

The Effect of Blood Ketone Concentration and Exercise Intensity on Exogenous Ketone Oxidation Rates in Athletes

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ABSTRACT

DEARLOVE, D. J., O. K. HARRISON, L. HODSON, A. JEFFERSON, K. CLARKE, and P. J. COX. The Effect of Blood Ketone Concentration and Exercise Intensity on Exogenous Ketone Oxidation Rates in Athletes. *Med. Sci. Sports Exerc.*, Vol. 53, No. 3, pp. 505–516, 2021. **Introduction:** Exogenous ketones potentially provide an alternative, energetically advantageous fuel to power exercising skeletal muscle. However, there is limited evidence regarding their relative contribution to energy expenditure during exercise. Furthermore, the effect of blood ketone concentration and exercise intensity on exogenous ketone oxidation rates is unknown. **Methods:** Six athletes completed cycling ergometer exercise on three occasions within a single-blind, random-order controlled, crossover design study. Exercise duration was 60 min, consisting of 20-min intervals at 25%, 50%, and 75% maximal power output (W_{Max}). Participants consumed (i) bitter flavored water (control), (ii) a low-dose β -hydroxybutyrate (β HB) ketone monoester (KME; 252 mg·kg BW⁻¹, “low ketosis”), or (iii) a high-dose β HB KME (752 mg·kg BW⁻¹, “high ketosis”). The KME contained a ¹³C isotope label, allowing for the determination of whole-body exogenous β HB oxidation rates through sampled respiratory gases. **Results:** Despite an approximate doubling of blood β HB concentrations between low- and high-ketosis conditions (~2 mM vs ~4.4 mM), exogenous β HB oxidation rates were similar at rest and throughout exercise. The contribution of exogenous β HB oxidation to energy expenditure peaked during the 25% W_{Max} exercise intensity but was relatively low (4.46% ± 2.71%). Delta efficiency during cycling exercise was significantly greater in the low-ketosis (25.9% ± 2.1%) versus control condition (24.1% ± 1.9%; $P = 0.027$). **Conclusions:** Regardless of exercise intensity, exogenous β HB oxidation contributes minimally to energy expenditure and is not increased by elevating circulating concentrations greater than ~2 mM. Despite low exogenous β HB oxidation rates, exercise efficiency was significantly improved when blood β HB concentration was raised to ~2 mM. **Key Words:** EXOGENOUS KETOSIS, KETONE MONOESTER, EXERCISE, EXERCISE INTENSITY, CYCLING EFFICIENCY

To meet energetic requirements, most body tissues draw upon the oxidation of carbohydrate- and/or lipid-derived substrates. In states of decreased carbohydrate availability

such as starvation or ketogenic diet, circulating ketone bodies—mainly β -hydroxybutyrate (β HB) and acetoacetate (AcAc)—are elevated and represent an additional major substrate (1). During starvation, the primary purpose of ketogenesis is to provide a supplementary substrate (to glucose) to support cerebral metabolism (2); however, most extrahepatic tissues, including skeletal muscle, have the capacity to oxidize ketones (1). Compared with other sites of peripheral ketone oxidation, notably the heart and kidney, skeletal muscle has comparatively low ketolytic enzyme activity (3). Furthermore, this varies widely within the spectrum of skeletal muscle phenotypes, with 3-hydroxybutyrate dehydrogenase (BDH) and 3-oxoacid-CoA transferase (SCOT) activity being, respectively, ~2.5 and ~1.5 times greater in slow-oxidative versus fast-glycolytic fibers (3). Nonetheless, given that skeletal muscle comprises ~40% of total body mass, it represents a potentially important site for the metabolic disposal of ketones.

The advent of ingestible ketones now facilitates the transient induction of ketosis minus the metabolic conditions required for hepatic ketogenesis (reduced carbohydrate stores,

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elevated circulating free fatty acids, and a low insulin/glucagon ratio) (1,4). Doing so creates a novel physiological state—“exogenous ketosis”—that potentially holds benefits for human metabolism and exercise performance (4,5). Ketone metabolism has energetic advantages versus lipid- and carbohydrate-derived substrates (4,6). Per mole, more energy can be liberated through the oxidation of β HB versus pyruvate. In addition, β HB oxidation exerts opposite redox effects on the respiratory chain electron carriers, NAD, and coenzyme Q, thus increasing free energy liberated by ATP hydrolysis (6). These effects are thought to explain the 25% improvement in hydraulic efficiency ($J \cdot mol^{-1} O_2^{-1}$) in the working rat heart perfused with a physiological ratio of ketones and glucose versus glucose alone (6). Although fat is more reduced than both pyruvate and β HB (thus, more energy can be liberated through its oxidation) (4), the ratio of O_2 consumed to ATP produced is less favorable. In part, this may explain why fat oxidation is relatively low at high exercise intensities (7). As such, meaningful contributions from exogenous ketone oxidation to energy expenditure in exercising skeletal muscle might result in improved efficiency, observed as greater power output for a given volume of oxygen consumed (4).

Improvements in energy efficiency attributable to ketone metabolism may have contributed to the increased endurance exercise performance observed in athletes supplemented with a ketone monoester (KME) (5). However, the ergogenic potential of exogenous ketosis remains to be determined, with studies also finding indifferent (8–11) exercise performance after β HB KME supplementation, and worse (12) after AcAc diester supplementation. Furthermore, the observed increase in endurance exercise performance may have resulted from ketosis-mediated alterations in the availability and catabolism of other substrates, most notably the “rationing” of intramuscular glycogen stores and concomitant increase in lipid oxidation in exercising skeletal muscle (5). In other words, ketone signaling may be a greater contributor to the observed improvements in exercise performance, rather than ketone oxidation.

There is limited evidence regarding the relative contribution of exogenous ketone oxidation to overall energy expenditure during exercise. Using adjusted indirect calorimetry equations (13), β HB oxidation during 40% and 75% maximal power output (W_{Max}) exercise has been predicted to account for 18% and 16% of total oxygen consumption, respectively (5). However, direct measures of ketone oxidation in exercising humans are warranted. In addition, factors regulating ketone oxidation during exercise are largely unknown. In particular, it is unclear how blood ketone concentration and exercise intensity affect exogenous ketone oxidation rates during exercise.

Therefore, this work sought to determine (i) the direct contribution of exogenous β HB oxidation to overall energy expenditure during exercise using a β HB stable isotope tracer, (ii) the effect of altering blood β HB concentration on exogenous β HB oxidation rates during exercise, and (iii) the effect of exercise intensity on exogenous β HB oxidation rates.

METHODS

Ethical approval. The study was approved by the Oxfordshire Clinical Research Ethics Committee (15/SC/0542). The study conformed to the standards detailed in the Declaration of Helsinki. All participants provided written informed consent.

Participants. Eligibility criteria were healthy men or women age 18–45 yr who were currently undertaking >6-h training per week in rowing, cycling, running, or triathlon. No participants had been following a ketogenic diet before enrollment in the study. Exclusion criteria included the following: cardiovascular disease, diabetes, or any other disorder that may influence their capacity to exercise; pregnancy; or current breastfeeding; or women not taking the oral contraceptive pill.

Baseline test. Between 1 and 2 wk before the first glycogen depletion visit, participants attended the laboratory at the Department of Physiology, Anatomy and Genetics, the University of Oxford, to undertake an incremental exercise test to volitional fatigue on an electronically braked bicycle ergometer (ergoselect 100; Ergoline GmbH, Bitz, Germany; Fig. 1). Participants arrived at ~0800 h having fasted overnight. Before commencing exercise, participants adjusted the saddle height and handlebar position according to personal preference. Bike geometry was recorded and kept constant for all subsequent study visits. The test began at 100 W (light intensity) and increased 25 W every 3 min. Two of the following criteria must have been attained to satisfy that exercise was undertaken to exhaustion: (i) volitional fatigue, (ii) an inability to maintain a cadence >60 rpm, (iii) the maximum heart rate (HR) attained was within 10 bpm of an age-predicted maximum ($220 - \text{age}$), or (iv) the maximum RER attained was >1.10. Participants were instructed to hold a cadence of 80–100 rpm during the test. The ergometer was set to the rpm-independent mode, meaning the power output was held constant during each 3-min interval regardless of the cadence held. Respiratory gases (volume of oxygen consumed ($\dot{V}O_2$), volume of carbon dioxide produced ($\dot{V}CO_2$), and minute ventilation (\dot{V}_E)) were measured continuously through indirect calorimetry. W_{Max} achieved during the test was calculated as $W_{Step\ N-1} + (\text{time elapsed}/180\ \text{s} \times 25\ \text{W})$ and used to determine exercise intensities (% W_{Max}) for exercise during subsequent study visits.

