Oral ketone supplementation: effect on cognitive function, physiology and exercise performance

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Abstract

Nutritional strategies play an important role in facilitating peak athletic performances and research has shown that a state of physiological ketosis, from either a ketogenic diet or ketone supplementation, may have several benefits for athletes. These ergogenic properties may stem from the thermodynamic advantages ketones possess over other energy substrates as well as their ability to preserve glucose stores. Physiological ketosis has also shown improvements in cognitive performance, particularly for those suffering from neurological disease. The following thesis uses an oral ketone supplement (sodium- and potassium-based β-hydroxybutyrate) to elevate blood ketone concentrations to assess the effects of supplement induced ketosis on exercise metabolism and cycling performance (study 1, Chapter 2), as well as its effect on cognitive performance in an active population (study two, Chapter 3). As part of study 1, Twelve participants took part in a double-blind, placebo-controlled, randomised crossover design. The research assessed the effect of supplement-induced ketosis on 4 minute all-out cycling performance which followed 90 minutes at second ventilatory threshold (VT2). Upon ingestion of a ketone supplement (KET: 30 ml of Ketoforce; Prototype Nutrition, IL, USA) or placebo (PLA: 3g table salt; NaCl), effect size (ES) analysis, revealed an *unclear* 2.3 ±4.8% (Δ mean ±90% Confidence Interval (CI)) change in power output during a 4-minute maximal cycling performance test (4PT) in the KET trial compared to PLA. Therefore no substantial performance outcomes came as a result of ketone supplementation despite a three-fold increase in blood β-hydroxybutyrate concentration (ES ±90%

CI= 3.02 ±0.8; *very large*) which was accompanied by a 2.2 ±1.9% increase in the respiratory exchange ratio (RER) during the submaximal exercise phase (ES = 0.51 ±0.4; *moderate*). During the 4PT increases in both VO_2 (2.4 ±3.3%; ES = 0.24 ±0.3; *small*) and RER (4.3 ±3.3; ES = 0.75 ±0.5, *moderate*) were evident during the KET trial compared to the PLA. Similarly in study 2, the effects of oral ketone supplementation on cognitive function were assessed. Using a single blind, placebo-controlled design cognitive function was evaluated through five tests intended to assess different components of neuro-muscular performance, reaction time, processing speed and memory recall: finger tap test (FTT), stroop test (ST), reaction time test (RT), monkey-ladder test (MLT) and one-card test (OCT). The reliability of each test was also assessed using a test-retest protocol. No statistically significant differences ($p > 0.05$) were observed in cognitive function between groups. ES analysis revealed *small* improvements in the KET trial for ST (ES = 0.34 ±0.4) as well as the MLT (ES = 0.34 ±0.4) with either *trivial* or *unclear* results for FTT, RT and OCT when compared to PLA. In conclusion, ingestion of an oral ketone supplement was shown to have no substantial impact on human performance as measured through 4-minute cycling performance and measures of cognitive function.

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Abbreviations

ATP - Adenosine triphosphate

4PT - 4 min performance test

BHB - β - hydroxybutyrate

C_2 – Carbon-dioxide

CHO - Carbohydrate

CI - Confidence Interval

CV - Coefficient of variation

ES - Effect size

FFA – Free fatty acid

FTT - Finger tap test

GLU - Glucose

HC - High carbohydrate

HF - High fat

KET – Ketone supplement

Kg - Kilogram

LCHF - Low carbohydrate, high fat

HIE - High intensity exercise

PLA - Placebo

MCT – Monocarboxylate transporter

MIE - Moderate intensity exercise

min – Minute

ml – Millilitre

MLT - Monkey-ladder test

mmol/L - Millimoles per litre

MTri – Medium-chain triglyceride

NAD+ - Nicotine adenine dinucleotide

O_2 – Oxygen

OCT - One-card learning test

PPO - Peak power output

RER - Respiratory exchange ratio

RPE - Rate of perceived exertion

RT - Reaction time test

ST - Stroop test

TEM - Typical error of measurement

VCO_2 - Volume of carbon dioxide

VO_2 - Volume of oxygen consumed

VO_2max - Maximal volume of oxygen consumed

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Thesis overview

The format of this thesis includes several chapters that are presented in the style of individual journal articles, and consequently, some information may be repeated. The thesis is comprised of four chapters. Chapter One contains a review of literature and introduces the reader to ketones as well as the concept of ketone supplementation. Chapter Two focuses on the effects of acute ketone supplementation on cycling performance and metabolism, while Chapter Three considers the effect of ketones (via supplementation) on cognitive function. Both Chapter Two and Chapter Three are presented in the style of individual journal articles and appear in the same format that they were submitted to scientific journals (currently under review). The final Chapter summarises the overall findings from the two experimental studies included in this thesis and provides both practical applications and suggested areas for further research.

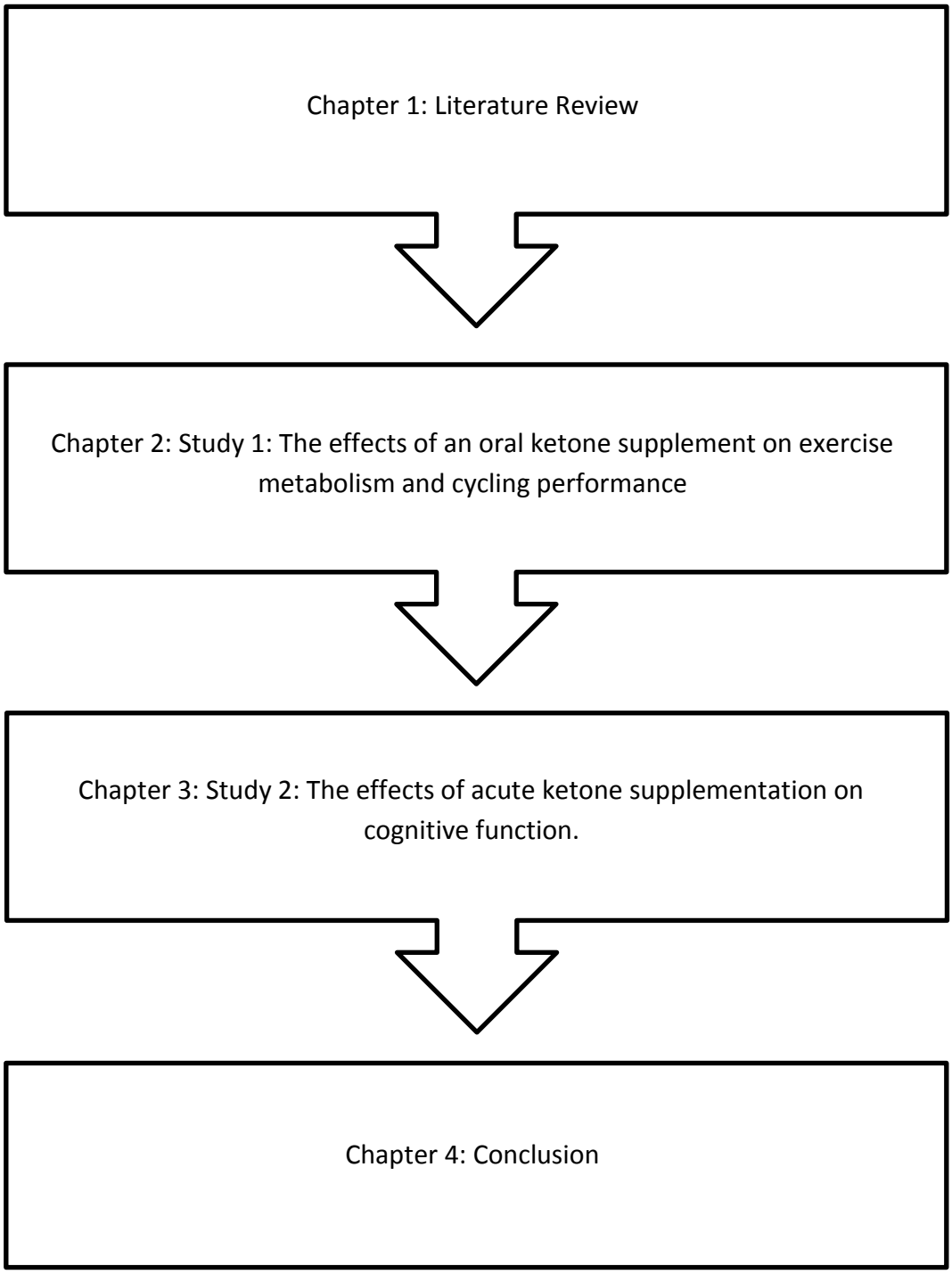


Figure 1. Schematic of the thesis structure

Chapter 1:

Literature Review

Introduction

Nutritional experts have conflicting views as to the importance of carbohydrates as a fuel source for peak athletic performance (Hargreaves, Hawley, & Jeukendrup, 2004). Contrasting views have led more athletes to experiment with diets that are very low in carbohydrate and high in fat, opposing some of the more traditional diet guidelines. Such a diet, known as a ketogenic diet, aims to elevate blood ketone concentrations through strict macro portions (~<50 g carbohydrates per day). Ketogenic diets have caught the attention of coaches, nutrition experts and scientists as these diets would theoretically allow athletes to tap into their relatively unlimited fat stores (~160,000 kJ fat compared to ~8400 kJ carbohydrate stored in the average male). For this reason, ketogenic diets may be of benefit in sports that are long in duration such as cycling, long distance/ironman triathlons and long distance running, as well as those where weight management is an issue, i.e. boxing, wrestling, rowing and cycling. Ketogenic diets have also been shown to aid the health of individuals in various disease states and studies on cognitive function may provide further reason for the promotion of a ketogenic diet for athletes (Reger et al.; Veech, Chance, Kashiwaya, Lardy, & Cahill, 2001).

A potential limitation of the diet is the depletion of glycogen stores, which has been shown to be detrimental to high intensity exercise performance (Bergström, Hermansen, Hultman, & Saltin, 1967). To combat this, there have been reports of athletes using a relatively novel ketone supplement. The use of ketone supplements may provide athletes many of the benefits associated with

elevated ketone levels without having to deplete glycogen stores and adhere to a strict ketogenic diet.

Indeed, there has also been speculation that members of the cycling peloton in the 2015 Tour de France have been supplementing with ketones to enhance their endurance performance (Farrand, 2015). The following review aims to analyse current literature concerning the elevation of blood ketone concentrations and shed light on a novel supplement now being used by athletes as an alternative to a ketogenic diet.

Ketones

Ketone bodies include three compounds; β-hydroxybutyrate (BHB), the major circulating form, acetoacetate and acetone. These compounds are small carbon fragments that are always present in the blood, however, their value can be increased through fasting, a ketogenic diet, after prolonged exercise or via nutritional supplementation (Clarke et al., 2012; Laffel, 1999; Owen et al., 1967). Veech (2004) states ketones as worthy of the title "super fuel" for their mitochondrial energy generation when compared to other common non-nitrogenous substrates. In essence, the production of ketones is a survival mechanism to produce molecules that mimic glucose from the breakdown of fat. The importance of ketones is due to their ability to be utilised as a fuel for the brain and without them humans would not survive in times of extreme starvation.

An increase in blood ketone concentrations >0.5 mmol/L is commonly referred to as physiological 'ketosis' – mentioned hereon in as ketosis (Krebs, 1966). Its name stems from the fact that when blood ketone concentrations are above 0.5 mmol/L there is a physiological shift in the body's energy regulation away from glucose and towards fat as a primary fuel (Veech et al., 2001). Blood ketone readings between 0.5 mmol/L and 5 mmol/L are considered by Veech et al. (2001) as optimal for performance, with anything up to 7 mmol/L considered to be within the normal range of blood ketone levels (without any pH change) resulting from the physiological response of carbohydrate restriction (Owen et al., 1967; Paoli, Bosco, Camporesi, & Mangar, 2015). In the past there has been fear amongst health professionals of ketosis as large increases in ketone bodies are linked to the potentially fatal diabetic ketoacidosis. Ketoacidosis occurs when severe insulin deficiency causes free fatty acids (FFA) to discharge out of adipose tissue and undergo transformation into ketone bodies at an uncontrollable rate (Veech et al., 2001). In this condition, ketone bodies can reach 25mmol/L in blood and the body becomes very acidic which can lead to disorientation or a loss of consciousness. Severe cases can produce coma and death (Veech, 2004). These high ketone concentrations are only possible with medical conditions and not through dietary intervention (Alberti, Johnston, Gill, Barnes, & Orskov, 1978).

Dietary ketosis: conflicting nutritional guidelines for exercise performance

There is conflicting opinion surrounding the role of ketones for exercise performance. To date, nutritional guidelines promote the ingestion of

carbohydrate prior to, and during intense exercise (Jeukendrup, 2014). It is for this reason athletes are routinely advised to ingest large amounts of carbohydrates during intensive training and racing. Guidelines for carbohydrate intake for athletes range from 5 to 7 $g.kg^{-1}$ body mass per day for general training needs; and 7 to 10 $g.kg^{-1}$ body mass per day for endurance athletes (Burke, Cox, Cummings, & Desbrow, 2001). Jeukendrup (2014) suggests that for optimal performance athletes must take in small amounts of carbohydrate for events less than 1 hr, 30 $g.hr^{-1}$ of carbohydrate during training sessions lasting between one to two hours, 60 $g.hr^{-1}$ of carbohydrate for events lasting two to three hours, and 90 $g.hr^{-1}$ for exercise of greater duration. However, Bosch, Dennis, and Noakes (1993) found carbohydrate loading does not necessarily result in greater carbohydrate stores, and excess carbohydrates only result in the storage and sparing of fat. Similarly, carbohydrate ingestion during exercise has been shown to slow down the rate of liver glycogen breakdown (Coggan & Coyle, 1991).

These findings identify the limitations surrounding carbohydrates and ask the question of whether the promotion of such large quantities of carbohydrate should be advised to fuel peak athletic performances during both training and competition. Several researchers dispute the need for carbohydrate to elicit peak exercise performance and these conflicting views have begun to reach mainstream media's attention (Lambert, Speechly, Dennis, & Noakes, 1994; Manninen, 2004; Phinney, 2004). Opposing those studies favouring low fat/high carbohydrate (HC) diets, several studies have found no clear advantage in carbohydrate consumption for submaximal endurance performance (Lambert et al., 1994; Langfort, Zarzeczny, Pilis, Nazar, & Kaciuba-Uścitko, 1997; Phinney,

Bistrian, Evans, Gervino, & Blackburn, 1983). These findings are likely due to the marked changes in rates of substrate utilization during prolonged, submaximal exercise following a high fat (HF) diet (Costill et al., 1977; Jenkins, Storlien, Chisholm, & Kraegen, 1988; Lambert et al., 1994). Lambert et al. (1994) and Phinney (2004) even argue that ketogenic diets, once adapted have benefits over and above a HC diet, such as their ability to tap into the relatively unlimited fat supply, their anti-inflammatory properties and lower blood lactate levels for the same given exercise intensity (Forsythe et al., 2008; Phinney, 2004; Zajac et al., 2014). Similarly, the ingestion of exogenous ketones has the ability to mimic several of these metabolic adaptations by increasing blood ketone concentrations (Clarke et al., 2012; Veech, 2014).

Ketogenic diet vs. ketone supplementation

When following a ketogenic diet there is an obvious absence of carbohydrate, whereby glucose can be synthesised by the breakdown of amino acids and fatty acids via a process known as gluconeogenesis (Owen et al., 1967). The body adapts to low muscular glycogen stores by increasing fat oxidation during low to moderate intensity exercise (Lambert et al., 1994; Phinney et al., 1983; Stellingwerff et al., 2006). The limitations of a ketogenic diet may result from the previously discussed low muscular carbohydrate stores and the commonly accepted degradation of physical performance during high intensity exercise. For this reason the interest in ketogenic diets is thought to be due to an up-

regulation in fatty acid oxidation (Bergström et al., 1967; Cameron-Smith et al., 2003). The diet is often described as difficult to adhere to due to its strict macro nutrient proportions and many find it unpalatable, making it a difficult lifestyle decision for athletes (Levy, Cooper, & Giri, 2012). The elevation of cholesterol levels, increased risk of dilated cardiomyopathy, as well as kidney issues may also be of concern for those following a ketogenic diet (Best, Franz, Gilbert, Nelson, & Epstein, 2000; Kielb, Koo, Bloom, & Faerber, 2000). Moreover, the strict nature of the ketogenic diet means that it is easy for individuals to fall out of ketosis.

To overcome many of these complications, nutritional ketone supplements, in the form of esters and salts, give athletes the ability to rapidly elevate blood ketone concentrations. Ketone supplementation allows individuals to achieve ketosis along with its claimed benefits, without having to comply with the strict ketogenic diet (Cox & Clarke, 2014; D'Agostino, Dean, Pilla, & Arnold, 2014; Veech, 2014). The safety and tolerability of ketone supplements has reported minor gastric discomfort. However, it is recommended that blood ketone concentrations are monitored to reduce the potential side effects but long term studies on the supplements safety are currently lacking (Clarke et al., 2012).

