Systemic, Cerebral and Skeletal Muscle Ketone Body and Energy Metabolism During Acute Hyper-D-β-Hydroxybutyratemia in Post-Absorptive Healthy Males

Kristian H. Mikkelsen, Thomas Seifert, Niels H. Secher, Thomas Grøndal, and Gerrit van Hall

Clinical Metabolomics Core Facility (K.H.M., T.G., G.v.H.), Department of Anaesthesiology (T.S., N.H.S.), Rigshospitalet, and Department of Biomedical Sciences (G.v.H.), Faculty of Health and Medical Sciences, University of DK-2100 Copenhagen, Denmark

Context: Ketone bodies are substrates during fasting and when on a ketogenic diet not the least for the brain and implicated in the management of epileptic seizures and dementia. Moreover, D-β-hydroxybutyrate (HOB) is suggested to reduce blood glucose and fatty acid levels.

Objectives: The objectives of this study were to quantitate systemic, cerebral, and skeletal muscle HOB utilization and its effect on energy metabolism.

Design: Single trial.

Setting: Hospital.

Participant: Healthy post-absorptive males (n = 6).

Interventions: Subjects were studied under basal condition and three consecutive 1-hour periods with a 3-, 6-, and 12-fold increased HOB concentration via HOB infusion.

Main Outcome Measures: Systemic, cerebral, and skeletal muscle HOB kinetics, oxidation, glucose turnover, and lipolysis via arterial, jugular, and femoral venous differences in combination with stable isotopically labeled HOB, glucose, and glycerol, infusion.

Results: An increase in HOB from the basal 160–450 μmol/L elicited 14 ± 2% reduction ($P = .03$) in glucose appearance and 37 ± 4% decrease ($P = .03$) in lipolytic rate while insulin and glucagon were unchanged. Endogenous HOB appearance was reduced in a dose-dependent manner with complete inhibition at the highest HOB concentration (1.7 mmol/L). Cerebral HOB uptake and subsequent oxidation was linearly related to the arterial HOB concentration. Resting skeletal muscle HOB uptake showed saturation kinetics.

Conclusion: A small increase in the HOB concentration decreases glucose production and lipolysis in post-absorptive healthy males. Moreover, cerebral HOB uptake and oxidation rates are linearly related to the arterial HOB concentration of importance for modifying brain energy utilization, potentially of relevance for patients with epileptic seizures and dementia. **(*J Clin Endocrinol Metab* 100: 636–643, 2015)**

Ketone bodies represent an alternative energy source when the carbohydrate intake is very low as during starvation when gluconeogenesis becomes limited, or when living on the ketogenic diet that typically consists of 88% fat, 10% protein, and only 2% carbohydrates. The ketogenic diet has been used for decades to treat central

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in U.S.A.

Received June 11, 2014. Accepted November 14, 2014.
First Published Online November 21, 2014

Abbreviation: AcAc, acetoacetate; HOB, D-β-hydroxybutyrate.

nervous system (CNS) disorders (1), has been proven successful in seizure management of juvenile and adult epilepsy (2), and has been implicated in improved motor scores in patients with Parkinson's disease (3) and cognitive function in Alzheimer's disease (4). In addition, exogenous provision of ketone bodies has been shown to decrease glucose and fatty acid levels in humans and thus has the potential as an adjunct in the management of hyperglycemia and hyperlipidemia in, for example, patients with type 2 diabetes despite the ketone bodies' ill reputation due to the complication of ketoacidosis in patients with type 1 diabetes (5–9). Unfortunately, both the ketogenic diet and ingestion of substantial quantities of ketone bodies to enhance their blood concentration are impracticable. In CNS disorders, the ketogenic diet is impractical because of its low compliance given that it is almost impossible to ingest pure fat-containing food items as it is unpalatable, the very low fiber intake causes constipation, and in children with epilepsy, growth disorders have been observed (10). Ingestion of large quantities of ketone body salts to enhance the blood concentration is also impractical because it causes gastrointestinal distress. However, ingestion of ketone monoesters was both safe and tolerable in healthy human volunteers with doses raising blood ketone body levels to approximately 1 mmol/L with a slow elimination time (11, 12). However, few human studies have determined ketone body kinetics of the brain (13, 14) and skeletal muscle (15–19) as the quantitative largest peripheral tissue that should guide oral ketone supplementation in CNS disorders. Moreover, several studies have investigated systemic human in vivo ketone body metabolism but lack the determination of glucose production and lipolysis (5–9). In addition, exogenous oral or iv provision of mainly hydroxybutyrate may have been affected by the fact that not the endogenous D-β-hydroxybutyrate (HOB) was provided but the racemic mixture of D- and L-β-hydroxybutyrate and/or in doses affecting the blood pH (6, 14, 20). Therefore, we investigated the metabolic effects of acute hyper-D-β-hydroxybutyratemia in healthy young males by infusing HOB at three consecutive increasing rates for 1 hour, eliciting an approximately 3-, 6-, and 12-fold higher HOB concentration compared with the basal overnight fasting level. We investigated the effects of these three levels of acute hyper-D-β-hydroxybutyratemia on the HOB systemic, cerebral, and skeletal muscle kinetics and the HOB conversion rate to the other abundant ketone body acetoacetate (AcAc) and their mutual oxidation rate; hence, ketone body oxidation rates. Moreover, we determined the role of acute hyper-D-β-hydroxybutyratemia on the endogenous glucose production and utilization and lipolysis.