Glycogen depletion visits. Approximately 40 h before each study visit, participants undertook a supervised glycogen depleting exercise protocol on the same bicycle ergometer followed by a high-carbohydrate-content diet (~70% of total energy intake) and no additional exercise (Fig. 1). These visits sought to provide a consistent level of fatigue and similar intramuscular glycogen contents for each study visit. The exercise protocol consisted of alternating 2-min “work” and “recovery” intervals commencing at 90% and 50% W_{Max} , respectively. When the participant could no longer hold 90% W_{Max} for 2 min (volitional fatigue or inability to hold a cadence >60 rpm), the work intensity was reduced by 10%. This continued until participants could no longer maintain 60%

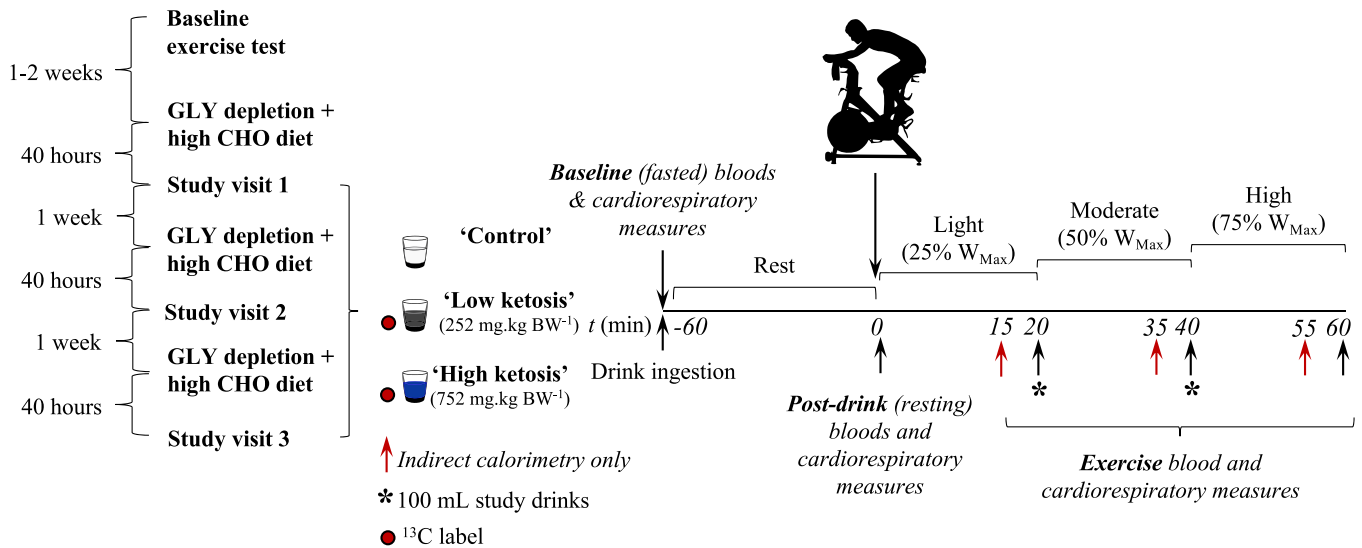


FIGURE 1—Study visit protocol. Participants ($n = 6$) attended the laboratory on three occasions to undertake incremental intensity cycling exercise in a random order, controlled, crossover fashion. Participants consumed either a calorie-free control drink (“control”), a low-dose KME drink ($252 \text{ mg}\cdot\text{kg BW}^{-1}$, “low ketosis”), or a high-dose KME drink ($752 \text{ mg}\cdot\text{kg BW}^{-1}$, “high ketosis”). KME drinks contained a ^{13}C tracer, allowing for the subsequent determination of exogenous βHB oxidation rates in collected respiratory gas samples. Approximately 40 h before all study visits, participants performed supervised glycogen (GLY) depleting cycling exercise followed by a high carbohydrate (CHO) content diet.

W_{Max} during work intervals. The number of completed repetitions was recorded. Participants were instructed to hold a cadence of 80–100 rpm, and the bicycle ergometer was set to the rpm-independent mode. This protocol reliably depletes intramuscular glycogen stores (14).

After each glycogen depletion visit, participants were instructed to maintain a high carbohydrate diet ($\sim 70\%$ of total energy intake) and refrain from further exercise. Glycogen depleting exercise immediately followed by a carbohydrate-rich diet is well observed to result in intramuscular glycogen “super-compensation” (15). Participants were asked to avoid foods naturally abundant in ^{13}C (e.g., corn sugars) and to refrain from alcohol. Participants kept a food diary between the glycogen depletion and study visits (~ 40 h) and were asked to closely match their food intake before each study visit.

Study visits. Each subject performed three exercise trials, separated by ~ 1 wk (Fig. 1). Participants reported to the laboratory at ~ 0800 h having fasted overnight. Upon arrival, a 22-gauge catheter (BD Venflon™ Pro Safety; BD, Wokingham, Berkshire, United Kingdom) was inserted retrogradely into a dorsal vein of the hand. A three-way tap (BD Connecta™ Plus Stopcock; BD) was attached, allowing for repeated blood sampling. The catheter was flushed with ~ 1 mL sodium chloride (Injection BP 0.9% w/v; Kent Pharmaceuticals Ltd, Ashford, United Kingdom) after each sample was taken. To achieve arterialized venous values, participants hands were heated for 2 min before blood was drawn (16). Baseline ($t = -60$ min) blood samples were drawn for the analysis of blood gases (1 mL, PICO50; Radiometer UK Ltd, West Sussex, United Kingdom) and blood metabolites (3 mL, BD Plastipak 3-Part Luer Slip Syringe; BD). Respiratory gases were measured via indirect calorimetry and respiratory gas samples were collected into 12 mL Exetainer™ tubes (Labco Ltd, Lampeter, United

Kingdom). Finally, resting HR and gastrointestinal discomfort, using scales previously described (17), were measured.

After completion of baseline measurements, participants consumed a 400-mL bolus study drink: (i) a calorie-free control drink (“control,” bitter flavored water), (ii) a “low”-dose KME drink (“low ketosis”; target blood ketone concentration of 2–3 mM), or (iii) a “high”-dose KME drink (“high ketosis”; target blood ketone concentration of >4 mM; Fig. 1). The order participants completed the three study conditions was randomly controlled, and participants were blinded to the condition they were undertaking. Participants then rested for 60 min before commencing exercise. After consumption of study drinks, subjects were asked to collect all urine in a provided container.

At $t = 0$ min, all baseline measurements (as described previously) were repeated. Participants then undertook 60 min of continuous ergometer cycling exercise, comprising 20-min intervals at 25% W_{Max} ($t = 0$ –20 min), 50% W_{Max} ($t = 20$ –40 min), and 75% W_{Max} ($t = 40$ –60 min) exercise intensities. Participants were instructed to maintain a cadence of 80–100 rpm, and the bicycle ergometer was set to the rpm-independent mode. Room temperature and airflow were consistent across study visits. Participants consumed water *ad libitum*. During exercise, indirect calorimetry ($\dot{V}\text{O}_2$, $\dot{V}\text{CO}_2$, \dot{V}_E), RPE, HR, and gastrointestinal discomfort measurements were made at $t = 15, 20, 35, 40, 55,$ and 60 min. Breath and blood samples were collected at $t = 20, 40,$ and 60 min. To ensure blood βHB concentrations remained stable throughout exercise, 100-mL study drinks were administered at the end of the 25% W_{Max} and 50% W_{Max} exercise intervals.

