Effect of acute hyperketonemia on the cerebral uptake of ketone bodies in nondiabetic subjects and IDDM patients

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Blomqvist, G., M. Alvarsson, V. Grill, G. Von Heijne, M. Ingvar, J. O. Thorell, S. Stone-Elander, L. Widén, and K. Ekberg. Effect of acute hyperketonemia on the cerebral uptake of ketone bodies in nondiabetic subjects and IDDM patients. Am J Physiol Endocrinol Metab 283: E20–E28, 2002. First published February 26, 2002; 10.1152/ajpendo.00294.2001.—Using R-[^1-11C]hydroxybutyrate and positron emission tomography, we studied the effect of acute hyperketonemia (range 0.7–1.7 mmol/ml) on cerebral ketone body utilization in six nondiabetic subjects and six insulin-dependent diabetes mellitus (IDDM) patients with average metabolic control (HbA1c = 8.1 ± 1.7%). An infusion of unlabeled R-[^3-11C]hydroxybutyrate was started 1 h before the bolus injection of R-[^1-11C]hydroxybutyrate. The time course of the radioactivity in the brain was measured during 10 min. For both groups, the utilization rate of ketone bodies was found to increase nearly proportionally with the plasma concentration of ketone bodies (1.0 ± 0.3 μmol/ml for nondiabetic subjects and 1.3 ± 0.3 μmol/ml for IDDM patients). No transport of ketone bodies from the brain could be detected. This result, together with a recent study of the tissue concentration of ketone bodies in the brain by magnetic resonance spectroscopy, indicate that, also at acute hyperketonemia, the rate-limiting step for ketone body utilization is the transport into the brain. No significant difference in transport and utilization of ketone bodies could be detected between the nondiabetic and the IDDM patients.

β-hydroxybutyrate; blood-brain barrier; positron emission tomography

KETONE BODIES SUPPLEMENT GLUCOSE as a fuel of the brain. The role of ketone bodies becomes important during special physiological conditions, such as long-term fasting. The resulting hyperketonemia is coupled to increased uptake and oxidation of ketone bodies in the brain. A parallel decrease in glucose oxidation maintains the total energy balance (8).

Type 1 diabetes in the poorly treated state is also characterized by hyperglycemia and hyperketonemia caused by insulinopenia. However, most type 1 diabetic patients also have 24-h levels of plasma ketone bodies during normal insulin treatment that are somewhat higher than those in nondiabetic subjects (7). Hyperketonemia will increase the uptake of ketone bodies in the brain, resulting in an increased oxidation of ketones. Such an increase would be additive to the excessive glucose uptake due to hyperglycemia and, unless counteracted by other regulation, would exacerbate the excess energy being delivered to the brain. Chronic hyperglycemia is known, at least in animals, to down-regulate glucose transporters in the brain (13), thereby decreasing the glucose load to the brain. Analogously, ketone uptake and oxidation in the brain could also be subject to regulation in diabetes, although, to our knowledge, this has not previously been tested in subjects with type 1 diabetes.

A method has previously been developed for measuring regional cerebral utilization of ketone bodies in humans with positron emission tomography (PET) using R-[^1-11C]hydroxybutyrate (β-[11C]HB) as tracer (1). The method was applied in studies of healthy male subjects at normoketonemia. The plasma concentration of R-[^1-11C]hydroxybutyrate (β-HB) was in the range of 0.02–0.09 μmol/ml. Three main features of ketone body utilization were observed. First, ketone body utilization was found to increase almost linearly with increasing concentration of ketone bodies in arterial plasma. Second, the uptake of ketone bodies could be well described by a model with a single rate constant, indicating that the uptake is essentially irreversible. Third, the tissue concentration of ketone bodies was found to be very low, suggesting that the transport across the blood-brain barrier (BBB) is the rate-limiting step for ketone body utilization.

As early as 1971, Daniel et al. (4) reported that the transport of ketone bodies across the BBB in rat is essentially irreversible. Together with other studies on rats, this led to the hypothesis (2) that the carrier for ketone bodies is reversibly used for brain-blood transport of pyruvate and lactate.

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The aim of the present study was to compare the ketone body utilization in nondiabetic subjects and subjects with insulin-dependent diabetes mellitus (IDDM) at hyperketonemia and to investigate whether the features of ketone body utilization previously observed at normoketonemia also persist at hyperketonemia.

MATERIALS AND METHODS

Subjects. The experimental procedure was approved by the Ethics and Radiation Safety Committees of the Karolinska Hospital and Institute. Before the subjects agreed to participate, they received written and oral information about the nature, purpose, and possible risks of the experiment. Six healthy male subjects served as a control group. Their mean age was 30.0 ± 7.4 yr (range 23–44 yr), and their body weight was 81 ± 10 kg. Six male patients with type 1 diabetes mellitus participated in the study. Their mean age was 32.0 ± 5.1 yr (range 23–38 yr), their body mass index was 24.5 ± 2.9 kg/m², and their body weight was 78 ± 10 kg. The age at onset of the disease was 15.3 ± 10.3 yr, and the duration was 16.3 ± 6.9 yr. All of them were treated with multiple insulin injections except one patient, who was treated with continuous subcutaneous insulin infusion. Their usual daily dose of insulin was 37.0 ± 4.5 U of short-acting and 19.2 ± 4.8 U of long-acting insulin. The level of glycosylated hemoglobin (HbA₁c) was 8.1 ± (SD) 1.7%. (The upper level of normal HbA₁c was 5.6%). None of the patients had experienced a serious hypoglycemic episode. Minimal signs of background retinopathy were present in four patients. One patient had macular edema but no other signs of nephropathy. Another patient was treated with antihypertensive drugs and a low dose of prednisolone because of glomerulonephritis due to systemic lupus erythematosus.

