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Acceptability and Tolerability of Ketone Supplements and Their Effects on Capillary Beta-Hydroxybutyrate Concentrations in Young Adults

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ACCEPTABILITY AND TOLERABILITY OF KETONE SUPPLEMENTS AND THEIR EFFECTS ON CAPILLARY BETA-HYDROXYBUTYRATE CONCENTRATIONS IN YOUNG ADULTS

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KINESIOLOGY

ABSTRACT

Increased concentrations of circulating ketones via ketogenic diet (KD) have been associated with beneficial effects on a number of chronic conditions, including obesity, fatty liver disease, refractory epilepsy, and neurodegenerative diseases. KD has been associated with low sustainability and compliance, which has led to the creation and commercialization of exogenous ketones. However, past studies examining the effects of exogenous ketones in humans are very limited. In this study, we compared two different commercially available exogenous ketones, a ketone monoester (KME) and a ketone monoester-salt mix (KME+S), at two different doses (5 g and 10 g ketones). Fourteen healthy young adults completed five study conditions (CON, KME_10g, KME_5g, KME+S_10g, and KME+S_5g) in randomized order. Circulating concentrations of plasma glucose and R-beta-hydroxybutyrate (R-βHB) were measured using a handheld ketone meter at baseline before drink consumption, then again at 15, 30, 60, and 120 minutes after ingestion. At 120 minutes, acceptability and tolerability was assessed using a symptom questionnaire. KME_10g achieved R-βHB concentrations of ~2.4 mM and KME+S_10g reached ~2.1 mM βHB. The 5-gram doses for both KME and KME+S achieved R-βHB concentrations of ~1.2 mM. There were negative correlations between R-ßHB and glucose at the 30-minute time point for all ketone study conditions except KME_10g. Both exogenous ketones used in the current investigation drinks had similar

tolerability, although decreases in appetite were more frequently reported for KME+S. The KME+S drink appears to be slightly more acceptable, due to the more frequently reported aftertaste for KME. Due to the expensive cost of KME products, consumers may find the KME+S drink to be more cost-effective than the KME drink. Additionally, lower doses may help to maintain cost-effectiveness without compromising desired outcomes when consuming exogenous ketones for general health purposes. Future studies should examine the magnitude and duration of repeated doses of exogenous ketones, as well as the effects of exogenous ketones in the fed state and in the context of different meal types to determine how consumers may utilize exogenous ketones on a daily basis.

Keywords: exogenous ketone, ketone ester, ketone salt, ketogenic diet, beta-hydroxybutyrate

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LIST OF ABBREVIATIONS

AcAc	acetoacetate
AUC	area under the curve
BAT	brown adipose tissue
BDH1	beta-hydroxybutyrate dehydrogenase 1
BIA	bioelectrical impedance analysis
BM	body mass
BMI	body mass index
CHO	carbohydrate
CON	control
CVD	cardiovascular disease
EE	energy expenditure
GRAS	Generally Recognized as Safe
HDAC	histone deacetylase
HDL-C	high-density lipoprotein cholesterol
KD	ketogenic diet
KDE	ketone diester
KE	ketone ester
KME	ketone monoester
KME+S	ketone monoester-ketone salt mix
KS	ketone salt

LDL-C	low-density lipoprotein cholesterol
MCT	medium-chain triglyceride
QUICKI	Quantitative Insulin Sensitivity Check Index
SCFA	short-chain fatty acid
SCOT	succinyl-coenzyme A-oxoacid transferase
T2DM	type 2 diabetes mellitus
βHB	beta-hydroxybutyrate

INTRODUCTION

Ketogenesis and Ketone Oxidation

For most of human existence, the acquisition of food presented challenges with recurring and sometimes extensive durations of undernutrition and starvation. Selection of genotypes capable of storing excess energy provided defense against starvation during prolonged periods of food shortage, establishing excess energy storage in adipose as a key determinant of survival. Classical experiments in humans show that during the first 24-28 hours of starvation, skeletal muscle and liver glycogen become depleted and the contribution of fatty acids to energy demand increases exponentially to decrease rates of gluconeogenesis and subsequently preserve skeletal muscle protein (Cahill, 2006). While lactate and glycerol from glycolysis and lipolysis contribute as substrate for gluconeogenesis, the primary substrate is amino acids derived from skeletal muscle proteolysis. Since continued degradation of skeletal muscle is crucial for activities related to food seeking and overall survival, gluconeogenesis significantly decreases within 48 hours of starvation. Fatty acids from adipose tissue lipolysis then provide an extensive substrate pool during post-absorptive metabolism and prolonged fasting/starvation (Owen 2005). Provided that limitations in energy were the primary determinant of survival, humans could survive for several weeks and perhaps months without food based on the energy density of fatty acids alone. However, fatty acid chain length is too large to cross the blood-brain barrier, which requires 16 and 18-carbon length fatty acids to be partially oxidized to shorter-chain, water-soluble four-carbon molecules referred to as ketones. As

fatty-acid-derived mitochondrial acetyl-CoA levels overcome maximal activity of citrate synthase or as oxaloacetate levels decline, acetyl-CoA exits the mitochondria and becomes partially oxidized to acetoacetate (AcAc) (with some spontaneous production of acetone following decarboxylation of AcAc) and beta-hydroxybutyrate (βHB) (Puchalska & Crawford, 2021). βHB is secreted from the liver in roughly a 4:1 ratio to AcAc in circulation. Circulating ketones enter extra-hepatic through monocarboxylate transporters 1 and 2 (MCT 1/2). βHB is initially converted to AcAc by the enzyme beta-hydroxybutyrate dehydrogenase 1 (BDH1) and eventually converted to acetyl-CoA by the enzyme succinyl-coenzyme A-oxoacid transferase (SCOT), the terminal enzyme required for oxidation of AcAc.

Ketones in Nutrient Signaling

In addition to their roles in energy metabolism, βHB and AcAc have emerged as signaling molecules and play a significant role in nutrient signaling. βHB, but not AcAc, exerts anti-lipolytic effects through activation of GPR109A (Plaisance et al., 2009; Tunaru et al., 2005), which inhibits lipolysis and decreases circulating non-esterified fatty acids as part of a negative feedback loop (Puchalska & Crawford, 2021; Puchalska & Crawford, 2017). Activation of GPR109A in retina suppresses secretion of a number of proinflammatory markers in diabetes while in macrophages, βHB exerts anti-inflammatory effects through activation of GPR109A and inhibition of the NLRP3 inflammasome (Benyó et al., 2005; Youm et al., 2015). Inhibition of the NLRP3 inflammasome reduces proinflammatory IL-1β, IL-18, and caspase-1 activation, independent of GPR109A (Youm et al., 2015). βHB is also a potent inhibitor of histone

deacetylases (HDACs) (Yan et al., 2022; Newman & Verdin, 2013; Newman & Verdin, 2014). Deacetylation of lysine residues on histones produces a compact chromatin structure on DNA resulting in lower accessibility of RNA polymerase and decreased gene expression, whereas HDAC inhibition increases acetylation and produces a less compact chromatin structure with increased expression of a number of regulators of glucose and lipid metabolism (Newman & Verdin, 2013; Park & Kim, 2020). AcAc was identified through ligand screening in a heterologous system as a ligand for GPR43 (Miyamoto et al., 2019). Short-chain fatty acids (SCFAs) such as butyrate and propionate regulate the receptor in enterocytes and adipocytes during the fed state, but during fasting or KD, SCFA concentrations decrease resulting in a metabolic switch and subsequent activation of GPR43 in adipocytes by AcAc. GPR43 activation leads to increased lipoprotein lipase activity which would be expected to enhance fatty acid uptake and oxidation. In fact, fat loss during seven weeks of KD was significantly lower in GPR43-/- mice compared to wild-type mice suggesting a key role for AcAc during KD and perhaps exogenous ketone administration (Miyamoto et al., 2019). Oxidation of AcAc in macrophages has also been shown to decrease hepatic stellate cell activation, matrix deposition, and fibrogenesis, an effect that was lost with knockdown of SCOT (Puchalska et al., 2019).

Ketogenic Diet and Effects on Components of Energy Balance and Weight Loss

In 1921, R. M. Wilder proposed a high-fat (80-85% by kcals), low-carbohydrate (<5%), and low-protein (10-15%) ketogenic diet (KD) for treating epileptic seizures (Kim, 2017). Although a number of pharmacological agents have since been developed to control epileptic seizures, KD is still frequently used for the treatment of childhood

refractory epilepsy. In fact, Gilbert et al. (2000) found a 50% reduction in seizure control after 3-6 months on KD at a threshold of 4 mM βHB. Others showed (Tagliabue et al., 2011) that fat oxidation increased following a 6-month KD in patients with epilepsy. The increase in fat oxidation was identified as a main predictor of reduction in seizure frequency. Increased fat oxidation and observations of weight loss on KD led to interest in using the diet to produce or maintain body weight loss.

While dietary energy restriction is an effective strategy to produce weight loss, one study showed that most individuals regain 73.4% of their lost weight over the first 3 years and 80.1% within 5 years even in tightly controlled studies (Anderson et al., 1999). Weight regain has been attributed to a number of factors including reductions in all components of energy expenditure resulting from decreases in total and skeletal muscle mass and food intake. Several studies show that a standard KD produces weight loss (Batch et al., 2020; Bueno et al., 2013; Drabińska et al., 2021; Johnstone et al., 2008; Kong et al., 2020; Li et al., 2022; Murphy & Jenkins, 2019; Perticone et al., 2019; Saslow, et al., 2017; Sumithran et al., 2013; Yancy et al., 2004; Yuan et al., 2020), with exceptions (Groleau et al., 2014; Tagliabue et al., 2011). Others demonstrated weight loss with KD for approximately five months followed by subsequent weight regain (Ting et al., 2018). KD has been shown to decrease fat mass (Kong et al., 2020; Perticone et al., 2019), while others show that KD decreases fat mass while preserving lean body mass (Cohen et al., 2018; Jabekk et al., 2010; Kang et al., 2020). However, one study (Yancy et al. (2004) showed decreases in fat mass and, to a lesser extent, fat-free mass. On the other hand, when compared to a high-carbohydrate, low-fat diet, KD did not produce superior fat loss (Hall et al., 2016). In addition, when compared to a non-KD low-carbohydrate

diet, KD did not significantly differ in weight or fat loss (Johnston et al., 2006). These findings suggest that the weight loss may be similar on isocalorically reduced diets and that the composition may be less important with regard to weight loss. In addition, the findings that a non-KD low-carbohydrate diet produced similar reductions in weight and fat loss as a KD show suggest the interesting possibility that a common thread to weight loss is the expected reduction in circulating insulin concentrations.

Effects on Appetite and Energy Intake

Studies in rats show that peripheral administration of βHB decreases food intake (Langhans et al, 1983; Langhans et al., 1985a; Langhans et al, 1985b) KD has also been shown to decrease hunger and desire to eat (Gibson et al., 2015), and when compared to a high-protein medium-carbohydrate diet in obese men, KD lowered food intake and hunger to a greater extent (Johnstone et al., 2008), presumably by increasing circulating ketone concentrations. Similarly, Lodi et al. (2020) compared KD to a Mediterranean diet in overweight women. Higher βHB levels were negatively correlated with appetite, providing further support that increasing circulating ketones suppress appetite. Another study by Kong et al. (2020) found that a 4-week KD in overweight and obese women decreased leptin concentrations, due to a KD-induced decrease in adipose tissue. Leptin is a master regulator of adiposity level in the sense that leptin synthesis and secretion from adipocytes increases with increasing fat volume and decreases with decreasing fat volume. From a practical perspective, this means that during weight/fat loss, as adipocyte volume decreases, so does leptin synthesis and secretion resulting in lower activation of

the leptin long receptors in the hypothalamus of the brain and leading to increased desire to eat and decreased resting energy expenditure (Friedman & Halaas, 1998).

In line with this, previous findings suggest that KD may also produce short-term changes in energy expenditure (EE). A study by Hall and colleagues (2016) determined that overweight and obese participants who switched from a high-carbohydrate diet to an isocaloric KD experienced small increases in energy expenditure, sleeping energy expenditure, and decreased respiratory quotient. Another study suggested that, compared to a standard diet, KD increased the thermic effect of feeding and daily energy expenditure by 100 kcals/day or more (Bistrian, 2019).

