Exogenous Ketone Bodies and Endurance Exercise Performance: Is it Worth the Hype?

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KETONE MONOESTERS AND PRECURSORS DO NOT ENHANCE ENDURANCE EXERCISE PERFORMANCE: A SYSTEMATIC REVIEW AND META-ANALYSIS

Abstract

There has been much consideration over whether exogenous ketone bodies have the capacity to enhance exercise performance through altered substrate metabolism. This systematic review aimed to determine the effects of both ketone precursors and monoesters on endurance exercise performance. A systematic search was conducted in PubMed, SPORTDiscus, and CINAHL for randomized controlled trials investigating endurance performance outcomes in response to ingestion of a ketone supplement compared to a nutritive or non-nutritive control in humans. A meta-analysis was performed to determine the standardized mean difference between interventions using a random-effects model. Hedges' g and 95% confidence intervals (CI) were reported. The search yielded 569 articles, of which 8 were included in this review (80 participants; 77 men, 3 women). When comparing endurance performance amongst all studies, no significant differences were found between ketone and control trials (Hedges *g*=0.136; 95% CI, -0.195, 0.467; p=0.419). Sub-analyses based on type of endurance tests showed no significant differences in time to exhaustion (Hedges g=-0.002; 95% CI, -0.312, 0.308; p=0.989) or time trial (Hedges g=0.057; 95% CI, -0.282, 0.395; p=0.744) values. Based on these findings, exogenous ketone precursors and monoesters do not significantly improve endurance exercise performance. While all studies reported an increase in blood ketone concentrations after ingestion, ketone monoesters appear to be more effective at raising concentrations than precursors.

Keywords: exogenous ketones, ketosis, ß-hydroxybutyrate

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CHO: Carbohydrate

KB: Ketone Body

KD: Ketogenic Diet

KS: Ketone Salt

KE: Ketone Ester

KME: Ketone Monoester

GI: Gastrointestinal

AcAc: Acetoacetate

MCT: Monocarboxylic Acid Transporter

ßHB: Beta-hydroxybutyrate

TT: Time Trial

MCR: Metabolic Clearance Rate

Chapter 1: Introduction

There is a continuous pursuit for training, recovery, and nutritional strategies that effectively enhance physical performance to thus gain an advantage in exercise or sport. Nutritional approaches traditionally focus on intake before, during, and after training/competition in an attempt to optimize substrate metabolism so that individuals may achieve optimal performance. This is particularly useful for endurance or ultra-endurance athletes as well as intermittent or team sport athletes where endogenous fuel stores, and more precisely carbohydrates (CHO) may become a limiting factor due to the prolonged length of exercise (Cermak and van Loon, 2013). It is known that during endurance-type exercise, the body is fuelled primarily by glucose and fat; thus, research has mainly focussed on optimizing this ratio of macronutrients using techniques such as CHO loading or CHO feeding (Cermak and van Loon, 2013; Coyle *et al*., 1983). These strategies have become widely accepted as effective in slowing muscle glycogen depletion and delaying the onset of fatigue during prolonged exercise (Cermak and van Loon, 2013; Jeukendrup, 2004; Bergstrom and Hultman, 1967).

More recently, ketone bodies (KB) have generated considerable interest for performance enhancement. Interestingly, they have also been recently recognized as potential therapeutic agents in treating epilepsy, Alzheimer's, and Parkinson's disease (Hashim and VanItallie, 2014). While garnering much positive attention, it is also known that KBs are toxic in high amounts. A build-up of these metabolites can lead to acidosis, which most often manifests in individuals with diabetes as diabetic ketoacidosis. Nonetheless, KBs, which are generated in the liver, have the capacity to act as an alternative energy source for most extra-hepatic tissues, including the brain, under circumstances such as prolonged fasting or starvation (Laffel, 2000; Robinson and

Williamson, 1980). KBs have thus been proposed as an alternative fuel for the body when CHO is otherwise unavailable (Laffel, 2000).

Given their ability to act as a substrate, much research has been conducted to determine the efficacy of KBs for fuelling exercise. Initially, studies exposed participants to the ketogenic diet (KD) to assess the effects of endogenous KBs only (Zinn *et al*., 2017; Burke *et al.*, 2017; Shaw *et al.*, 2019). In this particular case, the diet consists of extremely low CHO intake and contrastingly high fat consumption, which accordingly leads to an increase in the production of KBs (Westman *et al.*, 2007). This strategy has been studied in both strength-based athletes (Vargas-Molina, *et al.*, 2020; Urbain *et al*., 2017; Kephart *et al.*, 2018) and endurance athletes (Zinn *et al.*, 2017; Burke *et al.*, 2017; Shaw *et al.*, 2019) with results showing that endogenous production of KBs through the implementation of a KD does not result in improved physical performance. In fact, it may even be detrimental to performance, specifically for endurance activities. This is likely due to the severe restriction of CHO intake causing reduced liver and muscle glycogen stores which are ultimately required to sustain performance at the high intensities that are of the utmost importance in competitive endurance settings (Burke *et al*., 2017). Moreover, KDs can be difficult to adhere to long-term and with the overwhelming evidence pointing to the necessity for CHO, it appears that KD-induced KB production is not adequate for optimal endurance performance.

These findings have led to the development of KB supplements. These supplements include ketone salts (KS), ketone precursors, and ketones esters (KE), which are further divided into ketone monoesters (KME) and ketone diesters. All types allow for research in individuals without compromising CHO stores, unlike the KD (Clarke *et al.*, 2012). The use of supplements has been a valuable research tool for examining the effects of KBs on exercise performance

because participants can reach higher blood KB levels without having to adhere to a specific diet that may cause unfavourable changes to energy stores.

Research Problem

While this area of inquiry is still relatively new, there is a steadily growing body of literature accumulating on exogenous ketones and endurance performance. A systematic review of the current literature would be beneficial to determine the trend of outcomes across studies as well as inform future directions of research in this area. Previous systematic reviews have assessed the effects of all forms of exogenous KBs on endurance performance (Margolis and O'Fallon, 2020; Valenzuela *et al.*, 2020). However, results show that ketone diesters and KS are less effective at raising KB concentrations in the blood and may also cause more gastrointestinal (GI) distress (Stubbs *et al.*, 2017; Evans *et al*., 2016; Leckey *et al*., 2017). Therefore, a more specific and detailed review focussing only on ketone precursors and KME that take into account more recent studies published utilizing KME would be valuable to the field. Previous reviews have also lacked attention to other aspects of performance such as KB dose, concentration, GI effects, and time of administration. We aim to perform a systematic review on the effects of ketone precursors and KME on endurance performance. In addition, factors such as KB dose, concentration, and GI effects will also be investigated.

Chapter 2: Literature Review

Substrate Use During Exercise

During endurance exercise activities, CHO and fat are the two primary sources of fuel oxidized by skeletal muscle tissue (Cermak and van Loon, 2013). As exercise intensity increases, the contribution of CHO to overall fuel oxidation becomes greater (Romijn *et al*., 1993; Jeukendrup and Jentjens, 2000). Specifically, during prolonged moderate to high intensity exercise (>65% VO_{2max}), CHO stored as muscle glycogen becomes the most important substrate source by contributing more than 50% of the total energy requirements (Cermak and van Loon, 2013). Therefore, endogenous glycogen availability is an important factor because when stores become low or depleted during exercise it leads to fatigue and performance impairments (Cermak and van Loon, 2013; Jeukendrup and Jentjens, 2000). Providing CHO during endurance exercise has been extensively studied and generally accepted to be beneficial for performance in endurance athletes (Coyle *et al*., 1983; Coggan and Coyle, 1985). Coyle *et al*. (1983) demonstrated this by examining the effect of CHO feeding during an exercise test at 74% VO_{2max} in endurance cyclists. On average, exercise time to fatigue was 23 minutes longer in participants who consumed CHO 20 minutes into the test than those who received a placebo. Coggan and Coyle (1985) further showed that cyclists who ingested CHO immediately following an exercise session to fatigue were able to continue cycling straightaway for another 26 ± 4 minutes compared to 10 ± 1 minutes with a placebo. In short, CHO provision can lead to increased glucose oxidation rates and may improve overall endurance capacity by preserving endogenous glycogen. However, benefits of ingesting CHO during exercise are limited by the rate of glucose absorption, which remains maximally at around 1.0 g/min as shown through isotopic labelling of CHO (Wagenmakers *et al*., 1993; Rehrer *et al*., 1992). Studies by Romijn *et al*. (1993) and van

Loon *et al*. (2001) show that at higher exercise intensities (75-85%), CHO oxidation, or utilization, can increase to between 3 and 4 g/min, thereby overshadowing absorption by a ratio of approximately 3 to 1 (Cermak and van Loon, 2013). Therefore, ingesting CHO at a higher rate than what can be absorbed will likely have no further favourable effects. In fact, it can even be detrimental to performance as large amounts of CHO may cause digestive disturbances such as cramping and/or nausea (Romijn *et al*., 1993). Given the high rates of utilization and the limited rate of absorption, finding alternative fuel sources that could help spare glycogen during exercise thus becomes physiologically attractive.

Ketone Bodies

Ketogenesis is the production of KBs that occurs primarily in the liver as a result of beta-oxidation (Robinson and Williamson, 1980). Fatty acids are broken down into acetyl-CoA within the mitochondria of liver cells and converted to the KB, acetoacetate (AcAc), through a series of enzymatic reactions (Cox and Clarke, 2014). As previously stated, KBs are a unique metabolite in that, unlike free-fatty acids, they can be oxidized by most tissues including the brain (Robinson and Williamson, 1980; Cox and Clarke, 2014). As water-soluble molecules, they circulate freely in the blood and cross cell membranes into various tissues via monocarboxylic acid transporters (MCT) (Evans *et al*., 2016; Bouteldja *et al*., 2014). Importantly, they have the capacity to act as an alternative energy source while simultaneously sparing CHO reserves (Cox and Clarke, 2014). KBs include ß-hydroxybutyrate (ßHB), AcAc, and acetone, though changes in ßHB concentration are most indicative of overall plasma KB concentration (Harvey *et al*., 2019), which typically resides around 0.1 mM (Robinson and Williamson, 1980). Certain physiological or pathological conditions, however, can cause a rise in KB concentration. This

heightened level of KB is considered a state of ketosis or hyperketonaemia, which is characterized by plasma KB concentrations that exceeds 0.2 mM (Robinson and Williamson, 1980). Physiologically this may accompany periods of fasting, prolonged exercise, or low-CHO diets where plasma KB concentrations can reach 1-5 mM (Robinson and Williamson, 1980; Cox and Clarke, 2014). However, KBs can also be dangerous in high amounts due to their acidic nature. Excessive accumulation of KBs can cause a shift in blood pH below the highly regulated normal limits of 7.35-7.45 leading to metabolic acidosis which, if left untreated, can result in severe neurological or cardiac complications or even death (Mitchell *et al*., 1995).