Drink preparations. A 600-mL study drink was prepared containing $252 \text{ mg}\cdot\text{kg BW}^{-1}$ for low ketosis and $752 \text{ mg}\cdot\text{kg BW}^{-1}$ for high ketosis of KME ((*R*)-3-hydroxybutyl (*R*)-3-hydroxybutyrate; TAS Ltd, Oxford, United Kingdom). Thus,

based on a mean participant weight of 79 ± 4 kg, the mean amounts of β HB delivered were 19.9 ± 1.0 g (95.5 ± 4.8 kcal) in the low-ketosis condition and 59.4 ± 3.0 g (285.1 ± 14.4 kcal) in the high-ketosis condition. The KME delivered the D- β HB isoform, as opposed to the less well-characterized L- β HB isoform (18) (all references made to β HB within this article are therefore related to the D-isoform only). Control study drinks were calorie-free, isovolumetric amounts of bitter flavored (product number 648352; Symrise, Holzminden, Germany) water. All drinks also included a sweetener (NutraSweet, Augusta, GA) to aid palatability. The dose response of KME drinks was predicted from previous work investigating its pharmacokinetics (19). The 600-mL study drinks were divided into a 400-mL bolus consumed before exercise ($t = 0$ min) and two 100-mL drinks consumed at $t = 20$ and 40 min.

To determine ketone oxidation rates, a ^{13}C -labeled KME was created. Ninety-nine percent U- ^{13}C -labeled ethyl acetoacetate was purchased from Cambridge Isotope Laboratories (Tewksbury, MA). Ethyl acetoacetate was used as the precursor for the synthesis of both U- ^{13}C R-1,3- β -hydroxybutyrate and U- ^{13}C 1,3 butanediol in separate fractions. Both fractions underwent a lipase reaction to produce 50% U- ^{13}C -labeled KME with labels present in 1,3- β -hydroxybutyrate and U- ^{13}C 1,3 butanediol "halves" of the final monoester (Document, Supplemental Digital Content 1, description of stable isotope preparation and synthesis, <http://links.lww.com/MSS/C114>). All syntheses was performed under good manufacturing practice conditions (Sterling Pharmaceutical Solutions Ltd, England, United Kingdom). Incorporation of small aliquots of the ^{13}C containing ester into unlabeled KME drinks, resulting in a relative enrichment of ~25%.

Blood sampling and analysis. Blood gases in 1 mL arterialized venous blood samples were immediately analyzed using a benchtop blood gas analyzer (ABL800; Radiometer UK Ltd). pH and bicarbonate (HCO_3^-) values were subsequently corrected using a custom MATLAB script (v2017a; MathWorks, Natick, MA), which accounted for the increased hemoglobin saturation in arterialized blood, as previously been described (8). The second blood sample (3 mL) was collected in tubes containing ethylenediaminetetraacetic acid (BD Vacutainer®; BD). Blood β HB was analyzed immediately from these samples using a portable analyzer (Freestyle Optium Neo; Abbott Laboratories Inc., Abbott Park, IL). Samples were then immersed in an ice bath until the end of the test. After completion of the visit, blood samples were centrifuged (3600 rpm for 10 min), and the plasma component was transferred to an Eppendorf tube and subsequently stored at -80°C until later analysis. Glucose, nonesterified fatty acids (NEFA), and lactate were assayed in thawed serum samples using a semiautomated benchtop analyzer (Pentra C400; Horiba Medical, Grabels, France).

Urine sampling and analysis. The total volume of urine was recorded, and a 1-mL aliquot was taken and stored at -80°C until later analysis. Urine β HB was analyzed using a semiautomated benchtop analyzer (Pentra C400; Horiba Medical).

Indirect calorimetry and HR measurements. Participants wore a snug, comfortable fitting face mask (Hans Rudolph Inc., Shawnee, KS) attached to an indirect calorimeter (Metalyzer 3BR2; Cortex Biophysik GmbH, Leipzig, Germany) for the continuous sampling of respiratory gases, displayed in real-time using Metasoft® (v7.9.1; Cortex Biophysik GmbH). For the measurement of HR, subjects wore a chest strap (T31, Polar® Electro, Espoo, Finland) that communicated directly to the Metalyzer unit via a receiver cable attached to the bicycle ergometer. Cardiorespiratory data were averaged over 5-s intervals in Metasoft®.

Respiratory gas sampling. Participants breathed into a mouthpiece containing a one-way valve connected to a Douglas bag. Duplicate respiratory gas samples were collected into 12-mL Exetainer™ tubes (Labco Ltd). Samples were kept at room temperature until subsequent analysis (see discussion hereinafter), which occurred within ~6 wk of collection.

Calculations of exogenous β HB oxidation rates. The ^{13}C enrichment in exogenous β HB was determined by gas chromatography–mass spectrometry after derivatization with a trimethylsilyl group (20). The tracer-to-tracee ratio in respired gases (TTr; $^{13}\text{CO}_2/^{12}\text{CO}_2$) was determined using previously published methods (21). Exogenous ketone oxidation rate (in grams per minute) was calculated by the following equation:

$$\text{exogenous } \beta\text{HB (g} \cdot \text{min}^{-1}) = \dot{V}\text{CO}_2 (\text{TTr CO}_2 \text{ Expired} - \text{TTr CO}_2 \text{ Background}) / (\text{TTr}_{\text{KE}} - \text{TTr}_{\text{Background}}) (1/k)$$

The molar mass of β HB was assumed to be $104.1 \text{ g} \cdot \text{mol}^{-1}$ and k = the volume of CO_2 produced by the oxidation of 1 g of β HB (0.86 L).

Calculations of whole-body efficiency. Energy expenditure was calculated according to the exercise intensity–dependent formulas proposed by Jeukendrup and Wallis (22):

$$\text{energy expenditure (kcal} \cdot \text{min}^{-1}) \text{ at 25\% and 50\% } W_{\text{Max}} = (0.575 \dot{V}\text{CO}_2) + (4.435 \dot{V}\text{O}_2)$$

$$\text{energy expenditure (kcal} \cdot \text{min}^{-1}) \text{ at 75\% } W_{\text{Max}} = (0.550 \dot{V}\text{CO}_2) + (4.471 \dot{V}\text{O}_2).$$

The delta efficiency, which represents the change in energy expenditure relative to the change in work accomplished (23), was determined by calculating the individual linear regression ($y = mx + b$) of the relationship between energy expended per minute versus work accomplished per minute (24). Delta efficiency was then calculated from the reciprocal of the slope of this relationship ($1/m$) and expressed as a percentage. Delta efficiency is thought to provide a more valid reflection of changes in muscular efficiency during exercise versus other commonly used measures (23).

RPE. Subjective scores of RPE ("What is your perceived exertion?"), breathlessness ("How breathless are you?"), leg discomfort ("What is your leg discomfort?"), anxiety of

breathing (“How anxious are you about your breathing?”), and anxiety of leg discomfort (“How anxious are you about your leg discomfort?”) were recorded in a random order on a linear scale of 0–10 (0 (none) to 10 (maximum)); scales are presented by Faull et al. (25).

Skeletal muscle biopsy. As the activity of BDH and SCOT (which catalyze the first and second reactions of ketolysis, respectively) is significantly greater in type I (slow-oxidative) versus types IIa and IIb skeletal muscle fibers (3), an individual’s muscle phenotype (relative proportion of each fiber) may theoretically influence exogenous ketone oxidation rate. As such, to provide context for exogenous ketone oxidation data, we performed a skeletal muscle biopsy and subsequently determined each participant’s muscle phenotype. A skeletal muscle biopsy of *vastus lateralis* was performed on participants before baseline measurements at the control visit using aseptic techniques previously described (5).

Muscle fiber type. The percentage of slow-oxidative fibers in muscle biopsy samples was determined using methods adapted from Koopman and colleagues (26). Frozen *vastus lateralis* samples were embedded in optimal cutting temperature compound (Tissue-Tek® OCT; Labtech, Heathfield, United Kingdom), sectioned (4 μm) using a Cryostat Microtome (OTF5000; Bright Instruments Ltd, Uxbridge, United Kingdom), and mounted onto slides (SuperFrost® Plus; VWR International Ltd, England, United Kingdom). Samples were fixed for 1 h using a 4% formaldehyde solution. Triton X-100 (0.5%) was used to make samples permeable, before being blocked for 1 h at room temperature (1% bovine serum albumin, 0.1% Triton X-100). Samples were incubated overnight with a primary antibody (antislowl skeletal myosin heavy chain, ab11083; Abcam, Cambridge, United Kingdom) diluted in the block solution (1:50). The primary antibody was internally validated on dissected rat soleus (high percentage of slow-oxidative muscle fibers) and anterior tibialis (high percentage of fast-glycolytic muscle fibers) muscle (Figure, Supplemental Digital Content 2, images of rat (A) soleus (extremely slow-oxidative phenotype) and (B) extensor digitorum longus (extremely fast phenotype) muscle stained with an antislowl skeletal myosin heavy-chain primary antibody, <http://links.lww.com/MSS/C115>) and has previously been validated in human skeletal muscle (27). The secondary antibody (antimouse IgG (whole molecule)–fluorescein isothiocyanate produced in goat, F0257; Merck, Darmstadt, United Kingdom) diluted in the block solution (1:200) was applied for 1 h at room temperature. Finally, 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) diluted in ddH₂O (1:1000) was added for 10 min to detect nuclei before a coverslip was applied.

