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Evaluation of a new handheld biosensor for point-of-care testing of whole blood beta-hydroxybutyrate concentration

用於全血羥基丁酸鹽濃度護理點測試的新型便攜式生物傳感器評估

Objectives. To evaluate performance characteristics of the newly available handheld combined glucose and ketone meter for beta-hydroxybutyrate measurement.

Design. Laboratory method evaluation.

Main outcome measures. Accuracy of beta-hydroxybutyrate measurement and effect of acetoacetate interference at clinically important beta-hydroxybutyrate levels.

Results. Deming regression analysis of beta-hydroxybutyrate measurements assessed by the ketone sensor and a laboratory enzymatic method revealed a coefficient of determination of 0.989 (P<0.001). Passing-Bablok regression analysis showed a linear relationship between the two methods, ie Y= –0.32+ 1.13X. The 95% confidence interval of the slope and y-intercept were: slope= 1.13 (95% confidence interval, 1.04 to 1.22); intercept= –0.32 (95% confidence interval, -0.59 to -0.06). The Bland-Altman plot showed a small proportional bias between the two methods. The mean bias ±2 standard deviations was between -0.53 and 0.67 mmol/L. Beta-hydroxybutyrate measurements made by the sensor were linear up to 6 mmol/L. Replicate analysis of two samples spiked with 3.6 mmol/L and 0.8 mmol/L of beta-hydroxybutyrate resulted in coefficients of variation of 3.3% and 13%, respectively. The presence of acetoacetate caused a negative interference in beta-hydroxybutyrate measurement. Beta-hydroxybutyrate recovery was 97.0% and 90.7% when the ketone body ratios were 6:1 and 3:1, respectively.

Conclusion. The analytical performance of the sensor, when operated according to manufacturer's instructions, could meet the needs of point-of-care beta-hydroxybutyrate measurement. Additional clinical studies are needed to assess the benefits of introducing such an assay in a clinical setting.

Key words:
3-Hydroxybutyric acid;
Ketones;
Point-of-care systems

目的：評估用於β羥基丁酸鹽測量的新型便攜式葡萄糖和酮組合計之操作特性。

設計：實驗室方法評估。

主要結果測量：β羥基丁酸鹽測量精確度和乙琰乙酸對臨床重要的β羥基丁酸鹽水平的影響。

結果：通過酮傳感器和實驗室酶方法的β羥基丁酸鹽測量的同類群回歸分析顯示，確定性係數為0.989 (P<0.001)。Passing-Bablok 回歸分析顯示這兩種方法之間存在線性關係：即 Y= –0.32+1.13X 。 95% 置信區間的斜率和 Y 截距是：斜率 =1.13 (95% 置信區間， 1.04 至 1.22)；截矩 =-0.32 (95% 置信區間， -0.59 至 -0.06)。Bland-Altman 圖顯示這兩種方法間的小比例偏差。平均偏差 ±2 標準差介乎 -0.53 和 0.67 mmol/L 之間。由這種傳感器所做的 β 羥基丁酸鹽測量值為線性直到 6 mmol/L 。β羥基丁酸鹽濃度為 3.6 mmol/L 和 0.8 mmol/L 兩抽取樣品的複製分析結果，其方差係數分別為3.3%及13%。乙琰乙酸存在對β羥基丁酸鹽測量產生負干擾。當酮體比是 6:1 和 3:1 時， β 羥基丁酸鹽的恢復分別是 97.0% 和 90.7% 。

結論：按照廠家說明書指示使用，該傳感器分析性能可以滿足護理點 β 羥基丁酸鹽測量的需要。還需要進一步的臨床研究來評估在臨床中引入這種化驗的好處。

關鍵詞：
3 —羥基丁酸；
酮；
護理點系統

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Introduction

Ketone bodies, metabolic by-products of fatty acid metabolism, accumulate during periods of reduced glucose availability. Under such circumstances, ketone bodies act as an alternative metabolic fuel for the brain, heart, renal cortex, and skeletal muscle, thus, reducing tissue demand for glucose. Both physiological and pathological causes contribute to hyperketonaemia. Physiological ketone body production occurs in response to fasting, prolonged exercise, or a high-fat diet,[1] and generally results in a mild-to-moderate elevation in circulating ketone bodies. In contrast, pathological ketosis, such as occurs in diabetes mellitus, toxic ingestions of alcohol, salicylate overdose, and in some inborn errors of metabolism, can cause marked hyperketonaemia and disturbance in acid-base balance, resulting in ketoacidosis.