Studies of the pharmacokinetics of KB metabolism have primarily utilized isotope labelled AcAc and/or ßHB tracers to investigate KB dynamics in fasted or non-fasted individuals at rest, however rates of appearance (R_a), disappearance (R_d), clearance, production and/or utilization are inconsistently reported across studies. For example, Avogaro *et al*. (1990) used labelled AcAc and R-ßHB simultaneously and found a mean KB production rate of 206 ± 57 μmol/min/1.73m^2 along with a mean plasma clearance rate for AcAc and R-ßHB of 1996 ± 502 ml/min/1.73m^2 and 1443 ± 683 ml/min/1.73m^2, respectively. In another study by Beylot *et al*. (1986), total KB R_a using labelled AcAc ranged from 2.52 to 4.50 μmol/kg/min and 2.18 to 2.76 μmol/kg/min using labelled D-ßHB. Miles *et al*. (1983) report mean total KB production values of 1.5 to 2.2 μmol/kg/min, Fery and Balasse (1983), 3.46 μmol/kg/min, and Keller *et al*. (1981), 6 μmol/kg/min. Radioactive labelling of AcAc by Reichard *et al*. (1974) revealed AcAc and total KB oxidation rates were 98% of the production rates after participants fasted for 2-3 days. After 17-24 days of fasting, the mean maximum total KB production and oxidation rates were about 11 μmol/kg/min (150g/24h) and 9 μmol/kg/min (129g/24h), respectively. Ketonuria accounted for an additional 14g/24h leading to a difference between production and removal of about 7g/24h

(Reichard *et al*., 1974). Fery and Balasse (1985) found a maximal R_d of about 2.3 mmol/min/1.73m^2, which was attained at KB concentrations of 10-12 mM, or the equivalent to levels reached during prolonged fasting. They also determined an inverse relationship between the metabolic clearance rate (MCR) and plasma KB levels, further supporting the general finding that rates of appearance or production of KBs seem to exceed rates of disappearance or uptake. Hyperketonaemia resulting from fasting is primarily caused by an increased production of KBs, however this effect becomes amplified by a gradual limitation in the ability of tissues to remove KBs from the blood as concentrations rise (Fery and Balasse, 1985). The mechanisms behind this are, to our knowledge, still unknown however it is not unreasonable to assume this translates to exogenous ingestion of KBs as well. There are hypotheses that the impaired peripheral utilization of KBs helps to preferentially direct them to the brain (Balasse, 1979). Regardless, more tracer studies are required in individuals engaging in exercise of various intensities to determine maximal rates of absorption and utilization. If it is true that there is a maximum rate of utilization by peripheral tissues such as skeletal muscle, it would be interesting to compare to absorption rates as this may indicate limitations similar to what is seen in CHO absorption and oxidation.

Ketogenic Diet

As previously stated, a method of endogenously increasing levels of ketones is by adopting specific diet regimens such as the KD, which induces ketosis through an extended period of very low consumption of CHO and a contrastingly high fat intake (Westman *et al*., 2007). There are claims that KDs can improve physical performance in endurance exercise activities (McSwiney *et al*., 2018; Zajac *et al*., 2014). For example, Zajac *et al*. (2014) found an increase in relative VO_{2max} from 56.02 mL/kg/min to 59.40 mL/kg/min in experienced off-road

cyclists following a four-week KD intervention. However, there are also several studies that indicate an impairment in endurance exercise performance amongst participants adopting a KD (Zinn *et al*., 2017; Burke *et al*., 2017; Shaw *et al*., 2019). Zinn *et al*. (2017) found a mean decrease of 2 ± 0.7 minutes in time to exhaustion during an incremental cycle test of endurance athletes after a 10-week KD. Furthermore, Burke *et al*. (2017) studied the effects of a 3-week KD in professional race walkers and found significant increases in absolute oxygen cost, heart rate, and RPE at race speeds that translate to an overall reduced exercise economy. In other words, exercise at the same velocity measured prior to the KD elicited an increased energy demand. The increased energy demand was paired with a slower mean time (+23 s) for completion of a 10 km race walk compared to faster times in both the high CHO (-190 s) and periodized CHO (-124 s) groups (Burke *et al*., 2017). Therefore, it was determined that KDs are not an effective method for enhancing endurance exercise performance. These discoveries are likely what led to the development of KB supplements, or exogenous ketones.

Exogenous Ketones

As previously described, exogenous ketones have been a novel development for research on KBs and exercise performance. Important to note is the differences between the various forms: KME, KS, ketone diesters, and ketone precursors. Ketone esters are predominantly formulated as ßHB monoesters, given that ßHB is the most commonly occurring KB (Harvey *et al*., 2019). This monoester combines ßHB, an acid, with hydroxybutyl, an alcohol, and is easily broken down and absorbed in the small intestine and then further metabolized in the liver to provide two molecules of ßHB (Sivva *et al*., 2016). It is therefore very effective at elevating ketone concentrations in the blood. Research has shown that participants consuming this KME

can reach a 50% higher concentration than when consuming KS that contain equivalent amounts of ßHB (Stubbs *et al*., 2017). These salts are composed of KBs, again typically ßHB, bound to either sodium, potassium, or calcium salts (Stubbs *et al*., 2017; Evans *et al*., 2016). They often contain both *D*-ßHB and *L*-ßHB isoforms, the latter of which are less readily oxidized and therefore may not be as effective at increasing ketone concentrations (Stubbs *et al*., 2018). Given their composition, they are also more likely to cause GI distress, and potentially cation overload or acidosis if ingested in large quantities (Stubbs *et al*., 2017; Evans *et al*., 2016). Additionally, a lesser-known ketone diester combines a different KB, AcAc, with butanediol to deliver two AcAc molecules and one molecule of racemic ßHB once ingested (Stubbs *et al*., 2018). Studies examining AcAc diesters are scarce in the literature, likely because they are currently far less palatable and have lower GI tolerability than both the ßHB monoesters and KS (Leckey *et al*., 2017). There are a few select studies that utilize a ßHB precursor known as 1,3-butanediol. Similar to KME, 1,3-butanediol produces one molecule of ßHB and is easily absorbed. Previous systematic reviews included studies using all forms of exogenous ketones and determined largely null and even ergolytic effects in those using KS or ketone diesters (Margolis and O'Fallon, 2020; Valenzuela *et al*., 2020). For example, Leckey *et al*. (2017) discovered a 2 ± 1% impairment in 31 km time trial performance in participants consuming a ketone diester compared to placebo. Likewise, time to complete a 150-kJ time trial following 15 minutes of submaximal exercise was ~8% longer in participants consuming KS compared to control in a study by O'Malley *et al*. (2017). In contrast, one study from a series of experiments by Cox *et al*. (2016) demonstrated a 2% increase in distance travelled during a 30-minute time trial in participants consuming a combination of KME and CHO compared to an isocaloric CHO drink. In all of these studies, ingestion of the ketone supplement led to a suppression in the rise of plasma

glucose, free fatty acids, and lactate levels, indicating a possible reduction in glycolysis. It is unclear whether this suppression truly represents an impairment in glycolysis that could affect essential energy availability during endurance exercise. Regardless, all participants who consumed the ketone diester reported GI discomfort which could have contributed to impairments in performance (Leckey *et al*., 2017). As well, studies using KS often report lower peak ketone concentrations (~1-1.2 mM) than those using KME (~3 mM), which could also explain a lack of effect seen in participants consuming this type of exogenous KB.

Below is a schematic showing the pathway of the KME (R)-3-hydroxybutyl (R)-3-hydroxybutyrate once ingested. **Figure 1** also depicts the pathway of the ketone precursor 1,3-butanediol, which is a by-product of KME metabolism. To our knowledge, there is currently no data showing the exact absorption rates of KBs.

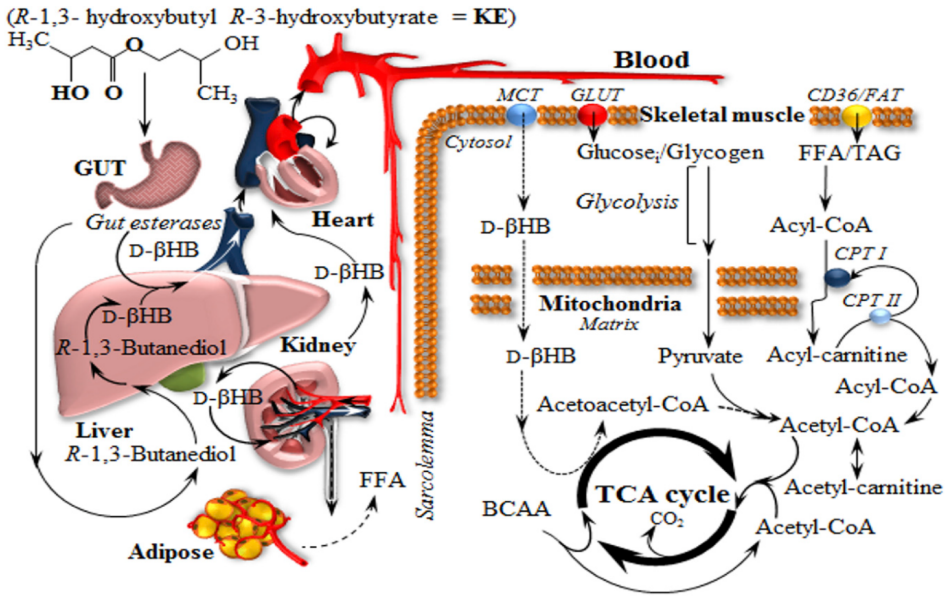

Figure 1: Pathway of ketone monoester (R)-1,3-hydroxybutyl (R)-3-hydroxybutyrate. Adopted from Cox *et al*. (2016). Ketone ester (KE) is hydrolyzed in the gut into *D*-ß-hydroxybutyrate and (R)-1,3-butanediol. Both are absorbed into portal circulation with (R)-1,3-butanediol undergoing first-pass metabolism in the liver to form *D*-ß-hydroxybutyrate. *D*-ß-hydroxybutyrate circulates in the blood and is transported into skeletal muscle mitochondria via monocarboxylate transporters (MCTs). Glucose is transported via GLUTs and free fatty acids by CD36/FAT

transporters. Within the mitochondria, all substrates are metabolized to acetyl-CoA and oxidized in the TCA cycle.

While the type of exogenous ketone is an important factor, there are other aspects that also need to be considered. For example, ketone dose, time of administration, the exercise intensity and duration, as well as effects on GI function are just some of these elements that may also impact performance. Previous reviews have indicated that it is challenging to make strong conclusions on the effects of exogenous ketones on performance due to the wide range of variability in methodologies across studies (Margolis and O'Fallon, 2020; Valenzuela *et al*., 2020). The following sections will further discuss these considerations.

Ketone Dose

In order to effectively assess whether KBs impact performance, it is necessary that the amount consumed is adequate to sufficiently raise plasma concentrations. Initially, it was unclear what this target concentration should be; likely higher than day-to-day levels of 0.1-0.4 mM but not so high that one risks the dangerous effects of ketoacidosis (Hashim and VanItallie, 2014; Laffel, 2000). In the past, ketoacidosis has been defined as KB levels in excess of 3 mM, however it is now known that levels can increase to 6-8 mM in individuals fasting for a prolonged period of time without causing harmful effects (Hashim and VanItallie, 2014; Laffel, 2000). As KB research has evolved and exogenous KBs have been added to the mix, the term "therapeutic ketosis" has emerged to describe the achievement of KB levels in the range of 2-7 mM, or comparable to those present when undergoing a fast or KD (Hashim and VanItallie, 2014). This has led to a recognized threshold of 2 mM and it is hypothesized that this

concentration needs to be reached or surpassed to realize any ergogenic potential of exogenous ketones (Clarke *et al*., 2012; Cox *et al*., 2016; Stubbs *et al*., 2018; Hashim and VanItallie, 2014).

Across the literature, ketone doses have ranged from approximately 300 mg/kg in studies using KS, to 500-700 mg/kg for those using ketone diesters, precursors, and some monoesters, and up to around 900 mg/kg in other studies using KME (O'Malley *et al*., 2017; Cox *et al*., 2016; Rodger *et al*., 2017; Waldman *et al*., 2018; Evans and Egan, 2018; Scott *et al*., 2018; Evans *et al*., 2019; Shaw *et al*., 2019; Poffé *et al*., 2020a; Poffé *et al*., 2020b; Poffé *et al*., 2020c). Margolis & O'Fallon (2020) found a significant relationship between dose and resulting blood or plasma concentration amongst studies using KS, precursors, diesters, and monoesters, however only 3 of the 10 studies included successfully surpassed the 2 mM threshold, all of which were studies using KME. While higher ketone doses may result in higher concentrations, tolerability must also be considered. KME tolerability was tested in individuals consuming 3 separate single doses of 140, 357, and 714 mg/kg and no adverse events were reported, suggesting good tolerability at these ranges (Clarke *et al*., 2012). The maximum concentrations achieved were 0.28, 1.00, and 3.30 mM, respectively. However, in this case the participants were at rest. The addition of exercise impacts tolerability as well as resulting concentrations because of increased KB oxidation. While there is currently no universally recognized optimal dose, the goal appears to be finding a balance between achieving therapeutic ketosis while maintaining overall tolerability in individuals ingesting the ketones.