Blood pressure was normal in all patients (<140/90 mmHg).

Experimental procedures. To obtain −1 mmol/l β-HB in the plasma at steady state, a primed infusion of a racemic mixture of unlabeled β-HB was started 1 h before the PET scan in all of the subjects. The proportion between the R and S forms was close to 1:1. The priming dose, given during the first 20 min (19), was twice the subsequent continuous infusion dose, which lasted to the end of the PET scan. The priming dose was 6 mg·kg⁻¹·min⁻¹ except in two cases (IDDM patients), when 3 and 4.5 mg·kg⁻¹·min⁻¹, respectively, were administered.

The PET experiments on the nondiabetic subjects were performed after an overnight fast ~12 h after the last meal. The subjects with diabetes mellitus reported to the ward at the Department of Endocrinology at 8 PM on the day preceding the study. Before dinner at around 7 PM, they had injected their usual dose of short-acting insulin. Administration of subcutaneous insulin was then discontinued, and an intravenous infusion of insulin was started [50 U Actrapid Human (Novo Nordisk) in 250 ml of 0.9% saline solution]. This infusion was adjusted to maintain blood glucose levels between 6 and 12 mmol/l during the night and during the PET experiment. Before the PET measurements, with the subject under local anesthesia, a catheter was placed in the left brachial artery for arterial blood sampling. A cannula was placed in the right brachial vein for injection of the tracer.

β-[¹¹C]HB was synthesized in a two-step stereo-specific synthesis starting with carrier-added [¹¹C]cyanide and R-propylene oxide. The total synthesis time, including high-performance liquid chromatography purification, was 45–50 min from the end of trapping. The radiochemical purity of the products was >99% (20). The amount of β-[¹¹C]HB administered was 100–400 MBq.

Immediately before the PET scan with β-[¹¹C]HB, a transmission scan for determination of the attenuation correction was performed. The PET scan after the bolus administration of β-[¹¹C]HB was performed during a 10-min period, between 45 and 55 min after start of the infusion for one of the nondiabetic subjects, and between 60 and 70 min for the remaining five healthy volunteers and all of the six IDDM patients. The PET camera used was the ECAT EXACT HR. The in-plane and axial resolutions are ~3.8 mm and ~4.0 mm (full width at half maximum), respectively (21). The measuring time was divided into 6 frames of 10 s, 3 frames of 20 s, 2 frames of 1 min, and 3 frames of 2 min; in all there were 14 frames. The camera was run in 2D mode in all experiments except three. The results from these three runs in 3D mode did not differ from the others. The radioactivity in arterial blood was sampled in 1-s intervals by an automatic blood sampling system. To reduce the blood loss, the sampler was used only during the first 5 min after the bolus injection. The withdrawal rate was 5 ml/min. During this time, five 2-ml samples of arterial blood were drawn manually, and during the remaining 5 min another four 2-ml samples were drawn manually. These samples were measured in an NaI well counter that had been cross-calibrated against the positron camera before the measurement. An aliquot (0.5 ml) was taken from each sample and measured in the well counter to determine the radioactivity concentration in whole blood. The remaining part of each blood sample was centrifuged, and the radioactivity in an aliquot of plasma (0.5 ml) was also measured in the well counter. Samples were also taken at timed intervals to measure the arterial concentrations of β-HB, glucose, lactate, glycerol, and insulin, and also to determine hematocrit, pH, and standard bicarbonate concentration. The different concentrations were determined by standard methods, as described by Grill et al. (6).

Data processing. The images of the radioactivity concentration in the form of matrices with 128 × 128 pixels for each of 47 slices, with a center-to-center distance of 3.125 mm, were reconstructed by standard software provided by the manufacturer.

To calculate the rate of uptake of β-HB, the time course of β-[¹¹C]HB in the arterial plasma, the “input function” is required. The manual samples were taken from a three-way connector at the catheter inserted in the artery. From the same connector, blood was also pumped through the blood sampler. To be able to compare the measurements made with the well counter and the blood sampler, the transport time in the catheter from the connector to the blood sampler has to be accounted for. The delay was ~10 s with the withdrawal rate used (5 ml/min). The camera and blood data were synchronized by correcting for the difference in arrival times of the radioactivity at the brain and at the blood sampler. The dispersion of the input function in the catheter and in the peripheral artery was corrected for with the aid of previous measurements.

On the basis of manual measurements, the ratio between the radioactivity concentrations in the plasma and in the whole blood was determined as a function of time. In accordance with the previous study (1), the time dependence of this ratio could be well described by a straight line with a slope insignificantly different from zero. For the nondiabetic subjects, the intercept was found to be 1.21 ± 0.05 and the slope 0.00016 ± 0.00012 (average ± SD). For the IDDM patients, the intercept was found to be 1.19 ± 0.09 and the slope −0.00004 ± 0.00033. With use of this ratio, together with the two calibration factors, blood sampler against well
counter and well counter against camera, the time course of $\beta$-[11C]HB in arterial plasma was estimated second by second from the whole blood measurements of the blood sampler. To obtain the plasma concentration in the whole 10-min interval, the manual measurements of $\beta$-[11C]HB in the plasma performed after the blood sampler measurements were added. The time course of the arterial plasma concentration of $\beta$-[11C]HB was used as input function in the kinetic analysis.

