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ORIGINAL RESEARCH

No Benefit of Ingesting a Low-Dose Ketone Monoester Supplement on Markers of Cognitive Performance in Females

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Abstract
Exogenous ketones have shown potential to mitigate cognitive decrements in states of fatigue, but studies in females are limited. Following a familiarization session and a baseline session without a mental fatiguing protocol (MF), 12 females completed two experimental sessions, consisting of a battery of cognitive tests (psychomotor vigilance test (PVT), task-switching, incongruent flanker) performed before (PRE) and after (POST) MF. In a counter-balanced crossover design, a R-βHB (R)1,3-butanediol ketone monoester (KME, ~ 188 mg·kg^{-1} body mass) or placebo (PLA) was ingested before MF. Markers of cognitive performance (speed and correct responses per second), blood β-hydroxybutyrate, glucose, and lactate, and subjective markers of perceived cognitive load and fatigue were collected at PRE and POST. From baseline measures, KME ingestion significantly increased blood β-hydroxybutyrate ($P < 0.001$; ~ 1.8 mM), decreased glucose ($P < 0.001$; ~ 0.6 mM), and attenuated a ~ 34% rise in lactate compared to PLA ($P = 0.04$) during POST testing. MF significantly increased perceived cognitive workload and fatigue for both experimental trials in comparison to the control ($P < 0.05$) but did not impair any of the cognitive variables assessed (all $P > 0.05$). Although changes in blood markers are similar to those observed in previous KME investigations, compared with PLA, KME ingestion did not affect cognitive performance following a MF protocol in females.

Keywords Ketosis · β-hydroxybutyrate · Cognition · Reaction time · Mental fatigue

Introduction

The human brain is unique in that it accounts for ~ 20% of total body energy demands and yet only accounts for ~ 2% of total body mass (Holliday, 1971). Under standard living conditions, the brain meets its energetic demands at rest primarily via glucose oxidation (Liang et al., 2018). However, during periods of carbohydrate restriction (< 50 g carbohydrate per day) or caloric restriction (< 500 kcal per day), prolonged endurance exercise, or uncontrolled diabetes, lipid-derived metabolites termed "ketone bodies" (KB) are produced in the liver and can serve as an alternative substrate for the brain and other extrahepatic tissues (Robinson & Williamson, 1980). These KB, primarily acetoacetate and R-β-hydroxybutyrate (βHB), are readily oxidized in the mitochondria for energy provision and unlike glucose, are taken up by the brain in direct proportion to their circulating concentrations (Courchesne-Loyer et al., 2017). Indeed, in a metabolic state where the presence of KB is elevated (i.e., ketosis, > 0.5 mM), the brain may increase its reliance on KB oxidation by nearly ~ 60% for meeting its energetic demands (Owen et al., 1967). However, interventions for inducing nutritional ketosis through dietary changes such as a low carbohydrate high-fat ketogenic diet can produce undesirable side

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effects, such as dyslipidemia, gastrointestinal (GI) distress, and decreased exercise "efficiency" (Burke et al., 2017; VanItallie, 2003). Therefore, alternative methods which elicit hyperketonaemia may be the provision of an exogenous ketone supplement, irrespective of carbohydrate or caloric restriction.

Over the past decade, exogenous ketone supplements have garnered the attention of athletes and coaches alike for their ability to induce acute ketosis and reduce an individual's dependency on endogenous carbohydrate stores without dietary alterations (Evans et al., 2017). Moreover, exogenous ketones have shown the potential to serve as an ergogenic aid, specifically for enhancing endurance performance (Cox et al., 2016), although most data to date have demonstrated either null findings or decrements to performance (Evans et al., 2022). More recent findings have identified a role for exogenous ketone supplementation and attenuating a decline in markers of cognitive performance (e.g., executive function and reaction time) in male sport athletes (Evans & Egan, 2018; Quinones & Lemon, 2022), increasing cerebral blood flow and improving correct responses in obese individuals (Walsh et al., 2021), and maintaining mental alertness in ultra-marathon male runners (Poffé et al., 2023). Currently, it appears that supplementing with exogenous ketones, like the ketone monoester (KME), may play a more prominent role in the preservation of cognition following physical or mental fatigue (MF) (Evans & Egan, 2018; Quinones & Lemon, 2022; Poffé et al., 2023), unless the individual is suffering from metabolic disruption (Walsh et al., 2021) or cognitive impairment wherein ketone supplementation may provide an improvement in the absence of fatigue. Although the mechanisms explaining the aforementioned improvements are still being fleshed out, it is possible that by supplying the brain with an alternative substrate, βHB may improve cognitive processes such as attention, memory, and executive function (Poff et al., 2020; Poff et al., 2021).

