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Second International Huaxia Congress of Endocrinology
14-17 December 2001

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PL1 Cardiovascular risk in diabetes mellitus: genetic association approaches

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It is well established that diabetes mellitus is closely associated with higher prevalence of cardiovascular disease. Diabetes mellitus is a complicated metabolic disorders that renders the diabetic subjects suffered from both macrovascular (coronary, cerebral, peripheral vascular diseases) and microvascular (nephropathy, retinopathy, neuropathy) diseases. People with diabetes are two to four times more likely to develop these diseases compared to people without the conditions. Recent evidence, however, suggested that it is possible to prevent or delay these complications by vigorous glucose control, blood pressure control and lipid controls.

Since diabetes mellitus is such high risk for development of cardiovascular disease as well as poor prognosis after having myocardial infarction. Based on recent report from treatment guidelines of ATP III from National Cholesterol Education Program, it is therefore suggested that treat diabetes mellitus as those nondiabetic subjects who has had coronary heart disease. For every subjects with diabetes mellitus, the goal of LDL cholesterol is less than 100 mg/dl. In addition, those subjects with metabolic syndrome should be treated vigorously by diet and life style modifications in order to prevent it become diabetes mellitus or having cardiovascular disease.

Diabetes mellitus is very prevalent in subjects with coronary heart disease. Based on data collected from Taichung VGH, it showed that almost 40% of subjects with coronary heart disease by angiography having diabetes (32% with history of diabetes mellitus while 7.3% were diagnosed newly). Furthermore, nearly 50% of them having glucose intolerance if we added those with impaired fasting glucose (11.1%). These high prevalence profiles are also recently reported from western societies. We had previously demonstrated that aggressive treatment of LDL cholesterol down to 80 mg/dl in order to see improve endothelial dysfunction in subjects with type 2 diabetes mellitus.

Candidate gene approaches to study genetic markers that might responsible for cardiovascular diseases in subjects with type 2 diabetes mellitus were on-going. Among them are genes involving insulin secretion, insulin resistance, renin-angiotensin system, glucose and lipids metabolism and endothelial function.

PL2 PPARα, and insulin resistance

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Peroxisome proliferator activated receptors alpha (PPARα) are transcription factors belonging to the nuclear receptor superfamily which are activated by fatty acids and their derivatives. PPARα is mainly expressed in tissues having a high metabolic rate such as liver and muscles and in the cells of the atherosclerotic lesion. PPARα regulates genes involved in lipid metabolism, haemostasis and vascular inflammation, making it a candidate gene for risk of dyslipemia, atherosclerosis and coronary artery disease.

A broad spectrum of compounds can serve as PPARα ligand and activate the receptor. After activation, PPARα binds, upon heterodimerisation with RXR, to specific response element in the promoter of target genes, thus regulating the transcription of these genes. PPARα activation have also recently been shown to modulate gene transcription by negatively interfering with other transcription factor pathways in a DNA binding-independent manner.
In human vascular cells, interference with the nuclear factor NF-κB represses cytokine-induced activation of a number of inflammatory genes such as VCAM-1, COX2, IL-6.
PPARα activators furthermore improve glucose homeostasis and influence energy metabolism by enhancing fatty acid flux and degradation in the liver. These observations indicate a modulating role for PPARα in the pathogenesis of age-related disorders predisposing to atherosclerosis.

PL3 Novel mechanisms of steroid and thyroid hormone action

WW Chin

(No abstract available)

PL4 Prognostic Factors of Patients with Papillary and Follicular Thyroid Carcinomas in Taiwan

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Background and Objectives: Although there are many factors which affect postoperative serum thyroglobulin (Tg) levels, such levels having been previously used to detect the recurrence of papillary and follicular thyroid carcinomas. In this study, we wish to elucidate the significance of postoperative Tg levels for patients with clinical presentations of papillary and/or follicular thyroid carcinomas. **Methods:** In order to collect data pertaining to thyroid cancer patients who were treated in Chang Gung Medical Center in Linkou, Taiwan, records relating to a total of 847 pathologically-verified papillary or follicular thyroid cancer patients, all of whom received total thyroidectomy and thyroid remnant ablation with radioactive iodide (^{131}I). The patients were categorized into three different groups based upon postoperative Tg level. Group A patients were classified as those demonstrating a one-month postoperative Tg level less than 1 ng/mL. Group B patients were classified as those displaying a one-month postoperative Tg level greater than or equal to 1 ng/mL but less than 10 ng/mL. Group C patients were classified as those exhibiting a one-month postoperative Tg level greater than or equal to 10 ng/mL. **Results:** Of the patients in group A, none presented with distant metastases at the time of diagnosis or during the follow-up period. For group B, 6 patients (1.8%) died from thyroid cancer. In this group, tumor size was an important factor in cancer-related mortality, diagnostic clinical class, and follow-up status. Of the patients in group C, 49 of the 491 (10.0%) patients died from thyroid cancer. Among the patients in group C, age, histopathological type, stage of diagnosis, and follow-up Tg values were important factors. Among groups A, B, and C, there were 161 (95.8%), 253 (76.4%), and 129 (37.1%) patients, respectively, revealing a disease-free status at the end of 1998. **Conclusion:** Postoperative serum Tg levels can be used as a prognostic factor for papillary and follicular thyroid cancer patients.

PL5 The role of parathyroid hormone in the pathogenesis and treatment of osteoporosis

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The aging process is associated with increases in the circulating concentration of parathyroid hormone (PTH). This increase in circulating PTH has been viewed by some as detrimental to bone in view of the known effects of PTH to cause bone resorption, especially at cortical sites such as the forearm. On the other hand, others have viewed the age-related increase in PTH as beneficial because of the known effects of PTH to be anabolic at cancellous sites in the vertebral spine. These two views will be summarized at the time of the lecture. Perhaps the most compelling observations arguing for a beneficial effect of PTH on the aging skeleton comes from a number of studies directed as the potential use of PTH as a therapy for osteoporosis. Animal data have confirmed that low dose, intermittent administration of PTH is associated with selective anabolic effects whereas continuous infusion of PTH leads to catabolic effects. This observation had led to an experimental design in which PTH is administered to human subjects once daily in doses that do not regularly lead to adverse events such as hypercalcemia. Two-year studies using PTH alone have been directed at postmenopausal osteoporosis (PMO) in women and at men with idiopathic osteoporosis. In both women and men, PTH leads to marked increases in bone density at the lumbar spine and also significantly in the hip. In PMO, PTH is associated with significant reductions in vertebral and nonvertebral fractures. When PTH is used in combination with an antiresorptive agent, like estrogen, the markedly positive effects on bone mass are also seen in PMO and in those with glucocorticoid induced osteoporosis (GIO). After PTH is withdrawn but estrogen therapy is maintained, the gains in bone mass are maintained. It is not known whether gains in bone mass are maintained if PTH were to be withdrawn without maintenance therapy with an antiresorptive agent but preliminary data suggest that the gains in bone mass begin to be lost. It has been possible to obtain bone biopsy samples in PMO and in men with idiopathic osteoporosis before and after therapy with PTH. When subjected to histomorphometric analysis, the post-treatment biopsy samples show marked increases in cortical thickness and significant improvements in indices of trabecular connectivity. Thus, concerns that PTH may causes cortical thinning do not appear to be substantiated with bone is analyzed directly. On the contrary, the changes in the cortical wall thickness argue for an anabolic effect here also. From animal studies it would appear that the increase in cortical wall thickness is due, in part, to the effect of PTH to stimulate periosteal apposition at a rate that is greater than its effect to cause endocortical resorption. The associated increase in cross-sectional diameter should strengthen bone even though cortical bone density does not change. In all studies reported to date, PTH appears to be well tolerated. The era of anabolic therapy for osteoporosis is at hand with PTH being the first to be tested sufficiently rigorously to demonstrate its efficacy.

PL6 Role of cystic fibrosis transmembrane conductance regulator (CFTR) in spermatogenesis

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It has been known that mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) gene is associated with a reduction in male fertility. To establish whether CFTR is functionally expressed in the testis, we subjected spermatogenic cells from rat testes to analysis of CFTR mRNA, protein and channel activity. CFTR mRNA was detected in the testes of mature but not immature rats using RT-PCR analysis. Western blot analysis performed with CFTR specific antibody revealed immunoreactivity in the membrane extract of spermatogenic cells. Immunohistochemical studies localized CFTR in round and elongated spermatids, but not in the fully developed spermatozoa. Using whole-cell patch clamp technique, we recorded an inward current activated by intracellular cAMP (100 mM) in round spermatids. The current displayed a linear I/V relationship and was inhibitable by 500 mM diphenylamine-2-carboxylate (DPC). Transfection of the rat germ cell CFTR cDNA into HEK 293 cells expressed a cAMP-activated chloride current with CFTR characteristics. The current was completely blocked by the antispermatogenic agents 1-(2,4-dichlorobenzyl)-indazole-3-carboxylic acid, lonidamine (500 μM) and 1-(2,4-dichlorobenzyl)-indazole-3-acrylic acid, AF2785 (250 μM). Further work showed that CFTR interacts with aquaporin-7 to bring about volume contraction in spermatids during spermiogenesis and that the indazole carboxylic acid derivatives inhibit spermiogenesis by inhibiting CFTR.

PL7 Direct Neuroregulation of the Anterior Pituitary

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The current theory of the regulation of mammalian anterior pituitary by the nervous system is based on the discoveries of Geoffrey Harris about half a central ago. The essence of the theory is that the anterior pituitary is regulated by hypothalamic hormones released at the median eminence and carried via the portal vessel to the gland, rather than by nerve fibers innervating the anterior pituitary. Admittedly there are a small number of nerve fibers in the anterior pituitary, they are vascular in nature and do not take part in direct regulation of the secretory activities of the gland. In the past decade or so, it has been shown that actually there is a direct neural regulation of the mammalian anterior pituitary. The main lines of evidence are as following:

1. The presence of nerve fibers in the vicinity of the anterior pituitary gland cells. Substantial amounts of nerve fibers have been demonstrated in the human, monkey, dog, and rat anterior pituitary, weaving their way among the gland cells. Most of the nerve fibers contain substance P and calcitonin-gene related peptide. Tyrosine hydorxylase-, ChAT-, and GABA-immunoreactive nerve fibers have also been identified in small numbers.
2. Formation of synapses between nerve fibers and the gland cells. Typical synapses have been identified in the anterior pituitary of the dog and rat. In the dog, synapses have been demonstrated on the corticotrophs and somatotrophs. In the rat, synapses have been found on every type of the secretory gland cell, most frequently on lactotrophs. There are around 12,000 terminals in an anterior pituitary of the rat.
3. Changes in plasma hormone levels may induce active changes of the nerve fibers. It has been found that in the adrenalectomized or gonadecotmized rats there is active sprouting of the nerve fibers in the anterior pituitary, and there is indication that the sprouting nerve terminals specifically favor the gland cells related to the hormone manipulated. In female rats, the number of the nerve fibers fluctuates with estrous cycle.
4. Stimulation of the nerve fibers may modulate release of anterior pituitary hormones. *In vitro* stimulation of the nerve fibers have induced changes in the release of ACTH and prolactin.

The evidence so far has been accumulated proved unequivocally the presence of direct neural regulation of the anterior pituitary. A hypothesis of neural-humoral dual regulation of the anterior pituitary has been postulated.

PL8 GROWTH HORMONE TREATMENT IN CHILDHOOD

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Pituitary derived growth hormone (GH) and subsequently recombinant GH have been used to treat children with short stature for over 40 years. At present, licensed indications for GH therapy in childhood include GH deficiency, Turner syndrome and growth failure due to chronic renal failure. Very few of the recommendations regarding GH treatment in childhood are based on high quality randomized control trials. The consensus guidelines for the diagnosis and treatment of growth hormone deficiency in childhood and adolescent recently issued by the Growth Hormone Research Society will be helpful to guide clinicians in the investigations and treatment of children with suspected GH deficiency.

The diagnosis of GH deficiency should be based on auxology, biochemical assessment of GH deficiency based on the measurements of serum IGF-I, IFGBP-3 and the GH response to two provocative stimuli (with sex steroid priming preferably) and evidence of structural involvement of the hypothalamus and pituitary gland. Children with GH deficiency should be treated with GH in a dose of 0.1 IU/Kg/day. Final height correlates significantly with pretreatment chronological age, height SDS and predicted adult height, and duration of therapy, birth length, height SDS and age at the start of puberty, and serum GH binding protein concentration. The management of GH therapy through puberty remains controversial. The use of escalating doses of GH and LHRH agonists during puberty could be considered in selected patients with GHD. With early diagnosis and treatment, a mean adult height of –1 SDS is to be expected. Untreated Turner girls have an adult height which is 3.1-3.3 SDS below the population mean. GH therapy results in an increase of between 3-6 cm in final adult height. Predicators of response include weekly GH dosage, weight SD, age and oxandrolone therapy. The causes of growth failure in children of chronic renal failure are multiple. Despite normal serum IGF-I levels, IGF-I bioavailability is low due to an excess of IGF binding proteins. Short-term randomized trials have proven the GH treatment is efficacious in promoting growth in children with chronic renal failure before transplantation. The mean final height of GH treated children was 1.6 SD below normal but 1.4 SD above the standardized height at baseline in one study.

Normal children with short stature treated with growth hormone for 3 to 5 years will result in an improvement in the final height by 3-6 cm. The use of GH in short normal children has remained an ethical dilemma in paediatric endocrinology. With the abundant supply of recombinant GH, growth hormone has been used to treat short children with Noonan syndrome, Prader Willi syndrome, Down syndrome, X-linked hypophosphataemic rickets, Russell-Silver syndrome, thalassaemia major and those born small for gestational age. Efficacy is still in question and such children should only be treated with GH under an experimental protocol and treatment should be closely supervised by a paediatric endocrinologist.

PL9 Nature History and Genetics of Type 2 Diabetes

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In the study of nature history of type 2 diabetes, several line of evidence indicating that insulin resistance is the primary defect initiating the path-physiological process leading to the development of glucose intolerance. A common pathophysiological model for the nature history of type 2 diabeted invokes several metabolically distinct stages, which unfold over many years . An early stage is defined by normoglycemia in the presence of insulin resistance. The insulin resistance might have either a genetic or an acquired basis, of which obesity is the most typical accompanying feature. At this stage, because the β-cells in the islet can compensate the insulin resistance by argument insulin secretion, the body can maintain a state of glucose homeostasis. A later stage is characterized by impaired glucose tolerance (IGT), in which fasting glucose is normal but post-prandial glucose escapes the control of circulating insulin due to the relative insulin deficiency and abnormality of the insulin secretion pattern which are most likely due to the intrinsic defect in the islet β-cells. The final stage is the disease itself, in which most subjects have circulating insulin but in quantities that are insufficient to maintain fasting normoglycemia. The relative insulin deficiency of type 2 diabetes results from inadequate b-cell function, which occurs on the background of prolonged peripheral insulin resistance. Thus, type 2 diabetes refers to a group of disparate metabolic diseases, which are typically characterized by insulin resistance in peripheral tissues, together with impaired insulin secretion from pancreatic β-cells.

There are a number of lines of evidence that indicate the importance of genetic factors in the development of Type 2 diabetes. They include data from the twin studies, family studies, studies among different ethnic groups living in the same environment and admixture studies.

In the last two decades, great effort has been taken in identifying genetic determinant of type 2 diabetes . Except for a few genes (*HNF4A, GCK, HNF1A, IPF1, TCF2*) which cause rare forms of maturity onset diabetes of the young(MODY) and some rare genetic defects causing type 2 diabetes, such as mutations in *IR, LMNA, GLU2, IRS1, IRS2 , NEUROD1, LEP, LEPR, PPARG* and *mitochondrial tRNA (Leu-UUR).* But gene defects contributing to the cause of majority of type 2 diabetes are still at large. Recent success of identifying *CAPN10* gene variants as a major determinant for the genetic susceptibility to type 2 diabetes in Mexican American population had enlighten the hope of identifying the distinct genetic defect from the mapped chromosomal regions in linkage with type 2 diabetes as a result of genome scan approach.

We have learned, from the past experience of looking for the genetic determinants of type 2 diabetes ,that the type 2 diabetes is a very heterogeneous and complex disease. The complexity of type 2 diabetes is related to factors such as genetic heterogeneity, interactions between genes, and the modulating role played by the environment.

Recent advance in defining the scope of un-classical forms of autoimmune diabetes such as LADA and a form of type 1 diabetes no showing known autoimmune markers has further blurred the artificial boundary between type 1 and type 2 diabetes. Since the type 1 and type 2 diabetes are naturally not mutually exclusive, it is possible that a subtype of type 2 diabetes is the result of the interaction between genes leading to slowly autoimmune destruction of islet and genes leading to insulin resistance.

With the advance of Human Genome Project and the availability of a relatively complete sequence of the human genome, the amount of genetic information that can be used to evaluate hypotheses for the genetic basis of type 2 diabetes will increase substantially. To make sense of human type 2 diabetes in the post-genomic era, it is essential to have well-defined phenotypes in addition to sufficient numbers of individuals with the appropriate pedigree structure from families and/or communities. In addition, the advance of genetic research technology such SNPs and gene-chip will greatly facilitate the endeavor of genetic research in the etiology of type 2 diabetes.

PL10 DESIGNING NEW TREATMENTS FOR DIABETIC COMPLICATIONS

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The major cause of morbidity and mortality in diabetic patients is due to dysfunctions of peripheral nerve, microvessels and cardiovascular disease. Hyperglycemia and insulin resistance are the two major causes of the neuronal and vascular complications that are specific to diabetic patients. In order to design new therapies, a clear understanding on the effects of hyperglycemia and insulin on neuronal and vascular cells is essential. However, this is often difficult since neuronal and cardiovascular tissues are not easily accessible. Thus, we have approached this problem by culturing vascular cells from retina, renal glomeruli, aorta, and myocardium. Using these cells we have found that insulin and hyperglycemia have a large number of actions which were previously unknown. For example, insulin can stimulate growth factors such as vascular endothelial growth factor (VEGF). This finding is important for collateral formation under hypoxia state as found in myocardium and wound healing which are known to be at risk in diabetic patients.

Using cultured vascular cells, we have found that hyperglycemia can activate protein kinase C (PKC), in particular, the β isoform. Once important changes such as PKC activation have been identified in aortic vascular cells, their physiological importance are confirmed in various vascular tissues in animal models of diabetes. For PKC activation, the β isoform was found to be increased in the retina, renal glomeruli, heart, and all vascular tissue studied so far in animal models of diabetes. To demonstrate clearly the role of PKC activation in the pathogenesis of diabetic complications, direct modulation of PKC β isoform either by pharmocological or molecular approach are necessary. For PKC β isoform activation, we synthesized an isoform specific inhibitor and made transgenic mice overexpressing or removed PKC β isoform to determine the role of PKC β isoform activation in the development of diabetic retinopathy, nephropathy, cardiac diseases and neuropathy. Thus, from the strength of the positive results from cultured vascular cells and animal models of diabetes, this PKC β isoform inhibitor is now in clinical trial for the treatment of retinopathy, macular edema, and peripheral neuropathy.

1.1 Regulation of the GnRH receptor gene

WW Chin

(No abstract available)

1.2 ROLE OF GnRH-I AND GnRH-II IN REPRODUCTIVE MEDICINE

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Gonadotropin hormone-releasing hormone (GnRH) is a key regulator of mammalian reproduction. Numerous GnRH analogs are now being used in many areas of reproductive medicine, such as assisted reproduction. Recently, a distinct gene encoding a second form of GnRH, termed GnRH-II, to distinguish it from the classical mammalian form (GnRH-I), has been reported in the human and other primates. GnRH-II, which was first identified in the chicken (cGnRH-II), has been identified in a wide variety of tissues in the human. To date, the biological function (s) of GnRH-II in the human have not been defined. In addition to its role in the pituitary, GnRH has been implicated as an autocrine or paracrine regulator in several extrapituitary tissues. In the human ovary, we have shown that GnRH directly inhibits progesterone production in granulosa-luteal cells. Recently, we have demonstrated the expression of GnRH-I, GnRH-II and their mutual receptor (GnRHR) in human granulosa-luteal cells, as well as in normal and neoplastic ovarian surface epithelium. Different GnRHR-mediated signaling pathways may be activated by GnRH, including coupling of GnRHR to a pertussis toxin-insensitive G protein ($G_{q/11\alpha}$), $G_{s\alpha}$ and $G_{i\alpha}$. Our analysis of the 5' end of human GnRHR gene revealed the presence of five consensus TATA boxes residing in close proximity to one another in a cluster-like arrangement. Primer extension experiments revealed five transcription initiation sites. The possibility of alternative use of promoters and transcription start sites may explain the differences exhibited in GnRHR expression levels and hence different GnRH binding affinities detected in different tissues.
[This research was supported by the Canadian Institutes of Health Research].

1.3 RECENT ADVANCES IN INDUCTION OF OVULATION

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Clomiphene citrate is still the first line drug for induction of ovulation in patients with polycystic ovarian syndrome (PCOS). In patients who failed to respond to clomiphene citrate, insulin sensitizers like metformin have been shown to be effective in inducing ovulation. Patients with PCOS usually have high basal levels of serum luteinizing hormone (LH). Down regulation with gonadotrophin releasing hormone (GnRH) agonists has been tried in these women to suppress the serum LH levels before stimulation with gonadotrophins. However, a recent meta-analysis did not show any clear advantage in the routine use of GnRH agonists in these women. New preparations of FSH including recombinant FSH are now available. Although the use of urinary or recombinant FSH has been associated with higher pregnancy rates in treatment with in-vitro fertilization when compared with human menopausal gonadotrophins (hMG), no such advantage has been shown in induction of ovulation in women with PCOS. Chronic low dose step-up protocols have been used for a long time with good results. Recent studies showed that step-down protocols are also effective. Although data are not conclusive, recent studies have raised concerns on the increased risk of ovarian cancers who have previously received fertility drugs. There is also the possibility of decreased implantation rates in women with high levels of serum oestradiol. Therefore, we should confine the use of gonadotrophins to the lowest effective dose and duration.

1.4 IMPORTANCE OF MALE ACCESSORY SEX GLANDS IN FERTILITY

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In the golden hamster, the major accessory sex glands (ASG) consist of the ampullary gland, seminal vesicles, dorsolateral prostate, ventral prostate and coagulating gland. Previously, we have observed developmental anomalies during the first half of gestation in embryos sired by males with some (AG or VP) or all ASG removed (TX). These include a delay in oocyte activation during fertilization, entry into the first S-phase, larger nucleolar size and volume at 4-cell sate, lower cell number at 72 h p.c., reduced implantation rate at 122 h p.c. and higher embryonic wastage. Exposure of sperm to secretions of the male accessory sex glands, in particular the ventral prostate, is important for the multiplication of cells during pre- and post-implantation embryonic development. Gas6 protein and up regulation of Gas6 receptor, *Rse*, was found in degenerating embryos sired by TX and VPX males.

Studies on ejaculated sperm from TX male hamsters showed a higher incidence and extent of single and double strand DNA breakage using single cell gel electrophoresis (or Comet) assay compared with the sham-operated control. Incubation of sperm with NADPH showed a dose-response relationship in terms of DNA breakage while ASG secretion was able to protect sperm from oxidative stress.

2.1 LOW HDL AS A CARDIOVASCULAR RISK FACTOR

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Low serum high-density lipoprotein cholesterol (HDL-C) is a strong predictor of coronary heart disease (CHD). It has been estimated that an increase of each 0.026 mmol/l would reduced CHD risk by 2 % in men and by 3 % in women. A low concentration of HDL-C has been defined as HDL-C less than 0.90 mmol/l in men and less than 1. 15 mmol/l in women. Low-C is the most common lipoprotein abnormality in the patients with CHD before the age of 60 years. Low HDL-C is a component of dyslipidemia patterns such as familial combined hyperlipidemia (FCHL) or an isolated familial trait. Low HDL/raised triglyceride trait is an inherent feature of diabetic dyslipidemia and insulin resistance. Consequently low HDL-C is the most common lipid CHD risk factor. CHD patients with low HDL-C have a different HDL subpopulation profile with deficiency of LpA-I particles. We have shown that the lowering of HDL-C in the affected members of low HDL families is mostly due to the decrease of the large particles as indicated by consistent decreases of HDL2-C, LpA-I and Apo A-I. The significance of low HDL-C as a predictor of early atherosclerosis is supported by a significant inverse correlation found between carotid artery intima media thickness and HDL-C, HDL2-C and LpA-I in asymptomatic members of low HDL families. Multiple mechanisms may explain how HDL slows the progression of atherosclerosis. It is well established that HDL has a critical role in reverse cholesterol transport. Recent discovery of the ATP-binding cassette transporter1 (ABC1) and SR B1 reverse transporter protein have markedly expanded our understanding of the regulation of reverse cholesterol transport. Other potential mechanism to explain the atheroprotective actions of HDL include its antioxidant activity, protective action against inflammation and anticoagulant activity. Recently two intervention trials have demonstrated that raising HDL cholesterol can markedly reduce CHD risk. VA-HIT trial reported 24 % reduction of CHD endpoint in men with low HDL treated with gemfibrozil. DAIS trial reported 40 % reductions of minimum lumen diameter and percent stenosis quantified in coronary angiography in Type 2 diabetic patients treated with fenofibrate. The cohort of AFCAPS/TexCAPS was characterized by low HDL values. AFCAPS/TexCAPS confirmed the treatment benefit of statins in low HDL population.

2.2 Clinical Significance of Hypertriglyceridaemia in Chinese

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Hypertriglyceridaemia is now a well established independent cardiovascular disease (CVD) risk factor. With meta-analysis of 17 population-based studies of triglyceride (TG) and CVD, Austin reported a 1 mmol/L increase in TG was associated with 76% increase in CVD risk in women and 31% increase in men. Among 3333 Hong Kong Chinese type 2 diabetic patients being followed up in clinic, 8.4% have history of non-fatal CVD. TG is one of the independent risk factors predicting CVD in both men and women. There is no consensus on the optimal cutoff in the definition of hypertriglyceridaemia. A continuum is, however, expected in the level of serum TG on CVD risk. In Hong Kong Chinese, 4.7% have their TG levels ≥2.3 mmol/L. Adverse effects of TG is multifactorial. Hypertriglyceridaemia is associated with many conventional CVD risk factors e.g. obesity, age, blood pressure, glucose intolerance. Among 2238 Chinese subjects, the odds ratio of hypertriglyceridaemia in diabetes vs. non-diabetes was 3.3 (5.6 in women, 2.6 in men). Hypertriglyceridaemia also implies an insulin resistant state. This was observed in Chinese subjects with isolated low HDL (il-HDL) i.e. HDL <0.9 mmol/L and total cholesterol <5.2 mmol/L, such that il-HDL with TG <1.7 mmol/L was determined by obesity only while il-HDL with coexistence of hypertriglyceridaemia was associated with higher fasting insulin level and insulin resistance (based on HOMA model). Tan has demonstrated that Chinese diabetic patients have increased level of small dense LDL. In multivariate analysis, TG was the most important independent factor accounting for the small dense LDL variability. On the other hand, we have previously demonstrated that 1 SD increase in TG in Chinese non-diabetic subjects was associated with 3.7% increase in fibrinogen while hyperfibrinogenaemia is itself an important CVD risk factor. It has also been reported that postprandial triglyceridaemia increases PAI-1 level and activates factor VII, hence increases the risk of clotting and thrombosis. In summary, hypertriglyceridaemia is associated with a higher CVD risk. It mediates its harmful effects through insulin resistance, small dense LDL, fibrinogen and other CVD risk factors.

2.3 ENDOTHELIAL DYSFUNCTION AND DIABETIC DYSLIPIDAEMIA

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Endothelial dysfunction is an early event in atherogenesis and precedes the thickening of the intima and the formation of atherosclerotic plaques. Endothelial dysfunction has also been implicated in the pathogenesis of both micro- and macroangiopathy in diabetes and has been consistently demonstrated in patients with type 1 and type 2 diabetes mellitus. Markers of endothelial dysfunction (eg. von Willebrand Factor, vascular cell adhesion molecules) are elevated in patients with diabetes mellitus and vasodilation mediated by endothelium-derived nitric oxide is impaired. The aetiology of endothelial dysfunction in diabetes is complex and it has been shown that more than one mechanism is involved. We and others have demonstrated that diabetic dyslipidaemia plays a significant role. The increase in the concentration of small dense LDL, enhanced susceptibility of LDL to oxidation and exaggerated postprandial lipaemia found in patients with type 2 diabetes mellitus are associated with impairment of endothelium-dependent vasodilation in these patients. Randomised placebo-controlled trials have shown that both atorvastatin and ciprofibrate are able to improve but not completely normalise endothelial function in patients with type 2 diabetes. Other factors that have been suggested to also contribute to endothelial dysfunction in diabetes include increased oxidative stress, formation of advanced glycation endproducts, insulin resistance, activation of protein kinase C and expression of various cytokines. We need a better understanding of the various mechanism(s) involved and the relative roles they play in causing endothelial dysfunction so that new therapeutic strategies can be developed.

2.4 GLUCOSE AND INSULIN REGULATION OF APOLIPOPROTEIN AI GENE EXPRESSION IS MEDIATED BY A SINGLE DNA MOTIF THAT BINDS SP1

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Apolipoprotein AI, apo AI is the major protein component of the anti-atherogenic high density lipoprotein, HDL particles. Atherosclerosis underlying ischemic heart disease, IHD is the number one cause of premature death in western societies. The inverse correlation between levels of apo AI and risk of IHD provides the rationale for studying apo AI gene regulation. Lower levels of apo AI/HDL in diabetics may give rise to the observed 2-3 fold higher incidence of IHD in these patients. We have previously shown that insulin stimulates but glucose inhibits apo AI gene transcription in Hep G2 cells. Our recent studies showed that the opposing effects of insulin and glucose co-localize to a single cis-acting element, the IRCE (-411 to -404). This GC-rich motif binds to the transcription factor Sp1 but not Sp2/3. That this motif alone could mediate the effects of both insulin and glucose was tested using a construct, pIRCE$_{MT}$-Luc. This construct contained a 41 bp DNA fragment spanning -425 to -385 of the rat apo AI gene linked to the metallothionein promoter and then fused to the reporter, luciferase. In Hep G2 cells transfected with pIRCEMT-Luc, 100 mU/ml insulin stimulated Luc-activity 2-fold, similar to the behavior of pAI.474-Luc, a construct containing the -474 to -7 fused to luciferase. As expected, the insulin mimetic, 5 mM of bpv also increased Luc-activity in cells carrying pIRCE$_{MT}$-Luc. Whereas, 22.5 mM glucose suppressed Luc-activity in cells transfected with pAI.474-Luc, glucose had no effect on Luc-activity in cells carrying pIRCE$_{MT}$-Luc. Insulin induction of apo AI involves a cascade of intracellular events mediated by kinase-activity of which 50% involves the PKC pathway. Not surprisingly, the phorbol ester, PDBu increased pIRCE$_{MT}$-Luc activity 3-fold. Furthermore, PDBu induction of Luc-activity in cells carrying pIRCE$_{MT}$-Luc was augmented in the presence of the phosphatase inhibitor, okadaic acid. Since Sp1 binds to the IRCE, we used site directed mutatgenesis to render positions 30, 47, 127 and 510 phosphorylation defective. Although single mutations did not block completely the inducing effects of PDBu, 3 of the 4 mutants attenuated stimulation. Together these findings show that the IRCE alone mediates insulin induction but not glucose repression. Sp1 mutants that are phosphorylation defective, attenuate the actions of PKC.

3.1 Clinical analysis and management of pheochromocytoma: experience from 296 cases in Peking Union Medical College Hospital (pumch)

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296 patients with pheochromocytoma were diagnosed and treated during the 50-year period from 1952 to 2001 (3 cases in 1952-1959, 10 cases in 1960-1969, 33 cases in 1970-1979, 74 cases in 1980-1989, 121 cases in 1990-1999, and 55 cases in 2000-2001) in Peking Union Medical College Hospital (PUMCH), Beijing, China. The aims of this study were to analyze their clinical characteristics, especially for the diagnosis and management of these patients with pheochromocytoma.

The study included 296 patients who was suffered from pheochromocytoma (168 men and 128 women), aged 7~75 years (mean age, 39.4 ± 14.3 years). There are 23 (8%) children and adolescent (£ 18 years). 206 cases (70%) arise from adrenal medulla: 75 left side (36%), 112 right side (55%), 19 bilateral (9%). The sites of 84 (28%) extra-adrenal paragangliomas include the paraaortic region (69 cases), the urinary bladder (9 cases) , the thorax (2 cases), the head and neck (4 cased). 6 cases showed adrenal medulla hyperplasia. There are 54 patients (18%) with malignant pheochromocytoma and 11 cases of multiple endocrine neoplasia type 2 (MEN-2). The pheochromocytoma was diagnosed by biochemical confirmation based on plasma catecholamine levels or 24-hour urinary excretion rates of catecholamines and their metabolites measured using biochemical methods or HPLC, and/or pharmacologic test, such as regitine test, glucagons test et al. Following biochemical confirmation non invasive imaging techniques such as CT and/or MR of the abdomen and ^{131}I-MIBG scintigraphy are performed to localize the tumors.

Surgery provides the definitive cure for pheochromocytoma. The preoperative preparation with alpha-adrenergic blocking drugs is very important. Routine preoperative pharmacologic blockade with phenoxybenzamine, an α-adrenoceptor blocker, opposes catecholamine-induced vasoconstriction. Most of the patients with pheochromocytoma take phenoxybenzamine, but Urapidil, another selective α_1-adrenoceptor blocker, was used by about 50 patients for preoperative preparation since 1994 year in PUMCH. We will compare the effects and dosage between these two drugs. Otherwise, 6 cases of malignant pheochromocytoma with or without metastasis were treated by ^{131}I-MIBG. Our experience shows ^{131}I-MIBG treatment prolongs survival and is effective in palliative treatment for malignant and all unresectable, ^{131}I-MIBG positive pheochromocytoma.

3.2 KLINEFELTER SYNDROME: OF MICE AND MEN

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Klinefelter Syndrome (KS) is a disorder in phenotypic males caused by an extra X chromosome (47 XXY) and found in 1:400-1000 live male births. The CNS manifestations occur early in life with germ cell loss in early childhood and androgen deficiency in the pubertal years. Mosaic (e.g. XXY/XY) and greater supernumerary (e.g. XXXY: XXXXY) forms of aneuploidy also exist. Many manifestations occur including hypogonadism, infertility, dyslexia and executive/behavioral disorders. The spectrum and characteristics of the disorder have been enumerated in a survey of over 100 affected males compared with age matched normal men, confirming the presence of many somatic abnormalities and cognitive dysfunction in specific brain domains. Many children with KS are mislabeled as attention deficit disorder; the majority of KS boys and men are dyslexic. The prevalence of homosexuality is increased although ascertainment bias may exist. Detailed cognitive testing has been performed and correlations with anatomical imaging of the brain by MRI and SPEC have been completed. A role for early testosterone replacement for learning disabilities has been proposed but objective data on outcome are not yet available. A mouse model has been developed in our laboratories demonstrating progressive loss of germ cells and Leydig cell hypertrophy and hyperplasia. Germ cells appear normal through day 5 of life with near absence by day 10. The testis histology of adult KS mice is similar to that of adult KS men. The trigger for germ cell apoptosis is not yet known. CNS pathology and behavior and cognitive assessment of XXY and XYY mice are pending.

3.3 FINE NEEDLE ASPIRATION IN THE DIAGNOSIS OF THYROID AND PARATHYROID DISORDERS

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Fine needle aspiration (FNA) cytology is a very useful method for the preoperative diagnosis of thyroid nodules, or differential diagnosis of various thyroiditis. It also could be used to diagnose parathyroid lesions under ultrasound-guidance. In thyroid lesions, we usually do FNA after thyroid ultrasonography. If the nodule is palpable, and appears as solid nodule or pure cyst, we do FNA using the Chinese brush-holding technique (Acta Cytol 33:939, 1989). If the nodule is small and impalpable, or partially cystic, we do ultrasound-guided FNA. The aspirate is smeared and stained using the method of Riu, which takes just 2 minutes. The cytological picture looks like that using May-Grünwald-Giemsa method. Papillary, medullary and anaplastic carcinomas are usually easily diagnosed by FNA. Follicular carcinoma and adenoma are not easily to be differentially diagnosed. Anaplastic carcinoma can mimic acute suppurative thyroiditis. Lymphoma sometimes looks like Hashimoto's thyroiditis. In addition to diagnosis, cytomorphology under light microscope or scanning electron microscope could be used to predict the prognosis, and to select methods of treatment (Anal Quant Cytol Histol 13:403, 1991, Acta Cytol 44:633, 2001). In the cytodiagnosis of parathyroid lesions, sometimes immunoperoxidase staining with PTH and thyroglobulin, or determination of PTH in the cystic fluid are necessary and helpful for the diagnosis.

3.4 Designing The Ideal Thyroid Hormone Replacement Therapy

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In adult humans, the molar ratio of thyroid hormones (TH), T4 and T3, secreted by the gland has been estimated as 14:1, and most circulating T3 comes from peripheral deiodination of T4. The most widely used preparation for replacement therapy, purified synthetic T4, can thus provide not only normal serum T4 levels, but also steady and near normal levels of serum T3. However, the concept of a direct correlation between plasma T3 levels and hormone action, mediated by T3 occupancy of nuclear receptors, has been modified in light of the demonstration that certain tissues generate a significant amount of T3 for local use. In rats, in most of the organs investigated, the contribution of T3 from local T4 appeared to be low as compared to T3 from circulation. On the other hand, CNS, thyroid gland, liver and brown fat derived 1/3 or more of their cellular T3 locally. The fact that the origin of tissue T3 varies from organ to organ suggests that this should be considered in designing optimal thyroid hormone replacement therapy. There are three tissue specific 3' and 3-deiodinases (D) involved in regulating the tissue supply of T3. Hypothyroidism stimulates type II D (DII) but decreases type I D (DI) activities in tissues. DI is stimulated by hyperthyroidism, is sensitive to PTU inhibition, and is expressed mainly in the liver, kidney and the thyroid in humans and rodents; much of the T3 generated is delivered to the circulation. In contrast, DII is relatively insensitive to PTU and is expressed in the CNS, pituitary, brown fat, placenta, skeletal muscle, heart and thyroid. DII is involved in generating tissue T3 for local use. The 3-deiodinase, DIII, is mainly for inactivation and degradation of TH. In thyroidectomized rats, it has been demonstrated that restoration of the euthyroid state in all tissues can only be accomplished by administering both T3 and T4. In a recent crossover study of hypothyroid patients, a combined T4 and T3 regimen resulted in improvement in mood and neuropsychological functions without abnormally suppressing serum TSH. In summary, in designing ideal thyroid hormone replacement therapy, we should consider:

1. The goal of treatment is to normalize thyroid status in all tissues, not merely normalize circulating TSH.
2. In the euthyroid state, a small but significant fraction of circulating T3 is derived from thyroidal secretion, and may be important in those tissues deriving most of their T3 from the circulation.
3. To achieve a fully euthyroid state,T3, preferably in a slow-release form, may be required in replacement regimens.

4.1 PHENOTYPE AND GENOTYPE OF LATE ONSET AUTOIMMUNE DIABETES

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The phenotypic features of latent autoimmue diabetes of adults (LADA) are highly variant in different ethnic groups. The mean age of onset of the disease was older in LADA than in type 1 diabetes (DM1), but younger than in type 2 diabetes (DM2). The age of onset in ninety-five percent of LADA in our patients was older than 22 years old. Furthermore, the age of onset<35years old accounted approximately for one fourth of our patients, a condition which has not been reported previously. Most authors agreed that LADA patients were nonobese. But in our patients, overweighted and obese (BMI≥25, and 27kg/m^2) accounted for 13.9% and 8.33%, respectively. Basal and stimulated C-peptide(FC-P and PC-P) in LADA group were remarkably lower than that in DM2, but higher than in DM1 group. FPG and PPG levels were parallel to C-P. The frequency of ketoacidosis was much lower in LADA than in DM1 (11.11% vs. 64.29%, P<0.01) and the median duration from the onset of the symptoms to ketosis and ketoacidosis was much longer in LADA than in DM1 group. The prevalence of GAD-Ab (positive rate) in clinically suspected LADA and DM2 was 51.43% and 16.3%, respectively.

In view of the immunological nature of the disease the search for genetic markers of LADA were mostly focused on HLA-II molecules. Such as DQ, DR, LMP2/LMP7, TAP1/TAP2, and CTLA-4 loci. In addition to DQ, DR loci which were related to some extent to the genetic susceptibility of LADA, the results of others were largely negative. The relation of the polymorphism of HLA-A molecules and LADA has not been reported previously. Our preliminary results showed that the frequence of A0205 loci was remarkably higher in LADA than in DM2 and normal controls (12.24% vs 5%, and vs 2.63%, respectively P<0.05; RP=4.65% 95% CI 1.14-18.98, P=0.0167). In contrast, the frequency of A30 loci in LADA was significantly lower than in DM2 and controls. Thus, the phenotypic features of LADA are somewhat between DM1 and DM2 with the onset of age in Chinese population being youger than in some other ethnic groups. Genetic characteristics of LADA are uncertain. A0205 may be related to the susceptibility of LADA, while A30 is probably the candidate of protective gene for LADA.

4.2 Islet Transplantation: Immunological and Functional Aspect

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A progressive destruction of insulin-producing pancreatic beta-cells leads type 1 diabetic patients to a life-long dependence on insulin therapy. The Diabetes Control and Complications Trial has shown that intensive treatment can only reduce, but not avoid, the occurrence of chronic complications. Moreover, such tight control is difficult to achieve and can result in frequent episodes of hypoglycemia. 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.

In contrast to the transplantation of vascularized whole or segmental pancreas, 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. To accomplish the islet transplantation, a healthy pancreas removed from a recently deceased donor is digested by collagenase and the islets are separated from the other pancreatic cells by density gradient. The islet cells are then injected into the portal vein of the liver and lodge in very small branches within the liver where they can sense blood glucose and secret insulin. From 1893 through 2000, a total of 493 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. Recently, Shapiro and his colleagues reported a 100% cure rate for type 1 diabetes with their "Edmonton protocol" for islet transplantation. This major breakthrough has caused a groundswell enthusiasm.

The reasons for islet allograft failure in the past include both nonimmunological (insufficient beta-cell mass and problems related to islet engraftment) and immunological (immune rejection, toxicity of immunosuppressants and autoimmune recurrence) factors. The shortage of human donor pancreases has led to a search for alternative sources, including animal islets, human pancreatic duct cells, genetically engineered insulin-producing cell lines and embryonic stem cells. These exciting studies hold the promise to expand the available islet tissues for future transplantation. Recently, newer immunosuppressants are available, and glucocorticoid-free immunosuppressive regimen, including sirolimus, tacrolimus and daclizumab, used in Edmonton protocol provided effective in preventing graft rejection and autoimmune recurrence of diabetes, with no apparent diabetogenic or toxic effects. Donor cells can be immunomodulated by depletion of antigen-presenting cells (APC) from islets or by methods that result in functional inactivation of APC. Another potential approach is the use of devices to isolate islets from cells and antibodies of the immune system (immunoisolation). The availability of new reagents that interfere with key pathways in the immune response, e.g., anti-CD154, CTLA4-Ig, and anti-CD45 RB, offer hope for the induction of donor-specific tolerance. Besides, tolerance induction can also be achieved by transplanting islets into immunoprivileged sites, such as thymus and testis.

In summary, islet transplantation has raised the hope for a cure for diabetes. Although its successful rate has markedly improved recently, the future application in clinical practice needs safer forms of immunosuppression and more sources of islet tissues.

4.3 TSH Receptor Antibodies in Pregnancy

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Autoimmune thyroid disease is a common condition affecting 2 to 4% of women of reproductive age group. The majority have Graves' Disease (GD), a condition due to autoantibodies developing against the TSH receptor. With the availability of more specific tests for characterization of the antithyroid antibodies, numerous studies confirmed the existence of heterogeneous TSH receptor antibodies (TRAbs). At anyone time, there may be presence of different stimulating and blocking antibodies which possess diverse behaviour with respect to their ability to bind to the TSH receptor and to stimulate thyroid cell functions. The changing activity or character of these autoantibodies may sometimes be associated with a parallel change in the clinical course of the patient, i.e. a spontaneous evolution from hyperthyroidism to euthyroidism or hypothyroidism. A change in specificity from stimulatory to blocking antibodies is observed in GD patients during pregnancy. Using various cell lines transfected with wild type human TSHR or TSHR-LH/CGR chimeras that have part of the TSH residues substituted with equivalent residues of LH/CGR, it was observed that the thyroid stimulatory antibody (TSAb) activity decreased whereas the thyroid blocking antibody (TBAb) activity increased progressively during pregnancy. All TBAbs had blocking activities against residues 261 - 370 of the C-terminus of the ectodomain. However, the majority of the TBAbs had a hybrid conformational epitope directed against N terminal residues 9 - 89 or 90 - 165 as well. Despite a change in the activity level, we did not observe any change in the epitope of either the stimulatory or blocking Abs as pregnancy advanced. The change in the specificity of TRAb from stimulatory to blocking activity and the appearance of TBAb may contribute to the remission of GD during pregnancy.

4.4 Cytokines regulate pituitary growth hormone gene transcription in vitro

JY Deng

(No abstract available)

5.1 Insulin resistance: link between diabetes and hypertension

P Raskin

(No abstract available)

5.2 Recent Changes in Diagnostic Criteria for Diabetes: Practical Implications

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In the late 1990's both the ADA and the WHO recommended changes to the diagnostic criteria for diabetes mellitus (DM).

The change common to both was reduction of the fasting glucose cut-off value. If venous plasma is used then the new cut-off value recommended for diagnosis of DM is 7.0mmol/L compare to 7.8mmol/L on previous criteria. Both organisations left the random, or 2 hour OGTT values (2HPG), unchanged at 7.8 mmol/L as the cut-off for diagnosing impaired glucose tolerance (IGT) and 11.1mmol/L as the cut-off value for diagnosing diabetes. In addition the ADA recommended the introduction of a new intermediate state of glucose intolerance based only on the fasting glucose - this is known as impaired fasting glucose (IFG) and is defined by venous plasma glucose values between 6.1-7.0mmol/L. This was backed by a recommendation from the ADA to avoid or restrict the use of OGTT. The new WHO criteria do not include this category and the WHO continues to recommend the use of OGTT.

With respect to China and other Asian populations these changes have a number of practical implications.

The reduction of the FPG value to 7.0 mmol/L will lead to increased diagnosis based on FPG alone and can reduce the need for OGTT. Previous studies of OGTT results in Chinese subjects indicated that only 40% of subjects with 2HPG>11.1mmol/L had FPG>7.8mmol/L. For FPG>7.0mmol/L this figure increases to 50%.

Thus it remains true that even with the new FPG criteria many subjects (circa 50%) with DM based on 2HPG will have FPG<7.0mmol/L. Assuming the validity of the 2HPG value, which is supported by epidemiology, this weighs in favour of the WHO criteria rather than ADA.

This also causes problems with the new category of IFG (ADA). In our experience many subjects with "IFG" will have 2HPG>11.1mmol/L and very few will have a normal 2HPG of <7.8mmol/L. Thus the category of IFG cannot safely lead to replacement of the OGTT in the Asian setting and the great majority of such subjects will have either DM or IGT.

Thus the new WHO criteria are helpful, although many subjects still require an OGTT, whereas use of the ADA criteria is likely to lead to underdiagnosis of DM in as many as 50% of cases.

At present HbA1c is not recommended as a diagnostic test for diabetes due to inherent variability, poor correlation with glucose values in borderline states, and lack of standardisation. However reported experience from both Hong Kong and Japan does indicate that combining an HbA1c with an FPG can be helpful as an initial testing procedure and may lead to reduction in the number of OGGT's otherwise required.

The recommended change to the diagnostic criteria was introduced along with a new classification which is aetiology-based and uses the terms Type 1 and Type 2 rather than "IDDM" and "NIDDM". It also recognises that all forms of DM have transitional phases such as IGT and that treatment and other modification can reverse the process. Again this change is generally helpful but can lead to practical difficulty with assignment of diagnosis of DM if single measurements at a point in time are used, especially during a changing situation. Also in Asian populations the distinction between Type 1 and Type 2 is sometimes difficult, especially in young patients. A recent report by the Japan Diabetes Society highlights these difficulties and suggests the use of the terms "normal, borderline and diabetic type" to cover situations where single measurements have been made. Final diagnosis will then depend upon persistence of this "type" - for example persistent hyperglycaemia meeting the criteria for "diabetic type" allows the diagnosis of diabetes.

5.3 A Genome-Wide Search for Type 2 Diabetes Susceptibility Genes in Chinese Hans

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Type 2 diabetes is a major health problem in many developed countries. In developing countries, like China, the prevalence of type 2 diabetes has significantly increased in recent years, especially with its economic booming(in China, the prevalence has almost tripled within the last fifteen years, from 1% to about 3%[1-4]). Although many efforts have been made in elucidating the etiology of type 2 diabetes, we still don't understand exactly its pathogenesis. However, it is now generally accepted that genetic susceptibility plays an important role in the development of type 2 diabetes, in addition to the environmental influences, and in most cases, genes involved in susceptibility to type 2 diabetes may be multiple, except for some rare forms of type 2 diabetes, such as the maturity-onset diabetes in young (MODY), in which the genetic factors have been identified as the mutation of HNF-4α(MODY1)[5], Glucokinase(MODY2)[6], HNF-1α(MODY3)[7], insulin promoter factor-1(MODY4)[8] and HNF-1β(MODY 5)[9]. The identification of the susceptibility genes for the more common form of type 2 diabetes has been shown to be much more difficult. Not only could the phenotype be largely influenced by many environmental factors such as nutrition status and life style, but also because of the heterogeneity and the ethnic variation of the genes involved in susceptibility to type 2 diabetes. Recently many groups have used family or sib-pair based linkage analysis and genome-wide scan to detect loci or chromosome regions that are linked to type 2 diabetes and had obtained some exciting results. Hanis et al found that locus D2S125, on chromosome 2q37, were linked to type 2 diabetes in a Mexican-American population[10]. Mahtani et al reported, on a data from a Finnish population, a locus near D12S1349 on chromosome 12 that were linked to type 2 diabetes in families with the lowest insulin levels[11]. Ji et al obtained evidence for linkage on chromosome 20q12-13.1 which was not caused by MODY1 gene[12]. Other loci on different chromosomes were also reported[13-18]. These results showed that the susceptibility genes could be very different in different populations. Comparison of data from different ethnic populations will thus give us a profound understanding of the genetic etiology of type 2 diabetes. However, until now most genome wide scan data come from Caucasian or American Indian populations, there is still very little data about East and Southeast Asia population. Here we report the result of a study for type 2 diabetes susceptibility genes in Chinese Hans population with the genome-wide scan method. Our result showed that loci on chromosome 9, and 20 may contain genes that contribute to type 2 diabetes susceptibility.

5.4 GENETICS OF EARLY ONSET TYPE 2 DIABETES

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Objective: The goal of this study was to determine the frequency of mutation in HNF-1α, a gene recently implicated as causing MODY, mitochondrial mutation, type 2 diabetes in early onset diabetes and to analyze the respective clinical presentations in an ethnically Chinese population in Taiwan. **Method:** We analyzed 45 unrelated subjects aged less than 35 who had early onset diabetes to test the possibility that mutation of the HNF-1α gene and mitochondrial gene were responsible for this disorder. The prevalence of DM in school children aged 6-18 was also studied. **Results:** The prevalence rates of DM in the school children aged 6-18 were 142 per million for boys and 204 per million for girls, and 172 per million for entire school children population. Obesity, age and family history of DM are strong predictors for DM in school children and adolescents in Taiwan. Type 2 diabetes can develop in children and adolescents, who tend to be obese with acanthosis nigricans. In additions, we have identified one patient with MODY had a novel missense mutation in exon 3 of the HNF-1α gene (Y218C). The MODY3 patient had lower BMI than NIDDM patients from the same families. The distinction between type- 1 and 2 diabetes may be difficult in certain situations, particularly in young adults.It has been suggested that the mutation in nucleotide 3243 of the mitochondrial DNA tRNA Leu(UUR) plays an important role in the pathogenesis of diabetes mellitus. A case of MELAS syndrome associated with diabetes and hyperthyroidism has been reported in Taiwan. **Conclusion:** The MODY3 and mitochondrial mutations are occasionally found in patients with early onset type-2 diabetes in Taiwan.

6.1 RECENT PROGRESS IN THE ELIMINATION OF IODINE DEFICIENCY DISORDERS IN CHINA

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IDD (iodine deficiency disorders) were recorded in ancient medical literatures for thousands of years which now are also one of major public health problem and continue to threaten the quality of life, human potential and social economic development in China. It is estimated that 700 million people are at risk for iodine deficiency. The epidemiological surveys revealed there were 35 million patients with endemic goiter, 250,000 typical cretinism and proportion of mild mental retardation (IQ 50-69) covered 5-15% of the children, and IQ deficit of 10 points in population living in IDD areas. Iodized salt program (KI 10-30ppm at production level) used to be the main strategy in moderate and severe IDD endemias. However, IDD was not completely under the control for a long time since lack of strong political will, unqualified iodized salt and no effective monitoring system.

Chinese Government has made the political commitment for the elimination of IDD by the year 2000, followed by the same commitment from each province, since A National Advocacy Meeting was held by State Council in Beijing in 1993. The State Council approved a new National IDD Control Program and a Regulation on Iodized Salt. USI (universal salt iodization) was adopted as major intervention and covered most of China. Health Education, social mobilization and multisectoral coordination play an important role in the sustainable elimination of IDD. The National and provincial Monitoring Systems are setting up and implemented once every two years since 1993 by using WHO/UNICEF/ICCIDD criteria for monitoring progress towards eliminating IDD. The current data indicate that IDD at national level is basically eliminated and demonstrated the rapid progress in the elemination of IDD since 1993 National Advocacy Meeting.

The incidence of iodine-induced hyperthyroidism (1-3 folds increasing) has been observed in some IDD areas after USI and the main reason is supposed to be relative higher level of iodine concentration at consumer level. The strategies are as follows: to stop iodized oil program in most of IDD regions where qualified iodized salt are available; and reduce iodine level at salt plants from 50 to 35mg/kg. The sustainable elimination is still the further challenge because there are half of provinces without reaching the elimination criteria. The developing provinces, "the hard to meet the goal", in particular the western parts of China, should be in the priority for National IDD Control Program.

6.2 Thyroid Cancers: Genetics and Gene Therapy

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In this talk, we will briefly review the genetic abnormalities associated with thyroid cancers. Genetic abnormalities that could serve as clinical markers for diagnosis and prognosis of thyroid cancers will be addressed. Molecular insight into how these genetic abnormalities contribute to the development and progression of thyroid cancers will be discussed. We will also discuss how current knowledge of thyroid cancer genetics may affect treatment options for patients with thyroid cancer. In particular, we will examine the possible strategies of gene therapy and their inherent limitations for patients with advanced thyroid cancers. Finally, we will explore the possibility of using sodium iodide symporter as a novel therapeutic gene for human cancers.

6.3 THYROID HORMONE RESISTANCE: FROM LABORATORY TO BED-SIDE

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Resistance to thyroid hormone (RTH) is a syndrome characterized by refractoriness of the pituitary and/or peripheral tissues to the action of thyroid hormone. Mutations in the thyroid hormone receptor β (TRβ) gene result in TRβ mutants that mediate the clinical phenotype by interfering with transcription of thyroid hormone regulated genes. To understand the molecular basis of this human disease, we generated mice with a dominantly acting mutation in the TRβ gene (TRβPV) via homologous recombination. PV was derived from a patient with severe RTH characterized by attention-deficit hyperactivity disorder, short stature, low weight, goiter and tachycardia. PV has a C-insertion at codon 448, which produces a frameshift of the carboxyterminal 14 amino acids of TRβ1, resulting in total loss of thyroid hormone (T3)-binding and transcriptional activation. TRβPV mice expressing a single PV allele showed the typical abnormalities of thyroid function found in heterozygous humans with RTH. Homozygous PV mice exhibit severe dysfunction of the pituitary-thyroid axis, hypercholesterolemia, impaired weight gain and abnormal bone development. This mouse allows us to ask questions that could not be addressed in humans. We identified abnormal expression patterns of several T3-target genes in tissues of TRβPV mice. The β-subunit and the α-glycosubunit of thyroid stimulating hormone were activated, whereas the T3-positively regulated hepatic genes were repressed in the face of elevated thyroid hormones. These findings, for the first time, uncover the molecular basis of the dominant negative action of mutant TR *in vivo*. We explored further the molecular events underscoring the intriguingly marked variability of resistance in different tissues in patients with RTH. We found that differential abundance of TRβ and TRα1 proteins in tissues mediate variable phenotypic expression in RTH. In tissues where TRβ is the major isoform, resistance to thyroid hormone is expected. In tissues where TRα1 is the dominant form, signs of thyrotoxicity may occur. Armed with this understanding, clinicians could devise better strategies when treatment of RTH patients is needed. TRβPV mice can be used for testing new drugs and trials of different treatment strategies of RTH. The generation of TRβPV mouse has brought laboratory findings closer to bed-side application.

6.4 TSH RECEPTOR GENE MUTATION AND PATHOGENESIS OF AUTOIMMUNE THYROID DISEASE*

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Objective: To investigate the association between the mutation of thyroid stimulating hormone (TSH) receptor gene and the pathogenesis of autoimmune thyroid disease. **Methods:** The whole extracellular domain of the TSH receptor gene from thyroid tissues of fifty patients with Graves disease(GD) was analyzed by using reverse transcription-polymerase chain reaction-single strand conformation polymorphism (RT-PCR-SSCP) and twenty-one normal thyroid tissues were analyzed as control. The exon 1 of TSH receptor gene in white blood cells from 146 patients with Graves dsease and 30 patients with Hashimoto thyroiditis was also analysed with PCR-SSCP. **Results:** It was showed from the analysis of thyroid tissues that there was abnormal mobility shift of SSCP in 11GD patients and 2 in control, and the abnormal mobility shift was same in GD patients and in control, the nucleotide sequence analysis showed the nucleotide position 660 (the third position of codon 187) has the T/C polymorphism. The codon is AAT or AAC separately, and both code Asn. No abnormal migration was found in the analysis of exon 1 of TSH receptor gene in white blood cells for both groups of Graves disease and Hashimoto thyroiditis. **Conclusion:** It is suggested from our study that the gene mutation of the TSH receptor is possibly not associated with the occurrence of autoimmune thyroid disease.

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7.1 STATUS OF DIABETES AND ITS COMPLICATIONS IN CHINA

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Objective: The Diabcare-Asia (China) study was carried out in 1998 as part of a multinational project, to provide an overview of diabetes management and diabetes complication status. **Methods:** A total of 27 clinics (in 10 government hospitals, 10 university hospitals and seven military hospitals) participated with data from 2430 diabetic patients. Type 2 diabetes patients constituted 93.7% of the population studied. **Results:** Overall, the patients had mean age (±SD) of 58.9 ± 11.4 years, diabetes duration of 8.2 ± 6.4 years and BMI of 23.7 ± 3.5 kg/m^2 (34% of patients with BMI >25 kg/m^2). Mean HbA_{1c} (assessed at a central laboratory) and FBG levels were 8.8 ± 2.1% and 9.1 ± 3.5 mmol/l respectively. Seventy-two percent of patients had HbA_{1c} ≥7.4% (≥1% above Upper Limits of Normal) and 55% had FBG >7.8 mmol/l. The majority had satisfactory levels of fasting lipids (75% had triglycerides <2.2 mmol/l, 90% had total cholesterol <6.5 mmol/l and 87% had HDL-cholesterol >0.9 mmol/l). Fifteen percent of patients had abnormal levels of protein (>500 mg/24h) in the urine, 2% had elevated serum creatinine levels and 29% had microalbuminuria. Cataract (31%), retinopathy (28%) and neuropathy (31%) were commonly reported diabetic complications. When patients were subgrouped by duration of diabetes: <10 years (33%) and ≥10 years (67%), significant ($p<0.001$) differences were seen between the subgroups in mean BMI, HbA_{1c} and FBG. Patients with duration ≥10 years had lower BMI (23.4 ± 3.4 kg/m^2) than patients with <10 years duration (23.8 ± 3.6 kg/m^2). In terms of mean HbA_{1c} and FBG, the levels were significantly higher in the ≥10 years subgroup compared to the <10 years subgroup: HbA_{1c}: 9.3 ± 1.8 % vs 8.6 ± 2.1%; FBG: 9.6 ± 3.5 mmol/l vs 8.9 ± 3.6 mmol/l. With longer duration of diabetes, there was a significant ($p<0.001$) increase in the proportion of patients with cataract [24% (<10 years) vs 46% ((10 years)], retinopathy [20% (<10 years) vs 43% (≥10 years)], neuropathy [26% (<10 years) vs 41% ((10 years)]. **Conclusion:** The data revealed suboptimal glycaemic control in more than half of patients studied. In patients with longer duration of diabetes, despite the relatively lower BMI, glycemic control was poorer and complications rates higher.

7.2 ALDOSE REDUCTASE AND DIABETIC COMPLICATIONS: STUDIES IN GENE KNOCKOUT MICE

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Objective: To investigate the role of aldose reductase (AR) in the pathogenesis of diabetic neuropathy. **Methods:** The mouse AR gene was inactivated by gene-targeted homologous recombination. Diabetes was induced by a single intraperitoneal injection of streptozotocin. Neuropathy was determined by measuring the nerve conduction velocity (NCV) of the sciatic nerve. Reduced glutathione (GSH) was measured by enzymatic method. **Results:** Two independent AR knockout mouse lines were developed. These mice showed no obvious abnormality other than mild polyuria and polydipsia. Diabetes significantly reduced NCV of the wildtype mice while there was no significant reduction of NCV in the diabetic AR deficient mice. GSH level was significantly reduced in the sciatic nerves of diabetic wildtype mice, but no change was observed in the diabetic AR deficient mice. **Conclusion:** These results indicate that AR plays a key role in the pathogenesis of diabetic neuropathy, and that the reduction of GSH in the diabetic nerve is most likely due to the reduction of glucose by AR.

7.3 MOLECULAR PATHOGENESIS OF DIABETIC VASCULOPATHY

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Hyperglycemia and insulin resistance are the two risk factors for the cause of micro- and cardiovascular diseases exist in diabetic patients. We have proposed that one major molecular pathway by which hyperglycemia (HG) is causing vascular disease in diabetic patients is due to the activation of diacylglycerol-protein kinase C signaling cascade (DAG-PKC). Over the last 10 years, a large number of reports have substantiated the findings that DAG-PKC pathways are activated in multiple vascular systems including retina, glomeruli, heart and other large vessels in animal models with diabetes and diabetic patients. In addition, we have shown that PKC β and δ isoforms are selectively activated by HG and diabetes. To substantiate this hypothesis, we and our collaborators synthesized a selective PKC β isoform inhibitor, which has shown in both diabetic patients and animals to reverse hemodynamic changes in the retina, glomeruli, and peripheral nerves. Phase III clinical trials using this specific PKC β inhibitor are now in progress. Our focus now is to determine which vascular and neuropathic lesions caused by diabetes is due to PKC activation. In addition, isoform specific actions due to β and δ isoforms have been detected in the vascular cells, but the distinctive pathways which are induced by PKC β and δ isoform is not known. Therefore, we have devised a whole host of methods including adenoviral vectors incorporating various wild types and dominant negatives of the PKC isoforms, and transgenic mice overexpressing or null mice of PKC β2 and δ mice in various vascular tissues and the heart. In addition, studies using 2D-gel and DNA microarray analysis are in process to identify selective protein targets of PKC β and δ isoforms in the vascular endothelium and contractile cells to determine the exact molecular mechanism by which these PKC isoforms are causing vascular complications in diabetes. We have also suggested that the mechanism by which insulin resistance is causing endothelial dysfunction and atherosclerosis is due to the selective loss of insulin's anti-atherogenic actions via the IRS-PI3 kinase-Akt eNOS pathway, whereas insulin's atherogenic actions mediated via the MAP kinase signaling pathway remain intact. This theory is supported by the studies in the Zucker obese and diabetic rats. New studies are now in progress to confirm this theory by directly measuring insulin's effects on microvessels of control and diabetic patients. We have also postulated that the development of insulin resistance in the endothelium of diabetic and insulin resistant patients is due to metabolic abnormalities which can activate protein kinase C. Preliminary studies have shown that PKC can inhibit insulin's actions on PI3 kinase and eNOS activation. Studies are now in progress to determine which PKC isoform is activated by FFA that can lead to insulin resistance in the endothelium to cause endothelial cell dysfunction. Transgenic mice overexpressing or knockout models will be made with respect to the PKC isoform induced by FFA in order to prove this hypothesis. These studies will provide a molecular explanation to explain the pathogenic role of HG and insulin resistance in the cause of diabetic vascular complications.

7.4 NON-ENZYMATIC GLYCATION AND DIABETIC COMPLICATIONS : CURRENT TREATMENT OPTIONS

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Non-enzymatic glycation is likely to play a major role in the pathogenesis of diabetic complications. Apart from intensive glycaemic control, possible strategies to reduce the impact of glycation include inhibitng the formation of advanced glycation endproducts (AGE), breaking AGE related crosslinks, reducing exogenous AGE intake, accelerating removal of AGE and modulating the effects of AGE on cellular functions. Clinical trials in humans have shown that aminoguanidine, an inhibitor of glycation, may be able to retard the development of diabetic nephropathy and retinopathy. Clinical trials on ALT-711, a crosslink breaker, appeared to reduce arterial stiffness. However due to the difficulty of obtaining tissue samples or administering therapeutic agents in the early stage of diabetes, primary prevention studies cannot be readily conducted in humans using these agents. Rodents have traditionally been used in laboratory research but their short life span and different anatomy make them unsuitable for the study of chronic diabetic complications. With these considerations in mind, we have maintained for about 10 years a group of baboons (Papio Hamadryas) with streptozocin induced type1 diabetes. These animals have a HbA1c level of about 10% and are treated with daily injections of long and short acting insulins. They receive regular examination for the detection of diabetic complications, including performance of renal and sural nerve biopsies, measurement of GFR and nerve conduction, and examination of the fundi by retinal photography. Intravenous ultrasound was also used to examine all the major arteries. Within a few years of onset of diabetes, these baboons have shown thickening of glomerular basement membrane, reduction of sural nerve fibre number and size, degeneration of epidermal small nerve fibres, increase in albuminuria and defects in autonomic function. After nearly 10 years of diabetes, these baboons have begun to develop overt proteinuria and diabetic nephropathy but as yet they do not have evidence of retinopathy or macrovascular disease. A subgroup of the diabetic animals were treated with aminoguanidine from the onset of diabetes and they have shown a reduction of renal and perineurium vascular abnormalities. These studies promise to define more precisely the importance of preventing glycation from the onset of diabetes.(Supported by the Rebecca Cooper Foundation and the Diabetes Australia Research Trust)

8.1 GROWTH HORMONE: A PANACEA FOR AGING?

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Aging is associated with reduced basal and stimulated growth hormone (GH) secretion. Certain aging related changes such as reduced muscle mass, increased abdominal adiposity, osteoporosis and atherogenic plasma lipoprotein levels are also found in adult GH deficiency, suggesting that GH may be useful as an anti-aging agent. This was supported by the uncontrolled study by Rudman et al in 18 older men with low serum IGF-1 levels, in which GH treatment for 6 months resulted in improved skin texture and bone mineral density. However, the benefit on bone could not be confirmed at 12 months, and the side effects became unacceptable to most patients. Elderly subjects appear to be very sensitive to the side effects of GH. Even at a low dose of 0.09U/kg thrice per week, the improvement in body composition in healthy elderly men was hampered by frequent complaints of lower limb oedema, arthralgia, hand stiffness, gynaecomastia and carpel tunnel syndrome. The gain in muscle mass is often not accompanied by increased strength and endurance, relative to resistance exercise alone. Consistent reductions in body fat are found in men only, with no preferential reduction in visceral fat. Significant lipoprotein changes have not been reported. While the benefit and safety of long-term GH replacement in healthy older people remain controversial, beneficial effects on physical function have been reported from short-term low dose therapy in elderly patients with hip fractures and, as an adjunct to dietary augmentation, in malnourished elderly subjects. The role of more physiological means of GH supplementation using oral GH secretagogues, including synthetic ghrelin, in combating frailty and maintaining physical independence in the elderly is under active research.

