Ketone body infusion abrogates growth hormone-induced lipolysis and insulin resistance

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Published in:
Journal of Clinical Endocrinology and Metabolism

DOI:
10.1210/clinem/dgac595

Publication date:
2023

Document version
Publisher's PDF, also known as Version of record

Document license:
Unspecified

Citation for published version (APA):
Ketone Body Infusion Abrogates Growth Hormone–Induced Lipolysis and Insulin Resistance


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Abstract

Context: Exogenous ketone body administration lowers circulating glucose levels but the underlying mechanisms are uncertain.

Objective: We tested the hypothesis that administration of the ketone body β-hydroxybutyrate (βOHB) acutely increases insulin sensitivity via feedback suppression of circulating free fatty acid (FFA) levels.

Methods: In a randomized, single-blinded crossover design, 8 healthy men were studied twice with a growth hormone (GH) infusion to induce lipolysis in combination with infusion of either βOHB or saline. Each study day comprised a basal period and a hyperinsulinemic-euglycemic clamp combined with a glucose tracer and adipose tissue and skeletal muscle biopsies.

Results: βOHB administration profoundly suppressed FFA levels concomitantly with a significant increase in glucose disposal and energy expenditure. This was accompanied by a many-fold increase in skeletal muscle content of both βOHB and its derivative acetoacetate.

Conclusion: Our data unravel an insulin-sensitizing effect of βOHB, which we suggest is mediated by concomitant suppression of lipolysis.

Key Words: ketone bodies, β-hydroxybutyrate, insulin sensitivity, glucose metabolism, growth hormone, free fatty acids

Abbreviations: βOHB, β-hydroxybutyrate; Akt, protein kinase B; CIS, cytokine-induced suppressor; EGP, endogenous glucose production; FFA, free fatty acid; Fv, fractional velocity; G0S2, G0/G1 Switch Gene 2; GP, glucose-6-phosphate; GH, growth hormone; GIR, glucose infusion rate; GS, glycogen synthase; HCA2, β-hydroxy-carboxylic acid 2; HEC, hyperinsulinemic-euglycemic clamp; LC-MS/MS, liquid chromatography–tandem mass spectrometry; mRNA, messenger RNA; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; Ra, glucose rate of appearance; Rd, glucose rate of disappearance; RER, respiratory exchange ratio; SOCS, suppressor of cytokine signaling; TCA, tricarboxylic acid.

Ketone bodies are produced by the liver from β-oxidation of fatty acids and serve as an alternative energy source in metabolically active tissues during stress and fasting (1). Interestingly, the salutary effects of calorie restriction on metabolic health are considered to be mediated in part by increased ketogenesis (2). Uncoupled lipolysis-driven ketogenesis, on the other hand, can be life-threatening in cases of diabetic ketoacidosis (3).

It is a consistent finding that ketone body administration in humans decreases circulating glucose and free fatty acid (FFA) levels (4–10). The underlying mechanisms are unclear, but some studies suggest an insulinotropic effect (4, 9, 10), whereas others indicate that ketone bodies lower endogenous glucose production (EGP) in the liver (6, 7, 11). It is also reported that oral ingestion of a ketone monoester increases glucose tolerance (6), but whether this is associated with enhanced insulin sensitivity remains to be established.

Growth hormone (GH) secretion increases during fasting and induces insulin resistance both in the liver and in skeletal muscle (12–15). The insulin antagonistic effect of GH is abrogated by suppression of lipolysis via administration of acipimox that activates the inhibitory G protein–coupled hydroxy-carboxylic acid (HCA)2 receptor (also known as PUMA-G or Gpr109) (16–21). This is of particular interest since the endogenous ligand for the HCA2 receptor is the ketone body β-hydroxybutyrate (βOHB) (17, 22).

We hypothesize that βOHB administration increases insulin sensitivity by suppression of lipolysis. To test this, we studied 8 healthy men on 2 occasions during a GH infusion to stimulate lipolysis in combination with βOHB or saline (control). Insulin...
sensitivity was measured by the hyperinsulinemic-euglycemic clamp (HEC) technique combined with infusion of a glucose tracer, and indices of lipolysis as well as βOHB uptake and insulin signaling were recorded in biopsies from skeletal muscle.

