Evaluation of exogenous ketone supplements

INTRODUCTION

The commercial availability of exogenous ketone supplements has created an increasing interest for their potential performance and therapeutic uses. (Egan & D'Agostino, 2016; Koutnik, D'Agostino, & Egan, 2019). The effects of metabolically active ketone bodies (KBs) in the form of ß-hydroxybutyrate (ßHB) and acetoacetate (AcAc) on various organ systems are well accepted. Including depression of lipolysis, glycolysis, and hepatic glucose production (Robinson & Williamson, 1980). However, their potential to alter substrate utilization during times of exercise has shifted attention toward the use in athletic performance (Cox et al., 2016; Evans & Egan, 2018). The ability of KBs to alter substrate preference during exercise can vary based off of nutritional status. Under fasting conditions, KBs contribute up to 10% of total energy in skeletal muscle during exercise (Balasse & Féry, 1989). While under acute supplementation from exogenous sources, this energy contribution can rise to 16 to 18 %. Provided that circulating ßHB is elevated into the 3 to 4 mM range (Cox et al., 2016). This increase in ßHB oxidation in working skeletal muscle coincides with the attenuation in glycolytic flux, as supported by the blunting of exercise-induced accumulation of plasma lactate and glycolytic intermediates, in addition to, an increase reliance on intramuscular triglycerides (IMTG) during exercise (Cox et al., 2016).

KBs are produced continuously under normal physiological conditions. Although they rarely reach concentration levels above 1 mM without nutritional manipulations. Particularly during times of prolonged low nutrient availability such as sustained fasting (> 16hrs), long-duration energy utilization absent of sufficient nutrient intake, or while consuming a

ketogenic diet consisting of very low carbohydrate (~5% kcal), low protein (~15% kcal) and high fat consumption (~80% kcal) (Robinson & Williamson, 1980). During the postprandial state, circulating KB concentrations are <0. 1 mM. Whereas hyperketonemia is generally accepted as KB concentrations above 0. 2 mM (Robinson & Williamson, 1980). Exogenous ketone supplements in a variety of forms can produce acute nutritional ketosis concentrations > 0. 5 mM (Cox et al., 2016; Evans & Egan, 2018; Volek, Noakes, & Phinney, 2015). The most potent variety of exogenous ketone supplements is the ketone monoester (KME; (R)-3-hydroxybutyl (R)-3-hydroxybutyrate). KME can elicit an increase in circulating ßHB of up to 6 mM 20 min post ingestion, if consumed in a fasted state (Cox et al., 2016; Stubbs et al., 2017). Decreases in plasma glucose, free fatty acids (FFAs), and triglycerides, are seen as ßHB concentration increases rapidly due to supplementation (Cox et al., 2016; Myette-Côté, Neudorf, Rafiei, Clarke, & Little, 2018; Stubbs et al., 2017). Exercise hinders the rise of circulating ßHB and can vary with exercise intensity. As seen during ingestion of KME (573 mg. kg $^{-1}$ body mass) prior to a 45 min period of constant load cycling at 45% and 75% peak power output (W_{max}). Which resulted in circulating ßHB of ~4. 0 mM at 45% and ~3. 0 mM at 75% (Cox et al., 2016). This suggests an intensity dependent method of KB uptake and utilization. Whereas, metabolism of KBs may hold a “ Hierarchical preference” over carbohydrates and fat, even at higher workloads that would be normally considered highly glycolytic (75% W_{max}) (Cox et al., 2016).

The possible detrimental metabolic effects of KME ingestion, such as the attenuation of plasma glucose and lactate concentrations has led to the co-

ingestion of KME and carbohydrates as a fueling strategy for exercise performance. This strategy created the first novel performance increase of 2% (411 ± 162 m

) in elite cyclists during a 30 min maximum distance time-trial ride, which occurred following a 1 h submaximal ‘ pre-load’ exercise (Cox et al., 2016). In contrast, a similar fueling strategy was utilized for a high-intensity shuttle run capacity test (~4 to 6 min), performed after a 75 min duration of intermittent running. Whereas the results showed no improvement between the KME co-ingestion and carbohydrates alone (Evans & Egan, 2018). While the first study theorized the increase in performance was due to glycogen sparing effect of KME (Cox et al., 2016). The second study however, theorized that the attenuation of glycolytic flux as ßHB concentrations increase in circulation could have been a major factor in the lack of improvement in that exercise model (Evans & Egan, 2018). It is well-established that long duration exercise with high intensity components (i. e. sprints) rely heavily on carbohydrate utilization (Hawley & Leckey, 2015). Therefore, any fueling strategy that can spare glycogen stores, while being able to maintain high intensity workloads interest both scientists and practitioners (Pinckaers, Churchward-Venne, Bailey, & Loon, 2017). In contrast though, if high intensity efforts are required and the attenuation of glycolytic flux caused by KME cannot be overcome, then there would likely be an impairment of performance (Hawley & Leckey, 2015). Furthermore, KME has the potential for cognitive performance improvements as well. It was recently observed in the previously mentioned intermittent running study, that preservation of executive function determined by a decision-