8.2 Insulin-like growth factor-I (IGF-I) and Cancer: Implications for GH Therapy.

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Growth hormone and IGF-I have independent and synergistic actions on cellular growth and metabolism, and are important in the regulation of normal human growth and development. Recent evidence suggest that GH and the IGFs may also be important in human cancer cell growth. The implications of these emerging studies on GH therapy, especially in adults, is uncertain. While it has long been known that GH and IGF-I have direct growth promoting effects on human cancer cells *in vitro*, the evidence *in vivo* has not been striking. Retrospective studies on patients with acromegaly have shown only a small (though significant) increase in colon cancer. However, recent preclinical studies on a rapid acting insulin analogue (Asp B10) with significantly higher affinity to the IGF-I receptor showed significant induction of rat mammary tumors (and caused discontinuation of development of the drug). Two large prospective epidemiological studies showed that a higher serum IGF-1 (within the normal range) was significantly associated with an increased risk of breast cancer and of prostate cancer. Pegvisomat (pegylated GH-receptor antagonist, B2036), which lowers circulating IGF-1, significantly inhibited the growth of breast, colon and prostate tumors in nude mice. Our own *in vitro* studies indicate that autocrine GH may be important in breast cancer cell growth. While these recent findings should not inhibit treatment of GH deficient children or adults in replacement doses, they should encourage some caution in the present enthusiasm for administration of GH to non-GH-deficient adults, especially in non-physiological doses. Greater understanding of the complexities of growth regulation, including the role of the IGF-binding proteins and the role of IGF-II in human cellular growth regulation, will be essential in resolving important controversies in this area.

8.3 ANDROGENS AND SARMS FOR THE ELDERLY MAN

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The decline of serum T in elderly men is associated with significant risk factors for frailty. Androgen replacement in elderly men improves libido but erectile dysfunction may persist. Androgens improve the sense of well being and mood. T replacement in elderly men may enhance spatial ability but insufficient data exists to determine if androgens improve cognition. There is abundant evidence to show that T replacement in elderly men increases lean body mass and decreases body fat. Whether this increase in lean mass translates to increased muscle strength, power or endurance is not known. T replacement also improves bone mineral density in older men but whether this will decrease fracture risk has not been proven. The available androgen replacement includes injectables, oral and transdermal preparations. T replacement in elderly men increases hematocrit and should be used with caution in men with elevated hematocrit. Occasionally this may result in sleep apnea in high risk patients. Most studies show decreases in HDL-cholesterol concentration after injectable T, while others report favorable effects of T on fibrinolysis and coronary artery dilation. In the 30 studies reported of androgen replacement in elderly men, there was no increase in PSA or prostate symptoms. These studies are not powered to address whether androgens will induce or promote growth of histologic cancer. Selective androgen receptor modulators (SARMs) act as co-activators or co-repressors at the androgen receptor and may show tissue specificity. SARMs could be designed to have benefits of androgens but without potential risks. Many SARMs are under development, screened by high throughput in vitro assays, undergone in vivo animal testing, and clinical studies are planned.

8.4 Androgen replacement in women

FCW Wu

(No abstract available)

9.1 Minimal Invasive Parathyroidectomy

LEE, CH

It is generally accepted that 85-90% of primary hyperparathyroidectomy (1°HPT) are caused by a single-gland disease, in which a routine 4-gland exploration is unnecessary , were the target able to be clearly identified before operations. $Tc^{99}m$ sestamibi parathyroid scan is known to be 95% specific for the localization of a hyperfunctioning parathyroid gland. Minimal invasive parathyroidectomy (MIP) were recently designed to remove the diseased gland thru a 2-3cm skin incision. A video-assisted endoscopic parathyroidectomy (VAE-PTX) , a radioguided PTX and a local exploration PTX are three types of MIP so far developed. In selected cases such as small sized, fresh patients with diseased gland located in the neck can be treated successfully.Our newly developed radio-sono-wire (RSW) directed PTX further simplified the local exploration for excision of the parathyroid adenomas. Not only the skin incision but the tissue damage were minimized. The operation can be completed within 30 minutes. There had been no complications. The successful rate was 90%. The serum Ca and i-PTH were all normalized on follow up. It is as easy and safe as excision biopsy for a superficial soft tissue tumor.
With the advancement of this surgical technique in combination with quick determination of serum i-PTH (QPTH) during operation (Biochemical frozen section), the principle for the surgical approach of 1°HPT is changed. An MIP may cure 65-70% of the patients leaving those with high QPTH and those with unlocalized diseased gland for open PTX.

9.2 Surgical Management of Thyroid Disorders: Current Approach

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In the last century, there have been significant advances in the surgical management of thyroid diseases and surgery is now the mainstay of treatment for thyroid malignancy as well as selected benign diseases. Fine needle aspiration of thyroid nodules for cytologic evaluation has become accepted as the initial diagnostic procedure of choice in patients with dominant or suspicious nodules. The longterm debate about total thyroidectomy versus unilateral lobectomy for well-differentiated thyroid carcinoma is ongoing and may never be settled without a prospective randomized trial. Demonstration of RET mutation in chromosome 10 has permitted genetic testing in families of all patients diagnosed with medullary thyroid carcinoma and prophylactic thyroidectomy is advised for cure. Although radioactive iodine ablative therapy is commonly used for Graves' disease, surgical management continues to be advantageous in certain circumstances. In addition, total or near-total thyroidectomy has definite advantagous over subtotal resection provided that complication rates do not increase. Thyroidectomy continues to produce a high rate of complications and medicolegal problems although excellent results of thyroidectomy have been reported in specilaized centres. Parathyroid autotransplantation has been shown to reduce the incidence of permanent hypoparathyroidism and intraoperative nerve monitoring may have a role in preventing recurrent nerve palsy. Patients requiring thyroidectomy should be cared for in high volume specialized centres where there should be increased quality of care with decreased costs and complication rates.

9.3 What's new in pre-operative endocrine imaging

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Pre-operative endocrine imaging aims at anatomical localization of the endocrine tumour to facilitate surgical planning. It may be applied prior to primary resection of the tumour, or reoperation for persistent or recurrent hormonal disorder. Modern imaging attempts at provision of both functional and morphological information. The trend is towards the use of non-invasive imaging, leaving the more invasive techniques for problem solving in need of additional information.

Morphologic imaging utilizes cross-sectional modalities to directly image the tumour. MRI is often the method of choice in view of its lack of radiation, multiplanar capability and superior soft tissue resolution. Technological advances in terms of multi-detector CT and faster MR imaging sequences permit more accurate delineation of small tumours. Pitfalls lie in tumour being too minute for current imaging resolution, ectopic tumours, incidentalomas, hyperplasia versus tumour, and benignity versus malignancy.

Functional imaging is utilized to lateralize or localize secretory tumours especially in patients with negative morphologic imaging. Recently introduced radioisotope tracers like sestamibi and somatostatin receptor are finding their role in preoperative imaging. Fusion of PET and CT images aims at better anatomical localization of functional tumours. Venous sampling like systemic sampling, inferior petrosal sinus sampling and arterial stimulated venous sampling is resorted to for specific conditions.

9.4 UPDATE ON THE RADIOTHERAPEUTIC MEASURES FOR ENDOCRINE TUMOURS

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Radiation therapy in the form of external radiation and unsealed radionuclides has an important role in the management of endocrine tumors. Recent advances in tumor imaging, radiation treatment planning and delivery, and radiopharmaceuticals have a significant impact on the efficacy and toxicity of localized and systemic radiation in the treatment of endocrine tumors.

For pituitary adenoma, radiation therapy is indicated when surgery cannot be performed because of medical conditions or patient refusal, when there is evidence of progression of tumor remnant after surgery, and in the presence of residual functioning tumor causing persistent elevated hormone after surgery. Using conventional radiation at a dose of 45-50Gy and fractional dose of 1.8-2Gy, the long term tumor control after surgery and postoperative radiation approaches 85 to 94%. For functioning adenoma, the circulating secretory hormones will undergo a slow but progressive decline over many years after radiation. Despite the excellent long term tumor control, development of radiation-induced late complications has raise concerns on the routine use of postoperative radiation in pituitary adenoma and the issue remains controversial. Review of updated data however suggests that risk of developing late complications may be much smaller with the use of modern radiation technique. In recent years, stereotactic radiosurgery has evolved as an alternative treatment modality for pituitary adenoma. Although clinical experiences on the use of radiosurgery for pituitary adenoma are still limited, it has been observed that a much faster and steeper fall in secretory hormone usually occurs after radiosurgery for functioning tumor as compared with conventional radiation, a feature which may be useful when a rapid fall of secretory hormones is desirable.

Systemic radiation therapy is achieved by administration of a radionuclide that becomes concentrated in an organ or site for sufficient time to deliver a therapeutic dose of radiation. The radionuclide usually emits beta particles with intense irradiation of the abnormal tissues. The most widely used radionuclide is I^{131} for hyperthyroidism and well differentiated thyroid cancer. Adjuvant radioiodine ablation of residual thyroid tissue is often done after surgery for well differentiated thyroid cancer to treat microscopic residue and to enhance the sensitivity of surveillance tests, which has been showed in restrospective studies to reduce recurrence and improve survival. Another radionuclide, I^{131}-meta-iodobenzylguanidine, has been used extensively for imaging of neural crest tumors. In patients with metastatic neuroendocrine tumors, treatment by I^{131}-MIBG often produces symptomatic and hormonal improvement with moderate tumor control. Recently systemic radionuclide therapy using radiolabeled somatostatin analogues have also been attempted with In ^{111} and Y^{90}, and trials of the use of this analogue in patients with advanced neuroendocrine tumors are ongoing.

10.1 FROM DIFFERENTIAL DISPLAY TO CLINICAL GENETICS — A PLAUSIBLE APPROACH TO UNDERSTAND PATHOPHYSIOLOGY OF OBESITY AND INSULIN RESISTANCE.

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Differentiation of 3T3-L1 preadipocytes into mature adipocytes is a well-characterized model system proven useful in studying cellular and molecular mechanisms of adipogenesis and insulin sensitivity. Among the genes that are differentially expressed, there are several genes that have been demonstrated to be important in the pathogenesis of obesity and type 2 diabetes. We have isolated 50~60 genes that were differentially expressed during adipocyte differentiation and found some of them were further regulated by insulin sensitizer. Here we demonstrated some of the examples relating to obesity, insulin sensitivity, and type 2 diabetes. The first is an adipose tissue-specific plasma protein, adiponectin, which has shown to modulate many biological processes. Low plasma levels of adiponectin were documented in subjects with obesity, diabetes mellitus or coronary artery disease. The second gene is SH3P12, an important adaptor for insulin-stimulated glucose uptake. Based on the findings, our hypothesis that adiponectin and SH3P12 might be genetic factors influencing body mass index (BMI) and insulin sensitivity was tested. We have isolated several SNPs and developed PCR-RFLP and/or heteroduplex analysis by DHPLC to determine the nucleotide variations at each locus. Some of these nucleotide polymorphisms revealed a significant correlation with BMI, plasma levels of fasting and two-hour post-glucose load insulin and insulin resistance index (IR). For example, adiponectin genotype was significantly related to FPI, 2h PI and IR. Also, the genotype frequency of T228A of SH3P12 also showed difference between the subjects with type 2 diabetes and obesity. Our results suggest that adiponectin and SH3P12, the two candidate genes found in differential display and both were upregulated by rosiglitazone, may be important and common genetic factors in controlling body weight and insulin sensitivity.

10.2 LEPTIN: A HORMONE THAT ONLY REGULATES ADIPOSITY?

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Leptin regulates energy balance by signaling the level of adiposity to the satiety and metabolism center in the hypothalamus. Further studies have revealed that leptin is a hormone with multifunctional actions. Particularly, it corrects the sterility of ob/ob mice independent of its weight reduction effect. In our experimental studies *in vivo*, intracerebroventricular infusion of leptin was proved to partially protect GnRH pulse generator from inhibition by morphine, as detected by volley frequency of multiunit activity recorded from bilateral electrode arrays implanted chronically in the mediobasal hypothalamus in the rhesus monkey. This observation is in concert with the result, that, *in vitro*, the GnRH secretion from GT-1 cell lines was stimulated when incubation with leptin at a concentration of 20ng/ml for 15 min. On the other hand, in our clinical studies, the leptin levels were significantly higher in 352 pregnant women as compared to 35 non-pregnant ones with matched age and BMI. In addition, the leptin levels in pregnant women were positively correlated to the systolic and diastolic arterial pressure. Leptin level in pregnancy eventually reached its highest level towards parturition and were correlated with BMI and serum prolactin during the whole pregnant term. The leptin levels in maternal blood (highest), cord blood, and amniotic fluid (lowest) were different, but there was a positive correlation between leptin levels of maternal blood and that of the amniotic fluid, and all these three levels had no relation to the sexes of the newborns. A cross-sectional study in 118 girls and 104 boys demonstrated that the leptin levels were continuously increasing in girls from Tanner Stage I to V, while in boys, it reached a peak during Tanner Stage II and declined thereafter. All these aforementioned clinical and experimental findings pointed to the same direction that leptin facilitates the reproduction-development related events, such as GnRH pulse generator firing, GnRH secretion, pregnant process, as well as pubertal development. It suggests that the leptinmay play a dual physiologic role which links reproductive function to the nutritional status.

10.3 EST Analysis of Human Adipose Gene Expression

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Objective: To obtain an overall view of the genes expressed in human adipose tissue and to discover novel genes potentially important in adipose biology and the pathogenesis of obesity and related disorders. **Methods:** We have sequenced 10,411 expressed sequence tags (ESTs) from an unnormalized human omental adipose tissue library. **Results:** Of all the sequenced ESTs, 8,986 ESTs (86%) matched 3,470 known genes. The rest 1,425 (14%) ESTs were not previously characterized and therefore considered novel. Among the most abundantly expressed genes are some housekeeping genes, such as translation elongation factor, translationally-controlled tumor protein 1 and beta-actin. Using digital Northern analysis we identified many known fat-predominant genes such as adipose-most-abundant gene transcript 1, lipoprotein lipase, CD36, perilipin, spot 14, fatty acid binding protein 4 and leptin, as well as some genes previously not known to be expressed in human fat tissue, including acetyl-coenzyme A carboxylase 2 (ACC2) and alcohol dehydrogenase 2 (ADH2). In addition, we have identified several uncharacterized fat-predominant gene/EST clusters whose functions are unknown, e.g., FEST1 (Fat EST1) and FEST2. Interestingly, FEST2 appears preferentially to be expressed in omental adipose tissue and is a secretory protein as demonstrated by in vitro analysis. **Conclusion:** Using high throughput EST sequence analysis, we have identified many genes expressed in adipose tissue that are either novel, or whose sequences were known, but not previously known to be expressed in adipose tissue. These findings will provide new insights into the molecular biology of adipose tissue and the pathogenesis of obesity.

10.4 Insulin resistance in the metabolic syndrome is related to visceral adiposity

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The insulin resistance or metabolic disease syndrome is a major factor in the increasing prevalence of type 2 diabetes and macrovascular disease. Recent studies have shown that insulin resistance occurs in those individuals who have increases in the visceral adipose tissue depot but not in those with comparable degrees of obesity who have primarily an increase in peripheral subcutaneous adipose tissue depots. The components of the insulin resistance syndrome similarly cluster with visceral (central) obesity. These data raise several interesting questions: What is unique about the visceral adipose tissue that accounts for its unique influence on insulin action at the level of the muscle? What factors determine the relative distribution of adipose tissue lipid storage between the visceral and subcutaneous depots?

Visceral adipocytes differ remarkably from subcutaneous adipocytes in their metabolic regulation and activity as well as in their anatomical relationships. The lipolytic response to catecholamines is greater in visceral adipocytes than subcutaneous ones leading to a greater release of free fatty acids and perhaps TNF alpha. Visceral adipose tissue stem cells are unresponsive to PPAR gamma agonists while the stem cells in subcutaneous adipose tissue are stimulated to differentiate into adipocytes. Glucocorticoid and androgen receptor mRNA are increased and steroid effects are greater in visceral than in subcutaneous adipocytes. Leptin mRNA and protein are greater in subcutaneous than visceral adipocytes. These are only a few of the known metabolic differences between the adipocytes in these depots.

The visceral adipose tissue pool drains directly into the liver. The percent lipid content of the liver and the serum triglyceride levels are closely correlated with the visceral adipose tissue volume. Similarly the muscle triglyceride content is more closely correlated to the visceral fat depot size than to the subcutaneous depot size. These data indicate that the products released from the visceral adipose tissue are more readily metabolized by the liver and can more directly influence the metabolism of the liver than those released from subcutaneous depots.

Adipose tissue releases a number of factors that have been shown to affect the intracellular insulin-signaling pathway. Free fatty acids and tumor necrosis factor alpha activate serine phosphorylation of IRS and PI-3 kinase molecules and that blocks their ability to be tyrosine phosphorylated. The result is an inability to transmit the insulin signal. Adipose tissue synthesizes a number of peptides, which have been implicated in either facilitating or blocking various components of the insulin action cascade.

There now appears to be ample evidence that some of the products of visceral adipose tissue are involved in the pathogenesis of the insulin resistance syndrome.

11.1 The DAX gene in endocrine disorders/ development

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The *DAX-1* gene, a 5 kb gene consisting of two exons, was mapped after studying a critical region of Xp21 in patients with a contiguous gene deletion syndrome in association with X-linked adrenal hypoplasia congenita, glycerol kinase deficiency and Duchenne Muscular Dystrophy, which overlaps a 160 kb locus associated with dosage-sensitive sex reversal. The DAX-1 gene was only cloned in 1994, and named as the human dosage sensitive sex reversal Adrenal Hypoplasia Congenita critical region on the X chromosome, gene 1 (DAX-1).

The *DAX-1* gene encodes a 470 amino acid protein, whose exact function is still unknown. However, the structure suggests that it is an orphan nuclear receptor, with an amino terminal which is proposed to regulate the transcription of target genes by binding to hairpin loop structures in DNA, and a carboxy terminus which has sequence homology to the ligand binding domain of other nuclear hormone receptors.

Data of the DAX-1 protein localization in embryonic tissues of the hypothalamus, pituitary, adrenals and gonads, in the rodent model and in human patients affected with DAX-1 mutations, suggests that DAX-1 has at least 4 important roles. The DAX-1 is involved in:

1. Adrenal development: It appears to be necessary for the development of the adult adrenal cortex; the absence of which results in adrenal hypoplasia and adrenal hypofunction. The onset of disease depends on the rate of regression of the fetal adrenal cortex, which may be controlled by other genetic or epigenetic factors.
2. Sex determination: The *DAX-1* gene is believed to be an anti-testes gene, which is important for ovarian development. Over-expression of *DAX-1* in the 46 XY male results in sex reversal in both the rodent model and in human patients.
3. Spermatogenesis: Male *dax-1* knock out mice are hypogonadal and infertile, despite having sufficient testosterone production for the formation of male internal and external genitalia. Their testes show progressive seminiferous tubule degeneration and loss of germ cells.
4. Regulation of gonadotropin secretion, as abnormalities of puberty have been described in X-linked AHC, in relation to the common association of hypogonadotropic hypogonadism, as well as rare associations with normal puberty and precocious puberty.

Current evidence suggests that DAX-1 is a transcriptional repressor of many genes in in-vitro gene expression studies. DAX-1 inhibits basal transcriptional activity of genes such as the stAR, P450scc, 3β-hydroxysteroid dehydrogenase and LHβ. In humans, DAX-1 antagonises the actions of sry in gonadal development. However, hypothetical models designed, suggest that DAX-1 is only one of several transcriptional factors, which regulate multiple functions in embryogenesis and physiology. The DAX-1 in X-linked AHC therefore provides a model of how a single gene abnormality can be associated with pathology in different physiological systems.

11.2 CONGENITAL ADRENAL HYPERPLASIA DUE TO 21-HYDROXYLASE DEFICIENCY: PROGRESS AND PROBLEM IN THE DIAGNOSIS AND TREATMENT

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Congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency (21-OHD) is the most frequent type of CAH, which accounts for over 90% of patients with CAH. 21-OHD is generally categorized into three forms, ie, salt-wasting (SW), simple virilizing (SV) and nonclassic (NC). SW and SV are also called classic 21-OHD. The incidence of classic 21-OHD discovered by the newborn screening programs was estimated to be 1 in 10 to 20 thousands. The incidence of NC is much higher than that of classic forms, it is one of the most common autosomal recessive disorders. Therefore, we think much more attention should be paid to this issue.

In this presentation, I would like: 1) to summarize some experiences in the diagnosis and treatment based on the clinical analysis of 175 cases of 21-OHD managed in this hospital since 1964; 2) to present our preliminary data on the genetic studies of 21-OHD done recently.

Among 175 cases of 21-OHD in our series, it was including 21 cases of SW (12.0%), 136 cases of SV (77.7%) and 18 cases of NC (10.3%). The ratio of male/female was 1:2.43 and this ratio in SW, SV and NC forms was 20:1, 30/106 and 1/17 respectively. The age of the patients at their first hospital visit was 5-28 days in SW, 0.9-31.8 yrs in SV and 16.4-38.5 yrs in NC.

The clinical manifestations of three forms were very different. Severe symptoms caused by hyponatremia were prominent in SW. Variety of virilization was the major abnormality in SV. Widespread pattern from almost normal sexual development to mild virilization showed in the cases of NC in this series.

To confirm the diagnosis of 21-OHD, corresponding hormone measurements were extremely important. Before 1980's, 17-ketosteroid (17-KS), 17-ketogenic steroids (17-KGS) and 17-hydroxycorticosteroids (17-OHCS) were performed in this hospital. After introduction of RIA in 1970's and chemiluminence immunoassay in late 1990's, the parameters such as cortisol in plasma and urine, ACTH, 17-hydroxyprogesterone (17-OHP), testosterone (T), renin activity (PRA), angiotensin II (ATII) and aldosterone (Ald) in plasma/serum gradually substituted for the formers. ACTH stimulation test was the key test for the diagnosis of NC form of 21-OHD. Middle dose dexamethasone

(Continue on page 32)

suppression test was used to differentiate 21-OHD from androgen secreting tumor. Abdominal CT or MRI showed clearly the significant bilateral adrenal hyperplasia in all cases who examined. Chromosome check-up should be done routinely, because it was useful to exclude other causes of sexual differentiation disorders.

All the cases of classic 21-OHD in this series received glucocorticoid replacement therapy. Hydrocortisone was the first choice. The routine dosage of this therapy was 20-40mg/d for adults and 10-20mg/m^2/d for children orally in 2 divided doses, usually 1/3 in morning, 2/3 in evening. In the situation of stress, the dosage was increased by 2-5 folds according to the degree of severity of the stress. Occasionally, cortisone, prednisone, even dexamethasone were used alternatively.

Early diagnosis and proper treatment was the key point for saving the life of SW 21-OHD patients during the newborn infancy. Gluco- and mineralo-corticoid should be used simultaneously besides replacement of liquid and electrolytes. In this series 21 cases of SW 21-OHD except one, whose treatment was refused by her parents, were all survived from the adrenal crisis.

Generally, during the replacement therapy, the levels of serum 17-OHP and T were dropped down to normal range. The signs of virilization could be improved. The second sexual characteristics for female patients appeared. For example, only 2 cases out of 87 female cases of SV had poor breast development (II stage) before treatment. However , all cases of these patients had much better breast development (III-V stage) when they reached the age of puberty. The menstruation was also significantly improved. 13 out of 18 cases of female and 1 case of male patients, who had married, had their own children.

Corrective surgery was necessary for female patients who had severe ambiguous external genitalia.

The problems were: 1) the final height was not significantly improved after replacement therapy; 2) the true premature puberty was developed in 16 cases (male 9, female 7) during the replacement therapy.

Molecular analysis of CYP21 gene was also carried out. The subjects included 32 cases of 21-OHD including SW 3, SV 27 and NC 2 cases, 5 pregnancies, and 30 normal controls. DNA was extracted from peripheral blood, amniotic fluid, cord blood or chorionic villi. Small gene mutations were detected with AS-PCR while gene deletion was analyzed with Southern blot. Point mutations at Exon4 and $Exon8_{318}$ were also detected with ASO probe dot blot hybridization. Results showed that mutation frequencies in affected alleles: deletion 18.8%, Exon4 39.1%, Intron2 15.6%, Exon7 4.7%, $Exon8_{318}$ and $Exon8_{356}$ 3.1% respectively, Exon3 and Exon6 1.6% respectively, Exon1 0%, and the undetectable accounting for 12.5%. The mutation frequencies in affected alleles were different from those in literatures, except gene deletion. The differences were most probably caused by the differences in clinical types among the subjects studied. All the detected parents were heterozygotes concordant with their children. ASO probe dot blot hybridization had the same results as AS-PCR. The prenatal genetic diagnosis methods seemed to be practicable clinically, but we should do much more efforts to make it more simple and more accurate.

11.3 INTRAUTERINE GROWTH RETARDATION AND HEALTH IN ADULT LIFE

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The "fetal origins" hypothesis postulates that conditions, most likely nutritional, "program" the fetus for the development of chronic diseases in adulthood. Many observational studies show that intrauterine growth retardation is associated with an increased risk of adult stroke, coronary heart disease, hypertension, insulin resistance and Type 2 diabetes. Experimental animal studies show metabolic, endocrine and structural changes, induced by nutritional insults during critical periods of early development, can have lifetime consequences.

Critiques written against this hypothesis mainly come from 3 perspectives: (1) the validity of the association between restricted fetal growth and the risk of these diseases; (2) the causal vs noncausal nature of the association; and (3) the quantitative importance of the association for public health.

Despite these concerns, the fetal origins hypothesis has been valuable in spawning basic scientific investigation to understand the mechanisms underlying the observed associations between restricted fetal growth and adult chronic diseases. Such as understanding may provide valuable clues both to optimizing fetal growth and to preventing these diseases, even if they are not casually related.

Data from local studies will be presented.

11.4 Proapective study of normal pubertal development in Daqi region

YF Shi

(No abstract available)

12.1 SEXUAL DIFFERENCES IN BONE METABOLISM AND MEASUREMENTS

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Objective: To investigate the age-related trends in bone markers, bone size and bone mass at lumbar spine and left hip in both genders aged 20 to 80 years. **Methods:** 750 females and 450 males recruited, from community were examined for 3 bone resorption markers inurine and 2 bone formation markers in serum in a cross-sectional fashion. Projectile bone areas (BA), femoral neck diameter, bone mineral content (BMC) and density (BMD) were measured. Lateral scans were obtained in 1/3 of the subjects to determine the proportion of BMC in vertebral body of L3. **Results:** Both females and males showed decreasing BMC and BMD with increasing age. However, although all the bone markers increased after menopause in females, they decrease with increasing age in males. Interestingly, higher bone marker values were associated with lower BMD in both genders, after age and weight adjustment. The diameter of femoral neck and the BA of lumbar spine vertebrae both increased with aging in males but not in females. The proportion of BMC located in the L3 vertebral body was less in females throughout the age spectrum. It was even less with increasing age in females, but not in males. **Conclusion:** The pathogenesis of age related bone loss is different in the two genders. Males seem to have relatively more formation deficit while females seem or have excessive bone resorption when they age. The female genders also had smaller increase of bone size than males, and less favorable distribution of bone mass of the lumbar vertebrae. All these factors may have played roles for the higher fracture rates in females.

12.2 EFFECT OF TETRACYCLINE-ESTRONE ON OVX RATS IN VIVO AND ONOSTEOCLASTIC LIKE CELL(OLC) FORMATION AND APOPTOSIS IN VITRO

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Objective: To investigate the effect of tetracycline-estrone on OVX rats and its preliminary effect on osteoclast in vitro. **Methods:** Sixty, three months old Wistar rats were randomly divided into five groups. Group S(sham-operated rats),group OVX(ovx rats), group E(OVX rats treated with estrone),group T(OVX rats treated with tetracycline), group TE(OVX rats treated with tetracycline-estrone). 13 weeks later, rats were euthanized, the detached femoral trabecular was studied by bone histomophometry while the bone marrow cells were cultured to investigate OLC formation and its apoptosis with the method of TRACP , TUNEL and Hoechst33258 stain. **Results:** tetracycline-estrone may effectively prevent trabecular bone lose after OVX. In vitro study showed that tetracycline-estrone may significantly inhibit OLC formation and stimulate its apoptosis. **Conclusion:** tetracycline-estrone may be a new choice for the treatment of postmenopausal osteoporosis.

12.3 Nutritional aspects of Osteoporosis : Studies in Southern Chinese

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Other than genetic factors, nutrition is one of three modifiable factors, together with physical activity and hormones, that determines bone mineral density. Plant-based diets, protein intake, isoflavone intake, calcium, phorphoms, magnesium, potassium, sodium, zinc, copper, manganese, vitamins D, C, and K have all been shown to affect either bone mineral density, attainment of peak bone mass, rate of bone loss, or risk of fractures. Studies in Southern Chinese have documented low calcium intake to be a risk factor for hip and vertebral fractures, while the beneficial effect of calcium supplementation have been studied in children and elderly women. Determinants of bone mass have been studied in children, young women, and the old-old population in Hong Kong. Calcium intake was a significant determinant only for women aged 21-40 years. The VDR polymorphism (BB genotype) associated with low bone mineral density was virtually non-existent in the Southern Chinese population. Although habitual calcium intake may be low, calcium absorption was higher in those children with habitually low intake compared with those with high intake, while calcium supplementation reduces the calcium absorption rate. Similar findings were observed in elderly osleoporotic women. Dietary soy isoflavone contribute to BMD in menopausal women, while vegetarianism is associated with poorer bone health in Southern Chinese in both Taiwan and Hong Kong. The positive nutritional features for bone health in this population include high consumption of fruits, vegetables, and soy products, and low prevalence of BB genotype of the VDR polymorphrism, while adverse features include low calcium, high sodium intakes, and possibly vegetarianism.

12.4 Osteoporosis in Men

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Osteoporosis is a public health problem in men. When a male enters the consulting room the doctor thinks about cardiovascular disease, lipids, hypertension, alcohol and tobacco abuse, prostate cancer, but not about loss of height, kyphosis, hypogonadism, and asymptomatic fractures. It is unlikely that hypertension or hypercholesterolemia would left untreated in a man discharged from hospital following a myocardial infarction, this is unthinkable. However, only 10-20% of women, and probably fewer men with osteoporosis and fractures are investigated or treated despite the fact that a prevalent fracture is a predictor of further fractures. The reason for this is historical; osteoporosis was unstudied by us as medical students, methods for measuring bone density were not available and no drugs were available to reduce fracture risk. So, osteoporosis and fractures were believed to be 'normal' ageing, an inevitable and untreatable consequence of ageing rather than a disease. This is wrong.

One third of all hip fractures occur in men. The mortality in men following hip fracture is twice that of women. The prevalence of spine fractures is similar to women, the incidence is half that of women except in 80+ yearolds where it is similar. The amount of trabecular bone loss is similar in men and women but there is less disruption of architecture as bone loss proceeds by thinning in men rather than increased resorption and trabecular perforation as seen in women. Greater age-related periosteal apposition in men compensates for bone resorption on the endocortical (inner) surface maintaining cortical thickness.

Men with spine fractures have reduced vertebral width, men with hip fractures have reduced femoral neck width; the result of reduced region specific growth, age-related periosteal apposition or both. Reduced vBMD is due to reduced accrual, excessive bone loss or both. Testosterone, growth hormone (GH), and insulin like growth factor 1 (IGF-1) deficiency may reduce periosteal expansion during growth and ageing (producing smaller bone size) and reduce bone formation during ageing in the basic multicellular unit (BMU). Estrogen deficiency may result in longer bones (due to delayed epiphyseal closure) and reduced trabecular thickening during growth, and increases remodeling during ageing. Secondary hyperparathyroidism in old age increases remodeling, endocortical resorption and intra-cortical porosity. Management of osteoporosis should focus on identifying removing causes for bone loss. There is now evidence that alendronate reduces spine fracture rates in men and some unpulbished work suggesting a reduction in spine fractures using intermittent PTH. At this time, the best treatment option for men with osteoporosis is the use of a bisphosphonate such as alendronate. Risidronate has been reported to reduce spine fractures in men taking corticosteroids. There is no evidence that calcitriol or fluoride reduce fracture risk in men. Testosterone should be considered if hypogonadism is confirmed. Vitamin D (ergocalciferol, not calcitriol) should be given if vitamin D deficiency (low 25 OHD3) is present.

01 ORAL SPRAY INSULIN IN PATIENTS WITH TYPE 1 DIABETES: COMPARISON WITH SUBCUTANEOUS INSULIN

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Objective: Insulin therapy by subcutaneous injection is the standard treatment for patients with Type 1 diabetes. Recently, a viable alternative has been developed in the form of an insulin-ejecting aerosol spray for oral insulin delivery. With this system insulin is delivered into the mouth at high speed and is absorbed through the oropharyngeal mucosa into the circulation. ***Methods:*** In the present study we aimed to evaluate the metabolic effect of oral spray insulin compared to subcutaneous insulin in patients affected by Type 1 diabetes. The study protocol was designed to compare blood glucose, insulin and C-peptide levels in 9 patients treated with subcutaneous or oral insulin on two consecutive study mornings. On day 1 patients were treated with their usual subcutaneous regular insulin regimen. On day 2 patients received oral spray insulin. In both mornings of days 1 and 2 patients received 125 mL of a standard meal. Patients were sampled up to 4 hours to evaluate blood glucose, plasma insulin and C-peptide. No basal insulin was administered to patients in the morning of the test. In 3 male patients affected by Type 1 diabetes (aged 28, 34, 35 years) pre-meal oral insulin treatment was prolonged for two consecutive days. In these patients we blood glucose levels were monitored throughout the day for three consecutive days by the recently developed glucose sensor monitoring system. ***Results:*** There were no significant differences in the blood glucose, insulin and C-peptide levels measured after treatment with subcutaneous insulin or oral insulin. In the 3 patients who received oral insulin for two days and who were monitored using the glucose sensor, no significant differences were observed in blood glucose levels with either oral or subcutaneous insulin.
Conclusions: We conclude that insulin via the buccal spray formulation is effective as the subcutaneous route in lowering blood glucose levels.

02 MUTATIONS IN THE HEPATOCYTE NUCLEAR FACTOR-1α GENE IN CHINESE MODY FAMILIES: PREVALENCE AND FUNCTIONAL ANALYSIS

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Maturity-onset diabetes of the young (MODY) is an autosomal dominant form of diabetes characterized by an early age of onset (usually <25 years). We screened for mutations in the hepatocyte nuclear factor (HNF)-1α (MODY 3) gene in 50 unrelated Southern Chinese families, which fulfilled the minimum criteria for MODY: two generations of type 2 DM with at least one member diagnosed under the age of 25 years. The 10 exons, flanking introns and promoter region of the HNF-1α gene were amplified by polymerase chain reaction and sequenced directly. Functional properties of the mutant proteins were investigated using site-directed mutagenesis and luciferase reporter assay. Six of the 50 (12%) subjects were found to have mutations, including one novel nonsense mutation Q176X, one novel intronic mutation IVS7-6 G→A and 4 reported mutations (frameshift mutation P379fsdelCT, nonsense mutation R171X, missense mutation G20R and P112L). The expression levels of wild type and mutant proteins in HeLa cells were similar except for R171X and Q176X which were not detected with the C-terminal antibody. The mutations locating in the coding region were found to have decreased trans-activating activity. The intronic mutation cosegregated with diabetes in the family, created a potential splice acceptor site and might alter the splicing of the HNF-1α mRNA. In conclusion, mutations in the HNF-1α gene appear to be an important cause of MODY in Southern Chinese. The mutations may affect normal islet function by altering the expression of the target genes.

03 TYPE 2 DIABETES IN CHILDREN (ANALYSSIS OF 34 CASES)

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Objective: To investigate the characteristics of type 2 diabetes in children. **Methods:** 34 cases of type 2 diabetes in children were analyzed since 1991—March,2001. **Results:** There were 16 boys &18 girls. Age range: 8 years—14 years 35.3% without diabetic symptoms. Obese children constituted 82.3%. Acanthosis Nigricans occurred in 9 cases. 55.9% had positive family history, most of them had parental &maternal patients, or more than 2 pts. in one family. DKA occurred in3 cases. **Complications:** Fatty liver 6 cases, hypertension 5 cases. **Treatment:** put on diet &exercise were main treatment, DKA had been treated with insulin, oral drugs usually used metformin. There were 2cases did not receive any drugs. **Conclusions:** Type 2 diabetes in children usually occurred in elder & obese children; Acanthosis nigricans are typical signs; Patients have strong positive family history; DKA occurred less than type I diabetes; Insulin dosage is lower with shorter course; Some patients could be put on diet & exercise along without any drugs; **Prevention:** Primary & middle school student should be screened every year especially those obese children or with positive family history; A scientific life style & nutritional knowledge should be understand by all the people.

04 Is It possible to Evaluate Pancreatic β-cell Function by Oral Tolerance Test?—Lessen from 468 Non-Diabetics of Pima Indians

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Objectives: To introduce a pair of insulin sensitivity and pancreatic ß-cell function (BCI) indices: an insulin action index, IAI=1/(FPGxFINS) and a modified β-cell index, MBCI=(FPGxFINS)/(PG2H+PG1H-2FPG), and to investigate if contribution of this pair of indices to changes of plasma glucose level were surpass that of some other commonly used pair of insulin resistance index (IR) and BCI indices. **Methods**:460 non-diabetics of Pima Indians (333 subjects of NGT and 136 subjects of IGT) were included. All of them were participants of a longitudinal study on DM. Insulin sensitivity (M, mg/kg/min) was evaluated by hyper-insulinemic euglycemic clamp technique. Other indices used in this paper were: Homa_IR, Homa_ β, ΔI30/ΔG30. Partial correlation, general linear regression and stepwise regression analysis were performed with SAS software. Power of each combination of IR and BCI indices were evaluated according to their contributions to change of glucose levels. **Results:** (1) After adjustment of influence of M, β-cell function evaluated by AIR, ΔI30/ΔG30, ΔI60/ΔG60 and MBCI in IGT+ NGT group were 70%,75%,65% and 51% vs 100% (p=0.0001), however Homa-β still failed to make this difference (89% vs 100%, p=0.057). (2) AIR, ΔI30/ΔG30, Homa-β and MBCI were all significantly correlated with PG2h in IGT group After the adjustment of M (r= -0.2965,-0.2999,-0.2917 and –0.3698,p=0.0001), however the result changed in NGT group (r=-0.0565 p=0.32, r=0.0103 p=0.86, r=0.2974 p=0.0001 and r=-0.4288 p=0.0001).(3)In general linear regression, M+AIR, M+ΔI30/ΔG30, M+Homa and **M+MBCI** could explain 0.21,0.18,0.21 and **0.36** change of PG2h. When replace M by Homa-IR, these pairs of IR and BCI could explain 0.21, 0.20 0.27 and 0.55 change of PG2h. **Conclusion: (**1)Homa insulin resistance index combined with AIR or Homa- β or ΔI30/ΔG30 could only explain 21% to 27% changes of PG2H, however an modified index of β-cell function, MBCI=(FPG×FINS)/(ΔG1H+ΔG2H), combined with IAI=1/(FPG×FINS) could explain about 55% changes of PG2H, which indicates it is possible to evaluate IR and β-cell function based on OGTT and MBCI is a better index of β-cell function than AIR or Homa_ β or ΔI30/ΔG30. (2) AIR,ΔI30/ΔG30 should not be used in NGT subjects because they were not significantly correlated with glucose changes after the adjustment of insulin sensitivity. (3) To evaluated β-cell function, influence of insulin sensitivity must be adjusted.

05 EFFECT OF NUCLEAR FACTOR-KAPPA B ON THE DIABETIC NEPHROPATHY

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Objectives: To study the effects of nuclear factor-kappa B(NF-κB) on the diabetic nephropathy(DN). **Methods:** The Wistar rats were divided into 3 groups, group A was the normal rats, group B the diabetic rats without any therapy, and the group C the diabetic rats treated with PDTC(inhibitor of NF-κB). At the 18th week, the kidneys were taken out from all the rats to measure the NF-κB activity by electrophoretic mobility shift assays(EMSA) , Ang II level, and the mRNA expressions of AT1R and fibronectin (FN); The creatinine of blood and urine, and the urine albumin excretion (UAE) were measured; The glomerular basement membrane (BM) thickening and mesangial matrix(MM) density(MM area/mesangial area) was observed. **Results:** NF-κB activity of renal tissue in group B was higher significantly than that in group A($P<0.01$), and in group C lower significantly than in group B ($P<0.01$). The clearance of creatinine in group B was lower significantly than that in group A ($P<0.01$), and in group C higher significantly than in group B ($P<0.01$). UAE, the expression of renal FN mRNA, GBM thickening and MM density in group B were higher significantly than those in group A ($P<0.01$), and in group C lower significantly than in group B ($P<0.01$). The renal Ang II levels in group C lower significantly than that in group B($P<0.05$), and there was no difference between in group B and in group A($P>0.05$). The expression of renal AT1R mRNA in group B was lower significantly than that in group A ($P<0.01$), and in group C showed a tendency to decrease when compared with in group B($P=0.088$). **Conclusions:** NF-κB is activated in the diabetic kidneys. The inhibition of NF-κB can retard the development of DN, decreases the Ang II level and expression of FN mRNA, also cause a tendency to decrease the expression of AT1R mRNA in diabetic kidneys. Our data suggest that NF-κB may play an important role in the development of DN.

06 GENE EXPRESSION PROFILING OF SKELETAL MUSCLES IN TYPE 2 DIABETIC PATIENTS

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Objective To explore the gene expression profiles of skeletal muscles in type 2 diabetic patients by fluorescence-labeled DD-PCR and expression cDNA microarrays. **Methods** Biopsies of muscle abdominis rectus were undergone in normal controls and type 2 diabetic patients who underwent elective abdominal surgery. Four 5'-arbitrary primers and 4 tetramethylrhodamine-labeled 3'-anchored primers were used in RT-DDPCR. The differentially expressed bands were reamplified, sequenced and analyzed by BLAST. The cDNA microarray spotted 2024 genes (doubled, 4096 spots) was applied for investigating the gene expression difference in m.abdominis rectus taken from normal controls and type 2 diabetics. **Results** Rab2 gene, a small GTP binding protein, along with 2 contractile proteins, nebulin and titin, was down-regulated in type 2 diabetic subjects by DD-PCR while 31 genes were differentially expressed in diabetic patients by microarrays. 19 of these genes were down regulated including glycogen phosphatase, stearoyl-CoA desaturase (Scd), protein kinase C delta type and skeletal muscle alpha-actin while other 12 genes were up-regulated including protein tyrosine phosphatase, the precursor of epidermal growth factor receptor, NADP(H):quinone oxireductase and collagen alpha-2 type I genes. **Conclusion** The application of DD-PCR and microarrays significantly facilitates the identification of new candidate genes and possible pathways that may be involved in the pathogenesis of type 2 diabetes mellitus.

07 THE FREQUENCY OF HIGH AVIDITY T CELLS DETERMINES THE HIERARCHY OF DETERMINANT SPREADING OF T CELL AUTOIMMUNITY IN NOD MICE

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Objective: To gain insight into the factors that determine the hierarchy of determinant spreading of T cell autoimmunity in NOD mice. **Methods**: We characterized spontaneous T cell autoimmunity to a panel of β cell autoantigens in type 1 diabetes-prone nonobese diabetic mice (NOD) by ELISPOT assays and their binding affinity to I-Ag7 by competitive binding assays. Furthermore, we determined the precursor frequency and functional avidity of the T cells recognizing these antigenic peptides by ELISPOT and lymph node T cell proliferation assays after immunization of in pro-diabetic NOD mice with a single antigenic peptide. **Results**: We observed that T cell autoimmunity gradually spreads from a β cell determinant, which had the largest precursor pool of high avidity T cells, to β cell determinants with progressively smaller and lower avidity T cell precursor pools in NOD mice. **Conclusion**: This correlation between the sequential development of spontaneous T cell autoimmunity and the frequency and avidity of autoantigen-reactive T cells suggests that the extent to which T cells were negatively selected by the self-determinants is the key factor determining the hierarchy of determinant spreading of T cell autoimmunity to β cell antigens in NOD mice.

08 VARIATION IN GLYCOSYLTRANSFERASE ACTIVITY IN THE HAMSTER OVIDUCT DURING THE ESTROUS CYCLE PARALLELS THE GLYCOSYLATION PATTERN OF OVIDUCTIN

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Objective: The protein and gene expression of hamster oviductin and the enzyme activities of several glycosyltransferases, which are responsible for assembling mucin-type carbohydrate chains, were investigated in the oviduct throughout the estrous cycle. **Methods:** The protein expression of oviductin was investigated by quantitative immunocytochemistry using a monoclonal and a polyclonal antibody prepared againstthe glycosidic epitope and a portion of a polypeptide core of hamster oviductin, respectively. The gene expression of oviductin was studied by Northern blot analysis. The enzyme activity of several glycosyltransferases including core 2 β6-GlcNAc-transferase, core 4 β6-GlcNAc-transferase and α2-fucosyltransferase was measured by HPLC and Dowex enzyme assays**. Results and Conclusion:** Results from our immunocytochemical studies using both monoclonal and polyclonal antibodies suggest that glycosylation of hamster oviductin is under hormonal control. Northern blot analysis revealed that the mRNA expression of the oviductin gene remained constant throughout the estrous cycle. Enzyme assays revealed that core 2 β6-GlcNAc-transferase activity, which is responsible for the addition of GlcNAc to core 1, Galβ1-3GalNAc, was regulated throughout the estrous cycle with high activities at proestrus and estrus and low activity at diestrus 1. An increase in glycosyltransferase activity at the time of ovulation suggests that glycosylation of hamster oviductin may be important in fertilization. (Supported by the Canadian Inst. of Health Research)

09 The role carbohydrate moieties of ZIF-1 in inhibiting spermatozoa-zona pellucida binding

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Objective: ZIF-1 is a glycoprotein in human follicular fluid that suppresses spermatozoa-zona binding, a critical step during fertilization. It is well accepted that mammalian gamete binding requires the appropriate recognition of specific carbohydrate sequences expressed on the zona pellucida. This study determines the role of carbohydrate moieties of ZIF-1 in inhibiting spermatozoa-zona pellucida binding.
Methods: Spermatozoa samples were obtained from men attending our infertility clinics. ZIF-1 was purified from human follicular fluid obtained from women during oocyte retrieval for assisted reproduction treatment. N-Glycosidase F Deglycosylation Kit (Roche) was used to deglycosylate the glycoprotein. The resulting carbohydrate moieties were purified by gel filtration. The effect of the carbohydrate moieties on spermatozoa-zona binding was determined by hemizona binding assay. The effect of various monosaccharides on the binding of ZIF-1 to spermatozoa was studied by competition-binding assay.
Results: Deglycosylated ZIF-1 did not inhibit the spermatozoa-zona pellucida binding. In fact, deglycosylated ZIF-1 did not bind to the treated spermatozoa. Among the various monosaccharides studied, mannose, fucose, acetylgalactosomine and acetylglucosamine compete with iodinated ZIF-1 for binding to human spermatozoa.
Conclusion: The carbodydrate moieties of ZIF-1 were important for the biological activity of the glycoprotein. Mannose, fucose, acetylgalactosomine and acetylglucosamine are likely to play a role in binding of ZIF-1 to the sperm membrane.
Acknowledgement
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010 DOWN-REGULATION OF A NOVEL TUMOR SUPPRESSOR GENE, PROTEIN TYROSINE PHOSPHATASE GAMMA (PTPγ), IN HUMAN BREAST BY ESTROGENICALLY ACTIVE AGENTS

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PTPγ is implicated as tumor suppressor gene. Zeranol (Z), a nonsteroidal estrogenically active growth promoter, is used in U.S. beef. **Objectives:** Investigating PTPγ expression by estradiol (E2) and Z; Examining the function of PTPγ. **Methods:** RT-PCR for evaluation of PTPγ mRNA in normal and cancerous human breast (nHBT, cHBT) and E2- and Z-treated nHBT; Immunohistochemistry for PTPγ in HBT; ^{3}H-thymidine incorporation for growth of MCF-7 stably transfected with PTPγ. **Results:** PTPγ mRNA was 50–60% lower in cHBT than in nHBT, and was suppressed by E2 and Z (30nM; 24h) in cultured nHBT by ~80%; PTPγ was immunolocalized to nHBT epithelium, and E2 or Z diminished PTPγ staining; PTPγ-transfected MCF-7 grew slower than mock-transfected cells. **Conclusions:** PTPγ is down-regulated by estrogenically active agents, and may be a biomarker for health risk from endocrine disruptors. PTPγ is a tumor suppressor gene in human breast. (Dept. of Defense Breast Cancer Res. grants DAMD8140 & DAMD0391)

011 MICROENCAPSULATION OF RAT TESTICULAR TISSUE: IN VITRO AND IN VIVO STUDIES

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Objective: To investigate the immunoisolation and the function, morphological changes of the microencapsulated rat testicular tissue. **Methods:** Using alginate beads (modified method) and diffusion rates were measured. The microencapsulated neonatal rat testicular tissue pieces were cultured and the testosterone concentration was measured. Those were transplanted into the peritoneal cavity of the emasculated Wistar rats. After 2 and 4 weeks, the microencapsules were taken out and the tissues were examined with electron microscope. **Results:** The microcapsules prevented large molecules (hemoglobin) permeation through the membrane, so it has immunoisolation property. but testosterone was diffused in and out freely. The testosterone concentration of the culture media were 16.14 ± 4.93 nmol/L and after HCG stimulation,the concentration of the testosterone of the encapsulated group was 28.9 ±13.4, while was higher than the nonencapsulated group. The encapsulated testicular tissues were taken out after transplantation and all showed survived well under morphological observation. **Conclusion:** Microencapsulation of the rat testicular tissue shows good immunoisolation function both through in vitro and in vivo studies and may be used for further research and in clinical practice.

012 EFFECTS OF FOLLISTATIN ON TESTOSTERONE SECRETION OF RAT LEYDIG CELLS IN VITRO

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Objective: Follistation (FS) was isolated and purified from porcine and bovine follicular fluid in 1987, and showed widely biological effects on hormone release of anterior pituitary. However, it is lack of identification of the role of FS on testicular function. The aim of this study is to investigate the effects of rhFS-288 on testosterone secretion of rat Leydig cells in Vitro. **Methods:** Male Wistar rats (B.W. 220-250g) were executed, and Leydig cells were isolated from testes by a discontinuous Percoll gradient procedure. Purified cells were inoculated into 24-well plate(10^5 cells/ml/well) and maintained for 24 hours in an incubator with temperature of 37°C and containing 5% of CO_2. rhFS-288 and Ca^{++} in concentration of 0.0, 0.1, 1.0, 10.0 and 100.0(ng/mL for FS, and mmol/L for Ca^{++}) were added to the well (n=4 in each concentration) separatively or jointly in both basic condition (without hCG) and stimulating condition (1.0 IU/Ml of hCG) to observe the changes of testosterone levels in media. **Results:** ① Testosterone release was significantly inhibited by rhFS-288 in dose of10.0and 100.0 ng/mL (both $P<0.05$). ② Calcium in dose of 10.0 mmol/L remarkably suppressed the testosterone secretion ($P<0.05$), and the suppressive effect was escaped during the calcium dose up to 100.0mmol/L($P<0.01$). Under stimulating condition, 1.0 and 10.0 mmol/L of Ca^{++} both inhibited testosterone Secretion (both $P<0.05$). Whereas, 100.0mmol/L of Ca^{++} relieved the inhibitory effect for some extent, and at that time the testosterone level was still lower than those in 0.0 and 0.1 mmol/L of Ca^{++}. **Conclusions:** ① rhFS-288 inhibits testosterone secretion of Leydig cells in vitro in a dose-dependent manner. ② Calcium is thought to be the second messenger of FS action. ③ The effects of FS on Leydig cells minght be a paracrine fashion.

013 EFFECTS OF ENDOTHELIN-1 ON RAT LEYDIG CELLS

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Objective: To investigate the mechanism of Endothelin-1 (ET-1) on testosterone (T) synthesis by rat Leydig cells. **Methods:** Purified rat Leydig cells were exposure to ET-1 (0.01~100 ng/ml) and hCG (0~100 ng/ml). Then hCG (1 ng/ml), inhibitors of PKC (GF 109203X 1 uM) and PKA (H89 10 nM), Ca^{2+} channel antagonist (nifedipine 100 uM) and Ca^{2+} (3 mM) were added to the Leydig cells treated with ET-1, respectively. After the cells were treated for 24 hs, the medium was collected and T was determined by RIA. StAR and P450scc mRNA expression levels in ET-1 treated cells were quantified by Northern Blotting and quantitative RT-PCT. **Results:** 1) ET-1 increased T secretion up to 3 fold of the control; 2) The secretagogue action of ET-1 was blocked by GF 109203X, but not by H89 and nifedipine; 3) the concentrations of calcium in the media did not modify T response to ET-1; 4) The mRNA expression levels of StAR and P450scc were increased by 55.6% and 51.5% respectively. **Conclusions:** 1) ET-1 increased T secretion at a dose-dependent manner; 2) The main signal cascade of ET-1 was PKC activation, but L-type Ca^{2+} channel was not involved. 2) ET-1 increased mRNA expression levels of StAR and P450scc.

014 ENVIRONMENTAL ENDOCRINE DISRUPTORS, TRIPHENYLTIN AND TRIBUTYLTIN, INDUCE LEYDIG CELL APOPTOSIS

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Objective: Triphenyltin (TPT) and tributyltin (TBT) are used in a variety of consumer products and industrial applications. Here, we investigate the apoptotic effect of TPT and TBT on rat Leydig cells cultured *in vitro*. **Methods:** (1) Leydig cells were treated with various concentrations of TPT and TBT ($2x10^{-8}$ M to $8x10^{-8}$ M) for a few days, and then the cell survival rate was determined using Trypan blue exclusion method; (2) Apoptotic effect of TPT and TBT on Leydig cell was determined by DNA ladder formation and Annexin V-GFP/PI staining of the cells; (3) To assess the roles of Ca^{++} and protein kinase C (PKC) in TPT- and TBT-induced apoptosis, cells were co-treated with TPT/TBT and Ca^{++} chelating agents EGTA or BAPTA, and then the cell survival and the intracellular Ca^{++} concentration were determined. **Results:** (1) A dose-dependent reduction in the cellular survival was observed at doses of TPT and TBT ranging from $2x10^{-8}$ M to $8x10^{-8}$ M. TPT and TBT appeared to be similar in the potency to reduce the cell viability. These effects were also time-dependent, which the cell survival was 73.7%, 49.7%, 42.6% and 30.4% after treatment with $4x10^{-8}$ M TPT for 24 h, 48 h, 72 h and 96 h, respectively; (2) The suppressive effect of TPT on cell survival was caused by apoptosis, as evidenced by DNA ladder formation and Annexin V-GFP/PI staining of the cells; (3) The apoptotic effect of TPT on Leydig cell might be mediated by intracellular Ca^{++} elevation because chelating agents EGTA and BAPTA partially blocked the apoptotic effects in the cells induced by TPT and TBT, and the intracellular Ca^{++} concentration increased after TPT and TBT treatment. **Conclusion:** Environmental endocrine disruptors TPT and TBT directly induce apoptosis in Leydig cells and these effects may be mediated by intracellular Ca^{++} elevation.

015 STUDY ON THE GENE EXPRESSION PROFILE OF ADRENAL CORTICOADENOMA WITH CUSHING's SYNDROME AND PHEOCHROMOCYTOMA AND CLONE NOVEL FULL-LENGTH cDNAs

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Objective: To explore the characteristics of the gene expression in the adrenal corticoadenoma with Cushing's syndrome (Cu) and pheochromocytoma (cdA) and their associated molecular mechanisms. **Methods:** cDNA libraries of human normal adrenal gland (AD), Cu and cdA libraries were constructed. Large-scale expressed sequence tags (ESTs) sequencing, In silico cloning and bioinformatics were used. **Results:** 2,089 and 4,115 ESTs were obtained from Cu and cdA libraries, respectively. Known genes, known ESTs and novel ESTs are 47%, 15% and 13% in Cu and 51.2%, 13.2%, 15.4% in cdA, respectively. Some synthetases such as 3bHSDII and P450c17A in steroidogenesis are expressed higher in Cu than in AD, especially 3bHSDII, but StAR is lower in Cu library. Compared with AD library, 45 and 126 genes were significantly different expressed in Cu and cdA libraries, respectively. Interestingly, some genes associated with cell division were high expressed in the tumors. Those involved in apoptosis and suppressing proliferation, which were expressed in AD, were not found in Cu and cdA libraries. 28 novel full-length cDNAs were cloned from the tumor libraries and also analyzed with bioinformatics. **Conclusions:** The genes involved in cell division and apoptosis, significantly different expressed in the tumors and AD libraries, may play an important role in the tumorigenesis. Some novel genes might be participating in the pathogenesis of the tumors.

016 EFFECTS OF TRANSFORMING GROWTH FACTOR α ON THE PROLIFERATION OF HUMAN PHEOCHROMOCYTOMA CELLS IN PRIMARY CULTURE

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Objective: To establish a primary culture of human pheochromocytoma cells, and observe the effects of transforming growth factor α (TGF α) on the cell proliferation. **Methods:** Pheochromocytoma cells from human pheochromocytoma tissue obtained during operation were isolated by collagenase I digestion and confirmed by paraformaldehyde-induced histofluorescence of catecholamine, dichromate staining and detection of catecholamine secretion in culture medium by HPLC. The cell proliferation was determined by MTT method after 24 hrs stimulation by various concentration of TGF α (1, 10, 100ng/ml). **Results:** Pheochromocytoma cells were round or polygonal, which had a marked tendency to grow in clumps, and had a rapid multiplication from 3 to 7 days after plating. Short processes were evident on 5-7 days, and thereafter elongated progressively. Finally the processes reached each other and became web-form. The primary culture cells survived for about 4 weeks. Norepinephrine, epinephrine and dopamine were detected in culture medium. Pheochromocytoma cells showed an intensive yellow-green fluorescence after exposure to paraformaldehyde vapor, and showed brown when stained with potassium dichromate. TGF α significantly increased cell number at the concentration of 10ng/ml by 56% over the control. **Conclusions:** The primary culture of human pheochromocytoma cells was established, and TGF α can stimulate the cell proliferation.

017 OVEREXPRESSION OF HUMAN PITUITARY TUMOR TRANSFORMING GENE INHIBITS TUMOR CELL GROWTH BY P21 $^{WAF1/CIP1}$ - DEPENDENT AND -INDEPENDENT MECHANISMS

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Objective: To investigate the inhibitory effect of human pituitary tumor transforming gene (hPTTG) on tumor cell growth. **Methods:** (1) Cloning of hPTTG by RACE and determination of the tumor cell expression by Northern blot; (2) Construction of pCMX-PTTG-GFP and pIRES*neo*-PTTG expression vectors; (3) Cell transfection and subcellular distribution of PTTG determined by confocal laser scanning microscopy; (4) Transfection of hPTTG into A549 and HeLa cells and then measurement of the cell growth properties; (5) Determination of mRNA and protein expression levels of p53 and p21$^{WAF\ 1/CIP\ 1}$ by Northern and Western blots; (6) Construction of p21$^{WAF1/CIP1}$ promoter controlled luciferase expression vector and analysis of luciferase activity. **Results:** (1) A 719-bp cDNA with an open reading frame of 609 bp encoding a 202 aa protein (GenBank accession number AF095287) was isolated from human pituitary tumors. A Northern blot analysis revealed that the strongest expression of hPTTG in THP-1 and Raji cells, and middle to weak expression in CEM, HeLa, AR230, DLA-1, H1299, HepG2 and A549 cells were detected; (2) The subcellular distribution of hPTTG was dependent on cell type, was predominantly nucleus in HeLa, Cos-7 and DU145 cells, and a diffuse distribution in both the nucleus and cytoplasm in A549, DLD-1 and NIH3T3 cells; (3) An overexpression of hPTTG significantly inhibited cell growth, which was determined by the adherent cell growth properties, colony formation in soft agar and [^{3}H]thymidine incorporation in HeLa and A549 cells; (4) The hPTTG overexpression increased both the p21$^{WAF\ 1/CIP\ 1}$ mRNA and protein levels in A549 cells, but not in HeLa cells; (5) The increased expression of p21$^{WAF\ 1/CIP\ 1}$ mRNA was regulated at transcription level and was independent on p53 because the luciferase activity was increased after the co-transfection of hPTTG and p21$^{WAF\ 1/CIP\ 1}$ promoter fragments with and without a p53 binding sequence. **Conclusion:** An overexpression of hPTTG inhibits the cell growth due to different mechanisms, which are p21$^{WAF\ 1/CIP\ 1}$-dependent and -independent.

018 EXPRESSION OF THE PITUITARY TRANSCRIPTION ACTIVATOR PIT-1 IN ESTROGEN-INDUCED RAT PROLACTINOMAS AND HUMAN PITUITARY ADENOMAS

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Objective: To study the role of Pit-1 in estrogen-induced rat prolactinomas and human pituitary adenomas. **Methods:** Estrogen-induced rat prolactinoma models were established. Human pituitary adenomas were obtained from 36 patients. The expression of rPit-1 mRNA in rat prolactinomas and the expression of hPit-1 mRNA in pituitary adenomas were studied using RT-PCR method. **Results:** rPit-1 mRNA levels in prolactinoma group were significantly greater than those in control group ($P<0.01$). Human pituitary adenomas consisted of 14 lactotropic adenomas, 6 somatotropic adenomas, 2 mixed somatotropic -lactotropic adenomas, 11 clinically non-functioning adenomas and 3 ACTH-secreting adenomas. hPit-1 mRNA expressed in all lactotropic, somatotropic and mixed somatotropic-lactotropic adenomas and in some non-functioning adenomas (9/11). No hPit-1 expression was found in ACTH-secreting adenomas. hPit-1 mRNA levels in lactotropic, somatotropic or mixed somatotropic-lactotropic adenomas were higher than those in non-functioning adenomas respectively ($P<0.05$ respectively). Serum prolactin (PRL) levels were positive related with hPit-1 mRNA levels ($\gamma=0.9256$, $P<0.01$) in PRL cell adenomas. **Conclusion:** 1.Pit-1 has a close relationship with PRL gene expression and has a role in estrogen-induced rat prolactinomas. 2. Cell type-specific expression pattern of Pit-1 mRNA in human pituitary adenomas demonstrates the role of Pit-1 in cytodifferentiation of human pituitary adenomas.

019 CELL TYPE-SPECIFIC EXPRESSION OF ESTROGEN RECPTORS α AND β IN HUMAN PITUITARY ADENOMAS

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Objective Estrogen receptor (ER) exists as 2 subtypes, i.e. ERα and ERβ, both of which have high affinity for their ligand, estradiol. In the present study, we examined the expression of ER subtypes in pituitary adenomas to disclose whether the expression of ER subtypes shows cell-type specificity. **Methods** We tested the expression of ERα, ERβ, Pit-1, SF-1, GnRH receptor, LHβ, FSHβ, GH, and PRL, as well as β-actin as a control by reverse transcription-polymerase chain reaction (RT-PCR). Adenoma tissues examined included 8 PRL-secreting adenomas from patients with prolactinoma, 14 GH-secreting adenomas from patients with acromegaly, and 18 clinically non-functioning adenomas from patients without excessive hormone secretion and endocrine manifestations. **Results** All 8 PRL-secreting adenomas expressed ERα predominantly; its expression level was 10^4 order higher than that of ERβ as evidenced by semi-quantitative PCR. Four adenomas from acromegalic patients, which expressed only GH but not PRL, expressed ERβ only or neither of ER subtypes. On the other hand, the rest of the adenomas from acromegaly which expressed both GH and PRL, expressed either ERα and ERβ. Eighteen clinically nonfunctioning adenomas could be classified into 7 GH/PRL adenomas, 8 gonadotroph adenomas and 3 adenomas having characteristics of both lineages, based on the expression of specific genes for each cell type as we reported. All 7 GH/PRL adenomas expressed ERβ, one of which expressed ERα as well. Among 8 gonadotroph adenomas, 4 expressed both ERα and ERβ, 2 expressed ERα only, and 2 expressed none of them. For the rest of 3 adenomas, either ERα and ERβ was expressed. **Conclusion** The expression of ER subtypes is linked to anterior pituitary cell lineage and the results indicated that switching or extinction of the ER subtype expression might occur during terminal differentiation of somatotroph/lactotroph lineage.

020 THE STUDY OF RET PROTO-ONCOGENE REARRANGEMENT IN PAPILLARY THYROID CARCINOMA

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Objective: To investigate the role played by ret oncogene rearrangement in the oncogenesis of papillary thyroid carcinoma(PTC). **Method:** 100 thyroid tissue samples were detected, including 37 of malignant thyroid tumors(27 of papillary carcinoma, 10 of other thyroid cancer), 33 of benign thyroid lesions and 30 of adjacent normal thyroid tissues. Multiplex PCR(MP-PCR) and identification PCR(ID-PCR) were performed with the first strand of cDNA. MP-PCR were performed to screen all the types of ret/PTC. ID-PCR were further performed for the samples which were suggested to habour a ret rearrangement because of the banding pattern of the MP-PCR using primers specific for ret/PTC-1, -2, and –3 respectively. Finally, sequencing analysis was performed on the ID-PCR product from ret/PTC-positive samples by automated direct sequencing. **Result:** The 15 samples which were suggested to harbour a ret rearrangement because of the banding pattern of the MP-PCR were further amplified with ID-PCR.The expected fragments for ret/PTC-1, -2, and –3 were detected in all the 15 samples. 11 of the samples harboured ret/PTC1, 3 were positive for ret/PTC3, and 1 for ret/PTC2. All the rearrangements were clearly identified by automated direct sequencing. All the 15 ret/PTC-positive tissue samples were histologically confirmed to be papillary thyroid carcinomas. Thus, the prevalence of the tumor-specific ret rearrangements in 27 patients with PTC in our study is 55.6%(15/27). **Conclusion:** ret rearrangement is a genetic event that characterizes papillary thyroid carcinomas(PTC). The detection of ret rearrangements will be very useful for the differential diagnosis of PTC. The novel MP-PCR method, adopted in the present study, proved to be be highly sensitive and specific to detect ret/PTC.