Time of Administration

It is essential that exogenous ketones are administered at a dose and time that optimizes the resulting blood ßHB concentration throughout the prescribed exercise, so that they are

available for ATP production if and when needed. It is also important to consider time of administration in relation to pre-exercise nutrition, other supplements such as CHO, and water intake to avoid GI distress. With longer duration exercise and larger doses of ketones, the majority of studies have divided the overall dose into smaller boluses (Cox *et al*., 2016; Evans and Egan, 2018; Scott *et al*., 2018; Evans *et al*., 2019; Shaw *et al*., 2019; Poffé *et al*., 2020a; Poffé *et al*., 2020b; Poffé *et al*., 2020c). This typically allows for sustained levels of ßHB concentrations throughout exercise while also imposing less of a load on the GI tract. All studies administering KME or precursors did so in either 2 or 3 boluses, with the first being consumed as early as one hour before beginning exercise or as late as 20 minutes prior (Cox *et al*., 2016; Evans and Egan, 2018; Scott *et al*., 2018; Evans *et al*., 2019; Shaw *et al*., 2019; Poffé *et al*., 2020a; Poffé *et al*., 2020b; Poffé *et al*., 2020c). Studies that saw the most success with a sustained rise in ßHB throughout the entire exercise session were Evans *et al*. (2019), Scott *et al*. (2018) and Poffé *et al*. (2020c). It should be noted that Scott *et al*. (2018), while demonstrating a sustained increase, only reached a peak of ~1 mM. This low concentration may be due to a lower dose of only 500 mg/kg or the possibility that the butanediol ketone precursor is less effective at raising blood ßHB concentrations. Evans *et al*. (2019) also only reached a peak of ~1.33 mM, though this could also be attributed to a lower dose (573 mg/kg) combined with a fed state of participants, as it has been shown that consumption of KBs immediately following a meal diminishes the resulting peak ßHB concentration (Stubbs *et al*., 2015). Poffé *et al*. (2020a; 2020b) both demonstrated successful increases in ßHB concentrations for the first half of their 180-minute intermittent exercise session, however concentrations fell below 2 mM for the final 120-200 minutes. These studies implemented some of the longest exercise sessions with the highest KME doses. It appears to be effective to split the dose up into several boluses and to

provide two doses in the hour leading up to the exercise session so that concentrations are well above the 2 mM threshold as activity commences. Cox *et al*. (2016) provided only one dose 20 minutes prior to exercise and ßHB concentrations dropped immediately upon commencement of the session, which is not ideal when the maintenance of higher levels throughout is desired. If participants in Poffé *et al*. (2020a; 2020b) had received one more bolus of KE at the 120-minute mark, this may have been enough to sustain concentrations above the 2 mM threshold for the entirety of the session. In both of these studies, participants were fed 2 hours prior to exercise and the first dose was provided 1 hour after the meal. Collectively, these data illustrate that a sustained increase in blood ßHB concentration for endurance exercise in a fed state is possible with a higher dose of KE, providing an adequate amount leading up to the exercise session, and ensuring boluses are administered in the second half of longer endurance sessions to maintain levels above the 2mM threshold. However, it is still unclear whether successfully maintaining these levels throughout an endurance exercise session leads to enhanced performance.

Exercise Test & Duration

There are further two other important considerations when investigating the effect of exogenous ketones on performance. First, at which intensity of exercise do they purportedly exert the most effects? Second, what duration of exercise is optimal to possibly observe the effects of KBs on performance? Research has suggested that athletically trained individuals may be more efficient at utilizing KBs both during and immediately after exercise (Fery and Balasse, 1983; Winder *et al*., 1974; Winder *et al*., 1975). It has been shown that exercise training leads to a higher expression of MCT transporters, which are responsible for the uptake of KBs into skeletal muscle (Evans *et al*., 2016; Fery and Balasse, 1983). Furthermore, a significantly smaller

post-exercise ketosis effect has been demonstrated in trained individuals, suggesting that they are able to oxidize KBs during exercise more effectively (Evans *et al*., 2016; Fery and Balasse, 1983). There are rodent studies suggesting that proportion of muscle fibre type also plays a role in KB uptake (Winder *et al*., 1974; Winder *et al*., 1975). Ketolytic enzyme activity as well as MCT transporter expression in rats appears to be highest in type I muscle fibres compared to intermediate and low levels found in type IIA and IIB, respectively (Winder *et al*., 1974; Winder *et al*., 1975). If these animal model results are transferrable to humans, it would suggest that KB uptake and utilization are likely highest in trained individuals with a high proportion of oxidative muscle fibers.

Exercise intensity was demonstrated to have an influence on KB metabolism by Cox *et al*. (2016) in their experiment consisting of 45 minutes of exercise at either 40% or 75% of W_{max} following 573 mg/kg KE plus CHO ingestion. They found that ßHB concentration was ~2 mM and ~3 mM lower than ketosis induced at rest at 40% and 75% $W_{max,}$ respectively, indicating that KB oxidation is intensity-dependent during exercise. They further estimated that ketone oxidation accounted for 16-18% of total oxygen consumption during the exercise session (Cox *et al*., 2016). However, in a recent study by Dearlove *et al*. (2021), it was shown that ketone oxidation contributed minimally (~4.46%) to overall energy expenditure during incremental intensity exercise. Participants consumed 400 of a 600 mL drink containing either 252 mg/kg (low-dose) or 752 mg/kg (high-dose) KE 1-hour prior to exercise, and then 100 mL at t+20 and t+40 minutes. The exercise session was 60 minutes in total (0-20 mins = 25% W_{max}, 20-40 mins = 50% W_{max}, and 40-60 mins = 75% W_{max}). Interestingly, they found that the high dose of KE, while effectively doubling blood ßHB concentrations (~2 mM vs. ~4.4 mM), did not significantly increase ßHB oxidation rates compared to the low dose (Dearlove *et al*., 2021).

Additionally, ßHB oxidation peaked in both the high-dose and low-dose during the 50% W_{max} exercise intensity. Dearlove *et al*. (2021) postulate that previous reports of ketone oxidation rates were overestimated due to the use of adjusted substrate oxidation equations from measured gaseous exchanges rather than whole body tracers. They further attribute the consistent oxidation rates despite higher circulating ßHB concentration to the metabolic flexibility of skeletal muscle, proposing that reliance will be on the oxidation of lipids in order to preserve ketone oxidation for cerebral metabolism. Ketone oxidation in skeletal muscle may increase between ~5 fold and ~11 fold during exercise compared to at rest, suggesting that it is not a matter of limited ketone transport or ketolytic capacity, but rather a tight regulation on skeletal muscle ketone oxidation in order to reserve energy for the brain (Balasse *et al*., 1978; Mikkelsen *et al*., 2015). Dearlove *et al*. (2021) also found a significant positive association between the percentage of type I fibers in *vastus lateralis* muscle biopsies and peak ßHB oxidation rate (r^2=0.696, p=0.039), further building upon the research by Winder *et al*. (1974,1975) which suggests that KB uptake may be optimal in endurance trained individuals. More research is required into total body ketone oxidation during exercise of different intensities. It may also be worthwhile to investigate how ketone oxidation may differ in keto-adapted individuals, or those who have chronically consumed exogenous ketones.

Cox *et al*. (2016), and Poffé *et al*. (2020b) also investigated KBs and muscle metabolism during exercise. Cox *et al*. (2016) found significantly higher intramuscular glucose and significantly lower glycolytic intermediates in the KE group (573 mg/kg) compared to a CHO or FAT group following 1 hour of cycling at 75% of W_{max}. Contrastingly, Poffé *et al*. (2020b) found no difference in muscle glycogen content between the KE (918 ± 102 mg/kg) and control group following 3 hours of intermittent cycling and a 15-minute TT. There are a few

factors that may be contributing to these alternate findings, including that the participants in the Cox *et al*. (2016) trial were in a fasted state. Also, importantly, the exercise session was only 60 minutes, compared to 195 minutes in the Poffé *et al*. (2020b) trial. In another one of the experiments by Cox *et al*. (2016), *vastus lateralis* muscle biopsies revealed changes in muscle metabolism and substrate utilization during one hour of steady state exercise at 75% W_{max}. It was shown that after KE ingestion, glycolytic intermediates including pyruvate, were decreased, suggesting that ketosis effectively suppressed skeletal muscle glycolysis despite a workload intensity that would normally be primarily glycolytic (Cox *et al*., 2016). This led to hypotheses surrounding exogenous KBs and whether they may induce a glycogen sparing effect during exercise. Theoretically, exercise may be sustained longer or with higher intensity when KB and CHO combined are utilized as fuel. Thus, exercise of longer duration (2-3+ hours) appears to be ideal for testing the efficacy of ketone supplementation. Very few studies have implemented exercise duration within this range thus far (Poffé *et al*., 2020a; Poffé *et al*., 2020b). Future studies should aim for a sufficient test of endurance with exercise >2 hours. Further intramuscular sampling of glycogen and glycolytic intermediates throughout exercise would also provide more knowledge on KB metabolism and its impact on performance.

Gastrointestinal Function

With any potential ergogenic aid supplied orally, there should be consideration for its effects on GI function. Consumption of any substance during moderate to high intensity exercise comes with the possibility of causing GI distress such as bloating, cramps, nausea, or vomiting. CHO are generally well tolerated by individuals, although there are many factors that contribute to tolerability, such as dose, substance form (i.e., liquid, solid, gel, etc.), time of administration,

and palatability. Recommendations for CHO intake is based on its oxidation rate, which is approximately 1.0-1.1 g/min for CHO at low to moderate exercise intensities, but can increase slightly with higher ingestion rates and/or exercise intensities (Cermak and van Loon, 2013; Jeukendrup and Jentjens, 2000; Wagenmakers *et al*., 1983; Rehrer *et al*., 1992). Ingestion of multiple transportable CHO have been shown to further increase oxidation rates by 20 to 50% (Cermak and van Loon, 2013). These findings are yet to be detailed for the use of exogenous ketones. Research shows that KME may be more palatable and well tolerated than KS or ketone diesters (Stubbs *et al*., 2017; Evans *et al*., 2016; Leckey *et al*., 2017), however optimal dose is still uncertain, as are oxidation rates of the different ketone forms. For example, in a study by Leckey *et al*. (2017) using ketone diesters, all 11 participants reported GI discomfort ranging from nausea to dry retching and vomiting, which appeared to negatively affect performance in the 31 km TT (KET; 2 ± 1 %, 58.2 s) compared to the control trial as no similar symptoms were reported after intake of the placebo. However, Poffé *et al*. (2020b) reported only a one-point difference in Likert scale rating for systemic, lower, and upper abdominal discomfort between ketone and control trials in participants consuming KME. Other studies utilizing KME or ketone precursors show mixed results of higher rates of GI symptoms in ketone trials in some, and no difference between trials in others (Evans and Egan, 2018; Scott *et al*., 2018; Evans *et al*., 2019; Shaw *et al*., 2019; Poffé *et al*., 2020a; Poffé *et al*., 2020c). Similar to guidelines for CHO consumption before or during exercise, it is likely that personal preference will play a role as not all individuals have the same intake tolerance. It also requires proper practice and may be geared towards well-trained athletes who are used to ingesting larger amounts of CHO during prolonged exercise at a higher workload (Cermak and van Loon, 2013; Jeukendrup and Jentjens, 2000).