making task was higher after exhaustive exercise during the KME condition (Evans & Egan, 2018). However, this remains to be confirmed in other exercise conditions.

Therefore, the aim of this present study was to investigate the effects of acute ingestion of an exogenous ketone monoester on physical and cognitive performance in elite cyclists in response to a ~48 min glycogen depletion phase followed by a 20km time trial.

What Are Ketone Bodies?

Ketone bodies (KBs) are water-soluble carbon-based metabolic substrates that come in 3 different forms (Acetoacetate (AcAc), acetone, and ß-hydroxybutyrate (ßHB)). AcAc and ßHB are short-chain, 4-carbon organic acids that are metabolically active substrates that enter the Krebs cycle to re-phosphorylate ATP. Acetone however, is generated by the spontaneous decarboxylation of AcAc and has a negligible energy contribution (Evans, Cogan, & Egan, 2017). They are created in hepatocytes from FFAs through a process called ketogenesis. To function as an alternative extra-hepatic fuel source to enter the Krebs cycle when glucose availability cannot meet the metabolic demand of the body.

The process of Ketogenesis is an evolutionary survival mechanism to provide a usable alternative substrate for the brain during times of starvation or very low carbohydrate availably. As KBs have the unique ability (like glucose) to pass through the blood brain barrier and supply the brain with fuel. KBs are predominately created from FFAs that are released from adipose tissue into circulation. The FFAs enter the liver via carnitine palmitoyltransferase (CPT1)

mediated transport and are then converted into fatty acyl CoA (FA-CoA), which undergoes ß-oxidation to become acetyl CoA (Ac-CoA). The creation of KBs via ketogenesis takes place in the liver. This process involves several sequential condensation reactions, beginning with a condensation reaction between two Ac-CoA molecules by mitochondrial thiolase activity of Ac-CoA acetyltransferase (ACAT). This forms the product acetoacetyl CoA (AcAc-CoA), which then undergoes a following condensation reaction with an Ac-CoA to create the six-carbon product ß-hydroxy-ß-methylglutaryl-CoA (HMG-CoA), catalyzed by the activity of HMG-CoA synthase (HMGCS). Finally, HMG-CoA lyase catalyzes the formation of AcAc (Evans, Cogan, & Egan, 2017).

While a small portion of AcAc can enter circulation, majority is reduced to ßHB via 3-hydroxybutyrate dehydrogenase (BDH). This is due to an equilibrium constant that favors ßHB. Once KBs are created, they can enter circulation and are transported into mitochondrial and sarcolemmal membranes via a solute ligand carrier (SLC) protein 16A (SLC16A), which is a type of monocarboxylate transporter (MCT) (Evans, Cogan, & Egan, 2017). There are some amino acids such as, leucine, lysine, phenylalanine, isoleucine, tryptophan, and tyrosine that can become ketone bodies. However, these amino acid derived KBs contribute less than 5% to the total in circulation (Thomas et al. 1982). The process of ketogenesis is stimulated by an elevated glucagon-to-insulin ratio, as well as a decline in hepatic glycogen stores. Whereas an increase of KBs in the blood suppresses further ketogenesis, as well as reduced blood flow to the liver (Robinson & Williamson, 1980).

Availability of Ketone Bodies

Plasma levels of KBs reflect the overall balance of ketogenesis (hepatic production) and ketolysis (extra-hepatic breakdown and utilization). Plasma ketone values in a post-prandial state are <0. 1mM, whereas hyperketonaemia is accepted as a plasma concentration > 0. 2 mM (Robinson & Williamson, 1980). Endogenous hyperketonaemia occurs during physiological states and nutritional manipulations that result in reduced CHO availability, most commonly occurring during prolonged fasting (> 16hrs), starvation (> 7 days) and ketogenic diets (Robinson & Williamson, 1980; Laffel, 2000). During a prolonged period without caloric ingestion (fasting or starvation), glycogen stores in muscle and liver are depleted first. After which, FFAs are mobilized from adipose tissue and transported into the liver to undergo ketogenesis, to replace the demand normally met through glycolytic metabolism.