Materials and Methods

Subjects

Six male subjects participated in the study at an age of 25 ± 4 years, body weight of 81 ± 9 kg, and a body mass index of 23 ± 2 kg/m^2. Before the study the subjects underwent a medical examination demonstrating normal blood analyses. The subjects were physically active; not smoking or using medication, nor had a known history of medication/disease. The subjects were informed of risks and discomfort associated with the experimental protocol both orally and in writing as approved by the Ethical Committee for the region of Copenhagen (H-D-2008-115) in accordance with the Declaration of Helsinki.

Protocol

On the experimental day the subjects reported to the laboratory at 0800 h after an overnight fast (from 2200 h) and brachial, arterial, jugular, and femoral venous catherization was performed (21). Some 20 minutes after catheterization a primed continuous infusion via an antecubital vein was started of sodium HOB (prime, 1.5 μmol/kg; 0.1 μmol/kg/min), [6,6-$^2H_2$]glucose, (prime, 17.6 μmol/kg; 0.4 μmol/kg/min, [1,1,2,3,3-$^2H_5$]glycerol (prime 1.5 μmol/kg; 0.1 μmol/kg/min), and a priming bolus of $H^{13}CO_3$ (Cambridge Isotope Laboratories) (Figure 1). After a baseline period of 2 hours, HOB infusion via the antecubital vein was started at 4.7 μmol/kg/min (low HOB) for 1 hour; hereafter, the infusion rate was increased for 1 hour to 9.4 μmol/kg/min (medium HOB), and 18.8 μmol/kg/min (high HOB). The infusion rate of D-β-[2,4-$^{13}C_2$]hydroxybutyrate was increased 3-, 4.5-, and 8-fold to minimized enrichment differences during the hour of low, medium, and high HOB infusion, respectively. Blood and breath sampling and indirect calorimetry (Cosmed, Quark b2) were performed before and 90, 105, and 120 minutes after the start of the primed continuous tracer infusions and after 30, 45, and 60 minutes at each of the three levels HOB infusion.

Analysis

HOB and AcAc concentrations and enrichments were determined by gas chromatography, mass-spectrometry (GC-MS, Automass II, Finnigan). Glucose and glycerol concentration and enrichments were determined by liquid chromatography, tandem mass spectrometry (Cohesive TX-1-Sciex API3000) (23). Blood for blood gas variables was sampled with a gas-tight heparinized syringes and directly analyzed (ABL725, Radiometer). Blood and breath CO_2 enrichment were analyzed with gas chromatography, isotope ratio mass spectrometry (GC-IRMS, Finnigan Delta^{plus}, Finnigan MAT). Details of the above analysis can be found in Supplemental Materials. Blood flows were calculated according to van Hall et al (21).

Calculations

Whole-body measurements

The whole-body HOB, glucose, and glycerol rate of appearance (R_a) was calculated as:

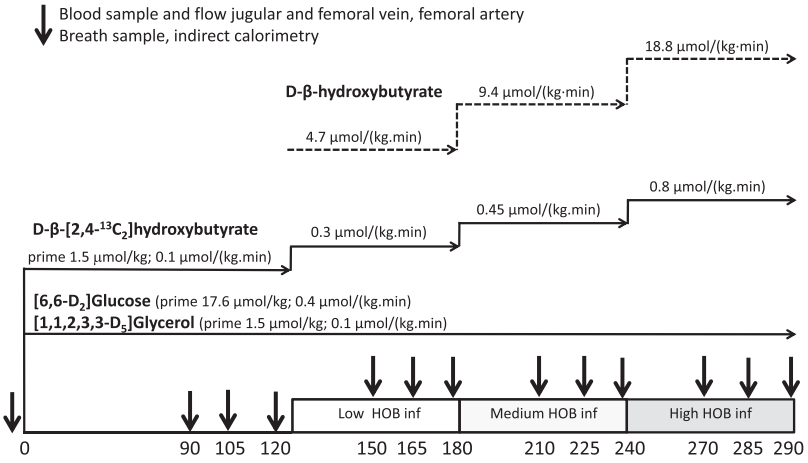

Figure 1. Schematic presentation of the experimental design. The male volunteers reported to the laboratory at 0800 h after an overnight fast (from 2200 h the previous evening). Around 0900 h, about 20 minutes after the cauterization procedures, the experiment was started (t = 0) with the primes and continuous infusion of the stable isotopes. HOB infusion started after 2 h, consecutively for 1 h at the low, moderate, and high HOB rate (low HOB inf, medium HOB inf, high HOB inf) to raise the arterial HOB concentration approximately 3-, 6-, and 12-fold.

$$\text{Total } R_a = R_d = F/E_a$$

where F is the isotopic infusion rate (μmol/kg/min) and E_a is the arterial isotope enrichment of the tracer/tracee ratio.

$$\text{Endogenous HOB } R_a = \text{Total HOB } R_a - \text{intravenous HOB infusion rate}$$

Cerebral and leg calculations

The net uptake of HOB, glucose and glycerol was calculated as

$$\text{Net uptake} = (C_a - C_v) \times \text{blood flow}$$

No differences between arterial and jugular and femoral venous HOB and AcAc enrichment was observed suggesting that there was no de novo synthesis or exchange reactions of HOB and AcAc in brain and skeletal muscle.

