Changes in cerebral blood flow and carbohydrate metabolism during acute hyperketonemia

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Hasselbalch, Steen G., Peter Lund Madsen, Lars Pinborg Hageman, Karsten Skovgaard Olsen, Niels Justesen, Søren Holm, and Olaf B. Paulson. Changes in cerebral blood flow and carbohydrate metabolism during acute hyperketonemia. Am. J. Physiol. 270 (Endocrinol. Metab. 33): E746–E751, 1996.—During starvation, brain energy metabolism in humans changes toward oxidation of ketone bodies. To investigate if this shift is directly coupled to circulating blood concentrations of ketone bodies, we measured global cerebral blood flow (CBF) and global cerebral carbohydrate metabolism with the Kety-Schmidt technique before and during intravenous infusion with ketone bodies. During acute hyperketonemia (mean β-hydroxybutyrate blood concentration 2.16 mM), cerebral uptake of ketones increased from 1.11 to 5.60 μmol·100 g⁻¹·min⁻¹, counterbalanced by an equivalent reduction of the cerebral glucose metabolism from 25.8 to 17.2 μmol·100 g⁻¹·min⁻¹, with the net result being an unchanged cerebral uptake of carbohydrates. In accordance with this, global cerebral oxygen metabolism was not significantly altered (144 vs. 135 μmol·100 g⁻¹·min⁻¹). The unchanged global cerebral metabolic activity was accompanied by a 39% increase in CBF from 51.0 to 70.9 ml·100 g⁻¹·min⁻¹. Regional analysis of the glucose metabolism by positron emission tomography (PET) using 2-[¹⁸F]fluoro-2-deoxy-D-glucose (FDG) as tracer and found a uniform decrease in glucose metabolism throughout the brain after 3.5 days of starvation, thus indicating no regional difference in ketone oxidation. This finding contrasts with the autoradiographic study in rats by Hawkins et al. (9), who found that neocortical regions in the rat brain were able to utilize ketone bodies to a greater extent than mesencephalic structures. Whether this discrepancy is due to species differences or to methodological differences remains unclear. With PET-FDG, the spatial resolution of previous studies may have prohibited the detection of small regional differences, but the use of scanners with higher resolution may allow this point to be clarified. With the use of a PET camera with higher spatial resolution and FDG as a tracer of regional glucose metabolism, we also sought to solve the question of regional differences in this metabolic shift.

MATERIALS AND METHODS

Eight healthy volunteers of normal weight were studied [mean age 24 ± 4 (SD) yr, 4 males, 4 females]. Before all investigations, oral and written consent were obtained, and the study was approved by the Central Ethical Committee in Denmark.

Protocol. Each subject was studied two times on 2 days separated by a 1- to 2-wk interval. A control study and a study during acute hyperketonemia were performed in random order. On both study days, subjects met in the morning after a light morning meal, and the studies were initiated in the postabsorptive phase 4–5 h after the morning meal. After placement of catheters and a subsequent resting period of 1 h, the subjects were placed physically relaxed in the supine position in a darkened room with eyes closed and padded.
Noise was kept at a minimum. In the hyperketonemia study, an infusion of β-hydroxybutyrate was started at time (t) = -140 min and was continued throughout the study. At t = -50 min, subjects were moved to the scanner couch, positioned, and a constant infusion of 133Xe was started at t = -30 min. After 30 min, at t = 0, the 133Xe infusion was terminated, and global CBF was determined by the Kety-Schmidt technique. rCMRGlue was simultaneously determined by PET-FDG after intravenous injection of FDC at t = 0.

β-Hydroxybutyrate infusion. Sodium β-hydroxybutyrate (no. 116501) was supplied by Sigma Chemicals (Poole, UK) and dissolved in sterile water, filtered, and tested for pyrogens to a final solution of 55 mg/ml. The pH of the final solution was 7.1. β-Hydroxybutyrate was infused in an antecubital vein at a rate of 4–5 mg/kg body wt 1 min¹, corresponding to an infusion of ~350 ml/h. The infusion was continued throughout the study, which lasted ~3–3.5 h. In the control condition, isotonic saline was infused at the same rate.

