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CURRENT OPINION

Exogenous Ketone Supplementation and Keto-Adaptation for Endurance Performance: Disentangling the Effects of Two Distinct Metabolic States

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Abstract
Ketone bodies (KB) provide an alternative energy source and uniquely modulate substrate metabolism during endurance exercise. Nutritional ketosis (blood KBs > 0.5 mM) can be achieved within minutes via exogenous ketone supplementation or days-to-weeks via conforming to a very low-carbohydrate, ketogenic diet (KD). In contrast to short-term (< 2 weeks) KD ingestion, chronic adherence (> 3 weeks) leads to a state of keto-adaptation. However, despite elevating blood KBs to similar concentrations, exogenous ketone supplementation and keto-adaptation are not similar metabolic states as they elicit diverse and distinct effects on substrate availability and metabolism during exercise; meaning that their influence on endurance exercise performance is different. In contrast to contemporary, high(er)-carbohydrate fuelling strategies, inducing nutritional ketosis is rarely ergogenic irrespective of origin and, in fact, can impair endurance performance. Nonetheless, exogenous ketone supplementation and keto-adaptation possess utility for select endurance events and individuals, thus warranting further research into their performance effects and potential strategies for their optimisation. It is critical, however, that future research considers the limitations of measuring blood KB concentrations and their utilisation, and assess the effect of nutritional ketosis on performance using exercise protocols reflective of real-world competition. Furthermore, to reliably assess the effects of keto-adaptation, rigorous dietary-training controls of sufficient duration should be prioritised.

1 Introduction

Endurance exercise requires high rates of sustained energy production, primarily via the oxidation of carbohydrate (CHO) and fat [1]; with exercise intensity positively associated with CHO utilisation [1]. Increasing blood ketone body (KB) concentration modulates substrate metabolism and oxidation during exercise, most notably by reducing CHO utilisation and free fatty-acid (FFA) availability, whilst providing a minor direct contribution of substrate into the tricarboxylic acid (TCA) cycle [2, 3]. Ketogenic diets (KD) and exogenous ketone supplements (EKS) increase blood KB concentration and may influence endurance performance; however, these two strategies are not equivalent as they exert two distinct metabolic states. Therefore, understanding how KD and EKS ingestion impact substrate metabolism is prudent to elucidate their application for endurance athletes.

2 Ketone Bodies and Nutritional Ketosis

Ketone bodies refers to acetoacetate (AcAc), acetone and β-hydroxybutyrate (βHB). However, only AcAc and acetone are ketones as they contain a carboxyl group with two hydrocarbons; βHB is technically a KB as the hydrocarbon atom is replaced by a hydroxyl group on the third carbon (Fig. 1). Compared with AcAc and βHB, acetone is largely excreted in the urine and breath and of negligible physiological importance during exercise [2]. Postprandial blood KB concentration is ~ 0.1–0.2 mM and varies depending on CHO availability [2]. Blood KB concentration > 0.5 mM is a commonly used threshold qualifying a state of nutritional ketosis or hyperketonaemia [4]; however, concentrations > 0.2 mM

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Key Points

Exogenous ketone supplementation and keto-adaptation are two distinct metabolic states characterised by diverse effects on substrate availability, metabolism and endurance performance.

During nutritional ketosis, the direct contribution of ketone bodies to exercising energy expenditure is likely minor, with ketone bodies instead exerting larger effects via modulation of carbohydrate and fat metabolism.

To date, inducing nutritional ketosis either via exogenous ketone supplementation or keto-adaptation does not appear to improve endurance performance beyond optimising CHO availability.

have also been proposed [2]. Ketone bodies can bypass the blood–brain barrier via passive diffusion [5] and enter extrahepatic tissues (e.g., brain, heart and skeletal muscle) via monocarboxylate transporters (MCTs) [6] to provide an alternative oxidative fuel source during periods of low CHO availability, such as starvation, fasting, prolonged exercise, or conformity to a ketogenic diet (KD) [2].

3 Ketogenic Diets and Endogenous Ketogenesis

Conforming to a KD increases blood KB concentrations to > 0.5 mM within days [2, 7] and can be sustained for weeks [7–9] to months [10, 11]. Ketogenesis occurs predominantly in hepatic mitochondria following β-oxidation of fatty acids to produce AcAc, the central KB in energy metabolism. The majority of AcAc is reduced to D-βHB, the primary circulating KB, thus shifting the blood AcAc:βHB ratio from 1:1 to–1:4 [2]. The ability for a fat-derived fuel to be used by the brain during periods of CHO insufficiency is critical to meet the brain's energy demand, thus negating the requirement for gluconeogenic protein catabolism [12, 13]. A KD is typically defined as < 50 g CHO day^{-1} or < 5% of energy intake (EI) from CHO, 15–20% of EI from protein and 75–80% of EI from fat [7–9, 14, 15]. The defining feature demarcating a KD from low(er)-CHO, high(er)-fat (LCHF) diets (~ 2.5 g CHO kg^{-1} day^{-1} or < 25% EI from CHO) is hyperketonaemia [4].

4 Defining Keto-Adaptation for Endurance Athletes

Keto-adaptation refers to the multi-organ and multi-system (physiological) adaptations exerted by ingesting a KD over several weeks-to-months [16]. There is no clear definition of what constitutes keto-adaptation or strategies for its optimisation, except adherence for at least 3–4 weeks and supplementation with sodium and potassium [17]. However, it is likely population-specific and exist on a continuum depending on the duration of dietary conformity. Studies investigating keto-adaptation in endurance athletes for at least 3 weeks demonstrate alterations to substrate availability and metabolism (Table 1), faecal [18] and oral [19] microbiome, and iron regulation [20, 21]; whereas, acid–base balance [22] and mucosal immunity [23] remain unaltered.

Depleted skeletal muscle [24] and hepatic [25] glycogen stores and an inability to maintain CHO oxidation rates [24, 26] may precede metabolic exhaustion during exercise. Therefore, the primary rationale for keto-adaptation is to reduce CHO-utilisation rates, whilst sustaining energy production, for a given exercise intensity. To achieve this, numerous adaptations occur that are synonymous with fat-adaptation (reviewed elsewhere [27–30] and are summarised in Table 1), which include: (1) increased fat oxidation [7–10]; (2) reduced blood glucose utilisation [8]; and 3) reduced skeletal muscle [8, 11] and hepatic [11] glycogen utilisation. Intermittent or continuous exposure to hyperketonaemia demarcates keto- from fat-adaptation; however, it remains uncertain—and contentious—what specific adaptations constitute keto-adaptation, their impact on endurance performance, and how these evolve with chronic KD ingestion (i.e., weeks vs. months vs. years). Furthermore, keto-adaptation is not a binary physiological state categorised by blood KB concentrations greater or less than 0.5 mM; rather, it is the ability to rapidly and efficiently increase ketogenesis and ketolysis relative to (lowering) CHO availability, whilst maintaining total substrate oxidation rates sufficient for energy requirements.

