Is capillary ketone determination useful in clinical practice? In which circumstances?

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SUMMARY
A new method is now available to measure capillary levels of 3-hydroxybutyrate (3HB), one of the three ketone bodies. It is a quantitative and enzymatic test that uses the same equipment as for home capillary blood glucose determination but with specific strips. In comparison to urine ketone test, there is no false negative or false positive results, it is highly correlate to standard automate assays and patients find it more acceptable. Clinical implementations of this new test begin to be reported. Some studies showed an advantage of ketonemia versus ketonuria measurement to detect and to treat diabetic ketoacidosis in the emergency room. In diabetic patients treated with continuous subcutaneous insulin infusion, ketonemia seems to be more relevant to detect lack of insulin. In the current care of patient with type 1 diabetes and especially in children blood ketone test is more effective than urine ketone test to prevent hospitalisation during sick days. For other situations such as diabetic pregnancy or type 2 diabetes, more data are needed to determine if capillary measurement of 3HB is really useful. This new test is easier and less unpleasant than doing urinary test but it is still far more expensive. Further clinical studies are needed to define whether self 3HB monitoring should substitute urinary test in outpatient care.

Key-words: Ketonemia · 3-hydroxybutyrate · Ketonuria · Ketoacidosis · Self-monitoring.

RÉSUMÉ
La détermination de la cétonémie capillaire est-elle utile en pratique clinique ? Dans quelles circonstances ?

La mesure des concentrations capillaires de 3-hydroxybutyrate (3HB), un des trois corps cétoniques, est maintenant disponible. Elle repose sur une méthode enzymatique quantitative réalisée par un appareil identique à un lecteur glycémique avec des bandelettes spécifiques. Comparativement au test urinaire, il n’y a pas de faux négatif ou de faux positif, les résultats sont hautement corrélés à ceux obtenus par automates standards et les patients la trouvent plus acceptable. Les intérêts cliniques de cette nouvelle méthode commencent à être rapportés dans la littérature. Certaines études ont montré un avantage de la mesure de la cétonémie par rapport à celle de la cétonurie pour détecter et traiter la céto-acidose diabétique aux Urgences. Chez les patients traités par pompe sous cutanée d’insuline, la mesure de la cétonémie capillaire semble plus intéressante pour détecter la carence en insuline. Dans la prise en charge courante des patients diabétiques de type 1, en particulier chez l’enfant, la mesure de la cétonémie capillaire est plus efficace que celle de la cétonurie pour prévenir une hospitalisation en cas d’affection intercurrente. Dans d’autres situations telles que le diabète au cours de la grossesse ou le diabète de type 2, des données supplémentaires sont nécessaires pour déterminer si la mesure capillaire du 3HB est vraiment utile. Ce nouveau test est simple et moins pénible que la réalisation des tests urinaires mais il est beaucoup plus cher. D’autres études sont nécessaires pour savoir si l’auto-surveillance du 3HB doit supplanter les tests urinaires dans le suivi ambulatoire des patients.

Mots-clés : Cétonémie · 3-hydroxybutyrate · Cétonurie · Céto-acidose · Auto-surveillance.
Until recently, only semi-qualitative urinary ketone measurement was available using strip tests. The detection of ketone bodies in the blood from finger prick offered new options for monitoring and treating diabetes. In this paper we will depict the method and evaluate the interest of capillary ketone measurement in comparison to urinary ketone measurement. We will try to propose clinical practice guidelines according to what have been reported in the literature.

Ketone bodies: physiology and pathophysiology

The term “ketone bodies” refers to three molecules, acetoacetate (AcAc), 3-hydroxybutyrate (3HB), and acetone. Acetoacetate accumulates during fatty acid metabolism under low carbohydrate conditions. 3-hydroxybutyrate is formed from the reduction of AcAc in the mitochondria (Fig. 1). These biochemical activities enable fat-derived energy to be generated in the liver and used by other organs such as brain, heart, kidney cortex and skeletal muscle when there is limited availability of carbohydrate or when carbohydrate cannot be used effectively.

*Ketogenesis* is the process by which fatty acids are transformed into AcAc and 3HB. This process takes place into the mitochondria of perivenous hepatocytes [1]. Fatty acid release by adipose tissue is stimulated by epinephrine and glucagon and inhibited by insulin. When glucose level is low (during fasting) or when insulin is lacking (poorly controlled diabetes) oxaloacetate (derived from pyruvate during glycolysis) is preferentially utilized in the process of gluconeogenesis, instead of condensing with acetyl-CoA. Acetyl-CoA accumulates due to the high level of fatty acid beta-oxidation and is then diverted to ketone body formation. Acetoacetate and 3HB are short chain (+-carbon) organic acids that can freely diffuse across cell membranes. Therefore, ketone bodies can serve as source of energy for the brain (which can not utilize fatty acids) and other organs. The control of ketogenesis depends on the glucagon-to-insulin ratio. A low insulin-to-glucagon ratio is associated with a stimulation of ketogenesis.