Slides were imaged using a DeltaVision widefield fluorescence imaging system (GE Healthcare, Amersham, Buckinghamshire, United Kingdom) consisting of a 10 \times /0.4 NA objective lens (Olympus Life Science, Southend-on-sea, Essex, United Kingdom) fitted to an Olympus IX71 inverted microscope stand. A Lumencor SpectraX LED light engine (Lumencor Inc., Beaverton, OR) with excitation lines at

395 and 470 nm and appropriate emission filters were used to sequentially image DAPI and fluorescein isothiocyanate fluorochromes on a CoolSNAP HQ2 CCD camera (Photometrics Inc., Huntington Beach, CA). The instrument was driven using the softWoRx software package (GE Healthcare) and subsequent image analysis performed in ImageJ (NIH, Bethesda, MD). Quantification of slow-oxidative fibers was performed through manual counting (minimum of 50 fibers) and expressed as a percentage of total muscle fibers. This was performed by a single, blinded investigator.

Statistics. Results are expressed as mean \pm SD. Significance was taken at $P < 0.05$. The effects of blood ketone concentration and exercise intensity on exogenous βHB oxidation rates were tested with two-way repeated-measures ANOVA (factors: condition (three levels) and time (five levels)). Simple linear regressions and repeated-measures one- and two-way ANOVA were used as appropriate for all other analyses. Sphericity and normality assumptions were tested in SPSS, v26 (IBM Corp., Armonk, NY), with all remaining analysis being performed in Prism v8.3 (GraphPad Software Inc., San Diego, CA). Where the assumption of sphericity was not met, a Greenhouse–Geisser correction was applied. Nonnormally distributed data were log₁₀ transformed. In such cases, all analyses were performed on transformed data sets. *Post hoc* comparisons were made using Tukey or Sidak multiple comparison corrections as appropriate.

RESULTS

Participants. Six athletes (5 men, 1 woman; age, 21 ± 1 yr; height, 186 ± 4 cm; weight, 79 ± 4 kg; $\dot{V}\text{O}_{2\text{ Peak}}$, 56.73 ± 4.25 mL \cdot kg⁻¹ \cdot min⁻¹; W_{Max} , 375 ± 65 W; training per week, 16 ± 6 h) completed the study. The participants’ primary sports were cycling ($n = 2$), running ($n = 2$), triathlon ($n = 1$), and rowing ($n = 1$).

Prestudy preparation. No order effects were observed for exercise performance (number of steps completed) in the glycogen depleting exercise, performed 40 h before study visits. Analysis of self-reported diet diaries showed that neither the macronutrient composition nor total energy intake for the 48-h preceding study visits differed between study conditions (Figure, Supplemental Digital Content 3, total energy intake and macronutrient composition of participants’ diets leading into each study visit, <http://links.lww.com/MSS/C116>). As requested, participants consumed a high-carbohydrate-content diet, representing $61.09\% \pm 10.44\%$, $64.16\% \pm 8.42\%$, and $57.09\% \pm 8.33\%$ of total energy intake leading into the control, low-ketosis, and high-ketosis conditions, respectively.

Blood βHB concentration. Main condition and time effects, and a condition–time interaction effect were present for blood βHB concentration (all, $P < 0.001$), which diverged in a dose-dependent manner after ingestion of the study drinks (Table 1). βHB concentration was approximately twofold higher in the high-ketosis (mean \pm SD of all postdrink values, 4.40 ± 0.35 mM) versus low-ketosis (1.96 ± 0.31 mM)

TABLE 1. Blood β HB concentration, exogenous β HB oxidation rate and contribution to total energy expenditure, and urine β HB.

	Predrink (Rest)		Post-Drink (Rest)		25% W_{Max}		50% W_{Max}		75% W_{Max}	
	BHB, mM	BHB, mM	BHB, mM	BHB Ox., g·min ⁻¹	BHB, mM	BHB Ox., g·min ⁻¹	BHB, mM	BHB Ox., g·min ⁻¹	BHB, mM	BHB Ox., g·min ⁻¹
Control	0.18 ± 0.04	0.20 ± 0.00	0.18 ± 0.04	0.009 ± 0.003	0.17 ± 0.05	0.087 ± 0.053 ^{a,c}	0.17 ± 0.05	0.098 ± 0.068 ^b	0.17 ± 0.05	0.098 ± 0.068 ^b
Low ketosis	0.20 ± 0.06	2.42 ± 0.53 ^a	1.83 ± 0.48 ^a	(2.47% ± 1.52%)	1.73 ± 0.65 ^a	(3.83% ± 2.12% ^b)	1.73 ± 0.65 ^a	(3.33% ± 2.01%)	1.87 ± 0.73 ^a	(2.48% ± 1.66% ^{c,d})
High ketosis	0.17 ± 0.05	4.90 ± 1.03 ^{a,e}	4.25 ± 1.65 ^{a,e}	0.012 ± 0.011 (3.37% ± 3.48%)	4.10 ± 1.38 ^{a,e}	0.079 ± 0.054 ^b (4.46% ± 2.71%)	4.10 ± 1.38 ^{a,e}	0.100 ± 0.058 ^{a,c} (3.56% ± 1.68% ^f)	4.33 ± 1.05 ^{a,e}	0.103 ± 0.044 ^b (2.58% ± 1.05% ^f)

Blood β HB concentration (in millimolar), exogenous β HB oxidation rates (in grams per minute), relative contribution of β HB oxidation to energy expenditure (given in parentheses), and urine β HB (in grams) are expressed as means ± SD ($n = 6$).

^aSignificant difference from control.
^bSignificant difference from postdrink (rest).
^cSignificant difference from 25% W_{Max} .
^dSignificant difference from 50% W_{Max} .
^eSignificant difference from low ketosis.

conditions at rest and throughout exercise. Blood β HB concentrations were unaffected by ingestion of the control drink. The consumption of 100-mL study drinks at the end of the 25% W_{Max} (20 min) and 50% W_{Max} (40 min) exercise intervals (see study protocol in Fig. 1) meant that blood β HB concentrations remained stable during exercise in the low- and high-ketosis conditions.

Gastrointestinal discomfort. A main effect of condition was present for nausea ($P = 0.009$), with *post hoc* analyses showing a tendency for this to be greater in the high-ketosis (mean ± SD, 2.2 ± 2.3 on a 0–10 scale) versus control condition (no reported nausea; $P = 0.053$). No differences were observed for heartburn, bloating, abdominal pain, dizziness, or headache.

Effect of blood β HB concentration and exercise intensity on exogenous β HB oxidation rate. Neither a main effect of condition nor a condition–time interaction effect was present for exogenous β HB oxidation rates (Table 1). Exogenous β HB oxidation rate was significantly associated with oxygen consumption ($R^2 = 0.402$, $P < 0.001$). This relationship remained significant when resting values were removed ($R^2 = 0.111$, $P = 0.048$; Figure, Supplemental Digital Content 4, associations between exogenous BHB oxidation rate and $\dot{V}O_2$ and individual BHB oxidation rates, <http://links.lww.com/MSS/C117>).

A main effect of time was observed for exogenous β HB oxidation rates ($P < 0.001$). In both the low- and high-ketosis conditions, oxidation rates rose significantly from rest to the 25% W_{Max} exercise interval (~7-fold increase) and from 25% W_{Max} to 50% W_{Max} exercise intervals (~1.5-fold increase), but thereafter plateaued (Table 1). Peak exogenous β HB oxidation rates were 0.103 ± 0.044 g·min⁻¹, observed during the 75% W_{Max} exercise interval. In both conditions, the relative contribution to energy expenditure during exercise was highest during the 25% W_{Max} (peak, 4.46% ± 2.71%) and lowest during the 75% W_{Max} exercise intensities (Table 1).