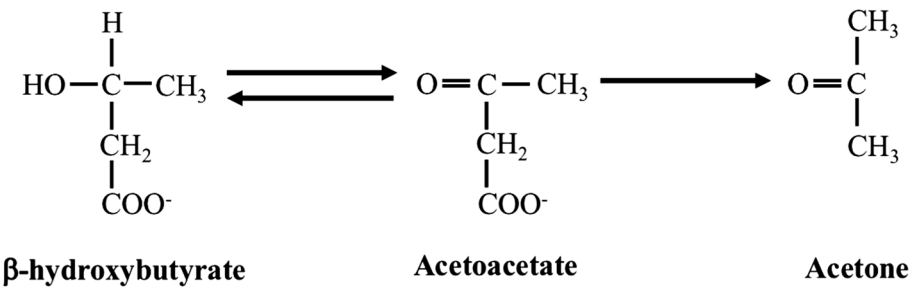

Fig. 1 Structure of ketone bodies

Table 1 Summary of metabolic responses during moderate-intensity (60–80% VO_{2max}) exercise between exogenous ketone supplementation and keto-adaptation (3-week minimum) compared with non-ketotic conditions

Metabolic variable	Exogenous ketone supplementation	Keto-adaptation
Indirect estimation of whole-body substrate oxidation	Indirect calorimetry is currently not validated to estimate CHO, fat or KB oxidation	CHO oxidation reduced by two-to-threefold [7–11]. Fat oxidation (including fatty-acid-derived KBs) increases two- to-threefold [7–11] Maximal reported fat oxidation rates of ~ 1.9 g min^{-1} at ~ 80% VO_{2max} in elite racewalkers [9] Indirect calorimetry is currently not validated to estimate KB oxidation
Direct measurement of KB oxidation	No evidence available	No evidence available
Blood FFA and glycerol concentration	Reduced blood FFA concentration following R-BD D-βHB monoester [55] and R,S-BD AcAc diester [53] ingestion; no evidence available for ketone salts or BD Reduced blood glycerol concentrations following R-BD D-βHB monoester ingestion [55]; no evidence available for R,S-BD AcAc diester, ketone salts or BD	Increased blood FFA [8, 10, 11] and glycerol [10, 11] concentrations
IMTG stores and/or utilisation	Increased IMTG utilisation following R-BD D-βHB monoester ingestion [55]; no evidence available for R,S-BD AcAc diester, ketone salts, or BD	No evidence available for keto-adaptation; however, IMTG stores appear to increase following ingestion of a high-fat diet [102]
Glycogen stores and/or utilisation	Reduced skeletal muscle glycogen utilisation following R-BD D-βHB monoester ingestion [55]; no evidence available for R,S-BD AcAc diester, ketone salts, or BD No evidence available for hepatic glycogen utilisation during exercise	Reduced skeletal muscle glycogen stores (~ 50%) and utilisation [8, 11]; however, a single study suggested no effect [10] No evidence available for hepatic glycogen stores; however, in most instances, depletion is obligatory to increase hepatic ketogenesis [2] Reduced hepatic glycogen utilisation [11]
Blood glucose concentration and utilisation	Blood glucose concentration reduced [55] or unchanged [92] following R-BD D-βHB monoester ingestion and reduced following R,S-BD AcAc diester ingestion [53]; no effect following ketone salt [37–39] or BD [44, 45] ingestion No evidence available for blood glucose utilisation	Blood glucose concentrations unchanged [7, 8, 10, 11], but may remain lower compared with CHO ingestion during exercise [9] Reduced blood glucose oxidation [8]
Blood lactate concentration	Blood lactate concentration reduced [55, 61] or unchanged [92] following R-BD D-βHB monoester ingestion and reduced following R,S-BD AcAc diester ingestion [53]; no effect following ketone salt [37–39] or BD [44, 45] ingestion	Blood lactate concentration increased [10, 11] or unchanged [7–9]
Acid-base balance	Reduced blood pH, increased H^+ ion and reduced HCO_3^- concentrations following R-BD D-βHB monoester ingestion [63]; no evidence available for R,S-BD AcAc diester, ketone salts, or BD	Blood pH, H^+ ion, and HCO_3^- concentrations unchanged [22]
Oxygen uptake and energy expenditure	Oxygen uptake unchanged [37–39, 44, 45, 53, 55, 92]	Oxygen uptake unchanged at exercise intensities < 65% VO_{2max} [7, 8]; increased oxygen uptake and energy expenditure at exercise intensities > 70%VO_{2max} [7, 9]
Heart rate	Heart rate unchanged [37–39, 44, 45, 53, 55, 92]	Increased heart rate [7, 9]

CHO carbohydrate, *KB* ketone body, *VO_{2max}* maximum oxygen uptake, *FFA* free fatty acid, *βHB* β-hydroxybutyrate, *BD* R,S-1,3-butanediol, *AcAc* acetoacetate, *IMTG* intramuscular triglyceride

The obligate duration of conforming to a KD to optimise keto-adaptation is uncertain. A minimum of 3–4 weeks appears necessary for performance [7–9]; however, whether ergogenic or ergolytic adaptations occur beyond this timeframe is unknown as studies of this duration either do not examine performance [10, 11], fail to rigorously monitor dietary intake and training load [14], or do not employ dietary standardisation prior to performance testing [15]. Therefore, to improve quantifying keto-adaptation in endurance athletes, the following variables should be reported: (1) dietary intake (refer to Mirtschin et al. [31] and Shaw et al. [7] for examples); (2) daily (morning) pre-exercise blood and/or urinary KB concentrations (to confirm dietary adherence); and (3) training load. Thereafter, cardiorespiratory parameters, ratings of perceived exertion (RPE), blood metabolites, and substrate oxidation during metabolic tests and/or performance tests can determine the utility of keto-adaptation and, potentially, time-course adaptations to KD ingestion. Noteworthy, steady-state whole-body carbohydrate and fat (not KB) oxidation can be calculated using indirect calorimetry without adjusting stoichiometric equations as fatty-acid-derived KBs are an intermediate metabolite of fat metabolism and contribute to total fat oxidation [32]—assuming insensible KB losses via the breath and urine and non-respiratory excretion of CO_2 are negligible, which may otherwise corrupt stoichiometric equations [33].