Overall, more research is required to determine current trends in GI tolerance across studies as well as establish more specific guidelines for KB consumption.

Summary

Performance enhancement is coveted by those in the world of exercise and sport. Current knowledge on nutritional strategies to improve performance is centred around substrate partitioning and the use of CHO and fat as fuel during exercise. There is an indication that CHO feeding can aid in sustaining endurance exercise, however, there are limits to CHO use in that it has a maximum absorption rate that is considerably lower than the rate of utilization during moderate to high intensity exercise (Wagenmakers *et al*., 1993; Rehrer *et al*., 1992). This has led to inquiry surrounding exogenous KBs as a fuel for endurance exercise. Previous studies have shown positive, null, and negative results with the use of various forms of exogenous KBs with no clear conclusion on their effects (Leckey *et al*., 2017; O'Malley *et al*., 2017; Cox *et al*., 2016; Rodger *et al*., 2017; Waldman *et al*., 2018; Evans and Egan, 2018; Scott *et al*., 2018; Evans *et al*., 2019; Shaw *et al*., 2019; Poffé *et al*., 2020a; Poffé *et al*., 2020b; Poffé *et al*., 2020c). The literature also lacks data showing the maximal rate of absorption and oxidation of exogenous ketones at rest and during exercise of various intensities. As with other ergogenic aids, there are many other factors to consider, such as dose, time of administration, duration and type of exercise, and GI effects. A review of the effects on endurance performance as well as attention to these factors would be beneficial for better understanding whether exogenous ketones have ergogenic potential and determining directions for future research.

Objectives and Hypotheses

Primary Objective

I. To determine whether exogenous ketone monoesters or precursors enhance endurance exercise performance (time trial/time to exhaustion) based on the current literature.

Secondary Objectives

I. To compare gastrointestinal effects between the ketone and control trials.

II. To assess the relationship between ketone dose and resulting circulating ßHB concentration.

Hypotheses

I. The current literature will show that neither ketone monoesters nor precursors exert a positive effect on endurance performance.

II. Gastrointestinal effects will not differ between ketone and control trials.

III. Higher ketone doses will result in higher ßHB concentrations.

Chapter 3: Methodology and Results

To avoid redundancy, the methodology and results sections are presented in the form of a paper, entitled: “Ketone Monoesters and Precursors Do Not Enhance Endurance Exercise Performance: A Systematic Review and Meta-Analysis”. The paper will be submitted for publication.

Acute Ingestion of Ketone Monoesters and Precursors Do Not Enhance Endurance Exercise Performance: A Systematic Review and Meta-Analysis

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KETONE MONOESTERS AND PRECURSORS DO NOT ENHANCE ENDURANCE EXERCISE PERFORMANCE: A SYSTEMATIC REVIEW AND META-ANALYSIS

Abstract

There has been much consideration over whether exogenous ketone bodies have the capacity to enhance exercise performance through altered substrate metabolism. This systematic review aimed to determine the effects of both ketone precursors and monoesters on endurance exercise performance. A systematic search was conducted in PubMed, SPORTDiscus, and CINAHL for randomized controlled trials investigating endurance performance outcomes in response to ingestion of a ketone supplement compared to a nutritive or non-nutritive control in humans. A meta-analysis was performed to determine the standardized mean difference between interventions using a random effects model. Hedges' g and 95% confidence intervals (CI) were reported. The search yielded 569 articles, of which 8 were included in this review (80 participants; 77 men, 3 women). When comparing endurance performance amongst all studies, no significant differences were found between ketone and control trials (Hedges *g*=0.136; 95% CI, -0.195, 0.467; p=0.419). Sub-analyses based on type of endurance tests showed no significant differences in time to exhaustion (Hedges g=-0.002; 95% CI, -0.312, 0.308; p=0.989) or time trial (Hedges g=0.057; 95% CI, -0.282, 0.395; p=0.744) values. Based on these findings, exogenous ketone precursors and monoesters do not exert significant improvements on endurance exercise performance. While all studies reported an increase in blood ketone concentrations after ingestion, ketone monoesters appear to be more effective at raising concentrations than precursors.

Keywords: exogenous ketones, ketosis, ß-hydroxybutyrate

Introduction

When it comes to exercise in a competitive or professional setting, research has focused on developing nutritional strategies that optimize fuel selection during training and competition, thereby allowing for optimal or even enhanced performance. The majority of these strategies revolve around the availability of carbohydrates (CHO), knowing that this is the primary energy source during high-intensity exercise and is required to sustain longer duration or endurance exercise of higher intensities (Cermak and van Loon, 2013; Romijn *et al*., 1993). Reductions in CHO availability (i.e. muscle and liver glycogen stores) are associated with fatigue and performance impairment. As such, an approach to spare endogenous CHO reserves is physiologically attractive (Cermak and van Loon, 2013; Jeukendrup and Jentjens, 2000). Examples of popular nutritional strategies include CHO loading and CHO feeding. However, given the limited storage and rate of absorption of CHO, one might ask whether another method may be a more efficient alternative (Coyle *et al*., 1993; Coggan and Coyle, 1985).

Ketone bodies (KB) have recently sparked interest as one such alternative to spare CHO. Produced predominantly in the liver through the process of ketogenesis, KBs (beta-hydroxybutyrate [ßHB], acetoacetate [AcAc], and acetone) are capable of acting as a substrate for most tissues including the brain, heart, and muscle when glucose is otherwise unavailable (Robinson and Williamson, 1980). Ketogenesis occurs more frequently under physiological conditions such as periods of fasting, prolonged exercise, or when adopting a very low-carbohydrate (ketogenic) diet (Robinson and Williamson, 1980; Cox and Clarke, 2014). Under all of these circumstances, KBs become a primary energy source as glucose is restricted or depleted. Ketosis, or hyperketonaemia, is achieved when plasma ketone concentrations exceed 0.2 mM, or higher than normal circulating levels of around 0.1 mM (Robinson and Williamson,

1980). Initially, research in this field concentrated on endogenous KB production by implementing the ketogenic diet and subsequently assessing exercise performance (Zajac *et al*., 2014; Zinn *et al*., 2017; Shaw *et al*., 2019; Harvey *et al*., 2019). It was quickly determined that reduced or depleted CHO stores, often observed in those adopting the ketogenic diet, are not beneficial to performance, despite the presence of KBs as an alternative energy source (Burke *et al*., 2017). In fact, it has shown to be detrimental to endurance exercise performance during which CHO availability becomes a limiting factor (Harvey *et al*., 2019).

Accordingly, the latest development of exogenous forms of KBs has given researchers the ability to investigate their effects without compromising CHO reserves (Clarke *et al*., 2012). In other words, they allow for the inclusion of glycogen replete individuals, which would otherwise not be possible with ketogenic diet-induced ketosis. There have since been claims that exogenous ketone metabolism is preferential over both CHO and fat by effectively suppressing glycolysis during exercise workloads that typically favour CHO oxidation (Cox *et al*., 2016). However, it is unclear whether this effect allows for a preservation of CHO stores to be utilized at a later time or instead creates an impairment of CHO oxidation during exercise. Given the increase in research on exogenous KBs, a systematic review would provide a comprehensive overview of observed effects compared to nutritive or non-nutritive control options.

It is important to note that these exogenous ketones come in various forms: ketone monoesters (KME), ketone diesters, ketone salts (KS), or ketone precursors. Ketone esters are predominantly formulated as ßHB monoesters, given that ßHB is the most commonly occurring KB (Harvey *et al*., 2019; Laffel, 2000). This monoester combines the acid ßHB, with an alcohol hydroxybutyl, to produce a compound that is easily broken down and absorbed in the small intestine and then further metabolized in the liver to provide two molecules of ßHB (Sivva *et al*.,

2016). It is, therefore, very effective at elevating ketone concentrations in the blood. Research has shown that participants consuming this KME can reach a 50% higher circulating concentration than when consuming KS that contain equivalent amounts of ßHB (Stubbs *et al*., 2017). These salts are composed of KBs, typically ßHB, bound to either sodium, potassium, or calcium salts (Stubbs *et al*., 2017; Evans *et al*., 2016). They often contain both *D*-ßHB and *L*-ßHB isoforms, the latter of which are less readily oxidized and therefore may not be as effective at increasing ketone concentrations (Stubbs *et al*., 2018). Given their composition, they are also more likely to cause gastrointestinal (GI) distress and even cation overload or acidosis in large quantities (Stubbs *et al*., 2017; Evans *et al*., 2016). Additionally, a lesser-known ketone diester combines a different KB, AcAc, with butanediol to deliver two AcAc molecules and one molecule of racemic ßHB once ingested (Stubbs *et al*., 2018). AcAc diesters have not been used in many studies, likely because they are currently far less palatable and have lower GI tolerability than both the ßHB monoesters and KS (Leckey *et al*., 2017). There are further select few studies that utilize a ßHB precursor known as 1,3-butanediol. This precursor produces one molecule of ßHB and is easily absorbed, similar to KME. Previous systematic reviews included studies using all forms of exogenous ketones and identified that the majority of studies using KS or ketone diesters had null or even ergolytic effects (Margolis and O'Fallon, 2020; Valenzuela *et al*., 2020). For the above-mentioned reasons, studies utilizing KS and diesters will be excluded from this review, making this the first systematic review of its kind to focus solely on the effects of ketone precursors and monoesters on endurance performance.

In theory, KBs are a practical alternative energy source with the potential to spare CHO reserves when consumed in an exogenous form. What is unknown is whether this novel development is truly worthwhile for use in endurance exercise endeavours. As such, this

systematic review will aim to determine whether exogenous KME or precursors have the capacity to enhance endurance exercise performance, specifically does consumption result in faster time trials or extended time to exhaustion. Secondary outcomes include assessing: 1-GI effects, 2-the relationship between ketone dose and resulting blood concentration, and 3-the relationship between ketone concentration and changes in performance.

Methods

Search Strategy

This systematic review was completed in accordance with PRISMA guidelines (Page *et al*., 2021) and was registered on PROSPERO (CRD42020218915). The literature search took place between November 2020 and May 2021 and included three different databases: PubMed, SPORTDiscus, and CINAHL. A search strategy was developed with the key terms 'humans', 'ketosis', and 'exercise performance' (See **Table 1**). Retrieved studies were first screened for inclusion by evaluating titles and abstracts by two independent reviewers. A third reviewer was consulted if there were any unresolved discrepancies. Next, studies were assessed in their full text by two independent reviewers for final inclusion in the review. There were no restrictions on publication date or language. Reference lists from the relevant publications were manually searched for any articles missed by the database searches.

Inclusion Criteria

Studies were eligible for inclusion in the review if they were randomized crossover or parallel controlled trials evaluating the effects of exogenous ketones on endurance exercise performance in humans. They also required a nutritive or non-nutritive control containing no

ketones. Only ketones in the form of either KME or ketone precursors that were ingested prior to and/or during the exercise session rather than postexercise (for recovery) or chronically (over the course of more than one day) were included. There were no restrictions on the ketone dose, study duration, sample size, or participant age, sex, body mass, or training status.

Exclusion Criteria

Studies were excluded from the analysis if they used animal models or ketogenic diets rather than exogenous ketones as the intervention. Studies using ketone diesters or KS were also excluded for reasons previously covered. Other reviews, commentaries, and editorial articles were not included.

Data Extraction

Data were manually extracted from the included studies onto a standardized excel spreadsheet. Sex, age, body weight, training status, and VO_{2max} were obtained from each study to provide descriptive characteristics of the participants. Performance test outcomes from the experimental and control trials were isolated for the meta-analyses. The type and dose of ketone supplement along with the peak blood concentration during the exercise session were recorded for regression analysis. The remaining data were extracted for descriptive purposes and included the mode of exercise, the exercise test and duration, the time of administration of the supplement, the control intervention, GI effects, and whether the participants were in a fed or fasted state for the exercise session.