**Kinetic analysis.** Because of the low uptake of ketone bodies in the brain, determination of regional ketone body utilization was not attempted in this study. Identification of brain regions in PET studies using $\beta$-[11C]HB requires auxiliary measurement with magnetic resonance imagery or some PET tracer with high uptake in the brain.

Three different methods of kinetically analyzing the data were applied. First, a single-tissue compartment model, the “1k model,” was applied, which assumes that $\beta$-[11C]HB is immediately metabolized after entering the tissue and that the labeled metabolites are irreversibly trapped during the measuring time (10 min). The model contains two unknown parameters, the vascular fraction of cerebral blood (CBV) and the parameter of interest, $K_{\text{keto}}$, the “accumulation rate constant” (see Eqs. A1 and A2).

Physiologically, there must be some concentration of ketone bodies in the tissue (18). The second model applied, the “3k model,” contains one reversible compartment for unmetabolized ketone bodies and one irreversible compartment for metabolites. This model has three rate constants (see Eq. A3). In the application of this model, CBV was not fitted, but the value obtained with the 1k model was used. $K_{\text{keto}}$ is obtained as $k_{1}k_{p}/(k_{2}+k_{3})$. Finally, we applied the Gjedde-Patlak analysis (5, 16). To reduce the influence of residuals in the dominant blood peak in the uptake, the data in the time interval 0–1 min were excluded from the fit of the straight line. The parameters $K_{\text{keto}}$ and $D_{\text{Vapp}}$ were obtained as the slope and y-intercept, respectively, of the fitted straight line. Also, in this case, the CBV value obtained from fit of the 1k model was utilized. Thus the applied models contain one, two, and three parameters in addition to CBV. For the 1k model and the Gjedde-Patlak analysis, the parameters were obtained by linear regression, whereas for the 3k model the parameters were estimated by using a nonlinear, iterative, least squares method (12). These models were also applied in the previous study at normoketonemia by use of the same tracer (1).

In all models, the rate of ketone body utilization, $\text{CMR}_{\text{keto}}$, is calculated as the product of $K_{\text{keto}}$ and the concentration of $\beta$-HB in the plasma, $[\beta$-[11C]HB]$_{\text{plas}}$. In the previous report (1), this quantity was denoted “primary $\text{CMR}_{\text{keto}}$.” Pure $\beta$-[11C]HB was injected, but in blood this tracer rapidly becomes a mixture of $\beta$-[11C]HB and [11C]acetoacetate ([11C]AcAc). These compounds are transported across the BBB with different rates, and therefore the measured $\text{CMR}_{\text{keto}}$ is a sum of the utilization rates of $\beta$-HB and AcAc. With the method used in this study, it is not possible to separate these two components. In the previous study (1), the two utilization rates were estimated with the aid of data from animal studies of ketone body utilization in combination with certain assumptions (3, 10). It has been found that the plasma concentration of [11C]AcAc in rats is very much lower than the concentration of $\beta$-[11C]HB (3, 10). If this is also the case for humans, $K_{\text{keto}}$ reflects mainly utilization of $\beta$-HB, and $\text{CMR}_{\text{keto}}$ is close to the rate of utilization of $\beta$-HB, $\text{CMR}_{\text{HB}}$. This picture is supported by a study by Hasselbalch et al. (9), in which the plasma concentrations of unlabeled AcAc and $\beta$-HB were measured in humans. The ratio between the found values is $0.065 \pm 0.074$ at normoketonemia and $0.111 \pm 0.035$ at hyperketonemia. In the present study, we have compared the overall ketone body utilization of nondiabetic subjects and IDDM patients with the aid of the primary parameters $K_{\text{keto}}$, $\text{CMR}_{\text{keto}}$, and $D_{\text{Vketo}}$, and we have not attempted to discriminate between the utilization of $\beta$-HB and that of AcAc.

The ketone bodies enter the tricarboxylic acid cycle via acetyl-CoA, and thereafter, tracer will be lost from the tissue mainly in the form of [11C]CO$_2$. In the previous study, it was estimated that this loss leads to an underestimation of $\text{CMR}_{\text{keto}}$ by $6\%$ when a period of 10 min is used for the parameter estimate. In the present study, we have not attempted to correct for this loss. We assume that the effect of the loss is the same for the nondiabetic subjects and the IDDM patients, and in the comparisons of the results with data obtained in the previous study, we also implicitly assume that the effect of the loss is the same at normo- and hyperglycemia.

For the kinetic analysis, routines developed in-house to use the MATLAB software were utilized.

**RESULTS**

Figure 1 shows the average $[\beta$-[11C]HB]$_{\text{plas}}$ as a function of time after start of the infusion for the nondiabetic subjects and the IDDM patients. As can be seen from Fig. 1, the start of the infusion (loading dose) caused an immediate, rapid rise of $[\beta$-[11C]HB]$_{\text{plas}}$, but after 30 min the rise was much less pronounced, and the concentration approached a constant level. As the error bars indicate, at a given time point $[\beta$-[11C]HB]$_{\text{plas}}$ varied considerably between the experiments. In contrast, for each separate experiment, the time variation of $[\beta$-[11C]HB]$_{\text{plas}}$ was small during the PET scan (60–70 min). In this interval, the difference between the lowest and highest value of $[\beta$-[11C]HB]$_{\text{plas}}$ within each particular experiment was $3.3 \pm (SD) 2.7\%$ for the nondiabetic subjects and $6.9 \pm 2.5\%$ and for the IDDM patients. In the same time interval, $[\beta$-[11C]HB]$_{\text{plas}}$ was found to be somewhat higher for the IDDM patients than for the nondiabetic subjects ($1.28 \pm 0.31$ and $0.98 \pm 0.33$.)
μmol/ml, respectively). In comparison, during the previous study, at normoketonemia [β-HB]_{plas} was 0.04 ± 0.03 μmol/ml. Thus, in the present study, [β-HB]_{plas} was, on the average, more than 20 times higher than in the previous study at normoketonemia.