5 Exogenous Ketone Supplementation

Exogenous ketone and ketogenic supplements induce nutritional ketosis within minutes without necessitating CHO restriction (reviewed elsewhere [3, 34, 35]). Their influence on performance is an area of interest [36] as they can elicit unique metabolic responses during exercise (Table 1). Ketone salts (KB + sodium, potassium, calcium, or magnesium), typically sold as a racemic mixture, increase blood D-βHB to ~ 0.3–1 mM [37–41]; however, they provide an undesirable salt load that may result in cation overload, acidosis, and gastrointestinal distress [42, 43]. Similarly, the racemic R,S-1,3-butanaediol (BD) increases blood D-βHB to ~ 1 mM [44, 45]; however, as a ketogenic supplement, the resulting R-BD and S-BD moieties require subsequent conversion to the isotopic enantiomers, D-βHB and L-βHB [46]. BD metabolism is rate-limited by the hepatic enzymes alcohol dehydrogenase and aldehyde dehydrogenase [47–50] (Fig. 2); therefore, blood BD accumulates when ingested in quantities beyond enzymatic saturation [51].

Ketone esters include the R,S-BD AcAc diester [52, 53] and R-BD D-βHB monoester [54, 55]. In humans, R-BD D-βHB monoester ingestion has attracted the most attention, with studies investigating its safety and tolerability [43, 54, 56], and pharmacokinetics [40, 54, 57], as well as its regulatory effects on blood glucose [58], appetite [59], inflammation [60], endurance performance [55, 61], RPE [62], acid–base balance [63], recovery [64, 65], and overreaching [66]. R,S-BD AcAc esters and R-BD D-βHB monoester are catabolised by carboxylesterases and esterases predominantly situated in the gastrointestinal tract [67], with the liberated BD metabolised as previously described. R,S-BD AcAc diesters increase blood D-βHB concentration to similar concentrations as racemic ketone salts and BD, whereas there is a significantly greater rise in blood AcAc concentration (~ 0.4 mM) [53]. In contrast, the R-BD D-βHB monoester increases blood D-βHB concentrations to 2–5 mM [40, 55, 57], which is attenuated when coingested with food [40], indicating that the contents of the gut may affect the digestion and absorption of EKSs.

6 Chirality of Exogenous Ketone Supplements

Multiple pathways exist for the absorption and metabolism of EKSs, particularly due to chiral differences of βHB (R- and S- can be interchanged with D- and L-, respectively (Fig. 2). The D-βHB derived from R,S-BD metabolism is identical to D-βHB produced via endogenous ketogenesis, whereas L-βHB is a by-product of fat metabolism present in trace and low amounts in blood [68] and extrahepatic tissue [69], respectively. In rats, the administration of R-BD and S-BD contributed to 86–98% and 47–75% of total ketogenesis (D-βHB + AcAc), respectively, indicating that S-BD has a significantly lower rate of conversion to D-βHB. D-βHB is rapidly catabolised to acetyl-CoA and adenosine triphosphate (ATP) via the TCA cycle in peripheral tissues, whereas L-βHB does not enter the TCA cycle and is converted by the liver to FFAs and sterols or acetyl-CoA prior to D-βHB or CO_2 (see Fig. 2) [70, 71]. The slow conversion and excretion of L-βHB means that it can remain present in the blood for up to 24 h, increasing the potential of blood L-βHB accumulation [40]. Speculatively, lowering of the D-βHB/L-βHB ratio may negate the D-βHB-induced suppression of glucose metabolism in muscle, as previously demonstrated within cardiac tissue in rats [69], thus preserving CHO-oxidation rates and performance during high-intensity exercise. However, further research is required to investigate the interaction of D- and L-βHB on substrate metabolism in exercising humans and how this changes relative to CHO availability.

7 Quantifying Ketosis

Various methods measure biological KB concentrations [72]. Dipsticks qualitatively or semi-quantitatively measure the presence of urinary AcAc and are a cost-effective option to confirm conformity to a KD [73]; however, they are unable to detect D-βHB and have a low sensitivity to acute shifts in blood KB concentration [72]. Whereas,