KD has also been shown to lower insulin concentrations and insulin-like growth factor in women with ovarian or endometrial cancer (Cohen et al., 2018) and overweight adults (Johnston et al., 2006). In individuals with type 2 diabetes mellitus (T2DM), KD lowered concentrations of insulin (Li et al., 2022) and glucose (Yuan et al., 2020). Others have shown that increasing ketone concentrations may regulate inflammation, as βHB can inhibit NLRP3 inflammasome activation by targeting potassium efflux (Hughes & O'Neill, 2018). This suggests that βHB may help to regulate general inflammation, which could be beneficial for conditions like obesity where NLRP3 dysregulation promotes inflammation.

Ketogenic Diet Effects on Blood Lipids and Cardiovascular Disease Risk Factors

The effects of KD on blood lipids are unresolved with some studies showing beneficial effects and others showing changes in blood lipid and lipoprotein characteristics that would traditionally be cause for concern. Bueno and colleagues

(2013) showed that KD decreased serum triglyceride (TG) concentrations. The diet also increased low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) concentrations compared to a low-fat diet. Others support these findings and show that KD increased LDL-C in normal-weight, young women (Burén et al., 2021) as well as in overweight men and women (Johnston et al., 2006). In patients with T2DM, one study found that KD decreased both HDL-C and LDL-C (Li et al., 2022), while another study found that KD increased HDL-C and decreased LDL-C (Yuan et al., 2020). In overweight participants with hyperlipidemia, KD increased HDL-C to a greater extent than a low-fat diet (Yancy et al., 2004). Due to the limited number of studies and contrasting findings, more studies are needed to further interrogate the effects of KD on LDL-C and HDL-C. Overall, KD consistently decreases serum TG concentrations with the exception discussed above, but effects on lipoprotein subfractions remain unresolved. Future studies are needed to investigate effects on lipoprotein particle size as LDL and HDL particle size are key components of cardiovascular disease (CVD) risk. For example, Griffin et al. (1994) showed that smaller LDL particles are more atherogenic than larger LDL particles regardless of cholesterol content. Similarly, large HDL particle size has been associated with lower CVD risk. These findings highlight the need for a thorough investigation of KD in a diverse population group to fully characterize responses on blood lipids and lipoproteins using sophisticated approaches that fully interrogate lipoprotein size and number before any conclusions are drawn regarding safety of KD. It will also be important to distinguish whether the types of fats employed in KD influence the outcome. For example, substituting unsaturated fats for

saturated fat in a KD has been shown to decrease circulating total cholesterol concentrations during KD (VanItallie et al., 2005).

Ketogenic Diet Effects on Neurodegenerative Diseases

Increasing circulating ketone concentrations may improve quality of life and health outcomes of patients with neurodegenerative diseases. Kashiwaya et al. (2020) demonstrated that R-βHB protected neurons from mitochondrial dysfunction in cell culture models of both Parkinson's Disease and Alzheimer's disease suggesting that ketones may provide a therapeutic effect for neurodegenerative diseases. According to Newman and Verdin (2013), βHB functions as an endogenous inhibitor of HDACs. HDAC activation has been shown to play a key role in regulating lifespan and impairing memory and learning in nematodes and mice (Peleg et al., 2010; Shao et al., 2020). βHB has also been shown to be a potent inhibitor of HDACs and could explain at least some of the beneficial effects of KD on outcomes related to dementia and other age-related diseases. A 28-day KD resulted in improved Unified Parkinson's Disease Rating Scale scores in five patients, suggesting that increased ketone concentrations may decrease disease severity (VanItallie et al., 2005). The potential of ketones to improve conditions such as Parkinson's Disease and Alzheimer's Disease remains promising.

Exercise Enhancement

In competitive endurance athletes, adapting to KD increased peak aerobic capacity but impaired performance due to increased rates of fat oxidation (Burke et al., 2017). However, a review by Kang et al. (2020) found that in normal-weight individuals

and athletes, KD of equal to or longer than three weeks did not appear to enhance endurance exercise performance. For resistance exercise, KD does not seem to have a negative effect on strength or power development, especially with increased protein intake. Similarly, KD maintains capacity for both endurance and resistance activities (Murphy & Jenkins, 2019). However, reduced carbohydrate (CHO) intake during KD may result in higher rates of perceived exertion and increased muscle fatigue following exercise. In healthy women, KD did not affect grip strength or time to fatigue but decreased cycling time to fatigue. Additionally, KD had an unfavorable effect on perceived exertion during exercise and throughout the day (Sjödin et al., 2020).

Sustainability and Other Safety Concerns with Ketogenic Diet

A primary concern associated with KD is low sustainability and compliance (Batch et al., 2020; Li et al., 2022; Murphy & Jenkins, 2019; O'Neill & Raggi, 2020; Paoli et al., 2011). KD has also been associated with minor adverse effects such as GI disturbances, hyperlipidemia, and lethargy (Cai et al., 2017; Yancy et al., 2004), although severe effects are extremely rare. Weight regain is often a potential problem when dieting for the purpose of weight loss, and this effect is also seen in KD. According to a review by Ting et al. (2018), KD may induce weight loss with a peak at approximately five months, but then weight may be slowly regained as described above. Safety concerns may also be associated with KD. As previously stated, KD may increase LDL-C, and additional studies are needed to examine long-term effects on CVD risk (Bueno et al., 2013; Burén et al., 2021; Johnston et al., 2006). Another concern associated with KD is its potential to exacerbate renal dysfunction, particularly in obese individuals (Schutz et

al., 2021). This effect might be explained by the increased intake of dietary protein alongside restricted CHO intake on KD – an effect that can be avoided by following a standard KD where protein intake is also limited. Poor adherence and potential adverse effects alongside the potential benefits of increasing serum ketone concentrations have led to the creation and use of exogenous ketones to increase circulating ketone concentrations without introducing a KD.

Exogenous Ketone Salts and Esters

Exogenous ketones are commercially available as ketone salts (KS), ketone esters (KE), or more recently as a mixture of KE and KS. KS contain a racemic mixture of R-βHB and S-βHB (1:1) that are covalently bonded to a cation such as sodium, potassium, calcium, or magnesium. Covalent bonding attenuates the decrease in pH and subsequent gastrointestinal distress while increasing absorption of the ketone. R-βHB is the primary physiological form produced in the liver with S-βHB produced at much lower levels (Cuenoud et al., 2020; Stubbs et al., 2017).

The two primary ketone esters that have been investigated over the past 10-12 years include R-1-3 butanediol-R-β-hydroxybutyrate (KME) and R,S-1,3-butanediol diacetoacetate (KDE). In both molecules, the butanediol ester is de-esterified by esterases in the small intestine. Following entry into the portal vein of the liver, R-1,3-butanediol is metabolized by alcohol and aldehyde dehydrogenases to R-βHB. While the fate of S-butanediol in the KDE is less clear, there is evidence that most of the molecule is eventually metabolized to R-βHB (Webber & Edmond, 1977).

There have been few studies examining the effects of KS in rodents or humans. Overall, findings from a systematic review and meta-analysis showed that KS increased serum βHB concentrations and produced reductions in serum glucose concentrations (Falkenhain et al., 2022). In one of the few long-term randomized, placebo-controlled clinical trials conducted with KS, the investigators compared the effects of a KS to placebo control in healthy male and female adolescents (10-17 years) with a body mass index (BMI) < 30 kg/m2 (Stefan et al., 2021). Participants were randomly assigned to the KS (3.75 g βHB) or placebo control twice per day, once in the morning prior to a meal and again 1.5 h after lunch for 90 days. A pilot study was also conducted with a cohort of six participants to examine the acute effects of a single dose of βHB (3.75 g) on circulating R-βHB concentrations. Fifteen minutes after consumption, mean circulating R-βHB concentrations rose to 0.7 ± 0.1 mM with similar concentrations at 30 minutes. At 60 minutes, circulating R-βHB concentrations were 0.4 ± 0.2 mM with only 2 out 6 participants maintaining a state of ketosis (R-βHB ≥ 0.5 mM). Long-term (90 days) study results showed that the KS had no effects on hematological safety markers, bone densitometry, emotional intelligence and psychological well-being, or cardiovascular markers of health. Furthermore, there were no within-group or between-group differences in body weight or body composition observed at completion of the study.

A subsequent study by the same group examined the effects of a racemic mixture of βHB salts in adults between the ages of 18 and 50 years to examine the effects of the KS on clinical chemistries and markers of blood glucose and lipid metabolism (Stefan et al., 2020). After 90 days on either the KS or placebo control, there were no differences between groups with regard to clinical safety markers that would indicate any serious

adverse effects of KS consumption or differences in blood glucose, blood pressure, immune status, bone densitometry, or psychological well-being.

Stubbs and colleagues (2017) examined the effects of approximately 12 and 24 g of KME with a racemic mixture of R-βHB and S-βHB salts. The KME increased circulating R-βHB concentrations to maximum circulating concentrations of 2.8 ± 0.2 mM with 24 g of the KME at 30 minutes that remained elevated up to 1 mM at 3 hours and subsequently returned to baseline by 4 hours. The lower dose of KME (12 g) increased average circulating R-βHB concentrations to 1.5 mM at 30 minutes that returned to baseline by 2 hours. The KS raised circulating R-βHB concentrations to 1.0 ± 0.1 mM to a similar extent at both doses with peak concentrations between 1 and 1.5 hours. The kinetic differences in the responses to the KME and KS are driven by the fact that the KS is composed of 50% R-βHB. As the authors acknowledge, it is unclear if the kinetic parameters would be similar if R-βHB were matched in the drinks. One of the primary purposes of the proposed investigation was to examine this question by administering the KME in comparison with a KME+KS mixture that produces or contains only the R-βHB enantiomer. The findings by Stubbs and colleagues also show that S-βHB remains in circulation for at least 8 hours but was no longer present at 24 hours providing further evidence that the metabolic fates of R- and S-βHB are different. This works supports that of Webber and Edmund, 1977, which shows that the S enantiomer has lower oxidation than the R enantiomer and is processed in different pathways (Stubbs et al., 2017). It is also possible that at least part of the total circulating S-βHB concentrations are eventually converted to the R enantiomer leading to the interesting possibility that administration of the S-enantiomer could provide a “sustained”

release of R-βHB. Another interesting finding from this study was that both the KME and KS produced a slight increase in plasma insulin after administration. While the mechanisms for this response or unclear, the authors suggest that ketones could stimulate or potentiate insulin secretion. For example, Biden and Taylor (1983) showed that ketones stimulated insulin secretion at glucose concentrations > 5 mM in isolated pancreatic islets.

Pre-clinical studies in mice show that a diet containing 28.6% KME (with an equivalent amount of CHO energy removed) produced a 16% decrease in energy intake and a 15% increase in resting energy expenditure, but no changes in 24-hour total energy expenditure (Srivastava et al., 2012). After the first 12 days on the diet, differences in body weight ceased with mice weighing approximately the same after 30 days on the diets. There were no differences in epididymal adipose tissue weight between groups, but brown adipose tissue (BAT) volume was significantly lower. BAT demonstrated a pattern of thermogenic activation as determined by mRNA and protein expression and FDG labeling. The KME groups also experienced a 73% increase in the quantitative insulin-sensitivity check index (QUICKI).

More recently, db/db mice with type 2 diabetes and heart failure were placed on KME for 4 weeks with 28.6% (by kcals) of the KME (Thai et al., 2021). The KME-treated animals had significant improvements in myocardial mitochondrial biogenesis, decreased oxidative and redox stress, and increased systolic and diastolic function compared to animals maintained on a control diet. Others have shown that the KME decreases oxidative stress in the mouse myocardium through pathways that seem to involve activation of the FOXO3/MT2 pathway by inhibiting HDACs (Ji et al., 2022).

Deacetylation of lysine residues on histones produces a compact chromatin structure on DNA resulting in lower accessibility of RNA polymerase and decreased gene expression, whereas HDAC inhibition increases acetylation and produces a less compact chromatin structure with increased expression of a number of regulators of glucose and lipid metabolism (Newman & Verdin, 2013). βHB, but not AcAc has been shown to inhibit HDACs in a number of tissues in glucose and lipid metabolism, aging, and cancer. These findings highlight the need for studies to examine the potential of the KME as a treatment strategy for a number of chronic disease conditions.