In theory both endogenous ketones and ketone supplements may spare glycogen (Robinson & Williamson, 1980), however supplements may enable athletes to do so without affecting glycogen stores. The difference stems from the way endogenous ketones are metabolised compared with exogenous ketones during ketogenesis and ketolysis. A diet induced ketosis forces the body to react to low glycogen stores by increasing FFA circulation. Upon an increase in FFA the body

produces ketones in the liver via a process known as ketogenesis (Laffel, 1999). In contrast, supplements ingested orally enter the gut where they are divided and then absorbed through the gut epithelium and monocarboxylate transporters (MCT). Ketones via supplementation, then take one of two paths into circulation, or undergo first-pass metabolism to ketone bodies in the liver (Cox & Clarke, 2014). From here both supplements and endogenously produced ketones take the same path. They are released into the blood stream where they can be metabolised by extra-hepatic tissue, or transported across the plasma and mitochondrial membrane by MCT's. A set of chemical reactions then allow ketones to enter the TCA cycle as acetyl-coA (Manninen, 2004). In these circumstances the brain has the ability to derive around two-thirds of its fuel from the ketone's BHB and acetoacetate (Bouteldja, Andersen, Møller, & Gormsen, 2014).

Therapeutic applications for the use of ketones

Interestingly, physiological ketosis through both ketone supplementation or a ketogenic diet may offer therapeutic benefits (Figure 2) in a variety of different disease states (Veech et al., 2001). Studies using ketone bodies have been shown to aid neurological health and improve certain metabolic disorders (D'Agostino et al., 2013).

Strong evidence exists for the use of a ketogenic diet to reduce seizure rates in those suffering from epilepsy (Kinsman, Vining, Quaskey, Mellits, & Freeman, 1992; Neal et al., 2008; Patel, Pyzik, Turner, Rubenstein, & Kossoff, 2010; Zupec-

Kania & Spellman, 2008). In one study of 600 patients following a ketogenic diet, it was found that almost one third of patients reported the cessation of seizures, another third reported a significant reduction in the number of seizures suffered, while the rest had no effect at all (Kinsman et al., 1992). In this study by Kinsman et al. (1992) ketone levels were analysed through urine, such measures have been shown to be a poor reflection of blood ketones therefore those who did not see improvements may not have elevated ketone concentrations to the necessary level. Consequently, one report suggests that blood concentrations of about 4 mmol/L are required to see effective improvements in symptoms related to epilepsy which is difficult to achieve through diet alone (Gilbert, Pyzik, & Freeman, 2000). The difficulty of raising ketone concentrations through a diet may explain the lack of improvement for some. The anti- epileptic properties of ketones, have been shown to suppress the expression of pro-apoptosis factors which strongly link to the enhanced rates of recovery from seizure episodes following a ketogenic diet and may explain the improvement for those following a ketogenic diet (Hallbook, Ji, Maudsley, & Martin, 2012).

Kashiwaya et al. (2000) suggests that mild ketosis might also be an effective therapy to improve Parkinson's disease. Ketones added to a culture of neurons from the hippocampal cells of embryonic rats seem to block the damage caused by the toxin 1-methyl-4-phenylpyridinium, MPP(+). In this circumstance, damage to cellular mitochondrial function is normally evident; however Kashiwaya et al. (2000) noted that ketones seemed to prevent the damage from occurring. Further studies have also shown that ketones have the ability to protect neurons from the effects of oxidative stress by reducing glutamate-induced free radical

formation and enhancing mitochondrial respiration in neocortical neurons (Kim et al., 2007; Maalouf, Sullivan, Davis, Kim, & Rho, 2007). This mechanism may, in part, contribute to the neuroprotective activity of ketones by restoring normal function in the face of oxidative stress.

Other positive results/biomarkers resulting from elevated blood ketone concentrattions have been found for those suffering from pathological conditions, such as diabetes, polycystic ovary syndrome, acne, cancer and the improvement of cardiovascular disease risk factors (Paoli, Rubini, Volek, & Grimaldi, 2013; Poff, Ari, Arnold, Seyfried, & D'Agostino, 2014; Veech, 2004; Westman, Yancy Jr, Mavropoulos, Marquart, & McDuffie, 2008; Yancy Jr, Foy, Chalecki, Vernon, & Westman, 2005; Zhou et al., 2007). These therapeutic effects, whilst having no direct implications for exercise performance, suggest that ketones should no longer be considered a disadvantaged fuel.

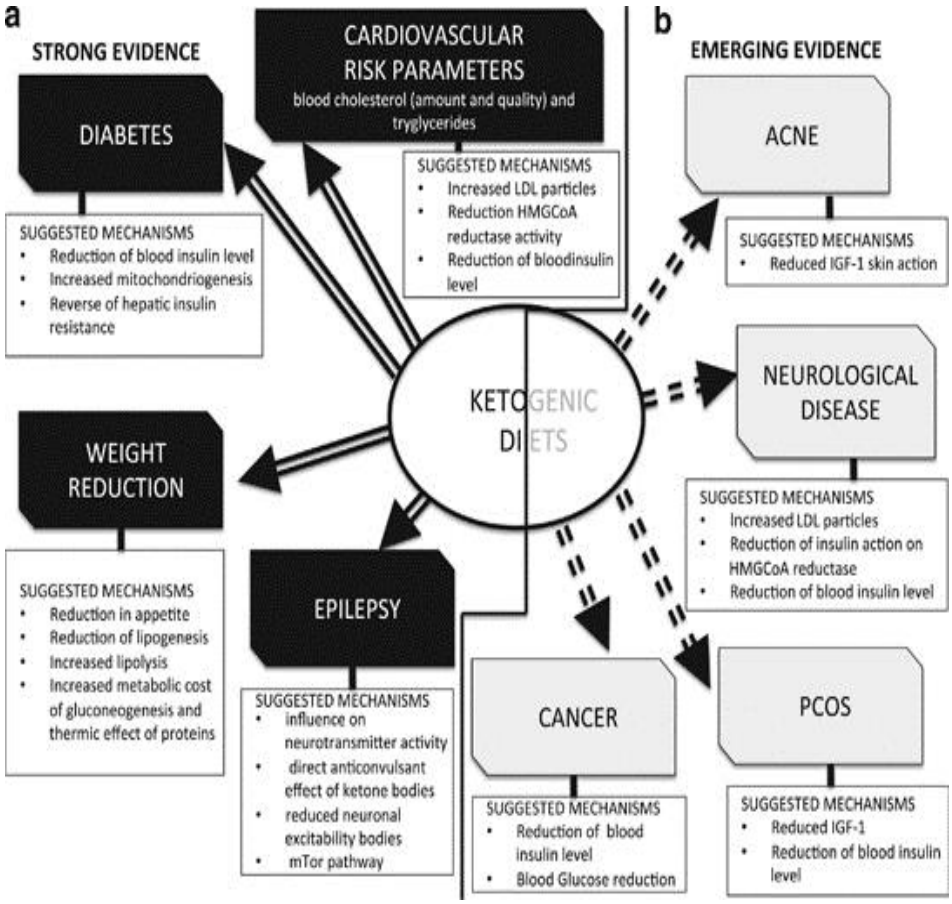


Figure 2. The suggested mechanisms for which there exists (a) strong evidence and (b) emerging evidence in regards to the therapeutic benefits of a ketogenic diet.

Obtained from: Paoli, A., Rubini, A., Volek, J. S & Grimaldi,K A. "Beyond Weight Loss: A Review of the Therapeutic Uses of very-Low-Carbohydrate (Ketogenic) Diets." European Journal of Clinical Nutrition, 67.8 (2013): 789-96.

Ketones: effect on cognitive performance

There is evidence to suggest that ketosis may have the ability to enhance cognitive function. Anecdotally an increase in ketones has been described by many to increase alertness and provide a stimulant like effect on the body (Brown, 2007). Research conducted thus far has shown improvements in cognitive function for those suffering from neurological disease.

Using an emulsified medium chain tryglicerides (MTri) to elevate blood ketone levels, Reger et al. (2004) reported that an increase in cognitive function was associated with higher ketone values ($p = 0.02$) in memory impaired adults. It is important to note that, when split into either APOE-ε4 or APOE-ε4+ genotypes, only APOE-ε4 allele showed improvement following MCT administration, whereas ε4+ subjects showed little change in performance. This finding may demonstrate the individual response to ketones on cognitive performance. Similarly Krikorian et al. (2012), using a diet that induced ketosis supplemented with MTri, found ketone levels were positively correlated with memory performance ($r = 0.45$, $p = 0.04$) in those with mild cognitive impairment. These findings were put down to the neurocognitive benefit of ketones in conjunction with, but also independent of, the effects on insulin - where there was a trend toward a moderate relationship between fasting insulin and memory performance within the low carbohydrate group. Whilst the majority of current literature focuses on individuals suffering from cognitive impairment, the findings from the above studies lay the grounds for future research on the ability of ketones to improve cognitive performance in healthy individuals.

The mechanisms responsible for improved cognitive performance are largely unknown and several advantages of ketones may exist. Mechanisms thought to enhance cognitive function may be due to the metabolic advantage held by ketones and their large anti-oxidant capacity (Veech et al., 2001). In an energy deficient state, ketones, over other fuels (including glucose), have been shown to be a more efficient fuel, providing a greater energy yield upon oxidisation (Sato et al., 1995). Therefore in conditions of hypoxia ketones may have applications for athletes by improving their ability to make better decisions during strenuous activity (Veech, 2004). Page et al. (2009) also demonstrated that BHB is comparable to glucose in its ability to support synaptic load. Synapse activity is necessary to produce complex behaviours and store memories, accordingly a reduction in synapse activity has detrimental effects on cognition (Mayford, Siegelbaum, & Kandel, 2012; Tampellini et al., 2010). Another theory for improved cognitive function comes from the enhancement of gene expression that encodes mitochondrial enzymes and energy metabolism in the hippocampus, a part of the brain important for learning and memory (Noh et al., 2004). The effect of ketones on neurodegenerative disease has been heavily researched, however little is known about the effects of ketones on cognitive performance in healthy individuals. However, the findings from the above studies lay the grounds for future research on the ability of ketones to improve cognitive performance in healthy individuals.

Ketones: effect on exercise performance

To our knowledge, there is no research to have focused on the use of oral ketone supplements for exercise performance. All literature directly relating to elevated blood ketone concentrations are from a diet-induced ketosis (Table 1). These studies have found mixed results, though vastly different methodologies have been used. In theory one would assume ketogenic diets to have the greatest effect on the aerobic energy system due to the diets sparing effects on glucose (Miller, Bryce, & Conlee, 1984). For this reason the majority of research has focused on the aerobic system, with just three studies on high intensity exercise performance and two studies focussing on strength performance. From earlier research regarding ketogenic diets and exercise, several potential benefits to elevated blood ketones concentrations have been found. One is that in conditions of hypoxia; ketosis may be beneficial due to the higher energy yield of ketones and this may translate to better performance at high altitudes, or cognitive function during maximal exercise but such claims remain untested (Veech, 2004). An additional advantage of utilising ketones during exercise may also be due to the reduced inflammation and the reduction of waste products, such as blood lactate concentrations (Forsythe et al., 2008; Masino, Kawamura Jr, Wasser, Pomeroy, & Ruskin, 2009; Zajac et al., 2014).

Ketogenic diets and aerobic exercise

Early research to assess the effects of ketogenic diets on exercise performance, showed the diet to be detrimental to performance (Galbo, Holst, & Christensen, 1979). The research by Galbo et al. (1979) showed that after 4 days of a ketogenic diet, participants (n = 7) reduced the time until exhaustion in a running test at 70% of $VO_{2\ max}$. Similar findings have been shown in other studies that have used shorter adaption periods of 14 days or less (often with carbohydrate restoration days). These studies have shown a trend for an increase in fat oxidation as well as a decrease in glycogen utilisation but in accordance with Galbo et al.'s study, have shown detrimental effects on performance (Burke & Hawley, 2002; Galbo et al., 1979; Stellingwerff et al., 2006).

In accordance to previous work, Phinney et al. (1980) agreed that short term fat adaptation decreased performance, but put forward the idea that a longer adaption period is necessary for the body to recover after the removal of carbohydrate from the diet. These views stem from research where participants were found to reduce their time until exhaustion after following a strict ketogenic diet for one-week, but improved their performance over time until the 6 week study concluded (Phinney et al., 1980). Further work has demonstrated that elite level cyclists were able to perform equally well after 4 weeks on a low carbohydrate, high fat (LCHF) diet when compared to a HC diet in terms of VO_{2max} and exercise time until exhaustion (Phinney et al., 1983). Subjects also increased fat oxidation, while reducing glucose oxidation and glycogen use after the ketogenic diet compared to the control diet. In his research, Phinney et al.

(1983) estimated that subject's blood glucose provided 28% of calories on the normal diet and 9% of calories on the ketogenic diet. It is worthwhile to note, the variation between participants was so great that only two improved their endurance performance substantially (by 57% and 30% respectively) and due to the small number of participants in this study, results may have been skewed. However the work by Phinney et al. (1983) questions previous views on the necessity of carbohydrates for exercise performance, specifically endurance exercise, and is unique due to the fact that it used a 4 week adaptation to the diet. The complexity of this research is highlighted through mixed findings and so far several studies have found no clear benefit to the use of a ketogenic diet to aid endurance performance (Goedecke et al., 1999; Lambert et al., 1994; O'Keeffe, Keith, Wilson, & Blessing, 1989).

Ketogenic diets and high intensity exercise

It is well known that as exercise intensity increases the body attains a greater proportion of its energy from carbohydrates (Bergman & Brooks, 1999). Even in a state of ketosis this has been shown to be the case and this is largely the reason for the promotion of carbohydrates during exercise (Lambert et al., 1994). However it has been shown that the glycogen sparing properties of ketones may allow glucose to be preserved for times of need and this finding questions several theories for carbohydrate loading (Robinson & Williamson, 1980). Studies that have included high intensity intervals as part of their performance assessment are currently limited, but demonstrate conflicting results.

Recent research by Rhyu and Cho (2014) using a parallel group design on trained taekwondo athletes found a significant improvement in 2000 m running performance after a similar weight loss in both diets. Positive effects on inflammatory cytokines where also evident after 3 weeks on a ketogenic diet compared with a normal diet (Iinterleukin-6, Interferon-γ, tumor necrosis factor-α). In the same study Rhyu and Cho (2014) found no difference in performance between groups in 100 m run time and Wingate cycling power output. Sawyer et al. (2013) had comparable findings in strength trained men (n = 16) and women (n = 15) where average power surprisingly increased from (811 ± 28W vs 850 ± 31W; mean ± SD for HC vs LC respectively) following a 30 second Wingate assessment after 7 days of a LC diet, however the increase was not considered significant. Similarly Lambert et al. (1994) reported that high intensity cycling (85% of peak power output unitl exhaustion) did not decline after two weeks following a ketogenic diet. A recent study by Zajac et al. (2014) revealed conflicting results during a 15min all-out time-trial following 90 min of submaximal exercise in 8 trained off-road cyclists. Zajac et al. (2014) found a decrease in high intensity exercise performance ($p = 0.04$) from 362 ± 16W to 350 ± 15W (mean ± SD) after the ketogenic trial. Before conclusions can be made, more trials are necessary to understand the mechanisms and factors responsible for the mixed findings of a ketogenic diet on high intensity exercise.

Ketogenic diets and strength performance

Only two peer reviewed studies have focused on athletic strength performance following a ketogenic diet. Research by Paoli et al. (2012) found no strength difference between gymnast's on a ketogenic diet compared to their 'normal' western diet. The study noted decreases in body weight and an increase in lean (muscular) body mass percentage occurred whilst following the ketogenic diet. The performance parameters for the different diets were all body weight exercises (hanging straight leg raise, ground push up, parallel bar dips, pull up, squat jump, countermovement jump, and 30 second continuous jumps). While the study claims no strength loss when on a ketogenic diet, the correct claim may be that there is no difference in power to weight ratio's between those on a ketogenic diet compared to their normal western diet. Using strength trained athletes Sawyer et al. (2013) reported similar findings in regards to max strength. Significant increases in performance were evident in the dynamometry handgrip, vertical jump and max squat. It is important to note research by both Sawyer et al. (2013) and Paoli et al. (2012) increased protein intake while on the ketogenic diet which can take one out of ketosis due to the insulin response of protein and its role in gluconeogenisis (Floyd Jr, Fajans, Conn, Knopf, & Rull, 1966), however with the intensity of training during the experimental trial, it is likely the diet induced a state of ketosis. Needless to say, current literature suggests that strength is not compromised when following a diet very low in carbohydrates.

Ketogenic diets and weight loss

Maintaining optimal body weight is important for many athletes to achieve success in their sport, and therefore a desirable weight can be difficult to achieve. Such reasons for athletes to reduce weight are often due to the need for a high power to weight ratio, or to make certain weight divisions. It is not uncommon for athletes in these situations to resort to rapid weight loss techniques or diets extremely low in energy. Such 'crash' diets can result in a loss of lean body mass and can impair physical performance (Turocy et al., 2011).

Studies have found very low carbohydrate/ketogenic diets to have better results for weight loss when compared to other diets higher in carbohydrate (Bazzano et al., 2014; Bueno, de Melo, de Oliveira, & da Rocha Ataide, 2013; Johnstone, Horgan, Murison, Bremner, & Lobley, 2008; Sondike, Copperman, & Jacobson, 2003; Stern et al., 2004). As such these diets may hold benefits for sports where weight control is important. The anti-obesity characteristics of ketone supplements have also demonstrated positive the mechanisms responsible for the weight loss is thought to be from a reduction in voluntary food intake and increase in resting energy expenditure (Clarke et al., 2012; Srivastava et al., 2012). In regards to a ketogenic diet, prior research has shown that if an adaptation period of at least two weeks is considered, no decrease in performance should be observed (Rhyu & Cho, 2014). Therefore a ketogenic diet may be a viable route for weight loss without any negative effects on performance (Paoli et al., 2015; Paoli et al., 2012).