As the timeframe without caloric ingestion increases, so does the demand for KBs. After 12-16 hours of fasting, plasma ketone levels rise to a few hundred micromolar. After 2 days of fasting, plasma ketone levels rise up to 1-2 mM (Robinson & Williamson, 1980) and continue to rise until a state of prolonged starvation is reached. Where plasma concentrations peak a 6-8 mM (Cahill, 2006).

Endogenous ketosis can also be achieved through a strict ketogenic diet. Instead of using a complete caloric restriction to enter ketosis, this diet utilizes a strategy of very strict CHO restriction (<5% total kcals from CHO) and a very high fat consumption (~80% total kcals from fat). This nutritional strategy effectively shifts the fuel for metabolic energy to ketone bodies and away from the glycolytic pathway. Plasma values above 2 mM can be

consistently reached when following this style of nutritional intake (Kim & Rho, 2008). Studies into keto-adaption have noted a performance drop in endurance within the first week of following a ketogenic diet, in both trained and untrained populations (Ma & Suzuki, 2019; Phinney, 2004). However, multiple studies have noted an aerobic performance increase during endurance exercise once keto-adaptation has occurred in both populations (Ma & Suzuki, 2019; Phinney, 2004).

Keto-adaptation is marked notably by an increase in peak fat oxidation and noted to increase up to 2. 3-fold in endurance trained athletes (Ma & Suzuki, 2019). Whereas an improvement in endurance time (65% of VO_{2max}) was noted after a 4-week adaptation period, with a significant decrease in mean RQ values among elite cyclists, respectively (0. 83, 0. 72) (Phinney, 2004). With this significant drop in RQ demonstrating that the athletes were almost completely fueled by fat oxidation. In addition to, having no significant effect overall on VO2 max or oxygen uptake per minute (Phinney, 2004). This ability for an individual’s substrate preference to adapt to fat oxidation as their primary fuel source can prove beneficial for both trained and untrained populations during times of low CHO availably, such as during prolonged endurance exercise. Due to the limited storage capacity of glycogen in skeletal muscle and the liver (~500g total) nutritional fueling strategies need to be implemented as glycogen stores can be depleted within 60-90 min of exercise, depending on intensity and/or training status (Bartlett, Hawley, & Morton, 2015

In comparison, the body has a great ability to store usable energy in the form of fat. As this form of energy storage is much more robust and the

average person carries about 10kg of adipose tissue (Ma & Suzuki, 2019). Through training an athlete can increase the amount of glycogen stores, especially when addition skeletal muscle is added. However, this slight improvement in glycogen stores may not overcome the additional energy that is required to move this added mass throughout long distance endurance event and might prove detrimental to performance. Other options to maintain glycogen stores during prolonged exercise include ingestion of simple CHO-based supplements, often in a gel or liquid form. Although, the rate of CHO uptake and utilization by the body in limited (~30-60g hr) (Bartlett, Hawley, & Morton, 2015). If no additional CHO-based supplements are added, the system will begin to produce ketone bodies in an effort to meet the metabolic demand, whereas levels of 1-2 mM can be reached (Koeslag, Noakes, & Sloan, 1980).

Recently, a new optimal fueling strategy for prolonged endurance exercise has been proposed through the use of exogenous KBs co-ingested with CHO. The creation of exogenous KBs has allowed for a unique manipulation in metabolic substrate availability during prolonged exercise. Due to its ability to elicit a rise in hyperketonemia up to 6 mM in as little as 20 min (Cox et al., 2016; Evans & Egan, 2018). Which allows for a high level of substrate availability for oxidation, in both CHO and KB.

Hyperketonemia from Supplements

Recent commercial availability of exogenous KB supplements in multiple forms has increased interest in their potential use in sports performance as an ergogenic aid (Pinckaers, Churchward-Venne, Bailey, & Loon, 2017).

Whereas these supplements provide an exogenous source of KBs (ßHB or AcAc) to elicit acute nutritional ketosis (~0. 5 to 6 h) (Clarke et al., 2012; Kesl et al., 2016). However, it has been shown that there are multiple differences between the type of KBs given (ßHB or AcAc) and its supplemented form (salt vs ester) (Evans, Cogan, & Egan, 2017).