Materials and Methods
Participants
The participants were lean men in the age range of 22 to 35 years with a mean ± SE body mass index of 23.3 ± 0.3. All participants were in good health according to a medical interview and a physical examination, including routine blood chemistry tests. Eligibility criteria included age over 18, male sex, absence of disease, and a body mass index between 19 and 25. The study was conducted in accordance with the Declaration of Helsinki II and approved by the regional ethics committee system. Oral and written consent were obtained from all participants before inclusion. The trial was reported at www.clinicaltrials.gov (NCT02655263).

Study Design and Protocol
The study was designed as a randomized, single-blinded, placebo-controlled, crossover study and carried out at the Medical Research Laboratory, Aarhus University, Denmark. All participants were examined on 2 occasions while receiving an intravenous GH infusion (Genotropin 30 ng/kg/min) together with infusion of either sodium-3-hydroxybutyrate (0.18 g/kg/h) (βOHB) or saline (control). The 2 visits were separated by a minimum of 4 weeks. The visit order was randomized using Random.org. The metabolic studies were performed between 8:00 and 14:00 hours (t = 0-360) after an overnight fast (12 hours). The participants were instructed to refrain from alcohol intake and vigorous exercise for 2 days before each study day. On each study day, an intravenous catheter for infusions was inserted into the antecubital vein, and a second catheter was placed in a contralateral hand vein, which was heated to obtain arterialized blood. GH and βOHB/saline were continuously infused from t = 0 to 360. A 3-hour basal period (0-180 minutes) was followed by a 3-hour basal period (t = 0-180 minutes) and the HEC (t = 270) to estimate energy expenditure and the respiratory exchange ratio (RER), as previously described (24).

Indirect Calorimetry
Indirect calorimetry was performed with a canopy system (Oxycon Pro; Innemedic) in the basal state (t = 150) and during the HEC (t = 270) to estimate energy expenditure and the respiratory exchange ratio (RER), as previously described (24).

Blood Samples
Plasma glucose levels were measured at bedside (YSI 2300 STAT Plus glucose analyzer; YSI). Commercial enzyme-linked immunosorbent assay kits were used to analyze plasma levels of insulin (Merkodia), glucagon (Merkodia, Uppsala, Sweden), glycerol (Cayman Chemicals), and cortisol (DRG Diagnostics), respectively. Serum FFA levels were determined by a colorimetric method using a commercial kit (Wako Chemicals). Serum GH concentrations were analyzed by a modified chemiluminescence technology (IDS-iSYS human GH; Immunodiagnostic Systems). Plasma epinephrine and norepinephrine concentrations were measured by electrochemical detection following high-performance liquid chromatography separation. C-peptide was determined using a commercial enzyme-linked immunosorbent assay kit (ALPCO). Serum concentrations of βOHB and β-hydroxy β-methylbutyrate (βHMB) were measured using hydrophilic interaction liquid chromatography–tandem mass spectrometry (LC-MS/MS) as previously described (25).

Urine Collection
Urine was collected in the basal period and during the HEC. βOHB was quantified in urine by LC-MS/MS as described previously (25) with minor modifications. Urine samples were diluted 1:1 with phosphate buffer (40 mM, pH 7.5) before βOHB was extracted with a mixture of methanol and acetonitrile followed by solid phase extraction using both polymeric strong cation exchange and strong anion exchange sorbents. Acetoacetate in urine samples was determined by headspace gas chromatography mass spectrometry as described (26) using lithium acetoacetate (Sigma-Aldrich) for calibration. For both βOHB and acetoacetate analyses, urine samples were diluted with water to match the measuring range of the analytical methods used.