If an athlete is looking to put on mass then evidence has shown that ketogenic diets will make this difficult due to the diets blunting effect on the IGF-1/AKT/mTOR pathway, therefore reducing the possibility of gaining muscle mass despite energy sufficiency (Sandri et al., 2013). Possible theories behind the effectiveness of ketogenic diets for weight loss include: a decreased appetite (Paoli, 2014); increased fat oxidation and reduced lipogenesis; a lower respiratory exchange ratio which could indicate better efficiency from the increased oxidisation of fats (Phinney et al., 1983); and the thermic effect of the ketogenic diet as well as the increased energy requirement from gluconeogenesis (Veech, 2004).

Table 1. Effect of low carbohydrate/high fat (LCHF) diet on exercise performance.

Author	Design	Duration of Adaptation Period	Macronutrient Proportions	No. of Participants	Sport	Training Status	Testing Protocol	Performance Outcome (mean)	Performance Effect
Galbo et al. (1979)	Crossover	4 days	Fat= 76% CHO= 10%	7	Running	Trained	70% VO_{2max} until exhaustion	HF:64 min HC:106 min	negative
Phinney et al. (1980)	Within subject design	6 weeks	1.2g protein.kg.day	6	Walking	Untrained	75% of VO_{2max} until exhaustion	HF: 249 ± 28 min Baseline diet – 168 ± 26 min	positive
Phinney et al. (1983)	Crossover design	4 weeks	(80% fat, 15% protein, <2% carbs)	5	Cycling	Well-trained	62-64% of VO_{2max} until exhaustion	HF: 151 ± 25 min HC: 147 ±14 min	positive
O'Keeffe et al. (1989)	Crossover design	1 week	<25% CHO	7	Cycling	Highly-trained	80% VO_{2max} until exhaustion	HF:60±12 min HC:113±2min	negative
Lambert et al. (1994)	Crossover design	2 weeks	Fat=70% CHO=7%	5	Cycling	Trained	85% PPO until exhaustion (HIE) + 50% PPO until exhaustion (MIE)	HF diet - MIE=79 min HC diet- MIE= 42.5 min	MIE = positive HIE = no effect
Goedecke et al. (1999)	Parrallel group design	15 day	HF Group Fat=69%, CHO=19% HC group CHO=53%	16 (8 control,8 HF)	Cycling	Trained	150 min @70% VO_{2peak} + 40 km TT	HF:69.3±2.7min to 63.4 ±1.9 min HC:69.9 ± 2.3min to 65.6 ± 2.3min	No effect

Abbreviations: HF = high fat; HC = high carbohdrate; CHO = carbohydrate; LCHF = low carbohydrate, high fat; HIE = high intensity exercise; MIE =moderate intensity exercise; PPO = peak power output, TT = time-trial.

Table 1. Effect of low carbohydrate/high fat (LCHF) diet on exercise performance (cont'd).

Author	Design	Duration of Adaptation Period	Macronutrient Proportions	No. of Participants	Sport	Training Status	Testing Protocol	Performance Outcome (mean)	Performance Effect
Paoli et al. (2012)	Within subject design	1 month	Fat=54.8±6.0% CHO= 4.5±0.5%	8	Gymnastics	Elite	Various body weight exercises	No significant change	No strength loss
Sawyer et al. (2013)	Within subject design	1 week	HF Group Fat=54%, CHO=5% HC group CHO=41%	16 men 15 women	Strength Trained	Trained	-Various strength exercises -Wingate anaerobic test	Significant improvements in handgrip, vertial jump, max squat	No strength loss
Zajac et al. (2014)	Crossover design	1 month	HF=70% Fat, 15 CHO	8	Cycling	Well-trained	Day 1- Incremental step test Day 2- 105min (90 min@85%LT,15 min@ 115%LT)	n/a	MIE = positive HIE = negative
Rhyu and Cho (2014)	Crossover design	3 weeks	HF Group Fat=55%, CHO=4.3% HC group Fat=30%, CHO=40%	16 (10 control, 10 HF)	Taekwondo	Trained	2,000 m sprint, Wingate test, grip force, back muscle strength, sit-up, 100 m sprint, standing broad jump, single leg standing	Significant improvement between trials in 2000 m run, No significant improvements in other assessments.	HIE = positive/no change No strength loss

Abbreviations: HF = high fat; HC = high carbohdrate; CHO = carbohydrate; LCHF = low carbohydrate, high fat; HIE = high intensity exercise; MIE =moderate intensity exercise; PPO = peak power output, TT = time-trial.

Limitations and future directions

Results from literature regarding elevated blood ketone concentrations and its effect on exercise performance are not unanimous. Reasons for this may be due to a large majority of studies not monitoring blood ketone concentrations, and although claimed as LCHF or ketogenic, they may not have induced a state of physiological ketosis. Another limitation to the above studies is their relatively small sample sizes, with the majority of studies only including < 8 subjects. From these studies individual responses to a ketogenic diet varied greatly which may have skewed results. Future work should attempt to take into account the possibility of responders and non-responders, and look to identify possible characteristics between these groups. The fact that athletes are already rumoured to be using exogenous ketone supplementation highlights the need for future research to understand the effects of the supplement on athletic performance. Research in the field may also help to improve our current knowledge regarding a diet induced ketosis.

Conclusion

Currently the effects of ketones on exercise performance are debated and research demonstrates conflicting opinions over the importance of carbohydrate to fuel athletic performance. The amount of carbohydrates required for performance, as well as general health, needs further attention and clarification. It can be concluded that a negative impact on performance can be seen when a ketogenic diet is followed for a short duration (<14days), however, positive

results are generally shown from literature using a longer adaptation period (Lambert et al., 1994; Phinney et al., 1983). Upon adapting to a ketogenic diet, the current review of literature suggests that several performance benefits may be seen in athletes taking part in aerobic exercise and/or those looking to reduce weight. The endurance benefits when following a ketogenic diet are likely to be due to the thermodynamic advantage of ketones over other nutritional substrates as well as the diets ability to alter metabolic economy through increased fat oxidisation and utilisation, resulting in glycogen sparing. The use of a ketogenic diet for weight reduction has confounding evidence but the exact mechanisms for the diets effectiveness are a topic of debate (Bueno et al., 2013).

Further research is needed to understand the effect of ketone supplements on both cognitive and exercise performance. Current literature demonstrates positive findings on cognitive performance in those suffering from neurodegenerative disease, but the role of ketones to enhance cognitive performance in healthy subjects is yet to be researched.

To our knowledge, currently no literature exists on the use of exogenous ketone supplementation and exercise performance. However, research in other fields gives evidence to suggest that the ingestion of a ketone supplement will likely alter an athlete's metabolic efficiency due to the high energy yield of ketones, but until experiments are conducted such claims remain unknown. Studies using nutritional ketone supplements may be useful to help fill the current knowledge gaps that exist in regards to fuel utilisation strategies for athletes. Research on the topic is of great importance due to claims that the supplement is already

being uses by professional athletes despite little being known about its benefits or limitations.

Chapter 2:

Study 1: The effects of an oral ketone supplement on exercise metabolism and cycling performance

Abstract

The purpose of the present study was to examine the effects of an oral ketone supplement on exercise metabolism and cycling performance. Using a double-blind, placebo-controlled, randomised, crossover design, 12 highly-trained cyclists (mean ± SD: age; 35 ± 8 y, mass; 74.5 ± 7.6 kg, VO_{2peak}; 68.0 ± 6.7 $ml.min^{-1}.kg^{-1}$) were supplemented with either 60 ml of an oral ketone supplement (KET: sodium- and potassium-based β-hydroxybutyrate; BHB) or a placebo formula (PLA: table salt; NaCl). Trials were performed on a cycle ergometer where participants cycled at a submaximal intensity (80% VT2) for 90-minutes, followed by a 4-minute maximal cycling performance test (4PT). During the work bout, the metabolic effects of the ketone supplement or placebo were assessed. Power output in the 4PT was 2.3 ±4.8% (Δ mean ±90% CI) greater in the KET (364 ± 58 W) vs. placebo trial (355 ± 46 W) and associated with an *unclear* effect on performance. Ingestion of the ketone supplement increased blood BHB concentrations more than two-fold compared to placebo (Effect Size (ES) = 3.02 ±0.8; *very large*). The increased BHB concentration was accompanied by a 2.2 ±1.9% increase in the respiratory exchange ratio (RER) during the submaximal exercise phase (ES = 0.51 ±0.4; *moderate*) and 4.3 ±3.3% increase during the 4PT (ES = 0.75 ±0.6; *moderate*). Submaximal VO_2 did not differ between trials, however, VO_2 was 2.4 ±3.3% greater during the 4PT phase in the ketone trial (ES = 0.24 ±0.3; *small*). In conclusion, ketone supplementation altered BHB concentrations, RER and VO_2 values during exercise, but had an *unclear* impact on 4-minute cycling performance.

Introduction

Low carbohydrate ketogenic diets increase blood ketone concentration levels and have the potential to enhance energy provision to working muscles for prolonged exercise performance (Cox & Clarke, 2014). However, the personal discipline required to achieve nutritional ketosis, by consuming less than ~50 g of carbohydrate per day, may limit its application in many athletes. Moreover, the inherent low carbohydrate consumption levels required to achieve ketosis have the potential to reduce an athlete's ability to perform high-intensity exercise (Havemann et al., 2006).

The majority of research examining fuel usage during exercise performance has failed to consider the role that ketones may have as a contributing substrate to the total energetic contribution. Ketones (acetone, acetoacetate, and β-hydroxybutyrate) are high efficiency substrates. For example, bomb-calorimetry work has shown that β-hydroxybutyrate (BHB) will generate 31% more energy per carbon molecule (C_2) than pyruvate (243.6 kcal/mole vs 185.7 kcal/mole, respectively). This highlights the substrate's potential to provide more potential energy to the electron transport chain (Veech, 2004). Similarly, the efficiency of ketones (hydraulic work/energy from O_2 consumed) have been demonstrated in the isolated perfused rat heart (Sato et al., 1995). These authors showed that the addition of ketone bodies (4 mmol/L BHB plus 1 mmol/L acetoacetate) to 10 mmol/L of glucose increased hydraulic work of the heart by ~25%, while the combination of insulin and ketones to the glucose increased efficiency by ~36% (Sato et al., 1995). Therefore, in theory oral ketone supplementation has the

potential to increase energy production in active skeletal muscle, either by reducing the VO_2 required during submaximal exercise, by eliciting enhanced energy production for a given VO_2 during maximal exercise, or a combination of both. The superior fuel efficiency of ketone substrates compared to glucose and its derivatives goes beyond the glycolytic pathway, and may be explained by two steps in the mitochondrial synthesis of ATP (Veech, 2004). As ketones are more reduced than pyruvate, a subsequent reduction in the mitochondrial NAD couple, and oxidation of the mitochondrial co-enzyme Q couple, results in an increased electron transport chain redox span, creating a larger $\Delta G'_{ATP}$ and resultant ATP generation (Cox & Clarke, 2014; Sato et al., 1995; Veech et al., 2001). Additionally, ketone bodies have been shown to act as signals that inhibit glucose and glycogen metabolism (Cahill Jr, 1976; Kashiwaya et al., 1994; Robinson & Williamson, 1980). The presence of ketone bodies therefore has the potential to spare carbohydrate stores during prolonged exercise, and aid subsequent high-intensity performance in sports like road cycling or triathlon (Lambert et al., 1994; Phinney et al., 1983).

While always present in the blood (at very low levels), blood ketone concentrations typically only rise in response to low blood glucose levels, such as during fasting, through dietary carbohydrate restriction (i.e., low carbohydrate ketogenic diets), or after prolonged exercise. In attempt to induce physiological ketosis, without the challenges associated with diet and exercise manipulation, a recently produced synthetic ketone supplement may offer the ability to rapidly induce ketosis (Veech, 2014). To our knowledge, we are unaware of research

that has examined the effect of such supplementation on exercise metabolism and performance in athletes. Therefore, the purpose of the current study was to assess the effects of an oral ketone supplement versus placebo on exercise metabolism over 90 minutes' submaximal cycling, and subsequent all-out 4-min exercise performance in highly-trained cyclists.

Methods

Participants

Twelve-highly trained male cyclists (mean ± SD: age, 35 ± 8 y; mass, 74.5 ± 7.6 kg; VO_{2peak}, 68.0 ± 6.7 $ml.min^{-1}.kg^{-1}$) volunteered to participate in this study. All testing took place during the competitive phase of the New Zealand cycling season where participants raced at either professional, A or B-grade level. Following an explanation of all procedures, risks and benefits, each participant gave their written informed consent to partake in the study, which was approved by the institution's Human Research Ethics Committee.

Experimental design

The study implemented a double-blind, placebo-controlled, randomised, crossover design that required participants to visit the laboratory on three separate occasions. Prior to taking part in the study, participants were required

to refrain from taking any nutritional supplements in the 4 weeks preceding the study, as well as during the course of the experiment. Participants were asked to document their food intake via a food diary and were instructed not to eat within the 2.5 h period before the test, and to repeat their diet in the 48 h period prior to each testing session. In the 24 h preceding each trial, participants were asked to refrain from strenuous activity and abstain from consuming caffeine on the day of the test. On the first visit to the laboratory, participants performed an incremental $VO_{2\ peak}$ test on their own bicycle attached to a Cyclus II ergometer (RBM elektronik-automation GmbH, Leipzig, Germany), where power output commenced at 150 W, and increased by 40 W every 4 min until volitional exhaustion. Immediately following the progressive exercise test, a familiarisation experimental trial was performed (described subsequently).

Exercise testing

The experimental trials, shown in Figure 3, were conducted on a Wattbike cycle ergometer (Wattbike Ltd, Nottingham, UK), set-up to replicate each participant's own bicycle. The 4-minute maximal performance test (4PT) on a Wattbike has been shown to be reliable for testing highly-trained athletes in a laboratory setting, with a test-retest coefficient of variation of ~2.7% (Driller et al., 2014). For each experimental trial, participants cycled for 90 min at 80% of their second ventilatory threshold (VT_2); referred hereafter as submaximal exercise), prior to a 4PT, separated by 2 min passive rest. During the 90 min submaximal cycling

phase, participants were asked their rating of perceived exertion (RPE) every 15 min (Borg, 1982).

Ketone supplementation

Twenty minutes prior to the trial, participants ingested either a ketone supplement (KET: 30 ml of Ketoforce; Prototype Nutrition, IL, USA) or placebo (PLA: 3g table salt; NaCL), diluted with 100 ml of sugar-free lemonade. A second dose of ketone supplement or PLA (as described) was ingested at the halfway point (45 min) during the 90 min submaximal cycling phase.

Blood sampling

Finger-tip blood samples were taken to assess blood glucose and blood BHB levels using a Freestyle Optium Blood Glucose Monitoring System (Abbot Diabetes Care, Oxon, UK). Blood lactate was measured using a Lactate Pro 2 Analyser (Arkray KDK, Shiga, Japan) alongside blood glucose and BHB measures at the end of the 90 min submaximal cycling test and 4PT.

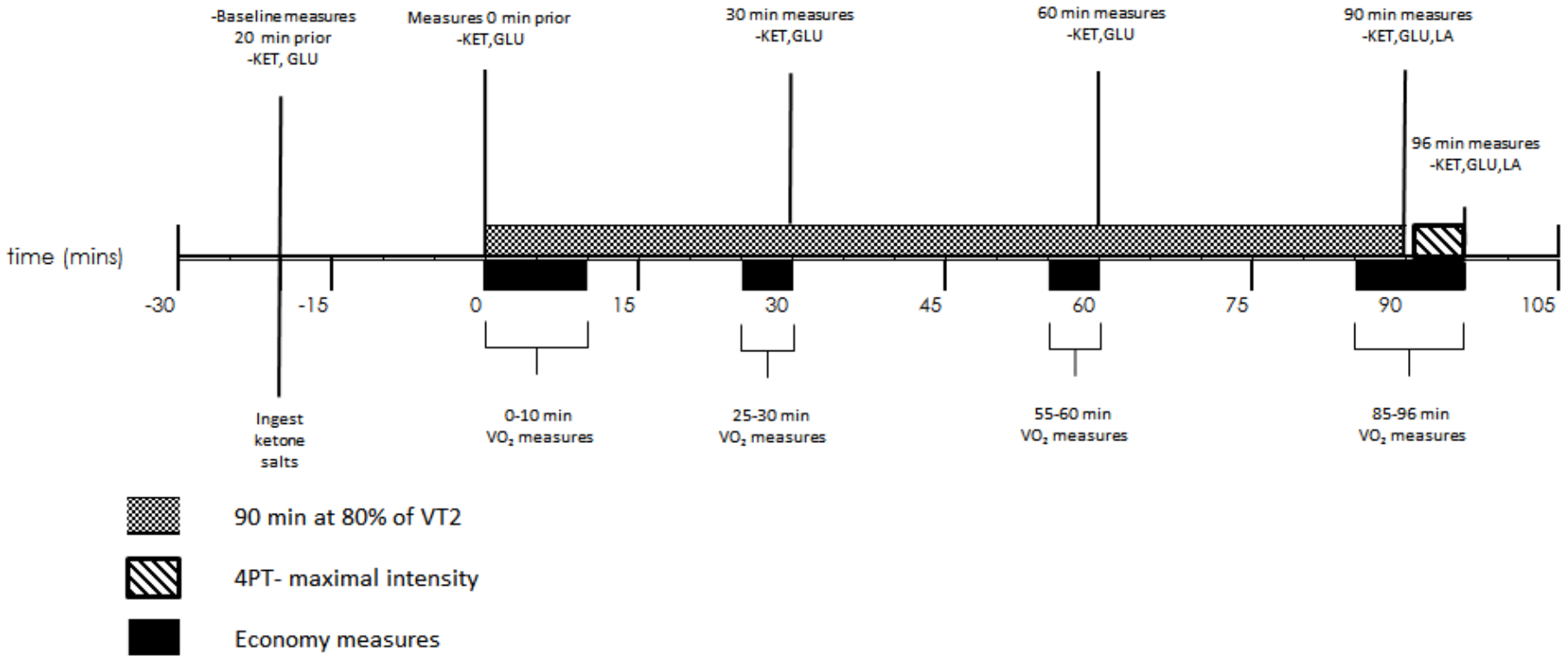


Figure 3. Experimental trial - timeline of events.
Abbreviations: KET= blood BHB concentration; GLU = blood glucose concentration; LA = blood lactate concentration.