These exogenous KBs can be delivered in two different forms, one of which is a ketone salt and the other is a ketone ester (Evans, Cogan, & Egan, 2017). The first is a KB (ßHB or AcAc) in its free acid form that is buffered with a salt (sodium/potassium/calcium). This form has received a lot of attention early on, due to it being more commercially available. However, most research has shown these to be relatively ineffective at rising ßHB alone (<1 mM) (Evans et al. 2018; Veech, 2004). Due to commercially available Ketone salts being a racemic mixture containing equal amounts of both ßHB isoforms (D-ßHB, L-ßHB). It is known that the D-isoform is found in circulation and acts as an oxidizable substrate. However, the L-isoform appears to not be ideal for oxidation as it remains in the system for up to 8 h and is excreted through the urine at higher rates than the D-isoform, showing its limited potential as a metabolic substrate. Further research into the L-isoform is needed as its role in physiological function remains unclear as it appears to be processed through alternative pathways (Stubbs et al., 2017; Webber & Edmond, 1977). Longer peak absorption times (~90 min), lower levels of ketosis and a high cation load from the salt component of the supplement (3. 2-6. 4g) has decreased research interests in sports performance, although therapeutic options are still being explored (Kesl et al., 2016; Stubbs et al., 2017).

The second type of exogenous ketones comes in the form of an ester. Currently there are two main forms of ketone esters discussed in literature, R, S-1, 3-butanediol acetoacetate diester (Kesl et al. 2016) and (R)-3-hydroxybutyl (R)-3-hydroxybutyrate ketone monoester (Clarke et al. 2012; Cox et al. 2016). Ingestion of either ester, results in acute nutritional ketosis (. 5-6 h) with a ßHB level greater than 1mM (Clarke et al. 2012; Kesl et al 2016). However, ingestion of the monoester elicits a greater rise in circulating ßHB. This rapid rise can reach 3 mM after 10 minutes and peak at 6 mM at 30 minutes post ingestion, when dosed at 573 mg. kg $^{-1}$ body weight (Cox et al. 2016). Studies into the acetoacetate diester are limited with only one major publication in human performance. Which was conducted in professional cyclists. Where a 2 ± 1% impairment during a performance time trial post acetoacetate diester ingestion was noted. Authors of the article sited gut discomfort and a higher perceived effort as to the cause of the slight decrease in performance (Leckey, Ross, Quod, Hawley, & Burke, 2017). This form is well tolerated in both humans and rodents and is non-racemic (Clarke at el. 2012; Cox et al. 2016; Kesl et al. 2016).

Along with eliciting a lower level of ketosis comes a high cation load due to the salt that is bound to the KBs and the higher consumption quantities to reach a level of hyperketonemia.

Though some rodent studies have shown improved ßHB levels with co-ingestion of medium chain triglycerides (C: 8, C: 10) to elicit a therapeutic glucose lowering effects (Kesl et al. 2016). Another down side of stimulating exogenous ketosis through salts is that ingesting large quantities are

impractical and could be harmful, due to gastrointestinal distress and the potential cation overload or acidosis (Veech, 2004).

Although similarly high levels of hyperketonemia can be achieved endogenously through prolonged fasts, they are not realistic ways to manipulate KB in athletic populations due to the high caloric demand

Current research into exogenous ketone bodies as an ergogenic aid for exercise has experience mixed results. A novel study by Cox et al. found a 2% (411 ± 162 m

) performance increase in elite cyclists. However, the ability of exogenous ketones to increase performance has not been seen in running based studies. Although an increase in physical performance was not shown, this study evaluated cognitive function pre and post exercise. Whereas, ketone bodies maintained a significantly higher level of executive function post exhaustive exercise, when compared to CHO (Evans & Egan, 2018). Therefore, it is the purpose of this study to further elucidate the impact of exogenous ketone ester consumption on cognitive and physical performance.

The first major published results of a performance boost provided by ketone esters, were that of a 2% (411 ± 162 m

) increase during a distance trial in elite cyclists. This was the final study in a series of research designs to further understand how nutritional ketosis effects fuel preference and exercise. With the previous studies in the series showing a reduced rate of glycogenolysis and lactic acid formation (Cox et

al., 2016). However subsequent studies have been unable to elicit an improvement in performance during a shuttle run to exhaustion, as well as, during a 10km time trial run (Evans & Egan, 2018; waiting to be published). Although an increase in physical performance was not shown, these studies did add a battery of cognitive tests pre and post exercise. In which, one study showed the ability to maintain a significantly higher level of executive function post exhaustive exercise, when compared to CHO (Evans & Egan, 2018). However, this novel finding of improved cognition was not replicated in their following study (unpublished). Research into the effect of ketosis on cognition has been examined in multiple studies, through animal models. One of the most influential studies, saw an increase in treadmill distance by 32% and an increase in completion time of an 8-arm radial maze by 38% in mice (Murray et al., 2016). It is known that Ketone bodies can provide energy to both skeletal muscle and neural tissue, in an attempt to save glucose-based fuel sources. So, the same mechanisms that can potentially lead to an improvement in physical performance, may at the same time lead to an improvement in cognitive function.