The relative cerebral and leg ketone oxidation was estimated from the $^{13}CO_2$ release. Despite the fact that [2,4-$^{13}C_2$]D-β-HOB was infused, a considerable and rapid label incorporation was found for AcAc and therefore the tissues $^{13}CO_2$ release is from both HOB and AcAc oxidation, albeit from the quantity and enrichment, most $^{13}CO_2$ originates from HOB oxidation. Moreover, not all ^{13}C-label from an oxidized ^{13}C-labeled substrate will appear directly as $^{13}CO_2$ for which a correction factor should have been applied (24, 25). The correction factor for ^{13}C-label retention is substantial for ^{13}C that enters in the 2 position of acetyl coenzyme A, but the quantity and time effects are largely unknown. Therefore, we chose not to correct for ^{13}C-label retention (24, 26).

$$\text{Relative ketone oxidation} = ([\{C_{v,CO2} \times E_{v,CO2}\} - \{C_{a,CO2} \times E_{a,CO2}\}/2]/[\{C_{a,HOB} \times E_{a,HOB} + C_{a,AcAc} \times E_{a,AcAc}\}/C_{a,HOB} + C_{a,AcAc}])$$

where $C_{v,CO2}$, $C_{a,CO2}$, $E_{v,CO2}$, $E_{a,CO2}$ are the blood CO_2 concentration and enrichment in tracer/tracee ratio in the veins and artery, respectively. The factor two is to account for two $^{13}CO_2$ appearing from the oxidation of one molecule of [2,4-$^{13}C_2$]HOB or AcAc.

Statistics

Statistics were performed on the average of three measurements made under basal condition and the three HOB infusion periods. Values in the graphs are presented as mean ± SEM for six subjects and statistical differences between intervention periods and the tissue substrate net uptake/exchange was analyzed by comparing mean values with zero were tested using the nonparametric Wilcoxon signed-rank test ($P < .05$). Tests and curve fitting (Figure 4) were performed with OriginPro 8.6 (OriginLab).

Results

Systemic, cerebral, and leg skeletal muscle ketone body kinetics

HOB infusion increased the plasma concentration of HOB (Figure 2A) and AcAc (Figure 2B), steady levels were achieved at the low and moderate HOB infusion rate within 30 minutes. The endogenous HOB R_a decreased with each increase in HOB infusion and reached zero at the highest HOB infusion rate (Figure 2C). At the low HOB infusion rate the AcAc concentration increased similarly as HOB but lagged behind more at the medium and high HOB infusions as depicted by the increased HOB/AcAc ratio (Figure 2D), implying that AcAc conversion from HOB could not keep up with the high HOB R_a. At the high HOB infusion rate the HOB/AcAc enrichment ratio reached unity (Figure 2D), meaning that all AcAc was derived from the infused HOB agreeing with the absence of any endogenous HOB production (Figure 2C).

No differences between the arterial, jugular, and femoral venous HOB and AcAc enrichment was found, suggesting that HOB and AcAc are not, or only to a minor extent, produced de novo or from exchange reactions in brain and skeletal muscle.

Cerebral HOB and AcAc uptake was only significant at the medium and high HOB infusion rates and was higher with the highest arterial HOB and AcAc concentrations (Figure 3A). However, the relative cerebral ketone oxidation, represented minimal quantities, was observed at all three HOB infusion rates (Figure 3, A and B) suggesting cerebral HOB uptake and oxidation at all HOB levels. Leg, skeletal muscle, HOB, and AcAc uptake was ob-

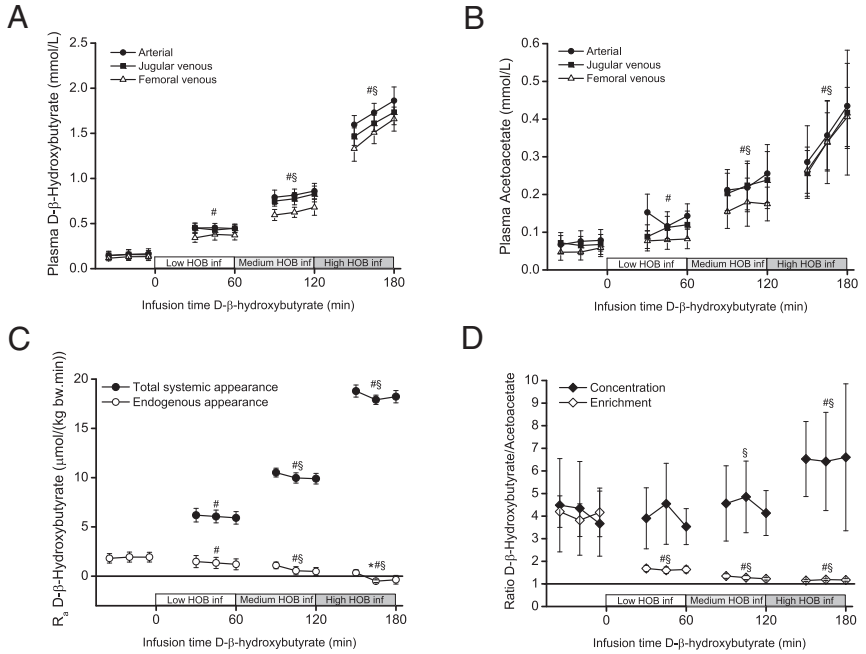

Figure 2. Systemic HOB and acetoacetate concentrations and kinetics in healthy males under basal condition and 1 h of acute low, medium, and high HOB iv infusion. #, Significantly different from the average basal period; §, Significantly different from the previous period; *, Not significantly different from zero.

served for all conditions. However, ketone relative oxidation only reached significance at the high HOB infusion rate (Figure 3, C and D). The contribution of the cerebral and total skeletal muscle HOB uptake to the systemic HOB disappearance was 8 ± 5% and 32 ± 8% under basal conditions and decreased during the high HOB infusion rate to 5 ± 2% and 15 ± 5%, respectively. Cerebral HOB uptake was linearly related to the arterial concentration when the nonsignificant basal HOB uptake condition was excluded (Figure 4A). HOB uptake by skeletal muscle showed exponential, saturation, uptake kinetics (Figure 4B).