Biopsies
Biopsies were collected at t = 120 (basal) and t = 360 (HEC). The subcutaneous adipose tissue biopsies were obtained from the periumbilical region by liposuction, and skeletal muscle biopsies were obtained from the vastus lateralis of the quadriceps femoris using a Bergström needle. Lidocaine was used as local anesthetic, the procedure was performed under sterile conditions and the biopsies were immediately washed free of blood, frozen in liquid nitrogen, and stored at −80°C until analysis.

Tracer Kinetics
A bolus (12 μCi) of [3-3H]-glucose (GE Healthcare) was given at t = 0 followed by a continuous infusion (0.2 μCi/min) at t = 0 to 360 minutes. In addition, [3-3H]-glucose was added to the glucose infused during the HEC to avoid rapid dilution (100 μCi [3-3H]-glucose in 500 mL 20% glucose). Specific activities of [3-3H]-glucose were measured in triplicate at the end of the basal period (t = 160-180 minutes) and the HEC (t = 310-330 minutes). Glucose rate of appearance (Ra) and glucose rate of disappearance (Rd) were calculated using the non–steady-state equation of Steele (23). EGP equals Ra in the basal period, whereas EGP during the HEC was calculated by subtracting GIR from Ra.

Glycogen Synthase Activity and Glycogen Content
GS activity was measured as previously described (27) in the presence of 1.67 mmol/L UDP-glucose. GS fractional velocity of the basal period (t = 160-180 minutes) and the HEC (t = 310-330 minutes). Glucose rate of appearance (Ra) and glucose rate of disappearance (Rd) were calculated using the non–steady-state equation of Steele (23). EGP equals Ra in the basal period, whereas EGP during the HEC was calculated by subtracting GIR from Ra.
Glucose-6-phosphate (G6P) and activity of GS independent of G6P (I-form) was determined in the presence of 0.02 mmol/L G6P. FV and I-form are given as percentage of maximal GS-activity assayed in the presence of 8 mmol/L G6P. Muscle glycogen content was determined in homogenates as glycans units after acid hydrolysis (28).

**Quantitative polymerase chain reaction (Pcr) analysis**

Quantitative polymerase chain reaction analyses were performed as previously described (31). β2 microglobulin was used as the housekeeping gene and β2 microglobulin mRNA levels were similar among all interventions. The following primer sequences were used: β2 microglobulin (GAGGCTATCC AGGCTACTCC, AATGTCGGATGGATGAAACCC, length 111), CISH (TTCCGGAATCTCTGGTTATGG, GC ATCTTTGCAAGGTTGTCG, length 69), SOCS1 (AC AGCCCATCGCCACATTC, CGAGGCCATCTCAGC TAAG, length 209), SOCS2 (TCCCTTCTAAGGTCGACC AAGAC, GCGACTTCAGCCTTTACATGCG, length 78), SOCS3 (GAGACTTCGATCCTGGGACA, GAAACTTG CTTGGTGTAC, length 131), PDE3B (TCTGAAACAC GCCGATTC, GACAGCCAAGCCATAAATCTCA, length 156), PTEN (GACAGGCTATCAAGAGATGC, TTC GAGAAGCTTACTCTCAGA, length 118), G0S2 (GA GACCCAGAGCCGATG, AGCCACCGCGAAGA G, length 137), FSP27 (CATTGGCTCTGTGAACGTGA, GGAGTGCCAGCTGATACG, length 154), PIK3r1 (GCACATTCCTGCTGGGAG, ATGTCTTACTCGCC GTCCA, length 77), PDK2 (AGGCTACACATGTC TAGTGC, AGTCGACCGCTGATTTCT, length 80), and PDK4 (ATTATAAGAATCTAGTGGGC, GCGGT GATTTTCTCAAGG, length 151). The fold change was calculated using the 2^ΔΔCt method, where ΔΔCt is the difference between the average cycle threshold (Ct) for the target gene and the housekeeping gene at time x, minus the difference between the average Ct for the target gene and the housekeeping gene in the basal state at the control day.