021 The *MEN1* polymorphism D418D is associated with sporadic primary hyperparathyroidism

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Background: Sporadic primary hyperparathyroidism (pHPT) occurs separately and in several hereditary disorders including multiple endocrine neoplasia type 1 (*MEN1*). Irradiation to the neck, female sex and age are well-identified risk factors that predispose to pHPT. The *MEN1* gene is the most commonly deranged gene in parathyroid adenomas and contains several polymorphisms including D418D with a prevalence of roughly 50%.
Methods: We genotyped 162 pHPT patients and controls. 114 of the pHPT patients and controls were recruited from a health-screening and were subjected to measurement of bone mineral density (BMD) at the lumbar spine, femoral neck, and total body.
Results: The prevalence of each genotype, i.e. MM, Mc, and cc, was for all pHPT patients: 62%, 29%, and 9%; and for all controls: 32%, 43%, and 25% ($p<0.0004$). In the screening-recruited controls, but not in pHPT patients, the MM genotype was also associated with higher total body bone mineral density (BMD) ($p= 0.01$) and BMD in the femoral neck ($p= 0.02$).
Conclusions: We report that the MM genotype is overrepresented in pHPT patients compared to controls, suggesting a novel marker for pHPT. Furthermore, the MM genotype was associated with higher BMD in the femoral neck and in the total body in the screening-recruited controls.

022 Efficacy of Somatuline in active acromegaly

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Objective: To study the efficacy of Somatuline in active acromegalic patients in 3 months therapy. **Methods:**15 active acromegalic patients were investigated, 5 females and 10 males, aged 24-60(42.1±9.0) years old. 11out of 15 were newly diagnosed and 4 out of 15 had been treated with transphenoidal pituitary adenomectomy.Their GH levels were failure to suppress GH test,and serum IGF-1 levels were above 120ug/L.The MRI were presented as macroadenomas in 11 out of 15 patients. Somatuline 30 mg was administered by intramuscular injection every other week for 2 weeks. Serum GH and IGF-1 levels were measured by radioimmunoassay. **Results:** (1) After first injection, serum GH and IGF-1 levels were decreased by more than 25% in 14 and 12 patients respectively. The serum GH levels were decreased from 50.03±61.89ng/ml in base line to 21.01±30.04 ng/ml in the 6th week and 24. 32±40.67 ng/ml in the 12th week respectively. The IGF-1 levels were decreased from 763.19±455.83 ng/ml in base line to 400.04±319.78ng/ml in the 6th weeks and 382.32±366.83 ng/ml in the 12th week respectively. (2) The symptoms and signs were improved in all patients. (3) The size of the tumors was reduced in 12 out of 15 patients on MRIs. (4) All of the patients felt slight abdominal discomfort at first 2-4 days. The routine blood tests were normal. ECGs were normal.The constructed function of gallbladder were obviously depressed after therapy. **Conclusion:**Somatuline can markedly decreased the serum GH and IGF-1 levels and improved the clinical symptoms and signs in active acromegalic patients.It showed good safety in 3 months therapy.

023 The Etiology and Clinical Characteristics of 408 Cases With Central Diabetes Insipidus

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Objective:To clear the clinical characteristics of central diabetes insipidus.
Methods: The clinical data of 408 cases with central diabetes insipidus (CDI) in Peking Union Medical Collage Hospital(PUMCH) between 1956 and 2000 were retrospectively analyzed. **Results and conclusions**:The results showed that the most common age of onset of CDI was between 8-12 years old in children and 25-35 years old in adults. The causes of CDI are various. Idiopathic CDI was about 52% and CDI caused by tumor in sella region was about 33% in children and in which geminoma is the most common one, and 22% in adults. The percent of trauma induced CDI were more common in adults, about 11%,while it was about 5% in children. All the patients suffered from histiocytosis X were children. When CDI children are accompanied with growth failure of GH deficiency, it is more likely that the patient has an occupying lesion in sella area. If the patient suddenly drinks less water, it may indicate that hypofunction of adrenal cortex has occurred. The MRI examination was the most valuable examination for any tumors in sella region. It should be followed-up every 3 to 6 months for the patients with negative MRI findings. Then positive findings may be found up to 86% within the following 2 years. Our results indicate that periodic clinical follow-up with serial brain MRIs are essential to find a final correct lesion in sella region that was firstly diagnosed as idiopathic DI. The prognosis of the patients with CDI after ADH replacement is satisfactory. For those with brain tumors, if radiation and/or operation therapies were promptly performed, the long-term survival rate may reach 80%.

024 THE EFFECTS OF THYROID HORMONE ON THE PITUITARY-TESTIS AXIS IN VITRO

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Objective: Thyroid hormone is one of the main hormones that control development and metabolism. The phenomenon that male patients with thyroid dysfunction often suffered from hyposexuality and impotency showed that thyroid hormone has some effects on reproductive endocrine system, but the mechanism is still unknown. The present study aimed to discuss the impacts of thyroid hormone on pituitary-testis axis via hyper- and hypo- thyroxinemia animal model. **Methods**: Hyperthyroxinemia and hypothyroxinemia rat models were induced by injecting thyroid hormone and methimazole (ip) for 3 weeks respectively. The rat's pituitary cells were cultured and exposed to a series of concentrations of T_3 (0--10 ug/ml) with or without GnRH. At same time, the rat's Leydig cells were also cultured and exposed to a series of concentrations of T_3 (0--10 ug/ml) with or without hCG. Basal and challenged rLH , rFSH and testosterone levels were measured. The distinction of pituitary cells and Leydig cells' secretion in vitro was evaluated between different thyroid function groups. **Results**: High concentrations of T_3 (1 and 10 ug/ml) showed inhibiting the secretion of rLH ($p<0.05$), but had no influence on the rFSH. The rLH secretion of pituitary cell in both hyper- and hypo- thyroxinemia rats was impaired. There was no significant difference in rFSH secretion between groups. Normal Leydig cells didn't response to the stimulation of T_3. However, an impaired secretion ability of T in basal or in stimulated conditions was found in the Leydig cells from both hyper- and hypo- thyroxinemia rats ($p<0.01$).**Conclusion**: Both hyper- and hypo-thyroxinemia inhibited the secretion of rat pituitary. It seemed that thyroid hormone have a direct effect on the pituitary. The Leydig cells from normal rats had no response to thyroid hormone in vitro, but those from hyper- or hypo- thyroxinemia rats showed impaired testosterone secretion. The phenomenon was more obvious in hypothyroxinemia rat .

025 HYPERGLYCEMIA (HYPOGLYCEMIA) AFFECTS THE EXPRESSION PROFILING OF THE HYPOTHALAMUS –PITUITARY-ADRENAL AXIS

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Objective: To study the gene expression profiling of the hypothalamus-pituitary-adrenal axis by hyperglycemia and hypoglycemia. **Methods:** Adult male monkeys were prepared to be the models of hyperglycemia and hypoglycemia with streptozotozin and insulin respectively. Hypothalamus, pituitary and adrenal were isolated 8-12 hours after hyperglycemia (BG>30mmol/L) or hypoglycemia (BG<1.5mmol/L). Total RNA was prepared and hybridized to 14,000 clones mainly from hypothalamus, pituitary and adrenal tissues. Genes were considered up- or down-regulated if the fold change was greater than 2 or less than 0.5. Dot blot (95 samples) and *in situ* hybridization were carried out to identify the changes of those genes up- (>2) and down-regulated (<0.5) by hyperglycemia and hypoglycemia (bi-directional). **Results:** 141 clones were bi-directionally regulated. Among them, 38 genes were known genes (i.e. PPARr). 2 full-length new genes were cloned (AD00y AF329101 and GMRP-1 AF329102). 255 clones were up-regulated by hyperglycemia and slightly decreased (0.5-0.99) by hypoglycemia; 101 clones were up-regulated by hyperglycemia and slightly increased (1.0-1.5) by hypoglycemia. The result of the dot blot and *in situ* hybridization confirmed most of the bi-directional regulations. **Conclusions:** Hyperglycemia (hypoglycemia) plays an important role in the regulation of gene expression profiling in hypothalamus-pituitary-adrenal axis, especially the genes related to the glucose metabolism. For instance, the uncoupling protein-3 gene was bi-directionally regulated in adrenal, coincident to the report of Dr Havel in his study to the hypothalamus. To illustrate the structures and functions of the bi-directionally regulated genes may contribute to the further understandings to the diabetes mellitus and other diseases.

026 The mechanism by which IFN-γ increases growth hormone promoter activity in IM-9 cell line

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Objective: Human lymphocytes express and secrete GH, and lymphocyte-derived GH could affect lymphocyte function by an autocrine or paracrine way. So the study of GH expression in lymphocytes may be of great importance for understanding the mechanism of the interaction between GH and immune system. Our previous work suggested that interferon-γ (IFN- γ) significantly increase GH promoter activity in human B-lymphoblast cell line IM-9. The objective is to explore the mechanism by which IFN-γ increase human growth hormone (hGH) promoter activity in IM-9 cell line. **Methods:** 1. Insert hGH gene promoter (-484~+2 bp) into pGL3-Enhancer vector. The recombinant plasmid was abbreviated as 484-luc. Then 484-luc was transfected into IM-9 cells. Fifty-six hours after transfection, IM-9 cells were treated with various cytokines, inhibitors and stimulators of intracellular signaling transduction pathway at different concentration. Four hours later, cells were harvested and lysed to measure luciferase activity. 2. Using pGL3-Enhancer vector, we constructed other four expression plasmids: 66-luc, 132-luc, 250-luc, and 380-luc, which contains the –66~+2 bp, –132~+2 bp, –250~+2 bp, and –380~+2 bp sequences of GH gene promoter, respectively. The above four constructs and 484-luc were transfected into IM-9 cells, respectively, to observe their response to the effect of IFN- γ increasing GH promoter activity. **Results:** 1. IFN- γ significantly increased luciferase expression of 484-luc that transfected into IM-9 cells in a dose-dependent manner. When it's concentration was 1000 U/ml, luciferase expression increased by 51%, whereas MAPK pathway inhibitor PD98059 significantly decreased luciferase expression by 48%. In addition, PD98059 completely abolished the stimulating effect of IFN-γ on luciferase expression. 2. IFN-γ (1000 U/ml) had no effects on the activities of luciferase expressed by 66-luc,132-luc, 250-luc and 380-luc that were transfected into IM-9 cells. **Conclusion:** The stimulating effect of IFN- γ on GH promoter activity in IM-9 cells is associated with intracellular MAPK signaling pathway and needs –484~–380bp upstream promoter sequence of GH gene.

027 Study on the Electro-physiological Activity of Arcuate Nucleus in Male Rabbits at Pre- and Post-Puberty Stage.

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Objective: To observe the electro-physiological activity(EPA) of the arcuate nucleus(ARC) in male rabbits at pre-puberty or post-puberty and if the electro-acupuncture(EA) affect the EPA of ARC. **Methods:** To record the dischargements of ARC in per- and post-puberty rabbits by nuclear microacupuncture. And to compare the EPA of ARC in these animals with those after 20 min EA in the points correlation to reproduction. **Results**: The EPA of ARC in 5 pre-puberty rabbits were showed fewer than those in the 5 post-puberty ones($P<0.05$). In 5 post-puberty rabbits experienced 20 min EA, the EPA of ARC were showed fewer than those in the same animals before EA($P<0.05$). But there's no significant difference of the diachargements in the pre-puberty rabbits after 20 min EA ($P>0.05$). **Conclusion:** The activity of ARC in hypothalamus could be detected by this microacupuncture method and testified by observing the variation of EPA of it. According to this way, we find that the activity of ARC, especially its frequency of dischargements , is related to the sexual development and mature. And we also find that EA in those points correlation to reproduction may affect the male hypothalamus-pituitary-testes axis.

028 THE EFFECTS OF HORMONE REPLACEMENT THERAPY ON ENDOTHELIAL FUNCTION IN WOMEN WITH TURNER'S SYNDROME

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Objective: To examine the effect of HRT on endothelial function in women with Turner's syndrome. **Methods:** Seven women with Turner's syndrome (mean age of 29.3 +/- 2.1 years) were studied. Forearm blood flow response to intra-brachial infusion of bradykinin, 10, 30, 100 pmol/min (endothelium-dependent vasodilator), glyceryl trinitrate, 4, 8, 16 nmol/min (GTN; endothelium-independent vasodilator), noradrenaline, 60, 120, 240 pmol/min (NA, alpha-adrenergic receptor agonist) and NG-monomethyl-L-arginine, 1, 2, 4 mol/min (L-NMMA; NO synthase inhibitor) was assessed by venous plethysmography. Subjects were studied on their usual HRT (Study 1), then after 6 weeks off HRT (Study 2) and finally after a further 6 weeks on HRT (Study 3). **Results:** Vasodilator response to bradykinin, expressed as the within-subject mean difference in area under the dose-response curve between Study 2 and Study 1, was significantly diminished (-744.2 +/-287.2, p=0.04), but improved 6 weeks after HRT recommencement. There was no significant change in response to GTN between studies. Vasoconstrictor response to L-NMMA was diminished in Study 2 compared to Study 1 (-100.4 +/- 35.4, p=0.039) and was restored after HRT recommencement (between Study 3 and 2, 117.5 +/- 69.3, p=0.17) whereas there was no significant difference in response to NA between studies. **Conclusion:** HRT leads to improved endothelial function in women with Turner's syndrome.

029 INVESTIGATION OF ANDROGEN RECEPTOR GENE EXPRESSION IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS

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Objective: To investigate androgen receptor (AR) mRNA expression in peripheral leukocytes of healthy men and women and in those of systemic lupus erythematosus (SLE) patients. **Methods:** AR mRNA was determined by RT-PCR and Northern blot, the androgen level in serum was detected by radioimmunoassay. Results: An AR cDNA fragment of the expected size of 390 bp was determined by RT-PCR. 9.4 kb AR mRNA was detected by Northern blot, which was consistent with AR mRNA in human prostate reported by Lubahn et al. The androgen level in SLE patients was lower than that in the control. With the final PCR product, we found that AR expression in SLE patient was much more than that in the normal control. **Conclusion:** By RT-PCR and Northern blot, we have demonstrated AR mRNA expression in human peripheral leukocytes. The effect of androgen on the function of the human peripheral leukocytes and the changes of AR in pathological stages will be investigated. The androgen level in SLE patients was lower than that in the control, but AR mRNA was converse; it may be the result of the up-regulation of AR, but the mechanism needs further investigation.

030 ESTROGEN (E2) AND THYROID HORMONE (T3) INTERACT TO REGULATE HYPOTHALAMIC GENE EXPRESSION AND FEMALE REPRODUCTIVE BEHAVIOR

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Objective: To investigate E2 regulation of hypothalamic gene expression and female reproductive behavior and its interaction with T3. **Methods:** Preproenkephalin(PPE) gene expression and female lordosis behavior in the rat were used as the model system. Transfection assays in cell cultures and gel mobility assays were used to identify functional estrogen response elements (ERE) in the rat PPE gene. PPE mRNA levels in the rat ventromedial hypothalamus (VMH) were analyzed by Northern blot, slot blot and in situ hybridization analyses. Lordosis behavior was analyzed in the female rats and the thyroid receptor (TR) null mice. **Results:** 1) functional EREs were identified in the rat PPE promoter region; 2) both ERalpha and ERbeta could mediate estrogen-induced PPE gene expression; 3) PPE mRNA levels in VMH were significantly decreased in ovariectomized (OVX) female rats, and increased by estrogen; 4) estrogen-induced PPE gene expression in cells and in VMH of OVX rats was diminished by T3; 5) the T3 inhibition may involve the squelching of cofactors; 6) T3 also inhibited estrogen-induced lordosis behavior; and 7) TRalpha1knockout reduced estrogen-induced lordosis behavior, while TRbeta knockout increased it. **Conclusion:** these data suggest that estrogen regulates PPE gene expression via its functional EREs, and the induction of PPE gene expression and lordosis behavior by estrogen is modulated by T3 in a TR isoform-specific manner.

031 HUMAN CHORIONIC GONADOTROPHIN (HCG) STIMULATES VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) PRODUCTION BY LEYDIG CELLS

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Objective: To examine whether the Leydig cell production of a potent angiogenic factor - VEGF, is under the trophic stimulation of hCG, and if so whether the effect can be mimicked by activators of the second messenger pathways involved in mediating hCG actions. **Methods:** Percoll-purified Leydig cells (LC) and mouse Leydig cell lines (TM3 and MLTC-1) were used, and they were studied at different time intervals following the exposure to hCG, 8-bromo-cAMP, forskolin and phorbol-12-myristate-13-acetate (PMA). The culture medium was collected for the measurement of VEGF by ELISA and total RNA was extracted from cells for the quantification of VEGF mRNA levels by Northern blot analysis. **Results:** hCG (0.001-1 U/ml) produced a modest dose-dependent stimulation of VEGF secretion from rat LC and MLTC-1 cells, reaching a maximum of ~50% above the basal levels. TM3 cells failed to respond to hCG (due to the loss of LH receptors) but gave dose-dependent increase in VEGF secretion following 12 h incubation with 8-bromo-cAMP (0.1-3 mM) and PMA (0.01-1 μM). Similar results were obtained from rat LC and MLTC-1 cells which gave maximum responses of >100% increase above the control. In line with the changes in peptide secretion, VEGF mRNA levels in MLTC-1 cells were upregulated ~1.5-fold after 2 h exposure to hCG (0.01-1U/ml). For TM3 cells, their VEGF mRNA levels showed time- and dose-dependent increases in response to forskolin (0.1-10 μM) and PMA (0.01-3 μM), with the later producing a greater stimulation. **Conclusions:** VEGF peptide secretion and mRNA expression in Leydig cells are stimulated by hCG which may produce its effect through both the protein kinase A and C pathways. The hCG-induced increase in VEGF production from Leydig cells could account for the increase in endothelial cell proliferation in the testis of adult rats after receiving hCG injection. **Acknowledgement:** Supported by RGC Grant (CUHK 422/95M).

032 Human oviductal cells produces three glycoprotein fractions that stimulate mouse embryo development

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Objective: The development of preimplantation (PI) mammalian embryos *in vitro* is inferior to that of *in vivo*. Lack of oviductal support is one of the possible reasons for the suboptimal embryo development *in vitro*. Coculture of embryos with oviductal cells suggests that paracrine pathway(s) exists between oviduct and PI embryos. We have demonstrated that coculture with human oviductal cells improves human and mouse embryo development in terms of increase in blastocyst formation, hatching and implantation rate, decrease in the incidence of cytoplasmic fragmentation and apoptosis. This study aims to study the embryotropic effects of three glycoprotein fractions from human oviductal cell conditioned medium on mouse embryo development.
Methods: Human oviductal cells were obtained from donated oviducts of hysterectomy patients and were cultured in DMEM/F12. The oviductal cell conditioned medium was fractionated by various liquid chromatographic systems. The purified fractions were tested on mouse embryos (MF1 x BALB/c) at different stages of development for different duration. Their effects on blastulation, hatching and allocation of cells to inner cell mass (ICM) and trophectoderm (TE) in the resulting blastocysts were determined.
Results: Three glycoprotein fractions named ETF-1, 2 and 3 with embryotropic effect on mouse embryo development were purified from the conditioned medium after human oviductal cells culture. ETF-1 and 2 affected the embryos at early cleavage stage and increased ICM cell number of the resulting blastocysts. ETF-3 stimulated the development of TE and had its maximal effects at around compaction. The enhanced development of TE after ETF-3 treatment led to larger expanded blastocysts with higher hatching rate.
Conclusion: Our studies showed that human oviductal cells improved mouse embryo development partly by the production of high-molecular-weight embryotropic factors. These factors had differential effects on mouse embryo development.
Acknowledgement: The Research Grant Council, Hong Kong (HKU241/95M) supports this research.

033 EFFECTS OF LEPTIN ON GONADOTROPIN-RELEASING HORMONE RELEASE AND GENE EXPRESSION IN HYPOTHALAMIC GT1-7 CELL LINE AND ITS RELATION TO CALCIUM INFLUX

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Objective: To investigate the effects of leptin on GnRH secretion and gene expression in GT1-7 cell line which was developed by targeting the SV 40 T antigen to GnRH neuron in a transgenic mouse. **Methods:** (1) GT1-7 cells were treated with leptin(2-2,000ng/ml) for 15, 30, or 60 min, GnRH released into the medium was assayed by RIA. (2) Cultured cells incubated with leptin were loaded with fluorescence probe Fluo-3/AM and $[Ca^{++}]_i$ was measured by laser scanning confocal microscope (LSCM). (3) Cells were incubated with leptin (20-200ng/ml) for 24hr, and RNA was extracted for Northern blot analysis. **Results:** (1) A directed effect of leptin on GnRH secretion was found, there was a significant stimulation of GnRH release at the dose of 20ng/ml after 15 min of incubation. (2) In the presence of extracellular Ca^{++}, Leptin 20ng/ml had an elevation effect on $[Ca^{++}]_i$.(3) Following an incubation for 24 hr of leptin, the GnRH mRNA levels showed a decline tendancy. **Conclusion:** Leptin appears to participate in the regulation of reproductive processes by acting directly on GnRH secretion and gene expression which seems related to free calcium influx.

034 CHANGES OF INCIDENCE OF HYPERTHYROIDISM IN AN AREA WITH BORDERLINE LOW-IODINE INTAKE BEFORE AND AFTER UNIVERSAL SALT IODIZATION PROGRAM: A 7 YEAR EVALUATION

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Objective: To evaluate that the effect of the change of iodine intake on the incidence of hyperthyroidism in an area with borderline low-iodine intake following universal salt iodization (USI). **Methods:** Urinary iodine excretion in school children was used for an objective index in the changes of iodine nutrition. A retrospective investigation for changes on the incidence of hyperthyroidism was carried out in a large enterprise before and after salt iodzation during the last 7 years. **Results:** Median urinary iodine excretion was increased from 64.5μg/L to 231.0μg/L after salt iodization. Before salt was iodized the incidence of hyperthyroidism was 11.8 [95% confidence intervals (CI) 6.2-21.0] per 100000 person-year in 1994 and 8.2 (95% CI 3.4-15.8) per 100000 person-year in 1995. After salt was iodized the incidence of hyperthyroidism were 22.2 (95% CI 13.8-33.2), 34.2 (95% CI 23.5-47.5), 31.4 (95% CI 21.0-44.0), 57.4 (95% CI 43.2-73.9) and 77.9 (95% CI 61.6-97.3) per 100000 person-year in 1996, 1997, 1998, 1999, and 2000 respectively. The relative risk (RR) for hyperthyroidism onset in population was 1.88 (95% CI 0.47-7.57), 2.90 (95% CI 0.8-10.49), 2.68 (95% CI 0.71-10.17), 4.86 (95% CI 1.50-15.75) and 6.60 (95% CI 2.21-19.68) after the USI in 1996, 1997, 1998, 1999, and 2000 respectively. The increase of the incidence of hyperthyroidism in female was much higher than that in male and most of them aged between 40 to 50 years. **Conclusion:** It suggests that there is an raised incidence of hyperthyroidism in studied area with rapid increment of iodine intake after the USI program. The iodine intake level is still in safe range for population in studied area. However, that iodine intake level has too rapid increment would contribute to onset of hyperthyroidism in an area with borderline low-iodine intake.

035 Therapeutic Outcomes of Four Immunosuppressants in Patients with Graves' Ophthalmopathy

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Objective: To compare the efficacy and tolerability of 4 immunosuppressants in patients with Graves' ophthalmopathy (GO)
Methods: Forty-three untreated GO patients were enrolled in this study. Severity of ophthalmopathy was at least equal to or more severe than Class 2 in NO SPECS classification. Those who could afford were randomly assigned to receive either cyclosporin A(CyA, n=8, 5~6 mg per kilogram of body weight per day) or mycophenolate mofetil (MMF,n=7,16~18mg per kilogram per day)therapy. The others were randomly assigned to be given either prednisone (n=13, finished in 11, 30~40mg per day for 4 weeks, 20mg for 4 weeks, and10 mg for 4 weeks) or tripterygium panterpene (T_{II}, N=15, 30~60mg per day) the therapeutic responses were quantitatively assessed according to Ophthalmopathy Index Scoring System from Given-Wilson (1989) with slightly modified. The improvement or progression of ophthalmopathy was defined if the variation, either increase or decrease of the score, reached 3 or more in opthalmopathy index .
Results: At the end of the course of 12-week therapy, 6(55 percent) of the 11 treated with prednisone improved, the remaining 5 lacked response. In T_{II} groups, 10(67 percent) of 15 responded to the therapy, 5(33 percent) had no change. There was no significant difference between the above 2 groups (P>0. 05). Three CyA-treated and 6 MMF-treated patients responded to therapy (38 percent vs. 86 percent;P<0. 05). None of all the patients in the 4 groups had worsening of opthalmopathy. In prednisone group, 3(23 percent) gained body weight by more than 5 percent, 1(8 percent) developed impaired glucose tolerance (IGT). One weight gaining patient and the subject in whom IGT emerged ceased the therapy. Correctable hypertension was found in 1(13 percent) of the CyA Group.
Conclusions: The study suggests that MMF may be more effective than CyA in treatment of Graves' opthalmopathy. T_{II} may have a equal effectiveness and perhaps a better tolerability than prednisone in the therapy.

036 CONTRIBUTION OF TAP GENE POLYMORPHISM TO PREDISPOSITION TO GRAVES DISEASE IN A SOUTHERN CHINESE POPULATION

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Objective: to examine the distribution of TAP gene polymorphism in a southern Chinese population and the association between TAP gene polymorphism and predisposition to Graves disease. **Methods:** we have performed TAP genotyping in 67 Graves disease patients and 69 healthy controls by amplification refractory mutation system (ARMS). **Results** : distribution of TAP genes in our healthy controls shows similar but some different finding compared with studies in other countries and other regions of our country, suggesting that the distribution of TAP genes might have ethnic or regional differences. The frequency of TAP1D haplotypes was significant higher in the healthy controls than in the Graves disease patients (RR=0.17, P<0.01), and the frequency of TAP1C haplotypes was significant lower in the healthy controls than in the Graves disease patients(RR=2.05, P<0.05). The frequency of TAP2A haplotypes was significant higher in the healthy controls than in the Graves disease patients(RR=0.46, P<0.05), and the frequency of TAP2F haplotypes was significant lower in the healthy controls than in the Graves disease patients(RR=9.95, P<0.05). **Conclusion:** TAP1D and TAP2A haplotypes might confer the protection against Graves disease while TAP1C and TAP2F haplotypes might confer the susceptibility to Graves disease.

037 CIRCULATING SOLUBLE FAS LIGAND, A CANDIDATE SERUM MARKER, CORRELATES WITH DISEASE ACTIVITY IN GRAVES' DISEASE

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Objective: To investigate the role of circulating sFasL and sFas in modulating disease activity of Graves' hyperthyroidism (GD). **Methods:** Serum samples were obtained from two groups of GD patients and one group of controls, including 22 untreated hyperthyroid GD patients with higher TRAb level (63.8±12.5 %, group I) and 22 treated euthyroid GD patients with lower TRAb level (7.9±5.9 %, group II). Detection of sFas/sFasL was performed using ELISA. Detection of apoptosis in Graves' thyrocytes culture by FACS analysis and Fas expression in frozen sections of Graves' thyroid by immunocytochemistry was done. **Results:** Serum levels of sFas were significantly higher in group I (1.56±0.26 ng/ml) than in group II (0.76±0.26 ng/ml, $p < 0.01$), and serum sFasL were higher in group I (0.153±0.018 ng/ml) than in group II (0.126±0.012 ng/ml, $p < 0.01$). A significant correlation was found between sFasL levels and TRÀb activity in all patients (r=0.69, $p < 0.01$). Apoptosis was found in 4.8±1.8% in Graves' thyrocytes culture, and Fas expression was detected on the primary culture and thyroid tissue from GD. **Conclusion:** It suggests that changes in sFasL levels and TRAb levels occur in parallel, increased serum sFasL in patients with GD may contribute to homeostasis in the thyroid. Serum sFasL may consider useful as a marker for predicting disease activity in Graves' hyperthyroidism.

038 THE EFFECT OF FCγ RECEPTOR ON THE PATHOGENESIS OF GRAVES DISEASE

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Objective: The essential reason of Graves disease is the anti-TSH receptor antibody (TSAb) existing in their sera. Recent studies showed that Fcγ receptor played a crucial role in a number of autoimmune diseases. In this study, we used TSH receptor expressing cells to immunize Fcγ receptor gene knockout mice to explore the roles of Fcγ receptor in the pathogenesis of Graves disease. **Methods:** Fcγ receptor gene knockout mice (C57BL/6 origin) and wild type C57BL/6 mice were immunized with hTSH receptor expressing cells (DAP3.WT). DAP3.WT cells were cultured in RPMI 1640 with 10% FCS. The cells were separated with trypsin and treated with 50μg/ml mitomycin C for 60 minutes. 1-2×10^7 DAP3.WT cells were peritoneally injected into mice and repeated per two weeks for total six times. Two weeks after final immunization, mice were killed for total thyroxine, TRAb and pathological examination. **Results:** The immunized Fcγ receptor gene knockout mice showed significant lower levels of thyroxine than those of immunized wild type control mice (2.2±0.31 vs 3.32±0.59μg/dl, $p<0.05$), but there was no significant change comparing to the thyroxine levels of non-immunized wild type control mice. The TRAb levels of the immunized Fcγ receptor gene knockout mice significantly increased compared to those of the immunized wild type mice (21.75± 8.21 vs 14.11±6.21, $p<0.05$). The thyroids of the immunized Fcγ receptor gene knockout mice did not change significantly compared to those of non-immunized wild type control mice. **Conclusion:** These results suggest that Fcγ receptor may be involved in the pathogenesis of Graves disease.

039 A New Model of Experimental Autoimmune Thyroiditis and Identification of Immunogenic Epitope on Mouse Thyroid Peroxidase (mTPO)

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Autoimmune Thyroid Disease (AITD) is a common condition affecting mostly female subjects. Thyroid peroxidase (TPO) is a well-characterized autoantigen in AITD. Autoantibodies and autoreactive T lymphocytes to TPO are believed to play a major role in the pathogenesis of Hashimoto's Thyroiditis (HT). To understand the mechanism of AITD and the role of TPO, we attempted to establish a mouse model of HT by immunizing C57Bl/6(H-2^b) x CBA (H-2^k) F1 mice with recombinant mTPO (rmTPO). To achieve this, we produced rmTPO ectodomain 1-837 from E. coli using pGEX-4T-1 expression vector. mTPO-GST fusion protein was induced to express at 116 kDa and mTPO ectodomain was isolated by GST-glutathione agarose beads column. rmTPO was recognized by anti-human TPO polyclonal antibody in western blotting analysis. To establish the mouse model of HT, mice were immunized with 10μg rmTPO in CFA adjuvant. At week 3, antibodies against mTPO were detected in all mice. At day 50, thyroiditis with infiltration of mononuclear cells and destruction of thyroid follicles were seen in 3 out of 5 mice. Moreover, 4 out of 10 mice showed decreased total T4 level with 51, 58, 59 and 59 pmole/L respectively, relative to 88 $\pm$ 14 pmole/L (mean $\pm$ SD) in control. To identify thyroiditogenic epitope, animals were immunized with a synthetic mTPO peptide a.a 587-606. This peptide sequence corresponds to human TPO a.a 599-617, which is reported to be a critical part of the conformational epitope within the human TPO molecule. Antibodies were detected in all animals immunized with the mTPO peptide, however the titer is much weaker when compared to mouse immunized with rmTPO ectodomain. Thyroiditis with infiltration was seen in 4 out of 6 animals, but there was no follicular destruction. None of the control mice immunized with adjuvant developed antibodies and thyroiditis. Immunotyping of the cellular infiltrate showed predominant CD4+ T cells and B220+ B cells. In conclusion, a model of HT induced with mTPO was established and a.a 587- 606 was identified to be one of the thyroiditogenic epitopes on mTPO.

040 EXPERIMENTAL STUDY ON EFFECTS OF THYROID HORMONE ON SIGNAL TRANSDUCTION SYSTEM IN CULTURED NEURONS

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Objective: Thyroid hormone(TH) plays a fundamental role in the differentiation and development of central nervous system. But the relationships between TH and signal transduction system are largely ignored . In this study, we employed the molecular biological techniques and neuron culture method to study the regulations of TH on signal transduction systems in rat brain, and the related mechanism at gene levels.**Methods:**(1).Cultured neurons from cerebral cortex of embryo rat, stimulated them by different concerntration of L-T3(0,0.5,5,50nM) for 5 days. Used the techniques of fluorescence-labelled DD-PCR to screen TH-regulating genes. (2).Established congenital hypothyroidism rat model, observed the changes of mRNA levels of these obtained genes in cerebral cortex during perinatal period by semiquantitative RT-PCR. **Results:**(1). Different concentrations of L-T3 in medium could induce differential expression of many genes in cultured neurons, six bands among which were confirmed by sequencing analysis as Krev-1, Human Acidic Protein 82KD, DnaJ-like Protein (Hsj-2), P28, and two novel gene fragments, among which Krev-1 is one component of ras signal transduction system, and P28 belongs to cis-Golgi signal protein. (2).Both TH deficiency and in excess could increase the level of Krev-1 mRNA. (3). TH deficiency could increase the expression of P28 gene, while excess of TH could decrease its transcription. (4).Congenital hypothyrodism could enhance transcription of Krev-1 gene in cerebral cortex of rat during perinatal period.**Conclusion:**Our studys show that TH has very important regulatory action on Krev-1 gene and cis-Golgi P28 in rat brain during the period of development.

041 INCREASE IN BONE MINERAL DENSITY DURING TESTOSTERONE THERAPY IN HYPOGONADAL MEN

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Objective: To study the effect of testosterone replacement on bone mineral density (BMD) in local Chinese male patients with hypogonadism. **Methods:** 31 male hypogonadal patients, aged 25-68 years (median 45.6 yr.) who received regular intra-muscular injection of testosterone propionate (sustanon) in QEH were recruited and underwent serial monitoring of BMD by Dual Energy X-ray Absorptiometry (Lunar). 10 patients had primary hypogonadism and 21 patients suffered from secondary hypogonadism. All patients had at least two measurements of BMD at intervals of 21.8±11.1 months and 13 patients had a third measurement at a further interval of 22.2±9.8 months. **Results:** At baseline, study subjects had a mean lumbar spine BMD of 0.872 g/cm^2 (T-score –2.62, Z-score –2.43). 19.4% (n=6) had history of fractures. An increase of 9.01±6.21% in spine BMD and 3.74±7.72% in femoral neck BMD was observed after a mean duration of 22 months of treatment. The annual rate of increase in spine BMD and femoral neck BMD was 5.96±4.93% and 2.05±3.91% respectively in the first two years of treatment. A further increase of 3.37±4.63% in spine and 1.29±5.26% in femoral neck BMD was observed with continuous treatment afterwards, i.e. the annual rate of increase in BMD decelerated to 1.40±2.76% and 0.82±4.54% after the first two years of androgen therapy. Regression analysis showed a strong negative association between the initial BMD and the rate of increase in BMD during androgen replacement therapy ($r = -0.68$; $P<0.0001$). **Conclusions:** Our study showed that androgen replacement therapy significantly increased BMD in Chinese hypogonadal men. The rate of increase in BMD was highest in the first two years of treatment and in those with lowest baseline BMD.

042 The Effect of Low Dose Nylestriol-Levonorgestrel Replacement Therapy on Bone Mineral Density in Women with Postmenopausal Osteoporosis

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Objective: To evaluate the effect of Compound Nylestriol Tablet on bone mineral density in women with postmenopausal osteoporosis. Compound Nylestriol Tablet, which contains 0.5mg of Nylestriol and 0.15mg of levonorgestrel per tablet, was permitted for clinical trial in postmenopausal osteoporosis. **Method:** An one year's clinical observation was performed in 191 eligible patients who were randomly divided into two groups. In group A, 119 patients were treated for one year with low dose Compound Nylestriol Tablet (one tablet per week) and in group B, 72 patients with placebo. BMDs of lumbar posterio-anterior, lumber lateral, hip total and forearm total positions were measured before and after treatment. **Results:** It showed that group A had a lower bone loss than that of group B significantly ($P<0.05$). There were no differences of other observed biochemical variables of serum total protein, albumin, globulin, ALT, ALP, calcium, phosphorus, uric acid, BUN, creatinine, or of urinary calcium, phosphorus, creatinine and hydroxyproline. No side-effects on uterus (size, endometrial thickness and vaginal exfoliated cells), or mammary glands (transillumination light scanning) were observed. All the patients did not have uterine bleeding or vertebral fractures. **Conclusion:** Low dose Compound Nylestriol Tablet is effective, safe and convenient in the treatment of postmenopausal osteoporosis.

043 NORMATIVE DATA OF QUS AT CALCANEUS IN CHINESE AND THE RELATIONSHIP BETWEEN THE MEASUREMENT AND BONE FRACTURE HISTORY

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Objective: To establish the normative data in Shanghai Chinese with QUS and to study the relationship between the measurement and bone fracture. **Methods:** 875 males and 1793 females were included for the measurement of the normative data and another 464 patients suspicious of osteoporosis were also detected with CUBA Clinical system (McCue, UK). Broadband Ultrasonic Attenuation (BUA) and Velocity of Sound (VOS) of the left calcaneus were determined. **Results:** In the adolescent aged from 13 to 20, BUA and VOS both increased with the age. No differences was found between the boys and the girls at each age until 20 years old(BUA 95±17 vs 86±14 dB/MHz, P <0.05, VOS 1710±37 vs 1706±37 m/s, P >0.05). In the population above 20, the people were divided into 6 groups every 10 years of age. The males' BUA of each group were 96±16, 89±15, 84±15, 83±15, 81±13, 76±14 dB/MHz, VOS were 1691±33, 1652±30, 1640±35, 1634±35, 1624±32, 1615±39 m/s, respectively. The females' BUA were 79±12, 78±13, 77±13, 69±14, 62±12, 51±15 dB/MHz,VOS were 1654±30, 1655±33, 1640±34, 1620±36, 1589±31, 1573±33 m/s, respectively. BUA and VOS decreased in both genders while aging, but the significant decrease of the measurements were in the different age of the males and the females. In the study of the relationship between the QUS and the bone fracture history, patients who ever suffered bone fracture had lower BUA or VOS (64±14 dB/MHz and 1604±34 m/s) than those of non-fracture patients (70±15 dB/MHz and 1620±38 m/s)(P <0.05). **Conclusions:** QUS is a useful method for evaluating the bone fracture risk.

044 SOUTHERN CHINESE PREMENOPAUSAL WOMEN WITH MATERNAL HISTORY OF LOW BONE MINERAL DENSITY HAVE LOWER PEAK BONE MASS

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Objective: To investigate the role of genetic and environmental impact on peak bone mass in premenopausal southern Chinese women. **Methods:** 464 premenopausal southern Chinese women had bone mineral density (BMD) of the lumbar spine and the hip measured by dual energy X-ray absorptiometry (DEXA, Hologic QDR 2000+). 265 of them had a maternal history of low BMD, which was defined as maternal T-score equal or less than 2.5 (Group 1). 199 women were daughters born to mothers with BMD T-score greater than –2.5 (Group 2). **Results:** The lumbar spine L1-L4 and total hip BMD in group 1 women were significantly lower than in Group 2 (L1-L4 BMD 0.958 +/- 0.117 g/cm^2 vs 0.987 +/- 0.116 g/cm^2, p=0.01; total hip BMD 0.816 +/- 0.143 g/cm^2 vs 0.841 +/- 0.103g/cm^2, p=0.04). There were no significant difference in the body weight, serum calcium, serum phosphate, serum total alkaline phosphatase, daily exercise level and daily calcium intake in women between the two groups. However, Group 1 women were significantly shorter (p=0.02) and older (p=0.001). Using analysis of covariance to adjust for age and body height, women in Group 1 still had significantly lower BMD at the lumbar spine (3%, p<0.05) and the hip (3.1%, p=0.05) when compared to those in Group 2. Body weight, maternal history of low BMD and serum total alkaline phosphatase were significant predictors of both lumbar spine and total hip BMD (multiple regression analysis). **Conclusion:** Genetic influence in terms of maternal history of low BMD is a major determinant of peak bone mass in Southern Chinese premenopausal women.

045 THE EFFECT OF PARATHYROID HORMONE(PTH) ON THE GENE TRANSCRIPT AND POLYPEPTIDE LEVELS OF INSULIN-LIKE GROWTH FACTOR-I(IGF-I) IN CULTURED RAT OSTEOBLASTS AND THE ROLE OF IGF-1 IN MEDIATING THE ANABOLIC EFFECT OF PTH

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Objective: The mechanism of the anabolic effect of PTH is not clear. Previous studies have shown that continuous PTH stimulation can increase the gene transcript and polypeptide levels of IGF-I in cultured rat osteoblasts(ROB), however, *in vitro* studies mimicking the pharmacokinetics of PTH were scarcely reported. **Methods:** The neonatal rat calvarial osteoblasts were divided into 3 groups: (1) Control group(Ctr): The cells were cultured in vehicle medium at the first 6h and subsequent 42h in a 48h-cycle; (2) PTH intermittently-stimulated group(Itm): The cells were cultured in PTH-containing medium at the first 6h but in vehicle medium at subsequent 42h; (3) PTH continuously-stimulated group(Ctu): The cells were cultured in PTH-containing medium at the first 6h and also subsequent 42h. The media in all groups were changed at 6h and 48h. The mRNA contents of type I collagen(Coll) and IGF-I within ROB were determined by reverse transctiptase-polymerase chain reaction(RT-PCR). The concentrations of procollagen type I carboxyterminal propeptide(PICP) and free IGF-I were measured by RIA and ELISA respectively. The calcium node in the cell layer was stained with Von Kossa's method and the total calcium content in it was analyzed by atomic absorptiometry. The cell proliferation was assessed by MTT assay. The goat anti-mouse IGF-Iantibody was added to the medium to block the activity of IGF-I. **Results:** The Coll mRNA content within ROB ,the concentrations of PICP and ALP in medium ,the number of calcium node and total calcium content in the cell layer of Itm were all higher than those of Ctr (p<0.05 or less), whereas these parameters of Ctu were all lower than those of Ctr(p< 0.05 or less). The cell numbers of Itm and Ctu were similar(p<0.05) and both were higher than that of Ctr(both,p<0.001). The IGF-I mRNA content of ROB was transiently elevated in Itm , whereas it was constantly elevated in Ctu. The free IGF-I concentrations in media of Itm at 0~6h,7~24h and 25~48h were all higher than those of Ctu (all,p<0.001). When IGF-I antibody was added to the medium,the ALP activities in Ctr and Itm decreased (p<0.05 and 0.001 respectively),while that in Ctu changed little(p>0.05).**Conclusion:** The anabolic effect of PTH *in vivo* could be mimicked in cultured ROB; IGF-I expressed by ROB might play an important role in mediating the anabolic effect of PTH; The changes of free IGF-I does not concur with that of IGF-I gene transcript level on the stimulation of PTH; Higher and lower levels of free IGF-I around ROB might be involved in the anabolic effect of intermittent PTH stimulation and decreased osteogenesis of continuous PTH stimulation respectively.

046 DETECTION AND CHARACTERIZATION OF ENDOTHELIN IN TRANSFORMED HUMAN OSTEOBLAST CELL CULTURE MEDIUM

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Objective: To detect and characterize the presence of immunoreactive endothelin (IR-ET) in transformed human osteoblast cell culture medium. **Methods:** IR-ET in conditioned media of transformed human osteoblast-like cells (HTb-96) collected at defined culture times was extracted by Sep-Pak C18 cartridges and then assayed by radioimmunoassay. Gel permeation chromatographic characterization of the IR-ET in culture medium was carried out using Sephadex G-25 superfine column chromatography. Fractionation of the IR-ET from the culture medium was carried out by Fast Protein Liquid Chromatography. **Results:** IR-ET was undetectable in the medium incubated at 0.5 and 1 hour and was 3.2 ± 0.2 fmol/105 cells (mean ± SEM, n=6) at 2 hr, 9.5 ± 0.5 fmol/105 cells at 6 hr, 19.8 ± 2.1 fmol/105 cells at 24 hr, and 23.7 ± 2.0 fmol/105 cells at 48 hr, respectively. Sephadex G-25 superfine chromatography and fast protein liquid chromatography studies showed that over 90% of IR-ET in the culture medium co-eluted with synthetic ET-1. **Conclusion:** These results show that ET-1 could be formed by transformed human osteoblasts. Further studies should be performed to elucidate the physiologic role of endothelins as possible autocrine, paracrine or endocrine factors in calcium and bone metabolism.

047 THE EXPRESSION OF SMAD INTERACTING PROTEIN (SIP)-1 AND ITS EFFECTS ON BMP SIGNAL AND MYOBLASTIC DIFFERENTIATION

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Objective: SIP-1 is a Smad-binding protein which belongs to transcription factor dEF1/Zfh-1 family, but its physiological function is not known. SIP-1 binds to 5'-CACCT sequence, which is a part of E-box, or the binding site for basic helix-loop-helix (bHLH) class transcription factors, and suppresses transcription. SIP-1 may, therefore, regulate the transcriptional activities of Smads as well as bHLH class transcription factors, such as MyoD that plays a key role in myoblasitc differentiation. **Methods:** In order to clarify the role of SIP-1 in osteoblastic and myoblastic differentiation, we examined the expression of SIP-1 in mouse osteoblastic cell line MC3T3-E1 and myoblastic cell line C2C12, treated with BMP-2 by Northern blot analysis. To analyze the effect of SIP-1 on BMP signal, we measured luciferase acitivity after co-transfection of MC3T3-E1 cells with full length SIP-1 and a BMP responsive element-containing reporter construct, 3GC2-lux, which is derived from Smad6 promoter. Similarly, in order to study the role of SIP-1 in myoblastic differentiation, we evaluated the promoter activity of muscle creatinine kinase (MCK), a muscle marker, by luciferase assay after the overexpression of full length SIP-1 in C2C12 cells. **Results:** We detected the expression of SIP-1 in both MC3T3-E1 and C2C12, which was induced by BMP-2. Enhancement of luciferase activity of 3GC2-lux by BMP-2 was almost completely abolished by the overexpression of SIP-1. SIP-1 also suppressed the activation of MCK promoter induced after myoblastic differentiation. However, SIP-1 had no effect on 25-hydroxyvitamin D-1a-hydroxylase promoter activity. **Conclusions:** SIP-1 is expressed in osteoblastic and myoblastic cells, and inhibits BMP signal mediated by Smads and myoblastic differentiation.

048

(Cancelled)

049 MOLECULAR CLONING AND IDENTIFICATION OF RELATIVE GENES DIFFERENTIALLY EXPRESSED IN THE ADIPOCYTES INDUCED BY PPAR γ 2

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Objective: To isolate and clone the genes related to adipocyte differentiation induced by peroxisome proliferator activated receptor γ 2(PPAR γ 2). **Methods:** The adipocyte differentiation model has been developed using retroviruses to express PPAR γ 2 in NIH3T3 cells. By utilizing mRNA differential display PCR (DD-PCR) technique, the mRNA prepared from the adipocyte model and the control cells were screened. The differential expressed genes found by DD-PCR were confirmed by semi-quantitative RT-PCR. The cell apoptosis rates of the two groups at the various differentiation stages were assayed using flow cytometer. **Results:** The lipid accumulation obviously existed in the PPAR γ 2-expressing NIH3T3 cells and these lipid-containing cells resembled the muture adipocytes. The typical differentiation-linked adipocyte genes, aP2 and leptin genes expressed in NIH3T3-PPAR γ 2 lipid-containig cells. Twenty cDNA fragments differentially expressed in the adipocytes induced by PPAR γ 2 were identified. The cDNA fragments' sequence demonstrated 8 known genes, one new gene, 2 ESTs and 7 new ESTs. UNR (Upstream of N-ras) gene was comfirmed to be up-regulated in the NIH3T3-PPAR γ 2 lipid-containig cells and apoptosis inhibitor 5 (API5) gene down-regulated using semi-quantitative RT-PCR. After 4 days of differentitation, the cell apoptosis rates of the NIH3T3-PPAR γ 2 lipid-containig cells were significatly higher than those of control cells. **Conclusions:** PPAR γ 2 plays an important role in adipocyte differentitation, it may enhance expression UNR gene and decrease the expression of API5 to regulate the adipocyte differnetitation.

050 THE EFFECT OF LEPTIN ON PROTECTING RAT ISLET β-CELL AGANGAINST LIPOTOXICITY INDUCED BY FREE FATTY ACID

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Objective: To investigate whether leptin could protect rat islet β-cell from lipotoxicity induced by free fatty acid. **Methods:** Pancreatic islets were isolated and randomly separated into 4 groups:Control group; Leptin group(Murine Recombinant Leptin 100ng/ml was added to the culture medium); FFA group(0.25mmol/L long chain fatty acid, in which oleate:palmitate=2:1 was added to the culture medium); FFA+Leptin group(both leptin and FFA was added). After cultured for 72 hours, TG content of islets、insulin releasing stimulated by glucose 、and the mRNA expression of fatty acid oxidase (CPT-I)、fatty acid synthase (ACC)、PPARα and PPARγ in islets were examined. **Results:**1) TG content was elevated in FFA group , and decreased in leptin group. 2) Leptin inhibited insulin secretion both in basal and glucose stimulating conditions. FFA stimulated insulin secretion in basal condition and inhibited it in glucose stimulating condition. 3) FFA could stimulate the mRNA expression of ACC and CPT-I and inhibit the mRNA expression of PPARγ and PPARα; leptin could stimulate the mRNA expression of CPT-I and PPARα and inhibit the mRNA expression of ACC and PPARγ. **Conclusion:** FFA could elevate the TG content in islets,and could stimulate insulin secretion in basal condition and inhibit it in glucose stimulating condition, so as to cause hyperinsulinmia; Leptin may regulate the expression of Fatty acid oxidase and synthase through the pathway of PPARα and PPARγ,It could decrease the TG content in islets and inhibit insulin secretion, so can protect islets from lipotoxicity induced by free fatty acid.

051 Efficacy and safety of sibutramine in Chinese obese patients

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Objective To test the clinical efficacy and safety of sibutramine in Chinese obese patients. **Design** Randomized, double-blinded, placebo-controlled study. Obese adults (BMI 27-45 kg/ m2) evaluated at six research centers were randomized to receive sibutamine 10 mg or placebo one tablet once a day with a controlled-energy diet for 24 weeks. **Results**: For intent-to-treat analysis, 125 sibutramine-treated subjects and 126 placebo-treated subjects were evaluated. After 24 weeks, sibutramine-treated patients lost more weight (mean± SD, 6.52±3.95kg) than placebo-treated patients (3.18±3.59kg, P=0.00)). Sibutramine-treated patients showed significant decreases (P<0.05) in serum levels of fasting glucose, 1 hour of postprandial glucose and the area under curve of serum glucose level in comparison with placebo-treated patients. Both groups had similar adverse–event profiles, the main adverse events of sibutramine-treated patients were headache,dizziness(18.4%),constipation(14.4%)and thirsty (13.6%),which were mostly mild and transient. There were no serious adverse events in both groups. **Conclusion**: Sibutramine in conjunction with a low-energy diet, produced greater significant weight loss than placebo during 24 weeks treatment. There was also an improvement in most serum glucose parameters. Sibutramine was well tolerated and offers a new approach to the management of obesity.

052 COMPARISON OF LDL SIZE BETWEEN OBESITY AND THOSE WITH NORMAL WEIGHT AND THE EFFECT OF ORLISTAT ON LDL SUBFRACTION OF CHINESE OBESITY IN A RANDOMIZED, DOUBLE-BLINC PLACEBO CONTROLLED CLINICAL TRIAL

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Objective: To compare LDL size between obesity and those with normal weight; to study the effect of orlistat on LDL subfraction of Chinese obesity as compared to placebo. **Methods:** 3-16% gradient polyacrylamide gel electrophoresis was used for the measurement of the size of LDL particles. The LDL particle was compared between 68 obesity (BMI 29.42±2.94kg/m^2) and 57 non-obesity(20.86±1.61kg/m^2). Then 68 obesity patients were randomized to orlistat or placebo treatment for 24 weeks. LDL particle size was measured before and after treatment. LDL size less than 25.5 nm was regarded as LDL type B or small, dense LDL. **Results:** The average size of LDL particle of obesity was significantly smaller than that of normal weight (25.48 nm vs. 26.48 nm, P<0.001); the percentage of LDL B type was significantly higher among obesity than that among normal weight (58.82% vs. 12. 96%, P<0.001). After 24 weeks treatment, the average LDL size was significantly increased (25.3 nm vs. 25.9 nm, P=0.001) in the orlistat group, but no significant change in the placebo group (25.9 nm vs. 25.6 nm, P=0.152). LDL B type was decreased from 60% to 28.9% in the orlistat group (P=0.003) and there was no change in the placebo group (56.5% vs. 56.5%, P=1.000). **Conclusion:** Obesity patients have smaller LDL particles compared with those with normal weight. The LDL particle size increased significantly after 24 weeks anti-obesity treatment with orlistat as compared to placebo. Anti-obesity treatment with orlistat may increase the LDL size and decrease the risk of developing cardiovascular disease.

053 ASSOCIATION OF CART GENE WITH OBESITY AND TYPE 2 DIABETES

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Objective: The objectives of the present study were to examine: 1.Polymorphism of CART gene in Chinese population;2.The association of CART gene polymorphism with obesity and type 2 diabetes;3.The relationship between CART gene polymorphism and clinic characteristics of obesity and type 2 diabetes.**Methods:** Screening for mutations in the entire coding region for the CART gene were performed by the polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) in 180 normoglycemic control subjects(76 normal weight and 104 obesity divided by BMI≥23kg/m^2) and 221 patients with type 2 diabetes.**Results:** Adenine deletion was identified at position 1475 nucleotide located at untranslation area of exon 3. In normal weight control, the frequencies of CART-A+ and CART-A- alleles were 84.8% and 15.13% respectively. The frequencies of CART-A+A+,A+A-, A-A+ genotypes were conformed to expectations of the Hardy-Weinberg rule. A deletion in CART is not associated with obesity. In obese subjects, the frequencies of CART-A+ and A- alleles were 82.69%, 17.31% respectively; the frequencies of CART-A+A+,A+A-,A-A- genotype were 67.3%, 30.8% and 1.9% respectively. In diabetic patients, the frequencies of CART-A+ and A-alleles were 84.6% and 5.4% respectively; the frequencies of CART-A+A+, A+A-,A-A- genotype were 71.9%,25.3% and 2.7% respectively.The frequency of the A deletion of the CART gene in diabetic patients did not differ significantly from normoglucemic control subjects. Diabetic patients with the A deletion had increased TC and LDL- C .The hyperlipoproteinemia could contribute to the increased incidence of cardiovascular complication.**Conclusion:** Adenine deletion of CART was identified in Chinese Han subject. A deletion in CART is not associated with obesity and type 2 diabetes. Diabetic patients with the A deletion had increased TC and LDL- C that could contribute to the increased incidence of cardiovascular complication.

054 Relationships between Plasma Membrane Glycoprotein Gene, β3 — AR Gene and Obesity Type 2 Diabetes, Insulin Resistance in Chinese Pedigrees and Population

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Objective: To explore the relationships between the plasma membrane glycoprotein (PC-1) gene , the β_3—adrenergic receptor(β_3—AR)gene and obesity, type 2 diabetes, insulin resistance. **Methods:** Eight pedigrees with obesity and type 2 diabetes, 53 non-obesity non-diabetic healthy individuals, 105 simple obesity subjects, 63 type 2 diabetic patients and 114 obesity type 2 diabetics have been detected with the technique of PCR-RFLP in glutamine for lysine 121 (Q for k allele) polymorphism in exon 4 of PC-1 gene and in codon 64 of the exon region representing the variation Trp 64 Arg of β_3—AR gene. **Results:** The frequency of PC-1 gene Q allele was 3%,18%,4% and 30% in control, obesity subjects, diabetic patients and obesity diabetic individuals respectively. For β_3—AR gene, to compare with the subjects of Trp 64 homozygsis group, the individuals with Arg 64 allele were much higher ($P<0.05 \sim P<0.001$)in maist to hip ratio (WHR)，mean blood pressure (MBP), fast and postprandial blood glucose (FBG、PBG), fast and postprandial serum insulin (FINS, PINS), fast and postprandial serum C-peptide (FCP, PCP)and much lower in insulin sensitivity index (ISI $P<0.01$)； In pedigree linkage analysis, the maximal LOD score of β_3—AR gene with obesity was 3.385109 ($\theta=0.00000$) at the mode of autosomal dominant in pedigree 3.**Conclusion:** The results suggest that (1). The PC-1 gene Q allele is a genetic marker susceptible to obesity, type 2 diabetes and insulin resistance. (2) The Trp 64 Arg variation might break the energy metabolic balance of the body and cause impaired of the function of uncoupling protein (UCP)and glucose transport-4 (Glut-4), leading to insulin resistance, obesity and type two diabetes.(3) The Trp 64 Arg variation have a cause effect significance of some familial obesity

055 STUDY THE EFFECT OF MHC CLASS II MOLECULES ON INITIATION OF GRAVES' DISEASE

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Objective Previous study the pathogenesis of GD focused on whether MHC class Ⅱ molecules aberrant expression by human epithelial thyroid cell in GD could present autoantigen such as TSHR to immune system. We immunized the three different H-2 mice with hM12 cells, which co-expressing MHC class Ⅱ molecules and hTSHR molecules, to development animal model of GD and to determine whether MHC class Ⅱ molecules expression by the human epithelial thyroid cell in GD would contribute to the pathogenesis of GD. **Method** Experimental groups of BALB/c mice ($H\text{-}2^d$), C57BL/6 mice ($H\text{-}2^b$) and C3H/He mice($H\text{-}2^k$) were immunized i.p. per 2 weeks with 2×10^7 hM12 cells for six times, while control groups were immunized with M12 cell. Five weeks after the final immunization, the thyroids were histologically examined, and serum samples were tested for anti-hTSHR autoantibodies and thyroid hormone levels. **Results** One BALB/c mouse in experimental groups developed Graves'-like disease, Total T_4 and T_3 levels of this mouse were above the upper limit of normal, TSAb activity was displayed in its serum. The thyroid histologically showed with the features of thyroid hyperactivity including thryocyte hypercellularity and colloid absorpation. Two C57BL/6 mice in experiment groups developed Graves' disease change. Total T_3 and T_4 levels of them were also above the upper limit of normal. TSAb was detectable, and the thyroid histologically showed thyrocyte hypercellularity. None of C3H/He mice developed Graves'-like disease. T cells of C57BL/6 mice could not recognize the hTSHR presented by hM12 cells because of different H-2 molecules. hTSHR expresed by hM12 cells may be treated by professional APCs of C57BL/6 mice, then presented to T cells and induced Graves' -like disease. This result indicated that occurrence of Graves'-like disease might not associate with the cells that used to immune animal express MHC Class Ⅱ molecules or not. **Conclusions** MHC class Ⅱ molecules expressed on human epithelial thyroid cell in GD might not be a pathogenic factor of GD. But a secondary phenomenon induced by other immune disturbance.

056 FULL-LENGTH CLONING AND FUNCTIONAL STUDY OF HUMAN TRIP15 GENE

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Objective: To clone full-length cDNA of human thyroid receptor interacting protein 15(hTRIP15) and explore its function. **Methods:** RT-PCR, Northern blotting, GST pulldown, yeast two-hybrid were used. **Results:** we have found in the pituitary library an EST sharing 73% homology with Drosophila alien. Full-length sequence of the gene was cloned and named as hTRIP15 with 1963bp coding a protein 433aa. It showed a homology of 87% with alien. In addition, an isoform with two -ag adapters at 3' end of exon 5 has been cloned. There is a difference of 7 aa between two isoforms. The expression of hTRIP15 has been demonstrated in many tissues by Northern blotting and semi-quantitative RT-PCR, it was especially highly in skeletal muscle, heart, kidney and some endocrine tissues. This gene has been localized on D15s146-D15s117 by Unigene search. It is positioned in the nucleus using GFP techniques. By means of yeast two-hybrid system, it was discovered that the concurrent rotation of rotating yeast strains the plasmids of TRIP-BD and TR-AD may activate the reporter genes expression and the interaction between hTRIP15 and TR could be suppressed in a dose dependent manner by T3. By using GST pulldown assay, it has been shown that both isoforms of hTRIP15 may interact with TR. Gel-shift assay demonstrated that hTRIP15 inhibited the binding of TR with TRE. N terminal 210 aa of hTRIP15 was able to interact with TR, but did not inhibit the binding of TR with TRE. In addition, hTRIP15 interact with YB-1 by yeast two-hybrid screening and was confirmed by GST pulldown. **Conclusion:** hTRIP15 can interact with TR and may be used as a corepressor in regulating the binding of TR with TRE. Since YB-1 may down-regulate the expression of MHC II antigen and TSH receptor in thyroid cells, it is suggested that hTRIP15 may take part in the regulation of thyroid function.

057 IMMUNE EFFECTS OF IODINE ON NORMAL HUMAN THYROCYTES AND AITD PERIPHERAL BLOOD MONONUCLEAR CELLS IN VITRO

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Objective: To investigate the effects of iodine on the expression of immune antigens on cultured normal human thyrocytes and the effects of iodine on apoptosis, proliferation and activation of peripheral blood mononuclear cell (PBMC) from autoimmune thyroid disease(AITD) patients in vitro. To elucidate the role of iodine on the pathogenesis of AITD at the level of target organ and peripheral immune system. **Methods:** Normal human thyrocytes were cultured in medium with potassium iodide. HLA-DR, CD40, B7.1 and Fas antigen expressions on thyrocytes were examined using immunofluorescence staining analyzed by flow cytometer. PBMC were separated from AITD patients, then cultured and stimulated with potassium iodide. The percentage of PBMC apoptosis was quantified using propidium iodine staining. The proliferation index of PBMC was quantified using ^{3}H-thymidine incorporation. Percentages of activated T helper and suppressor cells were also quantified by immunofluorescence staining. **Results:** Cultured thyrocytes expressed a mount of CD40. Iodide didn't stimulate CD40 and B7.1 expression alone, but upregulated CD40 expression stimulated by IFN-γ and B7.1 expression induced by IFN-α(P<0.05). Iodide had no influence on Fas and HLA-DR expression. Compared with normal person the percentages of apoptotic PBMC from AITD were significantly lower after culture. We found a significantly suppressed rate of apoptotic AITD PBMC after stimulated by iodide. Iodide significantly increased the proliferation response of AITD PBMC cultured with PHA (P<0.05). Both the percentages of activated T helper cells and T suppressor cells at the stimulation of PHA was upregulated by iodide (P<0.05). **Conclusion:** Our results suggest that iodine may play an important role in regulating immune reaction through regulating the expression of costimulators on thyrocytes within the thyroid gland. This may act via local cytokines. We also found that iodide suppressed apoptosis of AITD PBMC and stimulated their proliferation and activation. AITD PBMC can resist to apoptosis in vitro, which may play an important role in the development of AITD.

058 A COMPARATIVE SCREENING FOR AUTOIMMUNE THYROID DISEASE IN AREAS WITH DIFFERENT IODINE INTAKE

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Objective: To elucidate the relationship between iodine intake and autoimmune thyroid disease (AITD). **Methods:** A cross-sectional study was conducted in 3 rural communities of Pansan (iodine deficient), Zhangwu (iodine suitable) and Huanghua (iodine excessive). 4343, 7714 and 4230 inhabitants were questioned on thyroid disorders, as well as 1103, 1584 and 1074 among them were examined and sampled in Panshan, Zhangwu and Huanghua communities respectively. Sera TSH, FT3, FT4, TPOAb, TGAb and TRAb were examined. Urinary iodide and B ultrasound on the thyroids were also evaluated. **Results:** In Panshan, Zhuangwu and Huanghua, the median urinary iodide(MUI) was 103.1 μ g/L, 374.8 μ g/L and 614.61 μ g/L respectively (P<0.01). The prevalence of Graves'disease was 13.6‰, 12.0‰ and 11.2‰ respectively (P>0.05). The prevalence of subclinical hyperthyroidism is lower in Huanghua than that in Pansan and Zhangwu. The prevalence of positive TRAb and TPOAb in patients with subclinical hyperthyroidism is higher in Huanghua than that in Pansan and Zhangwu (P<0.01). The prevalence of Hashimoto's thyroiditis (HT) was 3.6‰, 10.1‰ and 14.9‰ in Pansan, Zhangwu and Huanghua respectively (P<0.05), and That of atrophic thyroiditis (AT) was 0.9‰, 6.9‰ and 13.0‰, respectively (P<0.05). The prevalence of either TPOAb or TGAb positivity was similar among the 3 communities (P>0.05), while the prevalence of elevated TSH in the TPO-Ab positive cases was significantly higher in Huanghua and Zhangwu than that in Panshan (P<0.01). **Conclusion:** The prevalence of HT and AT with hypothyroidism became higher along with the increased iodine intake. Cases with positive TPOAb were more likely to have hypothyroidism in areas with high iodine intake although there was no difference among the prevalence of positive TPOAb or TGAb in the 3 communities.

059 CLINICAL VERIFICATION OF METHIMAZAOLE OINTMENT ON HYPERTHYROIDISM THERAPY

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Objective: To evaluate the efficacy and safety of 5% Methimazole (MMI) ointmen objectively. **Methods:** 138 patients with Graves' disease aged from 18 to 65 were enrolled (untreated or stopped using ATD for more than 2 weeks). They were randomized into two groups (in a double-blind, double-modelling, parallel-matched trial) and treated for 12 weeks. Patients in ointment group (n=71) were treated with 0.2g 5% MMI ointment transdermally and placebo orally. Those in control group (n=67) were treated with placebo ointment and 10mg MMI (oral administration). All preparations were used three times daily. The dosage of MMI required to be reduced after biochemical euthyroidism. **Results:** The perfect control rate (clinical and biochemical euthyroidism) was 93.0% in ointment group and 86.6% in oral administration group ($P>0.05$). Incidence rate of systemic side effects including hepatic damage, leucocytopenia, and drug allergy in ointment group was significantly lower than that in oral administration group (2.78% vs 20.3%, $P<0.01$). Incidence rate of local side effects including pruritus, tighten, papules, desquamation, prick-pain were similar in the two groups (19.4% vs 14.5%, $P>0.05$), and maybe associated with the medium of ointment. However, local side effects were so light that patients can almost tolerate. **Conclusion:** 5% MMI ointment, with less systemic side effects, has similar efficacy on hyperthyroidism to oral preparation.