Quality Assessment

Cochrane's tool for assessing risk of bias was used to analyze the quality of the studies included in the review (Higgins *et al*., 2011). Ratings of low, high, or unclear risk of bias were assigned to each study for the following criteria: random sequence of generation and allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), and selective reporting (reporting bias).

Statistical Analysis

A meta-analysis was performed to assess the effects of exogenous ketone precursors or monoesters versus a control on endurance performance. This consisted of both an overall analysis of all studies as well as two separate sub-analyses consisting of ≥3 studies that measured performance using the same type of test. The standardized mean difference (Hedges g) between experimental and control trials and 95% CI were computed using a random effects model. The absolute difference in means of the experimental and control trials, the standard deviation of the difference of the paired measurements (s_d), and the standard error were used to compute the standardized mean difference. The s_d was calculated in one of 4 ways: (i) using Cohen's *d*, the s_d is equal to the mean of the paired measurements divided by Cohen's *d*, (ii) using the length of the confidence interval, the s_d is equal to the square root of the sample size multiplied by the length of confidence interval, which is then divided by two times the $t_{0.025}$ quantile from the t(n-1) distribution, (iii) using the p-value, the *t*-test statistic can first be determined, after which the s_d is equal to the mean of the paired measurements divided by the value of the *t*-test statistic, which is then multiplied by the square root of the sample size, (iv) using the correlation between the paired measurements, the s_d is equal to the square root of the

following: the standard deviation of one paired measurement (s_1) squared, plus the standard deviation of the other paired measurement (s_2) squared, minus two times the correlation multiplied by s_1s_2 (Hogg and Tanis, 2010). There was one study for which we were not able to compute the s_d, so we imputed the correlation of the paired measurements by using the median correlation from the remaining studies. The s_d was then computed the same way as above (iv). The standard error was calculated by dividing the s_d by the square root of the sample size. The I^2 statistic, which describes the percentage of variation across studies that is due to heterogeneity rather than sampling error (chance) was computed to assess heterogeneity between studies (Higgins and Thompson, 2002). Forest plots were generated to visualize the data. Meta-analyses were performed using the software Comprehensive Meta-Analysis (version 3.0) with a significance level of 0.05. SPSS software was used for all correlations. Spearman correlations were run for the comparisons between ketone dose and ßHB concentration and between percent change in ßHB concentration and percent change in performance with a significance level of 0.05.

Results

Study & Participant Characteristics

The literature search resulted in 569 retrieved articles, of which 8 studies met the criteria to be included in the systematic review (Cox *et al*., 2016; Evans and Egan, 2018; Scott *et al*., 2018; Evans *et al*., 2019; Shaw *et al*., 2019; Poffé *et al*., 2020a; Poffé *et al*., 2020b; Poffé *et al*., 2020c). A flow diagram of study retrieval and selection is presented in **Figure 1**. All participant and study characteristics are presented in **Table 2** and **3**. A total of 80 individuals (77 men, 3 women) participated in these studies, which were published between 2016 and 2020 and

included representation from the UK, Ireland, Belgium, and New Zealand. Sample sizes ranged from 8 to 12 participants. All individuals were trained athletes whose average age ranged from 25 to 38 years. Ketone supplements were provided in the form of KME or precursors only. The ketone dose ranged from approximately 500 to 922 mg/kg of body weight and the total exercise duration ranged between approximately 80 and 195 minutes of activity either in the form of cycling or running. Of the 8 studies, 3 required the participants to consume the ketone supplement and undergo the performance test in a fasted state (Cox *et al*., 2016; Scott *et al*., 2018; Shaw *et al*., 2019). Three different outcomes under the scope of endurance performance were identified across the 8 studies: 4 assessing time to exhaustion (TTE) (Evans and Egan, 2018; Poffé *et al*., 2020a; Poffé *et al*., 2020b; Poffé *et al*., 2020c), 3 assessing time trial (TT) completion for time (Scott *et al*., 2018; Evans *et al*., 2019; Shaw *et al*., 2019), and one assessing TT completion for distance (Cox *et al*., 2016). For this reason, separate sub-analyses were completed for the TTE outcome and the TT for time outcome.

Endurance Performance

All of the 8 studies assessed running or cycling performance. When comparing endurance performance amongst all included studies, no significant differences were found between the ketone and control trials (Figure 2; Hedges g=0.136; 95% CI, -0.195, 0.467; p=0.419) with evidence of moderate to substantial interstudy heterogeneity (I^2=56.888%, p=0.023). We further sub-analyzed results based on the type of endurance test. Four of the studies evaluated endurance by means of TTE (Evans and Egan, 2018; Poffé *et al*., 2020; Poffé *et al*., 2020; Poffé *et al*., 2020), 3 by means of time to completion of a TT (Scott *et al*., 2018; Evans *et al*., 2019; Shaw *et al*., 2019), and one by maximum distance achieved in a TT (Cox *et al*., 2016). TTE sessions

consisted of sprints to exhaustion (sprints lasted between approximately 50 seconds and 4 minutes), that immediately followed endurance bouts lasting between 80 and 195 minutes. No significant differences were found in endurance performance between the ketone and control trials based on TTE values (Hedges g=-0.002; 95% CI, -0.312, 0.308; p=0.989) with some evidence of heterogeneity (I^2=19.144%; p=0.294). TTs also followed pre-load endurance bouts with total exercise time lasting between 80 and 115 minutes. TT distances ranged from 5 to 10 km. No significant differences were found in endurance performance between the ketone and control trials based on TT values (Hedges g=0.057; 95% CI, -0.282, 0.395; p=0.744) with no significant evidence of heterogeneity (I^2=0%; p=0.664). One study was not included in the sub-analyses as there were no other studies assessing maximum distance in a TT as the endurance test (Cox et al., 2016). In this study, participants on average cycled 2% (411 ± 162 m) further in a 30-minute TT that followed 60 minutes of exercise at 75% W_{max}, after consuming a KME supplement compared to the control trial (Cox *et al*., 2016).

Ketone Dose & ßHB Concentrations

All studies reported peak blood or plasma ßHB concentrations during exercise of above 0.2 mM, indicating that participants reached a state of ketosis following supplementation. **Figure 3b** shows the correlation (r_s=0.68, p=0.06) between average mg/kg ketone dose and peak concentration. Overall, higher ketone doses tended to result in higher peak concentrations. **Figure 3a** shows the two studies utilizing ketone precursors have lower peaks than those using KME, despite one implementing a relatively high dose (Scott *et al*., 2018; Shaw *et al*., 2019). All but one of the studies using KME surpassed the hypothesized ergogenic threshold, reaching peak

concentrations well over 2 mM, whereas neither study using ketone precursors met this threshold.

In order to further investigate the relationship between ketones and performance, we compared the change in ßHB concentrations to the change in performance amongst the ketone trials in all studies. **Figure 3c** shows a weak correlation (r_s=0.048, p=0.91) between percent increase in performance of the ketone trial over control and the percent increase in blood ßHB concentrations.

Gastrointestinal Effects

Seven of the 8 studies assessed incidence of symptoms of GI distress following ingestion of the ketone supplements **(Table 4)**. Six of the seven studies reported higher levels of GI distress in the ketone trial compared to the control (Evans and Egan, 2018; Evans *et al*., 2019; Shaw *et al*., 2019; Poffé *et al*., 2020a; Poffé *et al*., 2020b; Poffé *et al*., 2020c), and the other indicated no significant difference in symptoms between trials (Scott *et al*., 2018). Two of the studies (Evans and Egan, 2018; Evans *et al*., 2019) used interviews to assess the presence or lack of presence of symptoms following the trials, which ranged from belching, cramps, flatulence, reflux, urge to defecate, and nausea, all of which were seen in both the ketone and control trials, to stitches, heartburn, and vomiting, which were seen only in the ketone trials. Three of the studies (Poffé *et al*., 2020a; Poffé *et al*., 2020b; Poffé *et al*., 2020c) used questionnaires which separated symptoms into systemic, lower abdominal, and upper abdominal categories and had participants rate each symptom on a scale of 0-8. One study (Scott *et al*., 2018) also used a Likert scale questionnaire, though participants simply rated their GI comfort on a scale of 0-10 at various timepoints throughout the trial, with 0 being very comfortable and 10 being extremely

uncomfortable. Finally, one study (Shaw *et al*., 2019) used a 27-item questionnaire which had participants rate the presence of symptoms from low to high. All but one study (Scott *et al*., 2018) recorded incidences of GI disturbances within 10 minutes of completion of each trial. One of the 8 studies (Cox *et al*., 2016) did not record any information regarding GI discomfort within the trials.

Quality Assessment

Cochrane's tool for assessing risk of bias revealed that the majority of the studies included in the review presented with low risks of performance, detection, attrition, and reporting bias while selection bias was largely unclear across the studies **(Table 5)**.

Discussion

The present meta-analysis results suggest that acute ingestion of exogenous ketones, both in the form of precursors and monoesters, do not significantly impact endurance exercise performance. This lack of effect exists when evaluating all studies together as well as through sub-analysis based on type of endurance test. Furthermore, correlational analyses did show a trend between ketone dose and the resulting peak in plasma concentrations ($p=0.06$), though this was not present between percent increase in ßHB concentrations and resulting percent changes in performance ($p=0.82$). Records of GI incidences indicate an increased number of GI symptoms reported in ketone trials compared to control in 3 studies and higher ratings of discomfort on a Likert scale in ketone trials amongst 3 of the 4 remaining studies. This suggests that GI symptoms or discomfort may be greater with ingestion of exogenous ketones compared to a control.

Previous reviews of the literature have examined all types of exogenous ketones and both endurance and power performance (Margolis and O'Fallon, 2020; Valenzuela *et al*., 2020). While Margolis & O'Fallon (2020) state the effects of exogenous ketones on physical performance to be inconclusive, Valenzuela *et al*. (2020) concluded that ketone supplementation has no effect on exercise performance. Still, both discuss the impact that different ketone types may have on performance, mainly through their ability to raise ßHB concentrations and their effects on GI function. Studies using KS and ketone diesters have shown consistently null or negative effects to date and were therefore excluded from the present review (Leckey *et al*., 2017; Evans *et al*., 2018; James and Greer, 2018; O'Malley *et al*., 2017; Rodger *et al*., 2017). Ketone precursors have lacked attention in the literature and were even grouped in with the ketone esters by Valenzuela *et al*. (2020). Our analysis suggests that the ketone precursor 1,3-butanediol, is not as efficient as KME at raising ßHB concentrations. Scott *et al*. (2018) and Shaw *et al*. (2019) reached peak concentrations of only 1.0 and 0.75 mM, respectively, compared to the average of ~2.7 mM amongst the studies using KME (Cox *et al*., 2016; Evans and Egan, 2018; Evans *et al*., 2019; Poffé *et al*., 2020a; Poffé *et al*., 2020b; Poffé *et al*., 2020c), which is far from the hypothesized ergogenic threshold of 2 mM (Clarke *et al*., 2012; Cox *et al*., 2016; Stubbs *et al*., 2018; Hashim and VanItallie, 2014). This smaller change in concentration is likely due to a difference in the delivery of ketone equivalents previously discussed by Stubbs *et al*. (2018). Ketone precursors deliver just one equivalent compared to the two provided by KME.

While no statistically significant relationship was found between dose and resulting peak concentration, this may be due to many factors, including the small number of studies, all with small sample sizes. Regardless, **Figure 3b** shows a clear trend in the data suggesting that higher doses lead to higher peak concentrations. Interestingly, this non-significant relationship persisted

when comparing only studies using KME (p=0.14). Also important to note, we found no significant association between percent increase in ketone concentration and percent increase in the ketone over control trial performance. This outcome suggests that increased levels of ketosis do not equate to larger improvements in performance, or more ketones in circulating concentration do not lead to a greater ergogenic effect. Though, this would be worthwhile to investigate in a larger sample of studies.