Systemic, cerebral, and skeletal muscle glucose and glycerol kinetics

The arterial glucose concentration decreased with each increase of the HOB infusion rate (Figure 5A). The glucose R_a also decreased with HOB infusion; however, the largest decrease (14 ± 2%; $P = .03$) was found going from basal condition to the low HOB infusion rate (Figure 5C). Glycerol concentration and R_a, a measure for lipolytic rate, were most decreased going from the basal condition to the low HOB infusion rate with 37 ± 4% ($P = .03$), albeit the high HOB infusion caused a further decrease in both glycerol concentration and R_a (Figure 5, B and D).

The cerebral glucose uptake was unchanged during the study whereas the basal leg glucose uptake was variable and absent at the high HOB infusion (Figure 5E). The leg glycerol release was substantially decreased with the low HOB infusion but decreased further to low levels at the high HOB infusion rate (Figure 5F). The decline of leg glycerol release was caused mainly by a decreased unidirectional glycerol release, suggesting either a reduction in the subcutaneous or intramyocellular triacylglycerol breakdown with HOB infusion.

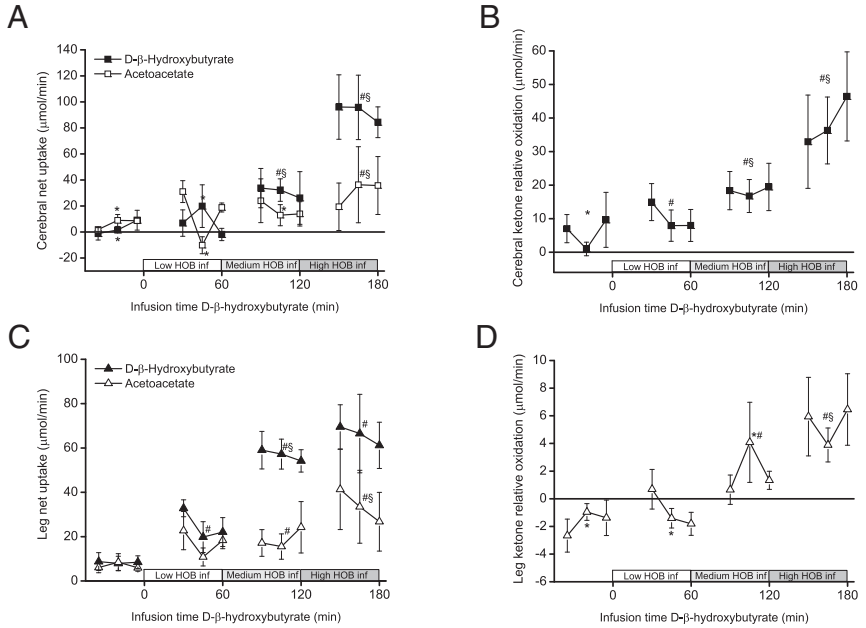

Figure 3. Cerebral and leg HOB and acetoacetate net uptake rates and relative ketone oxidation rates in healthy males under basal condition and 1 hour of acute low, medium and high HOB iv infusion. #, Significantly different from the average basal period; §, Significantly different from the previous period; *, Not significantly different from zero.

Insulin and glucagon concentrations

The arterial insulin and glucagon concentrations were unchanged after 1 hour at the low HOB infusion rate but at the medium and high HOB infusion rates, a decrease in insulin was observed, whereas there was a modest increase in glucagon (Figure 6).

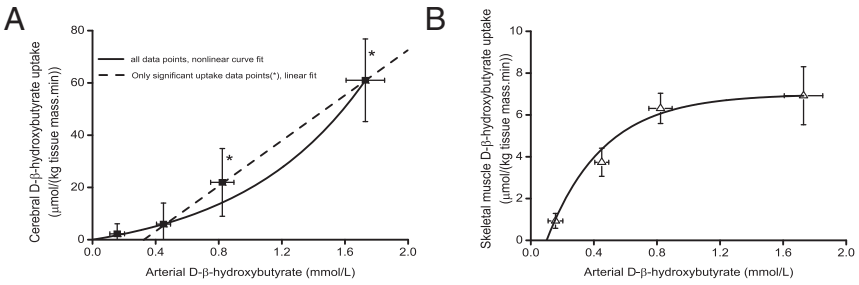

Figure 4. Relationship between the arterial HOB concentration and cerebral and leg, skeletal muscle, uptake rate per kg of tissue in healthy males. A simple exponential curve fitting was applied for all data, cerebral $y = 7.86 \times \exp(-x/-0.80) - 7.86$ and for skeletal muscle $y = -9.25 \times \exp(-x/0.35) + 7.00$. Since a significant cerebral uptake was only determined for the medium and high HOB concentrations (Figure 3A) a linear fit was made (dashed line), $y = 43.1(x) - 13.5$. Tissue masses used for the HOB uptake per kg tissue calculations were 1.5 kg as the average human brain weight and 8.1 kg for the leg skeletal muscle weight estimated from the total body skeletal muscle mass being in healthy young active males approximately 40% of the total body weight of which 25% is one leg muscle mass.