Urine β HB concentration. A main effect of condition was present for urinary β HB concentration ($P < 0.001$), which was greater in low-ketosis versus control condition ($P = 0.002$) and the high- versus low-ketosis condition ($P = 0.004$; Table 1).

Cardiorespiratory and RPE measures. As expected, there was a main effect of time for all measured cardiorespiratory and RPE values (all, $P < 0.001$), which rose in accordance with exercise intensity (Table 2 and Table, Supplemental Digital Content 5, compartmentalized perceptions (leg discomfort and breathlessness) to exercise exertion and emotional affective responses to these (anxiety of leg anxiety and anxiety of breathing), <http://links.lww.com/MSS/C118>). However, no main effects of condition nor condition–time interaction effects were present. Importantly, no within-condition differences were observed for $\dot{V}O_2$, $\dot{V}E$, HR, or RPE measures taken within the last 5 min of each exercise interval (e.g., $t = 15$ min vs $t = 20$ min for the light-intensity interval), indicating that athletes had achieved a physiological “steady state” at the point of respiratory gas collection for determination of exogenous β HB oxidation rates.

TABLE 2. Effect of blood β HB concentration and exercise intensity on cardiorespiratory measures and RPE.

Measurement		Control	Low Ketosis	High Ketosis
$\dot{V}O_2$, L·min ⁻¹	Predrink (rest)	0.31 ± 0.08	0.28 ± 0.07	0.31 ± 0.03
	Postdrink (rest)	0.30 ± 0.06	0.38 ± 0.11	0.37 ± 0.07
	15 min (25%)	1.62 ± 0.25	1.68 ± 0.35	1.64 ± 0.29
	20 min (25%)	1.55 ± 0.20	1.61 ± 0.25	1.70 ± 0.25
	35 min (50%)	2.60 ± 0.47	2.60 ± 0.46	2.64 ± 0.55
	40 min (50%)	2.65 ± 0.45	2.58 ± 0.39	2.66 ± 0.50
	55 min (75%)	3.78 ± 0.86	3.63 ± 0.80	3.75 ± 0.83
$\dot{V}CO_2$, L·min ⁻¹	Predrink (rest)	0.24 ± 0.08	0.22 ± 0.07	0.24 ± 0.04
	Postdrink (rest)	0.25 ± 0.06	0.28 ± 0.29	0.29 ± 0.05
	15 min (25%)	1.39 ± 0.33	1.37 ± 0.31	1.32 ± 0.32
	20 min (25%)	1.31 ± 0.30	1.30 ± 0.22	1.38 ± 0.27
	35 min (50%)	2.28 ± 0.46	2.20 ± 0.45	2.23 ± 0.51
	40 min (50%)	2.34 ± 0.43	2.16 ± 0.41	2.21 ± 0.49
	55 min (75%)	3.53 ± 0.87	3.34 ± 0.84	3.35 ± 0.86
$\dot{V}E$, L·min ⁻¹	Predrink (rest)	3.68 ± 0.80	3.48 ± 0.82	3.44 ± 0.72
	Postdrink (rest)	10.28 ± 1.58	8.76 ± 3.11	9.27 ± 2.11
	15 min (25%)	10.78 ± 2.17	11.34 ± 3.46	12.99 ± 1.91
	20 min (25%)	41.13 ± 5.02	42.05 ± 6.86	45.13 ± 9.40
	35 min (50%)	38.37 ± 5.30	41.09 ± 4.03	46.46 ± 8.77
	40 min (50%)	63.74 ± 8.39	64.94 ± 9.13	70.76 ± 11.27
	55 min (75%)	65.52 ± 5.43	64.57 ± 8.37	68.34 ± 19.56
HR, bpm	Predrink (rest)	104.59 ± 20.35	103.22 ± 22.06	105.27 ± 19.56
	Postdrink (rest)	107.84 ± 17.11	105.77 ± 19.31	112.34 ± 19.74
	15 min (25%)	97.40 ± 7.06	102.40 ± 8.41	104.40 ± 4.93
	20 min (25%)	98.33 ± 5.01	97.83 ± 12.04	104.17 ± 7.86
	35 min (50%)	125.83 ± 10.50	124.50 ± 13.49	128.17 ± 9.15
	40 min (50%)	125.83 ± 10.52	127.50 ± 10.29	127.83 ± 10.30
	55 min (75%)	156.83 ± 9.04	153.67 ± 11.78	153.00 ± 9.17
RPE (0–10)	Predrink (rest)	158.00 ± 8.79	156.33 ± 12.72	154.17 ± 8.38
	Postdrink (rest)			
	15 min (25%)	1.00 ± 0.82	1.50 ± 0.58	0.88 ± 0.25
	20 min (25%)	0.92 ± 0.66	1.42 ± 0.66	0.83 ± 0.26
	35 min (50%)	2.63 ± 0.75	3.25 ± 0.96	3.25 ± 0.87
	40 min (50%)	2.70 ± 0.84	2.60 ± 1.14	2.60 ± 1.08
	55 min (75%)	6.63 ± 0.48	6.75 ± 1.26	6.25 ± 0.87
60 min (75%)	6.33 ± 1.37	6.00 ± 2.00	5.58 ± 1.91	

Values are expressed as means ± SD ($n = 6$). Exercise measurements are presented as time point with the relative workload in parentheses.

RER and whole-body efficiency. There was a main effect of time ($P < 0.001$) and tended to be a main effect of condition for RER ($P = 0.051$; Fig. 2A). However, a time–condition interaction effect was not present. Exploratory *post hoc* comparisons of main condition effects showed that

RER tended to be lower in the low-ketosis versus control condition ($P = 0.051$). Delta efficiency also tended to be different between conditions (ANOVA, $P = 0.098$; Fig. 2B), with exploratory *post hoc* analyses showing that delta efficiency was significantly greater in the low-ketosis versus control condition ($25.94\% \pm 2.11\%$ vs $24.09\% \pm 1.91\%$, respectively; $P = 0.027$).

Plasma metabolite concentrations. There was a main effect of time ($P = 0.003$) and condition–time interaction ($P = 0.031$) effect for blood glucose concentration, which was significantly lower in the high-ketosis versus control condition at rest and after 25% W_{Max} exercise ($P = 0.036$ and $P = 0.035$, respectively; Fig. 3A). Significant condition, time, and condition–time interaction effects (all, $P < 0.001$) were present for blood NEFA concentration, which fell from ~ 0.6 to ~ 0.2 mM after ingestion of the KME drinks and was significantly lower at rest (postdrink) and throughout exercise in the low- and high-ketosis versus the control conditions (Fig. 3B). There were no differences in plasma NEFA concentrations between low- and high-ketosis conditions at any time point. There was a main effect of time for plasma lactate concentration ($P < 0.001$), but no condition or condition–time interaction effects.

Blood acid–base status. There were significant main effects of condition and time (both $P < 0.001$) for blood pH, which was reduced in the low-ketosis versus control condition ($P = 0.002$) and high- versus low-ketosis condition ($P < 0.001$; Fig. 3D). A significant negative correlation was present between blood β HB concentration and pH in the ketosis conditions ($R^2 = 0.455$, $P < 0.001$; Fig. 3E). In the high-ketosis condition, participants were mildly acidotic (pH 7.33 ± 0.03) before commencing exercise. As would be expected, a similar pattern was observed for blood HCO_3^- where main effects of condition ($P = 0.004$) and time ($P < 0.001$), and a condition–time interaction ($P < 0.001$) effect were present. Blood HCO_3^- was lower in the high- and low-ketosis conditions versus control at rest (postdrink) and throughout exercise (Fig. 3F).