Ketone bodies are produced within the mitochondria of hepatocytes. They include β-hydroxybutyrate (β-OHB), acetoacetate, and acetone, this latter being the least abundant. During ketosis, the magnitude of the rise in the concentration of individual ketones varies, thus, altering the ketone body ratio (KBR). The KBR is the ratio of β-OHB to acetoacetate concentrations in the blood. The KBR is normally 1:1, rising to 6:1 with prolonged fasting, and up to 10:1 in pathological ketosis.[1] Thus, β-OHB is the predominant ketone in most pathological states of ketosis. β-Hydroxybutyrate is formed from the biochemical reduction of acetoacetate and hence, the KBR is determined by the redox status within hepatic mitochondria. A high NADH/NAD+ ratio favours the formation of β-OHB. As the rate of ketone body production varies with factors such as period of fasting,[2,3] exercise, diet, as well as age, the reference interval for blood β-OHB is dependent on such physiological variables. The reference interval for β-OHB in adults has been quoted as up to 0.27 mmol/L.[4] However, Laffel et al[5] considered an elevated β-OHB level to be more than 0.5 mmol/L on random testing in the diabetic population studied.

Diabetic ketoacidosis (DKA) is the most common cause of pathological ketosis. In recent years, there has been evidence supporting the clinical utility of measuring β-OHB levels to assist the management of diabetes mellitus and DKA. On admission, β-OHB levels have been found to be markedly elevated (mean, 8.50 mmol/L[6]; mean, 9.29 mmol/L; range, 1.80-19.60 mmol/L[7]) in patients with DKA, with a typical KBR of 3:1.[6] Furthermore, during the course of treatment for DKA, β-OHB levels have been shown to correlate well with the resolution of acid-base disturbance, with ketoacidosis resolving in all patients with β-OHB levels <0.5 mmol/L, but not in patients with β-OHB levels >1.1 mmol/L.[6]

However, conventional bedside tests for urine and blood ketone assessment (eg Ketostix, Bayer Diagnostics, New York, US; Acetest, Bayer Diagnostics, New York, US) do not detect β-OHB. These tests are based on the nitroprusside reaction and react only with acetoacetate, and to a lesser extent, acetone. Misleading information may result from the use of such tests in the assessment of DKA. Such tests could fail to detect the presence of ketones when β-OHB is the predominant ketone (high KBR). Conversely, the opposite may occur during recovery from various ketoacidotic states, at which time β-OHB progressively decreases due to the increased clearance and reduced production of ketone bodies. In addition, with the reversal of the redox status within hepatic mitochondria, increasing amounts of β-OHB are oxidised to form acetoacetate. Consequently, despite normalisation of acid-base parameters, acetoacetate remains elevated.[6,8] Due to the increasing proportion of acetoacetate during DKA recovery, the nitroprusside test would become increasingly positive and thus, provide a false impression of worsening ketosis.[1]

Besides diabetes mellitus and DKA, β-OHB measurement is also useful in the diagnosis of other conditions, such as in salicylate overdose, the differentiation of alcoholic ketoacidosis from the ingestion of toxic alcohols,[2,3,9] as well as in the diagnosis of causes of neonatal hypoglycaemia.[10] In the past, β-OHB could only be measured by laboratory-based enzymatic methods which were not readily available in most laboratories. In recent years, handheld devices capable of point-of-care measurement of β-OHB, such as the combined handheld glucose and ketone (β-OHB) sensor (Optium, MediSense, Abbott Laboratories, Abbott Park, US) have become available.[11] This device may prove convenient for the home monitoring of both glucose and β-OHB by patients with diabetes, especially those with type 1 diabetes. In this study, the performance of the handheld sensor in terms of β-OHB measurement was evaluated.