Measurement of global CBF and global cerebral metabolism. Global CBF and metabolism were measured by the Kety-Schmidt (12) technique in the desaturation mode, using 133Xe as the flow tracer. Cerebral venous blood was sampled from a catheter inserted percutaneously low on the neck into the internal jugular vein. The catheter tip was advanced to the base of the skull, and the correct placement was verified as described elsewhere (8). Arterial blood was sampled from a catheter in the radial artery. Both catheters had the same dead space volume (1 ml). Measurements of global CBF were performed exactly as previously described in detail (14). In brief, the brain was saturated by an intravenous infusion of 133Xe dissolved in saline at a constant rate of ~15 MBq/min for 30 min. A 1.5-ml dead space volume was drawn simultaneously from both catheters immediately before 1 ml of blood was drawn into preweighed syringes. Blood samples were obtained at exact times as follows: t = 2, 1, 0, 0.5, 1, 2, 3, 4, 6, 8, and 10 min, where t = 0 denotes the time when the infusion was terminated. To avoid loss of 133Xe gas by diffusion, the syringes were reweighed immediately after each run and placed in sealed vials for counting in a well counter (COBRA 5003; Packard Instrument, Downers Grove, IL). During the CBF measurement, three paired samples of arterial and jugular venous blood were obtained for paired determinations of arteriovenous differences for oxygen (a-v O₂), glucose (a-v Glc), and lactate (a-v Lac). Arteriovenous differences for ketone bodies were measured two times and arteriovenous differences for pyruvate once. Arterial carbon dioxide tension (PaCO₂) was measured three times during the CBF measurement.

PET-FDG. We used the GE 4096+ Pet Camera (General Electric Medical Systems, Milwaukee, WI) yielding 15 consecutive slices with a slice thickness of 6.7 mm and a spatial resolution in the image plane of 6.7 mm. Slices were placed parallel to the canthomeatal (CM) line (a line through the lateral canthus of the eye and the external meatus of the ear) with midslice planes from 10 to 104 mm above the CM line. A transmission scan was performed immediately before the activity scan for attenuation correction. At t = 0, 185–210 MBq FDG in 10 ml saline were injected intravenously. Dynamic scanning was started at t = 0 with the following scan sequence: 10 × 6-s scan (0–1 min), 3 × 20-s scan (1–2 min), 8 × 1-min scan (2–10 min), 5 × 2 min (10–20 min), and 5 × 5 min (20–45 min). Blood samples (1 ml) were drawn from the radial artery with increasing time interval. Blood samples were immediately placed on ice, centrifuged, and 500 µl plasma were taken for gamma counting (COBRA 5003, Packard Instrument).

rCMRGlu was calculated from PET by the single scan approach first described by Sokoloff et al. (17) using the last scan (t = 40–45 min) corrected by rate constants and lumped constant determined from the dynamic scan sequence as previously described (8). Rate constants were calculated from six gray and six white matter regions of interest drawn by hand on three slices located ~43, 57, and 70 mm above the CM line. Region of interest analysis was performed with the use of the Computerized Brain Atlas (7) and internal ratios were obtained by normalizing regions to the weighted mean of all cortical regions.

Substrate analysis. Blood samples for determination of a-v O₂ and PaCO₂ were stored on ice and analyzed within 15 min for oxygen saturation, hemoglobin concentration, and PaCO₂ with an OSM3 and ABL apparatus (Radiometer, Copenhagen, Denmark). Blood samples for determination of plasma glucose and lactate concentrations were drawn into vials containing fluoride-EDTA, stored on ice for 5 min, centrifuged, and analyzed in duplicate within 15 min on a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, CA); the remaining blood samples for determination of pyruvate once. Arterial carbon dioxide tension (PaCO₂) was measured two times and PaO₂ was calculated using the hemoglobin concentration and oxygen saturation of hemoglobin in arterial and jugular venous blood. Cerebral a-v O₂ and PaO₂ were stored on ice and analyzed within 15 min on a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, CA); the remaining portions of these blood samples were stored at ~80°C until subsequently analyzed in duplicate with a YSI 2300 Lactate Analyzer (Yellow Spring Instruments, Yellow Springs, OH). Whole blood samples for determination of acetocetate and β-hydroxybutyrate were immediately deproteinized, stored at ~80°C, and later analyzed as described by Williamson et al. (21). Pyruvate was analyzed by standard enzymatic techniques.