060 CLINICAL EXPERIENCES AND PATHOLOGICAL MECHANISM OF INTERVENTIONAL EMBOLIZATION IN THE TREATMENT OF REFRACTORY GRAVES DISEASE

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Objective: To study the clinical experiences and pathological mechanism of interventional embolization in the treatment of refractory Graves disease. **Methods:** Selective arteriography followed by embolization of thyroid arteries were performed by Seldinger's technique in 22 patients who cannot tolerate oral anti-thyroid medications, radioiodine or surgery. The histomorphology of thyroid tissue removed by surgery was examined and diameters of arteries were measured with microscope calipers in those patients with huge goiter who received subtotal thyroidectomy after embolization. **Results:** 6 patients were cured by subtotal thyroidectomy after embolization. 16 cases were treated only with interventional embolization. Of whom 14 were cured, the other 2 patients were still on maintenance dose of anti-thyroid medication. At 6~50 months of follow up, all patients remained euthyroid and the size of thyroid glands had decreased remarkably by at least 1/3~1/2 of its original volume. Pathological examination showed that most thyroid arteries were embolized. Chemical inflammation, necrosis and fibrosis were observed. Average diameter of capillary network was 0.12~0.25mm. **Conclusion:** Embolic technique is a new effective, minimally invasive and safe method for the treatment of refractory Graves disease.

061 SEQUENCE VARIATIONS IN THE PROMOTER-PROXIMAL REGION OF PHOSPHOENOLPYRUVATE CARBOXYKINASE GENE (*PCK1*) IN DIABETES

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Objective: Genome mapping of type 2 diabetes had identified a linkage trend with chromosome 20, band q13, a region that includes *MODY1* and *PCK1* genes. Phosphoenolpyruvate carboxykinase is a rate-controlling enzyme in gluconeogenesis, playing a central role in glucose homeostasis. The expression of its gene (*PCK1*) in the liver is elevated in most forms of diabetes and its regulation is primarily at the transcriptional level, affected by hormonal and nutritional states. **Methed:** We screened a 579 bp segment (containing most of the regulatory elements) of the promoter-proximal region of *PCK1* for sequence variations by single-strand conformational polymorphism (SSCP) and direct-sequencing. A total of 137 subjects (52 affected and 85 unaffected members) from 18 kindreds were studied; initial scanning by PCR-SSCP of 4 overlapping fragments (188-209 bp) covering the 579 bp segment. **Results:** Results of the PCR-SSCP analysis showed no evidence of sequence variations in the -547 to -348, -434 to -246, and -174 to +32 segments. These segments contain the IRS motif, glucocorticoid response unit as well as thyroid hormone response elements. However, PCR products for the -322 to -137 segment showed different SSCP bands migration patterns. Direct-sequencing revealed a base variation at -232 (C/G) in both affected and unaffected subjects. The significance of this novel finding is not immediately apparent; however, this nucleotide is 3 bp downstream from the P3 element (cAMP response unit), an essential site for the CCAAT/enhance-binding protein b in the transcription of *PCK1* gene. **Conclusions:** the promoter-proximal region of *PCK1* is intact and mostly unchanged in Chinese diabetes patients. The novel finding of a single nucleotide variation next to the P3 element may suggest a possible association with increased expression of the *PCK1* gene product. Functional assays that incorporate this variation may draw further conclusions to its effect in *PCK1* gene expression.

062 THE ROLE OF CALPAIN10 GENE POLYMORPHISM IN THE GENETIC SUSCEPTIBILITY TO THE TYPE 2 DIABETES IN CHINESE POPULATION

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Objective: To test the hypothesis that Calpain 10 gene (CAPN 10) contributes to the genetic susceptibility of type 2 diabetes in Chinese population. **Methods**: SNP43 (G-A) and SNP 19 polymorphism were determined by PCR-RFLP method in 127 non-diabetes normal controls, 211 unrelated type 2 diabetic subjects , and a total of 801 individuals from 218 Chinese families affected with type 2 diabetes (including 597 type 2 diabetes patients, and 143 non-diabetes subjects). Statistical analysis including affected sibpair analysis were carried out to test the association or linkage of Calpain 10 gene with diabetes. **Results**: 1). The frequency of "G" allele of SNP43 were found to be significantly increased in 211 unrelated type 2 diabetes as well as 218 diabetic probands from type 2 diabetic pedigrees as compared with that in non-diabetic normal controls (0.92 vs 0.86, $P<0.05$ and 0.93. vs 0.86, $p<0.01$, respectively). 2). In affected Sibpair analysis, no evident of linkage was found between neither SNP43 or SNP19 and diabetes in 218 diabetic families. However, a stronger association was found between "G" allele of SNP43 and a subgroup of diabetic probands from type 2 diabetes pedigrees with Identity-by-decent (IBD) larger than 0.5 in SNP43. ("G" allele frequency was 0.95 in the subgroup, $p<0.00001$). **Conclusions**: in this study , we have observed association between "G" allele of SNP43 of calpain10 gene and diabetes as well as the association between "G" allele of Calpain 10 gene and the "evidence of linkage", therefore, the Calpain10 gene is a major determinant of genetic susceptibility to type 2 diabetes in Chinese population.

063 Effect of Orlistat on Cardiovascular Risk Factors and Insulin Sensitivity in Young Obese Chinese Type 2 Diabetic Patients

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Objective: We designed a pilot study to examine the effect of 6-month 120mg Orlistat *t.i.d.* treatment on body weight, cardiovascular risk factors and insulin sensitivity (IR) in 26 severely obese young Chinese type 2 diabetic patients (aged 37 ± 8 years, mean BMI of 34 kg/m^2) who failed to reduce weight despite having dietary counselling. **Methods:** Anthropometry, blood pressure, glycemic and lipid parameters were assessed; a standard 2-h oral glucose tolerance test (OGTT), a modified short insulin tolerance test (SITT) and DEXA were performed before and at the end of the treatment. **Results:** Following 6-month Orlistat treatment, these patients had significant reductions in weight (mean value of 4 %), waist circumference (4 %), % body fat (5 %), fasting glucose (17 %), HBA1C (10%), cholesterol (7 %), LDL-cholesterol (13 %), systolic (7 %) and diastolic (9 %) blood pressures. They also had significantly improved oral glucose tolerance (AUC, 1837±405 Vs 1591±454 mmol/l×min) and OGTT-derived IS (COMPOSITE, 6.3×/÷1.7 Vs 8.5×/÷ 2.1) as well as insulin resistance (HOMA, 42×/÷2 Vs 32×/÷2) indices. Moreover, univariate correlation analyses revealed that % weight loss subsequent to Orlistat treatment was significantly associated with % change in COMPOSITE (R=0.38) and SITT-derived IS indices including K_t (time for total glucose decrement, R=-0.45) and G_d (total 20-min SITT glucose decrement, R=0.71). **Conclusion:** Short term Orlistat treatment without conjunction of hypocaloric diet (primary care settings) significantly improved the cardiovascular risk factors and insulin sensitivity in this cohort of severely obese young Chinese Type 2 diabetic patients. The close relationship between weight loss and IS improvement was also emphasized.

064 PolyI:C Prevents Diabetes in NOD Mice

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Objective: To detect the effects of Polyinsinic polycytidylic acid (PolyI:C)on inhibiting diabetes ,insulitis and its possible mechanism. **Methods:** 64 female NOD mice were divided into two groups and administered PolyI:C 50ug and PBS respectively by intraperitoneal (i.p.) injection, one time every other day for 4 weeks. Then mice were injected with cyclophosphamide (300mg/kg body weight), 16h after cyclophosphamide administration ,detection of splenocytes apoptosis by DNA ladder qualitative analysis and by flow cytometry quantitative analysis.Other mice were observed for an additional one month to detect the effects of PolyI:C on inhibiting diabetes, insulitis and interleukin-10(IL-10),tumor necrosis factor-α (TNF-α)and interferon-γ (IFN-γ) mRNA expression in pancreatic tissue by RT-PCR technique. **Results:** Comparing with the control group, PolyI:C treatment clearly increased the percentage of apoptosis splenocytes(33.31%±3.95% vs control 22.53%±2.78%,P<0.05), and significantly suppress insulitis and diabetes (12.50% vs control 56.25%,P<0.05), Pancreatic tissue showed significantly lower levels of TNF-α and IFN-γ and higher levels of IL-10 in the PolyI:C group. **Conclusions:** PolyI:C can prevent cyclophophamide-induced diabetes in NOD mice. The protection may be independent of the presence of suppresser cells and may involve increased apoptosis of Th1 autoimmune effcetor cells. Furthermore, PolyI:C can change Th1 and Th2 cytokine in pancrease.The immune mechanism of PolyI:C is closed related to the change in IL-10, TNF-α and IFN-γ mRNA.

065 Aldose Reductase-Deficient Mice are Prevented form Reduced Glutathione Depletion in Sciatic Nerve Associated With Diabetes

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Objective: Exaggerated flux through the polyol pathway during hyperglycemia is thought to be the major cause of lesions in the peripheral nerve. The polyol pathway consists of aldose reductase (AR), which converts glucose to sorbitol with the aid of NADPH as a co-factor, and sorbitol dehydrogenase (SDH), which oxidizes sorbitol to fructose using NAD^+ as a cofactor. Evidences supporting the role of AR come from the animal studies where treatment with AR inhibitors (ARIs) led to the prevention of reduced glutathione (GSH) depletion, sorbitol accumulation and improvement in Motor Nerve Conduction Velocity (MNCV) deficits. However, the specificity of ARIs *in vivo* has been questioned. To clarify the role of these two enzymes in diabetic neuropathy, we adopted genetic approach. Previously, we have shown that overexpressing human AR (hAR) in lens of mice would led to further reduction of GSH in lens under hyperglycemia when compare with nontransgenice littermates. We suggested that the exaggerated flux through AR causing the depletion of NADPH may be the major culprit in the pathogenesis of this disease. **Methods**: We generated AR-deficient mice to study the effect of AR deficiency on the development of diabetic neuropathy. AR-deficient and wild type control mice were induced to become diabetic by streptozotocin injection (200mg/kg body weight). MNCV measurement, sugar osmolites analysis by high performance liquid chromatography (HPLC) and reduced glutathione (GSH) measurement had been performed in these animals. **Results**: There was no difference in MNCV between untreated AR-deficient and wild type mice. The diabetic wild type mice showed significant MNCV reduction compared to the normoglycemic littermates ($P<0.001$). However, the diabetic AR-deficient mice did not show reduction in MNCV when compare with normoglycemic littermates. Sorbitol level in the untreated AR-deficiet mice was significantly lower than the untreated wild type mice ($P<0.001$), but still, a trace amount of sorbitol was present in the untreated AR-deficient mice. More sorbitol was present in the diabetic AR-deficient mice when compare with the normoglycemic littermates, but the amount of sorbitol accumulated was significantly lower then that in the diabetic wild type mice ($P<0.001$). There was no difference in GSH level between untreated AR-deficient and wild type mice. The diabetic wild type mice showed significant GSH reduction when compare with the normoglycemic littermates ($P<0.001$). However, the diabetic AR-deficient mice did not show reduction of GSH when compare with the normoglycemic littermates. **Conclusion:** The present data suggest that the exaggerated flux through AR is the major contributor to the pathogenesis of diabetic neuropathy and GSH depletion caused by the exaggerated flux through AR is involved in the pathogenesis of this disease.

066 THE INHIBITORY EFFECTS OF ANTISENSE TGF-β_1 GENE ON THE MESANGIAL HYPERPLASIA BY UNILATERAL NEPHRECTOMY IN DIABETIC RATS

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Objective: To investigate the effect of antisense TGF-β_1 gene on the mesangial hyperplasia by unilateral nephrectomy in diabetic rats. **Methods:** Transfection with TGF-β_1 antisense oligonucleotide to the mesangial cells from unilateral diabetic rats with the lipofectamine was carried out by transgenic technique. The recombinant antisense TGF-β_1 retroviral vector plasmid by virus packaging cell PA317 was packed when the virus titer was also detected by NIH 3T3 cell, having the mesangial cells of unilateral diabetic rats infected with the highest titers virus supernatant. The TGF-β_1 mRNA expression of mesangial cells and other extracellular matrix such as collagen IV and fibronectin with RT-PCR was also measured, and the TGF-β_1 protein expression was measured with ELISA. **Results:** Both renal hypetrophy of mesangial cells and mesangial expansion of DN were inhibited by both antisence TGF-β_1 oligonucleotide and recombinant antisence TGF-β_1 retroviral expressing plasmid leading to a decrease not only in the expression of TGF-β_1 protein but also in the mRNA expression of TGF-β_1 mRNA. Furthermore, the matrix expression was also decreased, such as fibronectin and collagen IV. **Conclusion:** It suggests that TGF-β_1 antisense gene can prevent diabetic nephropathy progress.

067 ESTABLISHING THE GENE EXPRESSION PROFILE OF HUMAN NORMAL ADIPOSE TISSUE USING cDNA ARRAY

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Objective: To fully understand the physical function of adipose tissue and search novel obesity and insulin resistance related genes, the gene expression profile of human normal adipose tissue was established using cDNA array. **Methods:** ^{33}P labeled cDNA, derived from human normal adipose tissue total RNA , was hybridized to a cDNA array containing over 18, 000 human expressed sequence-tagged (EST) clones. The results were acquired and analyzed with several softwares. The EST was considered to be expressed in adipose tissue when the ratio of signal to noise was greater than or equal to 2. The known genes were divided into eight categories according to their putative function. The 400 EST clones with the highest signals on the membrane were further analyzed with bioinformatics. **Results:** (1) Totally, 8230 genes were expressed in normal adipose tissue. Known genes and known ESTs were 5200 and 3030, respectively. 289 clones were classified into known genes among the 400 ESTs strongly expressed in adipose tissue. Among them, less than 20%have previously been reported to be expressed in adipose tissue. (2) 62 secreted proteins, 129 receptor proteins and 74 transcription factors were found to be expressed in adipose tissue. (3) The chromosome localization of 400 genes strongly expressed in adipose tissue showed that relative abundance was significantly increased on chromosomes 1, 11, 19 and 22q compared to the expected distribution of the same number of random genes. **Conclusions:** Adipose tissue has an important secretory function and there is a complex regulatory network of autocrine/paracrine in the local region. The high expressed genes in adipose tissue were not an even distribution throughout the genome, which may provide clues to find novel obesity and insulin resistance related genes.

068 THE EFFECTS OF BERBERINE ON GLUCOSE METABOLISM AND LEPTIN, RESISTIN GENE EXPRESSIONIN ADIPOCYTE

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Objective: To observe the direct effects of berberine(BER) on glucose metabolism and leptin, resistin gene expression in 3T3-L1 adipocyte. **Methods**:1)Differentiated 3T3-L1 adipocytes induced by dexamethasone, insulin and isobutylmethylxanthine were used. The amounts of glucose disapppeared from the culture medium after incubation with drugs for 48h were determined as glucose consumed by the cells. 2)The uptake of 2-deoxy-[^{3}H]-D-glucose was used for the study of glucose transport. 3)Leptin and resistin gene expression were detected by RT-PCR. **Results**: 1)Differentiated 3T3-L1 cells after it had been induced for 8 days manifestated an adipocyte phenotype. Oil red staining showed that there was a great amount of fat in the cytoplasm. The insulin sensitivity increased markedly. 100pmol/L insulin increased significantly the amount of glucose consumption ($p<0.01$). Fat tissue specific genes leptin and PPAR-γ expression increased markedly. 2)In low glucose (5.5mmol/L) and high glucose(25mmol/L) culture medium, the glucose concentrations were significantly decreased by 1-100μmol/L BER while glucose consumption increased by 52-169% with BER ($p<0.001$). The glucose lowering effect of 10μmol/L BER was markedly stronger than that of rosiglitazone and gliclazide at the same concentrations. 50μmol/L BER enhanced significantly the glucose lowering effect of 100pmol/L and 100nmol/L insulin ($p<0.05$). 3)1-100μmol/L BER increased markedly glucose transport with dose-response($p<0.01$). 4)The results from RT-PCR showed that BER inhibited leptin and resistin gene expression. **Conclusion**: BER increases significantly the amount of glucose consumption and the rate of glucose transport, inhibits leptin and resistin gene expression. The implication of these results remains to be further explored.

069 FREQUENCY DISTRIBUTION AND ITS FEATURE OF PERCENTAGE OF BODY FAT BY BIOELECTRICAL IMPEDANCE ANALYSIS IN CHINESE IN SHANGHAI, CHINA

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Objective: Our former research had shown that there was a good correlation between predictions of body fat content by bioelectrical impedance analysis(BIA) and dual energy X-ray absorptionmetry(DEXA), the correlation coefficient was 0.79 in men and 0.86 in women respectively(P=0.0001). However, on accounts of its high cost of instrument and complexity, the use of DEXA in large population are limited. The purpose of this research was to measure the percentage of total body fat by BIA in an epidemiological study, as well as to study the frequency distribution and its feature of percentage of body fat in Chinese in Shanghai, China. **Methods:** From 1998.9-1999.9, a transactional survey of an urban population aged 40-70 years was performed in Huayang Community of Shanghai. BIA was applied to measure the body fat content in all 1522 subjects. **Results:** 1.Gender difference: The mean percentage of body fat was remarkably increased in women compared with men(34.37+/-0.23 vs 24.22+/-0.25, P=0.0001). After the subjects were divided into <10%, 10-<15%, 15-<20%, 20-<25%, 25-<30%, 30-<35%, 35-<40%, 40-<45% and 45%- subgroups according to their total body fat content, it could be noticed that the peak frequency distribution of percentage of body fat was concentrated in 20-<25% in men, but 30-<35% in women. 2.The trends of change in aged subgroups: among aged 40-50 years, 50-60 years, and 60-70 years subgroups, the percentage of total body fat in men were 23.72+/-0.38, 24.62+/-0.51, 24.53+/-0.42 respectively(P=NS); while in women were 33.05+/-0.33, 34.41+/-0.48, 36.26+/-0.43 respectively (P=0.0001). **Conclusions:** In Shanghai residents aged 40-70 years, average percentage of total body fat in men was about 24%, and 34% in women. Total body fat content was different with gender, which suggested that BIA could better represent the reality of body fat content.

070 ENVIRONMENTAL FACTORS AND GENETIC INFLUENCE OF ENDOTHELIAL NITRIC OXIDE SYNTHASE GENE POLYMORPHISM IN ESSENTIAL HYPERTENSION AMONG SOUTHERN CHINESE

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Objective: Nitric oxide is a potent physiologic vasodilator and hypertension develops in endothelial nitric oxide synthase (eNOS) knockout mice. It has also been shown that the allele frequency of a missense variant of a G-to-T substitution at codon 298 in exon 7 of the (Glu298Asp) eNOS gene differed significantly between hypertensive and normotensive (NT) French and Japanese individuals, though conflicting results have been reported. We therefore studied prospectively the role of this eNOS gene polymorphism in the development of hypertension in a group of non-diabetic Chinese subjects. **Methods:** Baseline anthropometric and biochemical parameters were measured in 644 non-diabetic subjects recruited from a community-based population study. Their glycaemic (WHO 1999 criteria) and blood pressure status (Hypertension: SBP $\geq$ 140/90mmHg or on treatment) were reassessed at 2 years. Genotyping of the eNOS polymorphism was performed using polymerase chain reaction amplification of genomic DNA followed by BanII and MboI restriction enzyme digestions. **Results:** At baseline, multiple logistic regression analysis showed that age (p<0.0001), body mass index (BMI)(p<0.0001) and Homeostasis Model of Assessment (HOMA) IR (estimate of insulin resistance) (p<0.01) were significant determinants of hypertension. There was no significant difference in the allele frequencies and genotype distribution of the Glu298Asp polymorphism among hypertensive subjects (n=171) (T:0.13) (GG/GT/TT=76.3%:21.3%:2.4%) when compared with NT subjects (T: 0.12) (78.0%:20.9%:1.1%). Of the 473 NT subjects at baseline, age, BMI but not the Asp containing variant were significant predictors of hypertension at 2 years. **Conclusion:** Development of hypertension in this group of Southern Chinese is predicted by age and BMI. Genetic influence of the Glu298Asp eNOS gene polymorphism may be overwhelmed by these environmental factors.

071 The relationship between insulin resistance and endothelium dependent vasodilation in obese subjects

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Objective: To study the relationship between insulin resistance and endothelium dependent vasodilation(EDV) in obese subjects. **Methods:** 10 non-diabetic obese subjects(OB) and 10 age matched non-obese controls were studied. All subjects were received following tests: ① OGTT and insulin releasing test; ② Acute insulin response(AIR) to an intravenous bonus of glucose(0.5g/kg weight); ③ euglycemic hyperinsulinimia clamp study to determine the peripheral glucose deposite rate(GDR) in steady state; √ EDV and endothelium-independent vasodilation(EIV) of brachial artery. **Results:** The FPG and 2hPG were normal in both groups, but the serum insulin concentration were robust elevated in obese group (0h: 34.5±11.7 vs. 14.8±2.0mU/L , 2h: 147.3±21.1 vs.29.6±10.1 mU /L, P<0.05). The AIR was enhanced and the area under the curve was larger in OB, the PG levels in 3, 5, 10 and 30 minutes were also higher in OB (P<0.05). The GDR were much lower in obese subjects (6.4±1.3 vs. 12.7±2.2 mg/kg/min P<0.01) and a inverse relationship between GDR and BMI was found in obese group. The EDV was impaired in OB (Δ% = 9.33±4.9 vs. 14.07±3.04, P<0.05), but EIV was no difference between two groups. **Conclusion:** The EDV is impaired in obese subjects even though the blood pressure, plasma glucose and cholesterol were normal which suggest that insulin resistance is an independent risk factor for macrovascular disease.

072 THE RELATIONSHIP BETWEEN FATTY ACID COMPOSITION IN SERUM AND INSULIN SENSITIVITY IN SIMPLE OBESE SUBJECTS AND HEALTHY SUBJECTS

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Objective: To investigate the relationship between fatty acid composition in serum, fatty acid indices such as PUFA/SFA, 20:4n-6/20:3n-6, 18:3n-6/18:2n-6, n-3PUFA/n-6PUFA and insulin sensitivity. **Methods:** Serum fatty acid composition, insulin, lipids, fasting plasma glucose were determined in 53 simple obese subjects and 30 healthy subjects. Blood pressure, height, weight, waist circumtance and hip circumtance were measured. BMI, WHR and QUICKI, an index of insulin sensitivity were calculated. The relations between variables and QUICKI were analyzed by multiple linear regression and comparison between two groups was performed by t-test. **Results:** ① Insulin sensitivity was negatively correlated with 14:0(r=-0.437, p<0.01), 16:0(r=-0.290, p<0.01), 16:1n-7(r=-0.309, p<0.01), SFA(r=-0.234,p<0.05), 18:3n-6/18:2n-6(r=-0.333,p<0.01); positively correlated with 18:2n-6(r=0.242, p<0.05), 20:4n-6(r=0.291,p<0.01),PUFA(r=0.324,p<0.01),PUFA/SFA(r=0.327, p<0.01). ② Compared to healthy subjects, PUFA /SFA in simple obese subjects was significantly lower (1.56±0.30 vs 1.74±0.37, p<0.05), while 18:3n-6/18:2n-6 was significantly higher (0.0113±0.0053 vs 0.0083±0.0035 p<0.01). ③ According to stepwise multiple regression, 20:4n6/20:3n6, PUFA/SFA were also significantly related to insulin sensitivity. **Conclusion:** In serum fatty acid profile, 14:0,16:0,16:1n-7,as well as PUFA/SFA, 20:4n6/20:3n6 were related to insulin resistance. The elevation of 14:0, 16:0, 16:1n-7 may impair insulin sensitivity, while increment of PUFA/SFA, 20:4n6/20:3n6 may be beneficial.

073 Bone Marker and Mineral Density in Hyperthyroidism of Premenopausal Women

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OBJECTIVE: Hyperthyroidism is associated with enhanced osteoblastic and osteoclastic activity, and patients frequently exhibit accelerated bone loss and high bone turnover. The aim of this study was to investigate the bone formation and resorption markers, quantitative ultrasound bone mineral density in premenopausal female with overt hyperthyroidism. **METHODS:** We investigated 53 Chinese premenopausal female patients, aged between 18 and 50 years, with overt hyperthyroidism before treatment. Patients were arranged Tc-99 thyroid scan and blood was drawn to measure the levels of serum T4, TSH, TBII by radio-immuno assay, and enzyme linked immunosorbent assay was used to measure bone-specific alkaline phosphatase (BALP) and urinary-deoxypyridinoline crosslinks (DPD) markers related to bone metabolism (Metra Biosystems, CA, USA), quantitative ultrasound of the dominant side calcaneus for speed of sound (SOS), broadband ultrasound attenuation (BUA), and stiffness were measured by the Achilles ultrasound bone densitometry (Lunar Corp., Madison, WI) and in 125 healthy volunteers as controls. **RESULTS:** Both bone formation and resorption markers were elevated, the BALP level was elevated by 285% and the urinary DPD level was elevated by 600% in hyperthyroidism, compared to controls both of markers were significantly increased ($P < 0.0001$). The QUS parameters SOS, BUA, and stiffness in hyperthyroidism patients were 1533±31 m/seconds, 111±12 dB/MHz, and 83±14%, respectively, were lower compared to controls. **CONCLUSION:** Our findings suggest that hyperthyroidism is associated with increased bone turnover, and bone mineral density is reduced in premenopausal women with overt hyperthyroidism before treatment.

074 INTERLEUKIN-1 RECEPTOR ANTAGONIST GENE POLYMORPHISM IN OLD-AGED MEN WITH OSTEOPOROSIS

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Objective : To investigate the frequency and pathgenesis of polymorphism ,numbers of 86-bp sequence repeats, in intron 2 of interleukin-1 receptor antagonist(IL-Ra) gene in old-aged men with osteoporosis from a population of China.**Methods**: The polymorphism of IL-Ra gene in the groups of 50 non-osteoporosis subjects,83 osteoporosis subjects in old-aged men and 35 early-aged men were detected by polymerase chain reaction (PCR).**Results:** The old-aged men had lower bone mineral density(BMD), IL-1Ra/IL-1 β ratio,serum levels of estradiol (E2), testolactone (T) ,blood bone glaprotein(BGP),but ,higher urine level of urine C-terminal telopeptide of type I collagen(CTX). A_2 allele(240bp,two repeats) was the less allele.The frequency of A_2 allele and genotypes with A_2 in control group were higher than that in osteoporosis group(0.190 vs. 0.096 , P=0.039; 0.360 Vs 0.193 , P=0.037,respectively).The A_2 allele has been associated with an increase levels of IL-1Ra,IL-1Ra/IL-1 β ,BMD **Conclusion**: The old men with A_2 were less susceptible to primary osteoporosis than that without A2 allele,which is associated ,at least to some extant,with the correct response of IL-1Ra to IL-1.

075 Association of Estrogen Receptor β Gene Polymorphisms with Bone Mineral Density in Southern Chinese Women

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Abstract. Estrogen has profound effects on bone metabolism. Cellular responses to estrogen are mediated by estrogen receptors (ERs), which belong to the nuclear receptor superfamilies. Because estrogen and its receptor play a critical role in the pathogenesis of osteoporosis, genetic variations in or adjacent to the estrogen receptor gene may be associated with variations in bone mineral density (BMD). The present study examined the correlation between a dinucleotide (cytosine-adenine; CA) repeat polymorphisms located in the flanking region of estrogen receptor β gene and BMD in 325 healthy southern Chinese women, aged 21 to 86 years. BMD at the lumbar spine and hip region were measured using dual energy x-ray absorptiometry (DEXA). The numbers of the repeats observed in our population ranged from 16 to 28, with the predominant allelic size being 154 (20 CA repeats) and 164 (25 CA repeats). Among the premenopausal women (n=120), subjects bearing at least one allele of 20 CA repeats had significantly higher BMD at L2-L4 lumber spine ($p<0.01$), total hip ($p<0.01$), femoral neck ($p=0.02$), trochanter ($p<0.01$) and Ward's triangle ($p<0.01$) region after adjusting for age, height, weight and estrogen exposure years. There were significant associations between the spine and hip BMD values and the number of alleles for 20 CA repeats. However, no association between ERβ gene polymorphisms and BMD was observed in the postmenopausal women (n=205) at any skeletal site. In conclusion, estrogen receptor β gene polymorphisms are associated with higher BMD at both hip and spine in premenopausal women, suggesting that ERβ gene may have a modulatory role in bone metabolism in young adulthood.

076 THE EFFECT OF INTERMITTENT INJECTION OF RECOMBINANT HUMAN PARATHYROID HORMONE (1-84) ON BONE MINERAL DENSITY AND HISTOMORPHOMETRY OF OVARIECTOMIZED RATS

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Objective: To observe the effect of intermittent injection of recombinant human parathyroid hormone (1-84) ($rhPTH_{1-84}$) on rat bone mineral density (BMD) and histomorphometry.**Methods:** 6-month-old virgin female SD rats were randomly assigned to 5 groups: (1) Ovariectomized for end point(OVXE, n=6), (2) Ovariectomized for PTH treatment (OVXEP,n=6), (3)Sham-operated for end point (ShamE,n=6), (4)Sham-operated for baseline(ShamB, n=5), (5)Ovariectomized for baseline(OVXB,n=6). The lumber and proximal femur BMD was measured by dual energy X-ray absorptiometry (DEXA) prior to operation, 3 and 4.5 months(m) after operation in OVXE, OVXEP and ShamE rats. During 3~4.5m after operation, OVXEP rats received daily subcutaneous injection of $rhPTH_{1-84}$ (20ug/100g BW,6 times/week),OVXE and ShamE received injection of vehicle; OVXE, OVXEP and ShamE rats at 4.5m, ShamB and OVXB rats at 3m after operation were sacrificed, the proximal tibiae were processed for histomorphometry, calcein was administered intraperitoneally at 6 and 2 days prior to killing to label the mineralizing surfaces of bone. **Results:**(1) During PTH/vehicle treatment, the lumber BMD in OVXEP had increased 15.7+/- 9.5mg/cm^2(mean+/-SD), while that of OVXE had decreased 13.8+/- 8.8 mg/cm^2, the changes between OVXEP and OVXE were significant($p<0.01$); The BMD of proximal femur of OVXE, OVXEP and ShamE all increased with similar extent($p>0.05$ between each two groups).(2) Bone histomorphometry analysis showed that the %TbAr of OVXEP was higher than that of OVXE($p<0.05$) and similar to that of OVXB($p>0.05$), the TbTh of OVXEP was higher than any other group(all, $p<0.05$), the TbN of OVXEP was slightly greater than that of OVXE($0.05<p<0.1$), the %LPM, MAR and BFR/BV of OVXEP were all higher than those of OVXE and ShamE($p<0.01$ or less), N of OC was not different among OVXE, OVXEP and ShamE. **Conclusion:** (1)$rhPTH_{1-84}$ could significantly increase the lumber BMD, maintain the teabecular area of tibital metaphysis and may have no effect on femoral cortical BMD in ovariectomized rats;(2)Intermittent treatment of PTH could promote the osteoblastic activity without influence on osteoclastic activity, this therapy increases bone mass mainly by increasing TbTh although it could slightly raise TbN.

077 The effect of estrogen on the change of osteoclast activity and bone histomorphometry in ovariectomiced rats

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Objective: To understanding further the mechanism of estrogen action on osteoporosis in ovariectomized rats by examining the difference of the formation of bone marrow stem cell derived osteoclast , the expression of IL-6, IL-6 receptor(IL-6R) and gp130 as well as bone histomorphometry. **Methods:** 3-month-old rats were divided randomly to three groups, etc: normal control, ovariectomized(OVX) and OVX plus estrodia(mg/kg)(OVX+E_2). Samples from bone marrow were used both for cell culture to induce osteoclast formation and RT-PCR to detect the expression of IL-6, IL-6R and gp130. Bone histomorphometry were determined by. **Results** Comparing to control group, the formation of osteoclast and IL-6, IL-6R were greatly increased in OVX($p<0.001$), and the increases were significantly inhibited by E_2 treatment. Bone histomorphometry analysis showed that estrogen treatment increased bone mass and the amount of trabecula by 129% and 132% respectively($p<0.05$). The activity of bone resorption decreased significantly and the rate of bone formation increased to 203%($p<0.05$). **Conclusion** Treatment ovariectomized rats with estrogen can significantly reduce the bone loss by inhibiting the increased osteoclat formation and activity. The effect of estrogen appears, at least partly, by inhibiting the expression of IL-6, IL-6R system.

078 STUDY ON EXPRESSION OF LEPTIN RECEPTOR (Ob-R) IN HUMAN OSTEOBLASTS

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Objective: It was studyed that the expression of leptin receptor (Ob-R) mRNA isoforms in human osteoblast and their binding characteristics with [^{125}I]-leptin. **Methods:** 1.Human osteoblasts, from discarded rib of an adult patient with bronchiectasis undergoing lung subsection, were cultured and identified by cell biological criteria. 2. Total RNA of human osteoblasts were isolated, and primers of long and short forms of Ob-R were synthesized. To amplify the isoforms of Ob-R mRNA, RT-PCR method was performed. 3. Leptin was labelled with ^{125}I by chloramine-T method and purified by PAGE. A receptoradioassay was performed to identify the binding of osteoblasts with [^{125}I]-leptin. **Results:** 1.Morphologically, cultured osteoblasts were triangle¸multilateral and formed multilayers. They also showed positive histochemical staining for alkaline phosphatase and immuno-fluorence staining for osteocalcin. Mineralization were also developed upon supplementation with ascorbate, β-glycerophosphate as indicated by Alizarin Reds. 2. Analysed by RT-PCR, both long and short Ob-R mRNA isoforms were detected in the primary, first, second and third passaged human osteoblasts respectively. 3. The percentage of ^{125}I-leptin binding to Ob-R on osteoblast were 16.7% +/- 2.3% and 6.1% +/- 1.4% without or with unlabelled leptin respectively. From the saturation experiments and by Scatchard plotting, a single class of high-affinity binding sites for leptin was identified with an apparent K(d) of 0.11 +/- 0.06 nM and a B(max) of 1.7+/-0.3 nM /10^6 cells respectivly. **Conclusion:** Results of this present study demonstrate that human osteoblast expresses both Ob-Ra and Ob-Rb, which could bind to [^{125}I]-leptin and be inhabited by cold leptin specifically. It indicates that osteoblasts are target cells of leptin, and possibly leptin could regulate osteoblast's growth, prolifiration and function and even bone remoldeling.

P1 THE MOLECULAR SCANNING ON GLUCOKINASE AND HEPATOCYTE NUCLEAR FACTOR-1ALPHA GENES IN TWO CHINESE MODY/NIDDM FAMILIES

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Objective: To explore the disease-related gene mutation(s) in type 2 diabetes (non-insulin dependent diabetes mellitus, NIDDM) families containing subjects with maturity-onset diabetes of the young (MODY). **Methods:** The microsatellite polymorphism genetic makers of glucokinase (GCK, MODY2) and hepatocyte nuclear factor-1 α (HNF-1 α , MODY3) genes were selected to perform the classical linkage analysis in two NIDDM/MODY families. Direct sequencing was employed to confirm the variation of nucleotide(s) in the promoter and all the exons of HNF-1 α gene, with which linkage analysis indicated the most likely correlation in these families. **Results:** The maximal LOD score was 3.584930(θ = 0.000000) for the HNF-1 α genetic maker with type 2 diabetes at the mode of autosomal dominant in family 50002. Direct sequencing displayed a heterozygous mutation in exon 5 , i.e. Tyr322Asn (TAT/AAT). The Direct linkage of diabetes with the mutation showed LOD score reching 3.60 when θ was set at zero at the mode of autosomal dominant. **Conclusion:** There is no enough evidence to demonstrate that the variation in or near GCK gene is the major cause of diabetes in the two families. The mutation Tyr322Asn(TAT/AAT) in exon 5 of HNF-1 α gene was supposed to have predisposition to MODY/NIDDM in family 50002.

P2 MITOCHONDRIAL DNA VARIATIONS IN TYPE 2 DIABETES IN TAIWAN

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Objective: Type 2 diabetes is sometimes transmitted in a mode of maternal inheritance, and mutations in mitochondrial DNA are thought to be responsible for the pathogenesis of the disease. The goal of this study was to determine the frequency of mitochondrial DNA NADH dehydrogenase (ND) 1 gene variations in type 2 diabetes in Taiwan. **Methods:** We analyzed 57 unrelated diabetic patients and 33 unrelated nondiabetic control subjects to test the possibility that variations of the ND 1 gene was responsible for type 2 diabetes. **Results:** Direct sequencing of the ND 1 gene revealed 6 homoplasmic substitutions at 3316 G/A, 3394 T/C, 3434 A/G, 3497 C/T, 3571 C/T and 4216 T/C. There are no significant differences in the prevalence of these substitutions between the diabetic patients and control subjects except the 4216 T/C. The 4216 T/C substitution causes amino acid replacement from hydrophilic neutral tyrosine to hydrophilic basic histidine and this variation was present in one diabetic patient not in control subjects. Substitution at 3394 T/C was seen in 5.3% of diabetic patients and 6.0% in control subjects in this study. **Conclusion:** Mitochondrial DNA NADH dehydrogenase (ND) 1 gene variation doesn't associate with type 2 diabetes in Taiwan.

P3 ASSOCIATION STUDIES OF THE THREE CANDIDATE GENES WITH TYPE 2 DIABETES MELLITUS IN A CHINESE POPULATION

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Objective: To explore the relationship between the polymorphisms of the selected short tandem repeats (STRs) of the candidate genes and type 2 diabetes mellitus (DM) in a Chinese population. **Methods:** STRs including D11S916 of uncoupling protein 3 (UCP_3) gene, binucleotide repeat (CA)n within introns 6 [HSLi6(CA)n] of hormone-sensitive lipase (HSL) gene and D20S501 of protein tyrosine phosphatase-1B (PTP-1B) gene polymorphisms were detected by performing polymerase chain reaction (PCR), polyacrylamide gel electrophoresis and silver staining in 106 patients with type 2 DM and 102 control subjects. **Results:** The allele distribution of UCP_3 and HSL gene differed significantly between patients with type 2 diabetes and control subjects (χ^2=26.12, P<0.005; χ^2=10.33, P<0.005, respectively). For UCP_3 and HSL gene , the frequencies of allele A6, A7, A8 and allele B9 were much higher in diabetic patients than in control subjects, while the frequencies of allele A1 and allele B5 were lower in diabetic patients than in control subjects. At D20S501 locus, The allele distribution of PTP-1B gene had no significant difference in two groups (χ^2=3.77, P > 0.05). **Conclusion:** Our date show that D11S916 of UCP_3 gene and HSLi6 (CA)n of HSL gene polymorphisms are associated with type 2 diabetes in Chinese suggesting that UCP_3 and HSL might represent susceptibility genes for type 2 diabetes. D20S501 of PTP-1B gene polymorphism is not associated with type 2 diabetes in Chinese.

P4 The association study of Intestinal Fatty Acid-Binding Protein gene polymorphism with Type 2 Diabetes Mellitus

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Objective: To investigate the contribution of Alanine to threonine substitution at codon 54 (Ala54Thr) polymorphism of the intestinal free fatty acid binding protein (FABP) gene to genetic susceptibility to type 2 diabetes mellitus in Chinese Han population. **Methods:** The cases consisted of 102 type 2 diabetic patients. 102 of their healthy spouses were chosen as controls. Genotype of the Ala54Thr polymorphism was analyzed by the polymerase chain reaction-oligonucleotide ligation assay. **Results:** The frequency of Thr54 alleles was not significantly different between type 2 diabetic patients and control subjects (29.4% and 30.4%, respectively); Thr/Thr genotype carriers has higher serum lipoprotein (a) (Lpa) concentration in control subjects (P=0.000); Although the subgroup carrying Thr/Thr genotype has higher OR value, there was no statistical significance (Thr/Thr vs.Ala/Ala: adjusted OR=2.94; 95%CI: 0.93-9.32 , P=0.067). **Conclusions:** It suggests that FABP gene is not a major gene for type 2 diabetes mellitus in Chinese Han population. Ala54Thr polymorphism of FABP gene may contribute to Lpa metabolism.

P5 Prevalence and Prediagnosis of Mitochondrial tRNA $^{Leu(UUR)}$ Gene 3243 A toG Mutation in 523 Type 2 Diabetes Families in Shanghai District

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Objective: To assess the incidence of A→G mutation at position 3243 of the mitochondrial tRNA $^{Leu(UUR)}$ gene in Chinese type 2 diabetes pedigrees .**Methods:** On the base of clinical phenotupe, 534 pedigrees of type 2 diabetes with diabetes with diabetic family history from out-patient clinic and ward were selected randomly to investigation. Some of them from the hospitals located in Shanghai peripheral region . Type 1 diabetic pedigrees had been excluded with identification of GAD of/and IA-2 antibodies. Screening of the mutation mentioned above for 523 probands and all members of its first degree relatives if proband with positive mutation was performed with PCR-RFLP. Direct sequencing was applied to confirm the mutation in some samples. **Results:** five families with mutation were identified by screening of 534 pedigrees , in which probands of four families and two diabetics of first-degree relatives, but not proband of the another family carried this mutation. The total incidence is 0.96%(5/523). The proband of this family was confirmed to be without mutation by PCR-RFLP repeatedly and direct sequencing after recollection of blood sample. **Conclusion:** the incidence of mitochondrial tRNA $^{Leu(UUR)}$ gene 3243 A→G mutation in 'chinese type 2 diabetic families from Shanghai and its peripheral region is 0.96%.Mutation inconsistency of proband with diabetic first-degree relatives exists certainly, which might be the result of mitochondrial heteroplasmy or mixture of MDM with common type of type 2 diabetes.

P6 STUDY ON ABNORMITY OF HNF-1α GENE IN MODY3

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Objective Maturity-onset diabetes of the young(MODY) is a heterogenous monogenic disease of NIDDM and autosomal dominant inheritance.MODY3 HNF-1a gene which have 9 exons had localized on 12q24.2.Some of susceptial special defected gene and mark site in NIDDN have been illuminated with the development of gene mapping, cloning and candidate cloning. The study included the attack level and clinical profiles of mutation in MODY3 and hepatocyte nuclear factor-1α (HNF-1α)in Asian (including Chinese and Japanese). **Method** We choose 500 MODY subjects with onset<30 years of age ,which contained 28 subjects from 3 generations in 7 families. The diagnosis standard is according to the standard of diagnosed and classified of diabetes of ADA(1997). Prepared Genome DNA with blood sampling of subjects, then DNA sequence analysed by polymerase chain reaction (PCR),DNA purified and the sequence was analysed(the reaction analysis by Applied Biosysstems DNA sequencers model 377),using flank sequences of HNF-1α gene 1-9 exons (9 pais)as primres. **Result** We screened 500 MODY subjects by polymerase chain reaction(PCR)and direced sequence of the products. Four new gene mutation of HNF-1α were found in 16 subjects, that is,Arg^{131}/Trp CGG→TGG in exon 2,Arg^{159}/Gln CGG→CAG in exon 2, Arg^{229}/stop codon CGA→TGA in exon 3,Arg^{271}/Trp CGG→GGG in exon 4.**Conclusion** The study indicated that there were four new gene mutations in 16 subjects by screened 500 IDDM subjects.9 subjects with changes of amino acid in 5 MODY families, which included gene products defects by the generation of stop codon .The decrease of HNF-1α expression could lead to functional defects of β cells and all subjects had incoordinate clinical blood glucose levels, some of whom developed to IDDM.

P7 THE RELATIONSHIP BETWEEN HLA-DM GENES AND TYPE 1 DIABETES IN CHINESE

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Objective To study the relationship between human leucocyte antigen(HLA)-DMA, DMB alleles and the susceptibility of type 1 diabetes in Chinese. **Methods** Genomic DNA were prepared from peripheral blood by standard method, then polymerase chain reaction and dot blot hybridization techniques were used to classify 4 DMA and 5 DMB alleles. **Results** DMA*0103, DMB*0103 alleles were significantly increased in the patients, (50% vs 8% P<0.01; 42.9% vs 21.8% P<0.01 respectively),while DMA*0102(10.8% vs 42%,P<0.01), DMB*0101 (48.6% vs 73.6% P<0.01) alleles were significantly less frequent in patients with type 1 diabetes. DMA*0101/0102(10.8% vs 37%,P<0.01), DMB0101/0101(7.1% vs 46%,P<0.01) genotype were significantly increased in controls , while DMA*0101/0103 (50% vs 8%)genotype were increased in the patients with the difference also being significant. **Conclusions** Our results showed that DMA*0103, DMB*0103 alleles confer susceptibility while DMA*0102, DMB*0101 alleles confer protection to type 1 diabetes. At the same time,DMA*0101/0102, DMB0101/0101 are protective genotypes while DMA*0101/0103 is susceptive genotype of type 1 diabetes in Chinese. Whether or not this relationship is secondary to disequilibrium with DRB1 or DQ B1 alleles is to be determined in the future.

P8 QUALITATIVE AND QUANTITATIVE ANALYSIS OF MITOCHONDRIAL DNA IN CHINESE PATIENTS WITH TYPE 2 DIABETES

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Objectives: Recent studies suggest that qualitative and quantitative change of mitochondrial DNA (mtDNA) may be associated with susceptibility to diabetes. We have investigated the mtDNA level in 152 Chinese Type 2 diabetic patients with disease duration ≤ 5 years (51with maternal family history of diabetes (FH), 51 with paternal FH and 50 with no parental FH), and 51 age and sex matched healthy control subjects. **Methods:** MtDNA levels was measured by quantitative PCR and corrected for 28S rRNA levels. **Results:** The relative mtDNA levels were similar in diabetic patients and control subjects (547 ± 249 vs 534 ± 318). Diabetic patients with FH had similar anthropometric and clinical characteristics compared with the patients with no FH. However, the latter group had significantly higher mtDNA levels (638 ± 259) than the groups with either maternal FH (533 ± 191, P < 0.05) or paternal FH (468 ± 264, P< 0.001 by ANOVA). In addition, mtDNA levels was inversely correlated with age and blood pressure in control subjects but mtDNA levels was positively correlated with age, age of diagnosis and lipid profiles in patients with maternal FH (all P< 0.05). Multivariant analysis showed that age was the only independent predictor of low mtDNA levels in control subjects. On the other hand, both maternal and paternal FH were independent predictors for low level of mtDNA amongst diabetic patients.We have also examined the 16189 T→C and 16223 C→T variations in 522 Type 2 diabetic patients and 173 healthy control subjects by PCR and M*nl*l digestion. Only the frequency of 16189 C variant was significantly higher in the diabetic than the control group (0.34 vs. 0.24, OR [95%Cis]=1.6[1.1-2.3]). In the diabetic group, the 16189 C carriers had significantly higher BMI than the T carriers (27.1±6.4 vs. 25.9±6.4 kg/m^2). The variant was not associated with anthropometric or clinical parameters in the control group.**Conclusions:**Our results suggest that FH and age may affect mtDNA levels. The opposite relationships between mtDNA levels and other clinical parameters in diabetic and control groups requires further clarification. Moreover, the mtDNA 16189 C variant is associated with Type 2 diabetes in Chinese.

P9 The changes of some cytokines in the newly diagnosed patients of type 1 diabetes mellitus

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Object: Tried to find out the profile of some cytokines and the relationship between cytokines and type 1 Diabetes mellitus. Analyze the relationship between cytokines and heredity, cytokines and metabolism. **Method and Subject**: 26 newly onset diabetes patients and 30 cases normal control. Six cytokines in serum of all subjects were measured and the ratios of CK from Th1 and Th2 were calculated. Values of HbA1c, fasting c-peptide and insulin of patients' was measured; Some HLA gene types of patients were defined. SPSS was used in Data analysis. **Result:** 1. IL-4 and IL-2 decreased in newly onset diabetes patients, IL-1β elevated. 2.There were positive lineal relation between IL-4 & IL-12, IL-1β & IL-12, IFN-γ & IL-12, IL-12 / IL-4 & IFN-γ / IL-4 3. There were positive lineal relationship between fasting insulin & IL-1β and fasting c-peptide & IL-12. 4.The value of TNF-α elevated in HLA-DQB1*0201 carrier compared to non-carrier. **Conclusion:** 1.Newly diagnosed diabetes patients have a defect of IL-4, IL-2 secretion and an elevation of IL-1β. 2.TH1 bias exited in tŷpe 1 diabetes patients. 3. There were not lineal relationship between Cytokines and indicators of metabolism. 4.HLA-DQB1*0201 carrier might be prone to elevated in TNF-α production.

P10 EXPRESSION OF CCR5, A Th1 CHARACTERIZED CHEMOKINE RECEPTOR INCREASED IN TYPE 1 DIABETES MELLITUS

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Objective: To investigate the role of chemokine receptor CCR5 and CCR3 which are the characteristic surface marker of Th1 and Th2 respectively in the development of type 1 diabetes mellitus. **Methods:** Peripheral blood mononuclear cells(PBMC) were isolated from heparinized venous blood from 15 newly diagnosed type 1 diabetics and 10 healthy control and 10 type 2 diabetics. Cells were stimulated with PHA and cultured in 15%FCS-1640 medium. After 48 hours cells was stained with the FITC-anti-human CD4, CHROME-anti-human CCR5, PE-anti-human CCR3 and detected by immunofluroescent marker-flow cytometry. The supernatant of culture cells were harvested and assayed for the level of IFN-γ and IL-4 with ELISA. **Results:** The number of $CD4^+CCR5^+$ lymphocyte of type 1 DM was significantly higher than type 2 DM and control group($P<0.05$) while the expression of $CD4^+CCR3^+$ was lower than other two groups. The Level of IFN-γ was increased but IL-4 decreased in type 1 DM($P<0.05$). **Conclusion:** It suggests that CCR5 and CCR3 ,which paly an important role in lymphocyte migration and Th lymphocyte differentiation may contribute to the immune pathogenesis of type 1 DM and it may be useful in screening type 1 diabetic patients.

P11 ERYTHROCYTES GLUCOSE UPTAKE IN PATIENTS WITH DIABETES MELLITUS

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In many studies were shown that tissue glucose uptake was altered at the Diabetes Mellitus. Glucose uptake was observed in erythrocytes separated by centrifuged from blood taken from cubital vien in 38 patients with Diabetes Mellitus, and observed in vitro by incubation erythrocytes in potassium-phosphate buffer medium containing 10 mmol/l glucose. Glucose uptake was calculated as different of the glucose concentration into erythrocytes during of incubation time. The results show that erythrocytes basal glucose uptake was increase in patients with Diabetes Mellitus on 1.85 time ($P<0.05$) than nondiabetic subjects. Erythrocytes glucose uptake were increase in patients with IDDM on 1.7 time ($P<0.05$) and in patients with NIDDM on 2.24 time ($P<0.02$). Erythrocyte glucose uptake had correlation with glycemia level, had positive correlation with erythrocytes membrane osmotic resistance abilities. Glucose uptake in erythrocytes may be occurred only from membrane glucose transporter 1 (GLUT1). Authors proposed that metabolic disturbances at the Diabetes Mellitus could influence to GLUT1 function and erythrocytes glucose uptake. Improving of the metabolic abnormalities may lead to normalizing of the tissue glucose uptake at the Diabetes Mellitus that can help to preventing of progression of the diabetic complications.

P12 EXPERIMENTAL STUDIES ON REPORTER GENE EXPRESSION DRIVEN BY PEPCK PROMOTER ADJUSTABLE TO INSULIN

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Objective:.To study the effect of phosphoenolpyruvate carboxykinase(PEPCK) gene promoter on regulation of reporter gene transcription. **Methods:**Firstly, Construction and identify of recombinant firefly luciferase reporter plasmid pGL2-PEPCK-Luc. Secondly, the recombinant pGL2-PEPCK-Luc and the control plasmid pSV-β-Galactosidase were co-transfected to rat hepatoma cell line(CBRH7919) by Lipofectin. And conformed that the luciferase could be expressed in hepatoma cell transfected by pGL2-PEPCK-Luc. Finally, to investigate the transcriptional regulation of PEPCK promoter in hepatoma cells transfected by reporter gene. **Result:** 1, CAMP and DEX stimulated PEPCK promoter obviously at 30min and reached their maximal effect one hour later. At the dosage mentioned before, no difference of effects on gene transcription was found between CAMP and DEX, but they exerted accumulative effects on PEPCK promoter. 2, At different concentrations (10^{-11} M, 10^{-10} M, 10^{-9} M, 10^{-8} M) in physiological range, insulin exerted suppressive effect on PEPCK promoter , and the suppressive effect was dose independent. 3, At different concentrations (0mg/dL, 50 mg/dL, 180 mg/dL and 500 mg/dL), glucose didn't exert any effects on PEPCK promoter within one hour. 4, At different levels of glucose concentration (0mg/dL, 50 mg/dL, 180 mg/dL and 500 mg/dL), glucose had no interaction with insulin. No influence of glucose was found on suppressive effect of insulin exerting on PEPCK promoter. **Conclusion:** With the aid of measuring luciferase activity, we conformed that there is a perfect feedback mechanism for PEPCK promoter in hepatoma cell. Our results provide a promising experimental basis for insulin release from hepatic cell controlled by PEPCK promoter physiologically.

P13 PROGRESSION OF THE ONSET OF TYPE 1 DIABETES AND β CELL APOPTOSIS IN FEMALE NOD MICE WITH SPONTANEOUS DIABETES

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Objective: To observe the progression of β cell apoptosis and the expression of TNF-α, IFN-γ, iNOS, Fas, Fas ligand in the islets of female NOD mice. **Methods:** After the pancreases were fixed and embedded at different week of age, insulitis was examined by HE staining, apoptosis by TUNEL reaction and the expression of antigens by immunohistochemistry, recorded the score of insulitis, the frequency of β cell apoptosis and antigen by semi-quantitative method, analyzed the association between them. **Results:** β cell apoptosis appeared two peaks , the first at week 3, which preceded the appearance of T cells (began at week 5) in the islets, the second at week 32, when the incidence of diabetes was maximal. There was statistically significant association between apoptosis and insulitis, blood glucose; FasL and blood glucose; IFN-γ, TNF-α and iNOS with age; iNOS with insulitis, blood glucose. **Conclusions:** It suggested that apoptosis is the mode of cell death responsible for β cell deletion, and there were multiple factors responsible for the apoptosis of β cells. Fas-FasL, NO and cytokines were effected in three independent ways. IFN-γ and TNF-α worked in different stage of the diabetes pathogenesis. IFN-γ played a significant role in the late stage (after week 20). TNF-α played role throughout the pathogenesis, but differ in young and adult mice.

P14 EFFECT OF LPS ON THE PERMEABILITY OF THE BLOOD-BRAIN BARRIER TO INSULIN

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Objective: To look at the effects of lipopolysaccharide (LPS) on the permeability of the blood-brain barrier (BBB) to human insulin in CD-1 mice. **Methods:** The mice were given an injection into the jugular vein of 0.2 ml of lactated Ringer's solution containing 1% bovine serum albumin and 2 x 10^6 cpm of ^{131}I-insulin. Some injections included ^{125}I-albumin 2 x 10^6 cpm or unlabeled human insulin. Blood was collected from the carotid artery. The brain/serum ratio of radioactivity was determined. **Results:** Intraperitoneal injections of LPS significantly increased the uptake by the brain of ^{131}I-insulin and disrupted the BBB to ^{125}I-albumin. After subtraction of the brain/serum ratio for ^{125}I-albumin, brain/serum ratios for insulin were increased: 10.38 ± 0.70 μl/g (LPS) vs 3.62 ± 0.27 μl/g (no LPS), $p<0.0001$, showing that LPS increased the uptake of insulin independent of BBB disruption. Pretreatment with indomethacin 10 min before LPS injections enhanced BBB disruption, but not insulin transport. Pretreatment with the nitric oxide (NO) synthase inhibitor aminoguanidine had no effect on insulin or albumin uptake, but pretreatment with NG-nitro-L-arginine methyl ester enhanced insulin transport, but not BBB disruption. **Conclusion:** LPS increases the saturable transport of insulin across the BBB independent of disruption and prostaglandins with potentiation by NO inhibition. Such increased transport could potentiate the central effects of insulin and so contribute to the peripheral insulin resistance seen with infection and inflammation.

P15 EXPERIMENTAL STUDY ON THE DEVELOPMENT OF INSULITIS BY NASAL ADMINISTRATION OF INSULIN TO NOD MICE

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Objective: To study the effects of nasal administration of insulin on insulitis in NOD mice. **Methods:** 14 female NOD mice were divided into two groups. Group 1 was nasally administered insulin 50 μg in 50 μl PBS and group 2 50 μl PBS only starting from the age of 5 weeks by the way of twice for the first week and then once weekly until the age of 15 weeks. IFN-γ and IL-4 of spleen cells were measured by ELISA, and the pathological changes of pancreas were examined. Moreover, FasL and Fas expression on islets were examined by immunohistochemical techniques. **Results:** Nasal adminstration of insulin could suppress insulitis. The level of IFN-γ was decreased and the level of IL-4 was increased in spleen cells of NOD mice administered with insulin. Fas expressed on islets of the mice suffering from insulitis only, whereas FasL expressed on islets of the mice administered with insulin but did not with PBS. **Conclusions:** Nasal administration of insulin suppresses insulitis in NOD mice. The decrease in Th1 response and down-regulation of proinflammatory cytokines as well as increase in Th2 response and anti-inflammatory cytokines may be involved in the mechanism. Nasal immune tolerance-induced expression of FasL may also play a role.

P16 EFFECT OF NITRIC OXIDE ON INSULIN ACTION IN STZ-INDUCED DIABETIC RATS

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Objective: to investigate whether nitric oxide(NO) replacement or nitric oxide synthase (NOS) inhibition influences *in vivo* insulin action. **Methods:** The NO donor, sodium nitroprusside(SNP, 3ng/kg/min), NOS inhibitor, NG-monomethyl-L-arginine (LNMMA,1mg/kg/min) or saline was infused constantly during the euglycemic clamp procedure in healthy and STZ-induced diabetic rats. Insulin was infused at a rate of 3.0 μU/kg/min in the awake condition. Plasma insulin levels during the insulin infusion were 30 μU/ml, and blood glucose of diabetic rats were clamped at 140 mg/dl by periodic adjustment of *i.v.* glucose infusion rate. **Results:** LNMMA significantly decreased (7.0±0.7 vs. 11.2±0.8 mg/kg/min, $p<0.05$) insulin-mediated glucose disposal rate (GDR) in diabetic rats, compared with saline infused diabetic rats. However, SNP resulted in a significant increase in GDR (21.6±2.0 vs.11.2± 0.8 mg/kg/min, $p<0.01$) in diabetic rats and the metabolic clearance rate for glucose (MCR) in diabetic rats infuced SNP reached to the 87% of levels of healthy rats. SNP and LNMMA did not affect GDR or MCR in healthy rats. **Conclusion:** It suggests that NO improves *in vivo* insulin action in diabetic rats.

P17 THE CYTOKINES PROFILE OF TYPE 1 DIABETES MELLITUS AT DIFFERENT STAGES

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Aim: Learn the relation between cytokines and the duration of diabetes. **Method:** Determined the levels of six cytokines in sera of all subjects and calculated 2 ratios of cytokines (IFN-γ/IL-4 and IL-12/IL-4). Group A: normal control 30 cases; group B: newly-diagnosed diabetes mellitus (DM) 26 cases; group C: long standing DM 20 cases; group D: newly-diagnosed Grave Disease 9 cases. **Results:** IL-1β of group B patients elevated, but group C and D decreased. IL-2 of group B patients decreased, but C and D elevated. The level of IL-4 was decreased in group B and C. The ratios of IL-12 / IL-4 and IFN-γ /IL-4 were elevated in group B and C. **Conclusion:** The level of IL-1β decreased with the course going on showed its decreasing followed the end of the death of the β cells. The decreasing of IL-2 was only in early stage. IFN-γ/IL-4 and IL-12/IL-4 were not recovery with the course going, it means the immune system of DM patients decline to a TH1 bias, maybe it is the bias that causes the onset of diabetes. There were high levels in most kinds of cytokines we determined on GD. The ratios of IFN-γ / IL-4 and IL-12 / IL-4 were not significant in GD when compared with group A. Our research on cytokines didn't show the TH2 bias in GD.

P18 EFFECTS OF CHLOROQUINE ON THE EXPRESSION OF INSULINASE OF INSULIN-RESISTANCE RATS

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Objective: To investigate effects of chloroquine on expression of insulinase gene (EIG) and insulinase protein (EIP) of liver of rats, and study the molecular biological mechanism of its ameliorating insulin sensitivity of the insulin-resistance(IR) rats. **Methods:** Wistar rats with high-fat feeding as IR animal model were studied (conventional chow fed Wistar rats as controls). EIG, EIP, insulinase activity of liver(IAL) were used. Average glucose infusion rate (GIR_{60-120}) measured with euglycemic-hyperinsulinemic clamp technique was used to estimate insulin sensitivity in IR rats and control rats.Moreover, effects of chloroquine on these indices were observed. **Results:** EIP,EIG were higher and GIR_{60-120}) lower in IR rats with high-fat feeding than those in the control rats with conventional chow feeding. As compared with IR rats with placebo (saline), EIG and EIP were decreased and GIR_{60-120} were increased in IR rats with administration of chloroquine Correlation analysis showed that IAL was positively correlated with EIG, EIP, and reversely correlated with GIR_{60-120} in IR rats and control rats with or without chloroquine. **Conclusion:** High-fat feeding can cause a increase in EIG and EIP of liver of Wistar rats. Chloroquine may decrease insulinase activity by inhibiting expression of insulinase gene and ameliorate insulin sensitivity in a degree.

P19 EPIDEMIOLOGY OF DIABETES IN PREGNANT RURAL TEHRANI WOMEN

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Objective: Pregnancy diabetes is one of the most prevalent metabolic diseases in the world. As the disease entails serious consequences for the fetus and the mother, screening is very important. This study, therefore, was carried out to determine diabetes prevalence among pregnant mothers in rural areas of Tehran. **Methods:** Altogether 820 pregnant mothers in their 20-28th weeks of conception, were selected by simple stepwise random sampling from among 108 villages in Tehran province. After filling out an individual questionnaire, anthropometric measurements were done and BMI calculated. Sedentary blood pressure was measured. An oral glucose tolerance test with 50g glucose was carried out. For screening glucose level of 140 mg/dl was taken as the cut-off point. Those with higher serum glucose levels were referred for 3-hour glucose tolerance test. Diagnosis criteria for pregnancy diabetes were those of NDDG. **Results:** Based on which 19 subjects (2.3%) were judged to be diabetic. Mean age of the diabetic subjects was 29.4±6.2 which was significantly higher than the normal subjects 24.2±6.2 ($P<0.001$). Mean BMI in the diabetes was 29±4.8 versus 26.5±4.5 in the healthy subjects ($P<0.02$). Diabetes was twice higher in women with the history of children with macrosomia and almost three times more prevalent in those with stillbirth. Positive familial diabetes history raised the chances of diabetes twofolds. Pregnancy diabetes was 4 times higher in those with 5 deliveries and more. Mean diastolic blood pressure was 64±14.6 mmHg in diabetics as opposed to 59±10.9 in the healthy ones ($P<0.05$), while no systolic difference was observed. Out of 106 subjects with positive screening test 18 (17%) were free of risk factors. Of 19 diabetic women 12 (63%) were below 30 years of age. **Conclusion:** Based on these findings it is suggested that screening be carried out, irrespective of risk factor signs, on all pregnant women, for diagnosis and treatment of the people.

P20 A COMMUNITY BASED DIABETES SURVEY IN HUANGPU DISTRICT

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Objective: To carry out a medical survey for investigating the prevalence of type 2 diabetes in WAITAN community, HUANGPU district. **Methods:** The survey was carried out in 1998 in 10025 residents aged 25 to 75 in community model (family as unit). Postprandial blood glucose level was used as screening test and OGTT was assigned to subjects with 2h postprandial glucose level=7.0mmol/L. **Results:** (1) The total number of diabetic patients found was 311 (81 male and 230 female), among them 80 cases were newly diagnosed as diabetes. (2) The total number of IGT cases was 80 (21 male and 59 female). (3) Among the 625 subjects with OGTT, there were 157 hypertensive patients in 311 diabetics, 39 hypertensives in 80 IGT subjects and 97 cases in 261 NGT subjects, with highest occurrence rate of hypertension in diabetes. (4) Also in subjects with OGTT, obese subjects were 118 in 311 diabetics, 40 in 80 IGT subjects and 92 in 261 NGT subjects, with highest occurrence rate in IGT subjects. (5) Among the diabetics, 10 couples were found as diabetic patients. (6) After standardization, the prevalence was 4.95%.**Conclusion:** The prevalence of diabetes in Shanghai increased rapidly with modernization of life style from 2.5% in 1994 to 4.95% in 1998. The occurrence rate of hypertension was highest in diabetics, and obesity in IGT subjects. Both the couples with diabetes could be explained by environment factors.

P21 CURRENT PREVALENCE OF IMPAIRED GLUCOSE REGULATION AND ITS METABOLIC DISORDERS IN SHANGHAI(1998-2000)—SURVERY OF HUAYANG COMMUNITY

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Objective: To investigate the current prevalence of impaired glucose regulation(IGR) and its frequency distribution in Shanghai, a city with tremendous economical development and rapid modernization in China. The relationship between IGR and other metabolic abnormality, such as hypertension and dyslipidemia were also determined. **Methods:** From Sept. 1998 to Aug. 2000, a cross-sectional survey with multiple-stage and random sampling was performed in Huayang community of Shanghai, China. 2978 residents aged 15-94 years(1214 men, 1764 women) were enrolled in this study. 75-gram oral glucose tolerant test(except those with a validated history of diabetes), serum insulin level, lipid profile, blood pressure were detected. IGR was diagnosed according to the WHO 1999 criteria. **Results:** 1. The current prevalence of IGR was 10.5%. After age stratification, that of IGR were 1.5%, 6.6%, 9.5% and 16.9% in 15-19yrs, 20-39yrs, 40-59yrs and 60yrs- subgroups. 2. IGR can be classified into three sub-types, including isolated impaired fasting glucose(IFG), isolated impaired glucose tolerance(IGT) and coexisting IFG and IGT(IFG+IGT). In this survey, the prevalence of IFG, IGT and IFG+IGT were 2.3%, 6.9% and 1.3%. 3.The frequency of hypertension in IFG, IGT; IGT+IFG were 35.7%, 49.7% and 62.2% respectively, while that of hypertriglyceridemia and/or lower HDL-cholesterol were 65.7%, 73.2% and 86.5%. **Conclusion**: 1.The current prevalence of IGR was 10.5% in Shanghai, China. 2. Among the IGR population, IGT is the most common type, which occupied 2/3 of IGR. 3. About 1/2 and 2/3 of the IGR population concomitantly had hypertension and dyslipidemia, more evident in those subjects with impaired glucose tolerance.