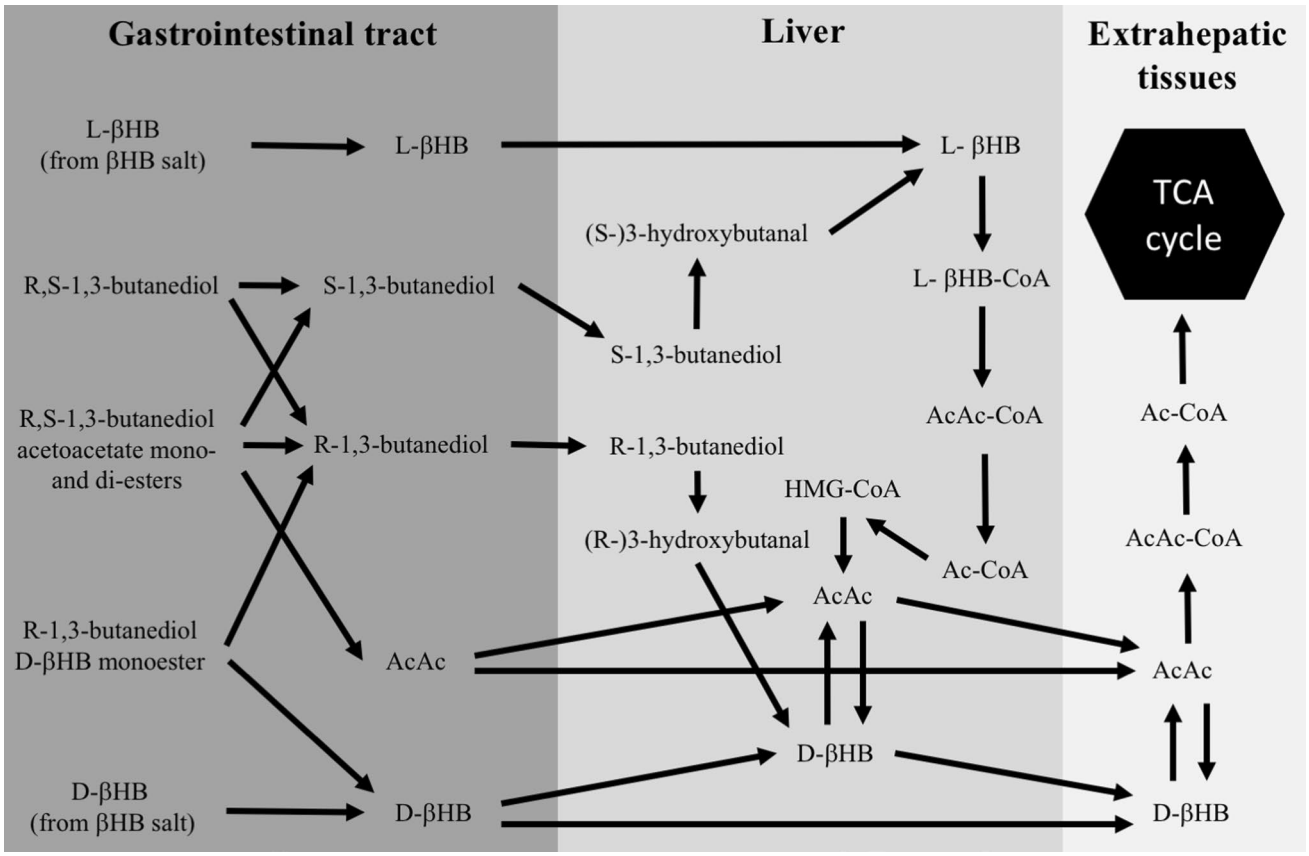

Fig. 2 A simplified schematic of the cleavage and major metabolic pathways of 1,3-butanediol-based ketone esters and ketone salts prior to oxidation in the tricarboxylic acid cycle within extrahepatic tissues. *βHB* β-hydroxybutyrate, *AcAc* acetoacetate, *HMG-CoA* 3-hydroxy-3-methylglutaryl-CoA, *AcAc-CoA* acetoacetyl-CoA, *Ac-CoA* acetyl-CoA, *TCA* tricarboxylic acid. Following oral ingestion, ketone esters and salts are cleaved and absorbed in the gut. R,S-1,3-butanediol is metabolised to R,S-3-hydroxybutanal prior to the ketone bodies acetoacetate, βHB, and acetone. Acetone is formed by the decarboxylation of AcAc and is not shown in the schematic as it does not contribute to energy production. Ketone bodies entering circulation are transported to extrahepatic tissues (e.g., skeletal muscle, kidney, brain, and heart), which enter via monocarboxylate transporters to be oxidised in the TCA cycle for energy production. Excretion of non-metabolised ketone bodies occurs via the faeces, exhalation by the lungs as acetone, or kidneys as AcAc and βHB

point-of-care, handheld monitors measure capillary whole-blood D-βHB concentration. Several point-of-care devices are available and widely used in healthcare settings for individuals with diabetes [72]. These devices specifically measure D-βHB, not L-βHB, by using D-βHB dehydrogenase coupled with electrochemical detection. However, compared with laboratory measures, point-of-care devices tend to have a higher coefficient of variation [74] and can overestimate blood D-βHB concentration by two-to-threefold [53, 75]; which makes it difficult to identify the optimal range of blood D-βHB concentration for endurance performance.

8 Limitations of Measuring Ketone Body Utilisation

The uptake and oxidation of KBs within skeletal muscle during exercise has been reviewed elsewhere [2, 3, 34]. Well-trained athletes have a greater capacity to oxidise KBs due to a higher abundance of MCTs [76] and ketolytic enzymes [77]. However, measuring KB oxidation is difficult as stoichiometric equations have only been validated for CHO and fat [78]. To estimate KB oxidation using indirect methods, further estimates are required for KB volume distribution values (i.e., total amount of KBs in the body divided by KB plasma concentration) and KB uptake into skeletal muscle [79]. For KB uptake, differences between the rates of KB appearance (i.e., the rate of hepatic ketogenesis during keto-adaptation or the rate of gastrointestinal absorption and/or BD conversion following exogneous ketone or ketogenic supplementation) and KB uptake must be known. Furthermore, not all KBs extracted from the blood are oxidised, as they can be stored in the form of D-3-hydroxybutyrylcarnitine (keto-carnitine), for which its role in energy production is uncertain [55, 80]. Insensible losses may also occur via the breath and urine [2].

A method proposed for estimating KB uptake into skeletal muscle following EKS ingestion uses incremental

area-under-the-curve of blood D-βHB concentration between resting and exercising conditions [55, 79]; however, during keto-adaptation, this strategy cannot be employed as rates of endogenous ketogenesis are unknown. In a study involving highly trained cyclists ingesting 0.573 g kg^{-1} of the R-BD D-βHB monoester, the contribution of D-βHB to energy expenditure was estimated to be 0.35 and 0.5 g min^{-1}, or 16 and 18% of oxygen uptake, at 45 and 75% W_{max}, respectively [55]. Blood D-βHB concentrations in the high-intensity trial were ~ 3 and 1 mM lower than the resting and low-intensity trials, respectively [55], demonstrating an exercise intensity-dependent effect on KB utilisation. Nevertheless, the accuracy of these calculations have not been confirmed using tracer techniques and are grossly higher than previous estimates using sodium AcAc infusion during moderate-intensity exercise [81]; therefore, indirect methods may overestimate KB oxidation and should be interpreted cautiously until validated.

9 Nutritional Ketosis and Endurance Performance

High-intensity (> 80%VO_{2max}) endurance performance up to ~ 3 h is CHO-dependent [82], but not necessarily limited by CHO availability [83]. Maximising energy production per unit of oxygen is important to performance; therefore, a shift towards fat oxidation may be ergolytic as the oxidation of fat compared with CHO results in a ~ 5% reduction in efficiency [84]. Whereas, at moderate intensities, depleting endogenous CHO availability can result in fatigue [85]; therefore, strategies to reduce the rate of endogenous CHO utilisation, whilst maintaining exercise intensity, are desirable. Ketone bodies downregulate skeletal muscle [55] and hepatic [86] glycogen utilisation and, as demonstrated in rodent skeletal and cardiac muscle models [87, 88], reduce pyruvate dehydrogenase complex (PDHc) activity; however, for the latter, evidence in humans following dietary intervention is currently unavailable. They also self-regulate their own production by suppressing adipocyte lipolysis [2], thus reducing FFA availability to exercising muscle. Ketone bodies may also be a more efficient fuel source than CHO and fat, as demonstrated in isolated rodent tissue models [89–91], with increased energy yield per C_2 unit [90] and greater Gibbs free energy of ATP hydrolysis [42]. Therefore, nutritional ketosis is frequently implicated in fuelling strategies for endurance performance; however, potential performance benefits and detriments exist at both high- and moderate-exercise intensities.