**Quantification of Acetoacetate and β-Hydroxybutyrate**

Quantification of acetoacetate and βOHb in skeletal muscle tissue was performed by a modified version of a previously published LC-MS/MS method (32). Freeze-dried muscle tissue samples (≈ 3 mg) were transferred into 2 mL tubes and added 50 µL of water, 400 µL extraction solvent methanol:acetone:nitrate:water (2:2:1 v/v/v), and 50 µL stable isotope labeled internal standards (20 µM of [13C4]-acetoacetate (in-house synthesized by alkaline hydrolysis of [13C4]-ethyl acetoacetate, Sigma-Aldrich) and [13C4]-βOHb (Cambridge Isotope Laboratories). The mixture was homogenized (Precelly evolution homogenizer, Bertin) using ceramic beads for 2 × 20 seconds, followed by ultrasonic bath for 30 seconds and a second round of homogenization for 2 × 20 seconds. The homogenate was centrifuged for 2 minutes at 10000g, and a 100 µL supernatant aliquot was diluted with 300 µL water. Separate pure calibrator samples in the concentration range 0.125 to 50 µM were prepared using reference compounds (lithium acetoacetate and sodium βOHb, Sigma-Aldrich).

A total of 10 µL of sample was injected into a liquid chromatography system (Waters UPLC) and the separation was performed with a UPLC HSS T3 column (Waters ACQUITY Premier HSS T3 1.8 µm, 2.1 × 100 mm) maintained at 30 °C. The flow rate was 0.3 mL/min initially with 100% mobile phase A (water:methanol 98:2 with 0.0125% acetic acid) that was changed through a linear gradient to 8% mobile phase B (methanol with 0.0125% acetic acid) over 3 minutes. From 3 to 3.5 minutes the gradient was changed to 90% B and maintained for 4.5 minutes, returned to 100% A, and allowed to equilibrate for 3 minutes. Samples were analyzed by a mass spectrometer (Waters Xevo TQ XS) using electrospray ionization in the negative mode. Compounds were detected in the multiple reaction monitoring mode with the transitions (m/z), acetoacetate (100.6 → 56.7, [13C4]-acetoacetate (104.8 → 59.6), βOHb (102.6 → 58.6), and [13C4]-βOHb (106.6 → 60.7).

Calibration curves were constructed by linear regression of the peak area ratio (analyte/internal standard) vs the nominal concentrations of the calibrator samples. The micromolar concentrations in extracted tissue homogenates were derived from the standard curve and then transformed into nanomolar per gram tissue using the specific input tissue weight in each sample extraction.

**Statistical Analysis**

The effects of βOHb infusion with time were analyzed by a repeated-measurement mixed-effects model analysis using the Restricted Maximum Likelihood method in STATA (version 14.2, StatA Corp). The model included intervention (βOHb vs saline), sample time, visit (first or second study day), and the interaction between intervention and sample time as fixed effects. Visit nested within subject was used as random effect, and time nested within visit was treated as repeated measurements using a covariance matrix with independent and identically distributed Gaussian residuals with one common variance within visit nested within subject.
Results
Circulating Hormones and Metabolites
As expected, comparable serum GH elevations were obtained in the 2 study arms (Fig. 1A). Circulating βOHB levels increased following βOHB infusion reaching a steady-state concentration of approximately 3.3 mmol/L (βOHB×time: P < .0001) (Fig. 1B). On the control day, serum βOHB levels were low although a minimal increase was seen at the end of the basal state followed by a decline during the HEC (P < .0001) (see Fig. 1B). Circulating levels of β-hydroxy-β-methylbutyrate were very low and decreased during the HEC in both study conditions (main effect of time: P < .0001) (Fig. 1C).

Plasma glucagon levels were stable in the basal state and became suppressed during the HEC, irrespective of βOHB infusion (main effect of time: P < .0001) (Fig. 1D). Circulating cortisol levels declined with time with no effect of BOHB (main effect of time: P < .0001) (Fig. 1E). Plasma concentrations of adrenaline and noradrenaline were stable throughout the day and were unaffected by βOHB infusion (Fig. 1F and 1G).