Association between muscle fiber type and β HB oxidation rate. There was a significant positive association between the percentage of slow-oxidative muscle fibers in

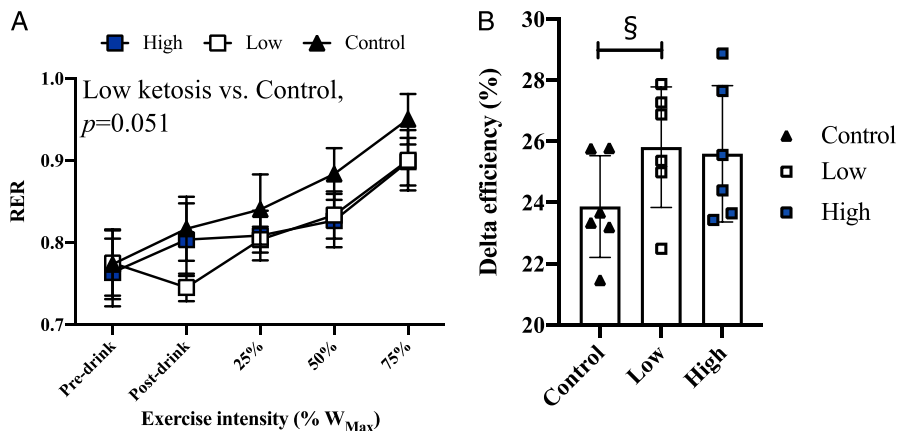


FIGURE 2—RER and exercise efficiency in the control, low-ketosis, and high-ketosis conditions. A, RER at rest and during exercise (mean ± SD). B, Delta efficiency (in percent), representing the relationship between energy expended per minute vs work accomplished per minute during exercise (mean ± SD). §Significant difference between control and low-ketosis conditions.

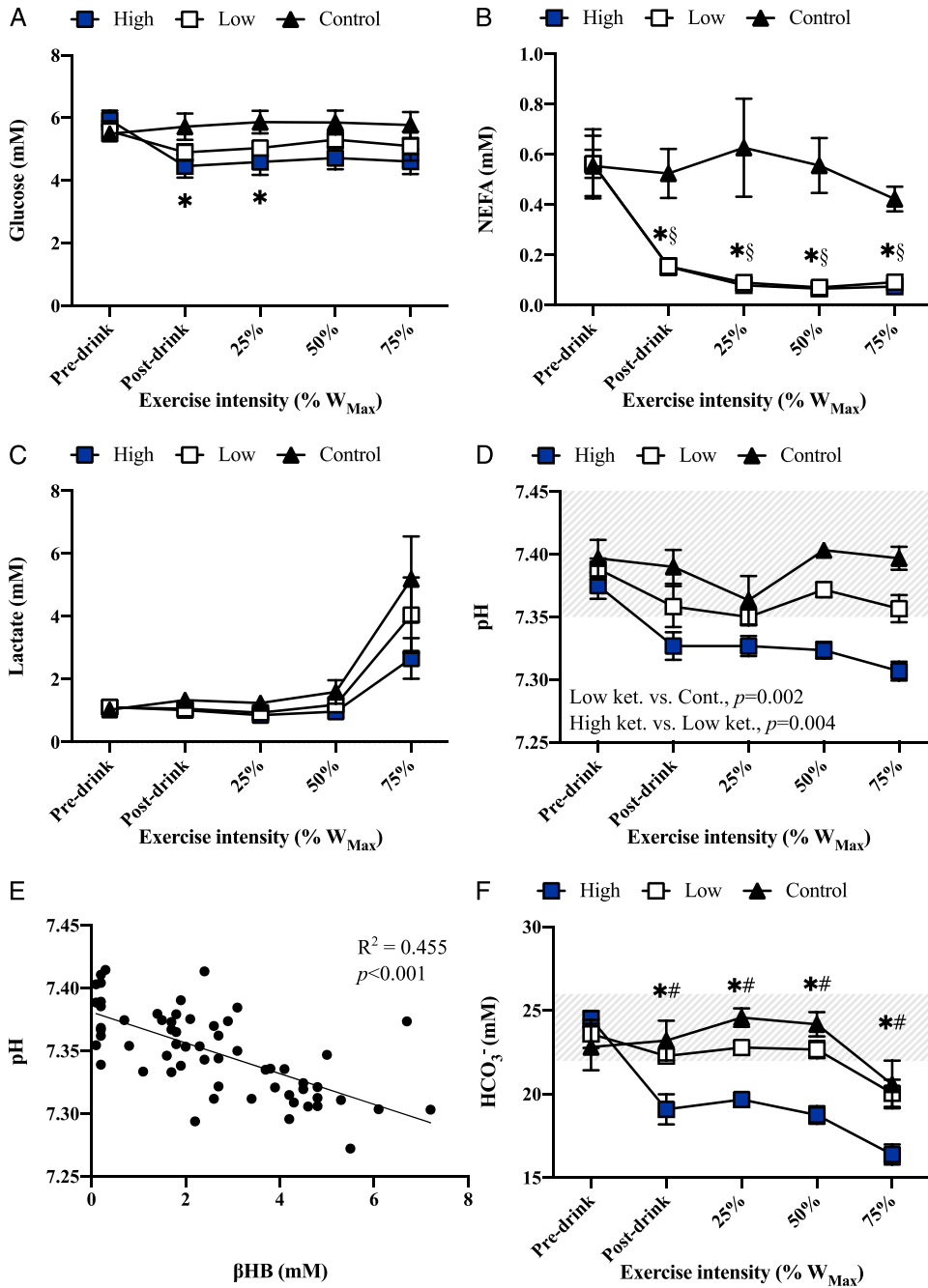


FIGURE 3—Blood metabolites and gases at rest and during cycling exercise in the control, low-ketosis, and high-ketosis conditions. **A**, Plasma glucose concentration (in millimolars) at rest and during exercise (mean \pm SD). **B**, Plasma NEFA concentration (in millimolars) at rest and during exercise (mean \pm SD). **C**, Plasma lactate concentration (in millimolars) at rest and during exercise. **D**, Blood pH at rest and during exercise (mean \pm SD). Shaded area represents the normal blood pH range. **E**, Relationship between blood β HB concentration and pH in the low- and high-ketosis conditions. **F**, Blood HCO_3^- concentration (in millimolars) at rest and during exercise (mean \pm SD). Shaded area represents the normal blood HCO_3^- range. *Significant difference between control and high-ketosis conditions. §Significant difference between control and low-ketosis conditions. #Significant difference between low- and high-ketosis conditions.

vastus lateralis muscle biopsy samples and peak exogenous β HB oxidation rate ($R^2 = 0.696$, $P = 0.039$; Fig. 4 and Figure, Supplemental Digital Content 6, example images of *vastus lateralis* samples, <http://links.lww.com/MSS/C119>).

DISCUSSION

The main findings of this study were that (i) increasing blood β HB concentration from ~ 2 to ~ 4.4 mM did not affect

exogenous β HB oxidation rates at rest or during incremental intensity exercise; (ii) the relative contribution of exogenous β HB oxidation to energy expenditure during exercise was low (2.48%–4.46%) and peaked during the 25% W_{Max} exercise intensity; and (iii) exercise efficiency (measured as delta efficiency) was improved when blood β HB concentration was raised to ~ 2 mM.

Exogenous β HB oxidation contributes minimally to energy expenditure during exercise. The peak

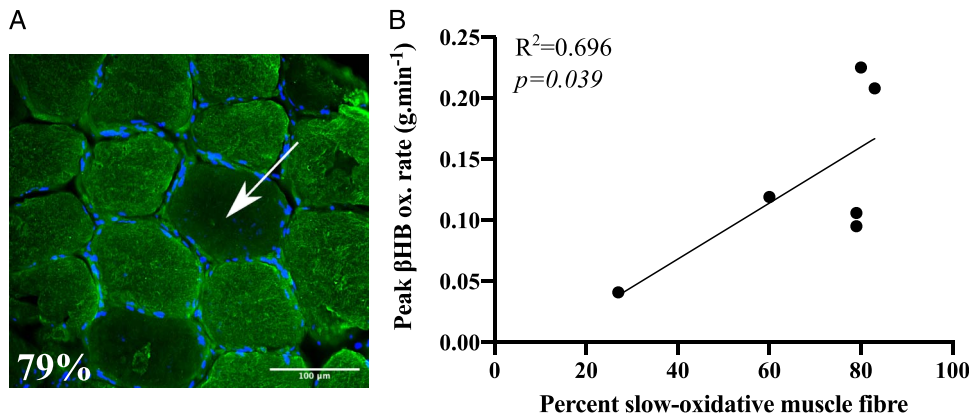


FIGURE 4—Association between skeletal muscle fiber type and peak exogenous β HB oxidation rate. **A**, *Vastus lateralis* biopsy sample stained with an anti-slow skeletal myosin heavy-chain primary antibody (fluorescent green). Nuclei are represented in blue (stained with DAPI). Example nonstained fibers (representing fast-glycolytic (IIa) and fast (IIb) fibers) are indicated with the white arrow. **B**, Relationship between peak exogenous β HB oxidation rate (in grams per minute) and skeletal muscle phenotype (in percent).

relative contribution of exogenous β HB oxidation to energy expenditure was $4.46\% \pm 2.71\%$. This is low compared with values reported for free fatty acids (31% at 40% \dot{W}_{Max}), other fat sources (27% at 40% \dot{W}_{Max}), plasma glucose (18% at 75% \dot{W}_{Max}), and muscle glycogen (58% at 75% \dot{W}_{Max}) during stepped-intensity cycling ergometer exercise (28). Thus, within the context of this study, exogenous ketones seem to contribute minimally to whole-body energy expenditure during exercise.