*Ketolysis* is the process by which ketone bodies are converted into energy that can be used as fuel for various intracellular metabolic activities. It occurs in the mitochondria of several extra hepatic organs [1].

*Ketosis* is a transient condition that is characterized by elevated serum levels of ketone bodies. The most common causes of ketosis are physiological responses to fasting (especially during infancy and pregnancy), prolonged exercise or a ketogenic (high-fat) diet [2, 3]. In infancy, children are more susceptible to physiological ketosis because of their lower hepatic glycogen stores. The most common pathological causes of ketosis are diabetes and toxics, especially binge drinking (alcoholic ketoacidosis) and salicylate overdose. However, in these situations, ketone body concentrations do not rise to very high levels [4].

**Mechanisms of diabetic ketoacidosis (DKA):** DKA is a serious acute metabolic complication of diabetes associated with elevated levels of ketone bodies in the blood to such a level that leads to acidosis. DKA is precipitated by omission or inadequate use of insulin, infection, new onset of diabetes, and concomitant affections (the stress induced by a surgery, hyperthyroidism…). These metabolic derangements are caused by an effective lack of insulin and simultaneous elevation of counter regulatory hormones such as glucagon, catecholamines, cortisol and growth hormone. Consequently, lipolysis in adipose tissue and ketogenesis in the liver are stimulated while lipid synthesis is inhibited, inducing a large quantity of circulating free fatty acids. In addition to the generation of abnormally high levels of ketone bodies in the blood, DKA is associated with an alteration in the ratio of 3HB to AcAc. This ratio rises to 3:1 or higher. A metabolic acidosis occurred since 3-hydroxybutyrate and AcAc are strong organic acids. The third ketone body, acetone, is formed from spontaneous decarboxylation of AcAc. Acetone does not contribute to metabolic acidosis since it does not dissociate to yield hydrogen ions. Acetone is fat soluble and is excreted slowly via the lungs. It generates the distinctive aromatic smell of the breath of patients with DKA.

**Measurement of ketone bodies in the urines**

In the seventies, home urine tests for ketone and glucose determination have been developed. In contrast to the striking advances in blood glucose monitoring, urinary ketone bodies evaluation did not progress over the last 30 yrs.
Commercial urinary ketone tests detect the presence of AcAc using a reaction that generates a coloured complex in the presence of alkali with nitroprusside. It is a semi-quantitative test that does not react in the presence of 3HB. Some commercial tests contain glycine and are able to detect both acetone and AcAc. There is no evidence to advantage one test to another.

Problems with the urinary tests:

It is usually recommended that patients with type 1 diabetes should test for ketone during acute illness or stress or when blood glucose levels are consistently elevated (e.g., 250 mg/dl [13.75.7 mmol/l]), during pregnancy, or when any symptoms of ketoacidosis, such as nausea, vomiting, or abdominal pain, are present. Patients perceive urinary ketone bodies testing to be an unpleasant and time consuming exercise [5, 6]. They need to go to the toilets and strips are often expired or stored incorrectly. As a result, the rates of non-compliance are very high. Even for the paramedical staff, it remains unpleasant because of urinary manipulation.

There are significant risks of false-negative and false-positive findings. In the presence of drugs containing sulfhydryl groups such as captopril, N-acetylcysteine, penicillamine, ketone tests based on nitroprusside reaction have been reported to give false positive results [7].

After ketoacidosis has resolved, AcAc can be detected in the urine while the ketone concentrations in the blood have returned to normal levels. False negative readings have been reported with nitroprusside test strips or tablets that have been exposed to air for a long time or have exceeded their expiry date. The ingestion of large amount of ascorbic acid has been reported to be associated with false negative values [8].