Methods

Ketone sensor operation

The handheld sensor is a pocket-sized device, designed for use in conjunction with glucose electrodes (MediSense Optium, Abbott Laboratories, Abbott Park, US) and β-ketone electrodes (MediSense Optium, Abbott Laboratories, Abbott Park, US) for the point-of-care measurement of glucose and ketones, respectively. This study evaluated the β-OHB measuring performance of the sensor only. The sensor is based on biosensor technology, with β-OHB quantified by the change in electrochemical state mediated by a redox mediator and NAD+/NADH during enzymatic (hydroxybutyrate dehydrogenase) conversion of β-OHB to acetoacetate.[11] Operation of the sensor was performed according to the manufacturer's recommendations. β-Hydroxybutyrate measurement involved applying either a fresh drop of capillary whole blood from a finger prick or 10 μL of fresh venous blood collected into heparin tubes, to the target area on the electrode within 30 minutes of collection. The β-OHB result was displayed after 30 seconds.

The performance of the sensor was assessed by analysing samples spiked with known amounts of β-OHB. The results were compared with an existing laboratory enzymatic method (Procedure No. 310-UV, Sigma Diagnostics; adapted onto Cobas Mira, Roche Diagnostic Systems, Basel, Switzerland).

Preparation of beta-hydroxybutyrate standards

Preparation involved dissolving D-β-OHB ((R)-(-)-3-hydroxybutyric acid, 29836-0; Sigma-Aldrich, St Louis, US) into normal saline to produce stock solutions with concentrations ranging from 10 to 80 mmol/L, in increments of 10 mmol/L. Solutions were allowed to stand at 4°C for 2 hours to ensure adequate equilibration. Venous blood with a low concentration of β-OHB (0.1 mmol/L) was collected from a volunteer into heparin tubes. The venous blood was immediately spiked with the appropriate solutions at 10% volume to produce whole blood samples containing β-OHB in concentrations ranging from 1 to 8 mmol/L. The blood samples were left to equilibrate for 1 hour at room temperature, according to the protocol of Abbott Laboratories. After equilibration, the samples were tested with the ketone sensor as soon as possible. After testing, the whole blood samples were centrifuged, and the resultant plasma was subjected to testing by the laboratory enzymatic method.

The precision of the sensor was assessed by replicate analysis (within 30 minutes) of two whole blood samples spiked with concentrations representing a mildly increased (0.8 mmol/L) and a moderately increased (3.6 mmol/L) level of β-OHB, respectively. Potential interference from the other predominant ketone body, acetoacetate, was assessed by analysing two whole blood samples spiked with 6 mmol/L of β-OHB (representing a markedly elevated level), and containing either 1 or 2 mmol/L of acetoacetic acid (A8509, Sigma-Aldrich, St Louis, US), giving a KBR of 6:1 and 3:1, respectively. Statistical analysis was performed by using Sigma Stat 2.03 (SPSS Inc., Chicago, US) and MedCalc 3.0 (MedCalc, Mariakerke, Belgium).

Results

The accuracy of the sensor β-OHB measurement was assessed by comparison with the laboratory enzymatic method. The Deming regression analysis showed a coefficient of determination of 0.989 (P<0.001). Passing-Bablok regression analysis[12] (Fig 1) showed a linear relationship between the two methods, ie Y= –0.32+1.13X. The 95% confidence interval (CI) of the y-intercept and slope were: intercept= –0.32 (95% CI, -0.59 to -0.06); slope= 1.13 (95% CI, 1.04 to 1.22). The Bland-Altman plot[13] (Fig 2) showed a proportional bias between the methods. The mean bias ±2 standard deviations was between -0.53 and 0.67 mmol/L. β-Hydroxybutyrate measurements made by the sensor were linear up to 6 mmol/L, beyond which a 'Hi' signal was displayed by the sensor. Replicate analysis of the two samples spiked with 3.6 mmol/L and 0.8 mmol/L