Previous work predicted between 16% and 18% of total oxygen consumed during cycling ergometer exercise (40% and 75% \dot{W}_{Max} , respectively) was attributable to β HB oxidation when similarly highly trained athletes were given a drink containing carbohydrate and $573 \text{ mg} \cdot \text{kg} \cdot \text{BW}^{-1}$ of the KME (5). In the present study, assuming the complete oxidation of β HB requires 4.5 mol of O_2 and that 1 mol of gas occupies 22.4 L (13), the peak contribution of exogenous β HB oxidation to $\dot{V}\text{O}_2$ was $\sim 4.5\%$. Without the capacity to directly trace whole-body exogenous β HB oxidation (as performed in this study), previous estimations utilized adjusted substrate oxidation equations from measured gaseous exchanges (13). Inherent to these equations is the assumption that β HB rate of disappearance from the blood (R_d ; difference in blood β HB area under the curve (AUC; in millimoles per liter per minute) at rest and during exercise) would accurately reflect exogenous β HB oxidation. However, a substantial proportion of the blood β HB R_d may have been attributable to intermediate metabolic processes, notably, the conversion of β HB to AcAc and its subsequent spontaneous decarboxylation to acetone. Assuming AcAc was subsequently oxidized, or that any acetone produced was excreted in respiratory gases, these intermediate metabolic processes would not affect overall conclusions regarding substrate metabolism (13). However, when there is an accumulation within nonblood body compartments or nonrespiratory gas excretion from the body of these metabolites (discussed further hereinafter), respiratory gas equations would be expected to overestimate exogenous β HB oxidation rate. This may account for some of the discrepancy

between findings here and those previously reported (5). However, it is important to note that other work using a ^{14}C AcAc tracer found that $\sim 10\%$ of CO_2 was attributable to ketone oxidation in exercising humans (29). Thus, oxidation rates here are also substantially lower than previous *direct* measures of whole-body exogenous ketone oxidation rates in exercising humans.

Whole-body oxidation accounted for $\sim 27.5\%$ (5.5 g) and $\sim 10.7\%$ (6.4 g) of β HB consumed in the low- and high-ketosis conditions, respectively. Assuming a β HB volume of distribution (V_D) of $0.2 \text{ L} \cdot \text{kg} \cdot \text{BW}^{-1}$ (20) (although values as high as $0.5 \text{ L} \cdot \text{kg} \cdot \text{BW}^{-1}$ have been reported [30]), at the end of exercise ($t = 60 \text{ min}$), the amount of β HB within the body fluids accounted for a further $\sim 15.5\%$ ($\sim 3.1 \text{ g}$) and $\sim 12.0\%$ ($\sim 7.1 \text{ g}$) of the β HB consumed in the low- and high-ketosis conditions, respectively. As in previous reports (18), the urinary excretion of β HB was minimal ($< 1\%$ in both conditions). Given the inhibitory effects of high circulating ketone concentrations on hepatic ketogenesis (1), we assume β HB measured in urine and blood was almost entirely derived from the KME drinks. Thus, $\sim 57\%$ of β HB in the low-dose KME ($252 \text{ mg} \cdot \text{kg} \cdot \text{BW}^{-1}$) and $\sim 77\%$ of β HB in the high-dose KME ($752 \text{ mg} \cdot \text{kg} \cdot \text{BW}^{-1}$) remained unaccounted for.

Within the mitochondria, β HB is dehydrogenated to form AcAc in a near-equilibrium reaction (1). Previous work has demonstrated an $\sim 4:1$ ratio of blood β HB to AcAc in healthy, overnight fasted subjects after ingestion of a $395 \text{ mg} \cdot \text{kg} \cdot \text{BW}^{-1}$ dose of the KME (18), consistent with that observed during an endogenously induced physiological ketosis (1). In addition, breath acetone, formed from the spontaneous decarboxylation of AcAc, was measured at 25 ppm after 1-h rest ($\sim 7.5 \text{ mg} \cdot \text{min}^{-1}$ acetone expelled, assuming a resting $\dot{V}\text{O}_2$ of $0.3 \text{ L} \cdot \text{min}^{-1}$), peaking at $\sim 70 \text{ ppm}$ ($\sim 21 \text{ mg} \cdot \text{min}^{-1}$) after 3 h (18). The slower rate of appearance (R_a) of acetone is likely attributable to sequestration in lipid stores, represented by an acetone V_D of $\sim 0.6\text{--}0.8 \text{ L} \cdot \text{kg}^{-1}$ (13). Taken together, these unmeasured ketones may account for a substantial proportion of the unaccounted exogenous β HB.

Doubling blood ketone concentrations did not result in greater oxidation. Exogenous ketone uptake in skeletal muscle is saturated at a relatively low circulating concentration in resting subjects (~ 0.8 mM vs a physiological plateau of ~ 7 mM) (31). Inactive skeletal muscle has relatively low energy demand; however, upon commencement of exercise, a large proportion of muscle may be activated (depending on the form of exercise undertaken), leading to a dramatic increase in blood supply to and energy flux within this tissue (32). Accordingly, exercise seems to “override” resting saturation kinetics: skeletal muscle ketone oxidation is between ~ 5 -fold (31) and ~ 11 -fold (29) higher upon commencement of exercise, resulting in a similar uptake per kilograms of tissue as the brain (31). This suggests that skeletal muscle ketone oxidation is not restricted by ketone transport and ketolytic capacity per se. Given this, in the present study, we postulated that ketone oxidation rates during exercise would be proportional to blood concentrations (i.e., significantly greater in the high-versus low-ketosis conditions).

In agreement with previous work (5,29), we found exercise stimulated an ~ 6 -fold increase in ketone oxidation in both low- and high-ketosis conditions. However, despite an approximate doubling of circulating β HB, oxidation rates were similar between conditions at rest and throughout all exercise intensities. Thus, we conclude that exercise but not circulating concentrations (at least > 2 mM) can stimulate an increase in ketone oxidation in skeletal muscle. This result is consistent with our observation that exogenous β HB is a minimal contributor to energy expenditure during exercise. As previously described, the primary purpose of ketogenesis is to provide a supplementary substrate to support cerebral metabolism when carbohydrate stores are challenged. Skeletal muscle is metabolically flexible and can meet most of its energy needs from the oxidation of abundant lipid stores. As such, it is logical that ketone oxidation in skeletal muscle is tightly regulated, thus preserving these respiratory fuels for the brain.

The greatest relative contribution of exogenous β HB oxidation is during light-intensity exercise. We previously reported that after ingestion of 573 mg·kg BW^{-1} KME, β HB AUC was significantly decreased at greater exercise intensities and subsequently, negatively correlated with oxygen consumption (5). In agreement with this work, here we found a significant association between exogenous β HB oxidation rate and $\dot{V}O_2$. Notably, this relationship remained significantly nonzero even when resting values were excluded (although the variation in exogenous β HB oxidation explained was substantially lower, representing just $\sim 11\%$). However, contrary to our previous report (5), exogenous β HB oxidation was not increased when the exercise intensity was raised from $50\% W_{Max}$ to $75\% W_{Max}$. As described, previous estimates of exogenous ketone oxidation relied on β HB R_d from the blood. It is possible that the inhibition of ketolysis downstream of the BDH reaction could be increased at higher exercise intensities, resulting in an accumulation of AcAc and, in turn, acetone. Inhibitory mechanisms may include a depletion of the succinyl-CoA pool, which is a substrate for the

SCOT catalyzed reaction, or product inhibition from increased acetyl-CoA (primarily derived from carbohydrate oxidation) on the acetyl-CoA acetyltransferase 1 catalyzed reaction. In such a case, changes in blood β HB R_d would overestimate β HB oxidation.