The blood samples used in the PET-FDG and CBF measurements contained gamma activity from both 133Xe [decay half-time (T½) = 5.29 days] and [18F]FDG (decay T½ = 110 min). Energy windows were set around the energy peak of each tracer [133Xe = 70–90 keV; [18F]FDG = 461–561 keV], and correction for cross talk between windows was applied. By extending the time interval from sampling of blood to the gamma counting of the blood samples, cross talk from the [18F] window into the 133Xe window decreased rapidly. The blood samples were counted continuously for the next 12 h, and the optimal counting time was found to be 8–10 h after injection, where the cross-talk correction from FDG into the 133Xe window was very small and no detectable diffusion of 133Xe out of the vials had occurred. However, it was found that the time interval between sampling of blood and counting of blood samples had no significant influence on the CBF calculation.

Calculations. CBF was calculated using the Kety-Schmidt (12) equation modified for the application of the method in the desaturation mode. The measured CBF values were corrected for the systematic overestimation of flow values due to incomplete tracer washout at the end of the measuring period (14). Cerebral a-v O₂ was calculated using the hemoglobin concentration and oxygen saturation of hemoglobin in arterial and internal jugular venous blood. Cerebral a-v Glc and a-v Lac were calculated from glucose and lactate concentrations in arterial and venous plasma. It is our experience that glucose and lactate determinations are more precise when performed on plasma than on whole blood, and the lactate and glucose concentrations presented in this study are plasma values corrected to corresponding whole blood values as described previously (13). Global metabolic rates (CMR) were calculated for each substrate according to the Fick principle, e.g., metabolic rate for oxygen (CMRO₂) = CBF × a-v O₂. A paired two-tailed Student's t-test was used in the statistical evaluation of the data.

RESULTS

Arterial concentrations, a-v differences, and metabolic rates for all brain metabolites measured are
shown in Table 1. During ketone body infusion, blood concentration of β-hydroxybutyrate increased sevenfold from 0.31 to 2.16 mM (P < 0.0001), and acetoacetate concentration also increased from 0.02 to 0.24 mM (P < 0.01) due to the conversion of β-hydroxybutyrate to acetoacetate in tissue. Blood concentrations of glucose, lactate, and pyruvate remained constant during acute hyperketonemia. Global CBF increased by 39% from a control value of 5.70 to 7.90 ml/100 g per minute during acute hyperketonemia (P < 0.02). The CBF increase was not due to changes in Pao2, pH, and HCO3 values at time of cerebral blood flow measurement (3 averaged measurements).

The present study demonstrates significant changes in brain metabolism and CBF when the blood ketone level is acutely increased in humans. The main findings are as follows: 1) a fivefold increase in β-hydroxybutyrate metabolism, 2) a 33% reduction in glucose metabolism, 3) a 39% increase in (CH3COOH) and 4) a small change in the regional pattern of (CH3COOH).

**DISCUSSION**

The present study demonstrates significant changes in brain metabolism and CBF when the blood ketone level is acutely increased in humans. The main findings are as follows: 1) a fivefold increase in β-hydroxybutyrate metabolism, 2) a 33% reduction in glucose metabolism, 3) a 39% increase in (CH3COOH), and 4) a small change in the regional pattern of (CH3COOH).