The KME received exemption and approval from the FDA for Generally Recognized as Safe status as a functional food for human consumption in 2014. While the number of published studies in humans is relatively sparse, most studies show that the KME is safe and well-tolerated with potential efficacy in areas particularly related to glucose metabolism. In one of the initial studies performed by Clarke and colleagues (2012) healthy male and female participants between the ages of 18 and 45 years received 140, 357, and 714 mg/kg body weight of the KME on separate occasions separated by at least seven days (it is not clear from the manuscript if the treatment order was randomized). At an average body weight of 74.7 kg, the amount of KME delivered would have been approximately 10.5, 26.7, and 53.3 g, respectively. Maximum concentrations of the KME were observed within 15 minutes following administration with a half-life of 6.5 minutes in females and 14 minutes in males illustrating rapid hydrolysis in systemic circulation. Blood R-βHB concentrations reached maximal concentrations of approximately 5 mM with the highest KME delivery (714 mg/kg body weight) with levels achieving approximately 1 mM concentration at the 357 mg/kg dose. There were

no treatment-related adverse outcomes reported following the single dose at any of the dosages provided. The authors did report that three daily doses provided over the course of 5 days using the same dosages for the single-dose study were associated with mild adverse events at the low and middle doses. Two participants reported adverse events at each of the two lower doses and included flatulence, nausea, headache, and euphoria. In contrast, the highest dosage produces adverse events in 12/12 participants which included flatulence, nausea, diarrhea, decreased appetite, headache, euphoria, and anxiety.

In two separate studies (Stubbs, 2018; Soto-Mota, et al., 2021), a single dosage of KME at approximately 25 g increased circulating R-βHB concentrations from 0.2 to 3.3 mM sixty minutes after consumption. The single dose of KME increased plasma insulin concentrations during the first 30 minutes, but the increase was 3-fold lower than the group receiving dextrose. Decreased postprandial concentrations of insulin, ghrelin, GLP-1, and PYY concentrations 2-4 hours were observed after consumption of the KME, compared to a group receiving an equivalent number of calories from a dextrose solution. The decrease in circulating GLP-1 concentrations was associated with reported hunger significantly lower 1.5 h after consumption (Stubbs et al., 2018). Soto-Mota et al. (2021) showed that 25 g of KME increased circulating R-βHB concentrations to 4.52 ± 1.23 mM. Administration of the KME decreased blood glucose concentrations at the dosage provided, but leucine blocked the decrease in blood glucose when administered with the KME. These findings suggest that KME-mediated reductions in muscle protein breakdown and subsequent reductions in leucine could reduce substrate availability for hepatic gluconeogenesis. Stubbs and colleagues (2017) provided approximately 10 and 20 g of KME resulting in R-βHB concentrations of approximately 1.5 mM and 2.9 mM

within 15 minutes that were sustained for approximately 2 hours in the lower dose and for up to 3 hours with the higher dose. Blood glucose concentrations decreased in a dose-dependent fashion and returned to baseline as circulating ketone concentrations decreased over time. Taken together, the acute increase in circulating insulin and reductions in hepatic gluconeogenesis are likely responsible for the reduction in circulating glucose observed with KME administration acutely. Additional studies are needed using more sophisticated approaches such as hyperinsulinemic-euglycemic clamps with stable isotopes to trace hepatic glucose rate of appearance and glucose disposal in extra-hepatic tissues to determine the specific contributions of hepatic and total body insulin sensitivity to KME administration.

Most studies conducted in humans today have examined extremely high doses in the ranges of 10 – 50 g as a single dose (Table 1). It is clear from the literature that there is a dose-dependent component to the circulating ketone response and other variables such as blood glucose, but it is unclear whether such high concentrations are necessary to produce benefit. In addition, the prohibitive cost of materials to produce ketone esters makes it unreasonable for most individuals looking to consume ketone esters for health or performance. Lastly, emerging formulations that integrate ketone esters and salts together within the same drink and the inclusion of R-βHB salts with medium-chain triglycerides (fatty acids) that are oxidized directly to ketones in the liver require further investigation. These studies are critical because exogenous ketones have shown the potential to treat or even prevent a number of chronic disease conditions. As we translate findings from the pre-clinical animal literature and piece together the wide range of frequencies and doses of exogenous ketones, it will be important to optimize the best types, frequencies, and

dosing regimens. In line with this, the purpose of the current proposal was to examine the responses to a single dose of the KME at lower doses (5 and 10 g) and to compare those responses to a KME + R-βHB (single enantiomer) mixture at 5 and 10 g doses. The primary outcome variables for the study were whole blood concentrations of R-βHB and glucose. Secondary outcomes included tolerability, acceptability, and assessment of adverse events. We hypothesized that the KME would raise circulating ketone concentrations to a greater magnitude and duration than a salt-ester drink and the placebo control drink. We also hypothesized that the group receiving the KME would have fewer adverse events, but lower acceptability of the KME compared to the KME+KS mixture.

Table 1

List of Research Studies That Investigated Exogenous Ketones in Humans

Reference	Intervention	KE Dose	Sample	βHB Concentration Reported at Rest (mM/L)
Bleeker et al., 2020	KME + CHO	395 mg/kg BM	N=5 patients with VLCADD	2.0 ± 0.2 at 30 min
Clark et al., 2021	KS	300 mg/kg BM of βHB	N=9 healthy, male young adults	0.88 ± 0.21 at 30 min
Clarke et al., 2012	KME + meal replacement drink	140 mg/kg BM	N=54 healthy adults	0.28 at 60-120 min
	KME + meal replacement drink	357 mg/kg BM	N=54 healthy adults	1.0 at 60-120 min
	KME + meal replacement drink	714 mg/kg BM	N=54 healthy adults	3.3 at 60-120 min
Cox et al., 2016	KME	573 mg/kg BM	N=6 male endurance athletes	~3.0 at 10 min to >5.0 by ~30 min
	KME	573 mg/kg BM	N=10 male endurance athletes	3.5 ± 0.3 at 10 min to >5.0 by ~30 min

	KME + CHO	573 mg/kg BM	N=8 male endurance athletes	~3.0 at 25 min
	KME + CHO	573 mg/kg BM	N=7 male endurance athletes	2.2 at 30 min
	KME + CHO	573 mg/kg BM	N=8 endurance athletes	~2.0 at 20 min
Cuenoud et al., 2020	KS	12 g of R-βHB	N=15 healthy adults	0.75 ± 0.06 at ~60 min
	KS	12 g of R+S-βHB	N=15 healthy adults	0.39 ± 0.03 at ~60 min
	MCT	15 g of MCT	N=15 healthy adults	0.54 ± 0.06 at ~60 min
Dearlove et al., 2019	KME	330 mg/kg BM	N=12 athletes	3.7 ± 0.3 at 60 min
Evans et al., 2019	KME + CHO	573 mg/kg BM	N=8 endurance runners	0.99 (0.85-1.14) at 30 min
Holdsworth et al., 2017	KME following glucose-depleting exercise	~573 mg/kg BM	N=12 male athletes	>4.0 by 30 min
Jo et al., 2020	KS + CHO	11.7 g of βHB	N=16 healthy, endurance-trained young adults	0.40 ± 0.3 pretreatment and 0.40 ± 0.4 posttreatment
Leckey et al., 2017	KDE + CHO prior to exercise	2 doses of 250 mg/kg BM	N=10 male cyclists	~0.5 following first dose, after 20 min of rest; ~0.8 following subsequent second dose and ~20 minutes of light exercise (45 min after first dose)
Løkken et al., 2022	KME	395 mg/kg BM	N=8 patients with GSDV	~3.39 ± 1.33 at 25 min
Myette-Côté et al., 2018	KME	482 mg/kg BM	N=20 healthy adults	3.2 ± 0.6 at 30 min
Myette-Côté et al., 2019	KME	482 mg/kg BM	N=15 adults with obesity	~2.5 at 30 min and ~3.0 at 60 min
Neudorf et al., 2020	KME	482 mg/kg BM	N=11 adults with obesity	1.99 ± 0.67 at 30 min and 2.96 ± 0.91 at 1 hr
Norwitz et al., 2020	KME + CHO	25 mL of KE drink, ~10.6 g of KME	N=14 patients with Parkinson's disease	3.5 ± 0.3 at 30 min

Plecko et al., 2002	KS	880-1000 mg/kg R+S-βHB BM per day for 5-7 months	N=2 6mo old infants with hyperinsulinemic hypoglycemia	up to 2.57 for Patient 1 and up to 2.75 for Patient 2
Poffé et al., 2020	KME + CHO	2 doses prior to exercise, first at 25 g and second at 20 g	N=12 male athletes	~2.0 at 30 min after first dose, ~3.0 at 1 hr after first dose and 30 min after second dose
Prins et al., 2020a	KS+MCT	7 g βHB + 7 g MCT	N=13 male runners	4.3 ± 0.2 at 30 min and 6.0 ± 0.2 at 60 min
	KS+MCT	14 g βHB + 14 g MCT	N=13 male runners	5.3 ± 0.2 at 30 min and 7.3 ± 0.2 at 60 min
Prins et al., 2020b	KS+MCT	300 mg/kg BM, containing 9 g βHB and 7 g MCT	N=10 male runners	~6.5 at 60 min
Rodger et al., 2017	KS	11.7 g of βHB	N=12 highly trained cyclists	~0.35 at 20 min
Soto-Mota et al., 2019	KME	25 mL of KME, 3 times daily for 28 days, drinks spaced out at least 4 hrs apart	N=24 healthy adults	4.1 ± 1.1 at 30 min after third drink of the day, averaged over 28 days
Stubbs et al., 2017	KS	141 mg/kg BM of R+S-βHB	N=15 healthy adults	peak of ~0.8 at 60 min
	KS	282 mg/kg BM of R+S-βHB	N=15 healthy adults	peak of 1.0 ± 0.1 at 90 min
	KME	141 mg/kg BM	N=15 healthy adults	peak of ~1.4 at 20 min
	KME	282 mg/kg BM	N=15 healthy adults	peak of ~2.8 at 30 min
	KME	395 mg/kg BM	N=16 healthy adults	~3.2 at 60 min
	KME + CHO meal	395 mg/kg BM	N=16 healthy adults	~2.1 at 60 min
	KME	3 doses of 387 mg/kg BM, at 3 hr intervals	N=12 healthy adults	3.8 ± 0.2 at 60 min after third drink of the day
	KME	continuous NG infusion at 4.4 mM/kg BM for 60 min, followed by 1.1 mM/kg BM over 8 hrs	N=4 healthy adults	2.9 ± 0.5 at 60 min, maintained at 2.0-3.0 over remaining 8 hrs
Stubbs et al., 2018	KME	1.9 kcal/kg BM	N=15 healthy adults	3.3 ± 0.2 at 60 min

Thompson & Neopocatych, 2020	KS	250 mg/kg BM	N=22 healthy females	0.5 ± 0.2 at 45 min and 0.4 ± 0.2 at 90 min
Vandoorne et al., 2017	KME + protein/CHO mixture following glycogen-depleting exercise	500 mg/kg BM at beginning of recovery period, followed by 250 mg/kg BM per hour, with protein/CHO recovery drinks every 30 min	N=8 healthy male adults	2.9 ± 0.3 at 30 min into recovery period, up to 4.3 ± 0.5 at 4 hrs into recovery period