Infusion of β-Hydroxybutyrate Suppresses Lipolysis
On the control day, GH infusion increased basal serum FFA levels, which reached a mean ± SEM peak of 0.7 ± 0.0 mmol/L at t = 180, whereas circulating FFA became markedly suppressed during βOHB infusion (βOHB×time: P < .0001) (Fig. 2A). Serum FFA levels were suppressed in response to the HEC on both study days. Basal glycerol levels displayed a similar pattern (βOHB×time: P = .0006) but a decline during the HEC was observed only on the control day (P < .0001) (Fig. 2B).

Urine Levels of β-Hydroxybutyrate (βOHB) and Acetoacetate Increase During βOHB Infusion
The mean (± SE) urinary levels of βOHB and acetoacetate were approximately 100-fold increased after βOHB infusion (data not shown).

Muscle Content of β-Hydroxybutyrate (βOHB) and Acetoacetate Increases During βOHB Infusion
The content of βOHB in muscle increased markedly during the βOHB infusion, both in the basal state and during the HEC (Fig. 3A). On the control day, muscle βOHB decreased during the HEC as compared to the basal state (βOHB×time: P < .01) (see Fig. 3A). Likewise, the muscle content of acetoacetate increased during the βOHB infusion, whereas no effect of the HEC was recorded (βOHB: P = .01) (Fig. 3B).

Infusion of β-Hydroxybutyrate Potently Increases Insulin Sensitivity
Overall, plasma glucose levels were comparable between interventions; however, at the end of the basal state (t = 180), a decrease in plasma glucose concentration was evident during βOHB infusion (P = .03) (Fig. 4A). Insulin levels in the basal period did not differ between the 2 study days, but during the HEC, circulating insulin levels were lower on the βOHB day (Fig. 4B).

The infusion of βOHB induced an approximately 100% increase in insulin sensitivity assessed by the GIR (Fig. 4C) and the M value (Fig. 4D) during the HEC (mean ± SE AUCGIR 522 ± 64 mg*kg⁻¹*min⁻¹ vs 1049 ± 145 mg*kg⁻¹*min⁻¹; P = .004; mean ± SE M-value 4.3 ± 0.6 mg*kg⁻¹*min⁻¹ vs 8.2 ± 1.1 mg*kg⁻¹*min⁻¹; P = .003). Adjusting the M value for the insulin concentration at the end of the HEC amplified the βOHB-induced increase in insulin sensitivity (mean ± SE adjusted M value 0.031 ± 0.004 mg*kg⁻¹*min⁻¹ vs 0.015 ± 0.002 mg*kg⁻¹*min⁻¹; P = .002). Glucose Rd (Fig. 4E) and EGP (Fig. 4F) in the basal state did not differ between control and βOHB, whereas the increase in Rd during the HEC was higher on the βOHB day as compared to the control day (βOHB×time: P = .002) (see Fig. 4E). EGP was suppressed during the HEC with no effect of βOHB (main effect of time: P < .0001) (see Fig. 4F).

β-Hydroxybutyrate Infusion Increases Energy Expenditure
Resting energy expenditure was similar between interventions, whereas energy expenditure during the HEC increased approximately 20% in response to βOHB (P < .0001) (Fig. 5A). The mean (± SE) RER in the basal state was unaffected by βOHB, whereas the increase in RER during the HEC tended to be more pronounced on the control day (P = .06) (Fig. 5B).

Insulin Signaling, Glycogen Synthase Activity and Glycogen Content in Skeletal Muscle is Unaffected by β-Hydroxybutyrate Infusion
The phosphorylation of Akt in skeletal muscle increased during the HEC with no effect of BOHB (time: P < .0001) (Fig. 6A). GS activity was measured both as activity ratio (Fig. 6B) and as FV (data not shown). Similar to Akt phosphorylation, GS activity increased during the HEC independently of βOHB (time: P < .0001) (see Fig. 6B). Glycogen content in the skeletal muscle was stable in the basal state and during the HEC, irrespective of the βOHB infusion (Fig. 6C).