9.1 Exogenous Ketone Supplementation and High-Intensity Endurance Performance

No beneficial performance effects during high-intensity exercise have been demonstrated following the ingestion of EKSs that increase blood D-βHB concentrations up to ~ 1 mM. Racemic ketone salts [38, 39] and BD [44, 45] exert no effect on oxygen uptake, blood glucose concentration, lactate accumulation, or RPE at exercise intensities > 70% VO_{2max}, and demonstrate no [39, 44, 45] or negative [38] effects on performance (Table 2). Whereas, the racemic R,S-BD AcAc diester, in conjunction with recommended CHO fuelling strategies, was shown to increase capillary blood D-βHB concentrations to 0.8–1.3 mM (serum D-βHB ~ 0.4 mM) and serum AcAc concentration to ~ 0.5 mM during a ~ 50 min cycling time-trial (TT) [53]. Blood glucose, free fatty acid, and lactate concentrations were lower than placebo [81], which was potentially due to the higher increase in serum AcAc concentration compared to the ingestion of βHB salts or BD alone, due to their cleavage from BD and immediate entry into circulation. In this case, however, performance was significantly impaired by ~ 2% (~ 3.7% reduction in average power output [339 ± 37 vs. 352 ± 35 W]), which was attributed to severe gastrointestinal distress [53] (Table 2). Therefore, it seems EKSs attaining blood D-βHB concentrations up to ~ 1 mM should be avoided for high-intensity endurance performance, particularly if exercise duration does not deplete CHO availability.

In contrast, ingesting the non-racemic R-BD D-βHB monoester increases blood D-βHB concentration to ~ 1–2.5 mM during high-intensity exercise [55, 61, 92]. In response, blood glucose, FFA and lactate concentration, and skeletal muscle glycogen utilisation decline, and intramuscular triglyceride utilisation appear to increase (Table 1). The coingestion of R-BD D-βHB monoester and CHO after an overnight fast increased distance cycled by ~ 2% during a 30 min TT preceded by 60 min at 75% of the maximal power achieved during the incremental test [55] (Table 2). However, R-BD D-βHB monoester ingestion with CHO fuelling strategies during the prior 24 h and exercise has failed to alter high-intensity exercise capacity in a run-to-exhaustion protocol preceded by 75 min of high-intensity intermittent running [61] and a 10 km time-trial running performance preceded by 60 min at 65% VO_{2max} [92] (Table 2). Similarly, maximal power output did not change following R-BD D-βHB monoester ingestion compared with placebo prior to an incremental exercise test, despite increased leg discomfort and the anxiety of breathing and leg discomfort [62] and increased acid–base balance [63] (Table 2). Therefore, ingesting the R-BD D-βHB monoester may not be ergogenic during high-intensity exercise.

9.2 Keto-Adaptation and High-Intensity Endurance Performance

Keto-adaptation tends to impair high-intensity endurance performance (reviewed elsewhere [27, 30]). However, after keto-adaptation periods of > 2–3 months, results are

Table 2 Studies investigating the effects of exogenous ketone and ketogenic supplementation on endurance performance and capacity

References	Study design and participant characteristics	Exogneous ketone supplementation protocol	Dietary standardisation	Performance protocol	Blood KB concentration range (pre- to post-exercise)	Performance outcome[a]
Cox et al. (2016) [55]	Crossover design; 8 (6 M, 2 F) highly trained cyclists; VO_{2max} 5.37 ± 0.3 (M) and 3.30 ± 0.01 (F) L min^{-1} (sex-specific relative VO_{2max} and body mass not reported)	Two doses of 0.287 g kg^{-1} R-BD D-βHB monoester (40% E) and CHO (60% E) ingested at 30 min pre-exercise and pre-TT (vs. energy-matched CHO only placebo)	Overnight fast; replication of preceding night's meal (unspecified); caffeine avoidance 24 h pre-exercise	Cycling; distance achieved during 30 min TT preceded by 60 min at 75% W_{max}	Plasma D-βHB 1.7–2.5 mM (laboratory analysis)	↑ 2 [~ 1.7]% distance (~ 411 m further); $p < 0.05$; ES = 0.15
Rodger et al. (2017) [39]	Crossover design; 12 highly trained male cyclists; VO_{2peak} 68 ± 6.7 ml kg^{-1} min^{-1}	Two doses of 11.7 g D,L-βHB salt ingested at 20 min pre-exercise and 45 min during exercise (vs. placebo)	Replication of 48 h dietary intake (unspecified) pre-exercise; caffeine avoidance 24 h pre-exercise; fast 2.5 h pre-exercise	Cycling; average power output during a 4 min TT preceded by 90 min at 80% VT_2	Capillary blood D-βHB 0.3–0.6 mM (point-of-care device)	↑ 3 [~ 2.8]% $W_{average}$ (~ 9 W higher); $p = 0.38$; ES = 0.19
O'Malley et al. (2017) [38]	Crossover design; ten recreationally active males; VO_{2peak} 45 ± 10 ml kg^{-1} min^{-1}	Single dose of 0.3 g D,L-βHB kg^{-1} salt (~ 24.9 g) ingested at 30 min pre-exercise (vs. placebo)	Overnight fast; replication of 24 h dietary intake (unspecified)	Cycling; time to complete and average power output during a 150 kJ TT preceded by 5 min at 30, 60 and 90% VT_2	Capillary blood D-βHB 0.7–0.9 mM (point-of-care device)	↓ 7 [~ 5]% performance time (~ 46 sec slower); $p = 0.03$; ES = − 0.82 ↓ 7 [~ 5]% $W_{average}$ (~ 16 W higher); $p = 0.03$
Leckey et al. (2017) [53]	Crossover design; 11 internationally competitive male cyclists; VO_{2peak} 71.4 ± 5.6 ml kg^{-1} min^{-1}	Two doses of 0.25 g kg^{-1} R,S-BD AcAc diester ingested at 50 and 30 min pre-TT (vs. placebo) in conjunction with 250 ml 6% CHO sports drink mid-TT	High-CHO meal and snack (total of 3 g CHO kg^{-1}) the previous night; breakfast 2 h pre-TT (2 g CHO kg^{-1}); 200 mg caffeine 2 h pre-TT; 27 g CHO and 50 mg caffeine 5 min pre-TT	Cycling; time to complete and average power output during a 31.17 km simulated World Championship TT course	Serum D-βHB ~ 0.4 mM (laboratory analysis); serum AcAc ~ 0.5 mM (laboratory analysis); capillary blood D-βHB 0.8–1.3 mM (point-of-care device)	↓ 2% performance time (~ 1 min slower); $p < 0.001$; ES = − 0.42 ↓ 4% $W_{average}$ (~ 13 W lower); $p < 0.001$
Evans et al. (2018) [37]	Crossover design; 11 trained male team sport athletes; estimated VO_{2max} 53.9 ± 2.2 ml kg^{-1} min^{-1}	Three doses of R-BD D-βHB monoester (0.375, 188 and 188 g kg^{-1}) ingested at 20 min pre-exercise, 30 and 60 min during exercise, respectively (vs. placebo), in conjunction with 1.2 g min^{-1} CHO during exercise	~ 6 g CHO kg^{-1} previous day; two high-CHO meals (total of 6 g CHO kg^{-1}) ingested at least 3 h pre-exercise; caffeine avoidance 24 h pre-exercise	Running; TTE alternating between 20 m at 55 and 95%VO_{2max} preceded by 5 ×intermittent high-intensity exercise	Plasma D-βHB 1.0–2.6 mM (laboratory analysis)	↓ 14 [4.2]% duration (~ 39 sec shorter); $p = 0.13$; ES = − 0.45