P22 Analysis And Evaluation Of Diabetes Mellitus In Elderly People By the Application Of The World Health Organization And American Diabetes Assocation Diagnosis Criteria

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Objective To evaluate the sensitivity and specificity of the World Health Organization(WHO) criteria of DM and American Diabetes Association (ADA) criteria of DM when they are applied to elderly people. **Method** 1,204 subjects without a previous history of DM aged 60 to 90 were selected , and grouped them into different glucose levels by WHO or ADA criteria and analyzed the concordance and the discordance between these populations . **Results** The prevalence of DM was 3.16% and 16.28% by FPG criteria and PG-2h criteria respectively. The sensitivity of diagnosed DM was 15.3% and specificity was 99.2% according to ADA criteria. The coincidence percentage under the two criteria was only 15.3% . The coincidence percentage under IFG and IGT was only 4.5% . The optimal FPG cut-point of diagnosed DM was 5.5 mmol/L in the elderly, which is affected by gender, age, body mass index (BMI) and the presence of hypertension. **Conclusion** There was lack of concordance between WHO and ADA criteria in the elderly. The ADA criteria could not replace for WHO criteria when it was used to diagnose DM patients. It might be suggested that the elderly people with 7.0mmol/L> FPG $\geqslant$ 5.5 mmol/l should receive standard 75-g OGTT.

P23 ANALYSIS OF THE CHANGES OF GLYCOMETABOLISM STATUS AFTER 5 YEARS ON 826 NON-DIABETES

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Objective: To investigate the changes of glycometabolism status after 5 years on 826 non-diabetes and the related factors. **Methods:** 826 subjects including 475 cases with normal glucose tolerance (NGT) and 351 cases with impaired glucose tolerance (IGT) who were identified by OGTT in a screening survey were followed after five years. OGTT was repeated. 1997 ADA criteria was used in the diagnosis of diabetes and IGT. The measurement of body mass index (BMI) and blood pressure were carried out in these people together with the determination of plasma insulin, serum cholesterol, triglyceride and urinary albumin excretion. Insulin resistance (IR) was assessed by Homa Model in order to investigate the influence of these factors on the development of DM. **Results:** (1) 77and 99 cases out of 475 normal subjects were diagnosed as IGT and DM respectively after 5 years. In 351 IGT group, 106 subjects developed as DM, 54 subjects reverted to NGT. (2) The body mass index at initial year (baseline) between subjects who became DM and who remained normal had significantly difference (25.31±3.67 vs. 23.88±3. 25, P<0.001). The former group also had higher insulin resistance (IR) (1.52±0.21 vs. 1.42±0.20, data was transformed to natural logrithm, P<0.001) and fasting plasma insulin (0.85±0.25 vs. 0.78±0.20, data was transformed to natural logrithm, P<0.01) compared with the normal group at baseline. (3) Logistic regression analysis demonstrated a significantly positive correlation between IR, BMI and the development of DM. **Conclusion:** Insulin resistance and obesity were the major risk factors and predictor of occurrence of diabetes mellitus. For all communities, either NGT or IGT, early control of body weight was an important way to prevent glucose tolerance from deteriorating.

P24 A SURVEY OF DIABETES MELLITUS IN BAOTOU IRON & STEEL COMPANY CHINA

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Objectives To investigate the prevalence rate of diabetes mellitus(DM),impaired glucose tolerance(IGT),impaired fasting glucose (IFG)in Baotou Iron & Steel Company and provide scientific evidences for prevention and intervention of DM. **Methods** Using 1997 ADA criteria, we investigated 20221 workers aged 20 years and over (male 15124, female 5097) in Baotou Iron & Steel Company. At the first screening, all subjects surveyed took orally 75 gram glucose and capillary blood glucose were measured before and 2 hours after taking orally 75 gram glucose. Subject with FBG>=6.0 or PG2h>=7.0, a 75 gram oral glucose tolerance test was performed. Blood plasma glucose were determined by glucose-oxidase method. Body mass index (BMI) and waist hip ratio (WHR) were calculated. **Results** The actual prevalence of DM,IGT and IFG were 2.97%,3.15% and 2.10% respectively in 20221 subjects aged 20 years over. The prevalence of DM, IGT and IFG standardized by age were 3.22%, 3.48% and 2.09% respectively. The prevalence rate of DM ,IGT and IFG increased with age and body mass index. The family history of DM and overweight were related to the prevalence of DM, IGT and IFG . The prevalence of DM, IGT and IFG were higher in the mental laborer than physical laborer, $p<0.01$. The prevalence of DM was higher in male than in female($p<0.01$). The prevalence of IGT was higher in female than male($p>0.05$), the prevalence of IFG was higher in female than in male($p<0.01$) The higher the education, the lower the rate of DM, IGT and IFG. The rate of DM, IGT and IFG in the high temperature worker has no significantly difference as compared with control group. The mean value of blood pressure were significantly higher in the persons with DM, IGT and IFG than normal controls, $p<0.01$. **Conclusion** Old age, overweight, obesity , family history of DM and low education are risk factors of DM, IGT and IFG. There is no impact of high temperature environment on the prevalence of DM, IGT and IFG.

P25 INFLUENCES OF THE MICROSOMAL TRIGLYCERIDE TRANSFER PROTEIN GENE POLYMORPHISM ON PLASMA LIPIDS AND LIPOPROTEINS IN SOUTHERN CHINESE

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Objective: Microsomal triglyceride transfer protein (MTP) is a heterodimeric lipid transfer protein. It plays a key role in the assembly and secretion of apolipoprotein B(apoB)-containing lipoprotein in the liver and intestine and its expression is regulated by insulin *in vitro*. A common functional polymorphism (-493G/T) in the promoter of the MTP gene has been shown to influence plasma concentration of VLDL and LDL in Caucasians. We have investigated the effect of this polymorphism on plasma lipids and LDL subfractions in patients with type 2 diabetes mellitus. **Methods:** The –493 G/T polymorphism was determined in over 300 healthy southern Chinese subjects and over 250 patients with type 2 diabetes mellitus by polymerase chain reaction amplification and restriction enzyme digestion. Small dense LDL was measured by density gradient ultracentrifugation. **Results:** The frequency of the T allele was similar in controls and type 2 diabetic patients (0.14 vs 0.17 respectively). Diabetic patients with the TT genotype had the highest concentration of small dense LDL (TT: 156.4 ± 54.9 mg/dl; GT: 118.9 ± 52.0; GG: 117.0 ± 49.4, $p<0.05$), whereas such a relationship was not found in the controls. **Conclusion:** The –493G/T polymorphism is associated with changes in small dense LDL concentration in patients with type 2 diabetes and we speculate this may be due to the interaction of hyperinsulinaemia/insulin resistance and the MTP polymorphism.

P26 APOLIPOPROTEIN E POLIMORPHISM IN TYPE 2 DIABETIC PATIENTS

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Objective: To investigate the apolipoprotein E (apoE) polymorphism in type 2 diabetic patients and its relationship to micro- and macrovascular complications. Methods: In this study, we tested apoE phenotype via DNA amplification and HhaI restriction method in 34 non-diabetic controls and 116 type 2 diabetics and the association between apoE phenotypes and micro- and macrovascular complications including nephropathy, neuropathy, retinopathy, hypertension, coronary heart disease and cerebral infarction. Results: The frequencies of E4 carrier, E3 carrier and E2 carrier were 18.10%, 68.10% and 13.80% in diabetics with no difference between the male and female subjects, no difference between diabetics and non-diabetic controls as well. The number of diabetic nephropathy was higher in E4 carriers and lower in E2 carriers. Further analysis demonstrated that the F-Ins and 2h-Ins levels were significantly higher in E4 carriers and the E2 carriers were of lower level of CH and LDL-CH than rest two. Conclusion: In type 2 diabetics, apoE2 had some protective effect on macro- and microvascular complications with lower concentration of CH and insulin, while apoE4 enhanced the risk of the complications with higher level of CH and insulin levels.

P28 A META-ANALYSIS REPORT: THE RELATIONSHIP BETWEEN CHILDREN'S INTELLIGENCE AND IODINE DEFICIENCY, SUPPLEMENT IODINE, EXCESSIVE IODINE

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Objective: To estimate the role and extent of iodine deficiency on mental development in children, how affection of iodine supplement on intelligence, furthermore to employ the relationship of intelligence and excessive iodine. **Methods:** Meta-analysis was applied to study 116 independent items from 60 published or non-published papers and reports. The references collected standard includes: Sample age declared by references is 5-15; Belong to comparison study; Children lived in iodine deficiency disorders (IDD) area and non-IDD; The social economic and culture development level in study group should be similar with the control group. **Results:** Over 97% of collected references relating to iodine deficiency-intelligence and iodine supplement-intelligence had been published in journal or academe proceeding. And respective 67% and 79% among those reports mainly involved severe IDD areas which were distributed in 18 and 14 provinces of mainland. The weighted average ES of damage effect on children intelligence by iodine deficiency achieved 0.69, which was equivalent to a marked drop in 10.4 IQ points (95% confidence interval (CI): 9.9-10.9IQ) of the children living in non-IDD. The weighted average ES of protective effect on children intelligence by supplement iodine reached to 0.81, that meant the IQ of children born after correction of iodine increased 12.2 points (95% CI: 11.5-12.9IQ) in comparison with those born at least one year before correction. Most of references about the relationship between excessive iodine and intelligence were gather from proceedings, the others from journal. They are located in some area of Shandong, Hebei, Shanxi and inner Mongolia. The mean ES of the role of excessive iodine on intelligence is 0.2 corresponding to 3.1 IQ points (95% CI: 2.0-4.2IQ). Hunter test of above there studies didn't been found any statistic difference at the level of P=0.05. **Conclusion:** Iodine deficiency plays a middle strength role in delaying brain development. Iodine deficiency leads children in IDD area to be at least 10 IQ points lose. Effective supplement iodine plays a remarkable strength role in protecting brain development. It can cause 12 IQ points increasing for children born after correction of iodine in IDD area. Excessive iodine has not show important role in children.

P29 A COMPARATIVE EPIDEMIOLOGY STUDY OF SERUM THYROTROPIN (TSH) LEVELS IN NORMAL SUBJECTS THREE DIFFERENT IODINE INTAKE AREAS

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Objective: To make a comparative epidemiological study on serum TSH level and its related influencing factors in normal subjects in three different iodine intake areas. **Methods:** In the areas of mild iodine deficiency, moderate iodine intake and excess iodine intake, residents were investigated randomly and tested by serum TSH (IMMULITE, the third- generation assay), TPOAb, TGAb, TG, urinary iodine and thyroid ultrasonic scanning. Those having histories of thyroid diseases, familial thyroid diseases or with positive TPOAb, TGAb , abnormal levels of TG or abnormal thyroid ultrasonic scanning were excluded. Then, 660, 728, 550 normal subjects were left respectively and the relationships between TSH levels and urinary iodine concentration, sex, age and TG levels were compared. **Results:** The median urinary iodine concentration of the above three normal groups were 121.17 μg/L, 384.92 μg/L and 621.03 μg/L respectively. The median serum TSH concentration in the area of excess iodine intake was significantly greater than the other two areas', and the lowest was in the mild iodine deficiency area ($P<0.01$). The median serum TSH concentration of female was higher than the male's in moderate and excess iodine intake areas ($P<0.01$), but there was no sexual difference in mild iodine deficiency area ($P>0.05$). The statistically significant differences between TSH levels and ages lied in moderate and excess iodine intake areas but not in mild iodine deficiency area. In all the three areas the serum TG and TSH concentrations showed statistically significant positive correlations. **Conclusions:** In normal subjects the increase of iodine intake may cause a raise of serum TSH level. The median TSH concentration of female is higher than male's when the iodine intake is up to some extent. The relationship between age and TSH are not identical in different iodine intake conditions, whereas TG and TSH have the significant positive correlation .

P30 COMPARATIVE SURVEY ON THE EPIDEMIOLOGY OF HYPERTHYROIDISM IN THREE RURAL COMMUNITIES WITH DIFFERENT IODINE INTAKE

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Objective: To investigate the prevalence of hyperthyroidism in three rural communities with different iodine intake after Universal Salt Iodination (USI) in 1996 and the impact on the incidence of hyperthyroidism by USI. **Methods:** Three rural communities were chosen for epidemiologic study. Inhabitants in Panshan did not used iodized salt whereas both those in Zhangwu and Huanghua had used iodized salt since 1996. We examined the thyroid function (TSH, FT3 and FT4, the functional sensitivity of the assay of TSH was 0.002mU/L, the reference range was 0.3-4.8 mU/L), thyroid autoantibodies (TPOAb, TGAb), urine iodine concentration and thyroid B ultrasound in 1103,1584 and 1074 subjects (>14 years old) in the three communities. We also collected the patients diagnosed as hyperthyroidism between 1991 and 1999 to calculate the average incidence of hyperthyroidism. **Results:** The median urine iodine concentration of Panshan, Zhangwu and Huanghua was 103 μ g/L, 375 μ g/L and 615 μ g/L(P<0.05), respectively. The prevalence of active hyperthyroidism was 1.6%, 2% and 1.2% (P>0.05) and the prevalence of subclinical hyperthyroidism was 3.7%, 3.9% and 1.1% (P<0.05) respectively. Comparing the yearly average incidence of hyperthyroidism of 1996-1999 with that of 1991-1995, we found a significant rise of the incidence in Panshan, a slight but insignificant elevated one in Zhangwu and a nearly unchanged one in Huanghua. **Conclusion:** 1.There was no difference in the prevalence of clinical hyperthyroidism in three communities with significant different iodine intake level. 2.The increased iodine intake is not the only reason for the increasing incidence of hyperthyroidism.

P31 PREVALENCE OF THYROID DYSFUNCTION AND THYROID AUTOIMMUNITY IN MEMBERS FROM MULTIPLEX FAMILIES WITH GRAVES' DISEASE AND THE EFFECT OF IODINE LEVEL ON THE INCIDENCE OF THE DISEASE

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Objective: In order to determine the prevalence of thyroid dysfunction and thyroid autoantibodies in members from multiplex families with Graves' disease (GD), and to evaluate the effect of varying amounts of iodine intake on the incidence of GD, 357 individuals from 56 multiplex GD families were study. **Methods:** Free T3, free T4, TSH, thyroid peroxidase antibody (TPOab), thyroglobulin antibody, and TSH receptor antibody were assayed for all the subjects, and 234 were also measured for urinary iodine excretion. **Results:** In the first-degree relatives of GD patients, the prevalences of subclinical hyperthyroidism, clinical and subclinical hypothyroidism were 5.2%, 1.0% and 1.4% respectively, all these patients were positive for thyroid autoantibodies. Antithyroid antibodies were detected in 68% of the euthyroid subjects of the first-degree relatives, among whom 22.7% were positive for TPOab, 16.0% were positive for TGab, and 58.2% were positive for TRab. The incidence of Graves' disease increased significantly when urinary iodine excretion reached 500-599μg/l. **Conclusion:** Relatives of GD patients from multiplex families had a significantly increased tendency to produce thyroid autoantibodies and to develop Graves' hyperthyroidism. Urinary iodine excretions of 500μg /L-599μg/L was a risk factor for high occurrence of GD.

P32 EPIDEMIOLOGY OF SCHOOLCHILDREN'S THYROID FUNCTION AND INTELLIGENCE IN THE AREAS OF DIFFERENT INTAKE OF IODINE

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Objective: To make a comparatively epidemiological research on thyroid function and intelligence of the schoolchildren at the mean age of 8.48±0.86yrs(x±s) in the areas of low ,moderate and excessive intake of iodine. **Methods:** In the area of low intake of iodine(Panshan in Liaoning province, median urinary iodine(MUI)was 99.23 μg/L), of moderate intake of iodine (Zhangwu in Liaoning Province, MUI 338.30 μg/L) and of excessive intake of iodine (Huanghua in Hebei Province, MUI 631.22 μg/L), 190,236 and 313 schoolchildren were respectively selected to take part in China Raven's Test and then 116,110 and 112 of them were made an examination on thyroid function, thyroid autoantibody(TAA) and MUI. **Results:** There were no significant difference in the incidences of overt hyperthyroidism, subclinical hyperthyroidism and overt hypothyroidism in theses 3 areas. But significant difference was found in the incidences of subclinical hypothyroidism (P=0.001) in theses 3 areas. The incidences of subclinical hypothyroidism in the areas of Huanghua and Zhangwu were 4.76 and 3.37 times higher than that in the area of Panshan. TAA were negative in all the schoolchildren with subclinical hypothyroidism except for one. No significant difference was found among the positive rates of thyroid peroxidase antibody (TPOAb) and thyroglubin antibody (TGAb) in these 3 areas. Serum thyroglubin(TG) values of Huanghua were markedly higher than those of the other two (P=0.0157) .Serum TG values of Zhangwu were higher than those of Panshan but with no significant difference. IQ values of the schoolchildren in Huanghua were markedly higher than those in Zhangwu (P=0.0012) .IQ values of the schoolchildren in Panshan were lower than those in Huanghua and higher than those in Zhangwu but with no significant difference. **Conclusion:** The increase of iodine intake may increase the risk of schoolchildren for subclinical hypothyroidism. In the area of excessive iodine intake, most of the subclinical hypothyroidism cases of schoolchildren are not of autoimmune origin. No obvious effect of excess iodine is found on mental development of schoolchildren.

P33 BIOLOGICAL AFFECTS AND CLINICAL MWANING OF HIGH IODINE ON HUMAN THYROID FOLLICLE EPITHELIAL CELL IN PRIMARY CULTRUE

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Objective: To investigate the relationship between iodine and thyroid disease, we designed iodine affecting on changes of thyroid follicle epithelial cell (TFC) in primary culture of secretory function and molecular level. **Methods:** We used TFC primary culture technique to culture TFC. Added different concentration of KI into the medium.,then detemined contents of T3, T4 and Tg in the supernate by RIA ; determined contents of IL-1a, IL-6 and cAMP in the supernate by ELISA. **Results:** The secretory contents of T3 and Tg were the highest level at 0.15ug/ml; The secretory contents of T4 was the highest level at 0.75ug/ml; there were a significance difference compared with control group. Our experiments proved that TFC can secrete a few contents of IL-1a and IL-6. High iodine concentrations can regulate the secretion of IL-1a and IL-6. The cAMP formation in TFC was also regulated by high iodine concentrations. **Conclusions:** High iodine is the induced-cause of hyperthyroidism, hypothyroidism and thyroiditis.

P34 A STUDY ON IODINE METABOLISM, THYROID FUNCTION AND GOITER FORMATIONIN RATS OF IODINE DEFICIENCY AND IODINE EXCESS

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Objective: To investigate the alterations of iodine metabolism, thyroid function and goiter formation caused by iodine deficiency and iodine excess. **Methods:** Wistar rats were divided into three groups of low iodine group(LI), high iodine group(HI) and normal iodine group(NI) as a control. All Rats were sacrificed at 12 and 24 weeks respectively. Thyroid weight and its morphology, iodine content in urine and thyroid tissue, T4 and T3 levels in serum and thyroid tissue were examined. Expressions of Tg and TPO mRNA in thyroid tissue were observed using RT-PCR method. **Results:** (1) Thyroid weight in LI group was markedly higher than that in other two groups and goiter was found in LI rats with a typical histology of follicle hyperplasia. Goiter was not found in HI rats and thyroid histology was similar to the NI rats. However, some enlarged thyroid follicles full of collagen and some follicles with obvious cellular hyperplasia were found at 24 weeks in HI tars. (2) Urinary iodine and tissue iodine in LI rats was very low. But urinary iodine in HI rats was much higher than the NI rats and the tissue iodine was similar to the NI rats. (3) Serum and tissue T4 in LI group was much lower than the other two groups, but serum T3 was increased at 12 weeks by the compensation and decreased markedly at 24 weeks. Serum T4 and T3 in HI group looked lower than the NI group with no statistical significance, but T4 in thyroid tissue was significantly lower than the NI group and T3 was almost normal. (4) Expressions of Tg and TPO mRNA in HI rats were decreased. But TPO mRNA expression in LI rats was increased and Tg mRNA expression decreased. However the Tg mRNA expression in LI group showed higher at 12 weeks and much lower at 24 weeks compared to the HI group. **Conclusion:** The findings indicated that iodine deficiency produced goiter and severe hypothyroidism. Long term of high iodine intake did not cause more storage of iodine in thyroid gland and extra iodine was excreted through urine by kidney. So iodine excess did not induce goiter formation in Wistar rats, but potential hypothyroidism may exist due to the homeostasis mechanism manifested by the inhibited expressions of Tg and TPO mRNA and decreased synthesis of T4 in thyroid gland.

P35 THE EXPRESSION OF mRNA OF GROWTH FACTOR AND NUCLEAR PROTOONACOGENE IN PAPILLARY THYROID CARCINOMA

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Objective To study the expression and relationship of mRNA of transforming growth factorteba1andteba2 (TGF-beta1, beta2), keratinocyte growth factor (KGF), type1 receptor of TGF-teba(TGF-betaR1),C-myc,C-fos,and C-jun in papillary thyroid carcinoma (PTC). **Methods** Three growth factors were analyzed by reverse transcript-polymerase chain reaction (RT-PCR), three nuclear protooncogenes and TGF-betaR1 by dot blot hybridization in 6 papillary thyroid carcinomas, 6 adenoma, 6 Hashimoto's thyroiditis and 6 normal control tissues obtained at surgery. **Results** mRNA of TGF-ß1 has a high expression in these four thyroid tissues (>5/6), the quantity of its expression is more enhanced in PTC than in other three tissues ($p<0.05$). mRNA of TGF-teba2 is expressed in all these tissues (2-5/6), and no quantity difference among them. We can't detect KGF in these four thyroid tissues. However, a sequence, which related with human normal keratinocyte subtraction library is amplified. Its function is unknown. It is expressed in these four thyroid tissues (2-5/6), more in adenoma and Hashimoto's thyroiditis than in normal control ($p<0.05$). There is no difference between PTC and normal control. Three oncogenes express in all of PTC (6/6), partly in adenoma and Hashimoto's thyroiditis (3-4/6), and less in normal tissues (2-4/6). In addition, the three oncogenes are mostly co-expressed in these tissues (77.2%). **Conclusion** Increased expression of mRNA of TGF-beta1 and decreased expression of TGF-betaR1may be involved in the pathogenesis of PTC. In addition, it can help establish a correct diagnosis. Co-expression of nuclearprotooncogenes C-myc,C-fos,and C-jun in PTC might be related to pathogenesis of PTC.

P36 EXPRESSION OF FOCAL ADHESION KINASE AND ITS CLINICAL SIGNIFICANCE IN THYROID PAPILLARY CARCINOMA

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Objective: To investigate the expression of Focal Adhesion Kinase (FAK) antibody in thyroid papillary carcinoma and its clinical significance. **Methods:** The expression of FAK antibody in 70 thyroid papillary carcinomas and adjacent noncancerous portions was investigated by Dako EnVison immunohistochemical technique. **Results:** The expression of FAK in thyroid papillary carcinomas was significantly higher than that in adjacent noncancerous portions ($P<0.001$). The expression of FAK was higher in the group of over 40-year-old than that in the group of under 40-year-old ($P<0.01$). There was a significant difference between cases with lymphnodes metastasis and those without metastasis ($P<0.05$). The level of FAK expression showed no relation with the gender of the patient and the size of the tumor. **Conclusions:** The FAK might be a transformation linked enzyme of tumors. The level of FAK expression might be a valuable marker for the carcinogenesis and FAK might play an important role in biological behavior of thyroid papillary carcinoma.

P37 TELOMERASE ACTIVITY IN THYROID BENIGN AND MALIGNANT TUMOURS

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Objective: To investigate telomerase activity in tissue and FNA samples of benign and malignant thyroid tumor. **Method:** Telomerase activity of tissue and FNA sample of thyroid tumor was examined by telomerase repeat amplification protocol assay (TRAP), including 18 cases adenomas, 4 cases goiters, 24 cases malignant tumors. **Results** Total of 18 adenomas, telomerase activity in the tissue and FNA samples of 17 cases were negative. All goiters were also negative. Telomarase activity was present in 13/14(92.86%) tissues and 11/14(78.51%) FNA samples of papillary carcinomas. In 8 cases of follicular carcinomas, 7 cases was positive(87.5%) in tissues, and 6 cases positive (75%) in FNA samples. Telomerase activity was all present in 2 cases of tissues and FNA samples of medullary carcinomas. In one word, the predictive value of positive (PV) of tumor tissue is 95.65%, the predictive value of negative is 91.3%, sensitivity 91.67%, specificity 95.45%. The PVpos of FNA samples is 95%, PVneg 80.76%, sensitivity 79.16%, specificity 95.45%. **Conclusion** Telomerase activity are present in the tissues and FNA samples of thyroid malignant tumour. Telomerase assay of FNA sample is useful method for diagnosing the benign or malignant thyroid tumor.

P38 THE EXPRESSION OF GONADOTROPIN-RELEASING HORMONE RECEPTOR IN HUMAN THYROID TUMOR

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Objective: To investigate the expression of GnRHR in thyroid tumor. **Methods:** Paraffin-embedded thyroid specimens from ten patients with thyroid carcinoma and ten patients with thyroid adenoma were retrieved from the pathological files (2000-2001) of Xijing Hospital. None of the patients underwent any chemical therapy. And pathologists confirmed all the specimens. ABC immunohistochemical method and image analysis were used to investigate the expression, distribution and relative quantification of GnRHR in the specimens included. **Results:** All the specimens that were examined showed positive staining of GnRHR. The cytoplasm of tumor cells were stained dark brown, with nucleus subtly stained or no staining at all. That was to say the positive substances distributed in cytoplasm of tumor cells. Image analysis suggested that the relative amount of GnRHR of adenoma was significant higher than that of the carcinoma ($P<0.05$). **Conclusion:** It suggests that GnRHR was expressed in thyroid tumor tissues, and might have regulatory effects on the tumor development by autocrine or paracrine.

P39 EXPRESSOIN OF CYCLIND1 AND pRb IN THYROID CARCINOMA AND THEIR CLINICAL SIGNIFICANCE

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Objective: To investigate the expression of cyclinD1 and pRb in thyroid carcinomas and their relationship with the malignant degree of the tumors. **Methods:** Immunohistochemistry Envision system method and monoclonal antibody of cyclinD1 and pRb were used in different thyroid tumors and normal thyroid tissues near carcinoma respectively. **Results:** There were statistically significant difference of the expression level of cyclinD1 in thyroid carcinomas and in the nontoxic goiters, Hashimoto's thyroiditis , normal thyroid tissues ($P<0.05$). The expression of cyclinD1 was positive in 2/11 nontoxic goiters, 2/12 Hashimoto's thyroiditis , 5/11 follicular adenomas, 28/40 thyroid carcinomas and 0/9 normal thyroid tissues near carcinoma. There were statistically significant difference of the negative expression level of pRb in thyroid carcinomas and in the nontoxic goiters, follicular adenomas ($P<0.01$, $P<0.05$). The expression of pRb was negative in 2/11 nontoxic goiters, 4/12 Hashimoto's thyroiditis, 4/12 follicular adenomas, 31/43 thyroid carcinomas and 3/9 normal tissues. **Conclusion:** It suggests that the expression of cyclinD1 may be helpful in predicting the malignant degree and types of thyroid pathologic histology; pRb seems to be applicable to the differentiation between follicular adenoma and thyroid carcinoma.

P40 THE CLINICAL UTILITY OF RECOMBINANT HUMAN TSH (rhTSH) STIMULATION IN THE MANAGEMENT OF FOLLICULAR-CELL DERIVED THYROID CARCINOMA (FCDC)

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Objective: To determine if rhTSH stimulated thyroglobulin (Tg) measurement and concomitant radioiodine whole body scanning (WBS) altered the management of patients with FCDC. **Methods:** 67 patients with FCDC underwent rhTSH stimulated Tg and WBS using the standard 2 dose protocol. 13 patients with Tg autoantibodies were excluded. Of the remaining 54 patients, 27 had undetectable Tg (<1.0 ng/ml) (Group A) and 27 had detectable Tg (>1.0 ng/ml) (Group B) on thyroxine suppression. A stimulated Tg of >2.0 ng/ml was used as surrogate for recurrent cancer or thyroid remnant. **Results:** The characteristics of Group A vs. B: female 77% both groups, average age 51 vs. 46 years, papillary cancer 85% vs. 81%, stage 1 disease 49% vs. 67%, total thyroidectomy 91% vs. 100%, and prior 131-I ablation 89% vs. 96%. In Group A, 6 (22%) patients had rhTSH stimulated Tg rise and 5 (19%) had positive WBS. Only one (4%) patient was concordant for both abnormalities and received further 131-I therapy. A second patient received 131-I based on a Tg rise to 7.8 ng/ml. 17 (63%) patients had both negative WBS and Tg. In Group B, 20 (74%) patients had positive Tg increment and 8 (29%) had positive WBS. 4 (15%) patients had concordant positive results for both Tg and WBS. 11 (41%) patients were treated further with 131-I based on the rhTSH testing, all except 2 showed positive uptake on post ablation WBS. Only 3 (11%) patients had concordant negative results. **Conclusion:** rhTSH testing does not alter the management of most FCDC patients with undetectable Tg on thyroxine suppression. A follow-up study is needed to evaluate if the 131-I therapy given had eradicated the residual cancer.

P41 EXPERTISE ON THYROID FINE NEEDLE ASPIRATION CYTOLOGY

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Objective: To review the results of 1007 thyroid fine needle aspiration cytology (FNAC) and to evaluate clinical values. **Methods:** To evaluate the cytologic results of 1007 FNACs. **Results:** In 1007 FNACs, 87.39%(880/1007) of patients had a definitive cytologic diagnosis: 147 hyperthyroidism, 365 Hashimoto's thyroiditis, 22 Hashimoto's hyperthyroidism, 22 subacute thyroiditis, 66 goiter, 29 goiter with cysticum, 61 follicular adenoma, 54 adenoma cysticum and other diseases. **Conclusion:** FNAC has a central role in the management of thyroid nodules. FNAC has a definitive diagnosis in Hashimoto's thyroiditis. FNAC can different hyperthyroidism from Hashimoto's hyperthyroidism, subacute thyroiditis from Hashimoto's thyroiditis. FNAC is safe, inexpensive, minimally invasive and highly accurate in the diagnosis of thyroid diseases.

P42 DIFFERENTIAL DIAGNOSIS OF THYROID ADENOMA AND CANCER BY USING DNA PLOIDY WITH THYROID BIOPSY

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Objective: To investigate the differential diagnosis of thyroid adenoma and cancer by using DNA ploidy with thyroid biopsy. **Methods:** 113 individuals with 53 thyroid adenoma,47 thyroid node and thirteen thyroid cancer were investigated and the DNA ploidy of tissue or liquid of thyroid biopsy under ultrasonic was analyzed using flow cytometer (FCM) and pathology was diagnosised. **Results:** The 51 patients show Diploid DNA and two patients show aneoploid DNA in 53 patients with thyroid adenoma. The 47 patients with thyroid node show diploid DNA. The specificity of DNA analysis in benign tumor of thyroid is 96.2% with FCM. The twelve patients show aneuploid DNA and one patients show diploid DNA in thirteen thyroid cancer. The sensitivity of DNA analysis in thyroid cancer is 92.3% with FCM. **Conclusion:** It suggests that flow cytometric DNA ploid analysis with thyroid biopsy may be a valuable adjunctive method in the early diagnosis of thyroid of benign and malignant tumor.

P43 THE STUDY OF THE CALCIUM LOADING CT STIMULATED TEST IN POSTOPERATIVE MEDULLARY THYROID CARCINOMA

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Objective: It is well known that serum calcitonin(CT) is a sensitive and specific marker for the diagnosis and monitoring of medullary thyroid carcinoma(MTC).Our objective is to assess whether the calcium loading CT stimulated test could improve the early relapse in postoperative MTC. **Method:** 1.Subjects : Studied 15 patients with postoperative MTC,ranging between 36～67yr. 2 the calcium loading CT stimulated test :A slow iv injection of calcium element(3mg/kg weight) was carried our for 10 min. Blood samples were drawn at 10min in 0, 10 ,20 ,30 min after injection. **Result:** After the calcium loading CT stimulated test, the serum peak CT were elevated in all patients, among of them 7 patients had normal basical CT and peck CT; the anothers had elevated basical CT,and peck value were much higher (with Somstostain receptor imaging detected those had increased tracer uptake). **Conclusion:** The serum CT should be determined in postoperative MTC regularly It's very necessary for those patients with elevated CT to perform the calcium loading CT stimulated test in order to detection the recurs earlier.

P46 EVALUATION OF SURGICAL TREATMENT IN PATIENTS WITH RENAL FORM OF PRIMARY HYPERPARATHYROIDISM (PHPT)

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AIM: Investigation of effectivness of surgical methods of treatment in PHPT complicated with nephrolitiasis. **Materials:** 33 patient mean age 33.8±1.9y (range16-56y) had been studied (10 men,23 women). Mixed forms of PHPT were 17,renal form were 16 patients. **Results:** Diagnosis of PHPT was established by measuring of PTH (111.8±9.5pg/ml vs 59.6±1.6,p<0,001 in controls) and ionized Ca (1.08±0.02 vs 0.95±0.05,p<0.001).They were elevated in 84.8% and 78.7% patients respectively. On the first day after surgery the level of PTH has fallen, to 42. 2±0.08pg/ml and ionized Ca fallen to 0.87±0.02. 23 Patients noted excretion of urinary concrements 0.8sm of size (69.6%). Transitory hypoparathyroidism had been seen in 17 patients (51.5%). 3 patients had received postponed autotransplantation of criopreserved parathyroid gland for permanent hypoparathyroidism. Chronic renal failures were not considered as contraindications for surgical treatment of PHPT.It was mandotory to remove nephrolyts from ureter prior to parathyroid operation.In 5(15.1%) patients kidney stones had gone away completely,and there were no relaps in 22(66.6%) patients.False relaps of nephrolitiasis had been seen in 2(6%) patients.1 patient had true relapses of nephrolityasis,1- had undergone per cutaneus remote litotription, 2(6%)nephrectomy due to calculos pionephrosis. **Conclusions:** Relapsing nephrolityasis with high levels of PTH and ionized Ca can be an indication for surgical treatment in PHPT. The effectivity of parathyroid surgery for renal forms of PHPT is as high as 87.7%.

P47 CENTRAL DIABETES INSIPIDUS IN ADULTS

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There have been reviews on central diabetes insipidus in children, but the clinical characteristics in adults are largely undefined. **Objective:** To study the etiologies and clinical characteristics of patients with adult onset central diabetes insipidus. **Methods:** We retrospectively reviewed the records of 37 patients with permanent central diabetes insipidus seen at our hospital from 1966 to 2001. **Results:** 28 patients had adult-onset (>/=16 year-old) central diabetes insipidus (18 female and 10 male). Median age at diagnosis was 21 years (range, 16 to 67). The median duration of follow-up was 13 years (range, 1-27 years). The commonest cause was idiopathic (14). 6 patients had pituitary or suprasellar tumors (1 ependymoma, 1 Rathke‘s cleft cyst), 4 had other intra-cranial tumors (1 pinealoma, 1 astrocytoma, 1 third ventricle tumor, 1 brain secondaries), 1 had empty sella syndrome, 1 had multiple sclerosis, 1 had history of childhood central nervous system infection, and 1 had traumatic intra-cranial haemorrhage. Polyuria and polydipsia were the commonest presenting symptoms (21). Anterior pituitary deficiency was found in 11 patients. Half of the patients had abnormal findings on MRI scans of the posterior pituitary gland. The clinical course was benign (the patients with multiple sclerosis and brain secondaries died).

P48 Minirin® treatment for Childhood Enuresis

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Summary: Enuresis refers to uncontrolled urine voidings during the day or at night. The symptom is commonly seen in children. Consequences of enuresis are signitican, including ……

Objectives: To evaluate some related factors that ……

Methods: 62 enuretic children attaining the endocrinology out-patient department in ……

Results:

1. After treatment, 8 cases of secondary enuretic patients were ……
2. The outcome of treatment was ……
3. 83.5% of patients with maximum bladder capacity greater than 8ml/kg were ……
4. The treatment outcom for patients with ……

Conclusion: The treatment outcomes of Minirin® in enuretic children were ……

P49 ASSAY OF KAL-1 GENE DEFECTS IN PATIENTS WITH KALLMANN SYNDROME

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Objective: To explore KAL-1 Gene Defects in patients With kallmann syndrome (the deletion of the 3'end of the gene and kAL-1 gene mutation). **Methods:** We use PCR techniqe to detect the large deletion of the 3'end of the gene . We use PCR-SSCP, to detect KAL-1 gene mutations **Results:** We examined the PCR products from 27 KS patients including 7 typical case (with anosmia).who all show negative results. We select exon6 for gene mutation study, by PCR-SSCP in 27 KS patients' genomic DNA. We found 3 cases are different from the control. Gene sequencing does not show any mutation in exon 6. **Conclusion:** 1.KAL-1 gene large deletions seems relatively infrequent.2. It indicates the genetic heterogeneity of KS. KAL-1 gene defects may present within exon6, or introns, KAL-1 gene mutation is extensive.

P50 SANDOSTATIN LAR THERAPY IN PATIENTS AFTER TRANSSPHENOIDAL NEUROSURGERY OF PITUITARY TUMOURS

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Objective: Sandostatin LAR consists of octreotide incorporated into microspheres of the biodegradable polymer poly (DL - lactide - Co - glycolide) D (+) glucose. Lancranijan et al. reported the effects of long term treatment with Sandostatin LAR in acromegalic patients. Long - term treatment with the 20 mg dose of Sandostatin LAR given once monthly delivered steady - state octreotide serum concentrations (1348 ± 483 μg/l) The aim of this study was to evaluate the clinical and hormonal effects of Sandostatin LAR therapy in patients after surgery of pituitary macroadenomas. **Material and Methods**: The effects of hormonal therapy were investigated in 10 patients after nonradical resection of pituitary macroadenomas: (4 patients with acromegaly, 3 patients with Cushing disease, 2 patients with prolactinoma, 1 patient with nonfunctioning adenoma). After surgery the cobaltotherapia was, applicated in all patients. Sandostatin LAR (Novartis) was administered in a dose of 20 mg i.m. once monthly during 6 months. Serum GH, IGF1, cortisol, PRL, LH, FSH and TSH concentrations were measured with RIA methods. **Results**: In patients with Acromegaly serum GH and IGF 1 were markedly elevated after surgery and cobaltotherapy. Sandostatin LAR therapy caused the graduate suppression og GH and IGF 1 release and after 3 months treatment, the GH and IGF 1 became normal. The clinical symtoms of acromegaly such as headache, arthralgia, fatigue and hyperhidrosis improved in all acromegalic patients. In patients with prolactinoma the combined therapy: Sandostatin LAR + bromocriptine was administered. The normalization of prolactin release was observed after 3 months of treatment. The normalization of cortisol release in patients with Cushing disease was found after 6 months therapy with Sandostatin LAR. The clinical symptoms of hiperprolactinemia and hipercortisolemia were also markedly diminished. In all patients we found the improvement of visual field abnormalities and the most of tumour remnants do not show volume progression in the control MRI. **Conclusions** :The beneficial effects were demonstrated during Sandostatin LAR therapy in patients with pituitary macreadenomas after nonradical surgery.

P51 IMPAIRRED CARBOHYDRATE METABOLISM IN PATIENTS WITH ACROMEGALIA IN THE REBUBLIC OF UZBEKISTAN

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BACKGROUNDS AND AIMS: Impaired carbogydrate metabolism(ICM) is one of the causes of progressive ability and life time reduction in acromegaly(AM) and its incedence is 54%,diabetes mellitus (DM) makes it up to 25%. We studied ICM in patients with AM and its interaction with the disease activity and duration as well as with age-sex parameter.**MATERIALS AND METODS:**study was conducted in 92 patients with AM ,38 males(41%) and 54 females(39%) among them,mean age of 45,4±2,6 years.Clinico-hormonal,ophthalmological and CT-NMR examination as well as OGTT wereperformed, grows hormohe(GH) secretion daily rhythm(9.00,11.00,13.00.15. 00,17.00)being assessed.The disease duration from 2 to 33 years.According to disease activity the patient were divided into the group withinactive stage and the group with active one.According to disease duration there were three groups: up to 10 years, 11-20 years. **RESULTS:** in 57 patients (61,9%) ICM,in 20 (47,3%) DM and in 37(64,9%) IGT were obserwed.Among them 42 patients (73,6%) were with AM ICM active stage, 15 (26,3%) with inactive one,IGT being observed confidently on active stage in 38 patiens (90,4%) versus 7 cases (46,3%) on inactive one ($p< 0,005$), while DM was seen in 8 patients (53,3%) with inactive stage versus 4 patients (9,52%) with active one ($p<0,01$).As to diesease duration ICM prevailed in the II group (41,3%,n=38) as compared to the I group (18,4%,n=17) with 2,1% (n=2) in the III group.**CONCLUSIONS:** Icm is a severe complication and condition morbidity aggravation. It is closely connected with the disease duration and activity.Overt form of ICM, such as DM can be seen in patients with disorder in insulin secretion central regulation and glucagon, as well as direct effect of GH.

P52 GROWTH HORMONE TREATMENT IN CHINESE GIRLS WITH TURNER SYNDROME-LOCAL EXPERIENCE

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Background: Growth Hormone (GH) is well accepted for treatment to promote height gain in patients with Turner Syndrome (TS). Sponsorship for its use in TS has been approved by the Hong Kong Hospital Authority since 1999.

Objective: To evaluate growth rate with recombinant GH treatment in Chinese girls with TS.

Methods: Growth data of girls with TS who were treated with GH for at least 2 consecutive years at Queen Elizabeth Hospital were evaluated. Assessment was based on both the Hong Kong normal girls' growth standards (NGGS) and the Hong Kong TS specific growth standards. GH was given at a dose of 1 IU/kg/week if the height SDS was less than -2.5. Oxandrolone was given at around 9 years old (0.0625 mg/kg/day) and hormonal induction of puberty after 15 years of age.

Results: Eleven girls with TS were treated with GH. The average age at enrollment was 10.3 +/- 4.3 years (range 3.3-15.8). Results were tabulated as below. The change in growth rate was comparable to that of other studies.

	Height SDS (NGGS)	TS-specific height SDS	Growth rate (cm/y)
Pretreatment	-3.1 +/- 0.6	-0.06 +/-0.7	4.2 +/-1.2
Year 1	-2.4 +/- 0.5	0.5 +/-0.6	7.2 +/-2.1
Year 2	-2.1 +/-0.6	0.8 +/-0.6	5.1 +/-1.7

Values are the mean +/- SD.

Conclusion: Although the response to treatment with GH varied, it was associated with significant gain in growth in Chinese girls with TS. Further study will be conducted to evaluate their final height.

P53 The association of pituitary specific transcription factor Pit-1 with growth hormone promoter activity in IM-9 cell line

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Objective: Pit-1, the pituitary specific transcription factor, plays a key role in pituitary growth hormone (GH) expression. Human lymphocytes also express and secrete GH. Here we studied the association of Pit-1 with GH promoter activity in IM-9 human B-lymphoblast cell line. **Methods:** 1. To observe if there's Pit-1 mRNA expression in IM-9 cells by RT-PCR. 2. Insert GH gene promoter (-484～+2 bp) into pGL3-Enhancer vector. The recombinant plasmid was abbreviated as 484-luc. 3. To observe the effects of Pit-1 over-expression and inhibited expression (by co-transfecting pcDNA3.1-Pit-cDNA or Pit-1 antisense oligonucleotides and 484-luc plasmid) on luciferase activity expressed by 484-luc. 4. Using pGL3-Enhancer vector, we constructed other two plasmids: 66-luc and 132-luc, which contains the –66～+2 and –132～+2 bp sequences of GH promoter, respectively. The above two constructs and 484-luc were transfected into im-9 cells, respectively, to observe the relative luciferase activities expressed by them. **Results:** 1. Pit-1 mRNA expression was observed by the method of RT-PCR in IM-9 lymphocytes. 2. Luciferase activities expressed by 484-luc in IM-9 cells were increased by 1.12 times and decreased 37% by Pit-1 over-expression and inhibited-expression, respectively. 3. Both the luciferase activities expressed by 66-luc (contains no Pit-1 binding site) and 132-luc (contains two Pit-1 binding sites more than 66-luc) were only 1% of that of 484-luc, and there was no significant difference between them. **Conclusion:** 1. There's Pit-1 mRNA expression in IM-9 cells. 2. Pituitary specific transcription factor Pit-1 is important, but not sufficient for the GH promoter activity in IM-9 B-lymphoblasts.

P54 MELATONIN MAY DIRECTLY REGULATE THE PANCREATIC ISLET

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Objective: To clarify whether pancreatic islets express mt_1 and / or MT_2 receptor subtype. **Methods:** Following termination of pregnancy (4~6 months), we collected pancreas. The mt_1 and MT_2 receptor subtype probes were labeled with digoxigenin (DIG) respectively. The *in situ* hybridization was made according to procedures. The total RNA of pancreas were extracted, reversibly transcripted, and amplified. Shanghai GeneCore Biotechnologies made the sequence analysis. The streptavidin-biotin method was applied to immununohistochemistry, with primary antibodies against human mt_1 and MT_2 receptors kindly donated by Prof. S.F. Pang. **Results:** Specific hybridization of DIG-labeled probes reveals mt_1-and MT_2-expressing cells in pancreatic islet, primarily located to cytoplasm and nucleus. RT-PCR analysis demonstrated mRNA expression of the mt_1 and MT_2 receptor subtype in pancreatic islet. Sequencing of RT-PCR production show that the DNA sequence of production is coincided with the sequence from Gene bank. Either mt_1 or MT_2 receptor subtype proteins were observed in pancreatic β cells, primarily located to cytoplasm and membrane with rare location in nucleus. No significant differences of locations were found between the mt_1 and MT_2 receptor subtype. **Conclusions:** It is suggested that the mt_1 and MT_2 receptor subtype coexist in pancreatic β cells that we have investigated, but it is still an open question that how melatonin regulates the pancreatic secretion.

P55 ESTROGEN RECEPTOR GENE POLYMORPHISMS AND BONE MINERAL DENSITY IN CHINESE POSTMENOPAUSAL WOMEN

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Objective: To investigate the relationships among the PvuII and XbaI polymorphisms of estrogen receptor (ER) gene and bone mineral density (BMD; z-score) in Chinese postmenopausal women. Methods: The BMD of lumbar-spine (L2-4) and femoral neck (FN) were measured by DEXA, the genotypes of ER gene were detected by PCR-RFLP method. Results: In 186 postmenopausal women (65.0±0.6 yr), there were PP (14.5%), Pp (50.0%) and pp (35.5%) 3 PvuII and XX (7.0%), Xx (27.4%), xx (65.6%) 3 XbaI RFLPs. The FN BMD of pp genotype was higher than that of Pp ($P<0.03$); L2-4 BMD was higher in XX genotype as compared with Xx ($P<0.0003$) and xx ($P< 0.0005$). Combining the PvuII and XbaI RFLPs, 7 genotypes were got: PPXX：5.4%, PPXx: 4.3%, PPxx: 4.8%, PpXX: 1.6%, PpXx: 23.1%, Ppxx: 25.3% and ppxx: 35.5%. It was found that women without Px haplotype (PPXX, PpXX, ppxx , n=79) had higher L2-4 ($P<0.03$) and FN ($P<0.05$) BMD than those with it (PPxx and PPXx, Ppxx , PpXx , n=107). Px haplotype was one of the independent predictors of L2-4 and FN BMD in a stepwise regression analysis model ($R2 = 0.426$ and 0.258, respectively). Conclusion: pp and XX genotype of ER gene may play a certain role in maintaining FN and L2-4 BMD , respectively. Px haplotype might exert some harmful effect on BMD, while the genotypes without Px haplotype be favorable to bone mass.

P56 AN ANALYSIS OF THE CORRELATION BETWEEN INTERLEUKIN-1 RECEPTOR ANTAGONIST GENE POLYMORPHISM AND BONE MINERAL DENSITY IN POSTMENOPAUSAL WOMEN

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Objective: To investigate the frequency distribution of interleukin-1 receptor antagonist(IL-1RN) genotype in Chinese postmenopausal women ,and to evaluate whether there is any association between interleukin-1 receptor antagonist genotype and bone mineral density (BMD).**Methods:** Polymerase chain reaction (PCR) and agarose gel electrophoresis were used to determine interleukin-1 receptor antagonist genotypes in 166 Chinese postmenopausal women.Their L2-4 lumbar and femoral neck BMD were measured by dual-energy X-ray absorptiometry(DEXA). **Results:** Three alleles were identified(A1=4 repeats,410bp;A2=2 repeats,240bp;A4=3 repeats,325bp),with three genotypes observed:A1A1(91%),A1A2(7.2%),A1A4(1.8%). The BMD at lumbar of different genotypes were (0.777+0.139) g/cm^2(A1A1), (0.719+0.10) g/cm^2(A1A2), (0.705+0.136) g/cm^2(A1A4).The BMD at femoral neck were (0.638+0.141) g/cm^2(A1A1),(0.627+0.086) g/cm^2(A1A2),(0.624+0.09) g/cm^2(A1A4) respectively.There was no significant relationship between interleukin-1 receptor antagonist (IL-1RN) genotypes and BMD either at the lumbar spine or the femoral neck(P>0.05).**Conclusion:** The frequency distribution of interleukin-1 receptor antagonist (IL-1RN) genotype in Chinese postmenopausal women was different from that in western countries,but was similar to findings in Japanese and Korean population.There was no correlation between IL-1RN genotypes and BMD in Chinese postmenopausal women.

P57 VITAMIN D RECEPTOR GENE POLYMORPHISM AND ITS RELATIONSHIP WITH BONE MINERAL DENSITY IN POSTMENOPAUSAL OSTEOPOROSIS WOMEN OF SHANGHAI

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Objective: To estimate the relationship between the vitamin-D receptor genotypes and bone mineral density in osteoporotic women in Shanghai. **Methods:** 102 postmenopausal women recruited from Rui-Jin Hospital were diagnosed osteoporosis according to WHO criteria. Their age was 63.3±7.7 years. BMD at the lumbar spine, femoral neck and Ward's triangle were measured with a modal dual-energy X-ray absorbtionmetry. The VDR gene was amplified by using a polymerase chain reaction. As similar to previous reports, detection of the *BsmI* site was achieved by amplifying a region spanning the sites. Producing a 800 base pair fragment. Detection of *ApaI* and *TaqI* sites was done by using a single amplification, producing a 740 bp fragment. The VDR genotypes were determined by the PCR-RFIP. **Results:** bb, aa and TT genotype were found in major in these women. Only one of BB, two of tt were found among these patients. Higher frequencies were found for 'B, a, T' alleles than 'B, A, t'. No significant difference of BMD was found among the three subgroups of bb, Bb and BB. **Conclusion:** BB and tt genotypes are quite rare in our population. The rareness of B and t alleles suggested that it is unlikely that they are important factors for the heredity of osteoporosis in Chinese women.

P58 A SIMPLE CLINICAL TOOL TO IDENTIFY ASIAN WOMEN WITH OSTEOPOROSIS.

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Objective: To develop and assess a statistical model for predicting osteoporosis (femoral neck BMD T < -2.5) in postmenopausal Asian women, using risk factors obtained by questionnaire. **Methods**: A simple index, the Osteoporosis Self-assessment Tool for Asians (OSTA) was developed based on multiple variable regression modeling in 860 women. **Results**: The full index with 11 variables achieved 95% sensitivity and 47% specificity. The area under the curve (AUC) was 0.85. Removing all variables except patient age and weight did not materially reduce predictive ability (sens = 91%. spec = 45%; AUC = 0.79), but substantially improved simplicity. To calculate the OSTA index, age (yr) was subtracted from body weight (kg) and the result was multiplied by 0.2 and truncated to yield an integer. For example, 0.2*(61 kg minus age 66) = -1. Three risk categories were identified. Almost two-thirds (61%) of high risk patients (representing only 8% of all women in the study, index < -4) had osteoporosis -- physicians might be advised to consider intervention and measure BMD. Among women with low risk (representing 40% of all women, index > -1), only 3% had osteoporosis, and BMD measurements are probably not necessary. Approximately 15% of the moderate risk women (index = -4 to -1) had osteoporosis; the decision to measure BMD for this category may vary by community, depending on available resources. **Conclusion**: this index had acceptable predictive ability and was easy to use. This free and simple tool could help clinicians actively assess osteoporosis and determine the need for intervention or BMD measurements before fractures occur.

P59 A SURVEY OF BONE MINERAL DENSITY IN WOMEN AFERR 40 YEARS OLD IN CHANGSHA COUNTRYSIDE

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Objective To investigate the age related bone mineral density and the prevalence of osteoporosis at different skeletal sites in women age after 40 in Changsha countryside. **Methods** 627 healthy women aged 40 to 85 from Changsha countryside were involved. BMD measurements were taken at various sites by Hologic QDR4500A dual energy X-ray absorptiometry (DEXA). **Results** . (1) Compared with 40~44 years old women, 75~85 years old women's total bone loss rates were highest at the Word's (56.62%) and lowest at the 1/3 site of the distalforearm (29.82~31.43%). (2) The rank of osteoporosis detection rate from high to low after age 40 was lateral lumbar spine (38.72%), 1/3 site of the distal radio(35.42%), ward's triangle (27.52%) and anteroposterior lumbar spine and femur neck or major trochanter (8.28% and 8.85%). (3) The prevalence of osteoporosis at least at one site of these women increased gradually with age. **Conclusion** the change of BMD and the incidence of osteoporosis of countryside women age after 40 were almost like the women in other profession and age after 40. For factors involved in BMD are so many, comparing between them is improper. The most sensitive sites for detecting osteoporosis are lateral lumbar spine, forearm and ward's triangle.

P60 HIGH DIETARY PHYTOSTEROLS INTAKE IS ASSOCIATED WITH HIGHER BONE MINERAL DENSITY IN POSTMENOPAUSAL WOMEN

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Objective: Recent studies have suggested that aminobisphosphonates could suppress bone resorption by inhibiting cholesterol biosynthesis pathway. Preliminary study showed statin group of cholesterol-lowering agent is associated with higher bone mineral density (BMD). Phytosterols are structurally very similar to cholesterol. Phytosterols have been regarded as cholesterol-lowering agents as they inhibit cholesterol absorption and influence cholesterol synthesis. Traditional Chinese diet is known to contain relatively high plant foods. Whether high habitual dietary phytosterols intake would influence BMD is unknown. **Methods:** 357 postmenopausal southern Chinese women were recruited to determine their dietary phytosterols intake by a food frequency questionnaire. Three major phytosterols, β-sitosterol, campesterol and stigmasterol were analyzed. BMDs at the lumbar spine and hip region were measured using dual energy X-ray absorptiometry (DXA). The subjects were analyzed according to tertiles of phytosterols intake. **Results:** Significant differences in the BMD values at the femoral neck (0.610 ± 0.107 vs 0.581 ± 0.099 g/cm², $p < 0.05$), trochanter (0.516 ± 0.108 vs 0.489 ± 0.102 g/cm², $p < 0.05$) and Ward's triangle (0.444 ± 0.144 vs 0.409 ± 0.126 g/cm², $p < 0.05$) were observed between the highest and the lowest intake of phytosterol after adjusting for age, height, weight, years since menopause, smoking, alcohol consumption, HRT usage and daily calcium intake. Women with the highest intake of phytosterols had significantly lower levels of serum total alkaline phosphatase (ALP) and osteocalcin (both $p < 0.05$). No significant difference in serum PTH levels was found between the various groups of phytosterols intake. **Conclusion**: postmenopausal women with habitual high dietary intake of phytosterols were associated with higher BMD value at the hip region and had lower levels of biochemical markers for bone turnover.

P61 CHANGES IN BONE-MINERAL-DENSITY AND BONE METABOLISM OF PATIENTS WITH GRAVES DISEASE

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Objective: The bone-mineral-density and markers of bone metabolism were measured and analyzed in 240 cases of Graves disease , which shed some light on the prevention and early treatment to metabolic osteopathy. **Methods:** 240 Graves cases and 140 normal cases were examined to make comparison. The bone-mineral-density of lumber vertebrae2-4 were measured with x-ray bone densitometer model DPX-1, Serum Ca, P, ALP and urinary Hyp were measured with routine method (RIA). **Results:** 1,Average bone-mineral-density in Graves group is lower than in control group, $p<0.01$.The occurrence rate of low bone-mineral-density is 62.8% in 240 cases .2,The average urinary Hyp in Graves group is higher than that in control group ,$p<0.05$. 3,The concentration of serum Ca, P, ALP are all in normal range. 4,There is no obvious correlation between thyroid function and bone-mineral-density. 5,The Graves cases with low bone-mineral-density appeared to be normal by routine X-ray examination. **Conclusion:**X-ray densitometry is high specific and reliable, so it could be a sensitive marker to diagnose osteoporosis at early stage of graves disease. It is essential to measure the bone mineral density as routines for patients with Graves disease and to pay attention to the changes of bone-mineral-density and bone metabolism.

P62 STUDY OF BONE MINERAL DENSITY IN PATIENTS WITH GRAVES DISEASE

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Objective: To investigate change of bone mineral density (BMD) in patients with Graves disease (GD). **Methods:** BMDs of right calcaneus were measured in 146 patients with GD and 120 normal controls by single-energy X-ray absorptiometry. **Results:** BMD is lower in patients with GD than in controls [BMD (341.10±60.35) mg/cm^2 vs (401.68±57.05) mg/cm^2, P<0.001]. Incidence of bone density decreasing is higher in patients with GD than in controls (78.08% vs 47.50%, P<0.001).Incidence of OP is higher in patients with GD than in controls (33.50% vs 7.50%, P<0.001). Incidence of bone density decreasing or OP is not different respectively in patients with GD or in controls either male or female. BMD and T-Score of subgroups in patients with GD were lower than those in controls, except that 51~60 years subgroups. In 21~30 years subgroups, male BMD (350.17±65.99) mg/cm^2 vs (454.20±41.66) mg/cm^2, P<0.001, female BMD (336.10±60.80) mg/cm^2 vs (373.93±36.27) mg/cm^2, P<0.05. In 31~40 years subgroups, male BMD (372.40±73.49) mg/cm^2 vs (430.87±46.75) mg/cm^2, P<0.05, female BMD (342.11±50.19) mg/cm^2 vs (385.40±34.45) mg/cm^2, P<0.01. In 41~50 years subgroups, male BMD (382.50± 67.68) mg/cm^2 vs (433.93±44.61) mg/cm^2, P<0.05, female BMD (312.42±43.93) mg/cm^2 vs (384.40±50.33) mg/cm^2, P<0.001. Logistic regression analysis showed that activity of hyperthyroidism is the risk factor of BMD decreasing in patients with GD (OR=2.8676, 95%CI 1.0993~7.5216). **Conclusion:** BMD is decreased in patients with GD. Incidence of OP is increased significantly, but the difference is not significant either male or female. Activity of hyperthyroidism is the risk factor of BMD decreasing in patients with GD.

P63 THE INVESTIGATION ABOUT THE RELATIONSHIP BETWEEN THE CONCENTRATION OF SERUM TESTOSTERONE AND BMC ON HEALTHY OLD MEN

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Objective: To investigate the relationship between the concentration of serum testosterone and bone mass (BMC) on old men. **Methods**: Using RIA to measure the concentration of serum testosterone and using SPA to measure the BMC on 364 healthy old men and 104 younger healthy controls. **Results**: 1, With age increasing, the levels of testosterone also are increasing. After reaching to peak of it, the levels of testosterone are decreasing with age. ($p<0.001$, $r=-0.39074$) 2, The peak of testosterone is located the group between 20~29 years old. 3, With age increasing, BMC was also increasing. After reaching to peak of BMC, BMC was decreasing with age. Age was significantly related with BMC. ($p<0.001$, $r=-0.23084$) 4, The level of serum testosterone was significantly related with BMC. ($p<0.03$, $r=0.1675$) **Conclusions**: The occurrence and development of osteoporosis in old men were significantly related to the level of serum testosterone. **Keyword**: male testosterone BMC

P64 IMMUNOASSAY FOR QUANTIFYING TYPE I COLLAGEN DEGRADATION PRODUCTS IN URINE ESTABLISHED AND EVALUATED

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Objective: To analyze bone resorption , we developed a assessment with a marker of collagen degradation , C-terminal telopeptide of type I collagen (CTX). The ELISA quantitatively determined degradation products of type I collagen in human urine. **Methods:** The antibodies are raised against synthetic peptide with an amino acid sequence specific for a part of the C-telopeptide of the ^{a}1 chain of type I collagen. It is a competitive-inhibition enzyme-linked immunosorbent assay. The measuring range was 0.1 -10mg/L with a detection limit of 80μg/L. Within-run and total CVs were 5.2%and 9.6%, respectively. Creatinine (Cr) were used to correct all urinary parameters. **Results:** We have used this ELISA to measured a sample of health people comprising 200,aged 1-82 yr. The result show that CTX in urine correlated with age . In children (2-4 yr), the level of CTX/Cr in urine is high (from 174.9mg/mmol to 469.74mg/mmol). Urinary CTX excretion increased with age in 5-16 yr. and After 16yr , it begin to decrease. Among 20-40, the CTX excretion didn't significantly increase. In the women, menopausal women's CTX in urine showed an increase compared to values in premenopausal woman, but not significant. After menopause, the level of CTX isn't significantly different from premenopausesal woman . CTX measured by ELISA and NTX (N-terminal telopeptide of type I collagen) measured by NTX kit (NTX , OSTCOMARK®) are highly correlated (n=238; r=0.71). **Conclusion :** These results indicate that CTX ELISA is useful for monitoring bone loss caused by bone resorption . CTX and NTX concentration in urine are highly correlated.

P65 CHANGES AND SIGNIFICANCES OF CARBOXYTERMINAL PROPEPTIDE OF TYPE I PROCOLLAGEN IN COMMON ENDOCRINISM

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Objective: To discuss the changes and significances of carboxyterminal propeptide of type I procollagen (PICP) in common endocrinism such as hyperthyroidism and diabetes mellitus. **Method:** A: Control group (n=39) age 56±17 years old; B: DM group (n=38) age 55±12 years old ;C: Hyperthyroidism group (n=14) age 56±17 years old. PICP were measured in fasting by radioimmunoassay technique. The results were compared among the 3 groups mentioned above. **Results:**(1) PICP in Group A was 121.87±54.41μg/L, Group B was 125.92 ±51.13μg/L, Group C was 234.43±147.52μg/L.(2)The difference of PICP level between Group A and Group B was not significant (P>0.05). But it was higher in women than that in men in Group B(P<0.05),whereas in Group A there had no difference between women and men.(3) PICP in Group C was higher than Group A and Group B(P<0.01). **Conclusion:** PICP concertration of blood was in hyperthyroidism increased obviously. PICP in DM and controls were not significant, but PICP concertration of blood in DM is higher in women than that in men. PICP in women could be effected by the level of estrogenic hormones, so when to diagnose osteoporosis with PICP , the difference of age and sex must be considered.

P66 The effects of different administration of human parathyroid hormone fragment (hPTH1-34) on human osteoblast-like SaoS –2 cells

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Objectives: To observe the effects of different hPTH1-34 administration on SaoS-2 cells, and to explore its possible signal transduction pathway and molecular biological mechanism. **Methods:**The cells were stimulated by 50 ng/ml hPTH1-34 for 1, 3, 6, 12, 24 and 48 hours in every 48hours differently in 8 days. Total RNA was extracted by Trizol kit. ALP, BGP and cAMP levels were measured by chemical method, radioimmunoassay and competitive protein binding method respectively. c-fos gene expression was semi-quantified by RT-PCR. **Results:** ALP level was higher in 1, 3 and 6 hours hPTH1-34 intermittent stimulation group, especially in 3 and 6 hours group compared with control and consecutive stimulation with time dependent effect ($P<0.001$,$P<0.05$). The cAMP level increased significantly in 3 and 6 hours hPTH1-34 intermittent incubation group with time-dependent effect ($P<0.01$ and 0.001 vs control and pretreatment). Intermittent hPTH1-34 stimulation had more effects on cAMP level than consecutive action ($P<0.001$). 1, 3 and 6 hours of hPTH1-34 Intermittent stimulation enhanced c-fos gene expression significantly with time-dependent effect. **Conclusion:** Intermittent hPTH1-34 stimulation had stronger effect on ALP activity, cAMP release, and c-fos gene expression than consecutive action, especially at 3-6 hours intermittent stimulation. The synchronous c-fos , ALP and cAMP response to intermittent hPTH1-34 stimulation suggested that hPTH1-34 affected Saos-2 cells though cAMP dependent PKA pathway and c-fos gene exert important effect.

P67 THE EFFECTS OF OSTEOGENIC GROWTH PEPTIDE C-TERMINAL DERIVATIVE (G35I) ON OSTEOCLAST INN VITRO

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Objective: To discuss the effect of G35I on forming of osteoclastic like cells(OLC) in vitro and investigate its effect on production of OPG mRNA by human OB. **Methods:** one-month old Wistar rats were killed and marrow cells from femurs and tibas were collected and suspended in αMEM containing $1,25(OH)_2D_3$ and dexamethasone. The cell suspensions were plated in 96-well dishes.G35I was given at different concentration. Cultures were performed for a week. The cells with more than three nucleus were calculated as OLC. Semiquantitative RT-PCR was used to test the mRNA amount of OPG in human osteoblasts treated with G35I at certain concentration for various time. **Results:** At the concentration of 10^{-7}mmol/L G35I may enhance the formation of OLC and decrease OPG mRNA amount in human osteoblasts. **Conclusion:** G35I may not only enhance the bone formation but also increase OLC formation at a relative higher conceration in vitro. And this effect may be achieved by the way of interfering the reaction of OPG and OPGL.

P68 THE ANALYSIS OF THE GENE MUTATION AT THE TRANSMEMBRANE DOMAIN OF FGFR3 NUCLEOTIDE 1138 IN ACHONDROPLASIA PATIENTS

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Objective : To investigate the mutation at the transmembrane domain of FGFR3 nucleotide 1138 site being the major pathologic mechanism of achondroplasia (ACH) and to evaluate the efficacy of DGGE method as a useful tool for screening the point mutations .**Methods** : The genomic DNA from 15 clinical diagnosed ACH patients where analysed by PCR — RFLP with SfcI and MspI restriction endonucleases and also enroled point mutation screening by PCR — DGGE technique . **Results** : The DNA of the control and the normal parents of the ACH patients sequence in the PCR — RFLP with both SfcI and MspI digestion products represent only a 164 band was detected . Positive findings with mutation at nucleotide 1138 G →A transition were found in 12/15 of the ACH patients as heterozygotes . They showed 3 fragments of 164 、 109 and 55 bp by PCR — RFLP with SfcI digestion . The negative results shown by MspI digestion for 1138 G → C transition detection .They showed a 164bp band . Using PCR — DGGE as a screening method , 12 patients with positive finding by PCR — RFLP analysis also revealed positive for point mutation showing 4 bands as heterozygotes . 3 negative sample with PCR — RFLP showed only same band as illùstrated by the negative control . Thus no point mutation was suspected in this amplified region .**Conclusion** : Nucleotide 1138 in transmembrane domain of FGFR3 gene is the hot — for mutation in ACH and being its major pathologic cause . PCR — DGGE technique is sensible and reliable for point mutation screening .