Another factor that affects resulting ßHB levels is whether the individual consuming the ketones is in a fasted or fed state. It is known that ingesting ketones immediately following a meal diminishes the peak blood ßHB concentration thereafter (Stubbs *et al*., 2017; Stubbs *et al*., 2015). With exercise, peak concentrations become further reduced due to the increase in metabolism (Cox *et al*., 2016). Amongst the studies included, Cox *et al.* (2016) achieved a peak ßHB concentration of about 2.5 mM in participants who were fasted overnight. Interestingly, the remaining two studies with participants in a fasted state achieved the lowest peak concentrations of ßHB (Scott *et al*., 2018; Shaw *et al*., 2019). These were also the only two to utilize ketone precursors, suggesting that ketone supplement type may have more of an impact on resulting concentration. Evans *et al*. (2019) performed the only study that utilized KME and did not achieve a peak ßHB concentration above the 2 mM threshold. The fed state of the participants likely played a role in these results, as Evans *et al*. (2019) and Cox *et al*. (2016) administered the same dose of the same KME, however, participants in the Cox *et al*. (2016) study were fasted (peak=~2.5 mM) whereas those in the Evans *et al*. (2019) study were fed (peak=~1.33 mM). Regardless, consumption of ketone supplements under fasted conditions is not practical for real-life competition where individuals follow nutritional routines meant to optimize their fuel stores (Burke *et al*., 2015). All four of the remaining studies (Evans and Egan, 2018; Poffé *et al*.,

2020a; Poffé *et al*., 2020b; Poffé *et al*., 2020c) managed to surpass the 2 mM threshold of ßHB concentration, with participants all fed 2-3 hours before the exercise session. Thus, it is possible to achieve target ßHB concentrations with appropriate timing and dosing even after meal ingestion. Based on recommendations for pre-competition nutrition (Burke *et al*., 2015), strategies like this could be further investigated in future studies.

While records of GI effects showed that ingestion of KME or precursors caused a larger number or a greater severity of GI disturbances than that reported in the control condition across the majority of studies, it should be recognized that only 7 of the 8 studies included data on GI comfort. What is more, 3 reported incidences of symptoms and 4 reported ratings of discomfort on a scale, making it difficult to compare results across all 7 studies. The data was not statistically analyzed due to a lack of information provided across studies and therefore any conclusions should be interpreted with caution. Fasted consumption of larger doses or poor palatability of the formulated drink may have contributed to an increase in symptoms. For example, Shaw *et al*. (2019) noted, all participants in their study disliked the taste of the ketone precursor. Overall uncertainty in effects on GI function is consistent with previous studies, which have found that it is primarily context-dependent (Stubbs *et al*., 2019). Dose, time of administration, and exercise duration and intensity are all factors that can affect the appearance and severity of GI symptoms, not unlike CHO consumption during exercise (Stubbs *et al*., 2019). Based on the included studies, it appears that higher doses equated to higher incidences of GI symptoms amongst the ketone trials, though more data is required to corroborate this statistically. Stubbs *et al*. (2019) found that symptom load and severity did not differ between drinks containing KME combined with CHO and isocaloric CHO when consumed during 195 mins of exercise, suggesting that KE and CHO together may improve GI tolerability.

Nonetheless, ketone ingestion will likely need to be adjusted based on individual preference, similar to other substances consumed before or during exercise.

Strengths and Limitations

The present systematic review followed current PRISMA guidelines and included both a meta-analysis and multiple supplementary regression analyses. We performed a search of numerous databases as well as hand-searched for additional studies to ensure an exhaustive group of studies was included. This systematic review is the first of its kind to assess only studies using KME and precursors. Although meta-analyses were performed, the small number of included studies and their small samples is a limitation and speaks to further research directions. Previous reviews encountered the same concerns. Margolis & O'Fallon (2020) included a total of 10 studies and Valenzuela *et al*. (2020), just 13. Given that this review intended to narrow the inclusion criteria based on type of ketone supplement and that this is still a relatively new area of research, the search resulted in an even smaller number of trials. We recognize that the inclusion of only 8 studies may have also influenced the heterogeneity testing, as it has been shown that chi-squared tests have low power in situations with studies that have small sample sizes or are few in number (Higgins and Thompson, 2002). Therefore, while our test shows a statistically significant result of substantial heterogeneity ($p=0.023$), this should be interpreted with caution. In fact, for this reason a p-value of 0.10 is often used, in which case our results become non-significant (Higgins and Thompson, 2002). Regardless, the included studies varied widely in aspects of their methodologies, making it challenging to compare or detect commonalities between trials. For example, an important element that we wanted to assess between studies was the timing of ketone dose administration because timing can potentially affect ßHB

concentration, GI function, and ultimately performance. **Appendix A** shows how greatly this aspect varied across the studies, with each one implementing a different method of administration. Not only did the dose vary, but so did the number of boluses that the overall dose was divided into, the percentage ratio of these boluses, and their time of administration related to the exercise session. Similarly, discrepancies in exercise duration and intensity, along with consistency of co-ingestion with CHO made it difficult to effectively compare the studies as a whole and, therefore became a limiting factor to this review.

Future Directions

Future studies on the use of exogenous ketone supplements for endurance exercise performance enhancement should take into account the efficiency of KME at raising blood ßHB concentrations over other ketone supplement types such as precursors, diesters, or salts. It has also been shown that sufficient levels can be reached with prior meal ingestion and/or co-ingestion with CHO. These aspects should be considered along with attention to palatability and time of administration to effectively standardize and assess the effects of ketone supplementation while maintaining principles that we know to be beneficial to performance. More consistency in methodologies across studies is required to make definitive conclusions. Additionally, studies could aim to include more female participants as there is currently a large sample size discrepancy in the sex of individuals being studied. Of 80 participants that took part in the eight studies included in this review, only 3 participants were female. This lack of equal representation of sexes is not uncommon in the field of exercise performance and creates more research questions regarding whether there may be potential differences in the effects of exogenous ketones on endurance performance between males and females. An equal distribution of male

and female participants in studies is a valuable addition to KB research that needs to be explored to fully understand exogenous ketone metabolism.

Conclusions

Based on the results from this meta-analysis, we conclude that acute ingestion of exogenous ketone precursors and monoesters do not significantly improve endurance exercise performance. The overwhelming majority of studies show null effects when compared to both nutritive and non-nutritive controls. Future studies may consider making trials more applicable to real-life competition by feeding participants beforehand and coordinating the time of administration to minimize GI disturbances. More research is warranted to strengthen conclusions and better understand exogenous ketone metabolism specifics during endurance exercise.

List of Abbreviations

CHO: Carbohydrate

KB: Ketone Body

AcAc: Acetoacetate

ßHB: Beta-hydroxybutyrate

KME: Ketone Monoester

KS: Ketone Salt

GI: Gastrointestinal

S_d: Standard Deviation of the Difference of Paired Measurements

TTE: Time to Exhaustion

TT: Time Trial

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Table 1. Exact search terms used for electronic searches of the following databases: PubMed, SPORTDiscus, and CINAHL. Within each database, search strategies were combined with AND.

Database	Search Strategy
PubMed	(Humans) OR (Men)) OR (Women)) OR (Athletes)) (Ketosis) OR (Ketone bodies)) OR (Ketone supplement)) OR (Ketone ester)) OR (Exogenous ketones)) OR (Ketone*) (Exercise performance) OR (Physical performance)) OR (Endurance)
SPORTDiscus	(Humans) OR (People) (Exogenous ketones) OR (Ketone*)) OR (Ketosis) (Endurance performance) OR (Exercise)) OR (Training)) OR (Running)) OR (Cycling)) OR (Time trial)) OR (Physical performance)
CINAHL	(Exogenous ketones) OR (Ketone bodies)) OR (Ketone esters)) OR (Ketone supplement) (Physical performance) OR (Endurance)) OR (Time trial)) OR (Running)) OR (Cycling)) OR (Training)) OR (Exercise)

Table 2. Characteristics of studies and participants included in the review. Values are means ± SDs. M, male; F, female.

Study	Reference	Sample Size	Population	Age (y)	Weight (kg)	VO_{2max} (mL/kg/min)
1	Cox et al. (2016)	8 (6 M, 2 F)	Elite endurance athletes	29.4 ± 1.0	84.9 ± 5.2	M, 5.37 ± 0.3 L/min F, 3.3 ± 0.1 L/min
2	Evans and Egan (2018)	11 (11 M, 0 F)	Team sport athletes	25.4 ± 4.6	78.6± 5.3	53.9 ± 2.2
3	Scott et al. (2018)	11 (11 M, 0 F)	Trained runners	38.0 ± 12.0	67.3± 6.5	VO_{2peak} = 64.2 ± 5.0
4	Evans et al. (2019)	8 (7 M, 1 F)	Trained runners	33.5± 7.3	68.8± 9.7	62.0 ± 5.6
5	Shaw et al. (2019)	9 (9 M, 0 F)	Trained cyclists	26.7 ± 5.2	69.6± 8.4	VO_{2peak} = 63.9 ± 2.5
6	Poffé et al. (2020b)	12 (12 M, 0 F)	Cyclists and triathletes	25.0 ± 6.0	72.0 ± 8.0	62.4 ± 6.6
7	Poffé et al. (2020c)	12 (12 M, 0 F)	Trained cyclists	26.0 ± 6.0	70.0 ± 7.0	62.5 ± 5.5
8	Poffé et al. (2020a)	9 (9 M, 0 F)	Trained cyclists	29.0 ± 5.0	71.0 ± 7.0	61.0 ± 2.9

Table 3. Ketone supplement, control intervention, and exercise parameters for each study included in the review. Peak [ßHB] signifies the highest concentration reached during exercise. Results indicate the effect of the ketone supplement ingestion on the endurance performance parameters. KE, ketone ester. TT, time trial. CHO, carbohydrate.

Reference	Type of Supplement	Dose (mg/kg)	Peak [ßHB] (mM)	Control/ Placebo	Mode of Exercise	Exercise Test	Exercise Duration (min)	Fed vs. Fasted	Results
Cox et al. (2016)	(R)-3-hydroxybutyl (R)-3-hydroxybutyrate KE	573	2.5	Isocaloric, taste matched CHO drink	Stationary cycling	1 hr 75% W_{max} + 30 min TT for distance	90	Fasted overnight	Positive
Evans and Egan (2018)	ß - hydroxybutyrate (R) 1,3-butanediol KE	750	2.61	Taste matched 6.4% CHO-electrolyte solution	Running indoors	Loughborough intermittent shuttle test	~80	Fed (3 hr before exercise)	Null
Scott et al. (2018)	1,3-butanediol ketone precursor	500	1.0	Isocaloric CHO drink	Treadmill running	1 hr 75% VO_{2peak} + 5km TT	~80	Fasted overnight	Null
Evans et al. (2019)	(R)-3-hydroxybutyl (R)-3-hydroxybutyrate KE	573	1.33	Tase matched 8% CHO-electrolyte solution	Treadmill running	1 hr 65% VO_{2max} + 10km TT	~90	Fed (2 hr before exercise)	Null
Shaw et al. (2019)	1,3-butanediol ketone precursor	700	0.75	Orange flavoured drink	Stationary cycling	85 min ~73% VO_{2peak} + TT equivalent to 7 kJ/kg	~115	Fasted overnight	Null
Poffé et al. (2020b)	(R)-3-hydroxybutyl (R)-3-hydroxybutyrate KE	918 ± 102	3.3	Taste matched collagen peptan and water	Stationary cycling	3 hr submaximal intermittent + 15 min TT + sprint to exhaustion	~195	Fed (2 hr before exercise)	Null
Poffé et al. (2020c)	(R)-3-hydroxybutyl (R)-3-hydroxybutyrate KE	726 ± 75	3.5	Taste matched collagen peptan and water	Stationary cycling	1 hr warm up + 30 min TT + sprint to exhaustion	~90	Fed (2 hr before exercise)	Null
Poffé et al. (2020a)	(R)-3-hydroxybutyl (R)-3-hydroxybutyrate KE	922 ± 85	3.0	Taste matched collagen peptan and water	Stationary cycling	3 hr submaximal intermittent + 15 min TT + sprint to exhaustion	~195	Fed (2 hr before exercise)	Null

Table 4. Method of measurement used and incidence of GI symptoms in participants in ketone trials compared to the control trials. N/A, not applicable, CON, control.