Discussion

The major findings of the present study are that in healthy postabsorptive young males: First, a relative small acute increase in the plasma HOB concentration from a basal level of 160–450 μmol/L via iv HOB infusion caused within 30 minutes a 14 ± 2% reduction in blood glucose appearance and a 37 ± 4% decrease in glycerol appearance, representative for lipolytic rate, in the absence of changes in both insulin and glucagon. Second, higher plasma HOB concentrations caused a further decrease in glucose appearance and lipolysis despite a decrease in insulin and an increase in glucagon that would have the opposing effect. Third, HOB inhibited its own production in a dose-dependent manner with complete inhibition at the highest HOB infusion rate attaining an arterial HOB concentration of 1.7 mmol/L. Fourth, cerebral HOB uptake and subsequent oxidation was linearly related to the arterial HOB concentration. Fifth, resting skeletal muscle HOB uptake was much lower than the cerebral HOB uptake when expressed per kilogram of tissue and showed saturation kinetics with little increase in the HOB uptake between 0.8 and 1.7 mmol/L.

Glucose production and lipolysis

It is the general finding that ketone body infusion decreases glucose and free fatty acid concentrations, but the mechanism is not well understood. Most human in vivo studies point at a direct metabolic action of ketone bodies whereas others claim a hormonally mediated effect (6). In the present study we observed a marked 14 ± 2% decrease in whole-body glucose appearances and a 37 ± 4% decrease in the rate of lipolysis within 30 minutes of an acute increase in the plasma HOB concentration from 160–450 μmol/L. These decreases occurred without a change in either insulin or glucagon, the most important hormones for control of liver glucose output and lipolysis, suggesting that these changes are directly affected by ketone bodies. A further, but in absolute terms smaller, decrease in glucose appearances and lipolysis was observed when the HOB concentration was increased to 1.7 mmol/L, suggesting no clear dose response of the HOB concentration on systemic glucose appearance and lipolysis. However, the lower insulin and higher glucagon levels at these higher HOB infusion rates would elicit a higher or maintained glucose appearance rate and higher rate of lipolysis, thus opposing the effect observed. Acute HOB elevation is also suggested to be insulinotropic (6), in contrast with the lower insulin concentration with increasing HOB concentration in the present study. However, to demonstrate the insulinotropic effect of ketone bodies, the ketone concentrations must be greater than 2 mmol/L and only manifested in the initial 5–15 minutes of an acute increase in ketone bodies whereafter insulin returned to basal levels (27, 28). Therefore, the observed changes in insulin and glucagon are most likely the result of the lower blood glucose concentration. Most importantly this study shows that even small increases in the plasma HOB concentration have the potential to reduce glucose levels and lipolysis considerably. Whether these potential therapeutic effects are long lasting and present in patient remains to be investigated, including the hormonal responses.

Systemic HOB and AcAc kinetics

The postabsorptive basal HOB concentration of 160 mmol/L and rate of appearance of 2 μmol/kg/min compare well with previous investigations (29–31). The low and medium HOB infusion rate elicited a stable HOB and AcAc concentration and HOB turnover rates between 30 and 60 minutes upon commencement of the HOB infusion. This was also the case in half of the volunteers for the high HOB infusion but in the other half a steady ± 20% increase in HOB concentration was observed over the last 30 minutes. This may imply that HOB clearance was reaching its maximal capacity at the high HOB infusion rate, not the least in the liver for its conversion to AcAc implicated by an increased HOB/AcAc ratio and in skeletal muscle (see discussion below). HOB inhibited its own production in a dose-dependent manner reaching complete inhibition at the high HOB infusion rate eliciting a HOB concentration of 1.7 mmol/L supported by the ob-