METHODS

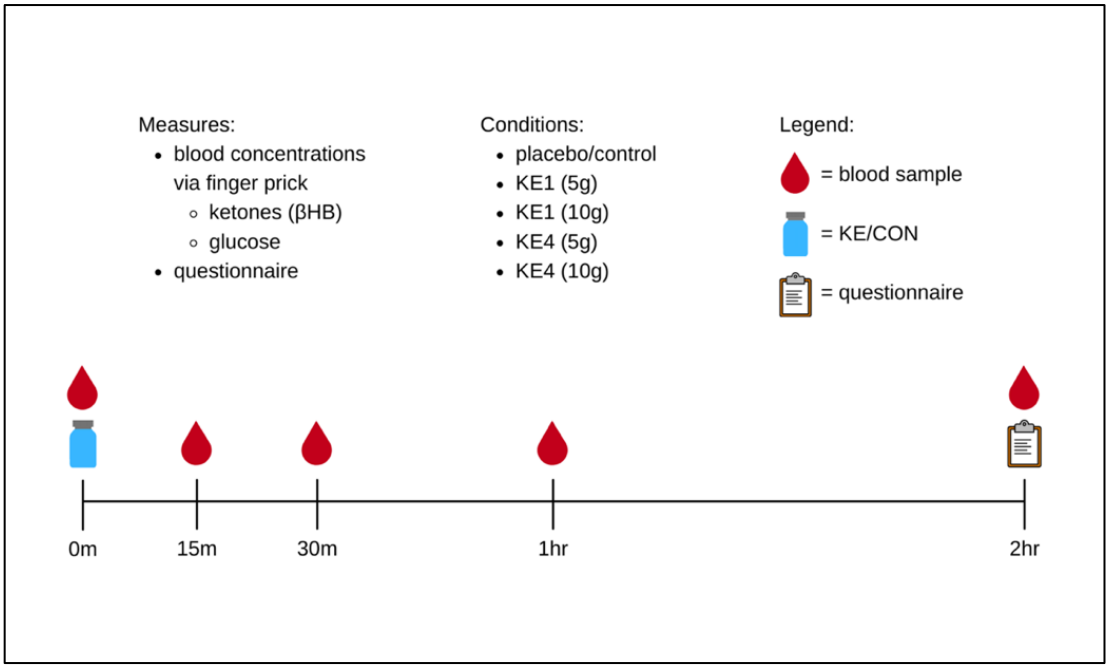
Participants

Fourteen participants between the ages of 18 and 25 years with a body mass index (BMI) between 18.5 and 29.9 kg/m2 were recruited for the study. Recruitment was conducted primarily by word of mouth with students on campus and speaking with classes on campus. Participants who were pregnant or trying to become pregnant, and those with pre-existing chronic disease conditions such as eating disorders, type 2 diabetes mellitus, coronary heart disease, or cancer were excluded from the study.

Study Design

The study is a randomized, single-blinded five-arm crossover design (Fig. 1). Plasma glucose and R-βHB concentrations were measured at baseline (time = 0) before ingestion of the placebo or ketone drinks at baseline. For each participant, the order of conditions was randomized using a number generator in Excel. The five study conditions included: 1) placebo control (CON); 2) 5 grams of KE1; 3) 10 grams of KE1; 4) 5 grams of KE4; and 5) 10 grams of KE4. Each condition was conducted following a 72-hour minimum washout period.

Figure 1
Study Flow Diagram



The exogenous ketones used in this study were purchased from Ketone-Aid (Falls Church, VA) and included a KME + KS mixture (KE1) and a ketone monoester drink (KE4). KE1 contained distilled water, a 5:6 ratio of KS:KME, monk fruit extract, natural flavors, and potassium sorbate. The KE4 drink contained water, ketone monoester, organic stevia, citric acid, allulose, natural flavors, and potassium sorbate. The placebo control was also manufactured by Ketone-Aid and consisted of distilled water, stevia, potassium sorbate, natural flavors, and denatonium benzoate. The drinks provided at each condition were diluted with water to a total volume of 110 mL for blinding and consistency.

Physiological Measurements

At the beginning of the first study visit, baseline measurements of body weight, height, body fat percentage, lean body mass percentage, waist-hip ratio, and personal demographic information (name, sex, age, race, and ethnicity) were recorded from each participant. Body weight was measured using a balance scale while height was measured using a stadiometer. Body composition was measured using bioelectrical impedance analysis (BIA, Omron, HBF-514C, Kyoto, Japan) while waist and hip measurements were conducted using a cloth tension tape measure. Waist circumference was measured at the narrowest part of the torso between the umbilicus and xiphoid process. Hip circumference was measured at the maximal circumference of the gluteus maximus.

On the day prior to each study condition, participants were asked to record all food and drinks consumed and encouraged to consume a similar diet before every visit. However, participants were not required to change their typical diet during the study. Participants were asked to fast for at least 8 hours prior to each visit. Participants were also told to avoid vigorous or planned physical activity on the morning of the study procedure.

Participants were administered KE1, KE4, or CON after obtaining baseline measurements of βHB and glucose (0-minute). Following ingestion, βHB and glucose were measured at 15, 30, 60, and 120 minutes for a total of five measurements. Capillary blood was measured using a commercially available ketone meter (Keto-Mojo, Napa, CA) which measures circulating R-βHB and glucose concentrations. At the end of each condition, participants completed a symptom questionnaire to report any adverse events and tolerability.

Statistical Methods

The primary outcomes were circulating R-βHB and glucose concentrations. Temporal responses over time (0, 15, 30, 60, and 120 min) were compared using a five condition (CON, KME_10g, KME_5g, KME+S_10g, KME+S_5g, and placebo control) x time ANOVA with repeated measures on time and condition. Total and incremental area under the curve (AUC) were calculated as previously reported (Matthews et al., 1990). Data are presented as means ± SD. Significance was set a priori at $P < 0.05$. The statistical analysis was conducted using SAS (SAS Institute Inc., Cary, North Carolina, v9.4). Responses to questions about tolerability and other qualitative criteria were analyzed by creating themes organized by frequency.

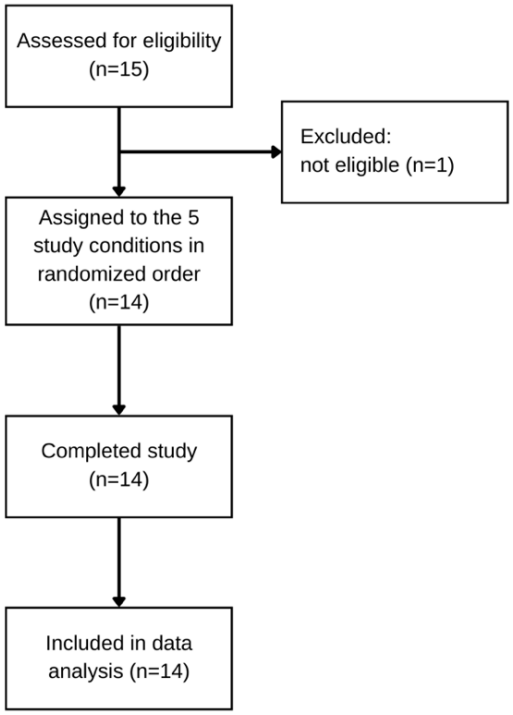
RESULTS

Participant Recruitment and Characteristics

Fifteen individuals volunteered for the study and were screened for eligibility with one excluded for not meeting BMI requirements (Fig. 2).

Figure 2

Participant Flow



An important outcome of this study was feasibility as it related to recruitment and retention of participants. While recruitment was challenging because of the amount of time required of each participant (~12 hours), coupled with no financial compensation,

participant retention was 100% with all 14 participants who joined the study completing each of the five conditions. Participant characteristics are shown in Table 2. The study group consisted of young adults between the ages of 18 – 25 years with six males and 8 females included in the analysis. Eight of the 14 participants identified as non-Hispanic white, one as non-Hispanic Asian, two as Hispanic white, one as Hispanic black, one as non-Hispanic African American, and one as non-Hispanic "Other"

Table 2

Participant Characteristics

Characteristic	Mean ± SD	Min	Max
Age (years)	21.0 ± 2.0	18.0	25.0
Height (m)	1.67 ± 0.09	1.55	1.84
Weight (kg)	69.7 ± 14.2	48.5	92.4
Fat (%)	28.1 ± 9.3	17.3	47.0
Lean (%)	33.1 ± 6.4	21.7	41.9
Waist (cm)	78.9 ± 10.5	67.0	94.0
Hip (cm)	99.7 ± 6.7	90.5	112.0
WHR	0.79 ± 0.08	0.67	0.91

WHR = Waist to hip ratio. Values are means ± SD along with minimum (Min) and maximum (Max) values.

Effects of KME and KME+S Drinks on Circulating R-ß-hydroxybutyrate and Glucose

There were no significant differences in R-ßHB and glucose responses or other variables observed between male and female participants; thus, all data is combined for

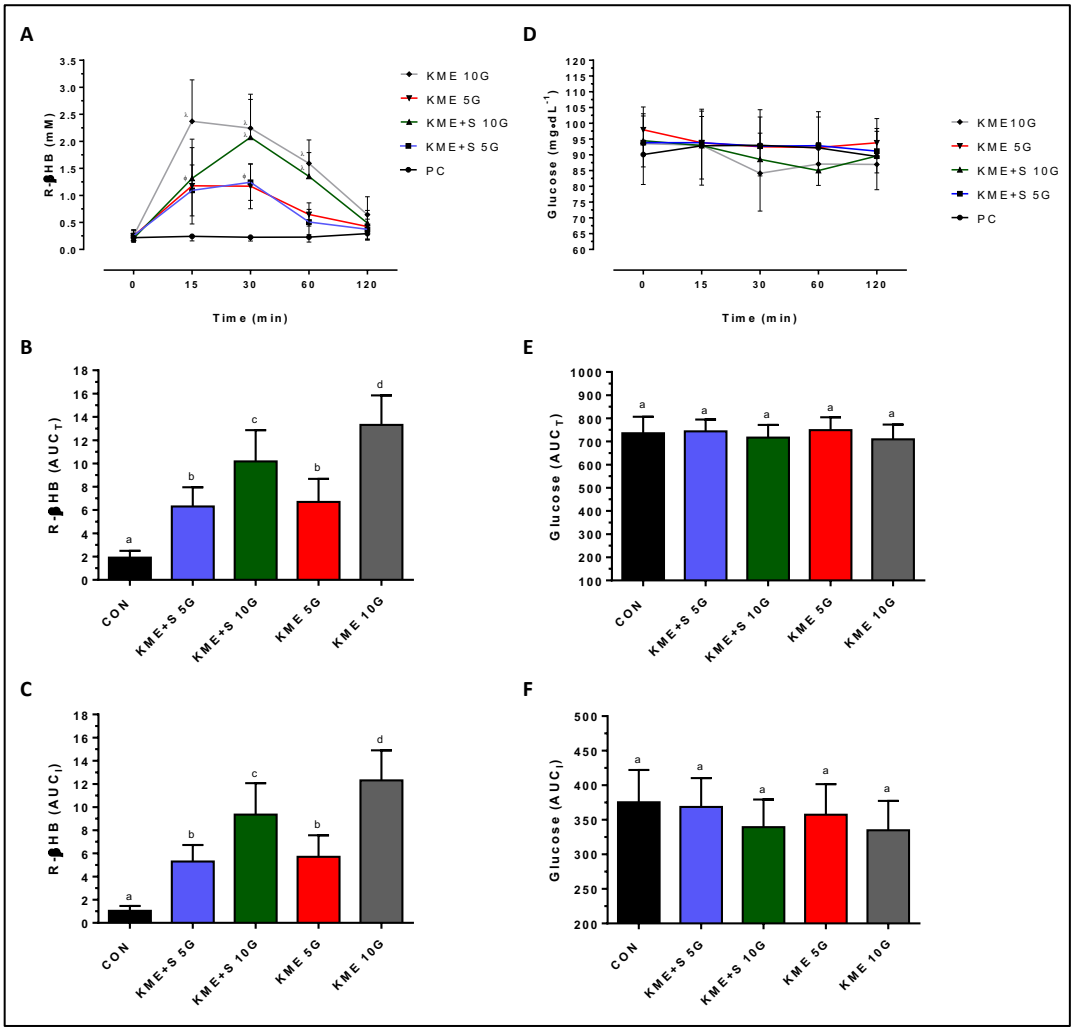
presentation purposes. R-ßHB concentrations were significantly higher than baseline for each condition ($P < 0.05$) after 15 minutes (Fig. 3A). There were no significant differences in the magnitude of response for the KME-5g, KME+S-5g, or KME+S-10g conditions at 15 minutes ($P > 0.05$). However, KME-10g was significantly higher than each of the other conditions ($P < 0.05$) reaching a peak concentration of 2.4 ± 0.1 mM at 15 minutes. In contrast, KME+S-10g reached its peak at 30 minutes (2.1 ± 0.1 mM) and was similar to KME-10g (2.2 ± 0.1 mM), $p = 0.23$. R-βHB concentrations for each of the treatment conditions declined by 60 minutes with only KME-10g and KME+S-10g remaining higher than CON ($p = 0.17$ and 0.05, respectively). R-βHB concentrations returned to baseline by 120 minutes with no significant differences among conditions. Calculated R-βHB total and incremental AUC (Fig. 3B and 3C) were not different as a results of similar baseline fasting concentrations. Each of the ketone drinks increased AUC compared to control with KME-5g and KME+S-5g producing similar results. KME-10g was significantly higher than KME+S-10g ($P < 0.05$) and each of the other conditions ($P < 0.05$ for all comparisons).