Study Reference	Method of Measurement	Results	Incidence of GI Symptoms in Ketone Trial (Compared to CON)
Cox et al. (2016)	N/A	N/A	N/A
Evans & Egan (2018)	Interview	Ketone – 9 of 11 participants reported symptoms CON – 4 of 11	Increased
Scott et al. (2018)	Likert scale questionnaire	GI comfort = 2 ± 2 out of 10 for Ketone and CON	No difference
Evans et al. (2019)	Interview	Ketone – 5 of 8 participants reported symptoms CON – 4 of 8	Increased
Shaw et al. (2019)	Questionnaire	Ketone – 5 of 9 reported low/moderate belching, 1 reported severe abdominal pain CON – no similar symptoms reported	Increased
Poffé et al. (2020b)	Likert scale questionnaire	Total GI discomfort (out of 96): Ketone = 13 ± 10 CON = 12 ± 12	Increased
Poffé et al. (2020c)	Likert scale questionnaire	Total GI discomfort (out of 96): Ketone = 12 ± 12 CON = 7 ± 12	Increased
Poffé et al. (2020a)	Likert scale questionnaire	Total GI discomfort (out of 96): Ketone = 16 ± 11 CON = 14 ± 13	Increased

Table 5. Risk of bias for publications included in the review. H = high, L = low, U = unclear.

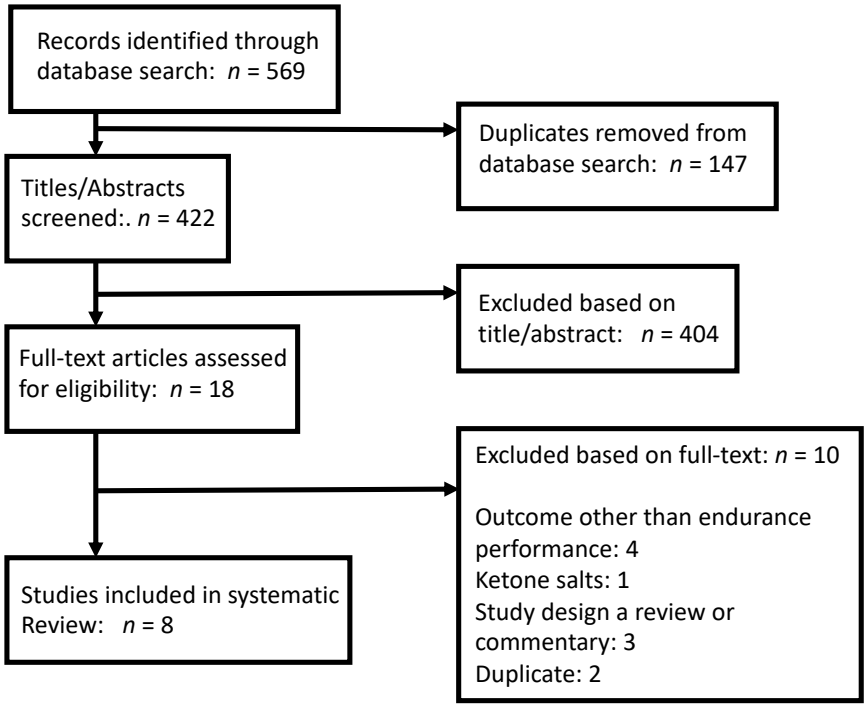
Reference	Selection Bias		Performance Bias	Detection Bias	Attrition Bias	Reporting Bias
	Random Sequence Generation	Allocation Concealment	Blinding of Participants/ Personnel	Blinding of Outcome Assessment	Incomplete Outcome Data	Selective Reporting
Cox et al. (2016)	L	L	L	H	L	U
Evans and Egan (2018)	U	U	L	L	L	L
Scott et al. (2018)	U	U	L	L	U	L
Evans et al. (2019)	U	U	L	L	L	L
Shaw et al. (2019)	L	U	H	H	L	L
Poffé et al. (2020b)	H	U	L	L	L	L
Poffé et al. (2020c)	L	U	L	L	L	L
Poffé et al. (2020a)	L	U	L	L	L	L

Figure Legends:

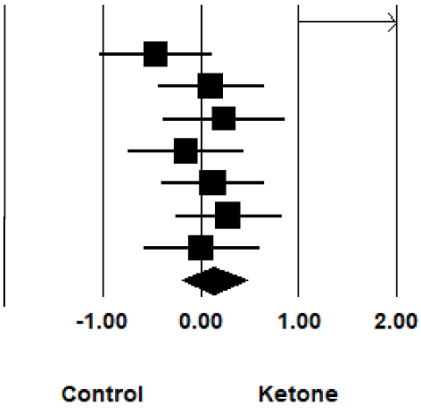
Figure 1. PRISMA flow diagram for study selection.

Figure 2. Effects of ketone supplementation on overall endurance performance. Each study and its corresponding statistics are listed, including Hedges g, 95% confidence interval (CI), p-value, and relative weight. Data is visually presented as a forest plot, with each square representing an included study and the diamond representing the overall analysis. Shapes lying to the left of the midline (0.00) indicate the results favour the control and those lying to the right of the midline indicate a favouring of ketones. The CI is represented by the lines extending from either side of each square or the lateral points of the diamond. The statistics of the overall analysis are: Hedges g=0.136; 95% CI, -0.195, 0.467; p=0.419; I^2=56.888%, p=0.023.

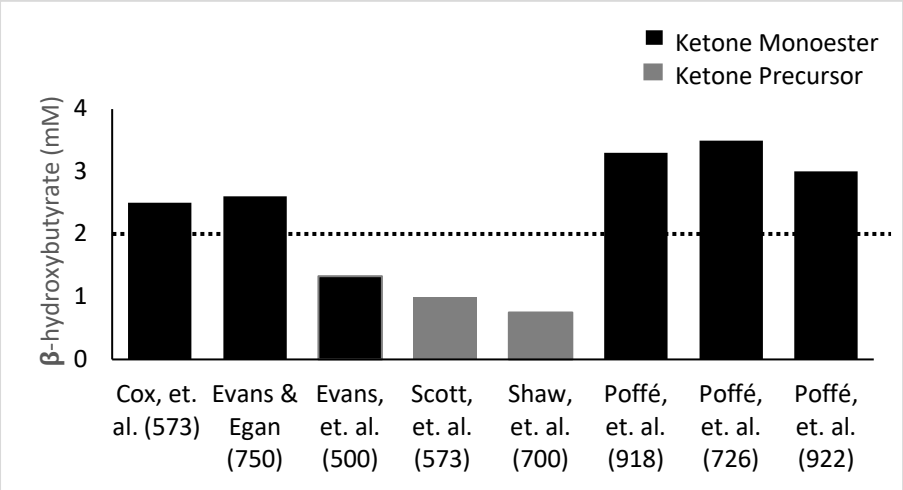
Figure 3. (a) Peak ßHB concentration in plasma or blood for each included study. Ketone dose is shown in brackets below each study (mg/kg). **(b)** Average ketone supplement dose (mg/kg) as a function of peak ßHB concentration (mM). Dotted lines represent the hypothesized threshold that must be reached in order to see ergogenic effects (2 mM) (Clarke et al., 2012; Cox et al., 2016; Stubbs et al., 2018; Hashim and VanItallie, 2014). r_s=0.68, p=0.06. **(c)** Percent increase from pre-ketone to peak blood ßHB concentration as a function of percent change in performance of ketone trial compared to control for each study. r_s=0.096, p=0.82.


Records identified through database search: *n* = 569
Duplicates removed from database search: *n* = 147
Titles/Abstracts screened:. *n* = 422
Excluded based on title/abstract: *n* = 404
Full-text articles assessed for eligibility: *n* = 18
Excluded based on full-text: *n* = 10
Outcome other than endurance performance: 4
Ketone salts: 1
Study design a review or commentary: 3
Duplicate: 2
Studies included in systematic Review: *n* = 8

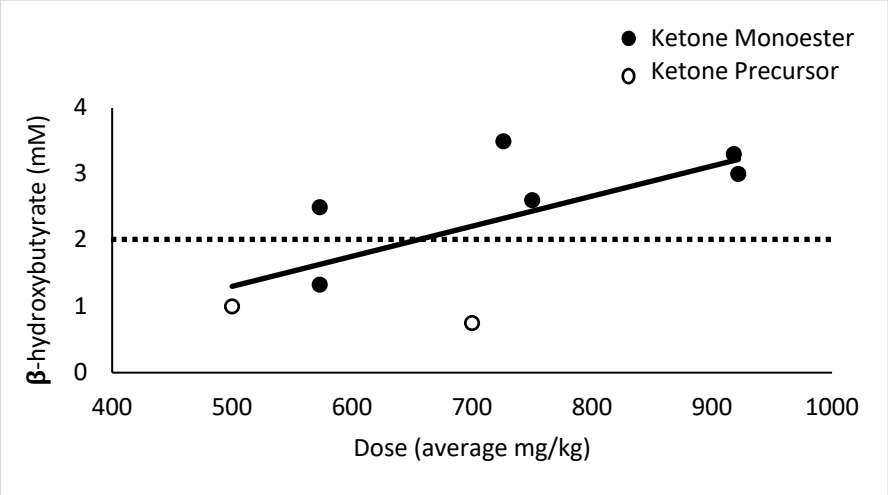
Study name	Hedges's g	Lower limit	Upper limit	p-Value	Relative weight
Cox et al. (2016)	2.265	0.996	3.535	0.000	5.23
Evans and Egan (2018)	-0.465	-1.044	0.114	0.116	13.39
Scott et al. (2018)	0.101	-0.446	0.649	0.716	14.01
Evans et al. (2019)	0.233	-0.394	0.859	0.466	12.52
Shaw et al. (2019)	-0.155	-0.750	0.439	0.609	13.10
Poffe et al. (2020b)	0.121	-0.407	0.650	0.653	14.38
Poffe et al. (2020c)	0.279	-0.259	0.817	0.309	14.19
Poffe et al. (2020a)	0.000	-0.590	0.590	1.000	13.18
	0.136	-0.195	0.467	0.419	



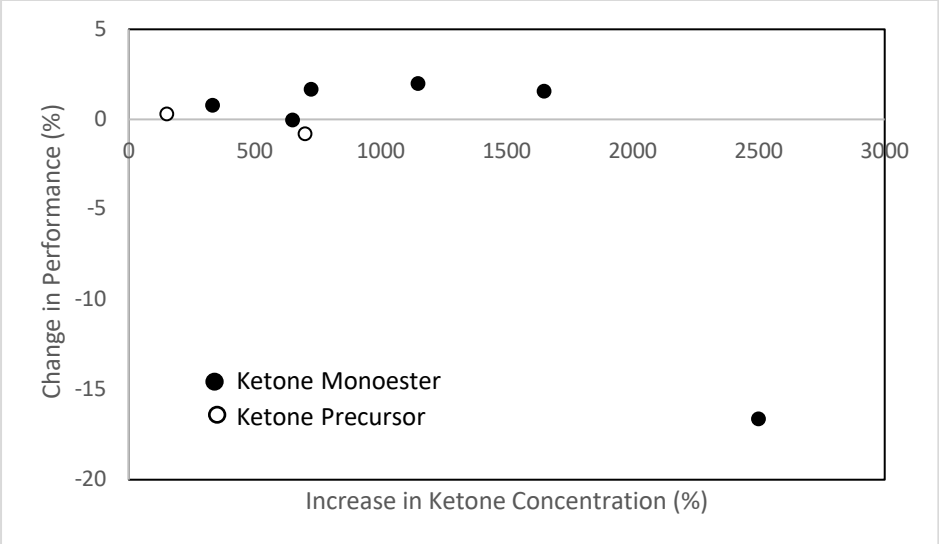
(a)