Growth Hormone (GH) Infusion Activates GH Signaling Irrespective of β-Hydroxybutyrate Infusion
Phosphorylation of the signal transducers and activators of transcription (STAT) 5, which is the canonical GH signaling pathway (33), was detectable in muscle to the same extent on the control day and after βOHB infusion (data not shown). Similarly, the mRNA expression of suppressor of cytokine...
Figure 1. Circulating hormones and metabolites. Circulating levels of A, growth hormone (GH); B, β-hydroxybutyrate (βOHB); C, β-hydroxy-β-methylbutyrate (βHMB); D, glucagon; E, cortisol; F, adrenaline; and G, noradrenaline in the basal state and during the hyperinsulinemic-euglycemic clamp (HEC) are presented as mean ± SE of raw data. Data were analyzed by repeated-measurement mixed-effects model analysis, and \( P \) values indicate interaction between βOHB and time and main effect of time.
signaling (SOCS) and cytokine-induced suppressor (CIS) proteins, SOCS1, SOCS2, SOCS3, and CISH, which are induced by STAT5 (33), did not differ between the 2 study arms (data not shown).

β-Hydroxybutyrate Infusion Decreases Expression of Muscle Pyruvate Dehydrogenase Kinase 4 Messenger RNA During the Hyperinsulinemic-Euglycemic Clamp

PDK2 and 4 phosphorylate and thereby inactivate pyruvate dehydrogenase (PDH) in skeletal muscle (34). The expression of PDK4 mRNA in skeletal muscle was similar between interventions in the basal state, whereas during the HEC, PDK4 mRNA expression decreased after βOHB infusion (control vs βOHB, P = .002) (Fig. 7A). In adipose tissue, the mRNA expression of PDK4 was lower during the HEC independently of βOHB (mean effect of time: P < .0001) (Fig. 7B). The expression of PDK2 mRNA was comparable between interventions and did not change with time in either skeletal muscle or in adipose tissue (data not shown).

Insulin-stimulated Expression of G0/G1 Switch Gene 2 (G0S2) Messenger RNA in Adipose Tissue Is Increased by β-Hydroxybutyrate Infusion

In the basal state, G0/G1 Switch Gene 2 (G0S2) mRNA expression in adipose tissue was similar on the 2 study days, whereas a statistically significant increase occurred during the HEC on the βOHB infusion day (βOHB x time: P = .002) (data not shown). The expression of adipose tissue phosphoinositide-3-kinase regulatory subunit 1 (PIK3r1) mRNA increased during the HEC on both study days (main effect of time: P = .0004) (data not shown). The mRNA expression of CIDEA, FSP27, PTEN, and PDE3B in adipose tissue was comparable between interventions and did not change with time (data not shown).
Discussion

The present study demonstrates that short-term βOHB infusion acutely increases insulin-stimulated glucose disposal during concomitant GH exposure in the presence of many-fold elevation in the skeletal muscle content of both βOHB and its derivative acetoacetate. We hypothesize that the underlying mechanism is inhibition of lipolysis by βOHB via the HCA2 receptor.

Ketone body administration lowers circulating glucose and FFA levels (4–11), and oral ingestion of ketone bodies increases glucose tolerance (6). This study documents that exogenous βOHB acutely increases insulin-stimulated glucose uptake assessed by the HEC. The use of a glucose tracer infusion allowed us to quantify EGP, which was unaffected by βOHB in contrast with previous studies showing reduced basal EGP (4, 5, 11). At the end of the HEC, we observed a lower insulin level during βOHB infusion indicating increased insulin clearance, which is noteworthy since reduced insulin clearance is a feature of insulin resistance (35).