Statistical analysis

Data are reported as means ± SD unless otherwise stated. Magnitude-based inferences were used to identify practically substantial differences between trials for each physiological and performance response during the experimental trials via analysis of log-transformed values, to reduce the bias arising from non-uniformity of error. Ratings of perceived exertion were analysed without log transformation. To make inferences about the likely range of the true effect, the uncertainty in the effect was expressed as ±90% confidence interval (CI). Effect sizes (ES) were calculated using Cohen's d, with an ES of <0.2 considered *trivial*, >0.2 *small*, >0.6 *moderate*, >1.2 *large* and > 2.0 *very large* (Hopkins, 1997). The effect was deemed unclear if its 90% confidence interval overlapped the thresholds for small positive and negative effects. The thresholds used to determine small positive and negative effects were and effect size of 0.2, or, where known, the value of the smallest worthwhile change (e.g. 0.3 of the CV = 0.81% for mean power) (Hopkins, 2006; Hopkins & Batterham, 2006).

Results

Time-trial performance

Power output in the 4PT was 2.3 ±4.8% (Δ mean ±90% CI) greater in the KET (364 ± 58 W) vs. PLA trial (355 ± 46 W) (Figure 4; Table 2). This result was associated with an *unclear* effect on performance when using 0.81% as the smallest worthwhile change.

Blood measures

Blood measured variables are shown in Table 2 and Figure 4. The ketone supplement (KET) trial revealed a three-fold increase in BHB levels (0.2 to 0.6 mmol/L) over the duration of the trial. Average baseline BHB concentrations for both trials were 0.2 ± 0.1 mmol/L (mean ± SD), however the ketone supplement increased blood BHB concentrations at a greater rate during exercise, resulting in a 157% ±63% compared to the PLA (Δ mean ± 90% CI; ES = 3.02 ±0.8; *very large*). Average blood lactate concentrations increased after both the submaximal (1.8 ± 0.7 mmol/L vs 1.6 ± 0.5 mmol/L for KET vs PLA) and 4PT (8.8 ± 3.2 mmol/L vs 7.6 ± 1.7 mmol/L for KET vs PLA respectively) exercise phases for the ketone trial but was *unclear* between conditions. Mean blood glucose concentrations at baseline were similar between trials (5.2 ± 1.7 mmol/L vs 5.4 ± 1.2 mmol/L for KET vs PLA; *unclear*). Average blood glucose during submaximal exercise was similar during the KET trial (0 min: 4.8 ± 0.7 mmol/L, 30 min: 4.4 ± 0.7 mmol/L, 60 min: 4.3 ± 0.6 mmol/L, 90 min: 4.1 ± 0.7 mmol/L) compared to PLA (0 min: 4.8 ± 0.7 mmol/L, 30 min: 4.4 ± 0.7 mmol/L, 60 min: 4.3 ± 0.6 mmol/L, 90 min: 4.1 ± 0.7 mmol/L) and between trial analysis was *unclear*. Blood glucose concentrations following the 4PT were also similar and *unclear* between trials (4.6 ± 0.9 mmol/L vs 4.6 ± 1.2 mmol/L for KET vs PLA, respectively).

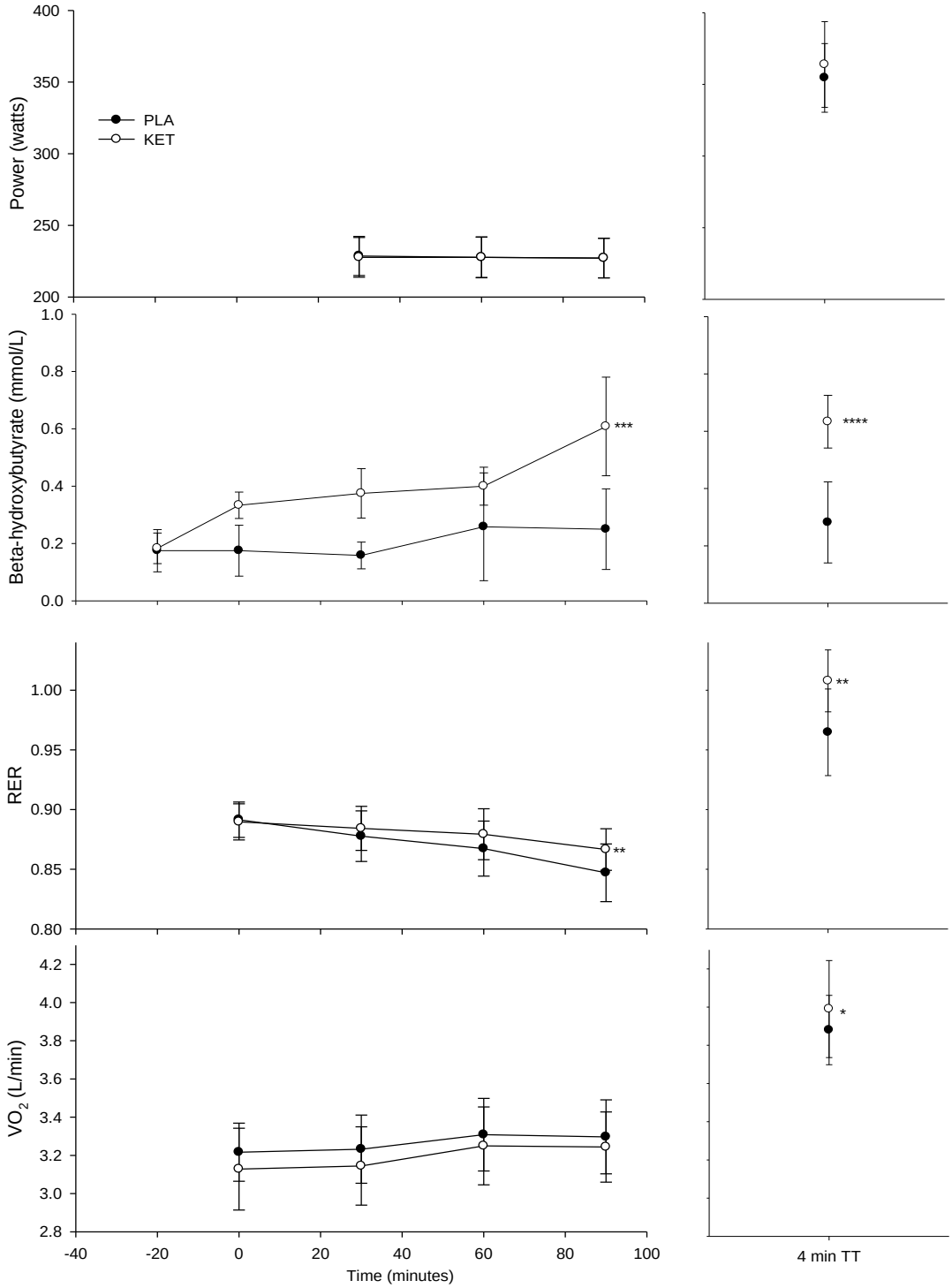


Figure 4. Key performance and physiological markers during the placebo (PLA) and ketone supplement (KET) trails.

The figure displayed represents average power at 30, 60, 90 min and following 4PT. β-hydroxybutyrate measurements were taken at baseline (20 min prior to exercise), 0, 30, 60, 90 min and after the 4PT. Both VO_2 and RER analysis take from 0-10 min, 25-30 min, 55-60 min, 85-90 min and during the 4PT. Error bars represent raw values as 90% CI. In terms of ES difference: *=Small ES, **=Moderate ES, ***=Large ES, ****= Very large ES.

Table 2. Values for the performance data and blood measure variables in the Placebo (PLA) and Ketone supplement (KET) trials

	PLA Mean ± SD	KET Mean ± SD	KET–PLA % ±90% CI effect size ±90% CI
Mean Power (W)	355 ± 46	364 ± 58	2.3 ±4.8 ES = 0.16 ±0.3 *Unclear*
4PT BHB levels* (mmol/L)	0.3 ± 0.3	0.6 ± 0.2	157 ±63 ES = 3.02 ±0.8 *Very Large*
Submaximal BHB levels** (mmol/L)	0.3 ± 0.3	0.6 ± 0.3	184 ±108 ES = 1.55 ±0.6 *Large*
4PT GLU levels* (mmol/L)	4.6 ± 1.2	4.6 ± 0.9	3.7 ±13.8 ES = 0.17 ±0.6 *Unclear*
Submaximal GLU levels** (mmol/L)	4.3 ± 0.8	4.19 ± 0.7	-3.7 ±6.5 ES = -0.21 ±0.4 *Unclear*
4PT lactate levels* (mmol/L)	7.6 ± 1.7	8.8 ± 3.2	9.7 ±29.0 ES = 0.37 ±1.1 *Unclear*
Submaximal lactate levels** (mmol/L)	1.6 ± 0.5	1.8 ± 0.7	6.7 ±26.0 ES = 0.19 ±0.7 *Unclear*

* = taken immediately post 4PT. ** = taken immediately post submaximal exercise bout (90min). Abbreviations: 4PT= 4 min performance test; BHB= β-hydroxybutyrate; GLU= glucose.

Gas exchange (table 3)

Mean RER was 2.2 ±1.9% higher in the KET (0.87 ± 0.05) compared to PLA trial (0.85 ± 0.03) during the 90 min submaximal exercise phase (ES ±90%CI = 0.51 ±0.4; *moderate*). Likewise, mean RER during the 4PT (1.01 ± 0.07 vs 0.96 ± 0.05 for KET vs. PLA, respectively) was 4.3 ±3.3% greater in the KET compared to PLA trial (ES = 0.75 ±0.6; *moderate*). Average VO_2 was lower in the KET compared to the PLA trial during the submaximal phase (3.19 ± 0.35 vs. 3.26 ± 0.33 L/min; 2.3% ±4.4%), but this difference was *unclear*. VO_2 was slightly increased (3.99 ± 0.49 vs 3.88 ± 0.35 for KET vs PLA) in the KET vs. PLA trial during the 4PT (ES = 0.24 ±0.3; *small*).

Perception

Ratings of perceived exertion was not substantially different between trials (13 ± 1 PLA, 13 ± 1; ES = -0.12 ±0.3; *unclear*).

Table 3. Values for the measured metabolic variables in the placebo (PLA) and ketone supplement (KET) trials

	PLA Mean ± SD	KET Mean ± SD	PLA–KET, % ±90% CI effect size ±90% CI
4PT RER	0.96 ± 0.05	1.01 ± 0.07	4.3 ±3.3 ES = 0.75 ±0.6 *Moderate*
Submaximal RER*	0.85 ± 0.03	0.87 ± 0.05	2.2 ±1.9 ES = 0.51 ±0.4 *Moderate*
4PT VO_2 (L/min)	3.88 ± 0.35	3.99 ± 0.49	2.4 ± 3.3 ES = 0.24 ±0.3 *Small*
Submaximal VO_2** (L/min)	3.26 ± 0.33	3.19 ± 0.35	-2.3 ±4.4 ES = -0.21 ±0.4 *Unclear*

*= average taken from the 85-90 min gas sample. **= average across all gas samples during the sub maximal bout. Abbreviations: 4PT= 4 min performance test; BHB= β-hydroxybutyrate.

Discussion

To our knowledge, this is the first study to examine the effects of an oral ketone supplement on exercise performance. The main finding was that acute oral ketone supplementation had an *unclear* effect on 4 minute all-out cycling performance, despite altering some of the physiological markers. However, ketone supplementation resulted in *very large* increases in blood BHB concentrations as well as a *moderate* increase in RER.

A possible reason for the *unclear* effect on 4 minute all-out cycling performance may be due to the need for higher blood ketone levels than what was achieved in the current study. BHB values above 2mmol/L have been reported in humans without side effects using a ketone ester (Clarke et al., 2012). Average blood BHB concentrations in the current study (0.63 mmol/L) peaked far below the levels described by Hashim and VanItallie (2014) to induce therapeutic ketosis (≥2 mmol/L). These higher blood ketone concentrations will result in the body utilising a greater proportion of BHB as fuel compared to glucose. There may also be possible advantages of ingesting glucose alongside ketones which has been shown to further increase hydraulic efficiency in the rat heart than ketones alone (Sato et al., 1995). Sato et al. (1995) demonstrated that the addition of ketone bodies (4 mmol/L BHB plus 1 mmol/L acetoacetate) to 10 mmol/L of glucose increased hydraulic work of the heart by ~25%, while the combination of insulin and ketones to the glucose increased efficiency by ~36% (Sato et al., 1995). The finding suggests that the inclusion of CHO into the ingested formula

may enhance the utilisation of ketone bodies and yield an opportunity to further increase exercise performance.

The increased RER, which coincided with the increased BHB concentrations during submaximal exercise, was in stark contrast to previous research resulting in similar blood ketone levels after adaptation to a ketogenic diet (Lambert et al., 2001; Phinney et al., 1980; Zajac et al., 2014). Phinney et al. (1980) demonstrated lower RER values during exercise following a ketogenic diet (RER = < 0.70) for 6 weeks compared to a pre-diet control (RER = 0.76). The different findings highlighted in the current study may be explained by the metabolism of exogenous ketones compared with endogenously produced ketones (Cox & Clarke, 2014). The creation of ketones via ketogenesis are due to the breakdown of adipose tissue to produce energy (Laffel, 1999). In contrast, supplements provide 'free' ketone bodies that are easily metabolised in the gut. Therefore the likely reason for the higher RER observed in our study may be because ketones, following oxidation, resemble the glucose RER output more so than fat (acetoacetate = 1.0, BHB= 0.89, acetone =1.0) (Frayn, 1983). These higher RER values may also suggest that ketones were oxidised in preference to available glucose. Indeed, ketone bodies are thought to act as signals limiting glucose and glycogen metabolism. This in turn spares glycogen for during high intensity exercise when it is needed (Cahill Jr, 1976; Robinson & Williamson, 1980). The higher RER, VO_2 and power output during the 4PT phase in the ketone trial in the current study supports this possibility.

The current study found a *small* increase in VO_2 during the 4PT as well as an *unclear* reduction in VO_2 during the submaximal exercise phase in the ketone trial compared to the placebo trial. Our results differed slightly from work by Sato et al. (1995) who demonstrated the efficiency of ketones compared to glucose in rats by showing a reduction amount of oxygen consumed (VO_2) during exercise at a fixed intensity. Therefore, we would have expected to see a clear decrease in oxygen consumption over the submaximal bout (Kashiwaya et al., 1994; Sato et al., 1995; Veech et al., 2001); however our finding was *unclear*. As discussed prior, blood ketone concentrations may not have been high enough to see a substantial change in submaximal VO_2.

It has been previously suggested that ketones are carried by the same monocarboxylate transporters (MCT) as lactate and therefore it is likely that well-trained athletes have already made the adaptations needed to utilise ketones at a greater rate (Pilegaard et al., 1999). For this reason an athlete who is able to exercise at higher rates of glycolysis should see greater performance benefits due to their ability to transport ketones across cell membranes at greater rates to be metabolized elsewhere (Halestrap & Wilson, 2012; Pilegaard et al., 1999). We can therefore postulate that well-trained athletes will have a greater improvement using ketone supplements than those who are less trained. In our study the two cyclists who were considered to be the most highly trained (as measured through VO_{2peak} and competition status), showed the greatest performance improvement - increasing power output by 15% and 19%

respectively. Pilegaard et al. (1999) had made similar observations when comparing the responses of highly trained vs lesser trained individuals.

Future research should explore the synergistic effects of carbohydrate and its effect on the utilisation of ketone bodies. Longer performance tests are also more likely to demonstrate worthwhile changes in exercise performance due to the potential glycogen sparing ability of ketones (Robinson & Williamson, 1980). It is also advised that future work should look to provide an equal calorie placebo so that comparisons can be made between energy sources to mimic real world athletic practices during racing situations. New research should also look to make comparisons between well-trained vs untrained populations and look at the individual variation found in this research.

In conclusion, the present study has shown that ketone supplementation increased blood BHB concentrations, but had an *unclear* effect on 4-minute cycling performance in highly-trained cyclists. These findings were attained alongside an increase in RER values during the entire exercise bout and an increase in average VO_2 during the 4-minute performance test.

Chapter 3:

Study 2: The effects of acute ketone supplementation on cognitive function.