Figure 2, A–D, shows examples of uptake curves and model fits for one control subject (Fig. 2, A and C) and one IDDM patient (Fig. 2, B and D). The results of applying the 1k model and the Gjedde-Patlak analysis to the data are displayed. Figure 2 illustrates that both models give satisfactory fits of the data over the whole time interval for both control subjects and IDDM patients. For the healthy control subjects, $K_{ket}$ was found to be 0.093 min$^{-1}$ with the 1k model, whereas for the IDDM patients $K_{ket}$ was found to be 0.091 min$^{-1}$ with the same model. When the 3k model was applied (not shown), the compartment of unmetabolized β-[11C]HB was always found to be small compared with the compartment of metabolized β-[11C]HB. Therefore, the fit is close to the one obtained with the 1k model, and consequently the corresponding values of $K_{ket}$ are also close to each other.

Figure 2, C and D, illustrates that the data in the Gjedde-Patlak plot are well described by a straight line over the whole measuring interval and that the intercept $DV_{app}$ is close to zero for all subjects. For many other tracers, it is possible to distinguish an initial phase in the Gjedde-Patlak plot, before the reversible part has reached equilibrium with the input function, but clearly such a phase cannot be distinguished in this study. For the two distributions displayed in Fig. 2, C and D, $DV_{ket}$ was found to be $-0.0011$ and $0.0017$, whereas $K_{ket}$ was found to be 0.093 and 0.089, respectively. The initially large scatter in the data points is
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due to difficulties in describing the dominant blood peak accurately in the model. The radioactivity concentration is measured in arterial blood. However, ~80% of the blood in the brain is venous, in which the tracer has a different time course than in arterial blood.

The F-test and the Akaike information criterion (AIC) were applied to discriminate between the models. When testing the 1k model against the 3k model, i.e., when testing whether the two extra parameters are needed, significance was reached in 4 experiments (2 control subjects and 2 IDDM patients) out of 12 (level of significance 0.05) when the F-test was applied, and the 3k model was better in 7 experiments (4 controls and 3 IDDM patients) according to the AIC. It should be kept in mind that the F-test is strictly applicable (i.e., provides correct levels of significance) only for hypotheses that are linear in the parameters to be fitted. Clearly, the statistical analysis gives no preference for any of the models. Visually, good fits are obtained in all experiments for all models. For each experiment, nearly the same values of $K_{ket}$ were obtained with the different models. Unless otherwise stated, only $K_{ket}$ and the corresponding CMR$_{ket}$ obtained with the 1k model are used in the following summary.

A summary of measured and fitted quantities (using the three models discussed) is presented in Table 1. For purposes of comparison, the corresponding quantities obtained in the previous study at normoketonemia by use of the same tracer (1) are also presented. Sample averages and standard deviations are given. The values are averages over the brain, because only average uptake curves have been used. It should be noted that the presented values of concentrations in the plasma, such as [$\beta$-HB], in Table 1 are averages over the two or three measurements made during the PET scan. In fact, most of the quantities stayed relatively constant over the whole time interval (70 min) with the exceptions of [$\beta$-HB], [glucose], and [glycerol]. [Glucose] fell 3–10% in the nondiabetic subjects and 15–35% in the IDDM patients in the time period before the PET scan, but it then stayed constant within a few percentage points during the PET scan. The concentration of glycerol fell rapidly during the first 30 min, from 0.044 ± 0.019 (average ± SD) and 0.031 ± 0.009 µmol/ml for nondiabetic subjects and IDDM patients, respectively. The averages reached in the time interval of 60–70 min are presented in Table 1. Attempts to fit the 3k model to the data often terminated with $k_2$ and/or $k_3$ at the allowed upper limit for these parameters ($50$ min$^{-1}$), and therefore only upper bounds of DV$_{ket}$ [$= k_1/(k_2+k_3)$] can be given.

Figure 3 shows CMR$_{ket}$ vs. [$\beta$-HB]$_{plas}$ for each individual in the two groups and also the corresponding data obtained in the previous study of nondiabetic subjects at normoketonemia. The displayed line is the result of a linear regression analysis of all data in the present and the previous studies. The slope, intercept, and $R$ value were found to be 7.9 ± 0.5, 0.39 ± 0.54, and 0.97, respectively. With data only from the present study, the three parameters became 6.9 ± 1.3, 1.7 ± 1.6, and 0.85, respectively. In the previous study, the