Glucose concentrations were not significantly different among conditions at any time point (Fig. 3D). Glucose AUC_T was also similar for each condition (Fig. 3E). Although glucose AUC_I was not significantly different among each study condition, KME-10g and KME+S-10g approached significance (Fig. 3F; $P > 0.05$). There were no correlations between glucose and R-ßHB at baseline, 15 minutes, 60 minutes, and 120 minutes for any of the conditions. However, at 30 minutes, there were significant negative correlations between glucose and R-ßHB for KME-5g ($r = -0.73$, $p = 0.0028$), KME+S-5g ($r = -0.57$, $p = 0.0325$) and KME+S-10g (-0.57, $p = 0.0271$) suggesting that

increasing circulating R-ßHB concentrations produce a delayed decrease in circulating glucose concentrations. Surprisingly, there was no correlation between circulating R-βHB and glucose with the KME-10g condition at any timepoint.

Figure 3

Effects of KME and KME+S Drinks on Circulating R-ßHB and Glucose



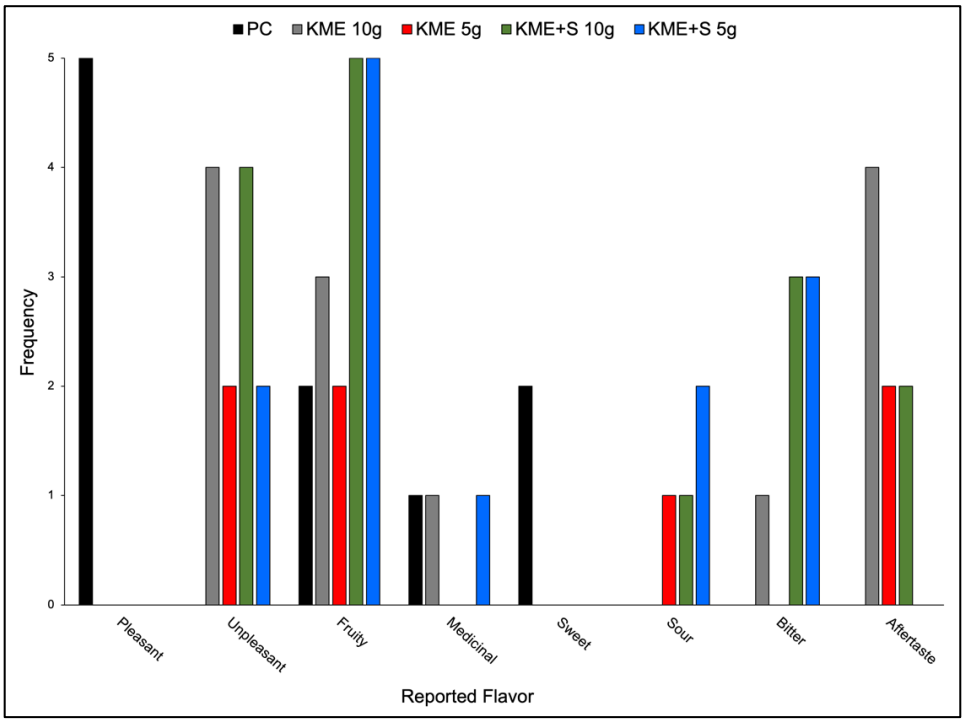
Note. (A) R-ßHB. (B) AUC_T of R-ßHB. (C) AUC_I of R-ßHB. (D) Plasma glucose. (E) AUC_T of plasma glucose. (F) AUC_I of plasma glucose. Symbols λ and Φ are used to group data points that are similar to each other and different from CON at each time point at $p < 0.05$ significance level. Comparisons that have the same letter are not significantly different.

Acceptability and Tolerability of KME and KME+S Drinks

Although taste was not quantitatively assessed, participants were encouraged to explain the flavor of each study drink either by writing in the "Additional Comments" section of the questionnaire or by verbally reporting to the research team; these data are shown in Fig. 4. Both KME-10g and KME+S-10g drinks were more frequently reported as being unpleasant, compared to the lower concentration drinks. Compared to KME, KME+S was more often reported as being "fruity" and "bitter," although aftertaste was more frequently reported for KME in a concentration-dependent fashion.

Figure 4

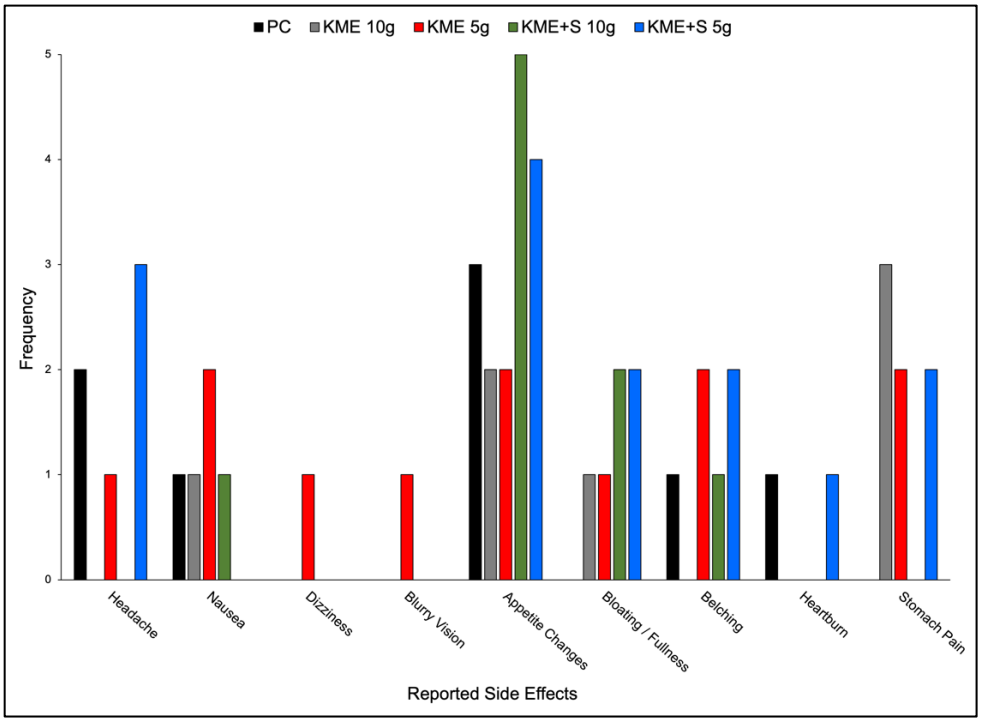
Frequency of Reported Flavors by Condition



All reported side-effects were reported as “mild,” except for one instance of nausea which was reported as “moderate” for KME-5g (Fig. 5). The most commonly reported side effects were appetite changes and stomach pain, as well as headache, nausea, belching, heartburn, and bloating to a lesser extent. Decreases in appetite were reported for each condition, but with greater frequency for KME+S. Dizziness and blurry vision were reported by one participant for KME-5g, but these symptoms quickly resolved within one hour.

Figure 5

Frequency of Reported Side Effects by Condition



DISCUSSION

The current investigation examined the tolerability and acceptability of ketone monoester (KME) and ketone monoester-salt (KME+S) drinks and their effects on circulating R-ßHB and glucose in healthy young adults. At the 10 g dose, the KME drink achieved ~13% higher R-ßHB concentrations than the KME+S drink at a similar concentration. A concentration-dependent effect was found for both exogenous ketones, and there was a discernible negative correlation between R-ßHB and glucose for each of the conditions except KME-10g. Although both exogenous ketone drinks had reported "unpleasant" flavors, the KME drink was less acceptable due to its more frequently reported aftertaste. Participants reported mild adverse effects such as gastrointestinal distress, headache, and decreases in appetite. Tolerability did not noticeably differ between exogenous ketone drinks, although "appetite changes" were more frequently reported for the KME+S drink. An additional purpose of the study was to compare the effects of two concentrations of two different exogenous ketones on the magnitude and duration of R-ßHB and glucose concentrations. The high concentration of the KME produced the greatest magnitude of R-ßHB concentrations compared to any of the conditions. These findings support our hypothesis that the KME drink would raise circulating ketone concentrations to a greater magnitude and duration than the KME+S and CON.

Past studies examining the effects of ketogenic diet have demonstrated its potential to improve a number of chronic health conditions such as refractory epilepsy, obesity, and neurodegenerative diseases. However, because of the high-fat (>80%) and low-carbohydrate composition of the diet, some have suggested that KD could raise circulating LDL-C concentrations and subsequently increase risk of cardiovascular disease, although this has not been substantiated. Furthermore, and despite the short-term success observed with KD, adherence is low (Batch et al., 2020; Li et al., 2022; Murphy & Jenkins, 2019; O'Neill & Raggi, 2020; Paoli et al., 2011).

The commercial availability of exogenous ketones provides an alternative approach to increase circulating ketone concentrations without extensive alterations to diet. However, studies are needed to investigate the acute, short-, and long-term effects of exogenous ketones, particularly as it relates to dosing, frequency of dosing, and duration of responses. While there is significant potential for exogenous ketones, characterization of the overall response to exogenous ketones are needed before they can be extensively deployed in humans.

Due to the costs of synthesizing ketone esters, the KME product has a higher cost than the KME+S product. Although the KME product achieved higher R-ßHB concentrations at the 10 g dose at 15 minutes, the KME+S product at 10 g produced similar R-βHB concentrations and was more acceptable to participants suggesting that the mixture offers a cheaper approach with the possibility of similar success compared with KME. This was also true for R-ßHB concentrations with the KME and KME+S drinks at

the 5 g concentration. Therefore, consumers utilizing the exogenous ketones in this study for general health purposes may find the KME+S drinks to be more cost-effective and tolerable.

Several studies have examined the effects of KME in humans (Table 1). The current study included a sample of healthy, normal-weight, young adults. A number of prior studies have examined the effects of KME in healthy adults (Clarke et al., 2012; Myette-Côté et al., 2018; Soto-Mota et al., 2019; Stubbs et al., 2017; Stubbs et al., 2018) as well as in trained adult athletes (Cox et al., 2016; Dearlove et al., 2019; Evans et al, 2019; Holdsworth et al., 2017; Poffé et al., 2020; Vandoorne et al., 2017). Other studies have investigated the effects of KME in participants with very long-chain acyl-CoA dehydrogenase deficiency (Bleeker et al., 2020), glycogen storage disease type V (Løkken et al., 2022), Parkinson's disease (Norwitz et al., 2020), and obesity (Myette-Côté et al., 2019; Neudorf et al., 2020).

Unlike the present study, some prior investigations added CHO energy alongside KME, typically for the purpose of exercise recovery (Bleeker et al., 2020; Clarke et al., 2012; Evans et al., 2019; Poffé et al., 2020; Vandoorne et al., 2017). When KME was combined with a meal replacement drink, βHB concentrations reached ~0.28 mM at 140 mg/kg body mass (BM), ~1.0 mM at 357 mg/kg BM, and ~3.3 mM at 714 mg/kg BM (Clarke et al., 2012). Without added CHO, βHB concentrations reached ~1.4 mM at 141 mg/kg BM, ~2.8 mM at 282 mg/kg BM, and ~3.2 mM at 395 mg/kg BM (Stubbs et al., 2017). In the current investigation, we achieved slightly higher concentrations of R-βHB with the KME-10g despite similar concentrations (10 g was equivalent to ~ 143.5 mg/kg and is similar to the lowest concentration used by Stubbs and colleagues, 2017). Cox and

colleagues (2016) examined the effects of KME at a dose of 573 mg/kg BM in endurance athletes. βHB concentrations exceeded 5.0 mM after 30 minutes following consumption of KME alone, but only reached up to ~3.0 following consumption of both KME and CHO energy. A study by Evans and colleagues (2019) also used a dose of 573 mg/kg BM KME combined with CHO energy in endurance athletes, and βHB concentrations reached approximately 0.99 mM by 30 minutes illustrating the βHB lowering effects of CHO during KME consumption. At the same dose, but without added CHO energy, athletes consumed KME after glucose-depleting exercise and demonstrated βHB concentrations of over 4.0 mM by 30 minutes (Holdsworth et al., 2017). Finally, at a dose of 482 mg/kg BM of the KME, βHB concentrations reached ~3.2 mM (Myette-Côté et al., 2018). Taken together, these findings suggest that there is a consistent concentration-dependent effect of the KME on circulating βHB concentrations. Future studies will be needed to more thoroughly understand the effects of meal composition and amount of energy consumed and their influence on circulating concentrations of βHB.