Abstract

Ketone supplements have been shown to improve cognitive performance in those suffering from states of neurological disease. However, research is yet to evaluate the effect that these supplements have on cognitive performance in healthy populations. The present single blind, placebo-controlled study examined the use of an oral ketone supplement on cognitive performance in 40 recreational athletes (22 male/18 female; mean ± SD: age; 30 ± 10 y). Individuals were randomly allocated into either the placebo (PLA, n=20) or ketone supplement (KET, n=20) group. Cognitive function was assessed through five tests designed to assess different components of neuro-muscular performance, reaction time, processing speed and memory recall; finger tap test (FTT), stroop test (ST), reaction time test (RT), monkey-ladder test (MLT) and one-card test (OCT), where test-retest reliability for each test was also assessed. No statistically significant differences ($p > 0.05$) were observed in cognitive function between groups. Effect size (ES) analysis revealed *small* improvements in the KET trial for ST (ES ±90%CI= 0.34 ±0.40) as well as the MLT (ES ±90%CI= 0.34 ±0.40) with either *trivial* or *unclear* results for FTT, RT and OCT when compared to PLA. Three of the five tests resulted in an acceptable test-retest reliability (< or ~10% CV). It is concluded that acute ketone supplementation has a limited effect on the cognitive performance tests used in the current study when performed by recreational athletes.

Introduction

For elite athletes, improvements in physical performance can be difficult to attain, therefore scientists and coaches are starting to recognise the importance that the neuromuscular system plays on athletic performance. For these athletes the ability to be able to make quick, accurate decisions may be the difference between first and second place. Consequently, athletes participating in many different sports look towards the use of ergogenic aids to facilitate mental alertness and subsequent athletic performances (Tokish, Kocher, & Hawkins, 2004). The ideas behind these ergogenic aids are to improve brain processing speeds, reaction time, neuromuscular performance, decision making and memory recall, in the hope of gaining an advantage over their competitors (Baker, Nuccio, & Jeukendrup, 2014). Anecdotally, the use of ketone supplements has been described by many to increase alertness and provide a stimulant-like effect on the body (Brown, 2007). Such claims are currently untested, although improvements have been reported in cognitive function for those suffering from various disease states (Paoli et al., 2013).

There is a plethora of research on the therapeutic benefits of ketones, many of which promote the ability of ketones to improve functions of the nervous system and improve certain metabolic disorders (D'Agostino et al., 2013; Masino et al., 2009; Paoli et al., 2013; Seyfried, 2014; Veech, 2004; Veech et al., 2001). Research has shown an elevation in blood ketone levels to enhance cognitive performance in those suffering from Parkinson's disease (Vanitallie et al., 2005), Alzheimer's disease (Krikorian et al., 2012; Reger et al., 2004) and epilepsy (Levy

et al., 2012). Using an emulsified MTri to elevate blood ketone levels, Reger et al. (2004) reported that an increase in cognitive function was associated with higher ketone values ($p = 0.02$) in memory impaired adults. Similarly Krikorian et al. (2012), using a diet that induced ketosis, it was found ketone levels were positively correlated with memory performance ($r = 0.45$, $p = 0.04$) in those with mild cognitive impairment. These findings from studies support the concept that ketones may offer a broad-range of neuro-protective benefits. Such research merits attention for ketones as a therapeutic option for neuro-muscular and cognitive performance.

While attention has been given to the effects of ketones on cognitive performance, little is known about the mechanisms responsible for the improvements in cognitive function. Page et al. (2009) put forward the idea that β-hydroxybutyrates ability to support synaptic load may be of importance due to the fact that synapse activity is necessary to produce complex behaviours and store memories, accordingly a reduction in synapse activity has detrimental effects on cognition (Mayford et al., 2012; Tampellini et al., 2010).

Ketones have also been shown to have neuro-protective properties although the mechanisms for the protection need further research (Gasior, Rogawski, & Hartman, 2006; Guzmán & Blázquez, 2004; Maalouf, Rho, & Mattson, 2009). The ability of neurons to resist metabolic stress may be a larger mitochondrial load and a more energy-efficient fuel (Sato et al., 1995). In combination, these factors may account for the enhanced ability of neurons to withstand metabolic challenges above what would ordinarily be beyond the resilience of the neurons

and result in cellular death. Another theory for improved cognitive function comes from the enhancement of gene expression that encodes mitochondrial enzymes and energy metabolism in the hippocampus, a part of the brain important for learning and memory (Noh et al., 2004).

In essence there is confounding evidence towards the cognitive sparing ability of ketones in those suffering from neurodegenerative disease; however it is yet to be demonstrated whether improvements can be seen in healthy recreational athletes. Numerous athletes have made the switch to ketogenic diets - low in carbohydrate and high in fat – in an attempt to elevate blood ketone concentrations and improve performance (Paoli, Bianco, & Grimaldi, 2015). The exact mechanisms for improved performance when following a ketogenic diet are unknown. Many believe the advantages of a ketogenic diet are due to the metabolic efficiency of ketones, and the ability of the diet to reduce inflammation (Cameron-Smith et al., 2003; Forsythe et al., 2008; Lambert et al., 2001; Sato et al., 1995). It is yet to be researched whether an elevation of ketone concentration via a ketogenic diet has the ability to improve cognitive function and therefore enhance sporting performance via improvements in cognitive performance.

Research showing improvements in cognitive performance has led to the development of products to elevate ketone levels. Means of increasing ketone levels include ketogenic diets, coconut oils, medium chain triglyceride oils and more recently, the production laboratory produced ketones, in the form of esters and salts. The purpose of the current study was to investigate the effect of

an oral ketone supplement (β-hydroxybutyrate in sodium and potassium form) on different measures of cognitive performance in healthy individuals.

Methods

Participants

40 recreational athletes volunteered for the current study (18 female, 22 male; mean ± SD: age; 30 ± 10 y). Following an explanation of all procedures, risks and benefits, each subject gave their written consent to participate in the study. Research was approved by the Institutions Human Research Ethics Committee.

Experimental design

The study implemented a single blind, placebo controlled, parallel group design whereby participants were randomly assigned to one of two conditions; placebo (PLA, n = 20) or a ketone supplement (KET, n = 20) trial. As part of the study, participants were required to attend the laboratory on a single occasion following a two hour fast. A requirement for those taking part was that they could not have taken any other nutritional supplement within one week of participating in the study. Participants were also required to refrain from strenuous activity (< 1 hour) or nutritional supplements (e.g. caffeine) considered

as potential ergogenic aids (< 12 hours) prior to the test. All experimental trials were conducted between the hours of 1300 and 1700 to limit the performance fluctuations caused by the time of day and influence of circadian rhythms (Blatter & Cajochen, 2007; Schmidt, Collette, Cajochen, & Peigneux, 2007).

Familiarisation/Reliability

To ensure that there was no learning effect during the experimental trial, participants performed four familiarisation trials of each test prior to the baseline trial. Each familiarisation trial was separated by ~3 minutes. Data from the familiarisation trials were collected in a subset of the population (n=30) to inform the test-retest reliability of each test in healthy participants.

Experimental trial

Following the familiarisation trials (~10 minutes), participants undertook baseline measures for all five of the cognitive tests. Upon ingesting either the ketone or placebo supplement, participants remained in a quiet, seated position for 45-minutes before the post-testing took place. Participants were not permitted to perform any physically or mentally challenging tasks during this time. The 45-minute period was implemented as it has been suggested that this is the optimal duration required for blood ketone levels to peak when using a similar dose of ketone supplements as the current study (D'Agostino, Arnold, & Kesl, 2014). The KET and PLA supplements were as follows:

KET: 30 ml of Ketoforce (Prototype Nutrition, IL, USA) diluted with 100 ml of sugar-free lemonade (The Coca-Cola Company, GA, USA).

PLA: 3 g of table salt (NaCL) diluted with 100 ml of sugar-free lemonade (The Coca-Cola Company, GA, USA).

Cognitive tests

The cognitive tests selected for the current study included five separate measures to assess neuromuscular performance, memory, reaction time, and brain processing speed. Tests were conducted on two hand-held computer tablets (Apple Inc., CA, USA; Samsung, South Korea). The following tests were used:

Finger Tap test (FTT)

The FTT is designed to assess simple motor speed and manual dexterity. The test is typically administered as part of a neurological or neuropsychological assessment and has been implemented in numerous scientific literatures (Carlier, Dumont, Beau, & Michel, 1993; Schear & Sato, 1989; Schmitt, 2013). The test required participant to rest the palm of their dominant hand beside the computer tablet and tap the screen as many times as possible in 10 seconds.

Stroop Test (ST)

The ST has been used widely in previous research because of its ability to assess cognitive flexibility, information processing speed, executive abilities and selective attention (Spooner & Pachana, 2006). The test required participants to indicate the colour of the font in the words 'RED' or 'GREEN' which can be written in either red or green font. The idea of the test was to correctly match the correct font colour with the corresponding word as many times as possible in 60-seconds. If the participant correctly matched the colour, then a point was scored, conversely a point deducted for an incorrect match. The ST test has been previously validated as a reliable tool for cognitive performance (Bajaj et al., 2014).

Reaction time test (RT)

The RT used a simple test whereby there was only one stimulus and one response. It required participants to tap the screen of the tablet as quickly as possible upon a change in colour from red to green. Each assessment consisted of five reaction tests and the median score was taken. RT tests have been validated as an accurate assessment of cognitive function ($p < 0.001$) in healthy adults (Jakobsen, Sorensen, Rask, Jensen, & Kondrup, 2011).

Monkey-ladder test (MLT)

The MLT is used to assess short-term working memory. Also named the 'limited-hold memory task' test, it stems from research where monkeys out-performed humans (Inoue & Matsuzawa, 2007). The test displayed a 5 x 5 grid containing several squares placed randomly around the screen. Initially these small blocks appear numbered (for 3 seconds). The numbers are then removed but the blocks remain in the same place. Participants must then click the blocks in the order that the numbers were displayed. The test will end when a participant makes three errors (wrong order) and the score given equates to the last level completed correctly.

One card learning test (OLT)

The OLT is another assessment designed to assess visual learning through a pattern separation model, which is part of the short-term memory function (Yassa et al., 2010). Theoretical models of pattern separation specify that information is organised in orthogonal and distinct non-overlapping representations so that that new memories can be stored quickly without interference (Norman & O'Reilly, 2003). A number of cards are revealed on the tablet screen whereby the subject must decide whether the card has been shown before and they respond by pressing "Yes" or "No". The assessment displays a total of 21 playing cards. Scores range from 21 (100% correct answers) to 0 (0% correct answers).

Statistical analysis

All data are reported as mean ± SD unless otherwise stated. The reliability data for each cognitive test were log-transformed and analysed using an Excel spreadsheet for reliability (Hopkins, Schabort, & Hawley, 2001). An individual's coefficient of variation (CV) was calculated as the SD of an individual's repeated measurement expressed as a percentage of his or her individual mean test score (Hopkins, 2000). Typical error is expressed as a CV% and as an absolute value along with upper and lower 90% confidence intervals (CI). An independent samples t-test was performed to examine the difference between KET and PLA trials for each test, with statistical significance set at $p < 0.05$ for all analyses. Effect sizes (ES) were calculated using Cohen's d, and were interpreted using thresholds of 0.2, 0.6, 1.2 and > 2.0 for *small, moderate, large* and *very large*, respectively (Hopkins 1997). An ES of 0.2 was considered the smallest worthwhile positive effect and an effect size of < 0.2 considered *trivial*. The effect was deemed unclear if its confidence interval overlapped the thresholds for small positive and negative effects (Hopkins, 2006).

Table 4. Reliability of cognitive assessments reported as typical error of measurement (TEM) and coefficient of variation % (CV) for each test. Data reported as means (90% Confidence Interval). Highlighted values represent the lowest TEM and CV for each test.

	FTT	ST	RT (ms)	MLT (level)	OCT
TEM 2-1	2.7 (2.3-3.5)	4.8 (4.0-6.1)	25.6 (21.3-32.3)	1.1 (0.9-1.4)	1.4 (1.1-1.7)
TEM 3-2	3.5 (2.9-4.6)	4.4 (3.7-5.6)	29.6 (24.1-38.8)	1.0 (0.8-1.3)	1.7 (1.4-2.2)
TEM 4-3	2.4 (1.9-3.1)	3.2 (2.6-4.1)	23.4 (19.0-30.9)	0.9 (0.7-1.2)	1.7 (1.4-2.3)
Mean TEM	2.9 (2.5-3.6)	4.2 (3.7-5.1)	26.3 (22.9-31.5)	1.0 (0.8-1.2)	1.6 (1.4-1.9)
CV 2-1	3.6 (3.0-4.6)	60.7 (48.4-82.0)	9.1 (7.5-11.6)	20.6 (16.6-27.4)	9.0 (7.4-11.5)
CV 3-2	4.6 (3.8-6.1)	31.7 (25.3-43.1)	11.1 (8.8-15.1)	21.3 (16.3-31.0)	11.8 (9.6-15.7)
CV 4-3	4.6 (3.7-6.1)	11.4 (9.1-15.4)	9.3 (7.4-12.9)	19.1 (14.6-28.1)	12.3 (9.8-16.9)
Mean CV	4.3 (3.7-5.2)	40.7 (34.3-50.4)	9.8 (8.4-11.9)	20.4 (17.2-25.6)	10.9 (9.3-13.5)

2-1, represents the reliability between tests one and two. 3-2, represents the reliability between tests two and three. 4-3 represents the reliability between tests 3 and 4. The lowest TEM's are identified by a shaded box.

Results

Reliability

The reliability of the cognitive assessments used in this study is presented in Table 4. The lowest typical errors of measurement were between tests 3 and 4 for the FTT, ST, RT, and MLT. From these results it is evident that these tests involve a learning effect. Only the OCT differed, where the lowest typical error of measurement (mean ±90CI: 1.4 ±1.2 cards) was between tests 1 and 2. Apart from the ST and MLT, other assessments showed acceptable levels of reliability (< or ~10% CV).

Cognitive function

There were no statistically significant improvements in cognitive performance following the KET trial ($p < 0.05$) when compared to PLA (Table 5 and Figure 5). Cohen's effect size analysis revealed a *small* improvement in the ST (mean ±90CI: -0.3 ±3.1; ES = 0.34 ±0.40) and MLT (0.5 ±0.6; ES = 0.34 ±0.40) for the KET trial, however these were the least reliable tests with the smallest CV being 11.4% and 19.1% for the ST and MLT, respectively. The FTT score improved in the KET trial compared to the PLA trial by an average of 0.9 ±2.7 taps (ES = 0.09 ±0.26; *trivial*). The difference between groups for the OCT and RT were deemed *unclear*.

Table 5. Pre- and Post- test scores for each group (KET and PLA – mean ± SD), with between group differences (mean ±90% confidence interval), statistical significance and effect size (ES).

	KET		PLA				
Test score	Pre	Post	Pre	Post	Δ KET- PLA (Mean ±90% CI)	P-value	Effect Size (ES ±90% CI)
FTT	77.0 ± 8.5	78.9 ± 6.8	77.8 ± 11.4	78.8 ± 11.9	0.9 ±2.7	0.57	0.11 ±0.3 *Trivial*
ST	26.7 ± 12.1	26.7 ± 8.4	29.7 ± 11.5	30.0 ± 13.7	-0.3 ±3.1	0.87	0.34 ± 0.4 *Small*
RT (ms)	264.3 ± 39.8	267.5 ± 48.8	265.8 ± 33.0	264.0 ± 27.6	5.0 ±12.5	0.51	0.09 ± 0.4 *Unclear*
MLT (level)	6.2 ± 1.6	6.4 ± 1.6	6.6 ± 1.23	6.3 ± 1.45	0.5 ±0.6	0.20	0.34 ±0.4 *Small*
OCT	15.3 ± 3.9	15.4 ± 2.4	16.9 ± 1.7	16.9 ± 2.2	0.1 ±1.3	0.95	0.20 ±0.5 *Unclear*

Abbreviations: finger tap test (FTT), stroop test (ST), reaction time test (RT), monkey-ladder test (MLT) and one-card test (OCT).