Table 2 (continued)

References	Study design and participant characteristics	Exogneous ketone supplementation protocol	Dietary standardisation	Performance protocol	Blood KB concentration range (pre- to post-exercise)	Performance outcome[a]
Scott et al. (2018) [44]	Crossover design; 11 trained male runners; VO_{2peak} 64.2 ± 5.0 ml kg^{-1} min^{-1}	Three doses of BD (0.25, 0.125 and 0.125 g kg^{-1}) and 60 g CHO ingested at 30 min, immediately pre-exercise and pre-TT, respectively (vs. energy-matched CHO only placebo i.e., ~ 110 g CHO)	Overnight fast; replication of 48 h dietary intake (unspecified) pre-exercise	Running; time to complete a 5 km TT preceded by 60 min at 75%VO_{2peak}	Plasma D-βHB 0.8–1.0 mM (laboratory analysis)	↑ 0.3 [~ 0.2]% performance time (~ 4 s faster); $p = 0.723$; ES = 0.03
Shaw et al. (2019) [45]	Crossover design; 9 trained male cyclists; VO_{2max} 63.9 ± 2.5 ml kg^{-1} min^{-1}	Two doses of 0.35 g kg^{-1} BD ingested at 30 min pre-exercise and 30 min pre-TT (vs. placebo)	Overnight fast; 6 g CHO kg^{-1} day prior to trial; caffeine avoidance 24 h pre-exercise	Cycling; time to complete and average power output during a 7 kJ kg^{-1} TT preceded by 85 min at 85%VT_2	Capillary blood D-βHB 0.4–0.8 mM (point-of-care device)	↓ 1% performance time (~ 13 sec slower); $p = 0.62$; ES = − 0.06 ↓ 1% $W_{average}$ (~ 4 W lower); $p = 0.50$
Faull et al. (2019) [62] and Dearlove et al. (2019) [63]	Crossover design; 12 (9 male, 3 female) trained endurance athletes (rowing, cycling, running or swimming); VO_{2max} 56.6 ± 2.6 ml kg^{-1} min^{-1}	Single dose of 0.33 g kg^{-1} R-BD D-βHB monoester ingested at 30 min pre-exercise (vs. placebo)	Overnight fast; additional dietary requirements unspecified	Cycling; maximal power output during an incremental exercise test commencing at 100 W and increasing by 25 W every 3 min until exhaustion	Capillary blood D-βHB 2.0–3.8 mM (point-of-care device)	↑ 1 [~ 0.1]% W_{max} (~ 4 W higher); $p = 0.31$; ES = 0.04
Evans et al. (2019) [92]	Crossover design; 8 trained endurance runners; VO_{2max} 62.0 ± 5.6 ml kg^{-1} min^{-1}	Three doses of R-BD D-βHB monoester (0.287, 0.143 and 0.143 g kg^{-1}) ingested at 30 min pre-exercise, 20 and 60 min during exercise, respectively (v.s. placebo), in conjunction with 1 g min^{-1} CHO during exercise	~ 6 g CHO kg^{-1} previous day; breakfast 2 h pre-exercise (1 g CHO kg^{-1}); caffeine avoidance 24 h pre-exercise	Running; time to complete a 10 km TT preceded by 60 min at 65%VO_{2max}	Plasma blood D-βHB 1–1.3 mM (laboratory analysis)	↑ 1 [~ 0.1]% performance time (~ 20 sec faster); $p = 0.48$; ES = 0.08

M male, *F* female, VO_{2max} maximal oxygen uptake, *BD* R,S-1,3-butanediol, *βHB* β-hydroxybutyrate, *E* energy, *TT* time-trial, *TTE* time-to-exhaustion, *CHO* carbohydrate, $W_{average}$ average power output, W_{max} maximal power output, *kJ* kilojoules, VT_2 second ventilatory threshold, VO_{2peak} peak oxygen uptake, *AcAc* acetoacetate, *ES* Cohen's d mean effect size (either calculated or obtained from publication), ↑ increased or ↓ decreased endurance/performance of exogenous ketone supplements versus control

[a][] Adjusted performance effects to average power output in time-trial as described in Braakhuis and Hopkins (2015) [103]

equivocal, as both negative [93] and no effects [15] were reported in recreational endurance athletes; albiet there is no published research in well-trained or elite athletes. The primary concern is lowering of CHO availability and oxidation via suppression of glycolysis and PDHc activity, which can manifest during metabolic testing as reduced respiratory exchange ratio (RER) at VO_{2max} (i.e. < 1.0) [7–9]. Keto-adaptation also increases energy expenditure and oxygen uptake beyond what can be explained by reductions in RER from pre- to post-adaptation (i.e., accounted oxygen uptake) when exercising at > 70% VO_{2max} [7]; suggesting increased mitochondrial uncoupling. In concordance, 31-day keto-adaptation reduced running speed at VO_{2max} in trained runners [7] and 3 weeks of keto-adaptation during intensified training in elite race walkers negated improvements in a 10-km TT performance compared with a high-CHO diet [9]. Therefore, increased fat and KB oxidation does not seem to compensate for attenuated carbohydrate metabolism following keto-adaptation, thus appearing to compromise high-intensity endurance performance.