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**Table 1. Primary and derived global parameters for ketone body utilization in the brain for nondiabetic subjects and IDDM patients**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Nondiabetic Subjects ($n = 6$)</th>
<th>IDDM Patients ($n = 6$)</th>
<th>Subjects From Previous Study* ($n = 5$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Quantities measured in arterial plasma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta$-HB, µmol/ml</td>
<td>0.98 ± 0.33</td>
<td>1.28 ± 0.31</td>
<td>0.043 ± 0.029</td>
</tr>
<tr>
<td>Range</td>
<td>0.46–1.32</td>
<td>0.94–1.74</td>
<td>0.020–0.093</td>
</tr>
<tr>
<td>Glucose, µmol/ml</td>
<td>5.0 ± 0.5</td>
<td>8.1 ± 1.4</td>
<td>5.5 ± 0.8</td>
</tr>
<tr>
<td>Lactate, µmol/ml</td>
<td>0.54 ± 0.12</td>
<td>0.50 ± 0.13</td>
<td>0.063 ± 0.014</td>
</tr>
<tr>
<td>Glycerol, µmol/ml</td>
<td>0.019 ± 0.008</td>
<td>0.016 ± 0.006</td>
<td>0.039 ± 0.009</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>5.2 ± 1.3</td>
<td>16.6 ± 9.3</td>
<td>12.7 ± 6.3</td>
</tr>
<tr>
<td>Range</td>
<td>3.4–6.7</td>
<td>10.0–35*</td>
<td>7–22</td>
</tr>
<tr>
<td>Hct, %</td>
<td>41.3 ± 1.4</td>
<td>40.8 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.42 ± 0.02</td>
<td>7.42 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>SBC, mM</td>
<td>27.5 ± 1.4</td>
<td>27.7 ± 1.4</td>
<td></td>
</tr>
<tr>
<td><strong>Primary and derived parameters from kinetic analysis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CBV (fraction $\bar{0}$–1)</td>
<td>0.034 ± 0.004</td>
<td>0.038 ± 0.003</td>
<td>0.0380 ± 0.0028</td>
</tr>
<tr>
<td>$K_{ket}$, 1k model§, min$^{-1}$</td>
<td>0.0082 ± 0.0016</td>
<td>0.0088 ± 0.0015</td>
<td>0.0114 ± 0.0012</td>
</tr>
<tr>
<td>CMR$_{ket}$, nmol·ml$^{-1}$·min$^{-1}$, 1k model</td>
<td>7.9 ± 2.7</td>
<td>11.0 ± 1.9</td>
<td>0.49 ± 0.31</td>
</tr>
<tr>
<td>$E_{ket}$†</td>
<td>0.019 ± 0.004</td>
<td>0.022 ± 0.004</td>
<td>0.0212 ± 0.0035</td>
</tr>
<tr>
<td>$K_{ket}$, min$^{-1}$, 3k model</td>
<td>0.0081 ± 0.0016</td>
<td>0.0086 ± 0.0014</td>
<td>0.0110 ± 0.0011</td>
</tr>
<tr>
<td>DV$_{ket}$, 3k model</td>
<td>&lt;0.003 ± 0.001</td>
<td>&lt;0.006 ± 0.004</td>
<td>0.0065 ± 0.0009</td>
</tr>
<tr>
<td>Tissue conch nmol/ml, ketones§§</td>
<td>&lt;3 ± 3</td>
<td>&lt;6 ± 4</td>
<td>0.28 ± 0.18</td>
</tr>
<tr>
<td>$K_{app}$, min$^{-1}$, Gjede-Parfalk model</td>
<td>0.0082 ± 0.0013</td>
<td>0.0088 ± 0.0012</td>
<td>0.0105 ± 0.0010</td>
</tr>
<tr>
<td>DV$_{app}$, Gjede-Parfalk model</td>
<td>0.0007 ± 0.0004</td>
<td>0.0019 ± 0.0018</td>
<td>0.0018 ± 0.0057</td>
</tr>
</tbody>
</table>

Values are averages ± SD over the sample. IDDM, insulin-dependent diabetes mellitus; $\beta$-HB, R-$\beta$-hydroxybutyrate; Hct, hematocrit; SBC, standard bicarbonate; 1k and 3k models, single and two-tissue compartment models, respectively; CBV, vascular fraction of cerebral blood; $K_{ket}$, accumulation rate constant; CMR$_{ket}$, cerebral metabolic rate of ketone body utilization; DV$_{ket}$, ketone body distribution volume; DV$_{app}$ apparent DV. *See Ref. 1. †One outlier (35), the rest in the range 10–16.5 (13.8 ± 2.5). ‡Net extraction of ketone bodies is calculated as $E_{ket}$ = $K_{app}$/CBF. In the previous study, cerebral blood flow (CBF) was measured. In the present study, CBF was assumed to be 0.6 ml blood·ml tissue$^{-1}$·min$^{-1}$, a value estimated by interpolation from the results by Hasselbalch et al. (9). §The tissue concentration of $\beta$-HB is calculated as DV$_{ket}$·[$\beta$-HB]$_{plas}$ = ($k_1/(k_2+k_3)$)·[$\beta$-HB]$_{plas}$.

* AJP-Endocrinol Metab • VOL 283 • JULY 2002 • www.ajpendo.org
same parameters were found to be $10.7 \pm 0.8$, $0.032 \pm 0.040$, and $0.99$, respectively. Thus there is a weak indication that the slope decreases with increasing $[\beta\text{-HB}]_{\text{plas}}$, which means that the relationship between $[\beta\text{-HB}]_{\text{plas}}$ and $\text{CMR}_{\text{ket}}$ is not perfectly linear over the range of $[\beta\text{-HB}]_{\text{plas}}$ in the present and previous studies (0.02–1.74 μmol/ml). The regression analysis of $\text{CMR}_{\text{ket}}$ vs. $[\beta\text{-HB}]_{\text{plas}}$ reveals no significant difference between the two experimental groups of the present study. For the nondiabetic subjects, the slope $7.1 \pm 2.1$ and intercept $0.9 \pm 2.2$ are obtained, whereas for the IDDM patients the slope $4.6 \pm 1.9$ and intercept $5.1 \pm 2.6$ are obtained.