It has previously been shown that infusion of βOHB per se does not affect insulin sensitivity assessed by the HEC, which supports our hypothesis that suppression of stimulated lipolysis is the mechanism underlying our observation (36). The present study design included GH as a means to stimulate

Figure 4. Infusion of β-hydroxybutyrate (βOHB) potently increases insulin sensitivity. Circulating levels of A, glucose and B, insulin in the basal state and during the hyperinsulinemic-euglycemic clamp (HEC) presented as mean ± SEM. C, Glucose infusion rate (GIR) presented as geometric mean ± 95% CI and D, M value. E, Glucose rate of disappearance (Rd) and F, endogenous glucose production (EGP) are presented as mean ± SEM. Data were analyzed by repeated-measurement mixed-effects model analysis and t test, and P values indicate interaction between βOHB and time, main effect of time, and difference between control and βOHB.
Figure 5. β-Hydroxybutyrate (βOHB) infusion increases energy expenditure. A, Energy expenditure and B, respiratory exchange ratio in the basal state and during the hyperinsulinemic-euglycemic clamp (HEC) are presented as mean ± SEM. Data were analyzed by repeated-measurement mixed-effects model analysis, and P values indicate interaction between βOHB and time and main effect of time.

Figure 6. Insulin signaling, glycogen synthase (GS) activity, and glycogen content in skeletal muscle is unaffected by β-hydroxybutyrate (βOHB) infusion. A, p-Akt Ser⁴⁷³/total protein kinase B (Akt) with representative Western blots; A, GS activity measured as activity ratio and C, glycogen content in skeletal muscle in the basal state and during the hyperinsulinemic-euglycemic clamp (HEC) are presented as geometric mean ± 95% CI. Data were analyzed by repeated-measurement mixed-effects model analysis, and P values indicate main effect of time.
lipolysis and induce insulin resistance, allowing us to unveil that βOHB potently suppresses lipolysis. Others and we have shown that administration of acipimox, which—like βOHB—is a ligand of the HCA2 receptor, suppresses lipolysis and abrogates GH-induced insulin resistance assessed by the HEC (19, 21, 37). Taken together, we find it plausible that βOHB increases insulin sensitivity in skeletal muscle via receptor-mediated suppression of lipolysis (Fig. 8).

Infusion of exogenous βOHB has a modestly alkalinzing effect, and pH in plasma typically increases 0.05 to 0.10 (4, 38). Although this theoretically could affect intermediary metabolism, a human study failed to observe any effects of a similar minute pH increment obtained with bicarbonate on leucine metabolism (39), and studies in rats revealed no effect of bicarbonate-induced pH increments on insulin sensitivity (40). Therefore, it seems unlikely that the pronounced increase in insulin-stimulated glucose disposal observed in the present study is caused by a small change in pH.

We recorded a pronounced increase in muscle βOHB after infusion, and the concomitant muscle increase in acetoacetate content suggests that βOHB was metabolized and, presumably, oxidized in the tricarboxylic acid (TCA) cycle, which is in accordance with data showing skeletal muscle as a major site of ketone body oxidation (11, 41). In support of this, we observed an increase in energy expenditure during βOHB. The RER during the HEC tended to be lowered by βOHB infusion; however, reliable estimations of substrate oxidation rates from indirect calorimetry in the presence of

Figure 7. β-Hydroxybutyrate (βOHB) infusion decreases expression of muscle PDK4 messenger RNA (mRNA) during the hyperinsulinemic-euglycemic clamp (HEC). PDK4 mRNA expression in A, skeletal muscle and B, adipose tissue in the basal state and during the HEC, are presented as geometric mean ± 95% CI. Data were analyzed by repeated-measurement mixed-effects model analysis, and P values indicate main effect of βOHB and time.

Figure 8. A model to suggest that β-hydroxybutyrate (βOHB) increases muscle glucose uptake via suppression of lipolysis. Based on data from the present study, we hypothesize that (1) infused βOHB (2) is taken up and metabolized in skeletal muscle and (3) suppresses lipolysis via binding to the HCA2 receptor. Infused growth hormone (GH) per se stimulates lipolysis and thereby insulin resistance (gray areas), but this is abrogated by (4) infused βOHB and the suppression of lipolysis and (5) free fatty acid (FFA) levels promote muscle glucose uptake.
ketone body abundance are not possible (42, 43). The low levels of circulating β-hydroxy-β-methylbutyrate observed in both study arms are indicative of low or suppressed ketogenesis. As regards glycogen synthesis and storage, we found that muscle glycogen content and GS activity were unaffected by βOHB, suggesting that glucose is metabolized rather than stored in skeletal muscle during βOHB infusion.