Collectively, these findings suggest a possible role for supplementing with KME such as in the preservation or attenuation of cognitive decline following physical or MF. However, while the aforementioned studies examining healthy cohorts used protocols which induced physical fatigue, the present study implemented a MF protocol while at rest to better examine the role of exogenous ketone supplementation effects on preserving markers of cognitive performance. Additionally, while limited to five investigations with male or mixed cohorts, we are unaware of any KME studies focusing specifically on cognitive performance in female cohorts. Therefore, this study sought to determine the effect of acute ingestion of a KME in the form of the R-βHB (R)1,3-butanediol on various markers of cognitive performance following a MF protocol in a group of females.

Materials and Methods

Participants

Twelve females (age, 23 ± 3 y; height, 164 ± 8 cm; body mass, 65.2 ± 12.7 kg; body fat, 22.0 ± 5.3%) gave written informed consent to participate after written and verbal explanations of the procedures were provided. Ethical approval was obtained from the University of North Alabama Institutional Review Board (IRB #: 2023-009) and was in accordance with the Declaration of Helsinki, with the exception of the study protocol not being registered in a trials database. Participants were either currently competing in NCAA Division I athletics (e.g., soccer, basketball, cross country) or actively training ~ 5 days per week, 45 to 75 min per day in running or cycling, and were classified as either Tier 2/Trained ($n = 4$) or Tier 4/Elite level athletes ($n = 8$) based on the recently proposed Participant Classification Framework (McKay et al., 2021). Four participants were taking combined (i.e., progestin and estrogen) oral contraceptives (brands: Cryselle and Blisovi) and eight participants were naturally menstruating (menstrual cycle length, 29 ± 1 d). The menstrual cycle phase was not controlled for in the present study.

Experimental Design

The present study was designed with criteria put forth by Betts et al. (2020) for best practice guidelines in sports nutrition trials and minimizing the risk of bias. Therefore, a two-condition (KME or placebo (PLA)), counter-balanced and cross-over design was used to examine the acute ingestion of a KME on a battery of cognitive tests via touch screen tablet, namely psychomotor vigilance (PVT), task-switching, and incongruent flanker tests as described further below. Participants visited the laboratory on four separate occasions over a 21-d period comprising one familiarization, one baseline, and two main experimental trials. During their first visit, each participant completed a battery of cognitive tests on three separate attempts, each attempt separated by a 2-min passive rest. During visit 2, participants completed the battery of cognitive tests before (PRE) and after (POST) a period of 30 min at rest without MF. During the main experimental trials (visits 3 and 4), after completing the cognitive tests at PRE, a MF protocol was performed and cognitive tests were again performed at POST. For the present study, MF was defined as any statistically significant decrement to a marker of cognitive performance from PRE to POST. Visits 3 and 4 were identical regarding pretrial preparation (standardized exercise and diet 24 h prior to each visit) and only differed in

the drinks ingested prior to the MF protocol. The primary outcome was a measure of executive function based on findings from a previous study observing that ingestion of KME attenuated decrements in executive function induced by physical fatigue associated with intermittent running exercise (Evans & Egan, 2018). Thus, we conducted an a priori power analysis in G*Power (version 3.1) for a repeated-measures design with the task-switching test as our primary dependent variable. Our power analysis indicated that a sample size of 8 would be sufficient to demonstrate a significant difference with an α level of $P < 0.05$, effect size *f* of 0.70, an expected power (1–β) of 0.80, and an assumed 0.5 correlation among repeated measures. Anticipating a 1:3 dropout in a worst-case scenario, we recruited 12 subjects for the present study. Secondary outcomes were changes in blood βHB, glucose, and lactate concentrations, along with subjective measures of mental fatigue and exertion.