**Alterations in cerebral carbohydrate metabolism.** Infusion with β-hydroxybutyrate caused a sevenfold increase in the blood level of β-hydroxybutyrate and a fivefold increase of CMR<sub>glc</sub>. The same correlation between the increase in blood ketone levels and the increase in cerebral ketone uptake after prolonged hypoglycemia has previously been observed in experimental studies (9, 16) and in humans (6, 8). Indirect evidence for acute cerebral oxidation of ketone bodies during hypoglycemia has been obtained both in animals and humans. In suckling weaning mice, Thurston
Table 3. Oxygen-to-glucose molar ratio and O$_2$ equivalents of glucose and ketone bodies before and during acute hyperketonemia

<table>
<thead>
<tr>
<th>Molar Ratio</th>
<th>Glucose</th>
<th>Ketones</th>
<th>Total</th>
<th>Measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.57 ± 0.47</td>
<td>145 ± 19 (97%)</td>
<td>5 ± 8 (3%)</td>
<td>150 ± 22</td>
</tr>
<tr>
<td>Hyperketonemia</td>
<td>7.91 ± 1.00$^+$</td>
<td>92 ± 18* (74%)</td>
<td>32 ± 12$^+$ (26%)</td>
<td>124 ± 12$^+$</td>
</tr>
</tbody>
</table>

Values are means ± SD. Values in parentheses are percentage of total. Molar ratio measured as ratio between oxygen and glucose metabolic rate; O$_2$ glucose (umol·100 g$^{-1}$·min$^{-1}$) = O$_2$ equivalents of total oxidation of glucose corrected for efflux of lactate and pyruvate (6 × (CMR$_{Glc}$ + CMR$_{Lac}$ + CMR$_{Py}$)/2), where CMR$_{Glc}$, CMR$_{Lac}$, and CMR$_{Py}$ are metabolic rates for glucose, lactate, and pyruvate, respectively; O$_2$ ketones = O$_2$ equivalents of total oxidation of β-hydroxybutyrate (β-OHB) and acetoacetate (AcAc; 4.5 × CMR$_{OH}$ + 4 × CMR$_{Ac}$). O$_2$ total = O$_2$ glucose + O$_2$ ketones = summed O$_2$ equivalents of glucose and ketone bodies; O$_2$ measured = oxygen consumption measured from CBF × arteriovenous O$_2$ difference. $^+$P < 0.005, $^*$P < 0.0005, $^+$P < 0.01; paired 2-Sample t-test.

et al. (19) reversed hypoglycemia-induced coma and sustained normal brain metabolism by infusion of β-hydroxybutyrate. In humans, ketone body infusion lowered the counterregulatory hormone response to hypoglycemia (1). However, also in humans, by infusing ketone bodies, Frolund et al. (5) found no changes in the hypoglycemia-induced neurohumoral response. Thus, while previous results are not clear and the studies only gave indirect data obtained during hypoglycemia, the present study, for the first time, provides direct evidence for changes in cerebral metabolism during acute hyperketonemia in normoglycemic humans. The acute hyperketonemia induced increase in CMR$_{OH}$ was counterbalanced by a 33% reduction of CMR$_{Glc}$, but no significant alterations in CMR$_{O2}$ were observed (144 ± 22 vs. 135 ± 22 umol·100 g$^{-1}$·min$^{-1}$, not significant). Thus, without changing the level of oxidative metabolism, acute hyperketonemia induced a partial substitution of cerebral glucose metabolism with metabolism of ketone bodies. The present study thus indicates that the human brain can use ketone bodies during normoglycemia and emphasizes that brain ketone body uptake is regulated by the availability of ketone bodies and not by glucose deficiency.

How the shift in brain substrate metabolism is brought about is not entirely known, but experimental studies have found metabolite changes compatible with inhibition of hexokinase, and phosphofructokinase, and thus inhibition of glycolysis (19). Inhibition of glycolysis could be brought about by an increase in citrate.