Infusion of βOHB induced a decrease in muscle PDK4 mRNA expression during the HEC, implying increased activity of the PDH complex, which promotes the entry of carbohydrate-derived pyruvate into the TCA cycle (44), whereas no βOHB-induced increase in muscle AKT signaling was evident. This is consistent with previous studies suggesting that GH-induced insulin resistance involves reduced PDH activity in skeletal muscle indicative of substrate competition between glucose and fatty acids at the entry of the TCA cycle (21, 45). Along this line, it is intriguing that glucose and βOHB appear to be taken up and metabolized simultaneously in skeletal muscle in the present context. Other studies, however, reported that intravenous infusion of lipids induced insulin resistance by reducing insulin signaling and action in human skeletal muscle (46–48). Indeed, in the context of GH-induced insulin resistance, the mechanisms may involve both substrate competition and impaired insulin signaling depending on the experimental context (21, 49).

At first glance, our observations contradict classic data by Randle et al (50) showing that βOHB suppresses glucose uptake and utilization in rodent heart and skeletal muscle in vitro. It is plausible that βOHB in the medium may suppress glucose uptake in vitro. Our study was in humans in vivo, and an antagonistic direct effect of βOHB on glucose uptake may have been obscured by the concomitant suppression of lipolysis. Furthermore, species-specific differences may apply.

We have previously reported that GH suppresses G0S2, which is an inhibitor of lipolysis in adipose tissue (20, 51–53), whereas insulin exerts the opposite effect (54). The present study suggests that βOHB potentiates the stimulatory effect of insulin on G0S2 expression, which may contribute to antilipolysis. In addition, the mRNA expression of Pik3r1, which encodes the regulatory p85α subunit of PI3K, was upregulated in adipose tissue during the HEC. PI3K and its downstream effectors, including AKT, are pivotal for the antilipolytic effect of insulin (55).

Certain limitations of this study merit attention. First, our model does not account for GH-independent effects of BOHB infusion. To substantiate causality and investigate the underlying molecular mechanisms, the metabolic effects of exogenous βOHB administration should be elucidated both in the presence and absence of GH-induced lipolysis. Second, although our results strongly suggest that βOHB is being metabolized in skeletal muscle, it remains to be verified by more direct methods. Assessment of enzyme activities including PDH as well as the use of fatty acid and ketone body tracers in combination with high-resolution respirometry are needed to map the changes in glucose, fatty acid, and βOHB metabolism. Finally, because only male participants were included, these results do not necessarily apply to women.

In conclusion, our study demonstrates that βOHB infusion during concomitant GH exposure acutely increases insulin-stimulated glucose uptake in skeletal muscle, which may explain the glucose-lowering effect of ketone body administration. The concomitant and marked reduction in circulating FFA levels suggests this is linked to inhibition of lipolysis via the HCA2 receptor. We hypothesize that this intrinsic effect of βOHB is concealed during endogenous ketosis, which is driven by low insulin levels and open-loop activation of lipolysis. Our observations add insight into the mechanisms underlying the glucose-lowering effects of ketone body administration.

Acknowledgments
We are grateful for the excellent technical assistance provided by Kirsten Nyborg Rasmussen, Elsebeth Hornemann, Lisa Buus, Annette Mengel, and Helle Zibrandtsen, Aarhus University Hospital; and Betina Bolmgren, University of Copenhagen. We also owe a great thanks to the participants for their commitment to this study.

Financial Support
This work was supported by the Danish Council for Independent Research (grant No. DFF-4004-00304).

Disclosures
The authors have nothing to disclose.

Data Availability
Data reported in this paper will be shared by the lead contact on request.

Clinical Trial Information
ClinicalTrials.gov registration number NCT02655263 (registered January 14, 2016).

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