P69 Lovastatin inhibit Insulin-like growth factor-1 expression on cultured human mesangial cells

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Objective Insulin-like growth factor-1(IGF-1) is a marker of glomerular hypertrophy in the early stage of diabetic rats. HMG-CoA reductase inhibitor have suppressive effect on transforming growth factor-β_1(TGF-β_1) expression in cultured mesangial cells. This study aimed to examine the effect of lovastatin on IGF-1 expression of mesangial cells. **Methods** Human mesangial cells were cultured in DMEM containing low glucose (5.6Mm), high glucose (30mM) with lovastatin(10μM) for 48 hours, respectively. IGF-1, TGF-β_1 mRNA expression of cultured mesangial cells were tested by RT-PCR and the concentrations of TGF-β_1, fibronectin, laminin, type IV collagen in supernatant were determined using RIA and ELISA techniques. **Results** The level of TGF-β_1, fibronectin, laminin, type IV collagen and the expression of IGF-1, TGF-β_1 mRNA increased in the mesangial cells cultured in high glucose as compared with low glucose. Both TGF-β_1 and IGF-1 mRNA expression in cultured mesangial cells in high glucose condition were suppressed by lovastatin. In the cultured mesangial cells with high glucose and lovastatin, the decrease of TGF-β_1, fibronectin, laminin and type IV collagen proteins were also observed as compared with cells in high level of glucose without lovastatin. **Conclusions** Lovastatin suppressed fibronectin, laminin, type IV collagen products and the expression of IGF-1, TGF-β_1 mRNA in cultured mesangial cells. This study suggests that the preventive effect of lovastatin on diabetic nephropathy result not only from the suppression of TGF-β_1, but also from its inhibitory effect on IGF-1 expression.

Key Words Lovastatin, Insulin-like growth factor, mesangial cell, diabetic nephropathy

P70 PREVENTION OF DIABETIC NEPHROPATHY IN EXPERIMENTAL RATS BY C-PEPTIDE

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Objective: To investigate the prevention effect of C-peptide on morphological and functional changes of diabetic nephropathy in STZ rats. **Methods:** The study include 4 groups of rats: 1) diabetic rats under insulin treatment with nearly normal blood glucose level, 2) diabetic rats under C-peptide treatment (130nmol/kg, sc, twice daily) with constant high blood glucose level, 3) diabetic rats without treatment with constant high blood glucose level, 4) age matched non-diabetic control rats. After 8 weeks of diabetes, urinary albumin excretion rate (UAER), glomerular volume [V(glom)], extracellular matrix area fraction of glomerular cross-section [S(matrix/glom)] and glomerular basement membrane thickness (GBMT) were measured. **Results:** C-peptide treated rats had lower UAER than non-treated diabetic rats with borderline significance (334μg/24h vs. 1028μg/24h, p=0.059), although the UAER still increased compared to well-controlled insulin group (72μg/24h, p=0.019) and normal controls (25μg/24h, p=0.001). V(glom) and S(matrix/glom) remained similar in C-peptide group, insulin group and normal controls which were approximately 50% and 70% respectively of the corresponding parameters in non-treated diabetic rats (p<0.001). The changing profile of GBMT in all groups was similar to that of UAER but with no statistical significance. **Conclusion:** The increase of UAER and the accumulation of glomerular extracellular matrix which leads to glomerular hypertrophy can be prevented by C-peptide treatment independent of metabolic control. C-peptide combined with insulin treatment may help retard the development and progression of diabetic nephropathy.

P71 The effect of combined C-peptide and insulin administration on renal function and morphological changes in STZ induced diabetic rats

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Objective: To investigate the the effect of combined C-peptide and insulin administration on renal function, and morphological changes in STZ induced diabetic rats**. Methods:** The STZ-induced diabetic rats are randomly divided into two groups: one group treated with insulin and C-peptide(INS+CP) group, (n=10),another group treated with insulin(INS group, n=8).the normal rats as control group(C group, n=11).Both INS+CP group and INS group are treated with porcine protamine zinc insulin to reach the expected blood glucose level at 10.0mmol/l under the strict blood glucose monitoring. INS+CP group is injected subcutaneous with C-peptide in a dose of 130nmo/kg two times a day, while the INS group is injected subcutaneous with the same volume saline as control. The experiment course is 8 weeks. At the end of experimemt ,The thickness of GMB, UAER, and were measured. Glomerular volume and the ratio of extracelluar matrix area to glomerular area were determined by Real Colour Image System. **Results:** No significant difference was seen between INS+CP group and INS group including the average blood glucose level during the experiment (mmol/l) (11.1±2.2 vs 11.4±2.5)、HbA1c(%)(4.76 ±1.06 vs 4.55±0.77).). The ratio of extracelluar matrix area to glomerular area(%) in INS+CP group was less than that in INS group（28.7±1.4） and (36.8±4.2) respectively, but there were no significant difference in glomerular volume (um3)(INS+CP group vs INS group: 0.713±0.041 vs 0.792± 0.221). The UAER(ug, described as median) at 8 week, was lower in INS+CP group) than that in INS group , (574ug/24h) and (750ug/24h),p<0.01, but there is no significant difference between the INS+CP group and C group.**Conclusions:** Tthe combination therapy with C-peptide and insulin can further reduce the accumulation of extracellular matrix and decreased the UAER than the mono-therapy of insulin.

P72 STUDY OF FN AND LN EXPRESSION OF THE RETINAL MICROVESSELS OF RAT DIABETIC RETINOPATHY

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Purpose: To elucidate the relationship between the expression of fibronectin(FN) and laminin(LN) and the pathogenesis of diabetic retinopathy. **Methods:** 20 male Wistar rats were randomly divided into both the diabetic retinopathy group(N=10, DR) and normal control group(N=10, NC). The retinal vessel whole mounts were used for visualizing the FN and LN expression and performing a different counts of pericytes and endothelial cells as well as pathological morphological observations. The peripheral blood was taken for rheological indexes. The expression levels of FN and LN were measured by computer image system. **Results.** In the DR group the expression level of FN and LN was significantly higher and the number of the pericytes and the proliferative endothelial cells was conspicuous decreased and increased respectively($p<0.001$), and the erythrocyte viscosity and plasma triglycerides had significant increase ($p<0.05$)compared to the NC group. **Conclusion:** The results indicated there existed a close relationship between the diabetic retinopathy and expression of FN and LN.

Keywords: diabetic retinopathy, rat retinal microvessels, LSAB immunostaining, FN and LN expression..

P73 THE EXPERIMENTAL STUDY OF THE RELATIONSHIP BETWEEN THE ENDOTHELIUM-DEPENDENT VASODILATION INDUCED BY INSULIN AND HYPERGLYCIMIA

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Objective: With a view of the mechanism of the dysfunction of endothelium-dependent vasodilation led by metabolic factors, a study of the effects of various concentrations of glucose, insulin on the levels of endothelial nitric oxide (NO) was carried. **Methods:** cGMP radioimmunoassay for endothelial cells (ECs) was used in this study as an indicator of NO production, and the levels of endothelial nitric oxide synthase (eNOS) mRNA were measured by semiquantiative RT-PCR. **Results:** Insulin (0.18nmol/L-6.0nmol/L) and glucose (20mM, 40mM) could stimulate concentration- and time-dependent cGMP production. High concentrations of glucose upregulated the level of eNOS mRNA, insulin had no effect on the expression of eNOS. Under elevated glucose, stimulation of cGMP levels by insulin (6.0nmol/L) were significantly decreased. **Conclusions:** Endothelium-dependent vasodilation induced by insulin is associated with endothelial NO production. Decreased Insulin sensitivity of endothelium is a part of insulin resistance. High concentrations of glucose probably inhibit insulin-induced endothelium-dependent vasodilation.

P74 INSULIN CAN MITIGATES THE EFFECT OF FREE FATTY ACID (FFA) TO CAUSE ENDOTHELIAL DYSFUNCTION IN RAT AORTIC RINGS

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Objective: To test whether FFAs directly and independently cause endothelial dysfunction and whether enhancing insulin action can mitigate FFA induced endothelial dysfunction. **Methods:** S-D rats underwent 4hr infusions (18μl/min) in four groups: Controls(C, n=5): saline alone; FFA(n=9): 20% intralipid 18μl/min + heparin 0.72IU/min; Insulin(I, n=5): hyperinsulinemic euglycemic clamp(insulin 20mU/kg/min); Insulin+FFA (I+FFA, n=10): hyperinsulinemic euglycemic clamp + FFA. Following infusion thoracic aortas were harvested and rings obtained. Rings were equilibrated in Krebs-Henseleit solution, constricted with $PGF2_{\alpha}$and then relaxed with increasing doses of acetylcholine (Ach) for endothelium dependent vasodilation (EDV) or sodium nitroprusside (SNP) for endothelium independent vasodilation (EIV). Vasorelaxation was expressed as % of maximal contraction. **Results:** EDV: C 15.0±3.2%, FFA 45.2±2.5%, (C vs FFA, p=0.002), I 19.4±1.8%,(C vs I, p=ns) and FFA+I 27.2±2.1% (FFA+I vs C p=0.02,vs FFA p=0.005). EIV response under all conditions were not different from controls. **Conclusions:** FFA cause endothelial dysfunction by a direct interaction with the endothelium. The data suggest that FFA may be proatherogenic and that hyperinsulinemia has salutary effects on endothelial function in the context of elevated FFA.

P75 Association Study on Type 2 Diabetic Macrovascular Disease and E-selectin ,GP Ia/IIa and MTHFR genes in Chinese

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Objective: To explore the association between 4 polymorphisms of 3 genes :E-selectin 561 A/C, GP Ia/IIa 807C/T, 873G/A, MTHFR (methyltetrohydrofolic reductase) 677C/T and diabetic macrovascular complications (cerebralvascular infarction and coronary heart disease) in Chinese. **Method**: Totally 416 Chinese subjects were divided into AS(atherosclerosis) group (216 patients with macrovascular disease), DM group (100 diabetics without any clinical macrovascular manifestations) and control group(100 subjects without diabetes and macrovascular diseases) according to respective criteria. The AS group was further divided into APOP (cerebralvascular infarction ,50 with and 61 without diabetes) subgroup and CHD(coronary heart disease, 48 with and 57 without diabetes) subgroup. Genotypes were determined by PCR/restrction enzyme digestion. **Results :** No association was found between E-selectin gene, GP Ia/IIa gene and diabetic macrovascular disease in Chinese. The frequencies of MTHFR TT genotype and T allele were much higher in macrovascular disease group. Stepwise logistic regression revealed that MTHFR gene were a potential contributor to both AS and APOP . Comparison of genotype frequencies between diabetics and non-diabetics within AS group and its subgroups also showed no difference. **Conclusion** :Our data suggested that MTHFR ,as an independent risk factor , contributes to the development of macroangiopathy (cerebralvascular infarction and coronary heart disease) whether with or without diabetes.

P76 SCREENING AND ANALYSIS FOR MYOCARDIOPATHOLOGIC RELATED GENE IN EARLIER PERIOD OF TYPE 2 DM

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Objective: To screen and analize myocardiopathologic related gene in earlier period of type 2 DM. **Methods:** (1) To develop a rat model of type 2 DM,SD rats were injected with a low-dose of streptozotocin(STZ)after high calorie was given.(2)After rat model ready-made, Glucose-insulin tolerance tests and immunohistochemistry with pancreas were performed.Dissected myocardium was observed by electron microscope. Myocardium was analized with fluorescence labelled mRNA differential display.Positive clones were BLAST after conformed by Northern blot.Via the application of bioinformatics, the function and structure of unknown ESTs were predicted. **Results:** (1) This model of type 2 DM was characteristic of decreased insulin sensibitity and little impaired insulin secretion.(2)The analysis of ultrastructure of myocardium verifyed diabetic cardiomyopathy.(3)12 positive clones conformed by Northern blot were carnitine palmitoyltransferase I, troponin T, mitochondrion ATP synthase A subunit and 9 novel ESTs which had submitted to Genbank named DHD1-9. DHD 5 which possessed an open reading frame was predicted to play a role of catalysis or signal conduction by bioinformatics analysis. **Conclusions:** The altered mRNA expression of these genes suggest that they are candidates for a role in the development of myocardiopathy prior to the ultrastructural alterations of myocardium in earlier period. Besides, the combination of dietary-induced insulin resistance and low-dose of STZ produce a relatively ideal animal model which simulates the metabolic of type 2 DM.

P77 The Study on the Relationship between the C-106T Poly-morphism in Aldose Reductase Gene Promoter Region and the Susceptibility of Retinopathy in type 2 Diabetes Mellitus

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Objective: To study the relationship between the C-106T polymorphism in aldose reductase (AR)gene promoter region and the susceptibility of retinopathy in type 2 DM of Han nationality in north China and the linkage of the C-106T polymorphism with (AC)n repeat polymorphic marker at the 5'end of AR gene. **METHOD:** 116 patients with type 2 DM were included. They were divided into no diabetic complication(NDC) group (66 cases) with the course of DM more than 10 years but free of diabetic microangiopathy and diabetic retinopathy(DR)group (50 cases) with DR developed within 5 years after DM to be diagnosed.. PCR were performed using genomic DNA as template to amplify a 236bp DNA fragment which included C-106T polymorphic site in AR gene promoter region. 8ul PCR products and 5U restriction enzyme Bf α 1incubated at 37°C for 6h. Genotypes were determined by electrophresis in 3% agarose gel and EB staining using gel documentation and analysis system. **RESULT:** Two alleles C/T and three genotypes CC,CT,TT were found in both NDC and DR group. The frequence of CC genotype was significantly higher in DR group than that in NDC group (64.0% vs 43.9%, $P<0.05$) and the frequence of CT genotype was significantly lower in DR group than that in NDC group (30.0% vs 54.0%, $P<0.01$). After analyzed together with the (AC)n polymophism at 5'-end of AR gene which was proved strongly associated with DR by our previous study, the results showed that frequence of Z-2/C haplotypes was significantly higher in DR group than that in NDC group (37.0% vs 3.0%, $P<0.001$) and the frequence of Z+2/C and Z+2/T paplotype was significantly lower in DR group than that in NDC group (11.0% vs 42.0%, $P<0.01$ and 2.0% vs 14.0%, $P<0.05$ respectively). **CONCLUTION:** In type 2 DM of Han nationality in north China, AR gene promoter region presents C-106T polymorphism . CC and CT genotype were significantly associated with the susceptibility of DR. Patients with CC genotype might increase whereas with CT genotype might decrease the susceptibility of DR. The C-106T polymorphism and the (AC)n polymorphism might be in linkage disequilibrium.

P78 THE EFFECT OF THE PLASMA HOMOCYSTEINE LEVEL ON TYPE 2 DIABETIC RETINOPATHY

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OBJECTIVE: An increased plasma homocysteine level is an important risk factor for vascular disease in the genernal population.However,the role of hyperhomocysteinemia in the development of type 2 diabetic retinopathy (DR) is still unknown.Therefore,our aim was to determine the relationship between the fasting plasma homocysteine levels and the presence of diabetic retinopathy in patients with type 2 diabetes . **METHODS**: The study group consisted of 55 cases of type 2 diabetic patients and 19 healthy persons as control(CON,12 men and 7 women) group.The DM group were subdivided into non-diabetic complications(NDC) group(39 cases,17 men and 22 women), DR group(16 cases,8 men and 8women). The renal function and the ratio of Alb/Cr were within normal range in all subjects.The presence and the grade of retinopathy were determined by a ophthalmologist. Plasma total Hcy was measured by automated high –performance liquid chromatography(HPLC) with reverse phase separation and fluorescence detection.**RESULTS:**Plasma tHcy levels were significantly different among DR, NDC and CON groups (F=2.405,p=0.031). Plasma total Hcy was significantly higher in DR group(14.7±5.28umol/L) than that in NDC(11.3±4.94umol/L) and CON group(9.65±2.66umol/L).There was no difference between NDC group and CON group. In DR group, plasma tHcy was significantly higher in proliferative diabetic retinopathy(PDR) group than that in background diabetic retinopathy(BDR) group.Hyperhomocysteinemia was defined as tHcy >14umol/L,was seen in 4 patients in PDR group,and the ratio was significantly higher than in BDR group. **CONCLUSION:** It was suggested that hyperhomocysteinemia may be a risk factor for the development and progress of type 2 diabetic retinopathy.

P79 THE RISK FACTORS OF DIABETIC NEPHROPATHY IN PATIENTS IDDM

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Diabetic Nephropathy is one of the terrible complications of the DM which development depended from many of the risk factors. 87 patients with Insulin Depended Diabetes Mellitus (IDDM) were observed. Patients were selected on three groups: without proteinuria, with proteinuria and without uremia, with uremia. Age of patients without proteinuria was significant bigger than in patients with proteinuria. But duration of disease no differ between the groups. Occurrences of diabetic nephropathy no develop in each of patients, but presence of nephropathy reflected on patient survival. On develop of nephropathy has relation the age when was manifested disease. In patients whom IDDM manifested before 15 years old frequency of Diabetic Nephropathy was significant higher than who's were disease manifestation after 15 years old. The glycemic control level has no differ between the groups in our study. Plasma insulin level no had difference into each group. Plasma aldosteron level was significantly decreased with increasing of renal damage, but arterial hypertension were higher even plasma aldosteron level was droped. Presence in heredity from hypertension more related to frequency of nephropathy than heredity from Diabetes Mellitus. Accompanying other kidney damages in patients with Diabetes Mellitus also has relation to development of nephropathy. In conclusion, on develop of nephropathy in patients with IDDM more influent the age of manifestation of IDDM (before 15 years old), heredity from hypertension, the presence of other kidney damages. So such patients need in a frequently control and early preventive intervention of nephropathy.

P80 MEASURE OF URINE ENZYME ACTIVITY IN EARLY DIAGNOSIS OF DIABETIC NEPHROPATHY

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Early diagnostic and preventive intervention of the diabetic kidney damage are important. Glomerular changes at the Diabetes Mellitus are well investigated. But tubular dysfunction and its role in early diagnostics of Diabetic Nephropathy are not clear enough. In 51 patients with Diabetes Mellitus (32 with IDDM and 19 with INDDM) were observed glomerular filtration rate, serum creatinin level, proteinuria level and indexes of tubular function from urine neutral alpha glycosidase, alkaline phosphatase and beta 2 micro globulin. Urine neutral alpha glycosidase activity, alkaline phosphatase and beta 2 micro globulin level were significantly higher in all patients than non-diabetic subjects. Excretion of urine enzymes raised accordingly to glomerular filtration rate, serum creatinin level, proteinuria level and stage of Diabetic Nephropathy. These date suggested that glomerular changes and tubular dysfunction are correlated at the Diabetes Mellitus. Thus urine neutral alpha glycosidase activity was raised in early stages, when no detected microalbuminuria and glomerular filtration changes. In conclusion measure of urine enzyme activity reflected both glomerular and tubular disturbances degree and may be usefull for determination, preventive intervention of the Diabetic Nephropathy in early stages.

P81 EARLY DETECTION OF KIDNEY DISEASE BY MICROALBUMINURIA IN CHILDHOOD AND ADOLESCENCE TYPE 1 DIABETES MELLITUS

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Objective. To evaluate the use of microalbuminuria in detecting renal disease in type 1 DM children and to study their clinical presentations. **Method** All diabetic patients in our Department were screened yearly for microalbuminuria since September 1995. An albumin excretion rate greater than 30 mg per day was considered as abnormal. Renal biopsy would be performed in those patients with persistent microalbuminuria i.e. at least 2 episodes in a 6-month period. **Result**. Eight patients (10.1% of 79 type 1 DM patients) were detected to have persistent microalbuminuria. All of them were Chinese with male to female ratio of 3:5. Their mean age at presentation of diabetes was 9.3 ± 2.4 years and the mean duration of follow up was 10.9 ± 4.4 years. Five patients consented to renal biopsy. Two patients showed histological changes of diabetic nephropathy and vasculopathy and one of them had coexisting IgA nephropathy. One patient had isolated mesangial proliferation with deposition of IgM. The remaining two biopsies did not show any abnormal histo-morphology. All eight patients were treated with enalapril, at an average dose of 15 mg per day (range 5-30mg per day). Microalbuminuria decreased from a mean of 367.64 mg/day pre-treatment (range 56-1415 mg/day) to 37.24 mg/day post-treatment (5-107.9 mg/day) with p value of 0.1. Mean systolic blood pressure decreased from 132 mmHg (pre-treatment) to 118 mmHg (post-treatment) with $p < 0.01$. The change in diastolic blood pressure was not significant. All patients had normal serum creatinine and creatinine clearance at presentation and serial yearly measurements did not show significant changes with p value of 0.33. **Conclusion** Coexisting immune complex glomerulonephritis was not uncommon in type 1 children and microalbuminuria is not solely a marker of diabetic nephropathy. Prompt identification by renal biopsy facilitated early diagnosis and intervention. Functional abnormalities, detected by means of microalbuminuria, could precede morphological changes in renal biopsy.

P82 FAMILIAL CLUSTERNG OF ABNORMAL URINARY ALBUMIN EXCRETION RATE IN CHINESE FAMILIES WITH TYPE 2 DIABETES AND RELATED FACTORS OF URINARY ALBUMIN EXCRETION RATE

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Objective: To study whether urinary albumin excretion rate (UAER) is a heritable trait in Chinese families with type 2 diabetes and its related factors. **Methods:** 534 individuals out of 149 type 2 DM families from Beijing area were investigated. Urinary albumin-to-creatinine ratio (ACR) was used as a measurement of microalbuminuric status. **Results:** ①The Ln(ACR) was significantly higher in diabetic siblings of microalbuminuric probands than diabetic siblings of normoalbuminuric probands (2.65 ± 1.07 vs 2.20 ± 0.51, $P=0.003$) after being adjusted for age, duration of diabetes, hypertension history, SBP, GHbA1c, cholesterol and HDL-C. ②In diabetic siblings or first-degree relatives, elevated ACR is associated with higher SBP and their proband's ACR level. No difference was found in Ln(ACR) between nondiabetic siblings or first-degree relatives of microalbuminuric probands and that of normoalbuminuric probands (2.03 ± 0.51 vs 2.06 ± 0.52, $P=0.901$ between two groups of nondiabetic siblings; 2.09 ± 0.54 vs 2.07 ± 0.60, $P=0.819$ between two groups of nondiabetic first-degree relatives, respectively). ③The heritability of ACR in diabetic first-degree relatives ranged from 14%-23%. ④The best genetic model for the familial segregation of ACR levels was multifactorial with evidence of a common major gene effect (estimated frequency 0.37). **Conclusion**: There is a familial aggregation of abnormal UAER in this collection of type 2 diabetic families and therefore ACR is a heritable quantitative trait. The genetic predisposition to abnormal UAER is expressed only in those patients who develop the type 2 diabetes. Segregation analyses suggested that the best model for ACR levels was multifactorial with evidence for a common major gene.

P83 ON THE FUNCTIONAL STATE OF KIDNEY TUBULAR PART IN TYPE II DIABETES

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Objective: To investigate the tubular functional states in diabetic patients with type II and its relation with the stage diabetic nephropathy and diagnostical importance of determining urine enzyme activity. **Methods:** The investigation have been carried out with 60 diabetic patients with insulin-independent diabetes mellitus. The complex investigation of the functional state of kidney was performed with all the patients that included clinical and biochemical analysis urine and blood. **Patients:** were divided into three group: the first group included the diabetic patients without proteinuria, second one included patients with proteinuria and without uremia, third one consisted from the patients with proteinuria and uremia. The control group included 10 persons without diabetis mellitus. **Results:** As a result of performed investigation we have established that the diabetic patients (in comparison with healthy people) have the tubular dysfunction which expressed by decreasing of creatinin clearance, by increasing of β-2- microglobulin excretion and the activity of alpha-glicosidase and urinary alkaline phosphatase. The direct correlation between the duration of diabetis mellitus and the tubular affection degree have been detected. **Conclusion:** Thus our investigation showed that the study of the urinary enzyme activity could enable one to obtain the additional information on the tubular affection at diabetes mellitus. Therefore it could be used as the diagnostical method for determining of the kidney affection degree.

P84 ADRENOCORTICAL AXIS AND LEPTIN IN RELATION TO DIABETIC NEPHROPATHY

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Objective: To assess evidence of adrenocortical axis activation and leptin level in Type 2 diabetic subjects with nephropathy. **Method**: In this pilot study, we examined the relationships between these hormonal parameters and diabetic nephropathy. Fasting plasma glucose, lipid profile, 9:00 am serum cortisol and leptin levels were measured in 34 Type 2 diabetic patients with nephropathy(DMN) (plasma creatinine 120-260 mcmol/l and spot urinary albumin creatinine ratio (ACR)> 25 mg/mmol), 12 diabetic subjects (DM) with normoalbuminuria (spot urinary ACR <3.5 mg/mmol and plasma creatinin<120 mcmol/l) and 34 non-diabetic subjects (EC) (fasting plasma glucose <5.5 mmol/l), all age matched and 57 young non-diabetic control subjects (YC). **Result**: All former 3 groups had similar mean age, BMI, total cholesterol, LDL-C and blood pressure. The young control group had the lowest values for all readings. Patients with diabetic nephropathy had lower HDL-C and higher TG than the two age-matched groups. Serum cortisol (DMN: 646±287, DM: 532±383, EC: 384±112, YC: 430±205 nmol/l) and leptin (DMN: 17.5±16.8, DM: 14.6±10.5, EC: 9.1±7.1 YC: 8.9±6.7 ng/ml) were higher in the diabetic nephropathy group than the two non-diabetic groups but there was no difference within the diabetic group. On multivariate analysis, ACR (Beta=0.450, p<0.001) and BMI (Beta=0.257, p=0.002) were independently associated with leptin (R2=0. 279, F=22.1, p<0.001) while fasting plasma glucose (Beta =0.280, p=0.003) was independently associated with morning cortisol (R2=0.078, F=9.15, p<0.001). **Conclusion**: These findings support the intimate relationships between obesity, leptin and albuminuria while activation of the adrenocortical axis had independent effect on the diabetic status.

P85 INTENSIVE CARE IN A CLINICAL TRIAL SETTING DELAYED RENAL DISEASE PROGRESSION IN TYPE 2 DIABETIC PATIENTS WITH NEPHROPATHY

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Background and Objective: Optimal control of BP and metabolic indices and also renin-angiotensin-system (RAS) inhibition reduce albuminuria and retard the progression of diabetic nephropathy (DN) to renal failure. The renoprotective effects of the newer ACEI, fosinopril and the AII antagonists, losartan and irbesartan in patients with DN and renal impairment were examined in randomized, controlled, multi-centered clinical trials, where Hong Kong was one of the centers. We hypothesized the intensive care received by patients in a clinical trial setting involving treat-to-target protocols with better drug compliance attenuated renal deterioration.
Methods: Data from these 3 trials at our hospital center were pooled: (1) fosinopril 10-20mg/d vs placebo (n=38); (2) irbesartan 75-300mg/d vs amlodipine 2.5-10mg/d vs placebo (n=10); and (3) losartan 50-100mg/d vs placebo (n=55). The rate of renal deterioration was expressed as the regression coefficient of the monthly plasma creatinine reciprocal (1/Cr*t). Using each patient as his/her own control regardless of treatment allocation, paired t-tests were performed to compare 1/Cr*t during the 6-24 months before and after recruitment into the trials provided that ≥3 plasma creatinine points were available for each period.
Results: 103 Chinese subjects were recruited and randomized at our center for one of these 3 studies among which 66 patients fulfilled this requirement (age 63.1±6.5; male 64%). The mean 1/Cr*t was reduced from 15.7 (±15.7) x10-5 $L \cdot mmol^{-1} \cdot month^{-1}$ to 6.9 (±6.5) x10-5 $L \cdot mmol^{-1} \cdot month^{-1}$ (56% mean reduction) after recruitment (P<0.001). Inspection of each patient's creatinine reciprocal-to-time plot revealed slope flattening in 44 (67%) of the patients following entry into the trials. In the losartan/placebo trial alone, 80% had their slopes flattened.
Conclusion: The proportions of slope flattening greatly exceed a 50% active drug to placebo ratio suggesting a renoprotective effect of entering the trial environment in addition to that of RAS inhibition from active drug allocation. This additional factor may be the more aggressive control of BP and metabolic indices associated with the intensive monitoring in a good clinical trial.

P86 LIMITED JOINT MOBILITY IN DIABETIC PATIENTS IS REVERSIBLE WITH CAPD

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Objectives: We have previously shown that limited joint mobility (LJM) was prevalent among diabetics. In the present study we examined: (1) its relationship with diabetic retinopathy and nephropathy, (2) reversibility with peritoneal dialysis in end stage diabetic nephropathy. **Method:** 244 diabetic outpatients were compared with 291 non-diabetic outpatients or staff volunteers. Demographic data, DM duration, the latest 3 Hba1c levels, fundoscopy, and 24-hour urine samples were collected. The maximal angle of extension at wrist, 3rd and 5th MP joints were measured using goniometer. Flexibility score was defined as 180- (mean maximal angle of extension) and LJM as having flexibility score > 1.64 SD below the mean of non-diabetic controls. T test and one way ANOVA were used. **Results & Conclusions:** The two groups were well matched for age, sex, BMI, smoking and drinking history. Although we did not find a correlation between LJM and diabetic retinopathy and nephropathy, we did show significantly higher joint flexibility scores in diabetic patients on continuous ambulatory peritoneal dialysis (CAPD) as compared with those not receiving CAPD. This finding of potential reversibility of LJM by CAPD supports the hypothesis that deposition of advanced glycosylation end-products (AGEs) in extracellular matrix of periarticular tissues underlies the pathogenesis of LJM, and AGEs accumulated in renal failure may be dialysable.

P87 HONG KONG DIABETIC PATIENTS' PERCEPTION OF ERECTILE DYSFUNCTION

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Aim: To survey the perception of erectile dysfunction (ED) and expectation on corresponding treatment among diabetic patients in Hong Kong. **Methods:** A cross sectional survey was done on 500 Chinese diabetic patients attending a diabetic clinic in Hong Kong. The patients were interviewed for their knowledge, the psychosocial impact and the expectation on treatment for ED, as defined in the National Institute of Health (NIH) Consensus Conference 1993. Diabetic complications and clinical data of patients were obtained from patients' medical records. **Results:** Excluding the incomplete questionnaires, responses from 486 subjects were included in data analysis. Concerning the knowledge of ED, 30.9% of the patients defined ED incorrectly, 15.6% of them had not heard of it at all, and 32.1% had no knowledge that ED was a complication of diabetes mellitus. For those patients with ED, 23.6% of them worried about the problem, 21.8% reported ED caused them low self-esteem, and 18.2% claimed their relationship with sexual partners was adversely affected by it. Regarding treatment, 8.8% of all patients had ever had discussion on ED with any medical personnel. Of those patients with ED, 7.4% had received corresponding treatment. Among 65.8% of the patients interviewed, information of ED was desired to be part of diabetic management. When asked in case they had the problem, 73.5% of them preferred to have their sexual partners accompanying them for corresponding treatment. **Conclusion:** Diabetes patients' knowledge on ED is inadequate and the care they received on ED is poor. Given that ED is common complication in diabetic patients in Hong Kong, more attention may be needed for the provision of proper knowledge, and treatment for the dysfunction and its associated psychosocial impact.

P88 The Study of Sensory Evoked Potentials in Patients With Type 2 Diabetes Mellitus 1. The Changes of Brain Stem Evoked Potentials (BAEP) in Type 2 Diabetics

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Objective: The study used BAEP test aim to evaluate the function of brain stem auditory pathway in patients with type 2 diabetes mellitus, and to establish a possible relationship with various disease clinical parameters and the changes of BAEPs. **Methods:** BAEP were examined in 30 type 2 diabetic patients (group DM) and in 30 age-and-sex-matched nondiabetic control subjects (group C). The parameters of BAEP were recorded and analysed by SPSS. **Results:** Abnormalities were found in 18 (60%) diabetic patients, including waveform disappearance, wave latency prolongation and wave amplitude reduction. Compared with control group, we found a significant higher latency ($p<0.05$) as well as a remarkable lower amplitude ($p<0.05$) of wave V. We also found significant elongations in the mean interpeak latencies of I-III, III-V, I-V ($p<0.05$). Furthermore, a positive correlation was found between the latencies of BAEP and the disease duration, renal function and the level of blood lipid (<0.05). **Conclusions:** The results suggested the pathological changes of peripheral and central nervous system in type 2 diabetic patients, and BAEP technique was a sensitive and helpful indicator of early diagnosis of diabetic neuropathy. Lowering high blood fat and protecting the renal function would have beneficial effects to prevent the impairment of auditory pathway in diabetics.

P89 The Study of Sensory Evoked Potentials in Patients With Type 2 Diabetes Mellitus 2. The Changes of visual Evoked Potentials (VEP) in Type 2 Diabetics

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Objective: The study used VEP test aim to investigate the lesion site and extent of visual center in patients with type 2 diabetes mellitus, and to establish a possible relationship with various disease clinical parameters and the changes of VEP. **Methods:** VEP were measured in 30 type 2 diabetic patients (group DM) and in 30 age-and-sex-matched nondiabetic control subjects (group C). The numerical value of VEP were recorded and analyzed by SPSS. **Results:** Abnormalities were found in 20 (66.7%) diabetic patients, including waveform disappearance, wave latency prolongation and wave amplitude reduction. Compared with control group, the mean P100 latencies were significantly increased ($p<0.05$), the amplitudes were lower, but had not significant value ($p>0.05$). Furthermore, a positive correlation was found between the latencies of VEP and the renal function and the level of blood glucose ($P<0.05$). **Conclusions:** The results suggested that the VEP technique was a sensitive index in detecting early signs of the diabetic neuropathy in central nervous system (CNS) in type 2 diabetic patients. Lowering high blood glucose and protecting the renal function would have beneficial effects to release the impairment of visual center in diabetics.

P90 The Study of Sensory Evoked Potentials in Patients With Type 2 Diabetes Mellitus 3. The Changes of Somatosensory Evoked Potentials (SEP) in Type 2 Diabetics

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Objective: The study used SEP test (after the stimulation of both median and tibial nerves) aim to evaluate the functional status of both specific somesthesia pathway and cerebral cortex in patients with type 2 diabetes mellitus, and to establish a possible relationship between various disease clinical parameters and the changes of SEP. **Methods:** SEP were measured in 30 type 2 diabetic patients (group DM) and in 30 age-and-sex-matched nondiabetic control subjects (group C). The numerical value of SEP were recorded and analyzed by SPSS. **Results:** SEP during median nerve stimulation were found abnormal in 20 (66.7%) diabetic patients, and 22 (73.3%) during tibial nerve stimulation. All abnormalities include waveform disappearance, wave latency prolongation and wave amplitude reduction. Compared with control group, median SEP showed significantly increased latency values and remarkably decreased wave amplitude in N9、N13、P14、N20、P25 ($p<0.05$) ,and the longer latency and lower amplitude in N35、P45,but had no significant value ($p>0.05$) . In tibial SEP the latencies of N9、P30、P38、P60 were increased significantly ($p<0.05$) as well as a significant lower wave amplitude in N9 and N60 ($p<0.05$) .Furthermore, duration of disease, the level of blood glucose and lipid and the renal function positively correlated with SEP abnormalities ($p<0.05$). **Conclusions:** The results suggested that the SEP technique could judge accurately the functional status of both specific somesthesia pathway and cerebral cortex in patients with type 2 diabetes, which was a sensitive indicator of early diagnosis of diabetic neuropathy. We also found in diabetics the neuropathy was more severe in the lower limbs than in the epipod and parallel with the duration of disease. Lowering high blood glucose and fat and protecting the renal function would have beneficial effects to relief the impairment of diabetic neuropathy.

P91 The Study of Evoked Potentials in Patients With Type 2 Diabetes Mellitus 4. The Changes of Event-Related Potentials (ERP) in Type 2 Diabetics

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Objective: The study used ERP test aim to investigate the lesion site and extent of cognitive functions in patients with type 2 diabetes mellitus, and to establish a possible relationship with various disease clinical parameters and the changes of ERP. **Methods:** ERP were measured in 30 type 2 diabetic patients (group DM) and in 30 age-and-sex-matched non-diabetic control subjects (group C). The numerical value of ERP were recorded and analyzed by SPSS. **Results:** Abnormalities were found in 11 (36.67%) diabetic patients, including wave latency prolongation and wave amplitude reduction. Compared with control group, the mean P3a, P3b latencies were significantly increased ($p<0.001$), the amplitudes were lower ($p<0.05$) Furthermore, a positive correlation was found between the changes of parameters and the level of blood glucose and fat ($P<0.05$). was not related to the duration of illness, **Conclusions:** The results suggested that the ERP technique was a sensitive index in detecting early signs of dysfunctions for the cognitive function and to discover the potential dementia in type 2 diabetic patients. **3.**Lowering high blood glucose and fat would have beneficial protecting the cognitive function to release the impairment of the brain in diabetics.

P92 Evaluation of baroreflex sensitivities and arterial blood pressure variability in patients with diabetic cardiovascular autonomic neuropathy

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Objective: To evaluate baroreflex function in patients with diabetes mellitus, and investigate the relationship of baroreflex sensitivities (BRS) and arterial blood pressure variability (BPV) to diabetic cardiovascular autonomic neuropathy. **Subjects and methods:** 22 healthy controls and 54 patients with diabetes mellitus were involved in the study. Diabetics were divided into two groups: group 1 (n=23) patients with diabetic cardiovascular autonomic neuropathy (CAN) and group 2 (n=31) patients without CAN. CAN was diagnosed according to the results of standard tests of cardiovascular autonomic reflex functions using computer-based technique. Arterial blood pressure (SBP and DBP), intra-beat-to-beat interval (IBI) were examined detected using Finapress-System for measurement of BRS, HRV and BPV. **Results:** Power spectrum of BPV and IBI, spectral BPV in the high frequency band excepted, were reduced significantly in patients with CAN compared with diabetics without CAN and healthy controls ($P<0.01$). There were significant reducations in BRS between patients with CAN (3.30 ± 1.48) and patients without CAN (7.86 ± 3.83, $P<0.01$), control subjects (10.87 ± 5.99, $P<0.01$) and between patients without CAN and control subjects ($P<0.05$). **Conclusion:** There are significant reducations in the arterial blood pressure variability and beat-to-beat interval variability in diabetics with CAN. Baroreflex function in patients is impaired. The reducation in BRS could be as a good method to detect earlier changes of cardiovascular autonomic functions in patients with diabetes mellitus.

P93 VALUE OF ELECTROPHYSIOLOGICAL TEST FOR DIAGNOSIS OF DIABETIC PERIPHERIAL NEUROPATHY

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Objective: To discuss the value of electrophysiological examinations for diagnosis of diabetic peripherial neuropathy. **Methods:** 122 patients with type 2 diabetes were divided into five groups according to the neuropathy screening, physical examinations and electrophysiological examinations. Five groups were non diabetic neuropathy, borderline, mild, moderate and severe neuropathy. **Results:** The motor conduction velocity of median nerve (MCVM), ulnar nerve (MCVU), post tibial nerve (MCVPT) and the sensory conduction velocity of median nerve (SCVM), superficial peroneal nerve (SCVSP) occupied 0.33798, 0.35497, 0.33361, -0.17224, -0.03298 in factor 1, respectively. MCVM, MCVU, MCVPT in moderate and severe neuropathy were lower than those in non diabetic neuropathy, borderline and mild neuropathy ($P < 0.001$). Moreover, moderate and severe neuropathy had significant difference in MCVM and MCVU ($P < 0.001$). SCVSP in severe neuropathy was significantly lower than that of others. ($P < 0.01$). **Conclusions:** Nerve electrophysiological test was a good method for diagnosis of diabetic neuropathy. MCVU was the best parameter. The stage of diabetic neuropathy was associated with both the number and the velocity of impaired nerves.

P94 SERIAL SHENSHENG RECIPE ON TYPE 2 DM AND ITS CHRONIC COMPLICATIONS

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Objective: To explore the clinical effect of serial Shensheng recipe on patients with type 2 DM and its chronic complications. **Methods:**112 patients with type 2 DM (including 40 with diabetic nephropathy and 52 with diabetic neuropathy) in observation group were treated with different Shensheng recipe respectively according to the complication. 30 type 2 diabetic nephropathy and 30 type 2 diabetic neuropathy in control group were given captoril, vitamin B1 and vitamin B12 respectively. Fasting and 2h blood glucose, liver and renal function, proteinuria, bladder residual urine volume and gastro emptying time were observed before and after the treatment. **Results:** Fasting and postprandial blood glucose were significantly decreased after the treatment, while proteinuria reduced, renal function improved in diabetic nephropathy, and the syndromes of neuropathy relieved in different degree in diabetic neuropathy, bladder residual urine volume decreased in bladder vegetative nerve dysfunction, and half gastro empting time fastened in diabetic gastroparesis, which were all statistically significant. **Conclusion:** It suggests that serial Shensheng recipe can effectively lower blood glucose, ameliorate the symptoms, reduce proteinuria, improve renal function and relieve diabetic neuropathy.

P95 ACUTE AND CHRONIC COMPLICATIONS IN PATIENTS WITH LATENT AUTOIMMUNE DIABETES IN ADULTS

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Objective: To study the clinic characters of patients with latent autoimmune diabetes in adults (LADA). **Methods:** Using case-control study, the pattern of the prevalence of acute and chronic complications was investigated in 41 LADA patients with serum antibodies to glutamic acid decarboxylase (GADA) and / or to islet cell cytoplasmic antigens (ICA) and 36 acute onset type 1 and 377 type 2 diabetes. **Results:** The mean age, BMI, fasting and postprandial C-peptide levels of the LADA patients were higher than that of type 1 diabetes and lower than that of type 2 diabetes. The mean duration of diabetes did not differ among the three groups. Of the LADA patients 29.3% had ketosis. Although the frequency of repeated ketosis in the LADA patients was similar to that in type 1 diabetes, the duration from diabetic onset to first ketosis in the LADA patients was much longer. None of the LADA patients had hyperosmolar nonketotic diabetic coma. The prevalence of retinopathy in the LADA patients was 19.51% [95% confidence interval (CI) 7.38-31.64], which was similar to that in type 1 diabetes. The prevalence of cataract in the LADA patients was 48.78% (95% CI 33.48-64.08), which was similar to that in type 2 diabetes. Less LADA patients had microalbuminuria, compared with the other groups. There was no difference in the prevalence of overt nephropathy, neuropathy and hyperlipemia among the three groups. The prevalence of coronary heart disease in the LADA patients was similar to that in type 2 diabetes. The prevalence of hypertension in the LADA patients was higher than that in type 1 but lower than that in type 2 diabetes. **Conclusion:** It suggests that the clinic characters as well as acute and chronic complication pattern in LADA patients are difference neither that in type 1 nor that in type 2 diabetes. Serum GADA and ICA positive are important markers for distinguishing LADA from type 2 diabetes.

P97 THE STUDY OF THE RELATIONSHIP BETWEEN THE CTLA-4 GENE POLYMORPHISM AND GRAVES' DISEASE

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Objective: To study the relationship between the polymorphism of the cytotoxic T lymphocyte antigen 4(CTLA-4) gene and genetic predisposition to Graves' disease(GD). **Methods:**92 patients with Graves'disease and 56 healthy subjects as controls were studied. The genomic DNA was extracted from peripheral leukocytes, then A/G polymorphism at position 49 in exon 1 of CTLA-4 gene was screened by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to analyse the genotypes and alleles frequencies. **Results:** The genotype frequencies in exon 1 of CTLA-4 gene were GG 22.8%,AG 50%,AA 27.2% in Graves' patients and 8.9%,46.4%, 44.6% in control subjects. The distribution of the genotypes differed between the two groups ($P<0.05$). Frequency of the G allele in exon 1 of the CTLA-4 gene was the 47.8% in patients and 32.1% in control subjects. A significant difference in the frequency of the G allele was detected in the two groups ($P<0.01$).While no difference in the distribution of the genotypes was found in patients with Graves' disease with respect to sex. **Conclusion:** A/G polymorphism at position 49 in exon 1 of CTLA-4 gene is associated with genetic susceptibility to Graves' disease, but the polymorphism is not correlated to sex in patients with Graves' disease. It may be meaningful to apply the analysis of CTLA-4 gene by PCR-RFLP to find the persons with higher susceptibility to Graves' disease.

P98 NO MAJOR SUSCEPTIBILITY LOCUS FOUND ON CHROMOSOME 20Q FOR CHINESE FAMILIAL GRAVES DISEASE IN THE NORTH

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Objective: In order to determine if there is a major susceptibility locus on the long arm of chromosome 20 for Chinese familial Graves' disease (GD), a linkage analysis was performed on a dataset of 56 multiplex GD families from the north part of China. **Methods:** Seven highly polymorphic microsatellite markers spanning the whole area of chromosome 20q were used. Microsatellite markers amplified with fluorescent-labled primers were separated on a MegaBASE 1000 DNA sequencer. Genotyping was performed using Genetic Profiler1.1 software. Tow-point Lod scores were computed using Linkage software, assuming both dominant and recessive models. For each model, three levels of penetrance were tested (30%, 60%, and 90%). All linkage analyses were performed assuming a population disease prevalence of 1%. Multipoint Lod scores and NPL scores were computed using the GeneHunter program. An affected-only analysis was also performed on the dataset. **Results:** Two-point Lod scores ($\theta=0$) and multipoint Lod scores of less than –2 were observed for all the markers studied, at all levels of penetrance, in both the dominant and recessive modes of inheritance. P values obtained for each NPL score were all larger than 0.05. Similar results were also obtained for the affected–only analyses. **Conclusion:** We obtained evidence against linkage for all the markers studied, consequently we can say that there was not a major susceptibility locus on chromosome 20q for Chinese familial GD.

P99 GENETIC MAPPING FOR THYROTOXIC PERIODIC PARALYSIS

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TPP is a complication of thyrotoxicosis affecting 25% of thyrotoxic southern Chinese male but not female patients. Paralysis due to hypokalemia typically occurs after a high carbohydrate meal. This episodic paralysis will remit with the control of thyrotoxicosis but may recur with relapse of the disease. Shifting of potassium into intracellular space results in hypokalemia and paralysis. Increased Na, K-ATPase activity has been observed in TPP patients when compared to thyrotoxic Graves' disease (GD) patients and normal controls. To study the association of Na, K-ATPase genotype with TPP, we evaluated TPP patients (n = 100) and compared them to male GD patients without TPP (n = 60) and normal male subjects (n = 70). A set of polymorphic microsatellite markers with a genetic distance < 10 cM was used to study the allelic distribution. Our results showed that there was no significant difference between the 3 groups in the allelic distribution for all markers associated with the Na, K-ATPase $\alpha 1$ (1p13), $\alpha 2$ (1q21-23), $\alpha 3$ (19q13.2) and $\beta 1$ (1q22-25), $\beta 2$ (17p13.1), $\beta 3$ (3q22-23) genes. There was also no association of CACNL1A3 (dihydropyridine sensitive calcium channel receptor gene, located at chromosome 1q32) with TPP. Mutation of CACNL1A3 gene is linked to Familial Hypokalemia Periodic Paralysis in Caucasians. On screening the X-chromosome, one of the alleles (size 289) of the marker DXS1214 in the region of Xp11.3-21 was observed to be more frequent in TPP subjects when compared to GD patients and normal subjects ($p < 0.05$), suggesting that this locus may be linked to TPP.

P100 The Function of Fas, Fasl, Bcl-2 in the Pathogenesis of Autoimmune Thyroid Disease

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Objective To investigate the expression and function of apoptosis-related protein: Fas, FasL, and Bcl-2 in the pathogenesis of autoimmune thyroiditis. **Methods** Immunohistochemical staining was performed on 20 thyroid follicular adenoma, 20 Hashimoto's thyroiditis(HT), and 20 graves' disease(GD). **Results** All the cases expressed fas, mainly on the cell surface and cytoplasm. FasL was found in all except one of the thyroid adenoma(TA). Thyroid follicles in HT samples exhibited strong staining for Fas ,in contrast to GD and TA that exhibited moderate Fas. Immunostaining for FasL was high in thyroid follicles but weak in infiltrating lymphocytes(ILC) of HT, moderate in all of GD, and minimal or no in TA. While Bcl-2 was only expressed 15 in HT,14 in GD, and 12 in TA. In HT, ILC stained strongly for Bcl-2 and weakly for FasL, in contrast to follicular cells stained weakly for Bcl-2 and strongly for FasL.In GD, Bcl-2 was moderate to strong similarly in follicular cells and ILC. And, it was weak to moderate in TA. **Conclusion** The expression of Fas , FasL ,Bcl-2 in Hashimoto's thyroiditis and graves' disease was nearly similar . FasL and Fas strong expression and Bcl-2 weak expression on the follicles in HT may induce apoptosis. These results provided further proof of the function of Fas and its ligand and Bcl-2 in the pathogenesis of autoimmune thyroid disease. The lymphocytes do not seem to be directly engaged in the process with their own FasL, but they may provide some cytokines that , in turn , up-regulates Fas and/or FasL leading to apoptosis.

P101 Effect of Interleukin-1, Interleukin-6 and Tumor necrosis factor- α on Apoptosis in Cultured thyrocytes from patients with Graves' disease

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Objective: To investigate the influence of cytokines on apoptosis in thyrocytes from patients with Graves'disease(GD) and study their functions in the pathogenesis of autoimmune thyroid diseases. **Methods:** Thyrocytes from GD were cultured in the absence or presence of $1\sim10^2$u/ml Interleukin-1(IL-1), Interleukin-6(IL-6) and Tumor necrosis factor- α (TNF- α). Apoptosis, Fas/sFas expression and Fas/sFas mRNA level in thyrocytes were detected by flow cytometry, ELISA and semi-quantitative RT-PCR. **Results:** (1) IL-1, IL-6 and TNF- α could significantly increased apoptosis($P<0.01$); (2) Fas expression and Fas mRNA level in GD thyrocytes were markedly increased by IL-1, IL-6 and TNF- α ($P<0.05$); (3)sFas expression and sFas mRNA level were also markedly increased by IL-1, IL-6 and TNF- α ($P<0.01$). **Conclusion:** IL-1, IL-6 and TNF- α could induce apoptosis in GD thyrocytes in vitro, suggesting that cytokines might participate in the regulation of thyroid homeostasis through Fas-induced apoptosis.

P102 THE EXPRESSION OF HUMAN THYROID PEROXIDASE EPITOPES GENE IN *E. COLI* AND THE PRELIMINARY CLINICAL APPLICATION

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Objective: To express hTPO epitopes gene and apply the product in clinical assay. **Methods:** hTPO epitopes gene was inserted into pGEX-4T-3 . The recombined expression plasmids were transformed into *E.coli* BL21 after valid linkage was verified by sequencing. Expression of hTPO epitopes gene was induced by IPTG. the product (fusion protein GST-hTPO) was purified and its immunoactivity was demonstrated . ELISA using GST-hTPO as antigen was established. In the same 10 AITD patients , Sera TPOAb were determined and HLA-DR antigen、dendritic cells and lymphacytes in the thyroid gland tissue were observed. **Results:** GST-hTPO acquired from procaryotic expression and purification possesses high purity and good immunoactivity .The CVs of ELISA established with GST-hTPO were between 5.93%~7.59%. A significant positive correlation was found between the data of TPOAb and TMAb level determined respectively by ELISA and RIA method. TPOAb level and the distribution of HLA-DR antigen and dendritic cells had the same ascending tendency following the aggravation of lymphacyte infiltration . **conclusion :** GST-hTPO, Products of genetic engineering , can be used to establish clinical assay of TPOAb . The correlation between the level of serum TPOAb and the detriment of thyroid tissue is showed.

P103 The Cloning of Extracellular Domain I, II,III gene of Intracellular Adhesion Molecule 1 and Expression in *E.coli* and the Preliminary Application

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Objective: To express the recombinant extracellular domain I, II,III of ICAM-1 in *E.coli* for preparing polyclonal antibody and establish the RIA of sICAM-1and preliminary application. **Methods:** The extracellular domain I, II, III gene of ICAM-1 was cloned into pUC18 and then subcloned into expressive vectors of pET28b,pET42a,pGEX-4T-3 separately. The best expressive vector was screened and expressive conditions were optimized. The recombinant ICAM-1(GST-ICAM-1) was purified and identified. The rabbits were immunized by GST-ICAM-1 and the affinity and titer of the antibody were identified. sICAM-1RIA was established and the serum sICAM-1 of 30 normal peoples and 35 Graves' disease(GD) patients were measured. **Results:** The recombinant pET42a was the best expressive vector and transformed into the host of *E.coli* BL21 (DE3), induced for 6 hours at 20°C by 0.5% lactose. The production rate of GST-ICAM-1 was 41.61% compare to total protein of *E.coli* BL21 (DE3) and 35mg/L LB media. The GST-ICAM-1 had the immune activity and molecule weight was 66.37kD. Affinity of ICAM-1 antibody was K= $0.43x10^9$ L/mol. Titer was 1:12000.The serum level of sICAM-1 in GD group (251.28±109.82 ng/ml) was higher than normal group(161.59±41.03ng/ml) (P<0.05) . **Conclusion:** We have established sICAM-1 RIA successfully and firstly by the antibodies prepared with GST-ICAM-1.

P104 DETECTION OF PROLACTIN mRNA AND PROLACTIN-LIKE SUBSTANCE IN THYROID GLANDS FROM PATIENTS WITH GRAVES' DISEASE

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Objective: To investigate local production of prolactin (PRL), an important immunomodulatory hormone, in thyroid glands from patients with Graves' disease (GD). **Methods:** The expression of PRL mRNA was examined by nonisotopic *in situ* hybridization in 6 GD thyroid glands, 5 control specimens (including 3 multinodular goiters and 2 samples of normal thyroid tissue adjacent to adenomas), intrathyroidal mononuclear cells isolated from 5 GD thyroid glands and primary thyroid follicular cell (TFC) cultures derived from 2 of the 5 GD glands. The presence of PRL-like substance was examined by immunohistochemistry in 7 GD thyroid glands and the 5 control specimens. **Results:** The expression of PRL mRNA was detected in 5 of the 6 GD thyroid glands studied. It was localized in infiltrating mononuclear cells and vascular endothelial cells adjacent to mononuclear cell infiltrates, but not in TFC. $(9.8\pm3.3)\%$ $(\bar{x}\pm s)$ of intrathyroidal mononuclear cells isolated from GD glands were found containing PRL mRNA. It was absent in the primary TFC cultures. Immunoreactive PRL was detectable in all 7 GD thyroid specimens studied and mainly distributed in infiltrating mononuclear cells and vascular endothelial cells adjacent to the infiltrates. It was also present in both TFC nearby the infiltrates and the local connective tissue. Neither PRL mRNA nor PRL-like substance was detected in any of the control thyroid specimens studied. **Conclusion:** PRL-like substance can be locally produced in GD thyroid tissue, which may play an important role in GD pathogenesis by autocrine and paracrine mechanisms.

P105 INTERLEUKIN-18 EXPRESSION IN THE THYROCYTES OF HASHIMOTO'S THYROIDITIS

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Objectives: To investigate whether interleukin-18 (IL-18), a novel pro-inflammatory cytokine, is expressed and localized in the thyrocytes of Hashimoto's thyroiditis (HT). **Methods:** Thyroid tissues were obtained from six patients with HT and six patients with subacute thyroiditis (ST) by using automatic biopsy device which was employed for pathological diagnosis. Control thyroid tissues were from six patients laryngectomized due to having laryngocarcinoma. Immunohistochemical staining and reverse transcription-polymerase chain reaction (RT-PCR) were used to detect IL-18 expression. **Results:** IL-18 expression was barely detectable in normal thyroid tissues. However, IL-18 expression was more abundant in the thyroid tissues of HT group than in those of ST group. The expression was mainly localized in thyroid follicular cells from HT patients and in the mononuclear-macrophages within sarcoid from ST patients. **Conclusion:** Our data demonstrate for the first time that IL-18 is expressed in the thyrocytes of HT. IL-18 may play an important role in the pathogenesis of HT.

P106 EXPRESSION OF TRAIL, TRAILR1 AND TRAILR2 IN HASHIMOTO'S THYROIDITIS

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Objective: To investigate the significence of expression of TNF-related apoptosis-inducing ligand(TRAIL),TNF-related apoptosis-inducing ligand receptor 1 (TRAILR1) and TNF-related apoptosis-inducing ligand receptor 2 (TRAILR2) in the pathogenesis and pathological changes in Hashimoto's thyroiditis(HT). **Methods:** Expression and distribution of TRAIL,TRAILR1 and TRAILR2 in thyroid tissues from 18 patients with HT were investigated by immunohistochemical and in situ quantification methods. **Results:** The relative amount of positive immunostaining for TRAIL,TRAILR1 and TRAILR2 in thyrocytes from HT and NTG patients were 0.0951±0.0185 and 0.0453±0. 0142, 0.0799±0.0195 and 0.0414±0.0199, 0.0841±0.0150 and 0.0421±0.0165, respectively. The relative amount of positive immunostaining for TRAIL,TRAILR1 and TRAILR2 in thyrocytes from HT patients were siginificantly higher than those from the nontoxic goiter(NTG) patients(P<0.05). Strongly positive immunostained thyrocytes of HT for TRAIL ,TRAILR1 and TRAILR2 mainly distributed in follicles adjancent to lymphocytic infiltrates. Staining of infiltrating lymphocytes for TRAIL, TRAILR1 and TRAILR2 was found to be rather weak or even negative. **Conclusion:** High characteristic expression of TRAIL ,TRAILR1 and TRAILR2 in thyrocytes from HT is observed. The immunostaining of infiltrating lymphocytes for TRAIL and TRAILR is rather weak or negative. Expression of TRAIL ,TRAILR1 and TRAILR2 by thyrocytes in HT may lead to their destruction through apoptosis .

P107 EFFECT OF TSH RECEPTOR ANTIBODIES ON H_2O_2 GENERATION IN HUMAN THYROID CELL

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Objective: to investigate biochemical mechanism of TRAb stimulating thyroid hormones (TH)synthesis in Graves' disease. **Methods**: Graves' immunoglobulins G(GIgG) was isolated from the sera of Graves' patients who were positive for TRAb by precipitation with 20%PEG. Generation of H_2O_2 (GH)in the cell culture was measured with homovanillic acid fluorescence assay. **Results**: 1. Stimulation of GIgG for 15 min, GH in thyrocytes began to increase, GH was gradually increased further for 30 and 45 min, ,and reached maximum at 45 min. Stimulation for 0、15、30、45、60 and 90min by GIgG, GH(nmol/min·mg protein) was 17.8±4.2、28.2±6.2、32.3±6.7、 38.6±6.9、 35.7±8.1 and 34.8±7.5，respectively. IgG obtained from healthy subjects did not stimulate GH. 2. The doses of GIgG from 0.1 to 2mg/ml, GH in thyrocytes was increased with the dose， at dose of 2mg/ml, stimulatory action of GIgG reached maximum. 3. PKA inhibitor and intracellular Ca^{++} chelator BAPTA-AM decreased GH 26.2% and 87.2% stimulated by GIgG, respectively, while tyrosine protein kinase inhibitor genistein had no effect on stimulation of GH by GIgG. **Conclusion**: H_2O_2 play a key role in TH biosynthesis, TRAb can stimulate GH in thyrocyte, it suggests that stimulation of GH may be an important biochemical mechanism for TRAb stimulating TH synthesis. blockage of phospholipid- Ca^{++} pathway may block main pathogenic action of TRAb.

P108 INDUCTION OF GRAVES' DISEASE WITH THYROTROPIN RECEPTOR EXPRESSING EUKARYOTIC CELLS IN IFN-γ AND TNF-α DEFICIENT MICE

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Objective: To explore the role of Th1/Th2 cytokine unbalance in the pathogenesis of autoimmune thyroid diseases. **Methods:** IFN-γ and TNF-α deficient (KO) mice from a BALB/c background were immunized with the TSH receptor expressing cells, DAP3.WT. 1-2x10^7 DAP3.WT cells were peritoneally injected into mice and repeated per two weeks for total six times. Two weeks after final immunization, mice were killed for total thyroxine, TRAb and pathological examination. **Results:** The immunized wild type control mice expressed higher levels of TRAb (10.38±5.82 vs 2.8±3.9) and mild hyperthyroxinemia (3.37±0.65 vs 2.49±0.53μg/dl), whereas the IFN-γ KO mice expressed lower levels of TRAb only (5.31±2.58). The levels of TRAb (8.11±3.65) in the TNF-α KO mice were lower than those in the immunized wild type control mice, but higher than those in the IFN-γ KO mice. Pathological studies of the thyroids showed that there was some hypertrophy of the thyroid epithelium and colloid absorption in the follicles in the immunized wild type mice, but not in the IFN-γ KO mice or TNF-α KO mice. **Conclusion:** These results suggest that IFN-γ, and to a certain extent TNF-α, plays a critical role in the pathogenesis of Graves' disease.

P109 EFFECT OF DIFFERENT SOLVENT ON THE ESTABLISHMENT OF ANIMAL MODEL OF GRAVES' DISEASE BY GENETIC IMMUNIZATION

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Objective: To compare the effect of different solvent on the establishment of animal model of Graves'disease by genetie immunization. **Methods:** Three groups of eight-week female C3H mice were used (A group with 100 μ g PCR™3-hTSHR cDNA in 25% sucrose, B group with 100 μ g PCR™3-hTSHR cDNA in 1 X PBS, C group with 25% sucrose alone). Injections were repeated at third and sixth week. Blood were obtained at 9th week and 11th week for T_4 and TSAb assay. Thyroids were removed for histological examination. Lymphocytes were separated from blood and spleen for CD4+, CD8+ T cell analysis. ^{3}H-TdR incorporation was also carried out to test the proliferation of T cells. **Results:** 6/13 mice at 9th week in A group, while 2/11 mice at 9th week in B group, developed mild hyperthyroidism with elevated T_4 levels, positive TSAb and thyroid hypertrophy. 4/13 mice at 11th week in A group developed mild hyperthyroidism, but no mice at 11th week in B group developed hyperthyroidism. CD4+ T cells increased and CD8+ T cells changed little in group A and B comparing to group C. Furthermore, CD4+ T cells increased more obviously in group A than in group B. ^{3}H-TdR incorporation indicated T cell proliferation in experimental group was higher than that of controls. In addition, in group A had more obvious proliferation than in group B. **Conclusion**: It's more effective to immunize mice with PCR™3-hTSHR cDNA in 25% sucrose than in PBS on establishment of Graves' diseses model.

P110 ESTABLISHMENT A CELL LINE CO-EXPRESSING MHC CLASS II MOLECULES AND HTSHR

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Objective Graves' disease (GD) is an organ-specific autoimmune disease. The immunological mechanisms leading to GD are not yet fully understood. The pathogenesis of GD is thought to be associated with HLA class Ⅱ molecules expression by human epithelial thyroid cell in GD. Using electroporation , we established a stable cell line co-expressing MHC class Ⅱ molecules and hTSHR, named hM12,for study the pathogenesis of GD.**Methods** M12 cells were transfected with pCR™3-hTSHR plasmids using electroporation. Cells were selected for neomycin resistance using G418, and positive cells were cloned by limiting dilution. Stable transfectants were selected by flow cytometry, RT-PCR and their ability to increase cAMP levels in the presence of bTSH and crude IgG of GD. **Results** Using limiting dilution, fourteen cell lines were obtained. Flow cytometry analysis showed that No.3、5、6、8、11 cells lines expressed hTSHR, which can bind with TRAb of GD. PCR products proved that hTSHR mRNA were transcribed in No.3、5、6、8、11 cell lines. Cell lines were stimulated by crude IgGs of GD and bTSH, cAMP increased significantly in culture supernatants of No.6 and No.8cells lines, it suggest that the hTSHR expressing on the No.6 and No.8 cell lines have the ability of native hTSHR. **Conclusion** We successfully established the cell lines that co-express MHC class Ⅱ molecules and hTSHR.

P111 PRIMARY INVESTIGATION OF THE SYNDROME OF INCREASED THYROID HORMONE AUTOANTIBODIES

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Objective: To study the methods of laboratory diagnosis and the effects of L-T_4 and/or MMF on the syndrome of increased-THAAb. **Methods:** 6 cases of increased-THAAb were selected, and 53 subjects with autoimmune thyroid disease (AITD) and 60 healthy subjects as control. The serum T_3Ab titer and T_4Ab titer are measured with radioimmunoprecipitation. Radiolabeled thyroid hormone is incubated with the patient's serum. The immune complexes are then precipitated with PEG and the radioactivity of the precipitate is determined as a proportion of the total added radioactive label. The results are expressed as the binding percentage of radiolabeled hormone (bound/total tracer%). After analyzed by RIA and ECLIA respectively, the serum FT_3 and FT_4 concentrations from a singal serum are compared. Two patients have been followed up for a long term. **Results:** The T_3Ab titer and T_4Ab titer were 0.718±0.225 and 0.652±0.213 in increased-THAAb group respectively, 0.034±0.004and 0.036±0.005 in group of AITD, 0.033±0.004 and 0.037±0.005 in healthy group. The THAAb titer was significantly higher in increased-THAAb group than that in AITD or healthy groups ($P<0.05$). In method of using a solid-phase radioimmune system, the presence of THAAb cause spurious elevation of the free thyroid hormones than the results measured by ECLIA or PEG-RIA. The goiter disappeared and the clinical condition was improved after treatment with L-T_4 and/or MMF, but the autoantibody titer was not significantly reduced. **Conclusions:** The method of ECLIA combined with RIA is very useful for screening the patients suspected of having increased-THAAb. Radioimmunoprecipitation is a rapid and effective way to identify and eliminate the interference from THAAb. Approximately accurate values of the thyroid hormones are manifested through measurement of the serum, treated with PEG to eliminate THAAb. The medicine such as L-T_4 and/or MMF (mycophenolate mofetil), which partially improved the clinical manifestations and some biochemical indexes, may not resolve the problem of increased-THAAb from mechanism by which autoantibodies happened.

P112 THE CHANGE OF URINARY DEXYOBIDININE IN HYPEERTHYROID PATIENTS

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Objective: To study the changes of new bone metabolic index in hyperthyroid patients. **Methods:** 44 hyperthyroid patients, compared with 38 healthy controls having similar age and gender are under investigated. Serum calcium, phosphate, total serum alkaline phosphatase (ALP) were measured by BECKMAN automatic analyzer, osteocalcin (OC) and urinary dexyobidinine (DPD) were measured by RIA, and ELISA respectively. **Results:** Serum calcium concentrations did not differ significantly between the patients and controls($p=0.07$). Concentrations of serum phosphate, ALP and OC were significantly higher than those of controls ($p<0.01$, $p<0.01$, $p<0.01$ respectively). Urinary DPD concentrations were also significantly higher than those of controls ($p=0.0001$) and DPD were not significantly associated with age and duration. For serum ALP, OC and DPD, hyperthyroid patients showed a statistically significant increase of about 87%, 110% and 628% respectively.**Conclusion:** Hyperthyroid patients have higher bone metabolism resulting from high level thyroid hormones and accelerated bone loss are higher than accelerated bone formation. DPD is a sensitivity biochemical index of bone resorption.