![Fig. 1. Changes in pH, PCO$_2$, and arteriovenous differences for glucose (a-v Glc) and oxygen (a-v O$_2$) during infusion of β-hydroxybutyrate. Values are mean values of 8 subjects, scaled to 100% at start of infusion (time = 0). Decrease in a-v O$_2$ is presumably due to an increase in CBF, and uncoupling of a-v Glc and a-v O$_2$ 60 min after start of infusion indicates increased oxidation of nonglucose substrates. PET-FDG, positron emission tomography-[18F]fluoro-2-deoxy-D-glucose.](image-url)
metabolism after ketone body infusion

Rates (rCMRc, I,) in region normalized to weighted mean of all gray
glycolysis. In contrast to these experimental data, the
inhibition of pyruvate oxidation, which again inhibits
study, it was also observed that hyperketonemia caused
lism through the tricarboxylic acid cycle (16). In that
induced by the increased P-hydroxybutyrate metabo-
lates relatively smaller decrease in rCMRc, I, compared with mean
gray matter.

rCMRc, in mean gray matter). Increase in normalized value indi-
cates that the mesencephalon-to-cortex ratio
creased significantly by 8% during ketone body infu-
sion with autoradiographic data from rats (9), but the
increase in CBF was not due to alterations in Pao2, as
increase in CBF was not due to alterations in Pace, as
2 ml 100 g-1 min during hyperketonemia. This 39%
increase in CBF was not due to alterations in PaCO2, as
this variable was unchanged by hyperketonemia.
Likewise, the CBF increase cannot be explained by the
minor increase in pH, which, if anything, should lead to
a decrease in CBF.

Table 4. Regional changes in glucose metabolism after ketone body infusion

<table>
<thead>
<tr>
<th>Region</th>
<th>Control</th>
<th>Infusion</th>
<th>%Change</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orbitofrontal</td>
<td>1.03 ± 0.05</td>
<td>0.99 ± 0.09</td>
<td>-3.7 NS</td>
<td></td>
</tr>
<tr>
<td>Dorsolateral frontal</td>
<td>1.16 ± 0.12</td>
<td>1.15 ± 0.07</td>
<td>-0.1 NS</td>
<td></td>
</tr>
<tr>
<td>Temporal</td>
<td>1.03 ± 0.06</td>
<td>1.00 ± 0.07</td>
<td>-2.6 NS</td>
<td></td>
</tr>
<tr>
<td>Parietal</td>
<td>0.96 ± 0.08</td>
<td>1.01 ± 0.06</td>
<td>5.4 NS</td>
<td></td>
</tr>
<tr>
<td>Occipital</td>
<td>0.96 ± 0.06</td>
<td>0.93 ± 0.11</td>
<td>-2.6 NS</td>
<td></td>
</tr>
<tr>
<td>Nucleus caudatus</td>
<td>1.09 ± 0.04</td>
<td>1.12 ± 0.10</td>
<td>2.5 NS</td>
<td></td>
</tr>
<tr>
<td>Putamen</td>
<td>1.32 ± 0.17</td>
<td>1.33 ± 0.10</td>
<td>1.4 NS</td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td>1.09 ± 0.14</td>
<td>1.15 ± 0.12</td>
<td>5.7 NS</td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.73 ± 0.09</td>
<td>0.70 ± 0.09</td>
<td>-3.8 NS</td>
<td></td>
</tr>
<tr>
<td>Gyrus cingulus</td>
<td>1.03 ± 0.13</td>
<td>1.10 ± 0.11</td>
<td>8.1 NS</td>
<td></td>
</tr>
<tr>
<td>Pons</td>
<td>0.44 ± 0.09</td>
<td>0.44 ± 0.06</td>
<td>-2.3 NS</td>
<td></td>
</tr>
<tr>
<td>Mesencephalon</td>
<td>0.69 ± 0.08</td>
<td>0.75 ± 0.09</td>
<td>8.4 0.008</td>
<td></td>
</tr>
<tr>
<td>White matter</td>
<td>0.41 ± 0.05</td>
<td>0.39 ± 0.06</td>
<td>-3.0 NS</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. Values are regional glucose metabolic rates (rCMRc, I,) in region normalized to weighted mean of all gray matter regions (i.e., nucleus caudatus = rCMRc in nucleus caudatus/ rCMRc, in mean gray matter). Increase in normalized value indicates relatively smaller decrease in rCMRc, I, compared with mean gray matter. P < 0.01 was considered significant due to multiple comparisons. NS, not significant.