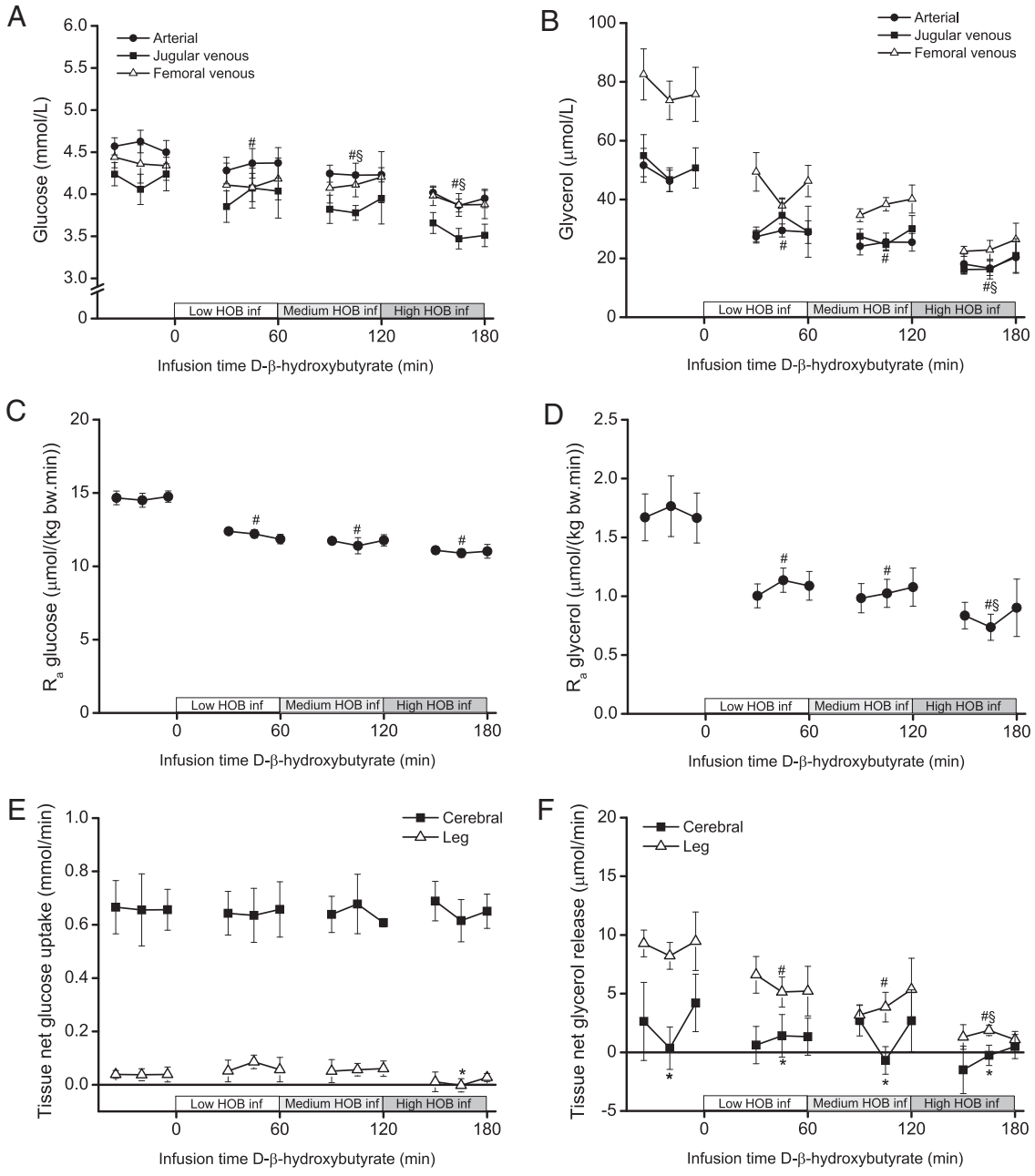

Figure 5. Systemic glucose and glycerol concentrations and rate of appearances and cerebral and skeletal muscle glucose and glycerol net uptake in healthy males under basal condition and 1 h of acute low, medium, and high HOB iv infusion. #, Significantly different from the average basal period; §, Significantly different from the previous period; *, Not significantly different from zero.

servation that AcAc enrichment reaching unity with HOB. Alternatively, the reduced lipolysis and thus fatty acid availability may have caused the inhibition. However, although lipolysis was reduced with HOB infusion, it was still at an appreciable level. Therefore, it seems that under postabsorptive conditions the endogenous HOB is inhibited by acute increases in HOB levels.

Cerebral and skeletal muscle HOB kinetics, oxidation, and effect on glucose uptake

It has been suggested that both brain and skeletal muscle may produce HOB and AcAc (19, 32). We cannot rule out that possibility, but the observation that the arterial, jugular, and femoral venous enrichment of both HOB and AcAc were not significantly different suggests that no, or to a minor extent, net production or exchange reactions occurred in brain and muscle. However, HOB was oxidized in both tissues and the first mandatory step in HOB oxidation is conversion to AcAc within the mitochondria. Therefore, undoubtedly AcAc is formed in brain and skeletal muscle but apparently not leaving the mitochondria and released into their venous drainage, ie, destined for oxidation, in postabsorptive healthy males under acute hyper-D-β-hydroxybutyratemia.

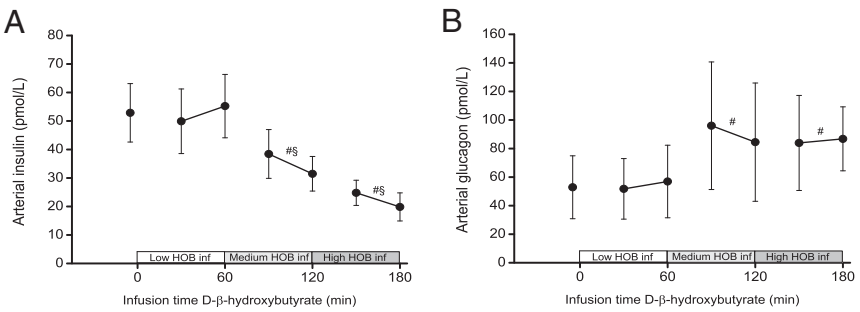

Figure 6. Plasma insulin and glucagon concentrations in healthy males under basal condition and 1 hour of acute low, medium and high HOB iv infusion. #, Significantly different from the average basal period; §, Significantly different from the previous period.