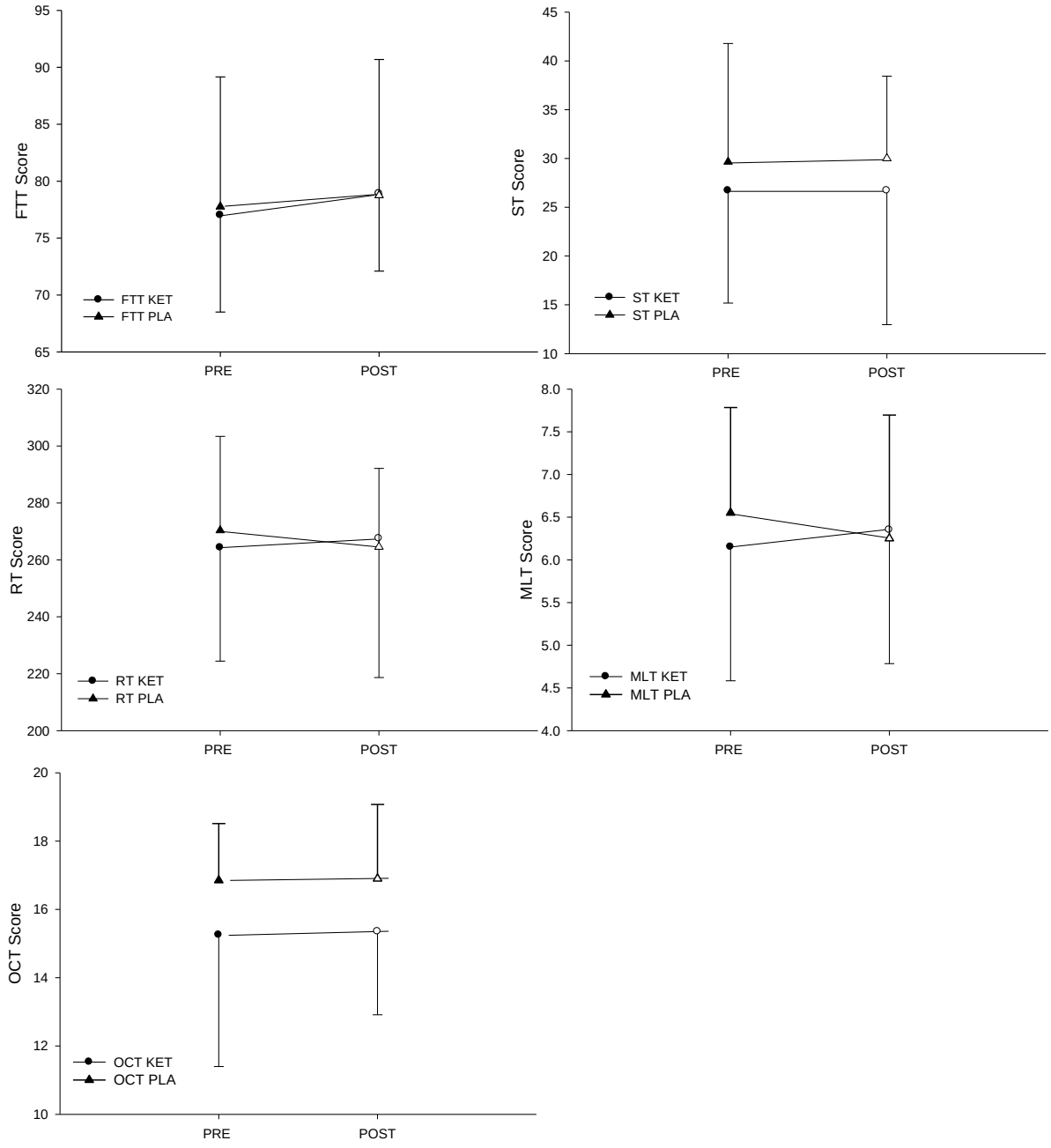


Figure 5. Average pre- and post- cognitive test scores for the ketone supplement (KET) trial vs placebo (PLA).

Error bars represent 90% confidence intervals (CI). FTT = Finger Tap Test, ST = Stroop Test, RT = Reaction Time Test, MLT = Monkey ladder test, OCT = One card learning test. * represents a *small* Effect Size.

Discussion

To the best of our knowledge the present study is the first to investigate the use of an acute oral ketone supplementation on cognitive function in healthy individuals. Our findings suggest that an acute ketone supplement has little effect on cognitive performance for healthy recreational athletes. Results for the current study showed s*mall* but not statistically significant improvements in both the ST and MLT, however, these two tests were shown to be the least reliable measures of cognitive performance according to our test-retest reliability analysis. The other assessments were deemed to have either *trivial* or *unclear* effects between the two groups.

Opposing our findings, prior research by Krikorian et al. (2012) and Reger et al. (2004) found higher ketone levels had a positive effect on cognitive performance ($p = 0.04$ and 0.02 respectively) for those suffering from memory impairment. An explanation for our findings to have differed from the above research may be because the tests used in our study were unable to place a large enough strain on the participants processing capabilities. Krikorian et al. (2012) believed that ketones may enhance the processing capacity when large cognitive loads are undertaken. Such a hypothesis could explain why healthy individuals did not improve cognitive performance after the KET trial. It is therefore presumed that the effects ketones demonstrate on those suffering from Alzheimer's disease and mild cognitive impairment are greater due to the relatively larger cognitive load placed on these populations (Krikorian et al., 2012; Reger et al., 2004; Roberts & Besner, 2005). The participants in the present study were deemed healthy with a

comparatively young average age and no history of cognitive impairment. A further possibility may be due to the fact that some of the tests used in the current study may not have had sufficient reliability to account for the sensitive changes between the supplement and placebo.

Further research should look to assess cognitive function during intense exercises bouts with the use of ketone supplements. Bough et al. (2006) discovered that consumption of a ketogenic diet may enhance the ability of neurons to resist metabolic stress via two factors: a larger mitochondrial load - through its ability to stimulate mitochondrial biogenesis - and a more energy-efficient fuel. Therefore a combination of these factors may account for the enhanced ability of neurons to withstand metabolic challenges (Bough et al., 2006). The exhaustive nature of exercise may increase the cognitive load in healthy individuals, allowing for a greater need to improve cognitive function. Alongside an increase in cognitive load, the lower requirement for oxygen during exercise when ketones are used as a fuel suggests that the supplement may yet improve the accuracy of decision making in sporting scenarios (Sato et al., 1995; Veech, 2004).

The average ketone levels described by Hashim and VanItallie (2014) to induce therapeutic ketosis are thought to be ≥2 mmol/L. A limitation of the current study is that blood ketone levels were not measured; therefore we cannot be certain that the supplement altered blood ketone concentrations and previous research suggests that the blood ketone concentrations would have peaked well below 2 mmol/L. Numerous means to elevate BHB concentrations now exist and

ketone esters may have the ability to elevate ketone levels beyond that of the ketone salt used in the current study.

In conclusion our research showed that ketone supplementation did not improve cognitive function in a healthy, active population. A possible reason for the lack of significant findings in the current may be related to the relatively low cognitive load placed on our participant's. The use of ketone supplements in healthy populations placed under high cognitive loads, such as that that occurs during intense exercise, warrants further research.

Chapter 4:

Conclusion

Summary

The two studies included in this thesis were designed to investigate an oral ketone supplement on measures of physiological, cognitive and exercise performance. The first study examined cycling performance following the ingestion of exogenous ketone supplements. The research found that ketone supplementation in highly-trained cyclists failed to show meaningful improvements in regards to performance. The ketone trial showed an *unclear* effect on power output (mean ±90%CI; 2.3 ±4.8%) during a 4-minute all out time-trial compared to the placebo. In regards to physiological markers, ketone supplementation increased blood BHB concentrations three-fold; subsequently increasing RER values by 2.2 ±1.9% during submaximal exercise compared to the placebo (ES = 0.51 ±0.4; *moderate*). During the 4-minute test, increases in both the average VO_2 (2.4 ± 3.3%, ES = 0.24 ±0.3; *small*) and RER were evident (4.3 ±3.3%, ES = 0.75 ±0.6; *moderate*) in the ketone supplement trial compared to the placebo. These metabolic changes warrant the need for further research regarding the use of ketone supplementation for sparing glycogen and may have real world applications for sports such as road cycling, where intermittent bouts of intense exercise are dispersed amongst long periods of low-moderate intensity exercise. The second study conducted in Chapter Three found that ketone supplementation did not improve cognitive function in recreational athletes. Cognitive function was assessed through five tests designed to assess different components of neuro-muscular performance, reaction time, processing speed and memory recall; finger tap test (FTT), stroop test (ST), reaction time test (RT), monkey-ladder test (MLT) and one-card test (OCT), where test-retest

reliability for each test was also assessed. No statistically significant differences ($p > 0.05$) were observed in cognitive function between groups. Effect size (ES) analysis revealed *small* improvements in the KET trial for ST (ES = 0.34 ±0.4) as well as the MLT (ES = 0.34 ±0.4) with either *trivial* or *unclear* results for FTT, RT and OCT when compared to PLA. Whilst our study results differ from others that have conducted their research on individuals who suffer from either cognitive impairments or neurological disease, we put forward two possible reasons that may explain the opposing results. One is that the cognitive load placed on individuals was not enough to see substantial improvements following ingestion of the ketone supplement. The second is that the improvement in cognitive function for those with memory impairments and neurological disease stem from the mechanisms responsible for these disease states of which little is currently known.

Practical applications

The evidence from this research does not support the use of ketone supplementation as an ergogenic aid to facilitate peak athletic performances. Whilst the research demonstrated alterations in physiological markers, such as an increase in blood BHB concentrations, and an increase in RER, there currently stands no evidence to suggest ketone supplementation plays a role in improving athletic performance. To the best of our knowledge the research in this thesis is the first to assess the effects of an acute oral ketone supplementation on short-

term, high-intensity cycling performance. Consequently the use of ketone supplementation to aid athletic performances of different durations and modalities should not be ruled out. Accordingly this research should act as a reference for future work in the area. The following recommendations are made for further studies:

Future research

Cycling performance

- Carbohydrates may further enhance the ability of ketone supplementation due to their synergistic properties; future research should include a formula that combines synthetic ketones and carbohydrates.
- Knowledge gaps remain plentiful in regards to a diet induced ketosis and further research using ketone supplements may facilitate our knowledge of endogenous ketones. However, for this to be effective, we must improve our understanding of the differences between exogenous and endogenous ketones regarding physiology and metabolism. Research in the field may improve the current strategies for utilising both endogenous and exogenous ketones for exercise performance.
- Individual responses in our research varied; therefore further research should look to see whether training status has an impact on results. Future work should attempt to take into account the possibility of

responders and non-responders, and look to identify possible characteristics between these groups

- Future research should aim to see whether there may be a dose-response relationship with ketone supplementation.
- Ketone-esters may have the ability to elevate blood BHB concentrations higher than ketone salts (as used in the current study). Therefore research using ketone-esters may have a greater impact on exercise performance.
- Much of the reported benefits of ketones for exercise performance are for ultra-endurance events due to their glycogen sparing ability. Future research should use longer performance tests to assess whether ketone supplementation can enhance performance for events longer in duration.

Cognitive performance

- Future work should look to conduct cognitive assessments in-between periods of high intensity exercise to assess whether ketones improve cognitive performance under conditions of hypoxia.
- To assess cognitive function, future work should use tests that are greater in difficulty to the ones used in our research. It is thought that both the reliability of the tests used in our research and relatively low cognitive load that these tests placed on individuals were not high enough to see worthwhile changes in performance.

References

References

Alberti, K. G., Johnston, D. G., Gill, A., Barnes, A. J., & Orskov, H. (1978). Hormonal regulation of ketone-body metabolism in man. *Biochem Soc Symp*(43), 163-182.

Bajaj, J. S., Heuman, D. M., Sterling, R. K., Sanyal, A. J., Siddiqui, M., Matherly, S., . . . Wade, J. B. (2014). Validation of EncephalApp, Smartphone-based Stroop Test, for the diagnosis of covert hepatic encephalopathy. *Clinical Gastroenterology and Hepatology.*

Baker, L. B., Nuccio, R. P., & Jeukendrup, A. E. (2014). Acute effects of dietary constituents on motor skill and cognitive performance in athletes. *Nutrition Reviews, 72*(12), 790-802.

Bazzano, L. A., Hu, T., Reynolds, K., Yao, L., Bunol, C., Liu, Y., . . . He, J. (2014). Effects of low-carbohydrate and low-fat diets: A randomized tria leffects of low-carbohydrate and low-fat diets. *Annals of Internal Medicine, 161*(5), 309-318.

Bergman, B. C., & Brooks, G. A. (1999). Respiratory gas-exchange ratios during graded exercise in fed and fasted trained and untrained men. *Journal of Applied Physiology, 86*(2), 479-487.

Bergström, J., Hermansen, L., Hultman, E., & Saltin, B. (1967). Diet, muscle glycogen and physical performance. *Acta Physiologica Scandinavica, 71*(2), 140-150.

Best, T., Franz, D., Gilbert, D., Nelson, D., & Epstein, M. (2000). Cardiac complications in pediatric patients on the ketogenic diet. *Neurology, 54*(12), 2328-2330.

Blatter, K., & Cajochen, C. (2007). Circadian rhythms in cognitive performance: Methodological constraints, protocols, theoretical underpinnings. *Physiology & Behavior, 90*(2–3), 196-208.

Borg, G. A. (1982). Psychophysical bases of perceived exertion. *Medicine & Science in Sports & Exercise, 14*(5), 377-381.

Bosch, A. N., Dennis, S. C., & Noakes, T. D. (1993). Influence of carbohydrate loading on fuel substrate turnover and oxidation during prolonged exercise. *Journal of Applied Physiology, 74*(4), 1921-1927.

Bough, K. J., Wetherington, J., Hassel, B., Pare, J. F., Gawryluk, J. W., Greene, J. G., . . . Dingledine, R. J. (2006). Mitochondrial biogenesis in the anticonvulsant mechanism of the ketogenic diet. *Annals of Neurology, 60*(2), 223-235.

Bouteldja, N., Andersen, L. T., Møller, N., & Gormsen, L. C. (2014). Using positron emission tomography to study human ketone body metabolism: A review. *Metabolism, 63*(11), 1375-1384.

Brown, A. J. (2007). Low-carb diets, fasting and euphoria: Is there a link between ketosis and γ-hydroxybutyrate (GHB)? *Medical Hypotheses, 68*(2), 268-271.

Bueno, N. B., de Melo, I. S. V., de Oliveira, S. L., & da Rocha Ataide, T. (2013). Very-low-carbohydrate ketogenic diet v. Low-fat diet for long-term weight loss: A meta-analysis of randomised controlled trials. *British Journal of Nutrition, 110*(7), 1178-1187.

Burke, L. M., Cox, G. R., Cummings, N. K., & Desbrow, B. (2001). Guidelines for daily carbohydrate intake. *Sports Medicine, 31*(4), 267-299.

Burke, L. M., & Hawley, J. A. (2002). Effects of short-term fat adaptation on metabolism and performance of prolonged exercise. *Medicine and Science in Sports and Exercise, 34*(9), 1492-1498.

Cahill Jr, G. F. (1976). Starvation in man. *Clinics in Endocrinology and Metabolism, 5*(2), 397-415.

Cameron-Smith, D., Burke, L. M., Angus, D. J., Tunstall, R. J., Cox, G. R., Bonen, A., . . . Hargreaves, M. (2003). A short-term, high-fat diet up-regulates lipid metabolism and gene expression in human skeletal muscle. *The American Journal of Clinical Nutrition, 77*(2), 313-318.

Cameron-Smith, D., Burke, L. M., Angus, D. J., Tunstall, R. J., Cox, G. R., Bonen, A., . . . Hargreaves, M. (2003). A short-term, high-fat diet up-regulates lipid metabolism and gene expression in human skeletal muscle. *American Journal of Clinical Nutrition, 77*(2), 313-318.

Carlier, M., Dumont, A. M., Beau, J., & Michel, F. (1993). Hand performance of French children on a finger-tapping test in relation to handedness, sex, and age. *Perceptual and Motor Skills, 76*(3), 931-940.

Clarke, K., Tchabanenko, K., Pawlosky, R., Carter, E., Knight, N. S., Murray, A. J., . . . Veech, R. L. (2012). Oral 28-day and developmental toxicity studies of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate. *Regulatory Toxicology and Pharmacology, 63*(2), 196-208.

Clarke, K., Tchabanenko, K., Pawlosky, R., Carter, E., Todd King, M., 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, 63*(3), 401-408.

Coggan, A. R., & Coyle, E. F. (1991). Carbohydrate ingestion during prolonged exercise: effects on metabolism and performance. *Exercise and Sport Sciences Review, 19*, 1-40.

Costill, D., Coyle, E., Dalsky, G., Evans, W., Fink, W., & Hoopes, D. (1977). Effects of elevated plasma FFA and insulin on muscle glycogen usage during exercise. *Journal of Applied Physiology, 43*(4), 695-699.

Cox, P. J., & Clarke, K. (2014). Acute nutritional ketosis: Implications for exercise performance and metabolism. *Extreme Physiology & Medicine, 3*(1), 17.

D'Agostino, D. P., Arnold, P., & Kesl, S. (2014). Compositions and methods for producing elevated and sustained ketosis: US20140350105 A1. Retrieved from http://www.google.com/patents/US20140350105

D'Agostino, D. P., Dean, J. B., Pilla, R., & Arnold, P. (2014). Use of ketone esters for prevention of cns oxygen toxicity: US20140073693 A1. Retrieved from http://www.google.com/patents/US20140073693

D'Agostino, D. P., Pilla, R., Held, H. E., Landon, C. S., Puchowicz, M., Brunengraber, H., . . . Dean, J. B. (2013). Therapeutic ketosis with ketone ester delays central nervous system oxygen toxicity seizures in rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 304*(10), R829-R836.

Driller, M. W., Argus, C. K., Bartram, J. C., Bonaventura, J., Martin, D. T., West, N. P., & Halson, S. L. (2014). Reliability of a 2-Bout exercise test on a Wattbike cycle ergometer. *International Journal of Sports Physiology and Performance, 9*(2), 340–345.

Farrand, S. (2015). *Tour de France: Froome hits back at doubts and innuendo.* Retrieved from http://www.cyclingnews.com/news/tour-de-france-froome-hits-back-at-doubts-and-innuendo/

Floyd Jr, J. C., Fajans, S. S., Conn, J. W., Knopf, R. F., & Rull, J. (1966). Insulin secretion in response to protein ingestion. *Journal of Clinical Investigation, 45*(9), 1479.

Forsythe, C. E., Phinney, S. D., Fernandez, M. L., Quann, E. E., Wood, R. J., Bibus, D. M., . . . Volek, J. S. (2008). Comparison of low fat and low carbohydrate diets on circulating fatty acid composition and markers of inflammation. *Lipids, 43*(1), 65-77.

Frayn, K. N. (1983). Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of Applied Physiology, 55*(2), 628-634.

Galbo, H., Holst, J., & Christensen, N. (1979). The effect of different diets and of insulin on the hormonal response to prolonged exercise. *Acta Physiologica Scandinavica, 107*(1), 19-32.