Arguably of more practical importance is the relative contribution of exogenous β HB oxidation to overall energy expenditure, which was low throughout exercise and declined with successive increments in exercise load. This observation is consistent with the increased importance of the ATP synthesized/oxygen consumed ratio (P/O ratio) at higher exercise intensities (7), which is greater for pyruvate versus β HB (4).

Exercise efficiency is increased during ketosis. Elevating blood ketone concentrations to ~ 2 mM resulted in an $\sim 7\%$ improvement in exercise efficiency versus the control condition. This effect was driven by small, nonsignificant reductions in both $\dot{V}O_2$ and $\dot{V}CO_2$ at the 50% and $75\% W_{Max}$ exercise intensities during ketosis, leading to an improvement in the ratio of the change of work accomplished per minute (which was fixed) and the change in energy expended per minute (i.e., delta efficiency [23]). β HB is more chemically reduced than pyruvate and its combustion increases the electron transport redox span, thus creating a greater $\Delta G'_{ATP}$ for the generation of ATP (4,6). In the working rat heart perfused with a physiological ratio of ketones and glucose versus glucose alone, these effects were reported to increase hydraulic efficiency ($J \cdot mol O_2^{-1}$) by 25% (6). Thus, a convenient explanation for the observed improvement in exercise efficiency is that exogenous ketone oxidation in exercising skeletal muscle resulted in lower oxygen consumption at the same (fixed) power output. However, it is unclear whether a peak $\sim 4.5\%$ contribution from exogenous β HB oxidation to energy expenditure would be sufficient to drive the observed improvements in exercise efficiency.

It is plausible that improvements in exercise efficiency resulted from ketosis-mediated alterations in carbohydrate and fat metabolism. However, the oxidation of fat seems to be augmented during exogenous ketosis (5), which has a lower P/O ratio versus carbohydrate (reflecting a reduction in the ATP produced vs oxygen consumed) (7) and would therefore be expected to lower exercise efficiency (33). Further work is required to both confirm the observed improvement in exercise efficiency during exogenous ketosis and determine the mechanisms contributing to this effect.

Skeletal muscle fiber phenotype may influence exogenous β HB oxidation rate. The activities of BDH and SCOT (which catalyze the first and second reactions of ketolysis, respectively) are, respectively, ~ 1.5 and ~ 2.5 times greater in slow-oxidative versus fast glycolytic rat skeletal muscle fibers (3). As such, it is postulated that the uptake and utilization of ketones are greater in athletes with a slow-oxidative skeletal muscle phenotype (34). In this study, we provide preliminary data supporting this hypothesis. Using simple linear regression, participants' muscle phenotype explained $\sim 70\%$ of variation in exogenous β HB oxidation. Although a plausible finding, these data should be interpreted

with caution as the association was driven by one participant with an extremely fast muscle phenotype (former national-level sprinter, undertaking training for 5- to 10-km running at the time of enrollment). Notably, this individual had an ~1.5-fold increase in blood β HB AUC and an ~2-fold increase in blood lactate AUC versus the mean for the remaining participants, and consistently lower blood pH. These results are consistent with a reduced uptake and oxidation of exogenous β HB in this athlete. Further work is warranted to test our preliminary results indicating that skeletal muscle phenotype is an important modulator of exogenous β HB oxidation in exercising humans.

Ketosis-mediated changes in glucose, nonesterified fatty acids, and lactate concentrations were not affected by elevating circulating ketone concentrations. Ketones have pleiotropic effects on metabolism, including acting as signaling molecules to alter the availability and catabolism of other substrates. Both β HB and AcAc are agonists for the hydroxycarboxylic acid receptor 2 on adipocyte cell membranes, which through a decrease in cyclic adenosine monophosphate signaling leads to a reduction in lipolytic rate and consequently, circulating NEFA and glycerol concentrations (35). Glucose concentrations also fall during exogenous ketosis (5), although the mechanisms have not yet been established. Ketone metabolism also inhibits glycolysis (5), potentially via glucose-fatty acid cycle-like mechanisms (36), which is commonly observed as a reduction in blood lactate.

Previous work has shown sequential decreases in blood glucose and glycerol concentrations with successive increases in blood ketone concentrations of ~0.5, 0.8, and 1.7 mM (31). Notably, the greatest decreases were observed from baseline (preinfusion) to ~0.5 mM. In this study, no differences were observed for glucose, NEFA, or lactate concentrations between blood ketone concentrations of ~2 and ~4.4 mM, either at rest or during exercise. Taken together, these results provide evidence for a blood ketone concentration threshold for ketosis-mediated alterations in circulating substrate concentrations, which is likely to occur <2 mM.

Acid-base regulation. Consumption of the KME drinks caused dose-dependent falls in blood pH (ketoacidemia). Notably, participants in the high-ketosis condition were acidotic before commencing exercise (pH 7.32). Furthermore, although blood buffering capacity corrected toward control values in the low-ketosis condition, HCO_3^- remained significantly depressed throughout exercise in the high-ketosis condition. Unlike previous work (25), ketoacidemia did not alter participants' perceptions of exercise exertion at any exercise intensity, nor were they affected by the degree of ketoacidemia induced (low vs high ketosis). We suggest that this was due to the comparatively lower exercise intensities and longer exercise intervals used in this study, with even minor changes in the relationship between time and power output having been shown to yield differences in the magnitudes of reported exercise exertion (37). Moreover, the rationale for including RPE measures within this study was to help ascertain (together with other cardiorespiratory measures) whether participants had

achieved a “steady state” at the end of each exercise interval, not to determine the effects of blood ketone concentrations on subjective perceptions of exercise exertion, which, given the design used (three conditions, six time points), the study was underpowered to achieve.

Limitations and future directions. It is important that exogenous β HB oxidation results presented here are interpreted in the context of an exogenously induced ketosis. It is very likely that whole-body oxidation rates during exercise would be different in the setting of a starvation- or dietary-induced ketosis. Participants recruited had been consuming a standard carbohydrate-rich diet before enrollment. Skeletal muscle is a highly plastic tissue, capable of altering its morphology and metabolism in response to a range of environmental stimuli, including diet (38). As such, it is possible that exogenous β HB oxidation rates and relative contributions to overall energy expenditure would be greater in individuals chronically exposed to hyperketonemia (“keto-adapted”), for example, through adoption of a ketogenic diet or even chronic consumption of exogenous ketones. Although preliminary data demonstrated that skeletal muscle phenotype influences exogenous β HB oxidation rates, further work in a wider population of athletes is warranted to test this association. Lastly, stable isotope tracers provide an invaluable tool for determining *in vivo* substrate oxidation. However, findings must be interpreted within the context of the inherent assumptions that apply to this methodology (e.g., that the ^{13}C -labeled KME will behave identically to the nonlabeled KME).

CONCLUSIONS

We report that exogenous β HB oxidation contributes minimally to energy expenditure at rest and during exercise in highly trained athletes. Furthermore, we find that oxidation rate is not affected by doubling blood β HB from ~2 to ~4.4 mM. Although peak exogenous β HB oxidation occurs at a light-moderate exercise intensity (50% W_{Max}), the greatest relative contribution is during light exercise (25% W_{Max}). Finally, despite low β HB oxidation rates, exercise efficiency was improved when blood β HB concentration was elevated to ~2 mM versus control.

K. C. is director of TΔS Ltd, a spin-out company of the University of Oxford, which seeks to develop and commercialize products based on the ketone monoester. D. D. is a current employee of TΔS Ltd and O. H. a former employee.

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The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation and do not constitute endorsement by the American College of Sports Medicine.

D. D., O. H., and P. C. were involved in the experimental design and data collection. D. D. performed data analysis and was responsible for the manuscript preparation. L. H. and A. J. assisted with data analysis and manuscript preparation. K. C. was involved in the manuscript preparation and support for the study. All authors reviewed the manuscript.

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