Measurement of blood 3 beta-hydroxybutyrate

Quantitative test for 3HB in the blood have been developed by two companies, using an enzymatic method. The first one is marketed by GDS Diagnostics (Elkart, IN) and uses a bench-top analyser. The GDS system determines 3HB levels on a drop of blood (25 l) in about 2 minutes with a detection range between 0 and 2 mmol/l. The need to dilute the sample with serum at a room temperature is deterrent to rapid and routine use. A second system is the Precision Xtra Advanced Diabetes management System (Abbott laboratories, MediSense Products, Bedford, MA) available on the market under the brand name MediSense Optium kit which uses a specific reagent strip for glucose and another for 3HB. This system can measure 3HB level on a finger stick blood specimen (5 l) within 30 seconds with a precise detection range between 0.1 and 6 mmol/l. The principle of the test is based on a reflectance reader able to measure the colorimetric reaction produced by the conversion of 3HB into AcAc in the presence of hydroxybutyrate dehydrogenase (HBDH) [1]. In the presence of 3HB, the HBDH-induced enzymatic reaction produces an electric current. The strength of current is proportional to the 3HB concentration in the blood. This method is reliable for hematocrite between 30% and 60%, temperature between 18 to 30 Celsius, until 2000 m of altitude and does not interact with anticoagulation treatment [1]. There is a high correlation between 3HB concentrations measure by capillary test and standard automate assay using the Bland and Altman analysis [9].

Capillary blood ketone determination seems to be more acceptable by patients [6, 10]. Moreover capillary 3HB test was found to be more sensitive than urinary nitroprusside test in the detection of under-insulinization states and avoidance of DKA [10, 11].

However, the cost of this new technology needs to be focus on. The urinary tests are very cheap: 6 euros for 50 strips. A kit of 8 Optium ketone strips is now available in France for 15 euros. There is another kit which contains 150 blood glucose strips and only 4 blood ketone strips for 60 euros. Patients are reimbursed under certain conditions (pregnancy, children and teenager under 18 yrs, patients treated with continuous subcutaneous insulin infusion). Strips are compatible with all glucose sensors MediSense Optium.

Implementations of quantitative capillary 3HB test in clinical practice

Is measurement of 3HB useful for the management of diabetic patients at the emergency room?

To detect DKA

Our group has recently compared ketonuria and capillary 3HB test to assess the severity of insulin deficiency in patients attending emergency room. The results showed that capillary 3HB test is more effective to detect patients who developed DKA or who need a hospitalisation [11]. A 3HB capillary value below 3 mmol/l enables to exclude anion-gap metabolic acidosis, and at 3 mmol/l and above predicts its existence with a positive likelihood ratio of 15.

In contrast, using urine dipsticks the best positive likelihood ratio with a three + reading is only 6. In fact, it is generally agreed that a likelihood ratio equal to or higher than ten often generates large and conclusive changes in post-test probability. Thus, specific management of hyperglycaemia in the emergency department should start when capillary ketone levels are equal to or higher than 3 mmol/l, without waiting for bicarbonate results. By contrast, this systematic short cut cannot be applied in the case of positive urine dipstick tests even when the reading is maximal (3 +). On the other hand, 3HB levels below 3 mmol/l do not justify meas-
asurement of blood bicarbonate to detect high-anion-gap metabolic acidosis since such a finding is very improbable while only the complete absence of ketonuria permits the existence of such an acidosis to be eliminated. The presence of ketone bodies in urine must lead to test for severe ketosis, which will sometimes involve unnecessary blood tests.

Schwab et al. have also reported that the positive predictive value of ketonuria is rather poor (35%) for the diagnosis of DKA and that it leads to unnecessary blood test and hence to additional costs that are not negligible [12].

To monitor the treatment of DKA

Some authors have shown the utility of 3HB for the management of DKA. Wiggam et al. [13] evaluated a protocol of insulin therapy adjustment to treat ketoacidosis based on 3HB levels compared to a protocol based on blood glucose levels. Based on 3HB levels, the resolution of ketosis occurred approximately 14 hours earlier than the conventional regimen. In this study however, no measurement of ketonuria was performed so that it was not possible to compare capillary 3HB with ketonuria in this situation.

Is measurement of 3HB useful in out patient care?

One investigation of 174 insulin requiring patients studied the utility of capillary 3HB testing in ambulatory population of children and adults [14, 15]. The patients found this new technology highly acceptable. During periods of disruption of treatment or episode of hyperglycaemia with symptoms of ketosis, 3HB levels rose consistently above 1 mmol/l. In order to define guidelines for the use of capillary ketone meter in clinical practice, Wallace et al. studied 22 patients with poorly controlled type 1 diabetes (mean HbA1c 10.4%) and 14 patients admitted with DKA. The data suggest that 3HB levels above 1 mmol/l require further action and those above 3 mmol/l necessitate medical review [16]. Thus, determination of serum levels of ketone bodies may serve as a metabolic marker of the degree of insulin deficiency [17].