References:

- Bartlett, J. D., Hawley, J. A., & Morton, J. P. (2015). Carbohydrate availability and exercise training adaptation: Too much of a good thing? *European Journal of Sport Science* , *15* (1), 3–12.
- Burke, L. M. (2015). Re-Examining High-Fat Diets for Sports Performance: Did We Call the ‘ Nail in the Coffin’ Too Soon? *Sports Medicine* , *45* (1), 33–49. https://doi. org/10. 1007/s40279-015-0393-9

- Cahill, G. F., & Owen, O. E. (1968). Starvation and survival. *Transactions of the American Clinical and Climatological Association* , *79* , 13–20.
- Clarke, K., Tchabanenko, K., Pawlosky, R., Carter, E., King, M. T., Musa-Veloso, K., … Veech, R. L. (2012). Kinetics, safety and tolerability of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate in healthy adult subjects. *Regulatory Toxicology and Pharmacology : RTP* , *63* (3). https://doi. org/10. 1016/j. yrtph. 2012. 04. 008
- Cox, P. J., Willerton, K., Kirk, T., McLure, S., Stubbs, B. J., Dearlove, D., & Clarke, K. (2015). Nutritional ketosis increases intramuscular fat oxidation during exercise. *Proceedings of The Physiological Society* , *Proc Physiol Soc 34* . Retrieved from http://www. physoc. org/proceedings/abstract/Proc%20Physiol%20Soc%2034PC217
- Cox, Pete J, & Clarke, K. (2014). Acute nutritional ketosis: implications for exercise performance and metabolism. *Extreme Physiology & Medicine* , *3* , 17. https://doi. org/10. 1186/2046-7648-3-17
- Cox, Pete J., Kirk, T., Ashmore, T., Willerton, K., Evans, R., Smith, A., … Clarke, K. (2016). Nutritional Ketosis Alters Fuel Preference and Thereby Endurance Performance in Athletes. *Cell Metabolism* , *24* (2), 256–268. https://doi. org/10. 1016/j. cmet. 2016. 07. 010
- Egan, B., & Zierath, J. (2013). Exercise Metabolism and the Molecular Regulation of Skeletal Muscle Adaptation. *Cell Metabolism* , *17* (2), 162–184. https://doi. org/10. 1016/j. cmet. 2012. 12. 012
- Evans, M., Cogan, K. E., & Egan, B. (2017). Metabolism of ketone bodies during exercise and training: physiological basis for exogenous

supplementation. *The Journal of Physiology* , *595* (9), 2857–2871. https://doi. org/10. 1113/JP273185

- Evans, M., & Egan, B. (2018). Intermittent Running and Cognitive Performance after Ketone Ester Ingestion: *Medicine & Science in Sports & Exercise* , 1. https://doi. org/10. 1249/MSS. 0000000000001700
- Féry, F., & Balasse, E. O. (1988). Effect of exercise on the disposal of infused ketone bodies in humans. *The Journal of Clinical Endocrinology and Metabolism* , *67* (2), 245–250. https://doi. org/10. 1210/jcem-67-2-245
- Maizels, E. Z., Ruderman, N. B., Goodman, M. N., & Lau, D. (1977). Effect of acetoacetate on glucose metabolism in the soleus and extensor digitorum longus muscles of the rat. *The Biochemical Journal* , *162* (3), 557–568. https://doi. org/10. 1042/bj1620557
- Murray, A. J., Knight, N. S., Cole, M. A., Cochlin, L. E., Carter, E., Tchabanenko, K., … Clarke, K. (2016). Novel ketone diet enhances physical and cognitive performance. *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology* , *30* (12), 4021–4032. https://doi. org/10. 1096/fj. 201600773R
- Robinson, A. M., & Williamson, D. H. (1980). Physiological roles of ketone bodies as substrates and signals in mammalian tissues. *Physiological Reviews* , *60* (1), 143–187.
- Winder, W. W., Baldwin, K. M., & Holloszy, J. O. (1975). Exercise-induced increase in the capacity of rat skeletal muscle to oxidize ketones. *Canadian Journal of Physiology and Pharmacology* , *53* (1), 86–91.