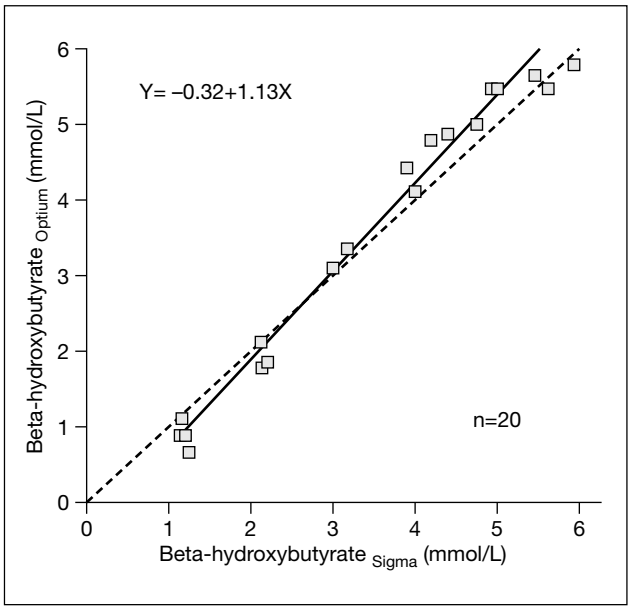

Fig 1. Passing-Bablok regression analysis: the beta-hydroxybutyrate concentration measured by the sensor (Optium) is plotted against the results of the laboratory method (Sigma)

of β-OHB resulted in coefficients of variation of 3.3% and 13%, respectively. In general, the presence of acetoacetate caused a negative interference in β-OHB measurement. β-Hydroxybutyrate recovery was 97.0% and 90.7% when the KBRs were 6:1 and 3:1, respectively, and was comparable to the recovery of β-OHB measured by the laboratory method (99.1% and 92.8%).

Discussion

Despite improvements in medical care, the mortality rate from DKA remains significant.[14] To aid prevention and early detection of DKA, the American Diabetes Association had previously recommended the testing of urine ketones "during periods of acute illness or stress, when blood glucose levels are consistently in excess of 300 mg/dL (16.7 mmol/L), during pregnancy, or when symptoms suggestive of ketoacidosis are present."[1] However, nitroprusside-based ketone tests for β-OHB provide unreliable information. On admission, when the KBR is high, and the acetoacetate concentration is low, the nitroprusside reaction may be falsely negative for ketones. With the use of nitroprusside-based ketone tests, increasingly positive urine ketone levels seen during recovery, along with the persistent elevation noted after recovery due to the persistence of acetoacetate, may result in unnecessary and dangerous increases in the dosage of insulin prescribed.[15] Other inherent problems of urine ketone testing include the risk of false-positive results in the presence of drugs containing sulphydryl groups, such as captopril, or N-acetylcysteine; as well as the chance of false-negative results if the test strips or tablets have been exposed to air for prolonged periods.[16] Consequently, the American Diabetes Association has subsequently revised their position on ketone testing:

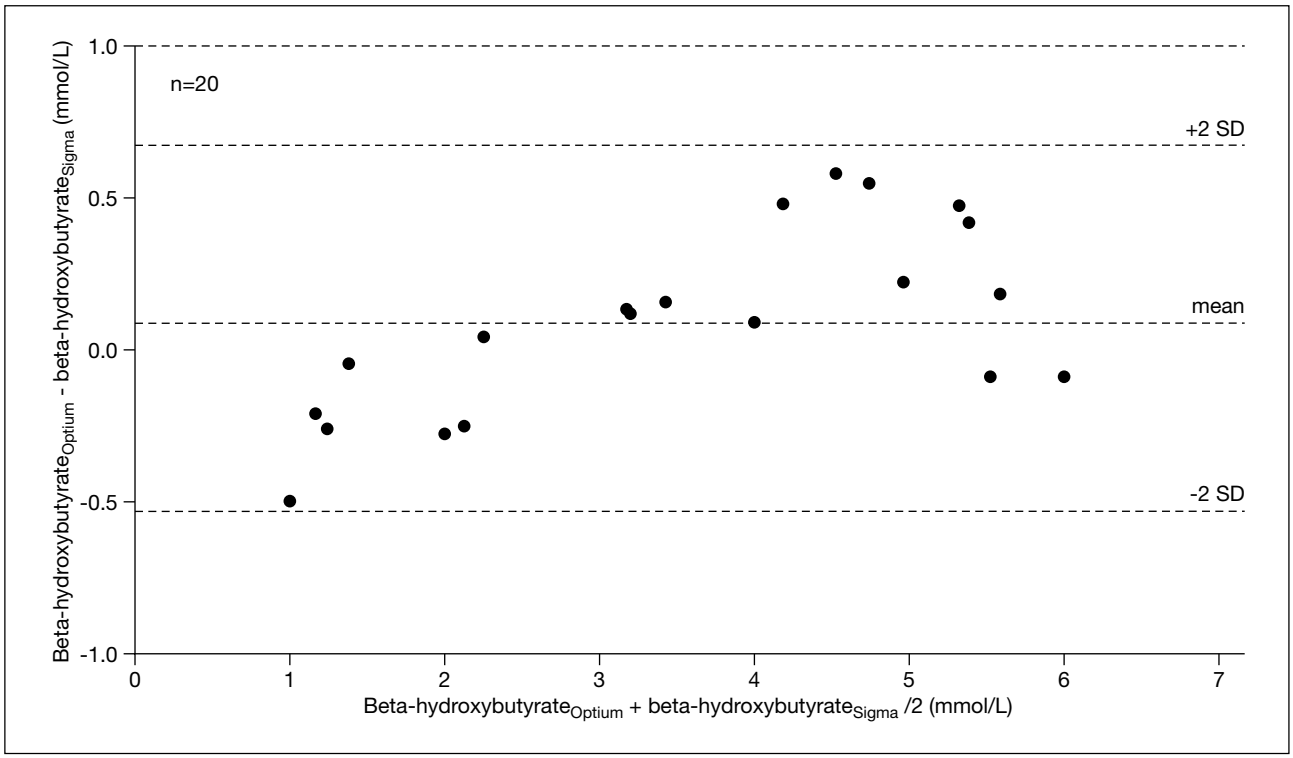

Fig 2. Bland-Altman plot: the difference in beta-hydroxybutyrate concentration measured by the sensor (Optium) and that of the laboratory method (Sigma) is plotted against the average of the two values

"Currently available urine ketone tests are not reliable for diagnosing and monitoring treatment of ketoacidosis. Blood ketone testing methods that quantify β-OHB…are now available. These may offer a useful alternative to urine ketone testing…."[17]

One such device, Ketosite (GDS Diagnostics, Elkhart, US) has been available for some time; however, this assay has a limited detection range (β-OHB, 0-2.0 mmol/L) and suffers from severe negative interference from acetoacetate.[7] On the other hand, evaluation of the recently introduced combined glucose and β-OHB sensor (Medi-Sense, Optium) in this study revealed that the sensor displayed a proportional bias when compared with the laboratory method. Precision was poorer at the lower end of the detection range, whereas it was acceptable at a moderately increased β-OHB concentration. The presence of acetoacetate resulted in minor negative interference, comparable to that seen with the laboratory method. The degree of deviation seen in performance of the sensor compared with the laboratory method is clinically insignificant, however. This is because the primary clinical use of a point-of-care ketone sensor is to distinguish a moderate-to-markedly elevated β-OHB level from that of normal, so that a self-monitoring patient is prompted to seek medical help, or an emergency diagnosis of ketoacidosis is made while confirmatory laboratory tests are being performed. The degree of proportional bias exhibited by the sensor would not result in the misclassification of a significantly abnormal β-OHB level. Consequently, the performance of the sensor could be judged as acceptable when used for the mentioned purposes, according to the manufacturer's guidelines. However, in view of the degree of bias and the poor precision at the lower end of the detection range, a borderline elevation of β-OHB displayed by the sensor should be verified by a laboratory method.

Previous studies assessing the clinical utility of β-OHB measurement in the management of diabetic patients have resulted in conflicting results.[6,7,18-20] Thus, clinical studies are needed to assess whether the use of this new sensor results in improved management of diabetic patients. Recently, Laffel et al[5,16] utilised this combined sensor for daily monitoring of β-OHB concentration in diabetic patients and reported an eight-fold increase in the risk of an elevated β-OHB level (of more than 1.0 mmol/L) in patients whose HbA_{1C} levels were greater than 8.5%. Besides diabetes mellitus, the sensor may also have applications in the management of patients with alcoholic ketoacidosis[2,3,9] or neonatal hypoglycaemia.[10] Relevant clinical studies in these populations are also indicated.

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