Gasior, M., Rogawski, M. A., & Hartman, A. L. (2006). Neuroprotective and disease-modifying effects of the ketogenic diet. *Behavioural Pharmacology, 17*(5-6), 431.

Gilbert, D. L., Pyzik, P. L., & Freeman, J. M. (2000). The ketogenic diet: Seizure control correlates better with serum β-hydroxybutyrate than with urine ketones. *Journal of Child Neurology, 15*(12), 787-790.

Goedecke, J. H., Christie, C., Wilson, G., Dennis, S. C., Noakes, T. D., Hopkins, W. G., & Lambert, E. V. (1999). Metabolic adaptations to a high-fat diet in endurance cyclists. *Metabolism, 48*(12), 1509-1517.

Guzmán, M., & Blázquez, C. (2004). Ketone body synthesis in the brain: Possible neuroprotective effects. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 70*(3), 287-292.

Halestrap, A. P., & Wilson, M. C. (2012). The monocarboxylate transporter family—role and regulation. *IUBMB Life, 64*(2), 109-119.

Hallbook, T., Ji, S., Maudsley, S., & Martin, B. (2012). The effects of the ketogenic diet on behavior and cognition. *Epilepsy Research, 100*(3), 304-309.

Hargreaves, M., Hawley, J. A., & Jeukendrup, A. (2004). Pre-exercise carbohydrate and fat ingestion: effects on metabolism and performance. *Journal of Sports Sciences, 22*(1), 31-38.

Hashim, S. A., & VanItallie, T. B. (2014). Ketone body therapy: From the ketogenic diet to the oral administration of ketone ester. *Journal of Lipid Research, 55*(9), 1818-1826.

Havemann, L., West, S., Goedecke, J., Macdonald, I., Gibson, A. S. C., Noakes, T., & Lambert, E. (2006). Fat adaptation followed by carbohydrate loading

compromises high-intensity sprint performance. *Journal of Applied Physiology, 100*(1), 194-202.

Hopkins, W. G. (1997). *A new view of statistics*. Retrieved from http://www.sportsci.org/resource/stats/

Hopkins, W. G. (2000). Measures of reliability in sports medicine and science. *Sports Medicine, 30*(1), 1-15.

Hopkins, W. G. (2006). *Analysis of a post-only crossover trial (Excel spreadsheet).* Retrieved from newstats. org/PostOnlyCrossover. xls

Hopkins, W. G., & Batterham, A. (2006). Making meaningful inferences about magnitudes. *International Journal of Sports Physiology and Performance, 1*(1), 50-57.

Hopkins, W. G., Schabort, E. J., & Hawley, J. A. (2001). Reliability of power in physical performance tests. *Sports Medicine, 31*(3), 211-234.

Inoue, S., & Matsuzawa, T. (2007). Working memory of numerals in chimpanzees. *Current Biology, 17*(23), R1004-R1005.

Jakobsen, L. H., Sorensen, J. M., Rask, I. K., Jensen, B. S., & Kondrup, J. (2011). Validation of reaction time as a measure of cognitive function and quality of life in healthy subjects and patients. *Nutrition, 27*(5), 561-570.

Jenkins, A., Storlien, L., Chisholm, D., & Kraegen, E. (1988). Effects of nonesterified fatty acid availability on tissue-specific glucose utilization in rats in vivo. *Journal of Clinical Investigation, 82*(1), 293-299.

Jeukendrup, A. (2014). A step towards personalized sports nutrition: Carbohydrate intake during exercise. *Sports Medicine, 44*(1), 25-33.

Johnstone, A. M., Horgan, G. W., Murison, S. D., Bremner, D. M., & Lobley, G. E. (2008). Effects of a high-protein ketogenic diet on hunger, appetite, and

weight loss in obese men feeding ad libitum. *American Journal of Clinical Nutrition, 87*(1), 44-55.

Kashiwaya, Y., Sato, K., Tsuchiya, N., Thomas, S., Fell, D. A., Veech, R. L., & Passonneau, J. V. (1994). Control of glucose utilization in working perfused rat heart. *Journal of Biological Chemistry, 269*(41), 25502-25514.

Kashiwaya, Y., Takeshima, T., Mori, N., Nakashima, K., Clarke, K., & Veech, R. L. (2000). D-beta-hydroxybutyrate protects neurons in models of Alzheimer's and Parkinson's disease. *Proceedings of the National Academy of Sciences of the United States of America, 97*(10), 5440-5444.

Kielb, S., Koo, H. P., Bloom, D. A., & Faerber, G. J. (2000). Nephrolithiasis associated with the ketogenic diet. *Journal of Urology, 164*(2), 464-466.

Kim, D., Y., Davis, L. M., Sullivan, P. G., Maalouf, M., Simeone, T. A., van Brederode, J., & Rho, J. M. (2007). Ketone bodies are protective against oxidative stress in neocortical neurons. *Journal of Neurochemistry, 101*(5), 1316-1326.

Kinsman, S. L., Vining, E. P., Quaskey, S. A., Mellits, D., & Freeman, J. M. (1992). Efficacy of the ketogenic diet for intractable seizure disorders: Review of 58 cases. *Epilepsia, 33*(6), 1132-1136.

Krebs, H. A. (1966). The regulation of the release of ketone bodies by the liver. *Advances in Enzyme Regulation, 4*, 339-353.

Krikorian, R., Shidler, M. D., Dangelo, K., Couch, S. C., Benoit, S. C., & Clegg, D. J. (2012). Dietary ketosis enhances memory in mild cognitive impairment. *Neurobiology of Aging, 33*(2), 425.e419–425.e427.

Laffel, L. (1999). Ketone bodies: A review of physiology, pathophysiology and application of monitoring to diabetes. *Diabetes/Metabolism Research and Reviews, 15*(6), 412-426.

Lambert, E. V., Goedecke, J. H., van Zyl, C., Murphy, K., Hawley, J. A., Dennis, S. C., & Noakes, T. D. (2001). High-fat diet versus habitual diet prior to carbohydrate loading: Effects on exercise metabolism and cycling performance. *International Journal of Sport Nutrition and Exercise Metabolism, 11*(2), 209-225.

Lambert, E. V., Speechly, D. P., Dennis, S. C., & Noakes, T. D. (1994). Enhanced endurance in trained cyclists during moderate intensity exercise following 2 weeks adaptation to a high fat diet. *European Journal of Applied Physiology and Occupational Physiology, 69*(4), 287-293.

Langfort, J., Zarzeczny, R., Pilis, W., Nazar, K., & Kaciuba-Uścitko, H. (1997). The effect of a low-carbohydrate diet on performance, hormonal and metabolic responses to a 30-s bout of supramaximal exercise. *European Journal of Applied Physiology and Occupational Physiology, 76*(2), 128-133.

Levy, R. G., Cooper, P. N., & Giri, P. (2012). *Ketogenic diet and other dietary treatments for epilepsy* (Cochrane Database of Systematic Reviews CD001903). Retrieved from http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD001903.pub2/epdf

Maalouf, M., Rho, J. M., & Mattson, M. P. (2009). The neuroprotective properties of calorie restriction, the ketogenic diet, and ketone bodies. *Brain Research Reviews, 59*(2), 293-315.

Maalouf, M., Sullivan, P. G., Davis, L., Kim, D. Y., & Rho, J. M. (2007). Ketones inhibit mitochondrial production of reactive oxygen species production following glutamate excitotoxicity by increasing NADH oxidation. *Neuroscience, 145*(1), 256-264.

Manninen, A. H. (2004). Metabolic effects of the very-low-carbohydrate diets: Misunderstood "villains" of human metabolism. *Journal of the International Society of Sports Nutrition, 1*(2), 7-11.

Masino, S. A., Kawamura Jr, M., Wasser, C. D., Pomeroy, L. T., & Ruskin, D. N. (2009). Adenosine, ketogenic diet and epilepsy: The emerging therapeutic relationship between metabolism and brain activity. *Current Neuropharmacology, 7*(3), 257-268.

Mayford, M., Siegelbaum, S. A., & Kandel, E. R. (2012). Synapses and memory storage. *Cold Spring Harbor Perspectives in Biology, 4*(6), a005751. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3367555/pdf/cshperspect-SYP-a005751.pdf

Miller, W. C., Bryce, G. R., & Conlee, R. K. (1984). Adaptations to a high-fat diet that increase exercise endurance in male rats. *Journal of Applied Physiology, 56*(1), 78-83.

Neal, E. G., Chaffe, H., Schwartz, R. H., Lawson, M. S., Edwards, N., Fitzsimmons, G., . . . Cross, J. H. (2008). The ketogenic diet for the treatment of childhood epilepsy: a randomised controlled trial. *The Lancet Neurology, 7*(6), 500-506.

Noh, H. S., Lee, H. P., Kim, D. W., Kang, S. S., Cho, G. J., Rho, J. M., & Choi, W. S. (2004). A cDNA microarray analysis of gene expression profiles in rat hippocampus following a ketogenic diet. *Molecular Brain Research, 129*(1-2), 80-87.

Norman, K. A., & O'Reilly, R. C. (2003). Modeling hippocampal and neocortical contributions to recognition memory: A complementary-learning-systems approach. *Psychological Review, 110*(4), 611-646.

O'Keeffe, K. A., Keith, R. E., Wilson, G. D., & Blessing, D. L. (1989). Dietary carbohydrate intake and endurance exercise performance of trained female cyclists. *Nutrition Research, 9*(8), 819-830.

Owen, O. E., Morgan, A. P., Kemp, H. G., Sullivan, J. M., Herrera, M. G., & Cahill, G. F., Jr. (1967). Brain metabolism during fasting. *Journal of Clinical Investigation, 46*(10), 1589-1595.

Page, K. A., Williamson, A., Yu, N., McNay, E. C., Dzuira, J., McCrimmon, R. J., & Sherwin, R. S. (2009). Medium-chain fatty acids improve cognitive function in intensively treated type 1 diabetic patients and support in vitro synaptic transmission during acute hypoglycemia. *Diabetes, 58*(5), 1237-1244.

Paoli, A. (2014). Ketogenic diet for obesity: Friend or foe? *International Journal of Environmental Research and Public Health, 11*(2), 2092-2107.

Paoli, A., Bianco, A., & Grimaldi, K. A. (2015). The ketogenic diet and sport: A possible marriage? *Exercise and Sport Sciences Reviews, 43*(3), 153-162.

Paoli, A., Bosco, G., Camporesi, E. M., & Mangar, D. (2015). Ketosis, ketogenic diet and food intake control: A complex relationship. *Frontiers in Psychology, 6*, 27.

Paoli, A., Grimaldi, K., D'Agostino, D., Cenci, L., Moro, T., Bianco, A., & Palma, A. (2012). Ketogenic diet does not affect strength performance in elite artistic gymnasts. *Journal of the International Society of Sports Nutrition, 9*(1), 34. Retrieved from http://www.jissn.com/content/9/1/34

Paoli, A., Rubini, A., Volek, J., & Grimaldi, K. (2013). Beyond weight loss: A review of the therapeutic uses of very-low-carbohydrate (ketogenic) diets. *European Journal of Clinical Nutrition, 67*(8), 789-796.

Patel, A., Pyzik, P. L., Turner, Z., Rubenstein, J. E., & Kossoff, E. H. (2010). Long-term outcomes of children treated with the ketogenic diet in the past. *Epilepsia, 51*(7), 1277-1282.

Phinney, S. D. (2004). Ketogenic diets and physical performance. *Nutrition & Metabolism, 1*(1), 2. Retrieved from http://www.biomedcentral.com/content/pdf/1743-7075-1-2.pdf

Phinney, S. D., Bistrian, B., Evans, W., Gervino, E., & Blackburn, G. (1983). The human metabolic response to chronic ketosis without caloric restriction: Preservation of submaximal exercise capability with reduced carbohydrate oxidation. *Metabolism, 32*(8), 769-776.

Phinney, S. D., Horton, E. S., Sims, E. A., Hanson, J. S., Danforth Jr, E., & Lagrange, B. M. (1980). Capacity for moderate exercise in obese subjects after adaptation to a hypocaloric, ketogenic diet. *Journal of Clinical Investigation, 66*(5), 1152-1161.

Pilegaard, H., Domino, K., Noland, T., Juel, C., Hellsten, Y., Halestrap, A. P., & Bangsbo, J. (1999). Effect of high-intensity exercise training on lactate/H+ transport capacity in human skeletal muscle. *American Journal of Physiology, 276*(2), E255-E261.

Poff, A. M., Ari, C., Arnold, P., Seyfried, T. N., & D'Agostino, D. P. (2014). Ketone supplementation decreases tumor cell viability and prolongs survival of mice with metastatic cancer. *International Journal of Cancer, 135*(7), 1711-1720.

Reger, M. A., Henderson, S. T., Hale, C., Cholerton, B., Baker, L. D., Watson, G., . . . Craft, S. (2004). Effects of β-hydroxybutyrate on cognition in memory-impaired adults. *Neurobiology of aging, 25*(3), 311-314.

Reger, M. A., Henderson, S. T., Hale, C., Cholerton, B., Baker, L. D., Watson, G. S., . . . Craft, S. (2004). Effects of β-hydroxybutyrate on cognition in memory-impaired adults. *Neurobiology of Aging, 25*(3), 311-314.

Rhyu, H.-s., & Cho, S.-Y. (2014). The effect of weight loss by ketogenic diet on the body composition, performance-related physical fitness factors and cytokines of Taekwondo athletes. *Journal of exercise rehabilitation, 10*(5), 326.

Roberts, M. A., & Besner, D. (2005). Stroop dilution revisited: Evidence for domain-specific, limited-capacity processing. *Journal of Experimental Psychology: Human Perception and Performance, 31*(1), 3-13.

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.

Sandri, M., Barberi, L., Bijlsma, A., Blaauw, B., Dyar, K., Milan, G., . . . Paoli, A. (2013). Signalling pathways regulating muscle mass in ageing skeletal muscle. The role of the IGF1-Akt-mTOR-FoxO pathway. *Biogerontology, 14*(3), 303-323.

Sato, K., Kashiwaya, Y., Keon, C., Tsuchiya, N., King, M., Radda, G., . . . Veech, R. (1995). Insulin, ketone bodies, and mitochondrial energy transduction. *The FASEB Journal, 9*(8), 651-658.

Sawyer, J. C., Wood, R. J., Davidson, P. W., Collins, S. M., Matthews, T. D., Gregory, S. M., & Paolone, V. J. (2013). Effects of a short-term carbohydrate- restricted diet on strength and power performance. *Journal of Strength & Conditioning Research, 27*(8), 2255-2262.

Schear, J. M., & Sato, S. D. (1989). Effects of visual acuity and visual motor speed and dexterity on cognitive test performance. *Archives of Clinical Neuropsychology, 4*(1), 25-32.

Schmidt, C., Collette, F., Cajochen, C., & Peigneux, P. (2007). A time to think: Circadian rhythms in human cognition. *Cognitive Neuropsychology, 24*(7), 755-789.

Schmitt, L. (2013). Finger-Tapping Test. In F. R. Volkmar (Ed.), *Encyclopedia of Autism Spectrum Disorders* (pp. 1296-1296). New York, NY: Springer.

Seyfried, T. N. (2014). Ketone strong: Emerging evidence for a therapeutic role of ketone bodies in neurological and neurodegenerative diseases. *Journal of Lipid Research, 55*(9), 1815-1817.

Sondike, S. B., Copperman, N., & Jacobson, M. S. (2003). Effects of a low-carbohydrate diet on weight loss and cardiovascular risk factor in overweight adolescents. *Journal of Pediatrics, 142*(3), 253-258.

Spooner, D. M., & Pachana, N. A. (2006). Ecological validity in neuropsychological assessment: A case for greater consideration in research with neurologically intact populations. *Archives of Clinical Neuropsychology, 21*(4), 327-337.

Srivastava, S., Kashiwaya, Y., King, M. T., Baxa, U., Tam, J., Niu, G., . . . Veech, R. L. (2012). Mitochondrial biogenesis and increased uncoupling protein 1 in brown adipose tissue of mice fed a ketone ester diet. *The FASEB Journal, 26*(6), 2351-2362.

Stellingwerff, T., Spriet, L. L., Watt, M. J., Kimber, N. E., Hargreaves, M., Hawley, J. A., & Burke, L. M. (2006). Decreased PDH activation and glycogenolysis during exercise following fat adaptation with carbohydrate restoration. *American Journal of Physiology-Endocrinology and Metabolism, 290*(2), E380-E388.

Stern, L., Iqbal, N., Seshadri, P., Chicano, K. L., Daily, D. A., McGrory, J., . . . Samaha, F. F. (2004). The effects of low-carbohydrate versus conventional weight loss diets in severely obese adults: one-year follow-up of a randomized trial. *Annals of Internal Medicine, 140*(10), 778-785.

Tampellini, D., Capetillo-Zarate, E., Dumont, M., Huang, Z., Yu, F., Lin, M. T., & Gouras, G. K. (2010). Effects of synaptic modulation on β-amyloid, synaptophysin and memory performance in Alzheimer's disease

transgenic mice. *Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 30*(43), 14299-14304.

Tokish, J. M., Kocher, M. S., & Hawkins, R. J. (2004). Ergogenic aids: a review of basic science, performance, side effects, and status in sports. *American Journal of Sports Medicine, 32*(6), 1543-1553.