Due to the high cerebral blood flow small arterial jugular venous difference are difficult to measure but the HOB fractional extraction of 11 ± 5% under basal condition with little change with increasing plasma HOB concentrations was similar as suggested previously from compiled data (28). As a result a linear relationship between arterial HOB concentration and cerebral uptake was observed (Figure 4A). Owens et al (13) showed an arterial HOB concentration of 6.7 mmol/L after 5–6 weeks of starvation in obese patients and a cerebral uptake of approximately 220 mmol/kg/min, which complies well with the relationship in the present study as it would give a cerebral uptake of 270 mmol/kg/min. In addition, acutely elevated HOB to 2.2 mmol/L via D,L-hydroxybutyrate infusion resulted in a cerebral HOB uptake of 56 mmol/kg/min also within the arterial cerebral HOB uptake relationship presented in this study (6, 14, 20). These studies suggest that cerebral uptake of HOB is linear over a substantial range of HOB concentrations and that chronic high levels of HOB do not enhance brain ketone utilization via increased enzymatic capacity and/or cerebral ketone transport capacity. Pan et al (33) showed with ^{13}C-magnetic resonance spectroscopy of [2,4-$^{13}C_2$]HOB that acute elevation of the plasma HOB concentration from 0.2 to approximately 2 mmol/L causes only a small increase in the brain HOB concentration from 0.16–0.24 mmol/L, respectively. This suggests minimal accumulation of ketone bodies in the brain, hence direct oxidation that was indirectly estimated to 45 mmol/min, which is similar to the direct measured ketone body oxidation in the present study (Figure 3B). The direct ketone oxidation rates presented are minimal rates because they should be corrected for ketones oxidized, but the ^{13}C of the [2,4-$^{13}C_2$]HOB not directly appearing as $^{13}CO_2$ due to exchange reactions in the tri-carboxylic acid cycle, TCA cycle, that may occur in substantial quantities, not the least in skeletal muscle, to produce other metabolites (24, 25). These TCA cycle reactions are more pronounced for ^{13}C-label in the 1 compared with the 2 position of acetyl coenzyme A (26) as is the case in the present study and in the study of Pan et al (33) using [2,4-$^{13}C_2$]HOB. However, it is unlikely, in contrast with muscle, that the brain provides metabolites in appreciable quantities for other tissues supported by the observation that little label from [2,4-$^{13}C_2$]HOB was found in glutamate and glutamine (22). Therefore, the cerebral ketone body oxidations rates are most likely only to a minor extent underestimated, and show a linear increase with arterial ketone body concentration and brain uptake. At the high HOB infusion the contribution of ketone oxidation accounted for approximately 5% of brain energy expenditure. This relative small contribution of ketone body oxidation to the total brain energy expenditure most likely explains the absence of a significant decrease in cerebral glucose uptake. In order for ketone bodies to contribute significantly to brain energy expenditure the arterial concentration must be increased considerably to reduced brain glucose utilization as shown for lactate (21).

HOB and AcAc uptake by skeletal muscle was lower than for the brain if expressed per kilogram of tissue (Figure 2B). The arterial HOB concentration vs uptake relationship showed saturation kinetics as previously suggested from compiled data (28). The measured basal HOB uptake was similar to the few studies that made measurements across the forearm or leg (15–18). However, active skeletal muscle has shown much higher HOB uptake for a given arterial HOB concentration (16). Indeed, in a pilot experiment (unpublished) during 2.5 hours of cycle ergometer exercise we observed that the muscle HOB uptake was approximately5-fold higher compared with resting muscle but similar to the brain if expressed per kilogram of tissue and its linearity with the HOB concentration found in the present study. Inactive skeletal muscle has a low energy expenditure so little need for energy and this may limit substrate uptake. Alternatively, upon exercise, blood flow to the muscles increases many fold and capillary recruitment thereby enhancing the HOB uptake capacity (34).

In conclusion, the present study provides evidence for that HOB is a potent inhibitor of systemic glucose appearance, lipolysis, and its own production upon acute increases in HOB concentrations in healthy postabsorptive males. Moreover, the cerebral HOB uptake and oxidation rates are substantial in the presence of ample blood glucose and linearly related to arterial HOB concentrations opening up for modifying brain energy utilization in disease.

Skeletal muscle HOB uptake and ketone body oxidation seems limited and much lower than that for the brain. However, active muscle shows a similar HOB uptake per kilogram of tissue as the brain, suggesting that skeletal muscle HOB uptake and oxidation is limited by other factors than the muscles' ketone body oxidative capacity per se. These results open for studies examining the possible therapeutic effects of iv HOB administration in the setting of diseases.

Acknowledgments

Address all correspondence and requests for reprints to: Gerrit van Hall, Clinical Metabolomics Core Facility, Rigshospitalet, section 7652, 9 Blegdamsvej, DK-2100, Copenhagen Ø, Denmark. E-mail: gerrit.van.hall@regionh.dk.

This work was supported by Lunbeck Fonden (R54-A5669).

Disclosure Summary: The authors have nothing to disclose.