Four studies have examined the effects of repeated doses of KME. When athletes consumed KME as two doses prior to exercise (25 g for the first dose and 20 g for the second dose), βHB levels reached ~2.0 mM after 30 minutes of the first dose and ~3.0 mM after 1 hour of the first dose and after 30 minutes of the second dose (Poffé et al., 2020). When healthy adults consumed 25 mL of KME three times daily over 28 consecutive days, βHB concentrations reached ~4.1 mM at 30 minutes after the third drink of the day (Soto-Mota et al., 2019). When healthy adults consumed three doses of 387 mg/kg BM, βHB levels reached ~3.8 mM at 60 minutes after the third drink of the day (Stubbs et al., 2017). Following glycogen-depleting exercise, healthy adults

consumed 500 mg/kg BM at the beginning of a 5-hour recovery period, followed by 250 mg/kg BM at each hour point (Vandoorne et al., 2017). Alongside the KME, participants consumed recovery drinks containing high CHO and protein every 30 minutes. βHB levels reached ~2.9 mM at 30 minutes into the recovery period and ~4.3 mM at 4 hours into the recovery period. These findings also support the need to explore the role of various forms of exercise and their interaction with diet to establish optimal dosing strategies.

Unlike the majority of prior studies, the present study takes a more practical approach to dosing exogenous ketones, as drinks were provided in absolute amounts of total ketones as opposed to being dosed according to individual body mass. While there are disadvantages to this approach, namely that differences in body mass are likely to affect circulating concentrations, this strategy more realistically models how a consumer may purchase and utilize ketone products. For reference, the dosage amounts in the present study were approximately 71.7 mg/kg BM and 143.5 mg/kg BM when accounting for the average weight of all participants.

Future studies should examine the magnitude and duration of repeated doses of exogenous ketones, as well as the effects of exogenous ketones in the fed state and in the context of different meal types. Such findings may demonstrate how consumers may better utilize exogenous ketones on a daily basis. Future investigations should consider using a Visual Analog Scale in order to better assess taste and acceptability, as well as effects of ketone drinks on appetite changes which were observed in this study for the KME+S drink.

CONCLUSION

Both exogenous ketones used in the current investigation drinks had similar tolerability, although the KME+S drink appears to be slightly more acceptable. When accounting for average body weight of all participants, the highest dose used in the present study was approximately 143.5 mg/kg BM. At this dose, the KME drink achieved βHB concentrations of ~2.4 mM and the KME+S mix reached ~2.1 mM βHB. Similar doses used in previous studies reached βHB concentrations of ~1.4 mM when participants consumed KME alone (Stubbs et al., 2017) and ~0.28 mM when participants consumed a meal replacement drink alongside KME (Clarke et al., 2012). Due to the expensive cost of "pure" KME products, consumers may find the KME+S drink to be more cost-effective than the KME drink. Additionally, lower doses may help to maintain cost-effectiveness without compromising desired outcomes when consuming exogenous ketones for general health purposes. While additional studies are necessary, it is possible that smaller, but more frequent, dosing could not only sustain higher concentrations but produce a scaffolding effect depending on when additional dosing occurs. Overall, exogenous ketones provide increase circulating ketone concentrations rapidly and transiently. It remains to be investigated whether exogenous ketone consumption produce convergent or divergent effects compared to ketogenic diet.

REFERENCES

Anderson, Vichitbandra, S., Qian, W., & Kryscio, R. J. (1999). Long-term weight maintenance after an intensive weight-loss program. *Journal of the American College of Nutrition*, 18(6), 620–627. https://doi.org/10.1080/07315724.1999.10718897

Batch, J. T., Lamsal, S. P., Adkins, M., Sultan, S., & Ramirez, M. N. (2020). Advantages and disadvantages of the ketogenic diet: A review article. *Cureus*. https://doi.org/10.7759/cureus.9639

Benyó, Z., Gille, A., Kero, J., Csiky, M., Suchánková, M. C., Nüsing, R. M., Moers, A., Pfeffer, K., & Offermanns, S. (2005). GPR109A (PUMA-G/HM74A) mediates nicotinic acid–induced flushing. *Journal of Clinical Investigation, 115*(12), 3634–3640. https://doi.org/10.1172/JCI23626

Biden, T. J., & Taylor, K. W. (1983). Effects of ketone bodies on insulin release and islet-cell metabolism in the rat. *Biochemical Journal, 212*(2), 371–377. https://doi.org/10.1042/bj2120371

Bistrian, B. R. (2019). Some musings about differential energy metabolism with ketogenic diets. *Journal of Parenteral and Enteral Nutrition, 43*(5), 578–582. https://doi.org/10.1002/jpen.1665

Bleeker, J. C., Visser, G., Clarke, K., Ferdinandusse, S., Haan, F. H., Houtkooper, R. H., IJlst, L., Kok, I. L., Langeveld, M., Pol, W. L., Sain-van der Velden, M. G. M., Sibeijn-Kuiper, A., Takken, T., Wanders, R. J. A., Weeghel, M., Wijburg, F. A., Woude, L. H., Wüst, R. C. I., Cox, P. J., & Jeneson, J. A. L. (2020). Nutritional ketosis improves exercise metabolism in patients with very long-chain acyl-CoA dehydrogenase deficiency. *Journal of Inherited Metabolic Disease, 43*(4), 787–799. https://doi.org/10.1002/jimd.12217

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. *The British Journal of Nutrition, 110*(7), 1178–1187. https://doi.org/10.1017/S0007114513000548

Burén, J., Ericsson, M., Damasceno, N. R. T., & Sjödin, A. (2021). A ketogenic low-carbohydrate high-fat diet increases LDL cholesterol in healthy, young, normal-weight women: A randomized controlled feeding trial. *Nutrients, 13*(3). https://doi.org/10.3390/nu13030814

Burke, L. M., Ross, M. L., Garvican-Lewis, L. A., Welvaert, M., Heikura, I. A., Forbes, S. G., Mirtschin, J. G., Cato, L. E., Strobel, N., Sharma, A. P., & Hawley, J. A. (2017). Low carbohydrate, high fat diet impairs exercise economy and negates the performance benefit from intensified training in elite race walkers. *The Journal of Physiology, 595*(9), 2785–2807. https://doi.org/10.1113/JP273230

Cahill, G. F. (2006). Fuel metabolism in starvation. *Annual Review of Nutrition, 26*(1), 1–22. https://doi.org/10.1146/annurev.nutr.26.061505.111258

Cai, Q. Y., Zhou, Z. J., Luo, R., Gan, J., Li, S. P., Mu, D. Z., & Wan, C. M. (2017). Safety and tolerability of the ketogenic diet used for the treatment of refractory childhood epilepsy: A systematic review of published prospective studies. *World Journal of Pediatrics: WJP, 13*(6), 528–536. https://doi.org/10.1007/s12519-017-0053-2

Clark, D., Munten, S., Herzig, K.-H., & Gagnon, D. D. (2021). Exogenous ketone salt supplementation and whole-body cooling do not improve short-term physical performance. *Frontiers in Nutrition, 8*. https://doi.org/10.3389/fnut.2021.663206

Clarke, K., Tchabanenko, K., Pawlosky, R., Carter, E., Todd King, M., Musa-Veloso, K., Ho, M., Roberts, A., Robertson, J., Vanitallie, T. B., & 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. https://doi.org/10.1016/j.yrtph.2012.04.008

Cohen, C. W., Fontaine, K. R., Arend, R. C., Alvarez, R. D., Leath III, C. A., Huh, W. K., Bevis, K. S., Kim, K. H., Straughn Jr, J. M., & Gower, B. A. (2018). A ketogenic diet reduces central obesity and serum insulin in women with ovarian or endometrial cancer. *The Journal of Nutrition, 148*(8), 1253–1260.

Cox, P. J., Kirk, T., Ashmore, T., Willerton, K., Evans, R., Smith, A., Murray, A. J., Stubbs, B., West, J., McLure, S. W., King, M. T., Dodd, M. S., Holloway, C., Neubauer, S., Drawer, S., Veech, R. L., Griffin, J. L., & Clarke, K. (2016). Nutritional ketosis alters fuel preference and thereby endurance performance in athletes. *Cell Metabolism, 24*(2), 256–268. https://doi.org/10.1016/j.cmet.2016.07.010

Cuenoud, B., Hartweg, M., Godin, J.-P., Croteau, E., Maltais, M., Castellano, C.-A., Carpentier, A. C., & Cunnane, S. C. (2020). Metabolism of exogenous D-beta-hydroxybutyrate, an energy substrate avidly consumed by the heart and kidney. *Frontiers in Nutrition, 7*(13). https://doi.org/10.3389/fnut.2020.00013

Davis, R. A. H., Deemer, S. E., Bergeron, J. M., Little, J. T., Warren, J. L., Fisher, G., Smith, D. L., Fontaine, K. R., Dickinson, S. L., Allison, D. B., & Plaisance, E. P. (2019). Dietary R, S-1,3-butanediol diacetoacetate reduces body weight and adiposity in obese mice fed a high-fat diet. *The FASEB Journal, 33*(2), 2409–2421. https://doi.org/10.1096/fj.201800821RR

Dearlove, D. J., Faull, O. K., Rolls, E., Clarke, K., & Cox, P. J. (2019). Nutritional ketoacidosis during incremental exercise in healthy athletes. *Frontiers in Physiology, 10*. https://doi.org/10.3389/fphys.2019.00290

Deemer, Davis, R. A. H., Gower, B. A., Koutnik, A. P., Poff, A. M., Dickinson, S. L., Allison, D. B., D'Agostino, D. P., & Plaisance, E. P. (2019). Concentration-Dependent effects of a dietary ketone ester on components of energy balance in mice. *Frontiers in Nutrition (Lausanne), 6*, 56–56. https://doi.org/10.3389/fnut.2019.00056

Drabińska, N., Wiczkowski, W., & Piskuła, M. K. (2021). Recent advances in the application of a ketogenic diet for obesity management. *Trends in Food Science & Technology, 110*, 28–38. https://doi.org/10.1016/j.tifs.2021.01.080

Evans, M., McSwiney, F. T., Brady, A. J., & Egan, B. (2019). No benefit of ingestion of a ketone monoester supplement on 10-km running performance. *Medicine & Science in Sports & Exercise*. https://doi.org/10.1249/MSS.0000000000002065

Falkenhain, K., Daraei, A., Forbes, S. C., & Little, J. P. (2022). Effects of exogenous ketone supplementation on blood glucose: A systematic review and meta-analysis. *Advances in Nutrition, 13*(5), 1697–1714. https://doi.org/10.1093/advances/nmac036

Friedman, J. M., & Halaas, J. L. (1998). Leptin and the regulation of body weight in mammals. *Nature, 395*(6704), 763–770. https://doi.org/10.1038/27376

Gibson, A. A., Seimon, R. V., Lee, C. M. Y., Ayre, J., Franklin, J., Markovic, T. P., Caterson, I. D., & Sainsbury, A. (2015). Do ketogenic diets really suppress appetite? A systematic review and meta-analysis. *Obesity Reviews, 16*(1), 64–76.