9.3 Exogenous Ketone Supplementation and Prolonged Moderate-Intensity Endurance Performance

Exogenous ketone supplementation has been tentatively linked with performance effects for endurance competition lasting several hours [94, 95]; however, no studies have examined their effect. Despite the potential for increased IMTG utilisation following R-BD D-βHB monoester ingestion [55], IMTG stores are depleted by ~ 50–70% during the initial 2–3 h of (fasted) exercise, which places greater reliance on adipose tissue lipolysis to maintain fat oxidation [96–99]. Since ketone ester ingestion suppresses adipose tissue lipolysis [53, 55], an important fuel source for prolonged events [100], CHO-oxidation rates could increase, thus accelerating fatigue. Moreover, endurance competition lasting several hours is highly influenced by gastrointestinal symptoms, which can be exacerbated by exogenous ketone supplementation [42, 45, 53, 61], thus preventing sufficient CHO and energy intake. Clearly, further research investigating the effects of EKSs on prolonged, moderate-intensity endurance performance is required.

9.4 Keto-Adaptation and Prolonged Moderate-Intensity Endurance Performance

Keto-adaptation may be ergogenic for prolonged, moderate-intensity endurance events limited by endogenous CHO availability; however, only a single study has investigated the effect of keto-adaptation on endurance performance or capacity lasting > 3 h [7] (Table 3). Arguably, the ergogenic effect of keto-adaptation may be greatest when CHO ingestion is restricted, potentially due to limited fuelling opportunities or in individuals who cannot tolerate CHO ingestion during competition [4, 30]. Following several weeks-to-months of ingesting a KD, fat oxidation (including fatty-acid-derived KBs) can contribute to > 70% of energy expenditure at 70–80% VO_{2max} [9, 11] and up to ~ 90% at 65% VO_{2max} [10], with rates invariably > 1 g min^{-1} and extending up to ~ 1.9 g min^{-1} [9]. However, when exercising ≥ 2 h without CHO ingestion, the rate of ketogenesis likely exceeds ketolysis as blood D-βHB concentrations rise > 1.5 mM [7, 9–11]. It is uncertain if increases in endogenous KB production result in corresponding direct increases in KB oxidation or, more likely, greater modulation of endogenous substrate metabolism, such as via effects on adipose tissue lipolysis, glycolysis, and/or PDHc activity.

In a seminal study, 4 weeks of keto-adaptation preserved mean time-to-exhaustion (TTE) at 62–64% VO_{2max} in five trained cyclists [8] (Table 3). However, the study design favoured keto-adaptation due to: (1) a single-arm design; (2) failure to implement performance nutrition strategies in the CHO trial; and (3) a large improvement in a single participant in the post- (keto-adapted) trial, thus distorting the results. A recent study aimed to address these limitations by investigating 31 days of keto-adaptation in eight trained runners using a randomised, repeated-measures, counter-balanced, crossover design [7] (Table 3). Mean TTE was preserved at 70% VO_{2max}, despite an increase in the rate of exercising energy expenditure [7], suggesting impaired exercise efficiency. Therefore, the ergogenic benefits of elevated fat and KB oxidation and the regulatory role of increased blood KB concentration on substrate metabolism are unlikely to outweigh high-CHO fuelling strategies; however, future research examining keto-adaptation, particularly for exercise durations > 4–6 h and in conjunction CHO ingestion before and/or during exercise, remains warranted.

10 Is There an Optimal Blood Ketone Body Concentration for Endurance Performance?

Optimal implies an ergogenic effect of KBs either via their regulation of or contribution to substrate provision for energy production; however, there is currently no clear benefit of nutritional ketosis for endurance performance following either exogenous ketone supplementation (Table 1) or keto-adaptation (Table 2). Published studies also contain small sample sizes (~ 8–12) and lack rigor to identify positive and negative responders due to the underlying noise of measurement error [101]. Blood KB concentrations also reflect the rates of ketogenesis (or KB appearance following EKS ingestion) and KB uptake by peripheral tissues, thus making interpretation difficult. Considering blood KB concentrations during keto-adaptation are metabolically

Table 3 Studies investigating the effects of chronic (3-week minimum) keto-adaptation on endurance performance and capacity

References	Study design and participant characteristics	Keto-adaptation protocol	Monitoring dietary compliance	Performance protocol and acute fuelling strategies	Blood KB range concentration (pre- to post-exercise)	Performance outcome[a]
Phinney et al. (1983) [8]	Single-arm, crossover design (order effect i.e., CHO-trial first); 5 trained male cyclists; VO_{2max} ~ 69.0 ml kg^{-1} min^{-1}	28 days; ~ 2% EI CHO and ~ 85% EI fat; normal training	Clinical ward study with all meals provided, allowing participants 1 meal away from the clinic per day; twice daily measurement of urinary acetone; twice weekly measurement of plasma D-βHB; daily weight	Cycling; TTE at 62-64%$VO_{2max;}$ overnight fast	Plasma D-βHB 1.28-1.45 mM (laboratory analysis)	↑ 3 [~ 0.2]% duration (~ 4 min longer); $p = 0.89^{\#}$; ES = 0.09
Burke et al. (2016) [9]	Unbalanced design (crossover of some participants); 21 elite male race walkers (n = 9 high-CHO, n = 8 periodised-CHO, n = 9 KD); average pre-diet VO_{2peak} for all groups ~ 64.2 ml kg^{-1} min^{-1}	21 days; ~ 4% EI CHO and ~ 78% EI fat; intensified supervised training	Training camp study with all meals provided; dietary intake recorded by registered dietitians (however, designated recording frequency unspecified); no monitoring of urinary or blood KB concentration during adaptation period	Racewalking; time to complete a 10 km TT with individualised (unspecified) pre-exercise fuelling strategies according to dietary allocation (i.e., fat fed in keto-adapted trial and CHO fed in CHO trial)	Capillary blood D-βHB 0.3-0.7 mM (point-of-care device)	↓ 2% performance time (~ 23 sec slower) for KD; $p > 0.05$; ES = 0.03; ES = -0.42 for mean pre–post change between KD and periodised-CHO diet ↑ 7% performance time (~ 190 sec faster) for high-CHO diet; $p < 0.01$ ↑ 5% performance time (~ 124 sec faster) for periodised-CHO diet; $p < 0.01$
Zinn et al. (2017) [93]	Single-arm, crossover design (order effect i.e., CHO-trial first); 5 recreationally trained athletes (1 M runner, 1F runner, 3 F cyclists); VO_{2peak} 44–54 ml kg^{-1} min^{-1} (visually extrapolated from results)	10 weeks; ~ 5% EI CHO and 66% EI fat; normal training	Free-living athletes; weekly dietary reporting; dietary counselling by registered dietitian only following participant-reported diet nonconformity; single weighed 1-day diet record in week 5 reported in results; capillary blood D-βHB concentration, albeit designated testing frequency unspecified	Cycling; TTE during an incremental exercise test commencing at 30 W and increasing by 30 W every 3 min	Not reported	↓ ~ 8 [~ 0.5]% duration (percentage change visually extrapolated from results; ~ 2 min shorter) for KD; $p = 0.004$; ES = − 0.53