References

1. **Hartman AL, Vining EP.** Clinical aspects of the ketogenic diet. *Epilepsia*. 2007;48:31–42.
2. **McNally MA, Hartman AL.** Ketone bodies in epilepsy. *J Neurochem*. 2012;121:28–35.
3. **Vanitallie TB, Nonas C, Di Rocco A, Boyar K, Hyams K, Heymsfield SB.** Treatment of Parkinson disease with diet-induced hyperketonemia: A feasibility study. *Neurology*. 2005;64:728–730.
4. **Costantini L, Barr L, Vogel J, Henderson S.** Hypometabolism as a therapeutic target in Alzheimer's disease. *BMC Neurosci*. 2008;9: S16.
5. **Senior B, Loridan L.** Direct regulatory effect of ketones on lipolysis and on glucose concentrations in man. *Nature*. 1968;219:83–84.
6. **Miles J, Haymond M, Gerich J.** Suppression of glucose production and stimulation of insulin secretion by physiological concentrations of ketone bodies in man. *J Clin Endocrinol Metab*. 1981;52:34–37.
7. **Henry RR, Brechtel G, Lim KH.** Effects of ketone bodies on carbohydrate metabolism in non-insulin-dependent (type II) diabetes mellitus. *Metabolism*. 1990;39:853–858.
8. **Beylot M, Chassard D, Chambrier C, Guiraud M, Odeon M, Beaufrère B, Bouletreau P.** Metabolic effects of a D-beta-hydroxybutyrate infusion in septic patients: Inhibition of lipolysis and glucose production but not leucine oxidation. *Crit Care Med*. 1994;22: 1091–1098.
9. **Féry F, Balasse E.** Effect of exercise on the disposal of infused ketone bodies in humans. *J Clin Endocrinol Metab*. 1988;67:245–250.
10. **Vanitallie TB, Nufert TH.** Ketones: Metabolism's ugly duckling. *Nutr Rev*. 2003;61:327–341.
11. **Clarke K, Tchabanenko K, Pawlosky R, et al.** Kinetics, safety and tolerability of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate in healthy adult subjects. *Regul Toxicol Pharmacol*. 2012;63:401–408.
12. **Clarke K, Tchabanenko K, Pawlosky R, et al.** Oral 28-day and developmental toxicity studies of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate. *Regul Toxicol Pharmacol*. 2012;63:196–208.
13. **Owen OE, Morgan AP, Kemp HG, Sullivan JM, Herrera MG, Cahill GF Jr.** Brain metabolism during fasting. *J Clin Invest*. 1967;46: 1589–1595.
14. **Hasselbalch SG, Madsen PL, Hageman LP, et al.** Changes in cerebral blood flow and carbohydrate metabolism during acute hyperketonemia. *Am J Physiol*. 1996;270:E746–E745.
15. **Hagenfeldt L, Wahren J.** Human forearm muscle metabolism during exercise. 3. Uptake, release and oxidation of beta-hydroxybutyrate and observations on the beta-hydroxybutyrate/acetoacetate ratio. *Scand J Clin Lab Invest*. 1968;21:314–320.
16. **Wahren J, Sato Y, Ostman J, Hagenfeldt L, Felig P.** Turnover and splanchnic metabolism of free fatty acids and ketones in insulin-dependent diabetics at rest and in response to exercise. *J Clin Invest*. 1984;73:1367–1376.
17. **Hartl WH, Jauch KW, Kimmig R, Wicklmayr M, Günther B, Heberer G.** Minor role of ketone bodies in energy metabolism by skeletal muscle tissue during the postoperative course. *Ann Surg*. 1988; 207:95–101.
18. **Elia M, Wood S, Khan K, Pullicino E.** Ketone body metabolism in lean male adults during short-term starvation, with particular reference to forearm muscle metabolism. *Clin Sci*. 1990;78:579–584.
19. **Avogaro A, Doria A, Gnudi L, et al.** Forearm ketone body metabolism in normal and in insulin-dependent diabetic patients. *Am J Physiol*. 1992;263:E261–267.
20. **Miles JM, Nissen SL, Rizza RA, Gerich JE, Haymond MW.** Failure of infused beta-hydroxybutyrate to decrease proteolysis in man. *Diabetes*. 1983;32:197–205.
21. **van Hall G, Strømstad M, Rasmussen P, et al.** Blood lactate is an important energy source for the human brain. *J Cereb Blood Flow Metab*. 2009;29:1121–1129.
22. **Pan JW, de Graaf RA, Petersen KF, Shulman GI, Hetherington HP, Rothman DL.** [2,4-$^{13}C_2$]-b-Hydroxybutyrate metabolism in human brain. *J Cereb Blood Flow Metab*. 2002;22:890–898.
23. **Bornø A, Foged L, van Hall G.** Glucose and glycerol concentrations and their tracer enrichment measurements using liquid chromatography tandem mass spectrometry. *J Mass Spectrom*. 2014;49:980–988.
24. **van Hall G.** Correction factors for ^{13}C-labelled substrate oxidation at whole-body and muscle level. *Proc Nutr Soc*. 1999;58:979–986.
25. **van Hall G, Sacchetti M, Rådegran G.** Whole body and leg acetate kinetics at rest, during exercise and recovery in humans. *J Physiol*. 2002;542:263–272.
26. **Wolfe RR, Jahoor F.** Recovery of labeled CO_2 during the infusion of C-1- vs C-2-labeled acetate: Implications for tracer studies of substrate oxidation. *Am J Clin Nutr*. 1990;51:248–252.
27. **Balasse EO, Ooms HA, Lambilliotte JP.** Evidence for a stimulatory effect of ketone bodies on insulin secretion in man. *Horm Metab Res*. 1970;2:371–372.
28. **Balasse EO, Féry F.** Ketone body production and disposal: Effects of fasting, diabetes, and exercise. *Diabetes Metab Rev*. 1989;5:247–270.
29. **Hall SE, Wastney ME, Bolton TM, Braaten JT, Berman M.** Ketone body kinetics in humans: The effects of insulin-dependent diabetes, obesity, and starvation. *J Lipid Res*. 1984;25:1184–1194.
30. **Féry F, Balasse EO.** Ketone body production and disposal in diabetic ketosis. A comparison with fasting ketosis. *Diabetes*. 1985;34:326–332.
31. **Beylot M, Beaufrère B, Normand S, Riou JP, Cohen R, Mornex R.** Determination of human ketone body kinetics using stable-isotope labelled tracers. *Diabetologia*. 1986;29:90–96.
32. **Nosadini R, Avogaro A, Saccà L, et al.** Ketone body metabolism in normal and diabetic human skeletal muscle. *Am J Physiol*. 1985; 249:E131–E136.
33. **Pan JW, Telang FW, Lee JH, et al.** Measurement of beta-hydroxybutyrate in acute hyperketonemia in human brain. *J Neurochem*. 2001;79:539–544.
34. **Clark MG, Rattigan S, Barrett EJ.** Nutritive blood flow as an essential element supporting muscle anabolism. *Curr Opin Clin Nutr Metab Care*. 2006;9:185–189.