Gilbert, D. L., Pyzik, P. L., & Freeman, J. M. (2000). The ketogenic diet: Seizure control correlates better with serum beta-hydroxybutyrate than with urine ketones. Journal of *Child Neurology, 15*(12), 787–790. https://doi.org/10.1177/088307380001501203

Griffin, B. A., Freeman, D. J., Tait, G. W., Thomson, J., Caslake, M. J., Packard, C. J., & Shepherd, J. (1994). Role of plasma triglyceride in the regulation of plasma low density lipoprotein (LDL) subfractions: relative contribution of small, dense LDL to coronary heart disease risk. *Atherosclerosis, 106*(2), 241–253. https://doi.org/10.1016/0021-9150(94)90129-5

Groleau, V., Schall, J. I., Stallings, V. A., & Bergqvist, C. A. (2014). Long-term impact of the ketogenic diet on growth and resting energy expenditure in children with intractable epilepsy. *Developmental Medicine and Child Neurology, 56*(9), 898–904.

Hall, K. D., Chen, K. Y., Guo, J., Lam, Y. Y., Leibel, R. L., Mayer, L. E., Reitman, M. L., Rosenbaum, M., Smith, S. R., Walsh, B. T., & Ravussin, E. (2016). Energy expenditure and body composition changes after an isocaloric ketogenic diet in overweight and obese men. *The American Journal of Clinical Nutrition, 104*(2), 324–333. https://doi.org/10.3945/ajcn.116.133561

Holdsworth, D. A., Cox, P. J., Kirk, T., Stradling, H., Impey, S. G., & Clarke, K. (2017). A ketone ester drink increases postexercise muscle glycogen synthesis in humans. *Medicine and Science in Sports and Exercise, 49*(9), 1789–1795. https://doi.org/10.1249/MSS.0000000000001292

Hughes, M. M., & O'Neill, L. A. J. (2018). Metabolic regulation of NLRP3. *Immunological Reviews, 281*(1), 88–98. https://doi.org/10.1111/imr.12608

Jabekk, P. T., Moe, I. A., Meen, H. D., Tomten, S. E., & Høstmark, A. T. (2010). Resistance training in overweight women on a ketogenic diet conserved lean body mass while reducing body fat. *Nutrition & Metabolism, 7*(1), 17.

Ji, L., He, Q., Liu, Y., Deng, Y., Xie, M., Luo, K., Cai, X., Zuo, Y., Wu, W., Li, Q., Zhou, R., & Li, T. (2022). Ketone body β-hydroxybutyrate prevents myocardial oxidative stress in septic cardiomyopathy. *Oxidative Medicine and Cellular Longevity, 2022*, 1–14. https://doi.org/10.1155/2022/2513837

Jo, E., Silva, M. S. C., Auslander, P. A. T., Arreglado, M. J. P., Elam, P. M. L., Osmond, M. A. D., Steinberg, M. R., & Wong, M. M. W. H. (2020). The effects of 10-day exogenous ketone consumption on repeated time trial running performances: A randomized-control trial. *Journal of Dietary Supplements, 19*(1), 34–48. https://doi.org/10.1080/19390211.2020.1838022

Johnston, C. S., Tjonn, S. L., Swan, P. D., White, A., Hutchins, H., & Sears, B. (2006). Ketogenic low-carbohydrate diets have no metabolic advantage over nonketogenic low-carbohydrate diets. *The American Journal of Clinical Nutrition, 83*(5), 1055–1061. https://doi.org/10.1093/ajcn/83.5.1055

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. *The American Journal of Clinical Nutrition, 87*(1), 44–55. https://doi.org/10.1093/ajcn/87.1.44

Kang, J., Ratamess, N. A., Faigenbaum, A. D., & Bush, J. A. (2020). Ergogenic properties of ketogenic diets in normal-weight individuals: A systematic review. *Journal of the American College of Nutrition, 39*(7), 665–675.

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. https://doi.org/10.1073/PNAS.97.10.5440

Kim, J.-M. (2017). Ketogenic diet: Old treatment, new beginning. *Clinical Neurophysiology Practice, 2*, 161–162. https://doi.org/10.1016/j.cnp.2017.07.001

Kong, Z., Sun, S., Shi, Q., Zhang, H., Tong, T. K., & Nie, J. (2020). Short-term ketogenic diet improves abdominal obesity in overweight/obese Chinese young females. *Frontiers in Physiology, 11*, 856. https://doi.org/10.3389/fphys.2020.00856

Langhans, W., Damaske, U., & Scharrer, E. (1985). Different metabolites might reduce food intake by the mitochondrial generation of reducing equivalents. *Appetite, 6*(2), 143–152. https://doi.org/10.1016/S0195-6663(85)80035-0

Langhans, W., Pantel, K., & Scharrer, E. (1985). Ketone kinetics and D-(−)-3-hydroxybutyrate-induced inhibition of feeding in rats. *Physiology & Behavior, 34*(4), 579–582. https://doi.org/10.1016/0031-9384(85)90052-6

Langhans, W., Wiesenreiter, F., & Scharrer, E. (1983). Different effects of subcutaneous D,L-3-hydroxybutyrate and acetoacetate injections on food intake in rats. *Physiology & Behavior, 31*(4), 483–486. https://doi.org/10.1016/0031-9384(83)90070-7

Li, S., Lin, G., Chen, J., Chen, Z., Xu, F., Zhu, F., Zhang, J., & Yuan, S. (2022). The effect of periodic ketogenic diet on newly diagnosed overweight or obese patients with type 2 diabetes. *BMC Endocrine Disorders, 22*(1), 34. https://doi.org/10.1186/s12902-022-00947-2

Lodi, A., Zarantonello, L., Bisiacchi, P. S., Cenci, L., & Paoli, A. (2020). Ketonemia and glycemia affect appetite levels and executive functions in overweight females during two ketogenic diets. *Obesity (Silver Spring, Md.), 28*(10), 1868–1877. https://doi.org/10.1002/oby.22934

Løkken, N., Storgaard, J. H., Revsbech, K. L., Voermans, N. C., van Hall, G., Vissing, J., & Ørngreen, M. C. (2022). No effect of oral ketone ester supplementation on exercise capacity in patients with McArdle disease and healthy controls: A randomized placebo-controlled cross-over study. *Journal of Inherited Metabolic Disease, 45*(3), 502–516. https://doi.org/10.1002/jimd.12484

Matthews, J. N., Altman, D. G., Campbell, M. J., & Royston, P. (1990). Analysis of serial measurements in medical research. *BMJ*, *300*(6719), 230–235. https://doi.org/10.1136/bmj.300.6719.230

Mikkelsen, K. H., Seifert, T., Secher, N. H., Grøndal, T., & van Hall, G. (2015). Systemic, cerebral and skeletal muscle ketone body and energy metabolism during acute hyper-D-β-hydroxybutyratemia in post-absorptive healthy males. The Journal of Clinical Endocrinology and Metabolism, 100(2), 636–643. https://doi.org/10.1210/jc.2014-2608

Miyamoto, J., Ohue-Kitano, R., Mukouyama, H., Nishida, A., Watanabe, K., Igarashi, M., Irie, J., Tsujimoto, G., Satoh-Asahara, N., Itoh, H., & Kimura, I. (2019). Ketone body receptor GPR43 regulates lipid metabolism under ketogenic conditions. *Proceedings of the National Academy of Sciences, 116*(47), 23813–23821. https://doi.org/10.1073/pnas.1912573116

Murphy, E. A., & Jenkins, T. J. (2019). A ketogenic diet for reducing obesity and maintaining capacity for physical activity: Hype or hope? *Current Opinion in Clinical Nutrition and Metabolic Care, 22*(4), 314–319. https://doi.org/10.1097/MCO.0000000000000572

Myette-Côté, É., Caldwell, H. G., Ainslie, P. N., Clarke, K., & Little, J. P. (2019). A ketone monoester drink reduces the glycemic response to an oral glucose challenge in individuals with obesity: A randomized trial. *The American Journal of Clinical Nutrition, 110*(6), 1491–1501. https://doi.org/10.1093/ajcn/nqz232

Myette-Côté, É., Neudorf, H., Rafiei, H., Clarke, K., & Little, J. P. (2018). Prior ingestion of exogenous ketone monoester attenuates the glycaemic response to an oral

glucose tolerance test in healthy young individuals. *The Journal of Physiology, 596*(8), 1385–1395. https://doi.org/10.1113/JP275709

Nebeling, L. C., Miraldi, F., Shurin, S. B., & Lerner, E. (1995). Effects of a ketogenic diet on tumor metabolism and nutritional status in pediatric oncology patients: Two case reports. *Journal of the American College of Nutrition, 14*(2), 202–208. https://doi.org/10.1080/07315724.1995.10718495

Neudorf, H., Myette-Côté, É., & P. Little, J. (2020). The impact of acute ingestion of a ketone monoester drink on LPS-stimulated NLRP3 activation in humans with obesity. *Nutrients, 12*(3), 854. https://doi.org/10.3390/nu12030854

Newman, J. C., & Verdin, E. (2013). Ketone bodies as signaling metabolites. *Trends in Endocrinology and Metabolism, 25*(1), 42–52. https://doi.org/10.1016/j.tem.2013.09.002

Newman, J. C., & Verdin, E. (2014). β-hydroxybutyrate: Much more than a metabolite. *Diabetes Research and Clinical Practice, 106*(2), 173–181. https://doi.org/10.1016/j.diabres.2014.08.009

Norwitz, N. G., Dearlove, D. J., Lu, M., Clarke, K., Dawes, H., & Hu, M. T. (2020). A ketone ester drink enhances endurance exercise performance in Parkinson's Disease. *Frontiers in Neuroscience, 14*. https://doi.org/10.3389/fnins.2020.584130

O'Neill, B., & Raggi, P. (2020). The ketogenic diet: Pros and cons. Atherosclerosis, 292, 119–126. https://doi.org/10.1016/j.atherosclerosis.2019.11.021

Owen, O. E. (2005). Ketone bodies as a fuel for the brain during starvation. *Biochemistry and Molecular Biology Education, 33*(4), 246–251. https://doi.org/10.1002/bmb.2005.49403304246

Paoli, A., Cenci, L., & Grimaldi, K. A. (2011). Effect of ketogenic Mediterranean diet with phytoextracts and low carbohydrates/high-protein meals on weight, cardiovascular risk factors, body composition and diet compliance in Italian council employees. *Nutrition Journal, 10*(1), 112. https://doi.org/10.1186/1475-2891-10-112

Park, S.-Y., & Kim, J.-S. (2020). A short guide to histone deacetylases including recent progress on class II enzymes. *Experimental & Molecular Medicine, 52*(2), 204–212. https://doi.org/10.1038/s12276-020-0382-4

Peleg, Sananbenesi, F., Zovoilis, A., Burkhardt, S., Bahari-Javan, S., Agis-Balboa, R. C., Cota, P., Wittnam, J. L., Gogol-Doering, A., Opitz, L., Salinas-Riester, G., Dettenhofer, M., Kang, H., Farinelli, L., Chen, W., & Fischer, A. (2010). Altered histone acetylation is associated with age-dependent memory impairment in mice. *Science (American Association for the Advancement of Science), 328*(5979), 753–756. https://doi.org/10.1126/science.1186088

Perticone, M., Maio, R., Sciacqua, A., Suraci, E., Pinto, A., Pujia, R., Zito, R., Gigliotti, S., Sesti, G., & Perticone, F. (2019). Ketogenic diet-induced weight loss is associated with an increase in vitamin D levels in obese adults. *Molecules (Basel, Switzerland), 24*(13). https://doi.org/10.3390/molecules24132499

Plaisance, E. P., Lukasova, M., Offermanns, S., Zhang, Y., Cao, G., & Judd, R. L. (2009). Niacin stimulates adiponectin secretion through the GPR109A receptor. *American Journal of Physiology-Endocrinology and Metabolism, 296*(3), E549–E558. https://doi.org/10.1152/ajpendo.91004.2008

Plecko, B., Stoeckler-Ipsiroglu, S., Schober, E., Harrer, G., Mlynarik, V., Gruber, S., Moser, E., Moeslinger, D., Silgoner, H., & Ipsiroglu, O. (2002). Oral beta-hydroxybutyrate supplementation in two patients with hyperinsulinemic hypoglycemia: Monitoring of beta-hydroxybutyrate levels in blood and cerebrospinal fluid, and in the brain by in vivo magnetic resonance spectroscopy. *Pediatric Research, 52*(2), 301–306. https://doi.org/10.1203/00006450-200208000-00025