Because children are more susceptible to ketosis, self blood ketone monitoring seems suitable for the management of their diabetes. In normal glycaemic state, 3HB concentrations can be up to 0.5 mmol/l [18, 19]. Laffel et al. have evaluated the interest of capillary 3HB testing during sick days in children [5]. Children (mean age around 14 years) were randomized in two groups. One group received recommendations about self capillary 3HB monitoring and supplementation in insulin accordingly. The other group was instructed to supplement insulin based on urine test results. After 6 months of follow-up, the group using 3HB measurements reduced the number of hospitalisation and emergency visit by 60% and 38%, respectively, compared to the ketonuria group. The authors concluded that sick days guidelines with careful monitoring of capillary glucose and 3HB associated with adequate supplemental insulin may prevent or reduce the occurrence of DKA episodes compared to ketonuria guidelines.

Is measurement of 3HB useful in patients treated with external pump therapy?

Krzentowski et al. [20] showed few years ago that an interruption in infusion of subcutaneous insulin infusion leads to an early and linear rise in plasma 3HB concentrations which is significant after 1h and average 1.3χ1.40 mmol/l after 6 h, emphasising the high risk of DKA in patients treated with external pump therapy. Therefore, self capillary 3HB seems to be relevant in such patients. In a population of 33 patients with type 1 diabetes treated with insulin lispro by continuous subcutaneous insulin infusion, Melki et al. (21) showed that 3HB level is a good marker to estimate the insulinopenia. In another study [11], 18 type 1 diabetic patients compared measurement of ketone in the urinary and in the blood, after interruption of continuous subcutaneous insulin infusion. The data showed that capillary ketonemia has higher sensitivity and negative predictive value (80 and 83%) than ketonuria (63 and 72%). For plasma glucose level > 250 mg/dl, plasma and capillary ketonemia were more often found positive (85 and 78% respectively) than ketonuria (59%; P = 0.017).

Is measurement of 3HB useful for the management of diabetic pregnancy?

The deleterious role of 3HB during the pregnancy is not clearly established. Physiological pregnancy is associated with two to three fold elevations in the maternal ketone body levels at baseline and with a rapid exaggerated rise in these levels in response to fast. Moreover ketone bodies cross the placenta freely. Monitoring 3HB in this situation of accelerated ketogenesis may thus be of interest. However, until now, no study answering the question of the utility of ketonemia measurement during diabetic pregnancy has been reported.

Is measurement of 3HB useful for the management of type 2 diabetes?

To our knowledge, only one study reported home capillary 3HB measurements in type 2 diabetes [17]. Capillary 3HB levels was found to be higher in well controlled type 2 diabetic subjects treated with diet alone or with sulfonylurea in comparison to those under insulin therapy. In all groups 3HB levels did not exceed 0.6 mmol/l. However, the real usefulness of capillary ketone testing in the management of type 2 diabetes has not yet been addressed.

Conclusion

Finger prickle ketone monitoring is more acceptable and accurate than urine testing. For reasons of cost, and because
Table I
Clinical guidelines based on ketonuria or capillary 3HB test in insulin treated patients.

<table>
<thead>
<tr>
<th>Ketonuria (stains)</th>
<th>Capillary ketonemia (mmol/l)</th>
<th>What to do</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trace</td>
<td>0.5-1</td>
<td>Check material, expired date of sticks</td>
</tr>
<tr>
<td>+</td>
<td>1-1.9</td>
<td>Check glycaemia and ketone one hour after</td>
</tr>
<tr>
<td>++</td>
<td>2-2.9</td>
<td>Supplemental insulin injection (3-5 U)</td>
</tr>
<tr>
<td>+++</td>
<td>3+</td>
<td>Supplemental insulin injection (6-10 U)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medical emergency Immediate medical review needed</td>
</tr>
</tbody>
</table>

urine dipsticks also provide information on the sediment (an urinary infection may be the cause of hyperglycaemia), it seems reasonable to recommend measurement of ketonemia in the emergency setting only in hyperglycaemic patients whose urine cannot be analyzed rapidly and accurately (risk of false negatives or positives) and those who have positive urine dipstick tests. The level of 3HB of 3 mmol/l enables to select patients at higher risk of DKA and may be propose as the threshold above which bicarbonate determination is needed. Capillary 3HB monitoring may be also of interest in children with type 1 diabetes because it reduces the number of medical visit and admission during sick days and in patients treated by external pump insulin infusion. As others [22], we propose clinical guidelines (Tab I) for the use of capillary 3HB testing. The interest of home capillary 3HB testing in the management of type 2 diabetes or diabetic pregnancy has not been proven yet.

References