR>555 with plasma aldosterone concentration >416 (plasma renin activity in $ng \cdot ml^{-1} \cdot hr^{-1}$; plasma aldosterone concentration in pmol/L). **Results:** Fifty-eight percent of subjects were on diuretic, 47% on beta-blocker, 42% on calcium-channel blocker, 15% on ACEI, 5% on alpha blocker, 5% on angiotensin II receptor blocker and 2% on methyldopa. Thirty subjects (32%) have positive ARR. The use of beta-blocker was statistically significant associated with positive ARR with p=0.004. Diuretic, calcium-channel blocker and methyldopa using did not have significant effect on ARR. ACEI and angiotensin II receptor blocker even when consider as a single group did not have significant effect on ARR. **Conclusions:** Beta-blocker using should be avoided when using ARR as a screening test for primary aldosteronism because it was significantly associated with false positive result.

P269 Prevalence of Primary Aldosteronism in Patients with History of Thiazide Diuretic Induced Hypokalaemia

Ace Yee LEE, Shing-chung SIU
Department of Medicine and Rehabilitation, Tung Wah Eastern Hospital, Hong Kong

Objective: To find out the prevalence of primary aldosteronism in a group of patient with thiazide diuretic induced hypokalaemia. **Methods:** Ninety-three subjects with hypokalaemia only when they were using diuretic were screened for primary aldosteronism by checking spot aldosteron to renin ratio (ARR). ARR were taken while they were continued on their usual anti-hypertensive medications. Twenty-four out of 30 subjects with positive ARR underwent oral salt loading test with measurement on urinary aldosterone excretion and plasma aldosterone concentration in order to confirm the diagnosis of primary aldosteronism. **Results:** Four subjects (4.3%) confirmed to have primary aldosteronism. There was no significant difference in blood pressure, baseline serum potassium and sodium level and development of hypokalaemia after salt loading between the subjects with primary aldosteronism and essential hypertension. **Conclusions:** The prevalence of primary aldosteronism was 4.3% in a group of subjects with thiazide diuretic induced hypokalaemia. We should not overlook this commonly encounter side effect of thiazide.

P270 Grass Carp Growth Hormone Receptor: Molecular Cloning, Functional Characterization, and Regulation of Transcript Expression at the Pituitary Level by Insulin-like Growth Factor

Gerald Francis BROWN, Hong ZHOU, Wendy K.W. KO, Anderson O.L. WONG
Department of Zoology, University of Hong Kong, Pokfulam Road, Hong Kong

Objective: In grass carp, growth hormone (GH) acts in an autocrine/pararine manner to stimulate GH secretion and GH gene expression in somatotrophs. To examine the signal termination mechanism for this unique "positive feedforward" phenomenon, we test the hypothesis that GH receptor (GHR) expression in the carp pituitary can serve as the target site of modulation by insulin-like growth factor-I (IGF-I). **Results:** Structural identity of grass carp GHR was established by molecular cloning. The GHR cDNA obtained is 2452 bp in size encoding a 605 a.a. receptor protein with the typical features of the GHR family. Using Northern blot, a single GHR transcript of ~4 kb in size was detected in the liver as well as in the pituitary. Functional expression of this newly cloned cDNA in CHO cells confirmed grass carp GHR could be activated by GH and functionally coupled to the $JAK_2/STAT_5$ signaling pathway. In grass carp pituitary cells, IGF-I treatment suppressed GHR transcript levels and blocked GH-induced GH mRNA expression. IGF-I inhibition of GHR mRNA expression could also be abolished by inactivating ERK1/2, PI3K and Akt with the respective pharmacological inhibitors. **Conclusion:** These results suggest that IGF-I may play a role in the signal termination of GH autoregulation in the carp pituitary by reducing GHR expression through activation of MAPK- and PI3K-dependent mechanisms.

P271 Regulation of Luteinizing Hormone Receptor Gene Expression in Grass Carp Pituitary Cells by Functional Interactions between Dopamine and Luteinizing Hormone

Cai-yun SUN, Gerald F. BROWN, Mulan HE, Wendy K.W. KO, Anderson O.L. WONG
Department of Zoology, University of Hong Kong, Pokfulam Road, Hong Kong

Objective: In Chinese grass carp, luteinizing hormone receptors (LHR) have been recently identified in the anterior pituitary and mediate the paracrine actions of LH to stimulate growth hormone (GH) synthesis and secretion in somatotrophs. To test if LHR expression can serve as the target site of modulation for the local action of LH, LHR gene expression was examined in grass carp pituitary cells. **Results:** The structural identity of grass carp LHR was established by molecular cloning. Functional expression of grass carp LHR confirmed that the receptor was specific for LH and functionally coupled to cAMP production and $[Ca^{2+}]i$ mobilization. In grass carp pituitary cells, LHR mRNA levels could be suppressed by LH or its functional agonist HCG. In contrast, basal levels of LHR mRNA were elevated by dopamine or its non-selective agonist apomorphine. These stimulatory effects could be mimicked by the dopamine D1 agonist SKF77434 through activation of AC/cAMP/PKA pathway. In grass carp somatotrophs, the opposite effects of HCG and SKF77434 on LHR mRNA expression could still be observed and the stimulatory action of SKF77434 was inhibited by HCG treatment. **Conclusion:** These results suggest that LHR expression in grass carp pituitary is under the differential control of LH and dopamine. In this case, dopamine stimulates LHR mRNA expression through D1 receptors coupled to cAMP-dependent mechanism and this stimulatory effect is under the negative regulation of LH. Functional interactions by the two endocrine factors may play a role in regulating somatotroph responsiveness to the paracrine actions of LH at the pituitary level.

P272 Community Integrated DM Service in Ngau Tau Kok GOPC

M. Mok, M. Ng, P. Lam, G. Kam, D. Chao, M.W. Tsang
Nurse Specialist, Union Christian Hospital, Hong Kong

Introduction: Community integrated DM service can provide holistic care to patients with type 2 diabetes. Joint efforts of Family medicine physician and diabetes nurse specialists can empower patient and achieve optimal metabolic control. **Objectives:** (1) To reduce Medical SOPD referral from GOPC. (2) To initiate insulin injection in GOPC setting. (3) To empower patient's self blood glucose monitoring. (4) To enhance quality of DM care in community. **Methods:** Patients with poor glycemic control on near maximum OHA or newly started insulin therapy; and/or poor treatment compliance were recruited. Diabetes nurse specialists provided comprehensive assessment and education; taught self-injection technique when indicated; and provided telephone follow-up for fine tuning of insulin dosage. Physician supervised and adjusted insulin according to patient's self-monitoring blood glucose results. Overall diabetic control evaluated. **Results:** A total of 51 patients had received this integrated DM service from January to August 2006. There were 22 male (43%) and 29 female (57%), patient's with mean age of 67.3 yrs old and mean duration of DM of 10 years (Table). **Discussion:** We have shown that initiation of insulin therapy in GOPC setting is feasible, ambulatory T2DM patients are willing to actively perform SMBG and glycemic control can be significantly improved through this CIDM service. Such model of ambulatory service can be extended to other GOPCs to improve the quality of community diabetes care and reduce unnecessary referral of uncomplicated diabetes patients to hospital.

	Before attending CIDM service	After attending CIDM service
Insulin injection	8 (16%)	20 (39%)
Insulin injection with active SMBG	1 (12%)	20 (100%)
OHA with active SMBG	8 (19%)	26 (84%)
*HbA1c % (mean)	9.1±1.23	7.8±0.64

Author Index