Poffé, C., Ramaekers, M., Bogaerts, S., & Hespel, P. (2020). Exogenous ketosis impacts neither performance nor muscle glycogen breakdown in prolonged endurance exercise. *Journal of Applied Physiology, 128*(6), 1643–1653. https://doi.org/10.1152/japplphysiol.00092.2020

Prins, P. J., D'Agostino, D. P., Rogers, C. Q., Ault, D. L., Welton, G. L., Jones, D. W., Henson, S. R., Rothfuss, T. J., Aiken, K. G., Hose, J. L., England, E. L., Atwell, A. D., Buxton, J. D., & Koutnik, A. P. (2020). Dose response of a novel exogenous ketone supplement on physiological, perceptual and performance parameters. *Nutrition & Metabolism, 17*(1), 81. https://doi.org/10.1186/s12986-020-00497-1

Prins, P. J., Koutnik, A. P., D'Agostino, D. P., Rogers, C. Q., Seibert, J. F., Breckenridge, J. A., Jackson, D. S., Ryan, E. J., Buxton, J. D., & Ault, D. L. (2020). Effects of an exogenous ketone supplement on five-kilometer running performance. *Journal of Human Kinetics, 72*(1), 115–127. https://doi.org/10.2478/hukin-2019-0114

Puchalska, P., & Crawford, P. A. (2017). Multi-dimensional roles of ketone bodies in fuel metabolism, signaling, and therapeutics. *Cell Metabolism, 25*(2), 262–284. https://doi.org/10.1016/j.cmet.2016.12.022

Puchalska, P., & Crawford, P. A. (2021). Metabolic and signaling roles of ketone bodies in health and disease. *Annual Review of Nutrition, 41*, 49–77. https://doi.org/10.1146/annurev-nutr-111120-111518

Puchalska, P., Martin, S. E., Huang, X., Lengfeld, J. E., Daniel, B., Graham, M. J., Han, X., Nagy, L., Patti, G. J., & Crawford, P. A. (2019). Hepatocyte-macrophage acetoacetate shuttle protects against tissue fibrosis. *Cell Metabolism, 29*(2), 383-398.e7. https://doi.org/10.1016/j.cmet.2018.10.015

Rodger, S., Plews, D. J., Laursen, P. B., & Driller, M. W. (2017). Oral β-hydroxybutyrate salt fails to improve 4-minute cycling performance following submaximal exercise. *Journal of Science and Cycling, 6*(1), 26-31.

Saslow, L. R., Daubenmier, J. J., Moskowitz, J. T., Kim, S., Murphy, E. J., Phinney, S. D., Ploutz-Snyder, R., Goldman, V., Cox, R. M., Mason, A. E., Moran, P., & Hecht, F. M. (2017). Twelve-month outcomes of a randomized trial of a moderate-carbohydrate versus very low-carbohydrate diet in overweight adults with type 2 diabetes mellitus or prediabetes. *Nutrition & Diabetes, 7*(12), 304. https://doi.org/10.1038/s41387-017-0006-9

Schutz, Y., Montani, J.-P., & Dulloo, A. G. (2021). Low-carbohydrate ketogenic diets in body weight control: A recurrent plaguing issue of fad diets? *Obesity Reviews: An Official Journal of the International Association for the Study of Obesity, 22*(S2), e13195. https://doi.org/10.1111/obr.13195

Shao, Peng, Q., Dong, M., Gao, K., Li, Y., Li, Y., Li, C.-Y., & Liu, Y. (2020). Histone deacetylase HDA-1 modulates mitochondrial stress response and longevity. *Nature Communications, 11*(1), 4639–4639. https://doi.org/10.1038/s41467-020-18501-w

Sjödin, A., Hellström, F., Sehlstedt, E., Svensson, M., & Burén, J. (2020). Effects of a ketogenic diet on muscle fatigue in healthy, young, normal-weight women: A randomized controlled feeding trial. *Nutrients, 12*(4). https://doi.org/10.3390/nu12040955

Soto-Mota, A., Norwitz, N. G., Evans, R. D., & Clarke, K. (2021). Exogenous d-β-hydroxybutyrate lowers blood glucose in part by decreasing the availability of L-alanine for gluconeogenesis. *Endocrinology, Diabetes & Metabolism, 5*(1). https://doi.org/10.1002/edm2.300

Soto-Mota, A., Vansant, H., Evans, R. D., & Clarke, K. (2019). Safety and tolerability of sustained exogenous ketosis using ketone monoester drinks for 28 days in healthy adults. *Regulatory Toxicology and Pharmacology, 109*, 104506. https://doi.org/10.1016/j.yrtph.2019.104506

Srivastava, S., Kashiwaya, Y., King, M. T., Baxa, U., Tam, J., Niu, G., Chen, X., Clarke, K., & 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. https://doi.org/10.1096/fj.11-200410

Stefan, M., Sharp, M., Gheith, R., Lowery, R., & Wilson, J. (2020). The effects of exogenous beta-hydroxybutyrate supplementation on metrics of safety and health.

International Journal of Nutrition and Food Sciences, 9(6), 154. https://doi.org/10.11648/j.ijnfs.20200906.13

Stefan, M., Sharp, M., Gheith, R., Lowery, R., & Wilson, J. (2021). The effect of exogenous beta-hydroxybutyrate salt supplementation on metrics of safety and health in adolescents. *Nutrients, 13*(3), 854. https://doi.org/10.3390/nu13030854

Stubbs, B. J., Cox, P. J., Evans, R. D., Cyranka, M., Clarke, K., & de Wet, H. (2018). A ketone ester drink lowers human ghrelin and appetite. *Obesity (Silver Spring, Md.), 26*(2), 269–273. https://doi.org/10.1002/oby.22051

Stubbs, B. J., Cox, P. J., Evans, R. D., Santer, P., Miller, J. J., Faull, O. K., Magor-Elliott, S., Hiyama, S., Stirling, M., & Clarke, K. (2017). On the metabolism of exogenous ketones in humans. *Frontiers in Physiology, 8*, 848. https://doi.org/10.3389/fphys.2017.00848

Sumithran, P., Prendergast, L. A., Delbridge, E., Purcell, K., Shulkes, A., Kriketos, A., & Proietto, J. (2013). Ketosis and appetite-mediating nutrients and hormones after weight loss. *European Journal of Clinical Nutrition, 67*(7), 759–764. https://doi.org/10.1038/ejcn.2013.90

Tagliabue, A., Bertoli, S., Trentani, C., Borrelli, P., & Veggiotti, P. (2011). Effects of the ketogenic diet on nutritional status, resting energy expenditure, and substrate oxidation in patients with medically refractory epilepsy: a 6-month prospective observational study. *Clinical Nutrition (Edinburgh, Scotland), 31*(2), 246–249. https://doi.org/10.1016/j.clnu.2011.09.012

Thai, P. N., Miller, C. v., King, M. T., Schaefer, S., Veech, R. L., Chiamvimonvat, N., Bers, D. M., & Dedkova, E. N. (2021). Ketone ester D-β-hydroxybutyrate-(R)-1,3

butanediol prevents decline in cardiac function in Type 2 diabetic mice. *Journal of the American Heart Association, 10*(19). https://doi.org/10.1161/JAHA.120.020729

Thompson, M., & Nepocatych, S. (2020). Beta-hydroxybutyrate (bhb) ketone salt supplement alters energy metabolism, blood glucose and ketone levels. *Medicine & Science in Sports & Exercise, 52*(7S), 1087–1087. https://doi.org/10.1249/01.mss.0000687488.43165.31

Ting, R., Dugré, N., Allan, G. M., & Lindblad, A. J. (2018). Ketogenic diet for weight loss. *Canadian Family Physician, 64*(12), 906.

Tunaru, S., Lättig, J., Kero, J., Krause, G., & Offermanns, S. (2005). Characterization of determinants of ligand binding to the nicotinic acid receptor GPR109A (HM74A/PUMA-G). *Molecular Pharmacology, 68*(5), 1271–1280. https://doi.org/10.1124/mol.105.015750

Vandoorne, T., de Smet, S., Ramaekers, M., van Thienen, R., de Bock, K., Clarke, K., & Hespel, P. (2017). Intake of a ketone ester drink during recovery from exercise promotes mtorc1 signaling but not glycogen resynthesis in human muscle. *Frontiers in Physiology, 8*, 310. https://doi.org/10.3389/fphys.2017.00310

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. https://doi.org/10.1212/01.WNL.0000152046.11390.45

Webber, R. J., & Edmond, J. (1977). Utilization of L(+)-3-hydroxybutyrate, D(-)-3-hydroxybutyrate, acetoacetate, and glucose for respiration and lipid synthesis in the 18-day-old rat. *The Journal of Biological Chemistry, 252*(15), 5222–5226.

Yan, A., Zhao, Y., Zhang, L., Liang, X., Zhang, X., Liang, F., Nian, S., Li, X., Sun, Z., Li, K., & Zhao, Y.-F. (2022). β-Hydroxybutyrate upregulates FGF21 expression through inhibition of histone deacetylases in hepatocytes. *Open Life Sciences, 17*(1), 856–864. https://doi.org/10.1515/biol-2022-0095

Yancy, W. S., Olsen, M. K., Guyton, J. R., Bakst, R. P., & Westman, E. C. (2004). A low-carbohydrate, ketogenic diet versus a low-fat diet to treat obesity and hyperlipidemia: A randomized, controlled trial. *Annals of Internal Medicine, 140*(10), 769–777. https://doi.org/10.7326/0003-4819-140-10-200405180-00006

Youm, Y.-H., Nguyen, K. Y., Grant, R. W., Goldberg, E. L., Bodogai, M., Kim, D., D'Agostino, D., Planavsky, N., Lupfer, C., Kanneganti, T. D., Kang, S., Horvath, T. L., Fahmy, T. M., Crawford, P. A., Biragyn, A., Alnemri, E., & Dixit, V. D. (2015). The ketone metabolite β-hydroxybutyrate blocks NLRP3 inflammasome–mediated inflammatory disease. *Nature Medicine, 21*(3), 263–269. https://doi.org/10.1038/nm.3804

Yuan, X., Wang, J., Yang, S., Gao, M., Cao, L., Li, X., Hong, D., Tian, S., & Sun, C. (2020). Effect of the ketogenic diet on glycemic control, insulin resistance, and lipid metabolism in patients with T2DM: A systematic review and meta-analysis. *Nutrition & Diabetes, 10*(1), 38. https://doi.org/10.1038/s41387-020-00142-z

APPENDIX

PROJECT APPROVAL LETTER FROM THE UNIVERSITY OF ALABAMA INSTITUTIONAL REVIEW BOARD

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
Office of the Institutional Review Board for Human Use

470 Administration Building
701 20th Street South
Birmingham, AL 35294-0104
205.934.3789 | Fax 205.934.1301 |
irb@uab.edu

APPROVAL LETTER

TO: Bolyard, Michaela

FROM: University of Alabama at Birmingham Institutional Review Board
Federalwide Assurance # FWA00005960
IORG Registration # IRB00000196 (IRB 01)
IORG Registration # IRB00000726 (IRB 02)
IORG Registration # IRB00012550 (IRB 03)

DATE: 22-Sep-2022

RE: IRB-300009075
IRB-300009075-004
Acceptability and Tolerability of Ketone Supplements and their Effects on Capillary beta-hydroxybutyrate Concentrations in Young Adults

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The IRB reviewed and approved the Initial Application submitted on 08-Sep-2022 for the above referenced project. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services.

Type of Review: Full (Institutional Review Board 01 (UAB))
Determination: Approved
Approval Date: 21-Sep-2022
Approval Period:
Expiration Date: 20-Sep-2023

Documents Included in Review:

- IRB EPORTFOLIO
- IRB PERSONNEL EFORM

To access stamped consent/assent forms (full and expedited protocols only) and/or other approved documents:
1. Open your protocol in IRAP.
2. On the Submissions page, open the submission corresponding to this approval letter. NOTE: The Determination for the submission will be "Approved."
3. In the list of documents, select and download the desired approved documents. The stamped consent/assent form(s) will be listed with a category of Consent/Assent Document (CF, AF, Info Sheet, Phone Script, etc.)