The relationship between plasma concentration and utilization is more clearly seen in Fig. 4, which shows $K_{\text{ket}}$ as a function of $[\beta\text{-HB}]_{\text{plas}}$ for all experiments in the present and the previous studies. $K_{\text{ket}}$ and $[\beta\text{-HB}]_{\text{plas}}$ are measured independently of each other, and therefore, from a statistical point of view, the data in Fig. 4 are easier to handle than the data in Fig. 3, where the $x$- and $y$-variables have a factor ($[\beta\text{-HB}]_{\text{plas}}$) in common. Clearly $K_{\text{ket}}$ has a tendency to decrease with increasing $[\beta\text{-HB}]_{\text{plas}}$. Linear regression gives the slope $-0.0025 \pm 0.0006$, intercept $0.0114 \pm 0.0006$, and $R$ value 0.76 for all experiments. The slope is significantly different from zero ($P < 0.0005$). For this regression, all models gave consistent results. Figures 3 and 4 show that it is difficult to distinguish any difference between the nondiabetic subjects and the IDDM patients at hyperketonemia. When data from only the present study are used, the slope $-0.0023 \pm 0.0021$ and intercept $0.011 \pm 0.002$ are obtained for the nondiabetic subjects, whereas the slope $-0.0033 \pm 0.0017$ and intercept $0.013 \pm 0.002$ are obtained for the IDDM patients. With the present statistics, these regression lines are not significantly different from each other or from the regression line obtained from the combined data in the previous and present studies.

**DISCUSSION**

Tissue concentration of $\beta$-HB—choice of model. The $1k$ model gives a good fit of the uptake data for both nondiabetic subjects and IDDM patients, indicating that the uptake of tracer across the BBB is essentially irreversible. One parameter, $K_{\text{ket}}$, describes the uptake in the tissue well (Fig. 2, A and B). The successful fit of the uptake data with this model implies that attempts to fit the data with more complex models, such as the $3k$ model, result in unreliable parameter estimates. This situation is a general feature of tracer studies that use PET, because with this technique only the total radioactivity concentration can be measured, which implies that the compartmental structure that follows a nearly irreversible transfer is very difficult to resolve. In particular, the PET experiment alone gives unreliable information about $\text{DV}_{\text{ket}}$ and the related $[\beta\text{-HB}]_{\text{tiss}}$. In fact, as good fits as with the $1k$ and $3k$ models are obtained with a constrained $3k$ model that forces $\text{DV}_{\text{ket}}$ to be large.

The undetectable efflux of ketone bodies from the brain ($J_{\text{out}} = k_2 [\beta\text{-HB}]_{\text{tiss}}$) can be interpreted in two ways. 1) With very low $[\beta\text{-HB}]_{\text{tiss}}$, $J_{\text{out}}$ also becomes low, even if the transport capacity for ketone bodies out from the brain is appreciable, i.e., even if $k_2$ is appreciable compared with $k_3$. 2) If this transport capacity is drastically decreased, i.e., if $k_2$ becomes very low compared with $k_3$, $J_{\text{out}}$ becomes low even if $[\beta\text{-HB}]_{\text{tiss}}$ is appreciable. Clearly PET experiments using $\beta\text{-[13C]}\text{HB}$ cannot discriminate between the two possibilities. Magnetic resonance (MR) spectroscopy provides independent information about $[\beta\text{-HB}]_{\text{tiss}}$, which can be utilized for this discrimination.

Recently, $[\beta\text{-HB}]_{\text{tiss}}$ has been measured during acute hyperketonemia in humans using high-field MR spectroscopy (14). The $\beta$-HB concentration was found to be...
$0.23 \pm 0.10 \mu\text{mol/ml}$ when $[\beta\text{-HB}]_{\text{plas}} = 2 \mu\text{mol/ml}$. This measured $\beta$-HB concentration can be explained by contributions from blood and cerebrospinal fluid (CSF) spaces. Based on data obtained after prolonged fasting (11), the summed contribution of $\beta$-HB in blood and CSF was estimated by Pan et al. (15) to be $0.17 \mu\text{mol/ml}$ at the $\beta$-HB plasma concentration of $3.5 \mu\text{mol/ml}$, which is not significantly lower than the measured $\beta$-HB concentration in the tissue. This result indicates that the very low $J_{\text{out}}$ obtained in the present PET study at acute hyperketonemia is an effect of a very low $[\beta\text{-HB}]_{\text{tiss}}$ and, consequently, indicates that the transport of ketone bodies into the brain is the rate-limiting step. Nothing can be concluded concerning the capacity for efflux ($k_2$) of ketone bodies across the BBB.

In contrast to the study at acute hyperketonemia, results obtained with MR spectroscopy after 2 and 3 days of fasting (15) indicate that $[\beta\text{-HB}]_{\text{tiss}}$ is considerable, close to $0.4 \mu\text{mol/ml}$ at $[\beta\text{-HB}]_{\text{plas}}$ equal to $1 \mu\text{mol/ml}$, and close to $0.6 \mu\text{mol/ml}$ when $[\beta\text{-HB}]_{\text{plas}}$ is close to $2 \mu\text{mol/ml}$. Thus the utilization of ketone bodies in the brain is found to be different at acute hyperketonemia and after fasting. However, these results give no information about the efflux of ketone bodies from the brain. Lactate and $\beta$-HB share the same transport system (monocarboxylic acid transporters) across the BBB, and during fasting, the lactate concentration in the tissue increases from $0.69 \pm 0.17 \mu\text{mol/ml}$ at normoketonemia to $1.31 \pm 0.26 \mu\text{mol/ml}$ at hyperketonemia (15). Therefore, it is possible that, due to competition, the efflux of ketone bodies is suppressed in this state. In contrast, during acute hyperketonemia, the lactate concentration in the tissue was found to be $0.72 \mu\text{mol/ml}$ (15), close to the value found at normoketonemia. A PET study with $\beta$-[$^{11}\text{C}$]HB as tracer would be suitable for measurement of the efflux after fasting. If this process is appreciable, the $1k$ model should not give a good fit, the $3k$ model should give a $D_{\text{vet}}$ significantly larger than zero, and the distribution of data in the Gjedde-Patlak plot should deviate from a straight line during the initial time period.