Ketone Monoester
Ketone Precursor
β-hydroxybutyrate (mM)
0
1
2
3
4
Cox, et. al. (573)
Evans & Egan (750)
Evans, et. al. (500)
Scott, et. al. (573)
Shaw, et. al. (700)
Poffé, et. al. (918)
Poffé, et. al. (726)
Poffé, et. al. (922)

(b)

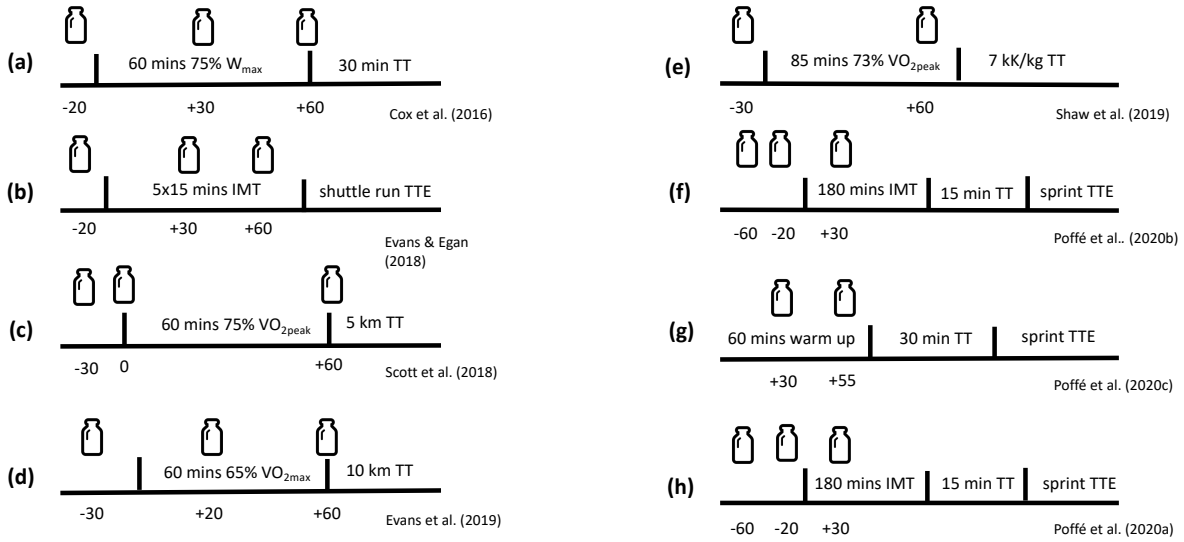

Ketone Monoester
Ketone Precursor
β-hydroxybutyrate (mM)
0
1
2
3
4
400
500
600
700
800
900
1000
Dose (average mg/kg)

(c)


Change in Performance (%)
5
0
-5
-10
-15
-20
0
500
1000
1500
2000
2500
3000
Ketone Monoester
Ketone Precursor
Increase in Ketone Concentration (%)

Appendices

Appendix A.



A1. Timeline of administration of ketone supplement doses for each study. Six of the 8 studies split the dose into 3 boluses, with 4 of the 6 administering them at a percentage ratio of 50:25:25 (a-d) and the other 2 at a ratio of 38:31:31 (f and h). The remaining 2 studies split the doses into 2 boluses at a ratio of 50:50 (e and g). , drink aliquots. TT, time trial. IMT, intermittent. TTE, time to exhaustion.

Chapter 5: General Discussion

In this thesis project, a systematic review was successfully conducted with multiple meta-analyses comparing endurance performance of individuals between ketone and control trials. This systematic review was the first of its kind to assess endurance performance in studies using only KME or ketone precursors. Given that previous systematic reviews allude to extensive variability across studies making conclusions difficult, our review offers a more narrowed approach by focussing on two types of exogenous KBs only (Margolis and O'Fallon, 2020; Valenzuela *et al*., 2020). This approach provided more specific results and allowed for easier comparison between KME and ketone precursors. Our research shows that neither KME nor ketone precursors enhance endurance exercise performance. Adapting and evolving the study during a global pandemic proved to be challenging. It was our original intention to investigate the ingestion of KME alone, CHO alone, and KME and CHO combined in female participants performing 120 minutes of cycling exercise. We hypothesized that there would be no significant improvements in performance in either the KME alone or KME and CHO combined conditions compared to the CHO alone condition. Although the final product of our research is different than what was originally intended due to adherence to public health guidelines, it will nevertheless be a valuable addition to the field on KBs and endurance exercise performance.

In the first systematic review published on exogenous ketones and physical performance, Margolis and O'Fallon (2020) suggested that KME are the best candidate moving forward based on their apparent superior ability to raise blood ßHB concentrations. They also suggested future studies to examine the effects of exogenous ketones in prolonged endurance events and assess GI distress. Valenzuela *et al*. (2020) further suggested more research is required on the use of exogenous ketones in endurance events lasting longer than one hour. We took all of these

considerations into account for our review. As previously stated, we included only studies using KME and precursors. Our review also included three studies that were not included in either of the previous reviews, two that implemented 3+ hours of consistent sub-maximal exercise and one that implemented 90 minutes of exercise (Poffé *et al*., 2020; Poffé *et al*., 2020; Poffé *et al*., 2020). This allowed us to give a more up-to-date analysis of the current literature and adhere to previous recommendations regarding longer endurance activities. We also recorded all measures of GI distress reported across the included studies to better recognize and estimate the general effects of exogenous ketones on GI function compared to control, which has not been done previously.

A systematic review, such as the one we conducted, is an overview of all available evidence regarding a specific research question (Greenhalgh, 1997). The main benefit of conducting a systematic review is that their conclusions are more reliable than that of a single study (Greenhalgh, 1997; Gopalakrishnan and Ganeshkumar, 2013). Other advantages include that the methods used to search for and select the studies reduce bias and therefore are likely to produce more reliable and accurate conclusions (Greenhalgh, 1997; Gopalakrishnan and Ganeshkumar, 2013). Reviews also make it easier for readers to comprehend large amounts of information in a succinct and summarized format (Greenhalgh, 1997; Gopalakrishnan and Ganeshkumar, 2013). A meta-analysis, which can stand alone or be included within a systematic review, adds even more reliability when combined, such as in the present review, as it uses statistical methods to summarize the results of two or more studies rather than descriptive only (Gopalakrishnan and Ganeshkumar, 2013). Moreover, systematic reviews and meta-analyses can help to identify knowledge gaps within a particular area of research (Greenhalgh, 1997; Gopalakrishnan and Ganeshkumar, 2013). For example, the present review not only led to the

conclusion that exogenous KME and precursors do not enhance endurance exercise performance, it also revealed a requirement for more investigation into the pharmacokinetics of exogenous ketones. The variation in ketone dose, time of administration, and exercise duration and intensity across studies led to the recognition that there are currently only a small number of tracer studies detailing the rate of appearance versus disappearance of exogenous ketones, and to our knowledge, no data clearly showing the maximal rate of absorption and oxidation of KBs. There is a further need for tracer studies in individuals during exercise. Féry & Balasse (1986) combined fasting and moderate intensity exercise in individuals and found that KB metabolism during exercise is a function of the initial degree of ketosis, with lower basal levels (below 0.6 mM) resulting in an increased R_a and MCR and levels exceeding 2.5 mM leading to a reduction in that same stimulatory effect. If levels reach between 3-4 mM this effect is, in fact, abolished completely. This disappearance of the stimulatory effect of exercise on MCR further suggests that at higher plasma levels, KBs may be preferentially utilized by non-muscular tissues such as, and likely primarily, the brain (Féry & Balasse, 1986). Regardless, without clear determination of maximal absorption and oxidation, it is difficult to standardize aspects such as dose and time of administration or make strong conclusions on the effects of exogenous ketones in comparison to other substances such as CHO. This review highlighted the need for future research in this area. Finally, systematic reviews also offer practical advantages, as they cost less to carry out and typically take less time to perform (Greenhalgh, 1997).

However, there are also limitations or disadvantages to utilizing systematic reviews and meta-analyses. Certain inherent flaws associated with them may include the location and selection of studies, heterogeneity, loss of information on important outcomes, inappropriate subgroup analysis, and duplication of publication (Gopalakrishnan and Ganeshkumar, 2013). As

previously discussed, inconsistencies in methodologies across studies can make it difficult to combine findings (Gopalakrishnan and Ganeshkumar, 2013). This inconvenience was not unlike previous reviews, as Margolis and O'Fallon (2020) and Valenzuela *et al*. (2020) both address heterogeneity in aspects such as ketone dose, ßHB concentration, physical test, and level of GI distress. We also noted variability in the time of administration of the ketone supplement as well as whether participants were in a fed or fasted state at the time of consumption. This is another challenge that comes with reviewing more recent literature, as accepted numbers and thresholds, such as ketone dose, exercise intensity, and duration have yet to be confirmed with more research, as stated above. Although our heterogeneity testing indicates a substantial amount of interstudy heterogeneity (I^2=56.888%), this is an improvement from the review by Margolis and O'Fallon (2020), which showed considerable heterogeneity across 8 studies assessing endurance performance using KS, KME, ketone diesters, and ketone precursors (I^2=93%). It is possible that the exclusion of KS and diesters and the inclusion of new studies that have not been previously reviewed led to overall less variation in effect size and direction. While the review by Valenzuela *et al*. (2020) reported no presence of heterogeneity (I^2=0%), this is generally unlikely as clinical and methodological diversity is inevitable in meta-analyses, meaning some level of heterogeneity is almost always present (Higgins and Thompson, 2002).

Conclusions

In conclusion, a meta-analysis of endurance performance across 8 studies showed no significant difference between ketone and control trials. In other words, current literature indicates no warranted use of exogenous KBs for enhancement of endurance exercise performance. This is consistent with our hypothesis, which was based on findings from previous

systematic reviews and an overall absence of research showing a clear mechanism to explain how KBs may enhance performance. There is still uncertainty as to whether the reduction in glycolysis displayed in some studies is in fact a glycogen sparing effect or instead an impairment in CHO use during exercise. More research is needed to better understand KB pharmacokinetics and metabolism during endurance exercise.

Available records of GI effects showed a higher amount of GI symptoms reported in ketone trials compared to control and higher ratings of discomfort on a Likert scale in ketone trials across the majority of studies. This is inconsistent with our hypothesis, though future studies should take into consideration other aspects that may affect GI function, such as dose, time of administration, concomitant meal ingestion, and familiarity or experience of the participant with KB consumption. Statistical analysis of GI effects would further aid in reliable conclusions.

Finally, our analysis showed a non-significant relationship between ketone dose and peak ßHB concentration, which was inconsistent with our original hypothesis. These findings differ from Margolis and O'Fallon (2020), who discovered a significant relationship between ketone dose and peak ßHB concentration amongst 10 studies. Though all of our findings must be interpreted with caution due to the small sample of studies, this indicates that more investigation should be focussed on the specifics of dosing.

Our review successfully assessed studies using only KME or ketone precursors and included more recent investigations that have not been a part of previous reviews. Our findings add valuable information to the field and simultaneously suggest directions for future research.

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