concentration and a decrease in ADP concentration induced by the increased β-hydroxybutyrate metabolism through the tricarboxylic acid cycle (16). In that study, it was also observed that hyperketonemia caused a marked increase of cerebral lactate efflux, indicating inhibition of pyruvate oxidation, which again inhibits glycolysis. In contrast to these experimental data, the unchanged level of cerebral lactate and pyruvate efflux observed during hyperketonemia in the present study can be taken to signify unaltered cerebral production of lactate and pyruvate. This observation is in accordance with data obtained during hyperketonemia after 3 days of starvation (8), indicating that inhibition of glycolysis is not related to inhibition of pyruvate oxidation. Whether the discrepancy of results is due to species difference or can be explained by methodological differences remains to be clarified.

Analysis of the regional changes in glucose metabolism revealed that the mesencephalon-to-cortex ratio increased significantly by 8% during ketone body infusion, which indicates a relatively smaller decrease in glucose metabolism in this region, signifying that mesencephalic structures do not oxidize ketone bodies to the same extent as the rest of the brain. This is in line with autoradiographic data from rats (9), but the reason for this regional difference in ketone body utilization capacity remains to be clarified.

Alterations in CBF. Despite the unchanged overall metabolic rate, infusion with β-hydroxybutyrate increased global CBF from a baseline value of 51 to 71 ml·100 g-1 min during hyperketonemia. This 39% increase in CBF was not due to alterations in PaCO2, as this variable was unchanged by hyperketonemia. Likewise, the CBF increase cannot be explained by the minor increase in pH, which, if anything, should lead to a decrease in CBF.

Theoretically, the increase in CBF could be due to a direct action of β-hydroxybutyrate on cerebrovascular smooth muscle, but no existing literature provides information on this point. Information on blood flow response to hyperketonemia in other organs is also sparse. Acute hyperketonemia increased human forearm blood flow by 15% (20), but the exact mode of action remains obscure. Because the CBF increase was parallel to a reduction of CMRGlut, it could seem similar to the inverse relationship between glucose metabolism and CBF, which has been demonstrated in hypoglycemia. Using single photon emission tomography in normal volunteers, Tallroth et al. (18) found a marked increase (~20%) in CBF 10 min after induction of acute hypoglycemia (mean plasma glucose 2.0 mM). Similar results have been obtained in rats by Bryan et al. (3), who induced a 140–240% increase in CBF by lowering the mean plasma glucose level to 1.5 mM. The CBF increase in the present study differs somewhat from the above studies in the sense that inhibition of glycolysis was not mediated by an acute lowering of plasma glucose, since there was ample glucose supply to the brain. Thus the CBF increase seems to be related to the decrease in glycolytic flux, in line with the experimental study of Breier et al. (2), who demonstrated significant CBF increases by blocking glycolysis by pharmacological doses of 2-deoxy-d-glucose. However, in contrast to studies in which severe glucopenia or blocking of carbohydrate metabolism may induce other and more profound metabolic changes in the tissue, the present study reveals that coupling of glycolysis and CBF is independent of the energy state of the brain or the total cerebral oxidative metabolism, because these did not change during hyperketonemia. This finding points to glycolytic intermediates as regulators of CBF, but the mechanisms behind the coupling of CBF and glucose metabolism remain unknown. Several other putative mediators have been proposed, including catecholamines and adenosine (10, 22), but none of these are likely to have played a significant role in the present study performed during normoglycemia, where no neurohumoral stress response or adenosine accumulation are expected to occur.

In contrast to the present data, after several days of starvation, no changes in CBF were observed, although glucose metabolism had decreased similarly to what was observed in the present study (8). It may be that a secondary downregulation of CBF takes place with time, but, since the mechanisms of the coupling still remain unclear, these speculations should be clarified in future studies.

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