It should be noted that the influence of AcAc has been neglected in the analysis. From the MR experiment, $D_{\beta\text{HB}}$ is obtained, whereas with PET, $D_{\text{vet}}$ has contributions from both $\beta$-HB and AcAc. Because $[\text{AcAc}]_{\text{plas}}$ is low compared with $[\beta\text{-HB}]_{\text{plas}}$ (2, 9, 10), it is likely that $[\text{AcAc}]_{\text{tiss}}$ is lower than $[\beta\text{-HB}]_{\text{tiss}}$. Furthermore, we have not corrected for loss of $[^{11}\text{C}]\text{CO}_2$ from the tissue. Such a correction cannot change the conclusions made, however, because it should give somewhat steeper slopes in the Gjedde-Patlak analysis, which in turn should tend to give even lower values of $D_{\text{app}}$. A low $[\beta\text{-HB}]_{\text{tiss}}$ value implies that the pool of unmetabolized $\beta$-HB in the tissue will rapidly reach equilibrium with $\beta$-HB in the plasma. Therefore, it is expected that the degree of steady state reached for $[\beta\text{-HB}]_{\text{plas}}$, shown in Fig. 1, reflects the degree of steady state reached for $[\beta\text{-HB}]_{\text{tiss}}$.

**Metabolic rate of $\beta$-HB—comparison between control subjects and IDDM patients.** The data in Table 1 show that the values of the rate constant for net utilization $K_{\text{ket}}$, estimated with different models, are close to each other. The good fits using the $1k$ model and the Gjedde-Patlak analysis show that, also at acute hyperketonemia, the rate of utilization is very close to the unidirectional influx of $\beta$-HB across the BBB. Therefore, in this case, influx, uptake, and utilization are synonymous.

Over the range of $[\beta\text{-HB}]_{\text{plas}}$ considered, $CMR_{\text{ket}}$ is found to be nearly but not completely proportional to $[\beta\text{-HB}]_{\text{plas}}$ for both nondiabetic subjects and for IDDM patients. Compared with the previous study at normoketonemia, $[\beta\text{-HB}]_{\text{plas}}$ is 23 times larger in this study for the nondiabetic subjects and 30 times larger for the IDDM patients, whereas the corresponding $CMR_{\text{ket}}$ values are only 16 and 22 times larger, respectively. $CMR_{\text{ket}}$ for the IDDM patients is, on the average, 39% larger than $CMR_{\text{ket}}$ for the nondiabetic subjects, but this difference can be explained by the fact that $[\beta\text{-HB}]_{\text{plas}}$ is, on the average, 31% larger for the IDDM patients than for the nondiabetic subjects. The regression analysis shows that the $K_{\text{ket}}$ values in the two groups are not significantly different from each other (Fig. 3). It should be noted that the $S$-isomer, included in the racemic mixture (≈50%) and used in the present study, may be metabolized somewhat less and might have a different fate compared with the $R$-isomer (17).

The results indicate that the net utilization of ketone bodies is far from saturation in both the nondiabetic subjects and the IDDM patients. The origin of the small deviation from strict proportionality between $[\beta\text{-HB}]_{\text{plas}}$ and $CMR_{\text{ket}}$ (decreasing $K_{\text{ket}}$) might be limitations in the transport capacity across the BBB. Another reason might be an increasing rate of loss of $[^{11}\text{C}]\text{CO}_2$ from the tissue with increasing $CMR_{\text{ket}}$. According to Hasselbalch et al. (9), cerebral blood flow (CBF) increases as an effect of acute hyperketonemia. CBF was found to increase from $0.51 \pm 0.09$ to $0.71 \pm 0.17 \text{ml·g}^{-1} \cdot \text{min}^{-1}$ at $[\beta\text{-HB}]_{\text{plas}}$ equal to $0.31 \pm 0.17$ and $2.16 \pm 0.42 \mu\text{mol/ml}$, respectively. Increasing CBF implies more effective removal of $[^{11}\text{C}]\text{CO}_2$ produced in the tissue, counteracting the increased rate of accumulation of tracer in the tissue due to increased metabolism and $[^{11}\text{C}]\text{CO}_2$ production. It is difficult to judge the net effect, but it cannot be excluded that hyperketonemia causes a larger proportion of the tracer to be lost from the tissue, which would explain the small decrease of $K_{\text{ket}}$ with increasing $[\beta\text{-HB}]_{\text{plas}}$.