P113 A PATHOLOGICAL STUDY ON CD44 AND E-CD IN AUTOIMMUNE THYROID DISEASE

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Objective: To investigate the effects of intercellular adhesion molecules CD44 and Epithelial Cadherin (E-CD) in the generation and development of Graves disease (GD) and Hashimoto' s Thyroiditis (HT). **METHOD:** A morphological study was performed on the various types of thyroid tissue of 53 GD and 52 HT of CD44, E-CD expression by histochemical and immunohistochemical techniques. **RESULLTS:** The observation on the number of CD44 positive immunoactive cell and CD44and E-CD positive follicular epithelium showed a same increase with degree of cellular infiltration from GD to HT. The highest peak of the former in P-type of HT, the latter in O-typpe of HT and showed descendent tendency in P-type. The positive expression of CD44 were distriduted on the cell membrane in GD, on the cytoplasm in HT; E-CD were distributed on the cytoplasm in GD and HT. **CONCLUSION:** The expression of intercellular adhesion molecular (CD44and E-CD) on the increase promote the contact between immunoactive cell and follicular cell——the fusion of cell membrane. These were advantageous in release of immunoactive material and being on "antibody-dependent cell-mediated cytotoxicity". The simulating follicular epithelium cell change from increase (hyperthyroidism in GD) to degeneration,atrophy and destruction (hypothyroidism in HT).

P114 THE ROLE OF VEGF AND cNOS mRNA IN THE ANGIOGENESIS OF AUTOIMMUNE THYROID DISEASE

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Objective: To investigate the role of VEGF and cNOS mRNA in angiogenesis of autoimmune thyroid disease (AITD). **Methods:** Human thyroid tissue of 45 Graves' disease(GD),41 Hashimoto`s thyroiditis(HT),14 HT complicated with papillary carcinomas and 10 normal thyroid tissues were obtained. The expression of VEGF and its receptor KDR were detected with S-P immunohistochemical method; in situ hybridization was used to observe the expression of cNOS mRNA;and CD34 immunostaining was used to measure the microvascular density (MVD).**Results:** The expression of VEGF in thyroid epithelial cells (TEC) was positively related to the MVD in GD,HT and HT complicated with papillary carcinomas ($P<0.05$). cNOS mRNA expression of GD,HT and HT complicated with papillary carcinoma was observed in the TEC, vascular endothelial cells and mononuclear phagocytes, and was higher than in normal thyroid. The expression of cNOS mRNA in endothelial cells was positively related to the MVD in GD and HT($P<0.05$).And the expression of VEGF in thyroid tissue was positively related to the expression of cNOS mRNA in endothelial ($P<0.05$). The MVD in the cases with positive expression of VEGF and cNOS was higher than in those with negative expression ($P<0.05$). **Conclusion:** It suggests that in AITD, the expression of VEGF and cNOS are associated with angiogenesis in thyroid. cNOS maybe mediate VEGF-induced angiogenesis.

P115 HYPERTENSION AND OBESITY IN DIABETIC AND NORMAL SUBJECTS

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Objective: As hypertension and obesity are the main risk factors in the development of cardiovascular diseases, their status were studied in normal and diabetic subjects residing in Islamshahr, 30 Km to the southwest of Tehran. **Methods:** 156 subjects who were recognized as diabetic in an earlier epidemiological survey along with 150 normal subjects were selected by random sampling. Height and weight were measured and BMI calculated. Waist to hip ratio was measured as the abdominal obesity indicator. **Results:** Out of 306 subjects 61% were females and 39% males, aged 31 t0 97 (47±11) years. WHR in diabetic and normal subjects was 0.98±0.07 and 0.87±0.07, respectively (P<0.01). There were no significant differences in mean ±SD BMI between the 2 groups. Systolic & diastolic BP in diabetic were higher than normal subjects (133±23 vs. 116±20 mmHg, P<0.001 and 79±13 vs. 68±13 mmHg, P<0.001) respectively. Eighty-nine percent of diabetic and 78% of normal subjects were moderately to severely obese. Diastolic pressure of over 90 mmHg was 25% in diabetic and 8% in normal subjects. 39% of the diabetic and 15% of normal subjects had systolic pressure over 140 mmHg. General obesity and abdominal obesity indicators were more pronounced in males than in females. There were no gender differences in systolic & diastolic BP. **Conclusion:** These findings confirm that obesity and hypertension are associated with diabetes in the community and it is recommended that they be remedied through the improvement of food habits and physical activity by educating the people, in order to prevent cardiovascular disorders.

P116 A STUDY ON RELATIONSHIP OF BODY FAT DISTRIBUTION TO INSULIN RESISTANCE, TYPE 2 DIABETES MELLITUS AND ESSENTIAL HYPERTENSION

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Objective: To investigate The relationship of body fat distribution to insulin resistance, type 2 diabetes mellitus and Essential Hypertension. **Methods:** By measurement of plasma insulin and C-peptide, and taking waist-to-hip ratio (WHR) as the index of body fat distribution, we studied 71 subjects divided into 5 groups. The groups of type 2 diabetes mellitus, hypertensive type 2 diabetes melltus, primary obesity, and hypertensive obesity consisted of 15 patients each, The remaining 11 subjects were controls. **Results:** 1 WHR in the hypertensive type 2 diabetes mellitus group was significantly higher than the type 2 diabetes melltus group, and that in the hypertensive obesities significantly higher than the primary obesities(P<0.05). 2. Insulin level and C-peptide during OGTT was the highest in the hypertensive obesities, high in the primary obesities, medium in the hypertensive type 2 diabetes mellitus group, low in the type 2 diabetes mellitus group, and lowest in the controls. Significant differences existed between every two groups(P<0.05). 3. There was no significant difference between every two groups of the ratio of insulin area to C-peptede area. 4. Last, upon glucose olad. The two groups with hypertension increased significantly in their average aterial blood pressure, while this did not happen in the other two groups. **Conclusion:** 1. Hypertensive obesities and hypertensive type 2 diabetes mellitus patients may have more severe IR. 2. An elevation in WHR was probably correlated to the decrease in the clearance of insulin by the liver. 3. Blood pressure regulations may be more sensitive to glucose load in the hypertensive groups than the non-hypertensive groups.

Key words: Body Fat Distribution, IR; NIDDM; Essential Hypertension; C-peptide

P117 THE STUDY OF RELATIONSHIP BETWEEN INSULIN RESISTANCE AND PLASMA vWF IN OBESITY

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Abstracts: Insulin resistance and endothelial dysfunction have been fond in obesity subjects. Moreover, endothelial dysfunction is associated with insulin resistance. Increases in plasma Von Willebrand factor (vWF) thought to a predictive marker of endothelial dysfunction have been reported in vascular diseases. **Objective**: To investigate the changes of body fat parameter, metabolic parameter, insulin resistance and vWF in plasma in obesity.To explore associating between endothelial repair and insulin resistance in obesity. **Methods:** vWF, BMI, waist, WHR, BP, lipid metabolic parameters, fasting insulin concentrations and insulin resistance index were analyzed in 59 obese (BMI≥25kg/m^2,AGE 65.0-20.4 year)subjects and 49 normal(BMI18.5-23kg/m^2, AGE 58.6-20.4year)controls. Insulin resistance was estimated by homeostasis model assessment (HOMA-IR). vWF was measured by ELISA. **Results:** Compared with control group, the obese subjects had a higher W, H, WHR ,BP, fasting insulin level and HOMA-IR value ($p<0.001$). The obese subjects had a higher vWF level than control ($P<0.05$). **Conclusion:** There were insulin resistance, abnormal body fat distribution, change of blood pressure and metabolic disorder in obese subjects. The increase of plasma vWF level in obese subjects indicated endothelium repair.

P118 THE ASSOCIATION BETWEEN SERUM TNF-α, LEPTIN LEVELS AND OBESITY

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Objective: To investigate the relationship between serum TNF-α, leptin levels and obesity. **Methods:** 60 DM patients were investigated and blood sampled at fasting and after taking 100g wheat flour 1 and 2 hours, 63 individuals as control group were given 75g glucose.7 parameters including BP, BMI, waist/hip ratio, lipid level, serum TNF-α, leptin and IRI were investigated. These two groups were further divided into subgroups in accordance with BMI. Analysis of correlation, Logistic regression and t-test were used between groups. **Results:** There were statistically significant difference of serum TNF-α and leptin in obesity and non-obesity patients (TNF-α 5.55± 0.69 pg/ml vs. 2.49 pg/ml±0.32pg/ml, $p<0.01$, leptin 12.33±1.54 ng/ml vs. 5.26±0.67 ng/ml, $p<0.01$). Serum leptin levels were significantly higher in female than in male(12.35±.0.91ng/ml vs. 4.54± 0.52ng/ml, $p<0.01$). There were correlation between TNF-α and IRI (fasting $r=0.43$, $p<0.01$; 1h $r=0.30$, $p<0.01$; 2h $r=0.35$, $p<0.01$), waist/hip ratio ($r=0.50$, $p<0.01$), cholesterol ($r=0.34$, $p<0.01$) respectively. A significant relationship between serum TNFα and leptin levels ($r=0.46$, $p<0.01$) was also found, but no difference of TNF-α and leptin in DM and non-DM patients. **Conclusions:** It suggests that serum TNF-α and leptin levels are correlated to obesity and distribution of body fat. TNF-α maybe directly act on the fat tissue and modulate the release of leptin. The intrinsic induction of insulin resistance may be due to the high levels of TNF-α and leptin..

P119 THE SERUM LEPTIN LEVELS AND THE EFFECT OF POLYMORPHISM OF PPARγ_2 (161 C→T) GENE ON SERUM LEPTIN LEVELS AND OTHER METABOLISM ITEMS IN SUBJECTS WITH DIFFERENT GLUCOSE TOLERANCE

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Objective: To study the serum leptin levels and the effect of polymorphisms of PPARγ_2 (161 C→T) gene on the metabolism items in subjects with different glucose tolerance. **Methods:** 218 subjects were divided into 3 groups(NGT, IGT and DM) according to their 75g OGTT, and divided into subgroups as obese and nonobese, male and female. The polymorphisms of PPARγ_2 (161 C→T) were analyzed by PCR-RFLP. The body weight, BMI, waist to hip ratio, blood pressure, FBS, CH, TG ,HDL, leptin, fasting insulin and HOMA IR were measured in all subjects. **Results:** The serum leptin was higher significantly in obese than nonobese in males and females of NGT. The serum leptin was not different among NGT, IGT, DM of non-obese men($p=0.109$),but in non-obese women, the leptin was 7.24(5.04,8.99), 7.81(5.15,10.22), 11.03 (7.07,14.34)ng/ml, $p<0.05$. By stepwise regression analysis, the BMI, fasting insulin in men, and BMI, fasting insulin ,TG in women were the independent affecting factors of serum leptin. In males,161T carriers of PPARγ_2 had higher BMI and insulin in NGT,IGT and DM. Female carriers had higher BMI in NGT,IGT and DM, higher leptin in NGT and IGT. **Conclusions:** 1.The affecting factors of human serum leptin levels included sex, body weight, BMI and insulin.2.In females, serum leptin levels was different among different glucose tolerance. 3.The gene polymorphism of PPARγ_2 (161 C→T) was related with increased BMI and serum leptin.

P120 LEPTIN, PROINSULIN, TRUE INSULIN AND OTHER METABOLIC PARAMETERS IN NON-DM SUBJECTS BASED ON POPULATION STUDY

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Objective: To assess the correlation of leptin with true insulin (TI), proinsulin(PI) and impaired insulin secretion (Higher fasting PI/TI ratios) and with insulin resistance (the HOMA model) and other metabolic parameters in non-diabetic subjects. **Methods:** All 965 non-diabetic subjects from a population of Beijing resident were studied and fasting blood sampled. The parameters measured include body mass index (BMI), waist and hip circumference, blood pressure (BP), plasma glucose, serum lipids, TI, PI and leptin concentrations. Leptin, PI and TI levels were detected by our sensitive and specific ELISAs. **Results**: Fasting leptin levels showed good correlation to BMI, WHR, BP and also triglyceride (all $p<0.01$). Leptin levels were significantly correlated with fasting TI ($r=0.50$, $P<0.001$) and PI ($r=0.47$, $P<0.001$) but inversely weakly with the PI/insulin ratio ($r= -0.15$, $P=0.055$) after adjustment for age, gender, and glucose. After further adjustment for BMI and waist-to-hip ratio (WHR), leptin levels remained significantly correlated with TI and PI, but with HOMA-R and BP in men (all $p<0.01$). **Conclusions:** TI and PI levels are positively related to leptin levels to some degree independent of obesity. Thus, subjects with increased insulin levels may be relatively resistant to the effects of leptin and a dysfunction of leptin-insulin axis. Leptin levels are not significantly associated with fast PI/TI ratio suggesting no relationship to impairment of insulin secretion. However, leptin associating with the markers of metabolic abnormalities suggested it may play a role in the etiology of the Metabolic Syndrome.

P121 The Relationship between Serum Leptin and Obesity

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Objective: to observe the relationship between leptin and BMI, insulin, lipid and so on in obese and non-obesity subjects complicated by and various disease.**Methods:**157 subjects were divided into obesity group (BMI≥25)and non-obesity group.(BMI<25). The FPG, leptin, CHO, TG, HDL, LDL, insulin and insulin sensitive index were determined, also the body weight and high. **Results:** The level of leptin in obesity group was higher then non-obesity group significantly. Leptin was related to BMI, SBP, DBP, CHO, LDL, TG, insulin and insulin sensitive index. In diabetes and IGT group, the serum leptin was higher in obesity group than in non-obesity group. It was positive correlation to HDL and insulin, but negative correlation to insulin sensitive index in obesity group. In non-obesity diabetic patients, the serum leptin was positive correlation to TG and LDL. There was no significant difference between control group in serum leptin. **Conclusion:** The level of serum leptin was affected by BMI, blood pressure, lipid metabolism, insulin and insulin sensitivity. The level of leptin was significantly higher in obesity then in non-obesity. It was related to BMI, There was no significant difference between various diseases in serum leptin.

P122 LOW PLASMA LEPTIN LEVEL IN PATIENTS WITH LYMPHOPROLIFERATIVE DISEASE.

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It has been reported that leptin – a protein secreted by adipocytes plays not only role in body weight regulation but is also involved in the enigmatic mechanism of immunity and hemopoesis. It has been shown that decrease of leptin level appears at leukopenia phase undergoing peripheral blood stem cell mobilization as well as is lower in bone marrow in patients with limphoproliferative diseases (LD)

The aim of study was to evaluate plasma leptin level in LD

Material and methods: 30 patients with diagnosis the following diseases : B – CLL 15, MM –8, NHL – 5, HD – 2. Plasma leptin levels were measured using RIA method. For statistical analysis T – Student test was used.

Results: In all patients with LD plasma leptin concentration was decreased. The plasma leptin level in all causes LD was significantly lower in comparison with the control group. We compared: MM vs control $p< 0.01$, LLC vs control $p< 0.01$, NHL vs control $p <0.01$. Lower leptin concentrations were observed particularly in LLC even in stage O (RAI)

Conclusion: Our results indicate that serum leptin concentration in LD is significantly lower than in the control healthy group. The relationship between stage of disease and plasma leptin concentrations require further investigations.

P123 LEPTIN LEVEL DURING THE PREGNANCY AND ITS RELATIONSHIP TO THE NEONATES

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Objective: To elucidate the changes of serum leptin during the pregnancy in Chinese women and its relation to the neonate sex. **Methods:** 352 women from Ruijin Hospital Obstetric Clinic were included in our study, aged from 22 to 44 and were divided into 4 groups according the gestational age(<20 weeks, 20-23.9 weeks, 24-27.9 weeks and >=28 weeks). The serum leptin was determined with radioimmunoassay. **Results:** In the 4 groups of women during pregnancy, the serum leptin were 13.89±5.74, 14.16±6.61, 14.51±6.68 and 15.59±7.89 μg/L, respectively. They were all significantly higher than the non-pregnant control women (P <0.05), and the concentration increased while the pregnancy progressing. The leptin reached 21.22±10.06μg/L at the time of parturition, ant it was much higher than the level during pregnancy. The leptin level possitively correlated to the gestational age, the body mass index, waist circumference, height of uterus bottom, systolic blood pressure and diastolic blood pressure (P <0.05 or P <0.01). No difference was found between the leptin levels during pregnancy between pregnant women grouped by the neonate's sex retrospectively after parturition. The leptin levels were 21.22±10.06, 11.47±8.06 and 2.81± 2.48μg/L in the maternal blood, umbilical blood and amniotic fluid, respectively, and possitive correlation was found between maternal blood leptin level and that of amniotic fluid(P <0.05). **Conclusions:** Leptin may be an important hormone regulating the energy balance during pregnancy.

P124 SERUM LEPTIN, INSULIN AND PROINSULIN LEVELS IN OBESE CHILDREN

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Objective: To investigate the metabolism changes in obese children and the relationship between obese children and metabolism syndrome. **Methods:** 198 obese children (BMI≥25) and 67 normal children (BMI 18.8~24) were chosen out of 2217 middle school students of 14-16yrs. The levels of serum leptin, insulin, proinsulin, glucose and lipid of these children were measured. **Results:** There was significantly gender difference of serum leptin levels in children of 14-16 yrs. The serum leptin level was higher in female than that in male. 2) The levels of serum leptin, insulin and proinsulin levels of obese children were significantly higher than those of normal children. 3) The blood glucose and TG levels of obese children were significantly higher than those of normal children, but serum HDL-C levels of obese children were significantly lower than those of normal children. **Conclusion:** The results suggested that there were leptin resistance, insulin resistance, function defects of β-islet cells and potential disorders of blood glucose level and lipid metabolism in obese children. Our study indicated that children obesity mould be related with earlier occurrence of type 2 diabetes.

P125 RELATIONSHIP BETWEEN CORD SERUM LEPTIN AND NEWBORN DEVELOPMENT

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Objective: tigate the effecting of cord serum leptin level to the development of the newborn. **Methods:** Serum leptin levels of cord serum from 98 normal birth weight, 10 low birth weight 20 high birth weight and their mother serum were determined by radioimmunoassay. Body height, body weight, serum fasting insulin and lipids were also determined. And relationship between leptin with all these factors were analysised. **Results:** Cord serum leptin level in normal birth weight was 7.88±4.02ng/ml, it was lower in low birth weight 2.34±1.62ng/ml, and higher in big baby 10.72±3.46ng/ml. But no difference between their mothers. Cord serum leptin levels were positive correlated with their birth weight, BMI, fasting insulin and triglicyride as well as their mothers. Cord serum leptin levels were positive correlated with the placenta weight too. However no correlation were found between cord serum leptin and their mother's serum leptin levels. **Conclusion:** 1) Cord serum leptin level was lower in low birth weight and higher in big baby. 2) Cord serum leptin levels were positive correlated with their birth weight, and placenta weight. Suggesting that leptin made important role in newborn development. 3) Cord fasting serum insulin and triglicyride may regulate their serum leptin levels. 4) Cord serum leptin were independed on their mother's serum leptin levels.

P126 EFFICACY AND SAFETY OF ORLISTAT IN CHINESE OBESE PATIENTS

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Objective To test the clinical efficacy and safety of orlistat in Chinese obese patients .**Design** Randomized ,double-blinded ,placebo-controlled study .Obese adults (BMI 25-40Kg/m2) evaluated at six research centers were randomized to receive orlistat 120mg 3 times a day or placebo one tablet 3 times a day with a controlled-energy diet for 24 weeks .**Results**:For intent-to-treat analysis , 295 orlistat-treated subjects and 148 plaebo-treated subjects were evaluated .After 24 weeks ,orlistat-treated patients lost more weight (6.05 ± 0.21kg)than placebo-treated patients (3.00 ± 0.29kg) ($p<0.0001$) .Orlistat –treated patients showed significant decrease ($p<0.05$) in waist circumstance and in the serum levels of total cholesterol ,low-density lipoprotein cholesterol , fasting glucose , postprandial glucose and HbA1C in comparison with placebo-treated patients .After treatment ,the level of diastolic blood pressure in orlistat-treated patients was lower than that in placebo-treated patients significantly ($p<0.05$).Both groups had similar adverse-event profiles ,the incidence of gastrointestinal events of orlistat-treated patients was higher which were mostly mild and transient .There were no serious adverse events in both groups.**Conclusion** : Orlistat in conjunction with a low-energy diet ,produced greater significant weight loss than placebo during 24 weeks treatment .There was also an improvement in most serum lipid ,glucose and blood pressure parameters .Orlistat was well tolerated and offers a new approach to the management of obesity.

P127 THE TREATMENT OF JUVENILE FATTY PATIENTS WITH LOW-MEDIUM CALORIC DIET AND MODERATE ATHLETICS AND METFORMIN

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Objective: To search an effective and safety treatment for juvenile fatty patients. **Methods:** All 24 juvenile fatty patients were given a low-medium caloric diet (about 1200kcal day^{-1}) and moderate athletics (slow running 6km day^{-1}) and grouped into A and B randomly according to their sex. The patients of group A took metformin 0.25 tid at 1-8 week and placebo 1 tablet tid at 9-24 week; whereas the patients of group B took placebo 1 tablet tid at 1-16 week and metformin 0.25 tid at 17-24 week. We measured all patients' height and weight and waist and hip, counted their BMI and waist-hip ratio (WHR) every four weeks, checked their fasting plasma glycemia (FPG) and insulin (FIns) and triglyceride (TG) and total cholesterol (Tch) and uric accid (BUA) and γ -GT every eight weeks. And we compared all above indexes at every stage respectively in each group or between two groups with q-test or t-test of statistics. **Results:** The BMI and WHR and FIns and TG and Tch and BUA and γ -GT of all patients were decreased ($p<0.05$). And the decrements of BMI and WHR and FIns and TG and γ -GT in group A was more significant than those in group B at 1-8 week ($p<0.05$), and this could last to 16 week ($p<0.05$) and disappear at 17-24 week after combining with metformin in group B ($p>0.05$). **Conclusion:** Low-medium caloric diet and moderate athletics is effective in treating juvenile fatty patients, this can be enhanced by metformin.

P128 Variations of serum soluble adhesion molecules in obese patients after sibutrimine treatment

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Objective: To investigate the concentration of serum soluble adhesion molecules in simple obese patients, and their variations after sibutrimine treatment. **Methods:** In comparison with 15 control subjects, the serum concentrations of E-selectin, ICAM-1 and VCAM-1 of 37 obese patients were measured with ELISA before and after sibutrimine treatment. According to the degree of weight loss, obese patients were divided into effective-weight-loss group and non-effective-weight-loss group. **Results:** (1). At the beginning of the study, E-selectin, ICAM-1 and VCAM-1 were significantly higher in 37 obese patients (both in effective-weight-loss group and in non-effective-weight-loss group) than in control subjects (2). After sibutrimine treatment, serum E-selection in effective-weight-loss group decreased significantly ($P<0.05$), but no significant change of ICAM-1 and VCAM-1 was observed ($P>0.05$). (3). There was no varition of serum E-selection, ICAM-1 and VCAM-1 in non-effective-weight-loss group after sibutrimine treatment ($P>0.05$). **Conclusion:** In simple obese patients, serum soluble E-selectin, ICAM-1 and VCAM-1 are increased; E-selection decreases in the obese patients with critical weight loss after sibutrimine treatment, which suggests the weight loss after sibutrimine treatment might counteract endothelium activation .

P129 EFFECTS OF SIBUTRAMINE ON BODY WEIGHT, BODY FATMASS AND METABOLISMS OF BLOOD LIPIDS AND GLUCOSEIN PATIENTS WITH OBESITY

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Objective: To evaluate the efficiency and safety of domestic sibutramine (produced by Sichuan Mingri pharmaceutical Co.) in the treatment of simple obesity. **Methods**: Double-blind, double-placebo, randomized controlled, multi-center clinical trial was made. 359 subjects were divided into group A、B and C respectively. Sibutramine tablets or capsules were administered 10 – 20mg/day for 24 weeks. CT scan was used to measure the intra or subcutaneous-abdominal fat areas (IAFA, SAFA) at L4-L5 level. DEXA was used to measure total body fat mass (TBFM). **Results**: 315 subjects continued to be followed for 24 weeks. Open-placebo showed group A was sibutramine tablet (n=107), group B was placebo (n=104), Group C was sibutramine capsule (n=104). Body weight (BW) loss was 4.86 Kg (6.42%) and 4.68 Kg (6.38%) in group A and C; BMI decreased from 29.71 to 27.77 and from 30.02 to 28.11 in group A and C; WHR from 0.92 to 0.89 in group A, 0.91 to 0.88 in group C . SAFA decreased 7.30% and 7.45%, IAFA decreased 19.21% and 16.98%, TBFA decreased 13.94% and 15.02% in group A and C respectively. FPG decreased from 5.69 to 4.85 mmol/L and from 5.38 to 4.69 mmol/L in group A and C respectively. FIns decreased from 20.98 to 14.75 mu/L and from 21.11 to 14.68mu/L, 2hIns decreased from 70.9 to 44.11 mu/L and from 73.13 to 41.93 mu/L in group A and C respectively. Serum TG decreased from 1.98 to 1.73 mmol/L and 1.84 to 1.67 mmol/L, Tch decreased from 5.09 to 4.75 mmol/L and from 5.06 to 4.46 mmol/L, HDL-ch increased from 1.13 to 1.28 mmol/L and 1.18 to 1.31 mmol/L in group A and C respectively. All the above parameters changed significantly in group A and C, but not in group B. ADR was 32.41%, 13.47% and 31.20% in group A、B and C. **Conclusion**: sibutramine tablet and capsule decreased BW and body fat mass especially intraabdominal fat, and also regulated blood glucose and lipids metabolisms properly.

P130 THE DIFFERENCE OF THE EFFECTS OF ORLISTAT ON BODY COMPOSITION IN OBESE DIABETIC AND NON-DIABETIC CHINESE ADULTS

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Objective: To examine the effect of 6-month treatment of Orlistat with eucaloric diet on weight and body composition in obese Chinese diabetic and non-diabetic subjects. **Methods:** Twenty-nine obese with type 2 diabetic patients (DM) (mean age: 36.0 ± 8.0 years and mean BMI 34.1 ± 4.6 kg/m^2) and 29 age-, sex- and BMI-matched obese non-diabetic subjects (non-DM) were recruited from the Diabetic Clinic. All subjects were treated with a dosage of 120mg Orlistat three times daily for 6 months with a eucaloric diet. Body composition was estimated by using dual-energy X-ray absorptiometry (DEXA) and skin-fold caliper. **Results:** Both DM and non-DM subjects showed a significant reduction in body weight (DM: -3.1 ± 0.5 kg, $p<0.001$; non-DM: -4.4 ± 0.6 kg, $p<0.001$), percentage of body fat (DM: -1.5 ± 0.2 %, $p<0.001$; non-DM: -1.8 ± 0.3 %, $p<0.001$), waist circumference (DM: -4.0 ± 0.6 cm, $p<0.001$; non-DM: -5.0 ± 0.6 cm, $p<0.001$) and hip circumference (DM: -1.8 ± 0.6 cm, $p<0.001$; non-DM: -4.3 ± 0.5 cm, $p<0.001$) within groups after the 6-month Orlistat treatment. However, the extent of the above changes was not significant between groups except for the hip circumference ($p<0.01$). Besides, there was no significant change in lean mass in both groups (DM: 0.1 ± 0.04 kg, non-DM: -0.7 ± 0.4 kg). **Conclusion:** Six-month treatment of Orlistat resulted in reductions in body weight and body fat in obese diabetic and non-diabetic subjects. The reduction in weight was mainly from body fat rather than lean body mass. The effects were similar in both DM and non-DM groups.

P131 Effect of Epinephrine on Aquaporin Adipose mRNA Expression in 3T3-L Cells

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Objective: Aquaporin adipose(AQPap) is a physiological glycerol channel specific to adipocytes, and it is expressed predominantly in adipose tissue. In this study we investigated the effect of epinephrine on expression of AQPap in cultured 3T3-L1 cell line. **Methods:** Total RNA was extracted from differentiated 3T3-L1 cells which was stimulated by 10^{-6}M,10^{-7}M,10^{-8}M,10^{-9}M epinephrine for 6 hours respectively. AQPap gene expression was detected by RT-PCR. **Results:** There was no significant change of AQPap mRNA levels under the treatment with different concentrations of epinephrine. **Conclusion:** Epinephrine, one of the lipolytic hormones, has no effect on the expression of AQPap mRNA levels.

P132 CLONING COMPLETE LEPTIN RECEPTOR CDNA WHICH POSSESSES LONG INTRACELLULAR DOMAIN (OBRB) FROM SD RAT AND CONSTRUCTING EUKARYOTIC RECOMBINANTS

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Objective: To provide a basis for approaching roles of OBRb in regulating metabolism. **Methods:** Total RNA was extracted from SD rat's hypothalamus. The complete OBRbcDNA was synthesized by RT-PCR. The first fragment is from the adenine of initiation codon to 1279;the second is from 1094 to 2685.Both of fragments were ligated into complete OBRacDNA. The third fragment is from nucleotide 2133 to stop codon of OBRbcDNA; it was ligated with complete OBRacDNA into complete OBRbcDNA. The nucleotide sequences were determinated by the dideoxynucleotide chain-termination method. The above complete OBRbcDNA was orientally ligated with pcDNA3 into pcDNA3OBRb. **Results:** Three fragments and their digestive products were consistent with theoretic values. The results of sequence analysis showed nucleotide 228 T→C, 515 T→C and 3051 C→A. The same results were obtained when the other one RT-PCR production with the same RNA were sequenced. The sizes of fragments derived from restrictive endonuclease digestion of pcDNA3OBRb were also consistent with theoretic values. **Conclusion:** Nucleotide 228 T→C and 3051 C→A are senseless change. The nucleotide 515 T→C makes amino acid Leu→Gln. These changes are possibly due to gene polymorphism, not due to misreading in RT-PCR. It is postulated that pcDNA3OBRb can express complete OBRb.

P133 EFFECTS OF INCREASED COMPLETE OBRB EXPRESSION IN SKELETAL MUSCLE CELLS ON GLUCOSE OXIDATION METABOLISM.

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Objective To observe the effect of leptin on glucose oxidation with increasing complete OBRb expression in cultured skeletal muscle cells. **Methods** Primary skeletal muscle cells were cultured by four groups. Group 1 is cells transfected with pcDNA3OBRb with complete OBRbcDNA, group 2 transfected with pcDNA3, and group 3 non-transfected (each group of cells exposed to leptin and regular insulin). Group 4 is cells without exposure to leptin and insulin. The pcDNA3OBRb and pcDNA3 were transfected into cells by clonfectin, respectively. Expression level of OBRbmRNA in transfeced cells was respectively tested by the semi-quantitative RT-PCR after 48 hours. Glucose oxidation was determined by radioactivity of collected $^{14}CO2$ (metabolite of D- [U-^{14}C] glucose) in three hours. **Results** The ratio of OBRb mRNA and β-actin mRNA is 1.22±0.10, 0.41±0.08 and 0.49±0.09 in Group 1, 2,3,respectively. OBRbmRNA in cells transfected with recombinants was significantly increased compared to the other groups of cells ($p<0.05$). Glucose oxidation in cells transfected with recombinants was also significantly increased when compared to the other groups of cells (all $p<0.001$). **Conclusion** Overexpression of complete OBRb mRNA improved glucose oxidation in cultured skeletal muscle cells.

P134 PREPARATIONS OF ANTIBODIES AGAINST HUMAN LEPTIN AND DEVELOPMENT OF NEW GENERATION ELISA FOR HUMAN SERUM LEPTIN

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Objectives: To prepare monoclonal (MCA) and polyclonal (PCA) antibodies against human leptin(hLEP) and to establish sensitive and accurate hLEP BA-ELISA. **Methods:** Use lymphocyte hybridoma technique and classic immunological methods to prepare MCA and PCA against hLEP, respectively. The antibodies were characterized, purified, and labeled with biotin according the relative methods. BA-ELISA for serum hLEP was developed by using the optimal conditions including the best paired solid and liquid phase antibodies. **Results:** We have successfully got rabbit PCA against hLEP and 7 hybridoma strains which can stable secrete MCA against hLEP. The titers for PCA and MCA were $0.2\sim0.5x10^4$ and $0.8\sim1.6x10^4$, respectively. Accurate and precise hLEP BA-ELISA was developed.The sensitivity was 0.15ng/ml.Detection limit ranged from 0.15 to 50ng/ml. Coefficients of variation (C.V.) for inter- and intra-assay were 3.7 to 7.8% and 5.5 to 9.8%,respestively.Recovery was 80.1~102.5% when 2ng/ml and 20ng/ml of hLEP was added. Dilution curve is parallel with standar curve. No cross-reaction with other hormones were found. Serum LEP levels were higher in female than male and in obese sudjects than normal subjects($P<0.001$).**Conclusion:** High qualitative MCA against hLEP were prepared and can be produced in large scale. The established hLEP BA-ELISA was sensitive, specific and accurate, it can be used for clinical and basic researches.

P135 THE EXPRESSION OF TNF-α CONVERTING ENYME, TNF-α, LEPTIN, LEPTIN RECEPTOR GENE IN OMENTAL ADIPOSE TISSUE OF OBESITY AND INSULIN RESISTANCE PATIENTS

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Objective: To study the effect of TNF-α converting enzyme(TACE)gene, TNF-α, Leptin and Leptin receptor gene in pathogenesis of obesity and insulin resistance.**Methods**:(1)Semi-quantitative RT-PCR was conducted to compare the expression of TACE, TNF-α, Leptin and Leptin receptor gene in omental adipose tissue of obesity and insulin resistance patients with normal individuals. **Results:**(1)In omental adipose tissue,the level of TACE, TNF-α, Leptin and Leptin receptor gene are all higher in obesity and insulin resistance patients than in normal controls.(2) TNF-α mRNA level was strongly associated with Leptin mRNA level($r=0.88, P<0.01$).**Conclusion:**(1) In obesity patients, the action of TNF-α in limiting fat accumulation may be abnormal and this could result in obesity;(2)TACE may play an important role in pathogenesis of obesity and insulin resistance through TNF-α,(3) Leptin resistance may exist in obesity patients,(4) TNF-α may increase Leptin gene expression in human; (5) TNF-α system may act through Leptin system to increase energy expenditure and decrease body weight in human;(6)Leptin could mediate one or more effects of the immune system on neuroendocrine function.

P136 ECG ABNORMALITIES (MINNESOTA CODE) ARE COMMON AMONG ASSYMPTOMATIC TYPE 2 DIABETES PATIENTS

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Objective: To estimate the prevalence of ECG abnormalities according to Minnesota Code in a diabetic population of a regional hospital and their risk factor profile. **Methods:** The resting ECG of consecutive 103 men and women referred to the Diabetic Clinic of United Christian Hospital from July 2000 to November 2000 were coded according to the Minnesota Code. The cardiovascular risk factors namely 1) overweight (BMI > 23); 2) smoking; 3) hypertension (BP > 135/85) and 4) hyperlipidemia (LDL-C > 3.40mmol/L or total triglyceride > 2.2nmol/L) were assessed. **Results:** ECG abnormalities were observed in 63 subjects (61.16%). The prevalence was higher among men (70.59% Vs. 51.92% in women). Among those classified to have ECG abnormalities. 45 subjects (87.3%) has at least 2 risk factors compared with 25 subjects or 62.5% in those without ECG abnormalities. **Conclusion:** The ECG abnormalities are rather common among the diabetic patients. These are especially true among those with additional cardiovascular risk factors. However, the prognostic values of these ECG abnormalities will require further investigation.

P137 The Epidemiologic Study Of Diabetes And Analysis Of The Prevalence And Incidence Of Coronary Heart Disease In The Elderly During 1996-2000

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Objective The aim was to determine the prevalence and incidence of type 2 diabetes mellitus (DM) and to evaluate the prevalence and incidence of coronary heart disease (CHD) in the elderly in Beijing. **Method** A survey was conducted among 2,239 old subjects aged 60 to 90 years old from 1996 to 2000. The risk factors of incidence in DM , IGT and incidence in CHD of different groups were analyzed with multiple logistic regression at the end of four observed years. **Results** The adjusted prevalence of DM increased from 15.8% in 1996 to 23.9% in 2000. The adjusted incidence of DM for the elderly population was 33.75 1000^{-1} $year^{-1}$. Logistical regression test showed FPG, PPG, MAP (mean arterial pressure) and BMI were risk factors related to incidence of DM. The incident of CHD were 113 cases(male 108, female 5). The incidence of CHD in IGT and DM group was 50.42 and 57.08 per 1,000 observation person-year, respectively. Both were significantly higher than that of NGT group (27.5/1,000 person-year). Logistical regression test showed DM, hypertension and age were risk factors related to incidence of CHD. **Conclusion** It could be concluded that the prevalence and incidence of DM in the elderly people was significantly higher than in general population. The incidence of CHD were associated with risk factors including impaired glucose metabolism, hypertension, obesity and age.

P138 INQUIRY INTO THE CLINICAL AND IMAGEOLOGICAL MANIFESTATIONS WITH FACTORS CONCERNED IN DIABETIC STROKE

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OBJECTIVE: To investigate the clinical characteristics of diabetic stroke and related factors thereby facilitating prophylaxis and treatment. **METHODS:** 90 stroke patients with one or more expressions pertaining to DM, male/female ratio 56 to 34,aged 42 to 83yrs with 53 individuals over 60(58.9%) and 2 cases over 80. All of them underwent CT scanning and 5 cases were by MRI(1 case received MRA) Blood chemistry hemorheology studies were performed accordingly. **RESULTS**: Clinically the majority of cases had long-standing history of cerebrovascular, cardic and renal troubles. CT and MRI features were divided into 4 groups: **1.** occlusive stroke, 75 cases of infarction, in which 50 were lacunar characterized by multiplicity, widespread distribution, e.g. basal ganglionic foci left 9, right 12, bilateral 24 with a total of 45, 43 in white matter, 2 in cerebellum and 8 in brain stem. **2.** hematomas, 8, the biggest one was $4.7\times1.2\times3cm^3$ in an old lady of 67.**3.** mixed stroke, 3 cases with both infarct and spotty bleeding. **4.** basivertebral insufficiency, 4 cases. Blood chemistry found FPG, TC, LDL-c and ηb markedly elevated in group, 2. In males the average age of lacunar cases(62.9 ± 7.24 yrs)was higher than others(57.7 ± 9.0yrs). The average age of lacunar female (67.09 ± 7.9 yrs) was markedly higher than that of males (57.7 ± 9.0 yrs). **CONCLUSION**: **1.** 90% patients of DM were more than 50 in age. **2.** Long standing DM with cerebrovascular troubles and elevated FPG, TC, LDL-c and blood pressure are prone to develop stroke at any time. **3.** The morbidity of cerebral infarction was higher than that of hemorrhage with the ratio about 2.6:1, lacunar infarction dominant and widely distributed.

P139 INFLAMMATION AND ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Objective: There is mounting evidence that inflammation plays a role in the pathogenesis of atherosclerosis and it has been suggested that endothelial dysfunction may be a possible link between inflammation and atherosclerosis. We have evaluated the relationship between C-reactive protein (CRP), a marker of systemic inflammation, and endothelial function in patients with type 2 diabetes mellitus. **Methods:** Endothelial function was assessed by high resolution vascular ultrasound in 80 type 2 diabetic patients and 80 age and sex-matched controls. High sensitivity C-reactive protein (CRP) was measured by immunoturbidimetric assay. **Results:** Diabetic patients had impaired endothelium-dependent (mean ± SD: 5.2% ± 2.5 vs 9.2 ± 4.3, $p<0.01$) and independent vasodilation (13.6% ± 4.9 vs 16.9 ± 5.5, $p<0.01$) compared to non-diabetic controls. Serum CRP was significantly higher in the diabetic patients than controls (median {interquartile range}: 1.75 mg/l {2.74} vs 0.86 {1.71}, $p<0.01$). Endothelium-dependent vasodilation correlated inversely with CRP ($r = -0.21$, $p<0.01$) whereas no correlation was found between endothelium-independent vasodilation and CRP. **Conclusion:** The association between the impairment of endothelium-dependent vasodilation and serum CRP concentration suggested that endothelial dysfunction in patients with type 2 diabetes mellitus might be partly related to inflammation.

P140 THE RELATIONSHIP BETWEEN PLASMA HOMOCYSTEINE AND MACROVASCULAR DISEASES IN TYPE 2 DIABETES

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Objective: To investigate the relationship between plasma total homocysteine(tHcy) and macrovascular diseases in type 2 diabetic(T2D) patients. **Methods :** We selected 191 T2D patients in our hospital from 2000 to 2001 and 23 healthy subjects. Venous blood was obtained after an overnight fast. Plasma was seperated and frozen at –20°C for batched analysis. Plasma tHcy concentration was determined by high-performance liquid chromatography (HPLC) method with fluorescence detection. Ultrasonographic scanning of the carotid arteries was performed, and the intimal-medial thickness(IMT) was measured. Plasma tHcy levels were compared among patients with IMT≥0.8mm 、IMT<0.8mm and control subjects. **Results:** Ln-transformed plasma tHcy levels in all diabetic patients and who without vascular complications were 2.41±0.33 and 2.26±0.30 respectively, both weren't different from control subjects (2.34±0.23),$p>0.05$. Ln-transformed plasma tHcy levels were higher significantly in diabetic patients with IMT≥0.8mm than who with IMT<0.8mm and controls (2.49±0.32vs2.25±0.27,2.34±0.23,$p<0.001$). Logistic regression analysis showed that plasma tHcy levels were associated with IMT (OR=1.365,$p=0.001$,95%CI 1.139—1.637) independent of age and fast blood glucose concentration. **Conclusions:** Plasma tHcy levels weren't higher in T2D than general population, but plasma tHcy levels were significant higher in diabetes with macrovascular diseases than those without macrovascular diseases. Plasma tHcy levels were independent factors of macrovascular diseases in T2D.

P141 EFFECTIVENESS OF AMLODIPINE VS LISINOPRIL ON BP CONTROL IN TYPE II DIABETES MELLITUS BY 24 HR BP MONITORING

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Background: Hypertension is a major determinant on diabetic nephropathy. **Objective:** To determine the effectiveness, tolerance and adverse effect of Amlodipine and Lisinopril. **Methods:** Randomized prospective study for 48 weeks. Amlodipine or Lisinopril ± Natrilix were titrated against blood pressure. 24 hour ambulatory BP monitoring were performed to assess overall BP control. **Results:** Total 57 patients were included. No major adverse effect reported. Both Amlodipine and Lisinopril were well tolerated. The mean systolic and diastolic BP (MSBP, MDBP) for Amlodipine and Lisinopril were 130.5/77.8 ± 10.0/8.1 Vs. 139.5/80.8 ± 18.9/10.6. The Amlodipine group has better MSBP p=0.016. The mean systolic BP load (MSBPL) (defined as percentage of SBP readings > 140mmHg daytime) is better in Amlodipine group VS. Lisinopril group (37.9% Vs. 38.4% p=0.047). The age adjusted MSBP control and MSBPL control were statistically significantly better in Amlodipine group (12/15 Vs. 6/12 p=0.021, 11/15 Vs. 5/16 p=0.018 respectively). **Conclusion:** With titration, both Amlodipine and Lisinopril can achieve target BP. However with 24 hr ambulatory BP monitoring, Amlodipine group was found to have better MSBP and MSBPL with or without age adjustment. Long term effect on renal protection await further study.

P142 ATORVASTATIN LOWERS C-REACTIVE PROTEIN AND IMPROVES ENDOTHELIAL DYSFUNCTION IN TYPE 2 DIABETES MELLITUS

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Objective: Endothelial dysfunction is frequently found in diabetic subjects. This study was performed to investigate whether atorvastatin was able to reverse endothelial dysfunction in type 2 diabetes and if so, whether the effect was due to its anti-inflammatory action. **Method:** Eighty patients (baseline LDL 4.37 ± 0.71 mmol/l) were randomised to atorvastatin (10 mg daily for 3 months followed by 20 mg daily for 3 months) or placebo in a double-blind study. Endothelial function was assessed by high resolution vascular ultrasound and high sensitivity C-reactive protein (CRP) by immunoturbidimetric assay. **Results:** Atorvastatin 10 mg and 20 mg lowered plasma cholesterol by 32.9% and 38.0%; triglyceride by 15.4% and 23.1%; LDL by 43.4% and 50.1% respectively. At 6 month, plasma CRP decreased in the atorvastatin group compared with baseline ($p<0.05$). Endothelium-dependent vasodilation improved in the atorvastatin group compared to the placebo group (6.5% ± 2.8 vs 5.0 ± 2.1, $p<0.05$). The percentage change in endothelium-dependent vasodilation at 6 month correlated with the percentage change in CRP ($r=-0.44$, $p<0.05$) but not with changes in plasma lipids. **Conclusion:** Treatment with atorvastatin in type 2 diabetes led to a significant improvement in endothelium-dependent vasodilation which might be partly related its anti-inflammatory effect.

P143 Improvement of glycemic control in Type 2 diabetic patients by addition of low-dose rosiglitazone to sulphonylurea therapy: A phase tow clinical trial in Chinese populations.

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Objective: To evaluate the efficacy and safety of low dose rosiglitazone therapy combined with sulphonylurea in patients whose type 2 diabetes is inadequately controlled with sulphonylurea alone.
Method: A multi-center, double-blind, randomized, parallel, with metformin-sulphonylurea controlled clinical trial. Patients were assigned to treat with 4mg/d of rosiglitazone plus previous dosage of sulphonylurea (n=102) or 0.25g of metformin plus previous dosage of sulphonylurea (n=96) for 12 weeks. Plasma glucose, glycosylated hemoglobin (HbA1c), insulin were tested and compared between baseline and week 12.
Results: Glycosylated hemoglobin levels, fasting plasma glucose (FPG) levels and insulin action improved significantly with sulphonylurea-rosiglitazone therapy and sulphonylurea-metformin therapy. The mean levels of glycosylated hemoglobin decreased by 1.09% and 0.95% in the 4 mg/d sulphonylurea -rosiglitazone group (test group) and 0.25g/bid sulphonylurea-metformin group (control group), respectively. Fasting and postprandial plasma glucose levels of the test group decreased by 2.52 mmol/L (25.0%) and 5.63 mmol/L (35.6%) compared with the baseline ($P<0.01$ for all). Of the control group, fasting and postprandial plasma glucose levels decreased by 1.76 mmol/L (17.7%) and 3.73 mmol/L (23.8%) compared with the baseline ($P<0.01$ for all). Increase in total and low-density lipoprotein cholesterol levels accompanied with increase in high-density lipoprotein cholesterol were observed ($P<0.01$ for test group taking rosiglitazone at week 12 vs baseline) in the test group. The proportion of patients reporting adverse experiences was comparable between two groups. The incidence of side effects was low and similar in test and control groups. There was no liver damage been found.
Conclusions: Our data suggest that combination treatment with 4 mg once-daily rosiglitazone plus previous dosage of sulphonylurea in patients whose type 2 diabetes is inadequately controlled with sulphonylurea alone can improve glycemic control, decrease insulin, hence improve insulin sensitivity, which was more effective than treatment with 0.5g twice-daily metformin plus previous dosage of sulphonylurea. This combination use was also safe and well tolerated.

P144 AN OBSERVATION OF DIFFERENT THERAPY MODELS OR TYPE 2 DIABETICS WITHE SECONDARY SULFONYLUREA FAILURE

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Objective: to investigate the intervention effects of 5 therapy models on Secondary failure of sulfonylures (SFS) in type 2 diabetics. **Methods:** 198 (Fasting blood glucose over 10mmol/L for two times in last 3 months) cases of type 2 diabetics with SFS were recmited into the study, their diet control, excise were fixed with sulfonylurea (Su) for over 1 years.Fasting blood glucose,post prandial glucose, Plasma insulin, CH, TG, LDL-C, HDL-C, BMI were checked befor and after 3 months intervention with 5 therapy models. An the patients were separeted to 5 therapy group by random.there were group I (Metformin only, 1.5g/d), group II (Su and metformin conbined, 1.5g/d), group III (small dose NPH plus metformin, 1.5g/d), group IV (NPH, metformin and acarbose combined), group V (NPH, metformin and Diamicron). **Results:** After 3 months intervation, the fasting blood and post prandial glucose showed significant decreases in all 5 therapy models group, post prandial glncose lowered greertly in group IV and fasting glucose decreased rapidly in group V, The decresing degree was in such a sequence: Group V, group IV > group III >group II >group I, Non significane differences were found in Fasting plasma insulin.CH, TG, LDL-C, HDL-C and BMI in 5 group. **Conclusion:** Among the 5 kinds of therapy models,those containing metformin had significant therapy effects, NPH combined with metformin had better effect,while metformin combined with NPH/ acarbose or dimicron showed the best effect.

P145 Management of insulin allergy: a prospective review of four patients

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Objective: To report 4 patients who suffered insulin local reaction and their management. **Background:** Local reaction due to insulin injection is quite common, they can be trivial such as mild itchiness which subsided spontaneously, to major problems like lipoatrophy or lipohypertrophy. Usual management included rotation of injection sites, avoiding injection of insulin immediately after taken from the refrigerator. In the past two years we had treated four patients with severe local reactions. They were characterized by intense local irritation, and large area of erythema immediately after insulin injection. The reaction persisted despite oral antihistamine and local steroid. **Method:** Four consecutive patients with severe local insulin reactions were studied prospectively. They had local reactions documented by various insulin preparations as compared to concurrent local saline injection at the opposite side of the abdomen. An alternative insulin preparation with no or least local reaction was used for desensitization program, which consisted of unit by unit titration of insulin every couple of days according to patients' response. **Results:** Three patients received insulin injection with no local reaction or mild irritation, one patient stopped insulin treatment because of good control.

Patients	Original preparation (per day)	Local reactions	Test result negative with	Final insulin preparation (per day)	Remarks
M/62	Monotard MC 10u	Erythema, irritation	Lispro	(back to OHA)	Hba1c 6.3 % with OHA, insulin stopped
F/68	Protaphane HM6u	Erythema, irritation	Lispro	Mixtard 30HM 72u	well
M/75	Protaphane HM42u Actarpid HM12u	Erythema, irritation	Humalog R	Humulin -N 58U Humulin -R 22u	Mild itchiness , no induration
M/44	Mixtard 30HM 30u	Erythema, irritation	Actrapid HM	Protaphane HM20u	Mild itchiness, no induration

Conclusion: In this small series the intermediate insulin was the commonest preparation that caused local reaction and this is consistent with published literature. The culprit is probably protamine contained in the intermediate insulin preparation. The exact incidence of protamine allergy is not known as the severity of local reaction varies. It can be a trivial discomfort to intense irritation with large erythema. Our experience suggested that diabetics with severe local insulin reaction can restart insulin with mild or no adverse reaction after carefully monitored desensitization program.

P146 A SURVEY OF DIABETES EDUCATION TO 102 PATIENTS WITH DIABETES MELLITUS

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A survey was carries out by questionary to estimate the levels of diabetes education in 102 patients with diabetes mellitus. One hundred and second patients with typ 2 diabetes mellitus treated with oral hypoglycemics were involved, of which 53 were male and 49 female, and age ranges were 25-76 years. The education involved in the questionary included diabetes symptoms, blood glucose analysis, diabetc complications, doctor selection, hospital selection, readings of diabetes education, diet and its constitution, excise, buying drug, drug treatment , follow-up, hypoglycemia, self-monitoring, preservation of case materials, etc. The score of education was calculated according to the aspects above. High score was 41-52,moderate score 26-40 and low score 2-26.The patients were divided into three groups of good control (30 cases), moderate control (38 cases)and bad control(34 cases) to order to observe whether the difference of the education was present among the patients. **Results:** The score of education was 37. 7 $\pm$0.869(x $\pm$sx) in the group of good control, 29.4$\pm$ 0.825 in the group of moderate control and 18.7 $\pm$1.731 in the group of bad control, respectively. The difference of the score was apparently significant among the three groups ($P<0.05$). The rate of obteining high score, moderate score and low score was 36.7%, 53.3% and 10%, respectively, in the group of good control, 10.5%, 60.5% and 28.9%, respectively, in the group of moderate control, and 5.9%, 17.6% and 76.5%, respectively, in the group of bad control. There was obvious difference in the grade of the score among the three groups ($P<0.05$). There was relationship between the score and education in 102 patients with diabetes mellitus. The score was not relative to the course of diabetes mellitus or complications whether the patients had. **Conclusion:** The results reveals that there was close relationship between diabetes education and the control of diabetes, indicating that it is important to improve diabetes education in prevention and treatment of diabetes mellitus.

P147 USING PDCA CIRCLE TO SET UP AND PRACTICE THE MODEL OF DIABETIC HEALTH EDUCATION

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Objective: Using PDCA Circle to Sett up, practice and evaluation the model of diabetic health education. **Methods:** We set up the group of diabetes education. The places of diabetes education were located at two special diabetic classrooms. Out-patient education: lectures and discussion; In-patient education: group education, individual education and self- education; post-hospital education: consulting on line. **Results:** We organized lectures 36 times and participants were 868. the average knowledge test scores was 74.4 and the pass rate was 86.3% in the out-patient education. 82.9% of hospitalized patients achieved excellent scores after education compared with 24.2% of the patients before education ($P<0.001$). For the self-care skill knowledge such as self-monitor of blood glucose and urine glucose, foot care were improved ($P<0.01$). consulting on line: 51.1% of patients' questions related to the treatments of diabetes and 20.7% of patients consulted the prevention and treatment of diabetic complications. It showed that we should pay more attention to these contents in diabetes education. **Conclusion:** Out-patient education courses provided a place of learning the diabetic knowledge for patients with diabetes. In-patient education was a good chance of learning the diabetic knowledge and improvement the ability of self-management. consulting on line was a convenient way of instruction the treatment and nursing for the post-hospital patients with diabetes.

P148 MODEL FOR MANAGEMENT OF DIABETES- SHARED CARE PROGRAM AND INTENSIVE INSULIN TREATMENT GROUP

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Objective: Diabetes is a massive public health problem. Nevertheless, its complications are highly treatable and preventable by early diagnosis, risk stratification, individualised therapy, treatment to target values and compliance reinforcement. Since 1995, our group has started a series of diabetes-related continuous quality improvement (CQI) programs with particular focus on communication, teamwork and education. **Methods:** ***1. Diabetes Shared Care Program.*** About 50 diabetic patients were assessed weekly using comprehensive structured protocols based on international guidelines and data computerisation with risk stratification. Approximately 30% of them who are clinically stable are discharged to community for shared care. They are empowered with knowledge and skills on self management, treatment goals and modes of follow up. To date, we have discharged over 1000 patients to the community or general medical clinics. ***2. Intensive Insulin Treatment Program.*** Over 30% of our patients have young onset of disease (<35 years), who pose particular therapeutic challenges with long disease duration, increased risks of complications, busy lifestyles with low incentives to comply to therapy. Since 1999, we run a 3-monthly 4-hour session involving 15-20 patients every time, co-ordinated by our diabetes nurses to manage these patients. Apart from intensification of medical therapy and education, peer pressures amongst patients have contributed to the substantial improvements in their metabolic control and self management. **Results and Conclusion:** In our shared care program, of the 600 patients discharged to the GOPCs between 1996 and 1998, patients who had repeat assessments at our Centre at 2-3 yearly intervals had on average 50% risk reduction in hospital admissions compared to those who did not return. For the intensive insulin treatment program, upon attending the clinic for at least 12 months, HbA_{1c} dropped from 11% to 8% in the 'poor control' group and from 7.2% to 6.8% in the 'fair control' group, which is translated into a 15-25% risk reduction in all diabetes-related clinical endpoints.

P149 BEHAVIOR PROBLEMS OF TYPE 1 DIABETES PATIENTS IN BEIJING DISTRICT

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Aim: To learn the behavior problems of children and adolescents with type 1 diabetes in Beijing district,and the difference between them and the healthy children and adolescent
Methods: Behavior problems were assessed in 150 patients with type 1 diabetes mellitus and 950 healthy children and adolescents using Achenbach Child Behavior Checklist (CBCL)and self-made questionaire of influential factors . SPSS soft package was used in data analysis.
Result: The rate of behavior problems was 22.6% in diabetes group,more than twofold higher than in the control group (10.2%).In diabetic subject , withdrawn and social problems were the mosr prevalent; In the control group, hyperkinetic and anxious/depressed were the main behavior problems.In diabetic subject ,the highest incidence rate were among the patients from 11 to 16years old.
Conclusion: In Beingjing district,type 1 diabetes have more behavior problems and the kinds of the problems were significantly different from the healthy.Monitoring may be particularly beneficial among the adolescents patients.

P150 A Study of Mechanism of immune prevention from diabetes by oral administration of insulin

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Objective: In order to find out the mechanism of immune prevention from STZ-insulitis and DM by oral administration of insulin. **Methods:** 20 healthy Kunming mice (equally divided into A and B groups) were ip with STZ (40 mg.kg^{-1}/d) for 5 days. Group A was pregavaged with porcine PZI (1mg. Per time.2time/w ×10 w), and group B was fed the same way with water as control. The T lymphocytes subsets in pancreatic islet, spleen and Fas/FasL expression on β cells as well as on infiltrating T cells in islets were investigated by immunochemistry method. **Result:** DM incidence and insulitis score in A and B groups were 10% vs 90% and 0-1 vs 2-4. In group B major subset of T cell was CD8+ T cells, but in group A in 90% mice appeared the normal pancreatic normal islet without T cell infiltration, the CD4+ and CD8+ islet-infiltrating T cells were expressed FasL , but Fas was only expressed on β cells in group B. **Conclusion:** Oral administration of insulin significantly suppressed recruitment and infiltration of CD8+ T cell subset and Fas positive expression on β cells, This protected β cells from destruction by apoptosis induced by interaction of Fas on β cells and FasL on active T cells.

P151 CONSTRUCTION OF A RECOMBINED HUMAN INSULIN EXPRESSION VECTOR UNDER THE CONTROL OF DOXYCYCLINE FOR INSULIN GENE THERAPY AND ITS EXPRESSION IN VITRO

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Objective: To construct a single plasmid vector madiating doxycycline-inducible recombined human insulin gene expression. **Method:** An expression cassette of rtTAnls driven by hCMV and a recombined human insulin expression cassette driven by a reverse poly-tetO DNA motif were cloned into a single plasmid vector (prTINS). prTINS and pLNCX were cotransfected into a myotube cell line (C2C12) and pLNCX vector were used as a control. After selection with G418, the transfected cells were induced with doxycycline at the concentrations of 0μg/ml, 2μg/ml and 10μg/ml. Cells after different days of doxycline incubation were harvested. RT-PCR was used to determine expression levels of recombinant insulin mRNA at the three doxycycline concentrations on day 5. Cell cultures and cell extracts were loaded to HPLC to collect the insulin parts as compared with insulin standards. The HPLC collections were analyzed with human insulin RIA kits. **Results**: Immune reactive insulin (IRI) was found increased at 24 hours of doxycycline incubation, and still increased at day 5. After withdrawn of doxycycline, IRI decreased sharply and was at baseline three days later. IRI and human insulin mRNA levels were positively related to different levels of doxycycline. A 25 fold of increase in IRI was found against background expression. **Conclusion:** Mature human insulin expression can be successfully regulated by doxycline and the background was very low. A more efficient tetracycline inducible insulin expression vector is under improvement and may provide a new approach to a controlled insulin gene therapy.

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P152 Antihyperglycemic Effect of Chitosan-microcapsulated Insulin in Streptozotocin Inducing Diabetic Wistar Rats

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Abstract Objective To observe the effect on lowering blood glucose (BG) of chitosan-microcapsulated insulin (insulin-microspheres) in diabetic rats by gavaging. **Methods** (1) Study insulin-microspheres shape and distribution by the scanning electron microscope in tissue of liver,spleen, and small intestine of normal rats by gavaging with insulin-microspheres. (2)The following groups of rats were studied: normal control rats (NC,n=5), streptozotocin-(STZ) inducing diabetic control rats(DC,n=6), STZ diabetic rats by gavaging with insulin-microsphere [120u/kg](DF,n=6), STZ diabetic rats treated with subcutaneous insulin injection [24u NPH/kg] (DT,n=6). Blood glucose was measured at 0~7 days after treating in rats. **Result** (1) The insulin-microspheres was found in small intestine, liver and spleen by scanning electron microscope at 1,2,3,7 day. (2) In DF group the average blood glucose level decreased significantly from 24.7±3.2mmol/l to 16.9±5.5mmol/l during the first day of treatment and reached the lowest level (12.1±5.7mmol/l) in the second day . From the third day ,the BG gradually elevated to the level before treatment . the blood glucose decreased 50.2% at most. In the DT group, the average blood glucose level decreased from 25.2±3.8mmol/l to 10.4±5.2mmol/l, and increased gradually from the 2nd day. The glucose level decreased 58.7% at most. There is no significant difference between the declined range of two groups. **Conclusion** Chitosan-microcapsulated insulin has an antihyperglycemic effect in STZ-inducing rats by gavaging.

P153 ADRENOMEDULLIN IN STREPTOZOTOCIN-INDUCED DIABETES.

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Objective: To study the changes in adrenomedullin levels in the plasma, the heart, blood vessels, the adrenal, the lung and the pancreas of streptozotocin-diabetic rats. **Methods**: Diabetes was induced by i.p. injection of 60 mg/Kg streptozotocin. Some of these rats were injected s.c. with 4 I.U. of insulin per day. At 4 weeks, rats were decapitated and adrenomedullin levels measured by RIA in the tissues mentioned above and compared with control. **Results**: There was an increase in adrenomedullin contents in the vasculature but a decrease in the left ventricle and both atria. Adrenal adrenomedullin contents also increased whereas plasma, lung and pancreatic adrenomedullin levels did not change. **Conclusions**: These results suggest that there may be changes in secretion and synthesis of adrenomedullin in the adrenal, the heart and the vasculature and confirm that pancreatic beta cells are not the major cells that produce adrenomedullin.

P154 THE STUDY OF SECRETION AND RESISTANCE OF INSULIN IN CHINESE AND JAPANESE GROUP IN NEWLY DIAGNOSED TYPE 2 DIABETIC PATIENTS

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Objective: To search for the clinical features of secretion and resistance of insulin and compare the difference between Chinese and Japanese groups in newly diagnosed type 2 diabetic patients. **Methods:** Diagnosed DM2 736 cases in Chinese group and 1011 cases in Japanese group were managed by the standard registration of diagnosed type 2 diabetic patients with the systematic documents which designed by National Diabetes Center of Kyoto National Hospital in Japan including the background factors and examination quotas relating to DM2. **Results:** In Chinese group officers and clerks were in the majority (60.00%), the followings were housewives(8.40%) and businessmen(3.90%), whereas in Japanese group administrative staffs and clerks (52.70%), housewives 11.10%, businessmen 13.90%. The data of two groups were quite close. Alcoholic cases were apparently higher in Chinese group($P<0.01$). Clinic examination criteria: Chinese group in BMI(25.40), insulin(14.40 mU/L), HOMA-R(6.64), dyslipidemia(61.50%) and hypertension(36.41%) were apparently higher than Japanese group(23.90, 7.56mU/L, 2.97, 39.7%, 33.40%). There was no difference in fasting blood sugar and hemoglobin A1c. **Conclusion:** The obvious difference of Chinese and Japanese groups is the resistance of insulin. Fasting plasma insulin and HOMA-IR are higher in Chinese group. These may relate to Alcoholic, BMI, Hypertension and dyslipidemia.

P155 STUDY ON THE DEGREE OF INSULIN RESISTANCE AND EARLY β CELL'S FUNCTION IN INSULIN RESISTANCE SYNDROME

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Objective : To study the insulin sensitivity and early islet β cell's function in simple obesity (Ob-NGT), normal glucose tolerance with hypertension (NGT-Hy), obesity with impaired glucose tolerance (IGT-Ob), hypertension with IGT(IGT-Hy) and T2DM. **Methods:** Insulin sensitivity index (SI) was assessed by Bergman's minimal model method with frequently sampled intravenous glucose tolerance (FSIGT) test in patients with Ob-NGT, NGT-Hy, IGT-Ob, IGT-Hy and T2DM as well as in the controls. Meanwhile the early β cells function was evaluated by the parameter Ip-b , which stands for the difference between the peak insulin value and basal insulin value (2-4 min) after glucose injection in FSIGT. **Results:** SI was significantly higher in the controls than that in the other five groups. The SI in NGT-Hy is significantly higher than that in the Ob-NGT ($p<0.02$). The Ip-b in control (70±48.56 mmol/L), NGT-Hy (69.36±46.53 mmol/L) was significantly higher than that in the IGT-Ob (50.17±47.14 mmol/L) and T2DM (15.8±25.79 mmol/L), but significantly lower than that in the Ob-NGT (158.27±107.27 mmol/L) and IGT-Hy (123.92±96.54 mmol/L). **Conclusion:** Insulin resistance is existed in the five morbidity groups; the early β cells function is still normal in NGT-hy, but is abnormal in the other four group, whether hyper- or hypo-function.

P156 THE ASSOCIATION BETWEEN FASTING SERUM LEPTIN AND SPECIFIC INSULIN, PROINSULIN AND INSULIN SENSITIVITY IN DIFFERENT GLUCOSE TOLERANCE INDIVIDUALS

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Objective: To investigate the relationship between fasting serum leptin and specific insulin, proinsulin and insulin sensitivity. **Methods:** 54 normal glucose tolerance(NGT) subjects,33 glucose intolerance(IGT)subjects and 47 newly discovered subjects with type 2 diabetes mellitus(DM) were included in this study. Their fasting serum leptin and 0, 1/2, 1, 2 hour specific insulin(SI) and proinsulin(PI) during oral glucose test were measured. **Results:** Fasting serum leptin of IGT group was higher than that of both NGT and DM group, there was no difference between NGT and DM group. The ration of SI to PI in DM group was higher than that of NGT and DM group.The insulin sensitivity index(ISI) of NGT group was higher than that of IGT and DM group, the ISI of IGT group was higher than that of DM group. The area under the curve (AUC) of SI in IGT group was higher than that of NGT and DM group, there was no difference between NGT and DM group. Sex and body mass index(BMI) were the most important factors that effect leptin, In addition to sex and BMI, Fasting serum leptin was also associated with 0-h SI in NGT group(P=0.047); Fasting serum leptin was associated with 0-h PI (P=0.007) , 2-h PI (P=0.001) , 2-h SI (P=0.006) and AUC (P=0.045) in NGT group; Fasting serum leptin was associated with 0-h PI (P=0.036) ,0-h SI (P=0.039) and 1-h PI (P=0.011) in DM group. There were no significant relationship between fasting serum leptin and ISI, as well as fasting leptin with PI/SI, in three groups. **Conclusion**: Hyperleptinemia and hyperinsulinemia were observed in IGT subjects. Disproportional hyperproinsulinemia was a pronounced characteristic of DM subjects, but their fasting leptin was near normal. The results implicated that during the process which IGT develops to DM, the change of PI and SI levels resulting from pancreas islet B cell dysfunction affects the regulation of fasting leptin.