Table 3 (continued)

References	Study design and participant characteristics	Keto-adaptation protocol	Monitoring dietary compliance	Performance protocol and acute fuelling strategies	Blood KB range concentration (pre- to post-exercise)	Performance outcome[a]
McSwiney et al. (2018) [14]	Parallel design; 20 trained male athletes (multiple endurance sports); average pre-diet VO_{2max} for both groups ~ 53.1 ml kg^{-1} min^{-1}	12 weeks; ~ 5% EI CHO and ~ 77% EI fat; normal training	Free-living athletes; weekly dietary counselling by researcher (qualification unspecified); single weighed 3-day diet record in week 12; no monitoring of urinary or blood KB concentration during adaptation period	Cycling; time to complete 100 km TT with individualised (unspecified) pre-exercise fuelling strategies according to dietary allocation; during exercise CHO 0 g h^{-1} in keto-adapted trial and 30-60 g h^{-1} in CHO trials	Fasting plasma D-βHB 0.5 ± 0.4 mM (laboratory analysis); pre- and post-TT circulating KB concentrations not measured	↓ 2% performance time for KD (~ 247 sec slower); ES = 0.13 ↑ 1% performance time for higher-CHO diet (~ 73 sec faster); $p = 0.057$ No difference between diets for mean pre-post change; $p = 0.057$; ES = − 0.20
Shaw et al. (2019) [7]	Crossover design; 8 trained male runners; VO_{2max} 59.4 ± 5.2 ml kg^{-1} min^{-1}	31 days; ~ 4% EI CHO and ~ 78% EI fat; normal training	Free-living athletes; compulsory daily contact and counselling with a registered dietitian; image-assisted, weighed diet record for 2 days in weeks 1, 2 and 3 and 5 days in week 4; daily urinary AcAc; day 3 and weekly capillary blood D-βHB	Running; TTE at 70% VO_{2max} (3-strike method) with pre-exercise fuelling strategies in preceding days according to dietary allocation; high-CHO (2 g CHO) or iso-energetic, high-fat (< 10 g CHO) breakfast ~ 2 h pre-trial; during exercise CHO 0 g h^{-1} in keto-adapted trial and ~ 55 g h^{-1} in CHO trials	Capillary blood D-βHB 0.6-1.7 mM (point-of-care device)	↓ 8% duration (~ 20 min shorter) for KD; $p = 0.36$; ES = -0.64 ↓ 3% duration (~ 6 min shorter) for higher-CHO diet; $p = 0.44$ No difference between diets for mean pre-post change; $p = 0.56$; ES = − 0.25

Table 3 (continued)

References	Study design and participant characteristics	Keto-adaptation protocol	Monitoring dietary compliance	Performance protocol and acute fuelling strategies	Blood KB range concentration (pre- to post-exercise)	Performance outcome[a]
Dostal et al. (2019) [15]	Parallel design; 30 recreationally active males; average pre-diet VO_{2max} for both groups ~ 45.1 ml kg^{-1} min^{-1}	12 weeks; ~ 8% EI CHO and 67% EI fat; 3–5 training sessions per week, including 2 supervised high-intensity sessions	Free-living individuals; open access to dietary counselling (qualification unspecified); daily dietary record using application (compliance unspecified); weekly monitoring of blood KB concentration	Running; TTE during an incremental exercise test commencing at 7 km h^{-1} and increasing by 1.5 km h^{-1} every 4 min; and TTE during a high-intensity intermittent exercise test consisting of 30 sec shuttles commencing at 8 km h^{-1} and increase by 0.5 km h^{-1} every 45 sec separated by 15 sec passive recovery; pre-exercise dietary standardisation unspecified	Pre- and post-TTE circulating KB concentrations not measured	Incremental exercise test ↑ 7 [~ 0.5]% duration (82 sec longer) for KD; $p = 0.005$; ES = 1.03 ↑ 7% duration (69 sec longer) for higher-CHO diet; $p = 0.018$ No difference between diets for mean pre-post change; $p = 0.53$; ES = 0.27 High-intensity intermittent exercise test: ↑ 18 [~ 1.2]% duration (158 sec longer) for KD; $p = 0.001$; ES = 1.93 ↑ 14 [~ 0.9]% duration (117 sec longer) for higher-CHO diet; $p = 0.001$; ES = 1.50

CHO carbohydrate, VO_{2max} maximal oxygen uptake, *EI* energy intake, *βHB* β-hydroxybutyrate, *KD* ketogenic diet, VO_{2peak} peak oxygen uptake, *KB* ketone body, *TT* time-trial, *TTE* time-to-exhaustion, W_{max} maximum power output, *ES* Cohen's d mean effect size (either calculated or obtained from publication), ↑ increased or ↓ decreased endurance/performance of post- versus pre-diet

[#] p value calculated using individual values reported within the study

[a] [] Adjusted performance effects to average power output in time-trial as described in Braakhuis and Hopkins (2015) [103]

regulated depending on energy demand and CHO availability [2], it may not be possible to identify an optimal range for endurance performance. In contrast, exogenous ketone supplementation can acutely manipulate blood KB concentration, which has prompted suggestion of an optimal blood D-βHB range of 1–3 mM [3]; however, 1 mM appears too low (Table 2). Therefore, for EKSs, further research exploring specific dosing strategies should consider the following: (1) blood KB concentration measurement technique; (2) blood D-βHB/L-βHB/AcAc concentration and ratio; (3) substrate availability and metabolism (e.g., fasted vs. fed vs. acute CHO fuelling strategies); (4) previous exposure to hyperketonaemia; (5) athlete training status; (6) physiological demands of competition; and (7) tolerability.

11 Conclusion

Exogenous ketone supplementation and keto-adaptation induce nutritional ketosis; however, they are not similar physiological states. Underlying alterations to substrate availability and metabolism differentiate the ergogenic potential for each and despite decades of research, it is only recently that their performance effects within ecologically valid contexts are being examined. Therefore, current recommendations to induce nutritional ketosis for endurance performance are unsubstantiated, but are an exciting area of future research.

Compliance with Ethical Standards

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