Hasselbalch et al. (9) studied cerebral uptake of ketone bodies before and during the infusion of $\beta$-HB in healthy volunteers. $\beta$-HB was infused during 140 min before the study. $CMR_{\beta\text{HB}}$ was measured by the Kety-Schmidt technique and found to be $11.1 \pm 12.1 \text{nmol·ml}^{-1} \cdot \text{min}^{-1}$ at $[\beta\text{-HB}]_{\text{plas}}$ equal to $0.31 \pm 0.17 \mu\text{mol/ml}$, and $56.0 \pm 22.5 \text{nmol·ml}^{-1} \cdot \text{min}^{-1}$ at $[\beta\text{-HB}]_{\text{plas}}$ equal to $2.16 \pm 0.42 \mu\text{mol/ml}$. The corresponding values for uptake of AcAc were $0.00 \pm 0.01 \text{nmol·ml}^{-1} \cdot \text{min}^{-1}$ and $24.9 \pm 4.17 \text{nmol·ml}^{-1} \cdot \text{min}^{-1}$, respectively. These
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The data are not directly comparable with our results; however, in agreement with the tendency observed in our study, the increase in CMRβHB (a factor of 5) was found to be somewhat smaller than the increase in \( \left[ \beta \text{-HB} \right]_{\text{plas}} \) (a factor of 7).

A major objective of the present study was to test whether chronic hyperglycemia and the tendency for hyperketonemia, typical for type 1 diabetes, would affect ketone body metabolism in the brain. This study does not provide evidence to support such a metabolic effect. The patients included in the study had average metabolic control, as assessed by their levels of HbA1c, and the results are therefore applicable to a majority of type 1 diabetic patients. To detect differences relating to chronic ketonemia, we would have had to recruit type 1 diabetic patients with markedly poor control. Such a study, however, was precluded by ethical concerns and by a scarcity of patients in the poor control category who were both willing and able to participate. Hence, we cannot exclude that worse metabolic control, i.e., more severe hyperglycemia and a stronger tendency for hyperketonemia than in the presently studied patients, might cause abnormalities in the uptake and metabolism of ketone bodies in the brain.

Conclusions. With plasma concentration of \( \beta \)-HB in the range 0.02–1.74 \( \mu \)mol/ml, the brain tissue was found to react to increased availability of ketone bodies by an increased net utilization of these compounds. There was no sign of saturation of this process. This holds true for both non-diabetic subjects and type 1 diabetic patients with average metabolic control. The transport of ketone bodies across the BBB is found to be essentially irreversible. Together with a recent study with MR spectroscopy showing low tissue concentration of \( \beta \)-HB (15), the findings of this study indicate that, also at acute hyperketonemia, the transfer from blood to brain is the rate-limiting step in ketone body utilization.

APPENDIX

Kinetic Models Used

In the \( k \) model, it is assumed that the transfer of \( \beta \)-[\( ^{11} \text{C} \) ]HB is irreversible and that the tracer is trapped in the tissue during the measuring time (10 min). The model contains a single parameter, \( K_{k}\), the accumulation rate constant, and the time course of the tracer in the tissue is governed by the simple expression

\[
C_{\text{tiss}}(T) = K_{k} \cdot \int_{0}^{T} C_{\text{in}}(t) \, dt \tag{A1}
\]

Here \( C_{\text{tiss}}(T) \) is the time course of the radioactivity concentration in the tissue outside the vascular volume, and \( C_{\text{in}}(t) \) is the input function (the time course of the tracer in plasma). \( C_{\text{tiss}}(T) \) is related to the total radioactivity concentration, \( C_{\text{tot}}(T) \), measured by PET

\[
C_{\text{tot}}(T) = \text{CBV} \cdot C_{\text{bol}}(T) + (1 - \text{CBV}) \cdot C_{\text{tiss}}(T) \tag{A2}
\]

\( C_{\text{bol}}(T) \) is the time course of the radioactivity concentration in whole blood, and \( \text{CBV} \) is the fraction (0–1) of the measuring volume that is vascular. The first term in Eq. A2 describes the time course of the tracer remaining in the blood, and the second describes the time course of the tracer that has entered the tissue. The extraction of ketone bodies is slow, and therefore a substantial part of the radioactivity remains in the blood during the measuring time, allowing a good estimate of the blood volume (Fig. 2).

In this model, the distribution volume of unmetabolized ketone bodies in the tissue, \( DV_{k} \), is expressed as \( k_{1} / (k_{2} + k_{3}) \), and the corresponding tissue concentration is \( \left[ \beta \text{-HB} \right]_{\text{plas}} \cdot DV_{k} \). The accumulation rate constant \( K_{k} \), \( k_{1} / (k_{2} + k_{3}) \), measures the efficiency of the metabolism of \( \beta \)-HB, and the corresponding CMR of \( \beta \)-HB is \( K_{k} \cdot DV_{k} \). The unidirectional rate of influx across the BBB, \( V_{\text{in}} \), is \( \left[ \beta \text{-HB} \right]_{\text{plas}} \cdot k_{1} \).

In the Gjedde-Patlak analysis, the operational equation is

\[
y = \frac{C_{\text{tiss}}(T)/C_{\text{plas}}(T)}{\text{DV}_{k} + k_{1} \cdot k_{2} / (k_{2} + k_{3})} = \frac{y}{x} = \text{DV}_{app} + K_{k} \cdot x \tag{A4}
\]

which gives \( K_{k} \) as the slope and the “apparent distribution volume,” \( \text{DV}_{app} \), as the y-intercept of a straight line fitted to the data (5, 16). Within the framework of the \( k \) model above, \( K_{k} \) and \( \text{DV}_{app} \) are expressed as \( k_{1} \cdot k_{2} / (k_{2} + k_{3}) \) and \( k_{1} \cdot k_{2} / (k_{2} + k_{3}) \), respectively.

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