Turocy, P. S., DePalma, B. F., Horswill, C. A., Laquale, K. M., Martin, T. J., Perry, A. C., . . . Utter, A. C. (2011). National athletic trainers' association position statement: safe weight loss and maintenance practices in sport and exercise. *Journal of Athletic Training, 46*(3), 322-336.

Vanitallie, T. B., Nonas, C., Di Rocco, A., Boyar, K., Hyams, K., & Heymsfield, S. B. (2005). Treatment of Parkinson disease with diet-induced hyperketonemia: A feasibility study. *Neurology, 64*(4), 728-730.

Veech, R. L. (2004). The therapeutic implications of ketone bodies: The effects of ketone bodies in pathological conditions: Ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 70*(3), 309-319.

Veech, R. L. (2014). Ketone ester effects on metabolism and transcription. *Journal of Lipid Research, 55*(10), 2004-2006.

Veech, R. L., Chance, B., Kashiwaya, Y., Lardy, H. A., & Cahill, G. F., Jr. (2001). Ketone bodies, potential therapeutic uses. *IUBMB Life, 51*(4), 241-247.

Westman, E. C., Yancy Jr, W. S., Mavropoulos, J. C., Marquart, M., & McDuffie, J. R. (2008). The effect of a low-carbohydrate, ketogenic diet versus a low-glycemic index diet on glycemic control in type 2 diabetes mellitus. *Nutrition & Metabolism, 5*(1), 36. Retrieved from http://www.biomedcentral.com/content/pdf/1743-7075-5-36.pdf

Yancy Jr, W. S., Foy, M., Chalecki, A. M., Vernon, M. C., & Westman, E. C. (2005). A low-carbohydrate, ketogenic diet to treat type 2 diabetes. *Nutrition &*

Metabolism, 2, 34. Retrieved from http://www.biomedcentral.com/content/pdf/1743-7075-2-34.pd

Yassa, M. A., Stark, S. M., Bakker, A., Albert, M. S., Gallagher, M., & Stark, C. E. (2010). High-resolution structural and functional MRI of hippocampal CA3 and dentate gyrus in patients with amnestic mild cognitive impairment. *Neuroimage, 51*(3), 1242-1252.

Zajac, A., Poprzecki, S., Maszczyk, A., Czuba, M., Michalczyk, M., & Zydek, G. (2014). The effects of a ketogenic diet on exercise metabolism and physical performance in off-road cyclists. *Nutrients, 6*(7), 2493-2508.

Zhou, W., Mukherjee, P., Kiebish, M. A., Markis, W. T., Mantis, J. G., & Seyfried, T. N. (2007). The calorically restricted ketogenic diet, an effective alternative therapy for malignant brain cancer. *Nutrition & Metabolism, 4*(5), 5. Retrieved from http://www.biomedcentral.com/content/pdf/1743-7075-4-5.pdf&embedded=true

Zupec-Kania, B. A., & Spellman, E. (2008). An overview of the ketogenic diet for pediatric epilepsy. *Nutrition in Clinical Practice, 23*(6), 589-596.

Appendices

Appendix 1 - Research consent form - Effects of an oral ketone supplement on exercise metabolism and cycling performance

Informed Consent form

Project Title: Effect of acute ketone supplementation on cycling performance and metabolism.

Principal Researchers: Shem Rodger, Dr. Matt Driller

This is to certify that I, hereby agree to participate as a volunteer in a scientific investigation as an authorised part of the research program of the Waikato University Sport and Leisure Department under the supervision of .

The investigation and my part in the investigation have been defined and fully explained to me by and I understand the explanation. A copy of the procedures of this investigation and a description of any risks and discomforts has been provided to me and has been discussed in detail with me.

- I have been given an opportunity to ask whatever questions I may have had and all such questions and inquires have been answered to my satisfaction.

- I understand that I am free to withdraw consent and to discontinue participation in the project or activity at any time, without disadvantage to myself.

- I understand that I am free to withdraw my data up until the point of analysis without disadvantage to myself.

- I understand that any data will remain anonymous with regard to my identity through a coding system. The data will be made publishable, so every effort will be made to ensure confidentiality, however this cannot be guaranteed.

- I certify to the best of my knowledge and belief, I have no physical or mental illness or weakness that would increase the risk to me of participation in this investigation.

- I am participating in this project of my (his/her) own free will and I have not been coerced in any way to participate.

Signature of Subject: _____ Date: ____/____/____

I, the undersigned, was present when the study was explained to the subject/s in detail and to the best of my knowledge and belief it was understood.

Signature of Researcher: _____
Date:___/____/____

Appendix 2 - Participant information sheet- Effects of an oral ketone supplement on exercise metabolism and cycling performance

Participant Information:

Dear participant,

You are being invited to take part in a research study, which will help the Waikato University Sport and Leisure Department determine the effect that ketone supplements have on the cycling performance. Before you volunteer to take part in the study please take the time to read the following information carefully and if there is anything that is not clear or you would like more information, please feel free to contact us.

Purpose

The aim of the study is to determine the effects of ingesting ketones as an ergogenic aid for cycling performance.

Significance

Theory indicates that ingestion of ketone supplement may have the ability to mimic the metabolic adaptations of a ketogenic diet a topic of interest for many high level athletes. This study concentrates on a new and exciting nutritional supplement that may enhance performance.

We will seek to answer the question of whether or not the use of such a supplement can alter an athlete's performance or metabolism. The significance of such a study will provide an insight into the use of an exogenous ketone for athletes competing in events between the 4 to 8 minute ranges.

To date, little is understood about the effect of ketone supplement on exercise performance. As such, the purpose of this study is to provide an insight into the supplements use prior to high-intensity exercise in well-trained cyclists.

Selection Criteria

A total of 12 trained cyclists who can commit to three testing sessions will be selected. Participants will have to be healthy and have no contraindications to vigorous exercise.

To be eligible in this study you must be:

- Aged between 18 and 50 years
- Have a $VO_{2\ max}$ >60ml.kg^{-1}.min^{-1}

And NOT have the following:

- Injury, illness or health issue which would disrupt the subject's performance eg. lower limb injury

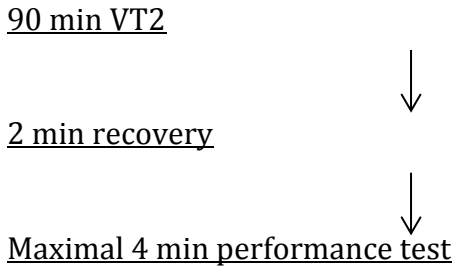
- Injury, illness or health issue which would endanger the subjects health eg. heart condition

If you meet all of these criteria then you can choose to participate in this research project.

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason

Protocol

Participants are required to attend the Waikato University laboratory at the Avantidrome in Cambridge on three separate occasions. One familiarisation session where a VO_{2max} test will be conducted and a practice exercise trial will be completed. One week after the familiarisation session participants will undergo the first experimental session, with the second experimental session taking place at least 48 hours after the first trial. Each participant will conduct an exercise trial taking the placebo and ketone supplement. The outline of the experimental session is highlighted below.



What you will gain from participating in the study?

As a participant, you will benefit from experience with the research process and gain knowledge about the area of research. You will be involved in innovative research, which will provide valuable information on sporting performance.

All information collected about you during the course of the research project will be kept strictly confidential. You will be identified by a code number and all personal information will be kept private. Although all measures will be taken to preserve anonymity, this cannot be guaranteed

Any inquiries regarding requirements and procedures used in this study are encouraged. Please contact me if you have any questions.

Researcher Contact Details

Shem Rodger
Post-graduate student
Waikato University, Sport and Leisure department.
Mob: [redacted]
Email: [redacted]

Appendix 3 - Research consent form - The effects of acute ketone supplementation on cognitive function

Informed Consent form

Project Title: The effect of ketone supplementation on cognitive performance.

Principal Researchers: Nicole Bates, Jeremy Spence, Shem Rodger, Dr. Matt Driller.

This is to certify that I, hereby agree to participate as a volunteer in a scientific investigation as an authorised part of the research program of the Waikato University School of Human Development and Movement Studies under the supervision of .

The investigation and my part in the investigation have been defined and fully explained to me by and I understand the explanation. A copy of the procedures of this investigation and a description of any risks and discomforts has been provided to me and has been discussed in detail with me.

- I have been given an opportunity to ask whatever questions I may have had and all such questions and inquires have been answered to my satisfaction.

- I understand that I am free to withdraw consent and to discontinue participation in the project or activity at any time, without disadvantage to myself.

- I understand that I am free to withdraw my data up until the point of recording without disadvantage to myself.

- I understand that any data will remain anonymous with regard to my identity through a coding system. The data will be made publishable, so every effort will be made to ensure confidentiality, however this cannot be guaranteed.

- I certify to the best of my knowledge and belief, I have no physical or mental illness or weakness that would increase the risk to me of participation in this investigation.

- I am participating in this project of my (his/her) own free will and I have not been coerced in any way to participate.

Signature of Subject: _____ Date: ___/___/___

I, the undersigned, was present when the study was explained to the subject/s in detail and to the best of my knowledge and belief it was understood.

Signature of Researcher: _____
Date:___/____/____

Appendix 4 - Participant information sheet- The effects of acute ketone supplementation on cognitive function

Participant Information:

Dear participant,

You are being invited to take part in a research study, which will help the University of Waikato Sport and Leisure Department determine the effect that ketone supplements have on cognitive function. Before you volunteer to take part in the study please take the time to read the following information carefully and if there is anything that is not clear or you would like more information, please feel free to contact us.

Purpose
The aim of the study is to determine the effects of ingesting a ketone supplement on cognitive function.

Significance

Numerous studies have indicated that elevated ketone levels have shown improvements in cognitive function in those suffering from mild cognitive impairments. To date, little is understood about the effect of ketone supplements on cognitive performance in healthy individuals although plethora of research has reported improvements in those suffering from neurodegenerative diseases such as Alzheimer's. As such, the purpose of this study is to provide an insight into the supplements used to enhance cognitive performance.

Selection Criteria
40-50 healthy participants who can commit to one 1-hour session will be selected.

To be eligible in this study you must be:
- Aged between 18 and 50 years

And NOT have the following:
- Injury, illness or health issue which would disrupt the subject's performance on an iPad with a touch screen. e.g. Injured finger, blind.

If you meet all of these criteria then you can choose to participate in this research project.

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason

Protocol

Participants are required to attend the University of Waikato laboratory at the Avantidrome in Cambridge on one occasion. At the beginning of this session three familiarisation trials will be conducted to ensure participants are aware of how the tests operate and minimise the possible learning effects. After this a baseline test is to be completed prior to ingesting the supplement. Upon ingesting the supplement, participants must wait 45 minutes before repeating the same cognitive tests. Experimental trials will include a memory test, stroop test and reaction time test. These tests are to be completed on an iPad.

What you will gain from participating in the study?

As a participant, you will benefit from experience with the research process and gain knowledge about the area of research. You will be involved in innovative research that will provide valuable information on performance in regards to cognitive function.

All information collected about you during the course of the research project will be kept strictly confidential. You will be identified by a code number and all personal information will be kept private.

Any inquiries regarding requirements and procedures used in this study are encouraged. Please contact any of us if you have any questions.

Research Contact Details

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Appendix 5 - Pre-Test Medical Questionnaire

First Name/s _____ Surname

_____

Date of Birth _____/_____/_____ Gender (circle) Male

Female

Please answer the following questions by circling the appropriate response, or filling in the blank.

1. How would you describe your present level of activity?
   Sedentary Moderately Active Active Highly Active

2. How would you describe your present level of fitness?
   Unfit Moderately Fit Trained Highly Trained

3. How would you consider your present body weight?
   Underweight Ideal Slightly Over Very Overweight

4. Smoking habits:
   Are you currently a smoker? **Yes** **No**
   How many do you smoke? ……per day

   Are you a previous smoker? **Yes** **No**
   How long is it since you stopped? ……..years

   Were you an occasional smoker? **Yes** **No**

   ……per day

   Were you a regular smoker? **Yes** **No**

   ……per day

5. **Do you drink alcohol?** **Yes** **No**
   If you answered **Yes**, do you have?

   An occasional drink A drink everyday More than one drink a day

6. **Have you had to consult your doctor in the previous six months?**
   If you have answered **Yes**, please give details…….
   ……
   ……

7. **Are you presently taking any form of medication?**
   If you have answered **Yes**, please give details…….
   ……
   ……

8. **As far as you are aware, do you suffer from or have you ever suffered from?(circle if yes to any)**

a. Diabetes	b. Asthma
c. Epilepsy	d. Bronchitis
d. Any form of heart complaint*	e. Raynaud's Disease
f. Marfans Syndrome*	h. Aneurysm/embolism*
i. Anaemia	j. Haemophilia*

Please continue filling form over the page.

9. ***Is there a history of heart disease in your family?** **Yes** **No**
10. ***Do you currently have any form of muscle or joint injury?** **Yes** **No**
11. **Have you had to suspend your normal training in the previous two weeks?** **Yes** **No**
12. **Please read and answer the following questions:**
    a. Are you suffering from any known serious infections? **Yes** **No**
    b. Have you had jaundice within the previous year? **Yes** **No**
    c. Have you ever had any form of hepatitis? **Yes** **No**

d. Are you HIV antibody positive? **Yes**

No

e. Have you ever been involved in intravenous drug use? **Yes**

No

f. For females, are you currently, or in the previous 6 months, pregnant? **Yes**

No

13. As far as you are aware, is there anything that might prevent you from successfully completing the tests that have been outlined to you?

If the answer to any of the above questions is yes then:

a. Discuss with the clinic personal the nature of the issue

Consent of Athlete/Participant

_____ _____/_____/_____

Athlete/Participant Signature Date

_____ _____

____/_____/____

Guardian name (required if age less than 16 yrs) Athlete/Participant Signature Date

_____ _____

____/_____/____

Witness name Signature Date

Appendix 6 - UOW laboratory informed consent form

I (print name) _____ consent to participate in physiological assessment on the following terms:

1. I have read the Explanation of Physiological Assessment Procedures attached and have understood what I will be required to do. I have had the opportunity to ask questions and received satisfactory explanations about the assessment/s to be conducted.

2. I understand that I will be undertaking physical exercise at or near the extent of my physical capacity and there is possible risk in the physical exercise at that level, such as episodes of transient light-headedness, fainting, abnormal blood pressure, chest discomfort.

3. I understand that this may occur although the staff in this laboratory will take all proper care in the conduct of the assessment, and I fully assume that risk.

4. I understand that I can withdraw my consent, freely and without prejudice, at any time before, during or after testing.

5. I have told the person conducting the assessment of any illness or physical defect I have that may contribute to the level of that risk.

6. I understand that the information obtained from the test will be treated confidentially with my right to privacy assured. However, the information may be used for statistical or scientific purposes with privacy retained.

7. I release this laboratory and its employees from any liability for any injury or illness that I may experience during the assessment as well as any subsequent injury or illness that is connected to or to any extent influenced by the assessment.

8. I will indemnify this laboratory in respect to any liability it may incur in relation to any other person in connection with the assessment.

9. I hereby agree that I will present myself for testing in a suitable condition and have abided by any requirements for diet and activity prescribed to me by laboratory staff.

_____ _____/_____/_____

Athlete/Participant Signature Date

_____ _____
____/_____/____

Guardian name (required if age less than 16 yrs) Athlete/Participant Signature Date

_____ _____
____/_____/____

Witness name Signature Date

From Australian Institute of Sport, 2013, *Physiological tests for elite athletes,* 2nd ed. (Champaign, IL: Human Kinetics).

Appendix 7 - Rate of perceived exertion scale

Rate of Perceived Exertion Scale

6	**No Exertion at All**
7	**Extremely Light**
8	
9	**Very Light**
10	
11	**Light**
12	
13	**Somewhat Hard**
14	
15	**Hard (Heavy)**
16	
17	**Very Hard**
18	
19	**Extremely Hard**
20	**Maximal Exertion**

Borg, G.A., (1982). Physiological basis of physical exertion. *Medicine and Science in Sport and Exercise*, 14, p 377.

Appendix 8 - Ethics approval

Dean's Office
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THE UNIVERSITY OF WAIKATO
Te Whare Wānanga o Waikato

MEMORANDUM

To: **Shem Rodger**

cc: Dr Matt Driller
Dr Karen Barbour

From: **Dr Nicola Daly**
Chairperson (Acting), Research Ethics Committee

Date: 3 November 2014

Subject: Supervised Postgraduate Research - Application for Ethical Approval (EDU087/14)

Thank you for submitting the amendments to your application for ethical approval for the research project:

The effect of ketone supplement on cycling performance

I am pleased to advise that your application has received ethical approval.

Please note that researchers are asked to consult with the Faculty's Research Ethics Committee in the first instance if any changes to the approved research design are proposed.

The Committee wishes you all the best with your research.

Nicola Daly

Dr Nicola Daly
Chairperson (Acting)
Research Ethics Committee

Appendix 9 - Ethics approval

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THE UNIVERSITY OF WAIKATO
Te Whare Wānanga o Waikato

MEMORANDUM

To: **Shem Rodger**

cc: Dr Matt Driller

From: **Professor John Williams**
Chairperson, Research Ethics Committee

Date: 9 June 2015

Subject: Supervised Postgraduate Research – Application for Ethical Approval (EDU038/15)

Thank you for submitting the amendments to your application for ethical approval for the research project:

The effect of ketone supplementation on cognitive function

I am pleased to advise that your application has received approval.

Please note that researchers are asked to consult with the Faculty's Research Ethics Committee in the first instance if any changes to the approved research design are proposed.

Professor John Williams
Chairperson
Research Ethics Committee