P157 EFFECTS OF GLYCEMIC CONTROL ON PANCREATIC β CELL FUNCTION AND INSULIN RESISTANCE IN TYPE 2 DIABETES

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Objective: To study the effects of glycemic control on β cell function and insulin resistance in type 2 diabetes with a longitudinal investigation. **Methods:** Type 2 diabetic patients with poor glycemic control (FSG ≥6.0mmol/l, PSG ≥8.0mmol/l,HbA_{1C} ≥6.2%) were selected. Besides anthropometric measurements, insulin tolerance test (ITT), standard steamed bread test (SSBT) , arginine stimulation test (AST) as well as fasting proinsulin (PI) assay were performed to measure β cell function and insulin resistance before and after glycemic control. Such indices as K_{ITT} in ITT, $AUC_{CP0\text{-}30MIN}$ and AUC_{CP} in SSBT , ACR_{ARG} in AST were used in the above measurements. **Results:** 30 subjects entered the study and 20 (11female and 9 male) finished the whole follow-up, with the median age 57years , the median duration 3.5 years and the median follow-up time 189.5days. In the end of the study ,glycemic control improved (HbA_{1C}9.75±1.72% vs 6.96±0.88%, P<0.01; FSG9.75±1.22 vs 6.40±1.49mmol/l,P<0.01; PSG20.28± 5.29 vs 15.89±4.53mmol/l, P<0.01) with no significant changes in anthropometric indices (BMI22.55+2.70kg/m^2 vs 22.59±2.24 kg/m^2, P>0.05; WHR 0.87±0.09 vs 0.86±0.08,P>0.05;SBP127.75±11.63mmHg vs 28.45±9.71mmHg, P>0.05; DBP87.5±5.34mmHg vs 79.15±5.35mmHg, P>0.05). Indices of β cell function used in SSBT and AST dramatically improved ($AUC_{CP0\text{-}30MIN}$22087±12691pmol/l vs 29723±12218pmol/l, P<0.01; AUC_{CP}144453±93744pmol vs 197726±79417pmol, P<0.01; ACR_{ARG}452±93pmol vs 814±125pmol, P<0.01). PI and PI/C decreased (PI36.26±5.02 vs 20.79±2.85 , P<0.01;PI/C:0.074±0.053 vs 0.025±0.015, P<0.01) and meanwhile K_{ITT} also increased. (0.73±0.20 vs 1.95±0.51, P<0.01). **Conclusions:** Glycemic control may improve pancreatic β cell function including glucose stimulated insulin secretion and non-glucose stimulated insulin secretion, and reduces the disproportionate PI secretion in type 2 diabetes. Similarly, glycemic control can ameliorate insulin resistance.

P158 Risk factors of diabetes mellitus in Guangdong population

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Objective: To investigate the risk factors of diabetes in Guangdong population. **Methods**: Using data from the 1998 Guangdong provincial diabetes interview survey, a population-based cross-sectional study. 11767 residents aged 20-74 years were enrolled (male 5462, female 6305,mean age 43.8±13.6 years). All subjects were conducted the 75-g oral glucose tolerance test and physical examination. Questionnaire included questions about physical activity, family history, economic state, average daily consumption of alcohol and cigarette, et al. **Results**: Age, family history, hypertension, physical activity, BMI, waist-to-hip ratio (WHR) and economic level associated with the diabetes risk. Multiple Logistic stepwise regression analysis shows the odd ratio of age>50 year is 2.0525 (1.5676~2.6874), family history is 3.2101(2.0191~5.1035), hypertension is 3.0875 (2.2456~4.2449), BMI≥25 is 2.5381(1.9052~3.3812), WHR≥0.9 is 2.2546 (1.6897~3.0083), and less physical activity is 1.6439(1.2169~2.2209), Low economic level is 1.230(0.878~1.723). **Conclusions**: Age, family history, hypertension, physical activity, BMI and WHR, but not economic level, are the most important predictive factors of diabetes in Guangdong population.

P159 THE RELATIONSHIP BETWEEN BODY FAT DISTRIBUTION AND INSULIN RESISTANCE IN TYPE 2 DIABETIC PATIENTS

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Objective: To compare methods evaluating body fat distribution and explore the relationship between body fat distribution and insulin resistance(IR) in type 2 diabetic patients. **Methods:** 41 type 2 diabetic patients were enrolled. LnIAI [Ln(1/fast insulinXglucose)] was used as insulin sensitivity index. Body composition was measured by: 1) body mass index (BMI), 2) waist, 3) waist to hip ratio (WHR), 4) skin-folds, 5) total body fat (%BF) measured by TANITA Analyzer, 6) %BF and regional body fat measured by dual-energy-X-ray-absorptionmetry (DEXA). Pearson correlation, multiple regression, cluster analysis and ANOVA were applied. **Results:** BF% measured by TANITA Analyzer were highly correlated with that by DEXA(r=0.839). LnIAI was most significantly associated with BMI (r=-0.480), In multiple regression, only BMI entered the equation. BMI was correlated with LnIAI only in the group of abdominal obesity (waist lager than 90cm in male, 80cm in female) (r=-0.488). When grouped by BMI and waist together, LnIAI of patients with BMI≥23 kg/m^2 and abdominal obesity together was statistically lower than those in other two groups (BMI ≥23 kg/m^2 or abdominal obesity alone). There was no correlation between skin-fold and LnIAI. **Conclution:**1. TANITA could be used in clinical practice. 2. BMI could be a better marker to reflect IR in type 2 diabetic patients. 3.Severe IR existed in type 2 diabetic patients with greater BMI and abdominal obesity than with either of them.4. There was little significance of measuring skin-fold.

P160 THE EFFECT OF EXERCISE ON THE METABOLIC OF LIPOPROTEIN AND INSULIN SENSITIVITY OF RATS INDUCED BY FAT-RICH-DIET

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Objective: To study the effect of exercise on the metabolic of lipoprotein and insulin sensitivity of rats induced by fat-rich-diet. **Methods:**69 male SD rats were randomly divided into control and experimental groups. After 12 weeks, the rats were randomly divided into①group H_1: were given fat-rich-diet ②group HL: were given basic-diet③group HHE: were given fat-rich-diet and taken exercise④group HLE: were given basic-diet and taken exercise simultaneously. **Results:**①After 12 weeks, there were statistically significant differences of Lee Index, FINS and IR between the experimental and control group. ②8 weeks of swimming and diet control can independently reduce Lee Index, FINS, IR and TG/HDL. FBG and TG were only reduced statistically in the rats taken exercise,while HDL was increased only in diet control group. **Conclusion:** The rat exercise and diet control can independently alleviate obesity, improve the insulin sensitivity and reduce TG/HDL of the rats induced by fat-rich-diet. Exercise can reduce the levels of FBG and TG, while diet control had more obvious effect on increasing the levels of HDL.

P161 THE EFFECTS OF AGING ON SERUM PROINSULIN, TRUE INSULIN LEVELS AND β-CELL FUNCTION.

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Objective: To investigate the changes of proinsulin (PI) , true insulin (TI) levels and PI/TI ratio in elderly subjects. **Methods:** 67 healthy older subjects (OL, age 60-78 yr, with normal glucose tolerance according to WHO criteria) and 60 younger control subjects (YC, age 30-49 yr) were studied.Serum levels of PI and TI during 2h-75g oral glucose tolerance test were measured by specific monoclonal antibody based amplified ELISAs. Blood glucose (BG) and serum lipid profiles were also analyzed. **Results:** The sex and body mass index were matched in the two groups. OL group had a little higher waist-to-hip ratio (WHR) and 2-h blood glucose after glucose loading. Both the fasting PI, PI/TI ratio (Geometirc means：5.2∝3.1pmol/l，P=0.009；0.21∝0.13，P=0.01）and 2-h PI, PI/TI ratio (Geometirc means：33.3∝20.5 pmol/l，P=0.02；0.29∝0.22，P=0.05）were significantly higher in OL group than in YC group, but after adjusting for WHR and 2-h blood glucose, the differences in the 2-h PI and PI/TI ratio were disappeared (P<0.05). However, TI levels between the two groups had no statistic difference. **Conclusion** The elevated fasting PI and PI/TI ratio as a feature of β cell dysfunction in type 2 DM did occurred in relation to aging. This may be a predisposing factor to the development of impaired glucose tolerance or type 2 DM in elderly subjects.

P162 CHARACTERISTICS OF INSULIN SECRETION AND INSULIN RESISTANCE IN SUBJECTS WITH IMPAIRED FASTING GLUCOSE

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Objectives The purpose of this study was to explore characteristics of beta-cell function and insulin resistance in subjects with impaired fasting glucose (IFG). **Methods** A total 3985 subjects (2664 males, 1321 females), aged 20-85 years, were studied. 75 g oral glucose tolerance test was performed after 10 h overnight fasting, and fasting insulin (FINS), 2 h insulin (2-hINS) levels were measured. These subjects were divided into six groups: normal glucose tolerance (NGT)(n=2588), (IFG) (n=272), impaired glucose tolerance (IGT) (n=449), IFG/IGT (n=116), newly diagnosed diabetes mellitus (DM1) (n=338), known diabetes mellitus (DM2) (n=222). We evaluated their insulin secretion and insulin resistance by IS=FINS/FPG, HBCI=20×FINS/(FPG-3.5), and Homa-IR=1/(FINS×FPG) and WC、BMI、SBP、DBP、CT、Tri、HDL-C. **Results** Homa-IR was higher in subjects with IFG, IGT and IFG/IGT than that in subjects with NGT(1.47±0.60, 1.39±0.58, and 1.70 ±0.61 vs 1.06±0.64, p=0.0001), but there was no significant difference between IFG and IGT(P=0.193). Subjects with IFG had higher FINS level than NGT (17.90±10.06 vs15.79±10.94mu/L, p=0.0001). There was no significant difference between IFG and NGT (P=0.063) for 2hINS. HBCI and IS were lower in subjects with IFG than subjects with NGT and IGT(HBCI 4.65±0.60 vs 5.27±0.76 and 5.49±0.79, p=0.0001), (IS 0.86 ±0.6 vs 0.99± 0.62 and 1.25±0.61 , p=0.04, p=0.0001, respectively). **Conclusion** The subjects with IFG might have hepatic insulin resistance and defection of insulin secretion. While the IGT might have peripheral insulin resistance and high insulin secretion. Both IFG and IGT presented as clinical features of the metabolic syndrome. IFG/IGT showed significant defection in insulin secretion. Compared with IFG and IGT, the defection in insulin secretion IFG/IGT approached diabetes mellitus.

P163 A STUDY OF THE VALUE OF OGTT-TEST, FINS, FCP OF THE FIRST DEGREE RELATIVE WITH FAMILY TYPE 2 DIABETES IN SHANNXI NORTH REGION

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Objective: To study the value of OGTT-test、FINS、FCP of the first degree relative with family type 2 diabetes in Shannxi north region. **Methods:** To excerpt 115 cases as the investigation grope from 78 families of the first degree relative with family type 2 diabetes, and without diabetes and IGT; and to excerpt 94 cases as the control grope from normal families in Shannxi north region. We divided in subsection with age in two gropes and determined the value of FPG、 FINS 、FCP、 SUA、2hPG、2hIHS、2hCP and calculated HOMA-IR and HOMA-B. **Results:** FCP increase in the investigation grope（P=0.038）. FCP、HOMA-IR、FINS、2hCP increase in the subsection of the first degree relative with family type 2 diabetes of younger than 40 years（P value is 0.028、0.039 and 0.003,respectively）. BMI and FINS (relate-coefficient is 0.5 and 0.253.P value is 0.001 and 0.006, respectively), SUA and value of waist/breech (r-coefficient is 0.385 and 0.461.P value is 0.001 and 0.006, respectively), SUA and BMI (relate-coefficient is 0.371 and 0.276.P value is 0.001 and 0.003, respectively), and SUA and FCP (relate-coefficient is 0.195 and 0.194.P value is 0.059 and 0.037, respectively) show positive correlation with correlation analysis in two gropes. SUA and FINS, SUA and HOMA-IR show no correlation with correlation analysis in two gropes. **Conclusion:** For the first degree relative with type 2 diabetes of younger than 40 years, they should be prevented and cured in earlier period as the high risk group of diabetes.

P164 THE RELATIONSHIP BETWEEN HYPERINSULINEMIA AND POLYCYTIC OVARY SYNDROME OF GIRL

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Objective: To investigate relationship between polycystic ovary syndrome (PCOS) and hyperinsulinemia. **Methods:** lo cases of girl age 15-19; amenorrhea for 0.5~4 yers, obesity slight acanthosis nigricans and hirsutism hypertesteromes.LH/FSH>2 hyperinsulinism insulin resistance (IR) lower insulin sensitivity index (IS).Ultrasonic examination indicated PCOS 8 cases, nomerl 2cases.Treated with oral acarbose, 4 cases of them were simultaneous oral metformin for 3~20 months. **Results:** Follow up 5~21 months body wight BMI decreased significantly.Serum testosterone (T) level declined from 1340± 218pg/ml before tratment to 482±195 pg/ml after tratment. Two hours OGTT indicated blood sugar nomerl. Serum insulin (INS) level declined form 277±136 μ Iu/ml to 61±47 μ Iu/ml. AUC dieclined from 338±174 μ Iu/ml.2h to 86±56 μ Iu/ml.2h; Is rised from –7.4±0.6 to –5.6±0.6 there changes were significant (P<0.01).8 cases resumed menses spontaneously period for 30~40 days and BBT record of 3 cases revealed ovulation period for three times; 2 cases failed to return spontaneous menses. **Conclusion:** the hyperimsulinism impeled ovarion stromal cells and interthecal cells to product androgen which hindered folliculi development; the strenuous efect of IGF-1(INS-Leke grouth factor) Caused folliculi systonic chage and couldn't meturate. The acarbose and metgomin declined the level of blood suger after eaten and impoved TR (insulin resistance), corrected hyperinsulinmia recovered the function of pitutaty-gonad axis.

P165 PHENOTYPES OF GLUCOSE TOLERANT SIBLINGS OF CHINESE YOUNG-ONSET TYPE 2 DIABETIC PATIENTS

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Objectives: We studied the phenotypes of the glucose tolerant siblings of young Chinese Type 2 diabetic patients (age of onset ≤40 years). Methods: Siblings of young Type 2 diabetic patients attending the diabetes clinic were invited to undergo detailed investigations and a 75g OGTT. Age and sex-matched control subjects were also recruited. Venous blood was taken for glucose, lipid, electrolyte and creatinine measurements. Plasma glucose and insulin were measured sequentially during the OGTT. Results: 157 normal glucose tolerant (NGT) siblings from 91 families and 32 controls were recruited. The siblings were more obese than the controls (BMI 23.9 ± 4.0 vs 21.8 ± 3.3 kgm^{-2}, p= 0.006) and there were more siblings with obesity (BMI>25 kgm^{-2}) than controls (32% vs 6%, p=0.003). We stratified the siblings into different categories of BMI (i.e. normal weight BMI < 23, overweight 25 >BMI>23 and obesity BMI>25 kgm^{-2}) and compared to the normal weight control subjects. Among the siblings, there was a progressive increase in systolic blood pressure, waist-hip ratio, plasma glucose, insulin, triglyceride, insulin resistance (HOMA model) and decrease in HDL cholesterol (Polynomial ANCOVA, p for trend < 0.005) from normal weight siblings, overweight siblings to obese siblings. When compared with normal weight controls, normal weight siblings had higher fasting plasma glucose (4.2 ± 0.5 vs 4.5 ± 0.5mmol/L, p<0.05), 15 minute glucose (5.8 ± 1.1 vs 6.6 ± 1.1mmol/L, p<0.05), area under curve (0-30minute) (173 ± 27 vs 190 ± 29min.mmol/L, p<0.05) glucose and plasma triglycerides (0.66 ×/÷ 1.44 vs 0.84 ×/÷ 1.61mmol/L, 95%CI).Conclusions: These findings suggested that metabolic abnormalities were present before potential evolution into glucose intolerance in the siblings of young Chinese Type 2 diabetic patients. The glucose tolerant-siblings of young-onset Type 2 Chinese patients were more obese than controls despite their young age. There was a progressive adverse trend of plasma glucose, insulin and lipid profiles with obesity. This adversity also existed in the normal weight siblings and could not be accounted by obesity alone. These data supports the concerted effects of genetic predisposition and environment impact (summation as obesity) in the progressive onset of metabolic syndrome.

P166 Regulation of Interleukin-6 Production by Cultured Human Thyrocytes

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Objective: To studide the modulation of interleukin-6 (IL-6) generation from human thyroid cells. **Methods:** Thyroid tissues were obtained at surgery from patients with Graves' disease. mRNA were extracted from primary human cultured thyroidal cells under stimulated and unstimulated conditions. IL-6 in the supernatant of cultured thyrocytes were detected with ELISA. **Results:** (1) Compare with the basal results , dexamethasone(10-103nmol/ml) could gradually suppresse IL-6 gene expression on human thyrocytes, while tumor necrosis factor-a(TNF-a) could obviously stimulate the production of IL-6 mRNA in the range of 10-1 -102u/ml. On the level of 102u/ml, Interleukin-1 (IL-1) increased the expression of IL-6, but the same effect could not be shown when IL-1 was 10-1 or 10 u/ml. Sodium iodine (NaI) did not affect IL-6 gene expression in the concentration of 10-8 to 10-4M. (2) ELISA results showed Thyrotropin (TSH,103 mu/L), TNF-α(10-1 u/ml), IL-1(10u/ml) and adrenaline(10 -5mmol/ml) increased the IL-6 levels in the supernatant of cultured thyroid cells, whereas dexamethasone (10 nmol/ml) suppressed IL-6 release from thyrocytes. NaI of 10-4M exerted no effect on IL-6. **Conclusion:** IL-6 produced by thyrocytes could be regulated by many factors that modulate thyroid functions in vitro as well as in vivo.

P167 CHARACTRIZATION OF THE SPATIAL DISTRIBUTION OF KREV-1 mRNA IN THE BRAIN OF NEONATAL RATS EITHER WITH HYPOTHYROIDISM OR EUTHYROIDISM BY IN SITU RT-PCR

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Objective: Krev-1 is a signal-transducting protein and we found, in our previous study, that the expression of Krev-1 gene in rat brain can be regulated by TH during the peroid of brain development. In this study, we intended to ascertain the spatial distribution of this gene in the brain of neonatal rat either with hypothyroidism or euthyroidism. **Methods:** 3 months old pregnant SD rats were randomly divided into two groups, one of which was given 2%PTU (150mg/kg·d) through intragastric administration to induce hypothyroidism, the other group that have not been given PTU was taken as the control.7 or 14 days after birth, the neonatal rats were decapulated and the brains were fixed in 10% neutral formaminde embodded in wax. In situ RT-PCR was run for for 20 cycles and the results were quantitatively analysised. **Results:**Krev-1 mRNA expresses at cerebrum, hippocampus, hypothalamic nucleus, thalamic nucleus, amygdaloid nucleus; with a higher expression at thalamic nucleus, hypothalamic nucleus, and amygdaloid nucleaus ($p<0.01$). Compared to the expression of Krev-1 gene in the brain of euthyroidism rats, the expression in the brain of hypothyroidism rats are more intensive at thalamic nucleus, hypothalamic nucleus, and amygdaloid nucleaus ($p<0.01$). **Conclusion:**Our findings show that Krev-1 is widely expressed in rat brain with a significantly higher expression at thalamic nucleus, hypothalamic nucleus, and amygdaloid nucleaus.

P168 SCREENING AND STUDYING THYROID HORMONE-RESPONSE GENES DURING RAT BRAIN DEVELOPMENT

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Objective: To screen thyroid hormone-response genes in perinatal rat brain, study the effects of thyroid hormone on their expressions and the related molecular mechanisms. **Methods:** Established congenital hypothyroidism rat model, screened thyroid hormone-response genes in cerebrum and hippocampus of perinatal rats by fluorescence-labeled DD-PCR analysis. Identified these genes by cloning, sequencing and database search in Gene Bank, further confirmed their differential expressions by Northern blot. Observed the effects of different doses of thyroid hormone and different duration of thyroid hormone deficiency on the transcriptional levels of these thyroid hormone-response genes by semiquantitative RT-PCR analysis in primary cultured cerebral neurons. Administered cyclohemixide to investigate possible regulatory mechanism. **Results:** Hypothyroidism could increase the level of Krev-1 mRNA, decrease the levels of DOC-2 and OSP mRNA during rat brain development. T4 supplement might restore their normal transcriptional levels. Both thyroid hormone deficiency and thyroid hormone in excess could increase the level of Krev-1 mRNA or decrease that of DOC-2 mRNA in primary cultured cerebral neurons. The expressions of Krev-1 and DOC-2 genes affected by thyroid hormone deficiency both appeared delayed changes and the regulatory effects of TH on their transcriptions could be blocked by cycloheximide. **Conclusion:** It suggests that thyroid hormone can regulate the expressions of DOC-2 , Krev-1 and OSP genes in rat brain during perinatal development. The abnormal expressions of these three genes may be partially responsible for neurological deficits in brain arising from thyroid hormone deficiency in the critical period of perinatal rats.

P169 IODINE REPLETION INCREASED THE INCIDENCE OF ATRIAL FIBRILLATION DUE TO THYROTOXICOSIS IN ISFAHAN, IRAN

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Objective: Thyrotoxicosis is one of the causes of atrial fibrillation (AF), a common cardiac arrhythmia. The management of atrial fibrillation due to or exacerbated by hyperthyroidism is difficult or impossible. In this situations clinical manifestations of thyrotoxicosis may be subtle, if present. So, hyperthyroidism should be diagnosed by measurement of serum thyroid hormone concentrations. Iran, had been considered iodine deficient until 1989. Thyrotoxicosis was observed in 3.7% of the patients with atrial fibrillation in university teaching hospitals in Isfahan, a centrally located city in Iran, just before extensive distribution of iodized salt. Repletion of iodine increases the rate of autoimmune thyroid diseases' such as Graves' disease and also it changes multinodular goiter to its toxic form. So, thyrotoxicosis, as a cause of atrial fibrillation is expected to be increased in iodine replete areas. This study was designed to evaluate the rate of thyrotoxicosis in patients with atrial fibrillation in the same hospitals after 9 years of iodized salt distribution.
Methods: In a case-control study with time ordered sampling, one hundred patients with atrial fibrillation were selected in the same university hospitals in 1998. One hundred age and sex matched patients taking similar drugs were chosen as control group. Those who were taking drugs with any effects on thyroid function tests, in both groups, were excluded. Serum thyroid hormone concentrations were measured by RIA and TSH by IRMA methods. Suitable statistical analysis tests were used in SPSS software. P- values less than 0.05 were considered statistically significant.**Results:**Eight percent (8%)of patients with atrial fibrillation had overt thyrotoxicosis versus one percent (1%) in control group (OR = 8.6, CI 95% = 6.5-10.7, P < 0.02). Thyrotoxicosis in patients with atrial fibrillation was 8 times higher than in control group. In comparison to pre-consumption period of iodized salt, it is increased more than two folds (8% vs 3.7%).**Conclusiom:** So, more attention should be paid to complications of iodine repletion, including thyrotoxicosis and its diagnosis, in atrial fibrillation. Although, it is not a reason to stop iodine supplementation.The benefits to the community from correcting iodine deficiency and avoiding its associated disorders far outweigh the damage from iodine-induced hyperthyroidism.

P170 QUANTITATIVE ANALYSIS OF MYOCARDIAL TISSUE BY USING ULTRASONIC BACKSCATTER IN PATIENTS WITH GRAVES' DISEASE.

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Objective: To evaluate the tissue characteristics of cardiomyopathy in Graves' disease patients. **Methods:**26 Graves' disease patients (aged37.38±9.88 years) and 17 normal subjects (aged 33.47±9.13 years) were selected. Integrated backscatter (IB), characteristics curve of IB in cardiac cycle and its variation (CVIB) in the ventricular septum, left ventricular anterior wall, lateral wall and posterior wall were analyzed in the short-axis views at the apical level, the papillary level and the mitral level by Hewlett Packard sonos 5500 ultrasound system with backscatter imaging system. **Results:** The shape of characteristics curve of IB in cardiac cycle was similar to sine wave both in patients and in normal subjects. The CVIB in all segment of left ventricular in Graves' disease was higher than normal（$P<0.05$）, but IB in all segment of left in Graves' disease was as same as normal（$P>0.05$）. **Conclusion:** The myocardial tissue density in Graves' disease was as same as normal, and the variation of myocardial tissue density in cardiac cycle in Graves' disease was higher than normal.

P171 INVESTIGATION OF THE RELATIONSHIP BETWEEN THE HLA-A, B, DR AND PATIENT WITH CHRONIC LYMPHOCYTIC THYROIDITIS (CLT)

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Objective: investigate the relation ship between the HLA and CLT. **Method:** HLA-A, B and DR antigen were determined in 60 patients with chronic proliferat lymphocytic thyroiditis(CLT) and 100 normal control, with used microlymphocytotoxin method for HLA-A, and B antigen; and DNA synthetic initiator with polymerase chain reaction method and PCR amplificate reagent casket for HLA-DR antigen. **Result:** The prevalence of HLA-DR_4, B_8, B_{22}, B_{35}, and A_2 in patients were significance higher than control(DR_4: 28.3% vs 14.7%; B_8: 11.6% vs 0%; B_{22}:30% vs 10%; B_{35} 21.6% vs 8%; A_2 60% vs 41%. P from<0.05-0.01). Howver the prevalence of HLA-B_{15} and DR_5 antigen in patient. Were significance lower than control (HLA-B_{15}: 0% vs 12%, P<0.01;DR_5: 20% vs 12 50%, P<0.05). **Conclusion:** Our date showed the prevalence of DR_9 and Bw_{46} antigen were no significance increase in patients. Additional, we observed the prevalence of DR_4, B_{22}, B_{35} and A_2 in CLT were significance higer than control(P<0.05-0.01). We consider, these high prevalence antigen may be the susceptibility genes of CLT, and those low prevalence antigen may be the protective genes of CLT.

P172 COMPARISON THE EFFECT OF THREE PLAMS IN SUBACUTE THYROIDITIS(SAT)

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Objective: study reduce the relapse rate and side effect of corticoid. **Methods:** We performed three plams (A: prednison 30-40mg/d, n=32; B: prednison 10-15mg/d with decotion n=34 and C: Decotion only, n=44 eath plam were 4 weeks/course)on 110 patients with SAT. **Result:** 1)the relapse rate was 45.4, 21.6 and 4.5% in A, B and C; (2)the side effect of corticoid was 36.4, 20.7 and 0% in A, B and C; (3)the mean time of fever reduce to normal was 10.1,16.3% and 27.3 days in A,B and C; (4)the mean time of nomal sedimentation rate (SR) was 18.5, 20.3 and 22 days in A,B and C. **Conclusion:** We consider the indacations for A high fever, severe neck pain an SR>80mm/h; for B was fever<38°C, moderate neck pain, SR 40-80mm/h; and for C was non conscious fever, mild neck pain, SR <40mm/h additional. the old age patient with hypertension or diabetes and patient with peptic nlcer, were first indicated for C, and the prednison administration may avoid as far as possible.

P173 APPLICATION OF PROPANOLOL IN THE TREATMENT OF HYPERTHYROID HEART DISEASE WITH HEART FAILURE

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Objective: To observe the dosage and effect of propanolol in treating 35 patients of hyperthyroid heart disease with congestive heart failure. **Methods**: The dosage of propanolol was determined by heart rate when ejection fraction (EF) of echocardiogram>50% and the effect digitalis preparation, diuretic and vasodilator was poor in treating hyperthyroid heart diseases with congestive heart failure. **Result**: There were 3, 18 and 14 patients with cardiac function of grade II, III and IV, EF 66.0%, 66.3% and 59.0%,and the dosage of propanolol were 35 mg/d, 55mg/d and 58mg/d, respective. The average recovery time of heart failure was 15.8 days (ranged from 1 to 60 days) and propanolol was of no side in the period of treatment. **Conclusion**: Propanolol can improve heart function and control the symptoms of hyperthyroidism of patients of hyperthyroid heart disease with congestive heart failure. It is safe to use propanolol in the patients with EF>50%.

P174 ALTERATIONS OF LIPIDIC METABOLISM IN PATIENS WITH HYPOTHYROIDISM

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The present study aimed at evaluating the possible alterations in the lipid profile associated with the hypothyroidism.

There was invastigated a lot of patients of Endocrinology Department, diagnosticated with hypothyroidism, during of 2000-th year; they were compared to healthy individuals matched for sex, age and body mass index. Patiens from both sexes were included. Both groups, were similar in relations to menopause, hormone therapy, family history of hyperlipidemia and life habits (sedentarism, alcoholism and cigarette smoking).

We noticed that patients with hypothyroidism had significantly higher total cholesterol, LDL-cholesterol, triglyceride, TC/HDL and LDL/HDL, when compared to the control group.

The results (expresed as the mean+SD) were analyzed by Student test.

There was an association between hypothyroidism and hypercholesterolemia and hypertriglyceridemia, wich can further increase the risk development of atherosclerosis.

P175 TWO PATIENTS WITH HYPOTHYROIDISM MIMICKING ISCHAEMIC HEART DISEASE

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Hypothyroidism may present with symptoms and laboratory abnormalities suggestive of cardiovascular disease. **Case 1.** A 64 years old obese gentleman complaining of two weeks of chest discomfort and exertional dyspnoea was admitted and was initially managed as ischaemic heart disease. Laboratory tests showed elevated serum CK of 3294U/L (normal 53-282), CKMB 3.8% and LDH 663U/L (normal 109-245). Total cholesterol 9.2 mmol/L. Echocardiography revealed normal LV function. On further questioning, the patient admitted to have generalized weakness, cold intolerance and other symptoms of hypothyroidism. This was confirmed by the elevated serum TSH 98.2mIU/L. Electromyography and muscle biopsy showed myopathic changes. Autoimmune primary hypothyroidism with myopathy was diagnosed. Three months after thyroxine replacement was started, his symptoms improved markedly and the serum CK, LDH and total cholesterol level returned to normal. **Case 2.** Another 56 years old obese lady with hypertension and hypercholesterolaemia was admitted because of chest discomfort. Lovastatin was given three days before admission. She was then managed as ischaemic heart disease. Serum CK was high (6633U/L) and was muscle in origin. Statin-induced myositis was diagnosed. CK remained elevated after lovastatin was withheld. Subsequent investigations showed TSH 66.34 mIU/L (0.32-5.00) and both anti-thyroglobulin & anti-microsomal antibodies were positive (1:6400 and 1:102400 respectively). Primary autoimmune hypothyroidism with myopathy was diagnosed. CK was normalized three months after thyroxine replacement therapy was given. **Conclusion:** Hypothyroidism may mimic cardiac problems. Persistently elevated cardiac enzymes with cardiovascular symptoms but without demonstrable myocardial damage should prompt consideration of hypothyroidism.

P176 THYROID SCINTIGRAPHY AND ULTRASONIC SCANNING IN THE EVALUATION OF CONGENITAL HYPOTHYROIDISM

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Objective: To evaluate clinical value of thyroid scintigraphy and ultrasonic scanning in the pathogenesis and prognosis of congenital hypothyroidism. **Methods:** By the end of 2000, 79 cases of CHT were found in Tianjin, 56 cases (26 male, 30 female) of them were investigated with Tc-99m for confirmatory diagnosis, 26 cases were examined with ultrasonography. 14 cases were rechecked with Tc-99m during the treatment. **Results:** The results of initial Tc-99m scanning in 56 cases showed: 26 cases (46.5%) with athyrea, 6 cases (10.7%) with hypoplasia, 18 cases (32.1%) with ectopic gland, 6 cases (10.7%) with goiter and uptake increasing. The first three results were due to thyroid abnormal growth, the last one was involved in hereditary abnormal synthesis of thyroid hormone. Among 26 cases checked by US, 6 cases (hypoplasia, ectopic gland and goiter each two) matched with clinical findings, 5 cases of the rest 20 (athyrea) were confirmed with thyroid tissue. In 14 cases scanned for second time, comparing with original scintigraphy, 5 cases with athyrea changed. 2 cases became hypoplasia, 3 cases became ectopic. It indicated some abnormal growth gland, with the age increasing, may partly restored function. **Conclusion:** The main cause of CHT is thyroid abnormal growth, Tc-99m scanning is a reliable modality to find the pathogenesis of CHT, the ultrasonography is a good complementary method, and it is vital for Tc-99m scanning to evaluate prognosis of CHT.

P177 ULTRASONOGRAPHIC EVALUATION OF ENDOMETRIAL GROWTH IN WOMEN UNDERGOING ASSISTED REPRODUCTION DURING THE LUTEAL PHASE IN FRESH AND FROZEN EMBRYO TRANSFER CYCLES

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Objectives: To compare the sonographic endometrial growth pattern in conception and non-conception cycles during the luteal phase of ovarian stimulation cycles for in-vitro fertilization (IVF) and spontaneous cycles of frozen embryo transfer (FET).
Methods One hundred and sixty cycles of women undergoing assisted reproduction treatment were evaluated in the luteal phase. Endometrial data were related to the day of luteinizing hormone surge (LH) or the hCG administration day (day 0). Ultrasonographic evaluation of endometrial thickness was performed using 5MhZ transvaginal probe on day 0, day 4 and day 10. Serum oestradiol (E_2) and progestrone (P) levels were measured on the corresponding days.
Results: In IVF (n=100), 23 conception cycles were compared with 87 non-conception cycle. There was no difference in the endometrial thickness, serum E_2 and P levels between conception and non conception cycles at all time points in the luteal phase. In FET cycles (n=60), a similar trend was observed on day 0 and day 4, between conception (n=13) and non-conception cycles (n=57). However, by the mid luteal phase the endometrium of conception cycles (12±2.3mm; mean ± SD) demonstrated a significantly ($P<0.05$) greater thickness than the non-conception cycles (10 ±2.9mm). Similarly, on day 10 serum E_2 (784±422 pmol/L) and P levels (83±30 nmol/L) in conception cycles were significantly higher than those in non-conception cycles (E_2 524±434 pmol/L., P 54±52 nmol/L). This pattern was not observed in IVF cycles. The sonographic thickness was similar at all time points in the luteal phase between conception and non-conception IVF cycles.
Conclusions: The pattern of endometrial development in IVF cycles was different from that in FET cycles. These differences in the late luteal phase can be related to the endometrial growth pattern, serum E_2 and P levels between conception and conception cycles.

P178 SATURATED FREE FATTY ACIDS INDUCE APOPTOSIS IN HUMAN GRANULOSA CELLS BY THEIR METABOLITES ACYL-COA

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Objective: Obesity is associated with insulin resistance and some reproductive abnormalities. Circulating free fatty acids (FFAs) are often elevated in obese subjects and are also closely linked to insulin resistance. In this study, we aimed to investigate the apoptotic effect of FFAs on human granulosa cells. **Methods:** (1) The granulosa cells were treated with various concentrations of saturated FFAs including palmitic acid (PA) and stearic acid (SA), and unsaturated FFAs including arachidonic acid (AA), oleic acid (OA) and linoleic acid (LA) for one to three days and then the cell viability was determined by trypan blue exclusion using a hemocytometer; (2) DNA fragmentation was examined by DNA ladder formation and Annexin V-GFP/PI staining of the cells; (3) To determine if the apoptotic effect of PA and SA was mediated by their acly-CoA metabolites and related to the increase of ceramide generation or NO production; (4) To measure the expression levels of apoptosis relative genes, Bcl-2 and Bax, by Western blot. **Results:** (1) PA and SA markedly suppressed the granulosa cell survival in a time- and dose-dependent manner. Unsaturated FFAs had little or no effect on the cell survival; (2) The suppressive effect of saturated FFAs on cell survival was caused by apoptosis, as evidenced by DNA ladder formation and Annexin V-GFP/PI staining of the cells; (3) The apoptotic effects of PA and SA were completely blocked by Triacsin C, an inhibitor of acyl-CoA synthetase and were unrelated to ceramide or NO production. In addition, acyl-CoA, pamitoyl CoA and stearoyl CoA markedly suppressed cell survival while arachidonic CoA had no such effect; (4) A Western blot analysis showed the apoptosis of the cells induced by PA to be accompanied by the down-regulation of Bcl-2 and the up-regulation of Bax. **Conclusion:** Saturated FFAs induce apoptosis in human granulosa cells due to the metabolite of the respective acyl-CoA form and these apoptotic effects may be a possible mechanism for reproductive abnormalities in obese women.

P179 PPARγ AND RXR LIGANDS INHIBIT THE AROMATASE ACTIVITY AND THE EXPRESSION OF P450AROM mRNA IN HUMAN GRANULOSA CELLS

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Objective: To investigate the effects of PPARγ and RXR ligands on aromatase activity (AA) and P450arom mRNA in human granulosa cells. **Methods:** (1) Measurement of aromatase activity and estrone (E1) production in granulosa cells before and after treatment with PPARγ ligands troglitazone (TGZ) and pioglitazone and RXR ligands LG100268 (LG) and other reagents; (2) determination of P450arom and PPARγ mRNA expression levels by RNase protection assay and P450arom transcription by nuclear run-on assay; (3) P450arom promoter II analysis. **Results:** (1) TGZ, pioglitazone, a native PPARγ ligand PGJ2 or LG alone significantly inhibited AA to about 45% to 54% of the baseline, whereas the combined treatment with TGZ, pioglitazone or PGJ2 and LG further inhibited AA to about 20% to 30% of the baseline. These inhibitory effects were dose- and time-dependent. E1 production in the media was also decreased by the same treatment; (2) The changes in P450arom mRNA levels were associated with comparable changes in AA. PPARγ1 was predominately expressed in the cells, but the expression level was not changed by the treatment. TGZ and LG regulated the P450arom mRNA at both the transcription level and post-transcription level based on a nuclear run-on assay and an RNA stability analysis; (3) The inhibitory effect of PPARγ and RXR on AA and P450arom mRNA was not mediated by the cAMP-PKA-CREB pathway that is usually implicated in the regulation of AA in granulosa cells because a) AA stimulated by forskolin was not inhibited by TGZ plus LG; b) the specific PKA inhibitor H89 could not block the inhibitory effect and c) the luciferase activity controlled by P450arom promoter II that contained CREB response element (CRE) was not decreased by the addition of TGZ and LG in transfected cells. **Conclusion:** TGZ plus LG inhibited AA and also decreased the P450arom mRNA level in granulosa cells, and the loss of P450arom mRNA expression was considered to be due to both the decreased transcription and rapid degradation of its mRNA.

P180 *IN SITU* MOLECULAR IMMUNOLABELING OF DNA AND CYTOCHEMICAL LABELING OF HYALURONIC ACID DURING NUCLEAR CHANGES IN APOPTOTIC GRANULOSA AND CUMULUS CELLS

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Objective: We examined the sequential changes that occurred in the nucleus of apoptotic granulosa and cumulus cells in the hamster ovarian follicles and the oocyte-cumulus complexes (OCCs), respectively. **Methods:** OCCs and ovaries were obtained from sexually mature golden hamsters, fixed in appropriate fixative, and then further processed for both L.M. and E.M. immunocytochemistry. The distribution of DNA and hyaluronic acid in the nucleus of granulosa and cumulus cells during various stages of apoptosis were studied using, respectively, the molecular biology-based *in situ* terminal deoxynucleotidyl transferase-immunogold technique and the hyaluronidase-gold approach. **Results:** Our results showed that the break down of the nucleus in apoptotic granulosa and cumulus cells follows a highly ordered manner. During the process, the nucleus is broken up into several apoptotic nuclear bodies each of which consists of two distinct subcompartments, namely, heterochromatin and euchromatin. Both DNA and hyaluronic acid were localized to the heterochromatin subcompartment in the apoptotic nuclear bodies. **Conclusion:** The localization of both DNA and hyaluronic acid to the same regions of the nucleus suggests the possibility that hyaluronic acid may play a role in nuclear function through the interaction with DNA-protein complex in the nucleosome. (Supported by the Canadian Institutes of Health Research)

P181 QUANTIFICATION OF TRANSFORMING GROWTH FACTOR b1 (TGF b1) mRNA EXPRESSION IN MOUSE PREIMPLANTATION EMBRYOS AND DETERMINATION OF TGFb RECEPTORS (TYPE I AND TYPE II) EXPRESSION IN MOUSE EMBRYOS AND REPRODUCTIVE TRACT

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We reported the quantification of TGFb1 mRNA in mouse embryos by competitive reverse transcription–polymerase chain reaction using RNA mimic. TGFb1 was first detected in the unfertilized oocyte, disappear after fertilization and expressed again at 2-cell stage (7.3±2.2 x 10-3 attomoles/embryo). It increases gradually and peaked at 8-cell stage (97.4±28.6 x 10-3 attomoles/embryo). The expression declines rapidly after morula stage and the concentration of TGFb1 mRNA at the blastocyst stage was 2.5±0.9 x 10-3 attomoles/embryo. The mRNA levels of TGFb1 at 8-cell and morula stages were significantly higher than that in other cell stages ($p < 0.05$). TGFb receptors [Type I (ALK-5) and Type II] were detected in 1-cell, 2-cell, morula and blastocyst stage embryos by immunocytochemistry. Northern hybridization and immunohistochemistry showed a constant expression of both TGFb receptors in the oviduct from Days 1-4 of pregnancy, whilst there was a marked increase in the expression of TGFb Type I receptor in Day 3 uterus. Expression of TGFb Type II receptor in the uterus remained unaltered throughout the studied period. These observations indicate that preimplantation mouse embryos produce TGFb1, and that the embryos as well as the reproductive tract are responsive to TGFb1 in the preimplantation period. This study strengthens the hypothesis that there is a close interaction between the preimplantation embryo and the reproductive tract via TGFb1.

P182 IMMORTALIZED AND PRIMARY HUMAN OVIDUCTAL EPITHELIAL CELLS PRODUCE ETF-3 THAT STIMULATE EMBRYO DEVELOPMENT

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Objective: To study the glycoprotein fractions 3 (ETF-3) secreted from immortalized and primary human oviductal epithelial cells and their embryotrophic activities on mouse embryo development. **Methods:** The primary human oviductal epithelial cells (OE) were immortalized (OE-E6/E7). By using Liquid Chromatography system, ETF-3 was derived from OE-E6/E7 (F3) and OE (P3). F3 and P3 were separated by electrophoresis under native and denatured conditions. They were then reconstituted with Chatot-Ziomek-Bavister (CZB) medium and used to culture Day 1 MF1xBALC/c embryos for 4 days. CZB medium alone culture was included as control. Embryo development was assessed at 2-cell, 4-cell, morula and blastocyst stages. **Results:** After electrophoesis, similar protein patterns were found in F3 and P3 under native and denatured conditions. Embryo culture showed that there are no significant difference in the cleavage rate among control, F3 and P3. However, the sizes of F3 and P3 treated blastocysts were significantly larger than control group (101±1.27mm and 105±1.17mm Vs 93±2.14mm, respectively). **Conclusion:** These results showed that F3 has similar embryotrophic avtivity as P3. F3 can be used for further characterization of the embryotrophic factors secreted from the human oviductal epithelial cells.
Acknowledgement: This study is supported by grants (HKU 241/95M, HKU 733/97M) from the Research Grant Council, Hong Kong

P183 CLINICAL COURSES OF ARTERIAL HYPERTENSION IN DIFFERENT ADRENAL GLAND TUMORS

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Investigations was carried out on 28 patients, age from 17 to 52 years, with arterial hypertension (AH) due to adrenal tumor. Were revealed hyperaldosteronism in 9 patients, pheochromocytoma in 4, Cushing syndrome in 8, adrenal carcinoma in 3, giant tumor with metastasis into liver, kidneys and intestine in 1 and adrenal incedentaloma in 4. All patients received complex investigation including clinical and hormonal with determination of the daily rhythm of secretion, electrolytes in blood and urine and computed tomography of the adrenals.
RESULTS: Typical crisis course of AH was observed in 14,2% with slight increase in catecholamine levels. Hypertonic crises with persistent AH were found in 28,5%, who had increase in plasma aldosteron and renin levels. The constant increase in AH without crises was noted in all the 16 cases, which was resis was resistant to hypotensive therapy. It should be underlined that the average fluctuations of the arterial pressure was from 150/80 till 300/180 mm Hg. However the average systolic pressure was 205 ±31,5, average diastolic pressure was 125±25,4 mm Hg. The very high levels of the arterial pressure were characterized for patients with pheochromocytoma, and relatively low pressure - for patients with Cushing syndrome. Computed tomography revealed that bilateral hyperplasia was in 8 (28,5%) cases, the right adrenal gland adenoma with the signs of Cushing syndrome in 3 (10,7%) cases left-side adrenal gland tumor in 17 (60,7%) cases, and in 5 cases tumor began from brain and proliferation into adrenal cortex.
CONCLUSION : Arterial hypertension of adrenal genesis occurs mainly among men and it is freqently accompanied by disorders in aldosterone secretion and affects the left adrenal gland.

P184 ADRENAL AUTOTRANSPLANTATION AFTER BILATERAL STAGED ADRENALECTOMY IN PATIENTS WITH ACTH-DEPENDENT CUSHING'S DISEASE

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Effectiveness of staged bilateral adrenalectomy with autotransplantation of adrenal gland tissue in patients with ACTH-dependent Cushing's disease has been analyzed. **MATERIALS AND METHODS:** Intraoperative autotransplantation of native adrenal gland tissue was performed in 9 patients. Bilateral staged adrenalectomy with autotransplantation was carried out in patients, after first unilateral adrenalectomy had been ineffective. Native adrenal gland tissue was autotransplanted into right forearm intraoperatively. **RESULTS:** Significant cortisol secretion has been showed on 7th day after autotransplantation of adrenal glands (401+39 nmol/L vs. 270±12,7 nmol/L, $P<0.05$). This significance was kept till 5-6 months after surgery and then tends to decrease. At 6th month cortisol was 248±14,4 nmol/L vs. 209±8,3 nmol/L ($P<0.05$) and at 12th month 193±9,8 nmol/L vs. 172±10,1 nmol/L ($P>0.05$) respectively. During first 6 months all patients were administered minimal hormonal replacement therapy of prednisolone in dose 2,5-5 mg/day and there were no need for mineralocorticoid administration. Then dose of prednisolone was increased up to 7,5-10 mg/day due to sighs of chronic adrenal insufficiency developed. **CONCLUSION:** Bilateral staged adrenalectomy with intraoperative autotransplantation of adrenal gland tissue in patients with ACTH-dependent Cushing's disease can contribute adequate functional activity of pituitary-adrenal axis during 6 months after surgery.

P185 PERSISTENT HYPERTENSION AFTER ADRENALECTOMY IN PATIENTS WITH CONN'S ADENOMAS

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Objective: to investigate the prevalence and factors affecting persistent hypertension after adrenalectomy in patients with Conn's adenomas. **Method:** 15 patients (7 males and 8 females) who underwent adrenalectomy for Conn's adenomas at UCH from January 1994 to June 2001 were studied retrospectively. **Results:** all 15 patients were surgically cured with normokalaemia. 6 patients (40%) were rendered normotensive while 9 (60%) had persistent hypertension. The number of anti-hypertensive medications in the latter group was reduced from 2 to 1 post-operatively. Both multi- and univariate analyses showed that the group with persistent hypertension differed significantly from the normotensive group by the age of onset (47.7±4.0 *vs* 36.0±4.7) and duration of hypertension pre-operatively (11.0±3.4 *vs* 3.5±1.3), but not by pre-operative blood pressure, serum K, plasma renin / aldosterone levels, 24 hr urine K excretion or tumour size. **Conclusion:** 60% patients had persistent but improved hypertension post-operatively. The age of onset and pre-operative duration of hypertension were predictive of persistent hypertension after adrenalectomty.

P186 THE EFFECTS AND SIGNIFICANCE OF ADRENOMDULLIN ON HYPOXIC PULMONARY HYPRETENSIONS

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Objective: Adrenomedullin (AM) is a newly discovered potent hypotensive peptide which is believed to play an important role for blood pressure control. They were studies effect of hypoxia on ADM release and ADM mRNA as well as ADMR mRNA expression . **Method**: We used cell culture, immunocytochemistry, radioimmunoassy, image analysis, and reverse transcription polymerizes chain reaction (RT-PCR) methods. **Results:** The expression of ADM mRNA and ADMR mRNA was released, More ADM was released in cultivated vascular endothelial cells. Extrinsic ADM hindered the proliferation of cultivated vascular smooth cells. After ADM receptor antagonist was given, effects of ADM were antagonized. **Conclusion:** Hypoxia induces and strengthens expression of ADM mRNA and ADMR mRNA with the increased release of ADM. ADM increase pulmonary vasoconstriction and pulmonary vascular remodeling during hypoxia. ADM may have a potential effect on the treatment of hypoxic pulmonary hypertension (HPH) of chronic cor pulmonale.

P187 IMMUNOHISTOCHEMICAL LOCALIZATION OF ADRENOMEDULLIN IN ADRENAL DISEASES

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Objective: Adrenomedullin(ADM) is a hypotensive peptide isolated from pheochromocytoma tissues. In order to identify pathophysiological effects of ADM on the etiology of adrenal diseases which can induce hypertension, the localization of immunoreactive adrenomedullin (ir-ADM) in tissues of human normal adrenal and different adrenal diseases was investigated by immunohistochemical staining. **Methods:** Peroxidase anti-peroxidase complex method was undertaken in tissues of human normal adrenal cortex (n=10) and medulla (n=10), pheochromocytoma (n=31), primary aldosteronism including adrenocortical adenoma (n=13) and hyperplasia (n=9), Cushing's syndrome including adrenocortical adenoma (n=12), hyperplasia (n=10), carcinoma (n=4) and non-functional adrenocortical carcinoma (n=7). **Results:** Positive immunohistochemical staining of ir-ADM was observed in all cases of normal adrenal medulla and 16 of 31 cases of pheochromocytoma. The intensity of immunohistochemical staining in the majority of pheochromocytoma was lower than normal adrenal medulla ($P<0.01$). The amount of cases with positive staining in paroxysmal hypertensive group were more than in sustained hypertensive group of pheochromocytoma ($P<0.05$). Negative immunohistochemical staining was found in human normal adrenal cortex and all adrenocortical diseases. **Conclusion:** It suggests that heterogeneous localization of ir-ADM in pheochromocytoma tissues may be correlated to the diversity of clinical manifestations in patients with pheochromocytoma and the pathophysiological effects of ADM on pheochromocytoma should be investigated.

P188 CONGENITAL ADRENAL HYPERPLASIA OF 21-HYDROXYLASE DEFICIENCY GENE MUTATION STUDY

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Objective: To detect 32 patients of 21-hydroxylase gene (CYP21) mutation. **Methodes**: Direct detected CYP21 in exon 3 300bp and in exon 6 600bp deletion by using polymerase chain reaction (PCR). Also detected CYP21 in intron 2 656, in exon 4 codon 172 and in exon 8 codon 356 point mutation by using polymerase chain reaction combining amplification created restriction site (PCR-ACRS). **Results**: 3 patients show CYP21 in exon 3 300bp, 3 patients show CYP21 in exon 6 600bp deletion, 3 patients show CYP21 in exon 3 300bp and exon 6 600bp deletion. 3 patients show CYP21 in intron 2 656, 2 patients in exon 4 codon 172, and 3 patients in exon 8 codon 356 point mutation. **Conclusion**: the two methods to detect CYP21 mutation are the most simplest way and practical.

P189 MISSENSE MUTATION $Arg^{96}\rightarrow Gln$ AND DELETION OF Asp^{487}-Ser^{488}-Phe^{489} CAUSE 17 α - HYDROXYLASE/17,20- LYASE DEFICIENCY

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Objective: To study the molecular genetic mechanism of a Chinese patient with 17α-hydroxylase/17,20-lyase deficiency. **Methods:** CYP17 gene of the patient was amplified with polymerase chain reaction(PCR) and performed the sequence analysis using the dideoxy terminator method. The corresponding exons of the parents were also amplified and sequenced to determine the zygosity and mutant source of the patient. **Results:** The analysis revealed the patient was a compound heterozygote, carrying two different inherited mutant alleles on CYP17 gene. One allele, from her mother, contains a point mutation Arg^{96}(CGG)→Gln(CAG); Another allele, from her father, contains a nine-base deletion at amino acid position 487~489（Asp-Ser-Phe） near the carboxyl-terminus of P450c17. **Conclusions:** The CYP17 gene of the patient(46,XY) with 17α-hydroxylase deficiency is a heterozygous mutation. The mutation changes the amino acid sequence of P450c17 enzyme, thus the enzyme activity is affected, which causes the disease probably. We suggest Arg^{96} is very important in the P450c17 enzyme structure and deletion of Asp^{487}-Ser^{488}-Phe^{489} may be a prevalent mutation causing P450c17 deficiency in Southeast Asia.

P190 RECOMBINANT HUMAN ENDOTHELIN-CONVERTING ENZYME EXPRESSING IN CHO-K1 CELLS

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Objective: To prepare enough endothelin-converting enzyme (ECE) for laboratory use. **Methods:** After total RNA was isolated from normal human adrenal tissue, a 1.6kbp of partial cDNA encoding human ECE was obtained by RT-PCR. This cDNA was used as a probe for screening HUVEC cDNA library, two types of cDNAs, PHECE1α and PHECE1β, corresponding to human ECE-1α and ECE-1β, respectively, were cloned, and were then subcloned into the expression vector PME18. The resultant expression plasmid constructs PME18 ECE-1α and PME18 ECE-1β, were transfected into CHO-K1 cell. **Results:** 1. The length of the two expression plasmid PME18 ECE-1α and PME18 ECE-1β is consistent to that of theoretically predicted. 2. The cDNA sequence of cloned ECE-1α and ECE-1β was identical to the former reported. 3. ECE activity from transfected CHO-K1 cells is 0.192±0.0018nmol/h.mg (mean±SD, n=4), while that from untransfected CHO-K1 cells is less than 0.0004nmol/h.mg ($p<0.001$). **Conclusion:** Human endothelin-converting enzyme gene was cloned and expressed in CHO-K1 cells successfully.

P191 Expression of Recombinant Human Insulin-Like Growth Factor-1 in Silkworm Larvae By a Baculovirus Vector

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Objective: To study the efficient expression and production of biologically active recombinant human insulin-like growth factor 1 (rhIGF-1) in the silkworm system by the vector of Bombix mori Nuclear Polyhedrosis Virus (BmNPV). **Methods:** A full length IGF-1 cDNA with the start codon at position –25 was inserted into the BmNPV genome under the control of the polyhedrin promoter. Silkworm larvae (JY1) were used as hosts for the infection with recombinant BmNPV /IGF-1. rhIGF-1 concentration were detected by ELISA, and Western blot was used to find the size of IGF-1, while MTT pathway was involved to study the bioactivity of rhIGF-1 by stimulation the growth of NIH3T3 cell line. **Results:** It was found that from 72h to 120h post infection, the concentration of IGF-1 in silkworm hemolymph was increased gradually(19.09-23.74ug/ml), and mature rhIGF-1 with a size of 7.5 kd was identified by Western blot analysis. It was also showed that rhIGF-1 has excellent bioactivity by stimulated NIH3T3 cell growth. Further verification was performed by specific inhibition using IGF-1 receptor antibody, IGF-1Ra. **Conclusion:** Human IGF-1 was highly expressed by the BmNPV expression vector, this production showed satisfied in bioactivity thus providing a very efficient way to produce soluble and biologically active mature rhIGF-1.

P192 STUDIES ON THE INTERACTING PROTEINS WITH INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-1 (IGFBP-1) BY YEAST TWO-HYBRID SYSTEM.

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Objective: To investigate those proteins that may be interacting with IGFBP-1. **Methods:** The human IGFBP-1 cDNA was isolated by PCR from fetal hepatic cDNA library and confirmed by sequence analysis. Then the proteins interacting with IGFBP-1 were screened from the fetal hepatic library by yeast two hybrid system. **Results:** IGFBP-1 showed the transcriptional activation. A mutant was designed and constructed by deleting 27 amino acid of the carboxyl end of IGFBP-1. The transcriptional activation was deleted by constructing the mutant. Seven genes, which encode proteins interacting with the mutant of the IGFBP-1, were found. 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:** These proteins interacting with IGFBP-1 may be new cues for investigating new biological activity and mechanism of IGFBP-1.

P193 STUDY ON GENE EXPRESSION PROFILING IN HUMAN INSULINOMA TISSUE

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Objective: To establish the gene expression profiling in human insulinoma tissue and search for the genes related to insulin secretion. **Methods**: Insulinoma tissue was obtained from a patient with insulinoma verified by pathologic examination. mRNA was seperated and purified. cDNA libraries were constructed. Expressed Sequence tags(ESTs) were obtained through sequencing reactions and analyzed by bioinformatics. [^{33}P]dCTP labeled cDNA derived from insulinoma mRNA was hybridized to a DNA array containing over 14,000 human genes and ESTs. The results were analyzed by ArrayGuage software. **Results**: Totally,we obtained 1852 clusters among which 1378 were derived from ESTs sequencing,975 from cDNA array,and 501 were found in both experiments. Among the 1852 clusters,known genes,known ESTs and novel ESTs were 633(34.18%), 846(45.68%) and 373(20.14%) respectively. The order of genes expressed in insulinoma by ESTs sequencing was human islet Langerhans regeneration protein gene(13 copies),insulin gene(11 copies) and WNTB2 gene(5 copies).Human Ly-6-related protein gene(S/N=25.8) followed by Human mRNA for carboxypeptidase E (S/N=25.3) was the most highly expressed by means of cDNA array.**Conclusion**: For the first time,we combine ESTs sequencing and cDNA array to establish gene expression profiling in insulinoma tissue. Human islet Langerhans regeneration protein is an important protein which can promote fetal pancreatic duct cells converting to pancreatic β cells and stimulate β cells growth.We speculate that Human islet Langerhans regeneration protein may play an important role in occurance of insulinoma and insulin oversecretion. WNTB2 is a kind of protein of WNT(wingless-type MMTV intergration site) family whose members are related to carcinogenesis and can stimulate cell growth.

P194 ZINC UPTAKE AND INDUCTION OF APOPTOSIS IN HUMAN PROSTATIC CELLS

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Objectives: to investigate the cell specificity of zinc uptake and the induction of apoptosis; to establish the mechanism (s) of apoptotic effect of zinc on the mitochondrial release of cytochrome c. **Methods:** Human prostate cell lines, e. g. normal cells (HPR-1), benign prostatic hyperplasia (BPH) and malignant cells (PC-3), were used. The zinc uptake was determined by TSQ assay. The effect of zinc on cell growth was determined by surviving cell numbers. Apoptosis was characterized by morphological features and DNA fragmentation. Western blot was employed to determine the levels of cytochrome c. **Results:** After zinc treatment, a significant higher levels of zinc was observed in the cytosol of PC-3 (4.3. fold vs. control) and BPH (2.7 fold vs. control) cells compared with that in HPR-1 (1.8 fold vs. control). The growth inhibitory effect of zinc was only detected in PC-3 and BPH cells (over 65% reduction of cell numbers vs. control). Zinc-induced apoptosis was evident in PC-3 and BPH cells by apoptotic bodies and DNA fragmentation, but not in HPR-1 cells. Western blot indicated that zinc significantly induced mitochondrial release of cytochrome c in PC-3 (2.0-2.9 fold vs. control) and BPH (1.4-2.3 fold vs. control)after 20' incubation with zinc, in contrast to that in HPR-1 (<1.1 fold vs. control). **Conclusions:** Zinc induces apoptosis in prostatic carcinoma and benign hyperplasia cells through the mechanism of zinc-induced mitochondrial release of cytochrome c, but not in normal prostate cells.

P195 The effects of cimetidine on abomasal secretion during pentagastrin stimulation in sheep

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In this study 10 Iranian cross-bred male sheep, between 2 to 3 year of age, weighing (58.85+14.69 kg) were used. An abomasal pouch was made in each animal and the effect of pentagastrin (4 mg/kg S.C.) and cimetidine in different doses (3.5 and 4.5 mg/kg I.M.) on abomasal secretion (volume, pH, total acidity and concentrations of pepsin, potassium, chloride and sodium) were assessed during feeding. The results showed that administration of pentagastrin (4mg/kg S.C.) caused a significant decrease in gastric secretion, total acidity and concentration of K+; and a significant increase in concentration of Na+ ($P<0.05$).

No significant changes were observed in pepsin , Cl-concentrations and pH level due to administrtion of pentagastrin ($P>0.05$). Administration of cimetidine in different doses (1and2) and various conditions (before and after abomasal stimulation with pentagastrin) showed similar effects on different factors mentioned above. Gastric secretion, total acidity and concentration of K+ decreased significantly whereas pH level and concentration of Na+ increased significantly at different hours after injection ($P<0.05$). However, concentrations of pepsin and Cl- sometimes showed a significant decrease which were not noticible. Separate administration of cimetidine at different doses (1and2) showed antagonistic properties began within 0.5 hour. Maximal effects for cimetidine (1and2) occurred within the 1.5 hour after injection.

Administration of pentagastrin after secretory inhibition by cimetidine (1and2) showed that their antagonism began within 0.5 hour and reached to a maximal level 1.5 hour postinjectionally. Administration of cimetidine (1and2) after secretory stimulation by pentagastrin showed; that their antagonistic properties began approximately within 0.5 hour.

P196 The Relationship of Polymorphism of the Cholesterol 7-alpha-hydroxylase Gene and the Concentrations of Low-density Lipoprotein Cholesterol in the population of the Chinese in Shanghai

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Objective: To investigated the difference of the polymorphism, an A to C substitution at the position of 278bp upstream of the translation initiation codon of CYP7 gene, between the obese and the general population. **Methods:** 68 individuals with BMI>25 and 92 with normal BMI were involved in this study. The variations of C→A substitution 278 bp upstream of the translation initiation in the DNA were identified by the method of restriction fragment length polymorphism (RFLP). Serum cholesterol, low-density lipoprotein cholesterol (LDL-C), triglyceride concentrations were determined. **Results:** There were 3 subjects of AA genotype, 59 of AC genotype, and 98 of CC genotype in this study, share 1.9%, 36.9% and 61.2%, respectively. It is differ from the report in Caucasus, but no significant differences between obese and non-obese subjects ($P>0.05$). There were only 3 AA genotype subjects in this study, we did not analyses their serum lipid profile to avoid the sampling error. The concentrations of LDL-C in CC individuals were significantly higher than in AC individuals. There were no significant difference in concentration of total cholesterol and triglyceride. The frequency with promoted serum LDL-C concentration in CC individuals was significantly higher than in AC individuals. **Conclusion:** The polymorphism in CYP7 contributes to heritable variation in serum LDL-C concentrations. The serum lipid profile of AA genotype and the difference of this polymorphism between Chinese and Caucasus remain elucidated in further study.

P197 DNA-BASED DIAGNOSIS OF FAMILIAL AMYLOIDOTIC POLYNEUROPATHY TYPE I IN A HONG KONG CHINESE KINDRED

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Familial amyloidotic polyneuropathy type 1 (FAP1, MIM 176300) is an autosomal dominant disease characterized by late onset progressive polyneuropathy, autonomic dysfunction, cardiomyopathy and gastrointestinal tract disorder. The disease is caused by transthyretin mutations. The transthyretin gene (*TTR*) spans 6944 nucleotides on chromsome 18q11.2 and consists of 4 exons and 3 introns. To date, over 80 *TTR* mutations have been reported, the majority of which is inherited in an autosomal manner and is related to amyloid deposition affecting predominantly peripheral nerve and/or the heart. To our knowledge, FAP1 was first reported in an extended Hong Kong Chinese kindred in 1989. In this family, three of the 4 histologically proven subjects have deceased. However the molecular basis of FAP1 in this family was not known. Pre-symptomatic carrier detection for FAP1 is not feasible because serum transthyretin concentrations in the probands are often normal. In this study, we have performed DNA-based diagnosis of FAP1 by restriction analysis and direct DNA sequencing in a symptomatic member of this family, who underwent a liver transplantation recently. DNA sequencing showed a cytosine for thymine substitution in the second base of codon 30, i.e., GTG→GCG and the creation of a novel *Hha*I restriction endonuclease site in exon 2. This mutation results in a substitution of an alanine for valine in the mutant TTR protein, i.e., Val30Ala (V30A). The same mutation was first reported in 1992 in a FAP family of German descent. In this study both restriction analysis and direct sequencing revealed the presence of V30A in one of the two asymptomatic siblings studied. DNA-based diagnosis of FAP1 enables us to make an accurate determination of carrier status, to perform prenatal diagnosis of this disease, and to instigate early therapeutic interventions, such as liver transplantation, to abort disease progression in pre-symptomatic carriers.

P198 POLYCYSTIC OVARIAN SYNDROME ASSOCIATED WITH WILSON'S DISEASE

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Polycystic Ovarian Syndrome is defined as menstrual irregularity and hyperandrogenism in a woman with no evidence of androgen-secreting tumor or congenital adrenal hyperplasia. A Chinese lady aged 31 years presented with recent weight gain, easy fatigue, slow reaction and tremor. She was obese with body weight of 78.5 Kg (BMI, 30.6 Kg/m^2). She had irregular menses and oligomenorrhoea for one year. She was also found to be hirsute. Her biochemical results were as follows: TSH, 1.11 mIU/L (0.35-5.50); Prolactin, 78 mIU/L (59-619); 24 hour urinary free cortisol excretion, 15 nmol/mmol crea (5-23); Testosterone, 6 nmol/L (0.5-2.6) E2, 395 pmol/L; LH/FSH ratio, 1.52. USG Pelvis was unremarkable and computerized tomogram of adrenals ruled out androgen-secreting tumor. Stimulated 17-OH Progesterone was 12 nmol/L excluding congenital adrenal hyperplasia. OGTT was normal. In addition to the features of PCOS, she was also found to have psychomotor retardation, intentional tremor and scanning speech. Physical examination revealed Kayser Fleisher ring at the cornea. Biochemical findings of reduced serum copper 3.2 umol/L (11.1-22.3), ceruloplasmin <30 mg/L (180-530) and increased urinary copper 3.28 umol/d (<1.20) confirmed the diagnosis of Wilson's disease. Literature review showed reports of association between hyperandrogenism and Wilson's disease. An interference of ovarian follicular aromatase activity due to copper intoxication is believed to be the cause of ovarian hyperandrogenism as a result of Wilson's disease.

P199 Patient with McCune-Albright syndrome caused by complex heterozygous missense mutation in the α subunit of the stimulating GTP-binding protein gene

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Objective: McCune-Albright syndrome(MAS)is a rare clinical disease caused by somatic mutations. The latest research revealed that MAS resulted from the somatic mutations of the α subunit of the stimulating GTP-binding protein. The classical of this pattern is the missense mutation of Arg201, which is replaced by either Cys or His. To detect the mutational sites of Gsα gene of the patients is a sensitive and reliable diagnostic mean.

Methods: In our study we examined the biochemical index, endocrine function index and carried out radiodiagnosis, osseous scanning, pathologic check-up of the younger patient. After extracted DNA from the blood and affected iliac tissue, we amplified the exon 8 and exon 9 of the Gsa gene, then subcloning, digesting by Alw I incision enzyme and sequencing in order to investigate the mutational state of this gene.

Results: (1) The patientshow polyostotic fibrous dysplasia, cafe-au-lait skin lesions. The AKP is distinctly increased, so are the urinary pyridol and erythrocyte sedimentation. GH and PTH are higher than normal. (2) We find the classical mutation existed in the position Arg201 that was replaced by Cys201 in both blood and pathologic osseous tissues. Interesting, we also discovered other three new mutational sites（E209G，GAC-GGG；T201I，ACC-ATC；I241V，ATC-GTC），and speculate that the new mutations maybe effect the structure and function of the key domain of the stimulating G protein.

Conclusions: The typical symptoms of MAS are polyosteotic fibrous dysplasia, cafe-au-lait skin lesions and sexual precocity accompanied with other hyperfunctional endocrine diseases. Extracting DNA from blood and pathologic osseous tissues in order to detect the mutation of Gsa gene is a reliable way for diagnosing the disease.

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