Cognitive Test Battery

The battery of cognitive tests (Soma Technologies, Lucerne, Switzerland) was administered via a touch screen iPad tablet (10th generation, Apple, Huntsville, AL) and lasted 11 min. Following an initial briefing by an investigator, participants sat on a stool throughout and the tablet was positioned at eye level ~ 1 m away. Participants were instructed to place their dominant index finger on a legend ~ 15 cm away from the screen and return it after each response. All testing took place in a dimly lit and quiet room, and participants were monitored through a window by an investigator during testing. An identical test battery was administered at PRE and POST in each trial. The test battery was chosen to (1) measure domains of low and high-level cognitive processing based on existing definitions (Chang et al., 2012) and (2) to replicate measures in past KME work where the ingestion of a KME exerted some influence on a marker of cognition (Evans & Egan, 2018; Poffé et al., 2023; Quinones & Lemon, 2022). Moreover, the following metrics were collected and used for statistical analysis from each cognitive task: (1) speed, defined as ($Mean_{step\ N1}$/reaction time), also known as reciprocal response time (Basner et al., 2011) and (2) responses correct per second (RCS).

Participants first completed a truncated PVT. While performance on the PVT has been used to assess MF, the normal length of completing it has also been criticized for inducing MF (Dinges & Powell, 1985) and therefore, we chose a truncated and reliable 5 min PVT (Basner et al., 2011). The test requires participants to respond to a visual stimulus by tapping a circle that appears in the middle of the screen at intermittent intervals. The stimulus was presented for 500 ms or until a response was given. The PVT is a low-level measure of cognitive processing, and is a measure of sustained attention, and measures the consistency with which participants respond to the visual stimulus.

The second task was a 3-min multitasking test and represents a high level of cognitive processing by measuring a participant's ability to task switch, also termed cognitive flexibility. Participants were presented with a number from 0 to 10 inclusive, in either a red or white font. For white numbers from 0 to 5 inclusive and red odd numbers, participants were instructed to tap a left-facing arrow displayed at the bottom left-hand corner. For white numbers from 6 to 10 inclusive and red even numbers, participants were instructed to tap a right-facing arrow displayed at the bottom right-hand corner. When incorrect responses occurred or a response was not given, an audible adverse buzzer would emit from the device.

The third task was a 3-min incongruent flanker test, which also represents a high level of cognitive processing by assessing a participant's ability to ignore task-irrelevant information. Participants were presented a series of five arrows, with four of the five arrows pointing in either the left or right direction. Participants were instructed to focus on the middle arrow when it appeared and ignore the two arrows immediately to the left and right of it. Whichever direction the middle arrow was pointing, participants were to tap at the bottom left or right-hand corner of the screen, the arrow that corresponded with the middle arrow's respective direction. When incorrect responses occurred or a response was not given, an audible adverse buzzer would emit from the device.

Pre-trial Preparation

All trials took place between 05:00 and 10:00 AM, separated by at least 72 h and at the same time of day ± 30 min, for each participant. All participants were asked to refrain from alcohol consumption 48 h prior to, caffeine ingestion 12 h prior to, and strenuous exercise for 24 h prior to each trial. Outside of these parameters, participants were asked to maintain their usual dietary and exercise habits throughout the period that they were active participants in the study. In addition, participants were instructed to arrive following an overnight fast of at least 6 h. During the familiarization trial, each participant provided a 24 h dietary recall to the principal investigator and was reminded 24 h beforehand to replicate this diet the day before each experimental trial (Kcal/day, 1876 ± 155; carbohydrate, 185 ± 25 g; protein, 95 ± 7 g; fat, 84 ± 20 g). Adherence to all requests for abstinence and diet replication were verbally confirmed upon arrival to each trial.

Baseline and Experimental Trials

Upon reporting to the laboratory, participants completed a series of health, menstrual cycle, and physical activity questionnaires, followed by the collection of body mass to the nearest 0.1 kg (Tanita Corporation, Japan), height to the nearest cm (Deteco, Webb City, MO), and body fat via 3-site (tricep, suprailiac, and thigh) skinfold measurements (Lange Calipers, Cambridge, MA). Body fat was assessed by the same trained investigator for all participants, and body fat was subsequently calculated using the Brožek formula (Brožek et al., 1963). Participants then completed a Likert-type gastrointestinal symptom questionnaire adopted from Stubbs et al. (2019), as well as an intrinsic motivation questionnaire regarding interest and enjoyment (McAuley et al., 1989). Regarding motivation, participants rated seven statements such as, "I will enjoy this activity" or "I would describe this activity as interesting" on a 7-point Likert scale, with anchors of 1 (not at all) and 7 (very true). To increase motivation, participants were offered the incentive of winning a smart watch for the best overall performance, defined as the participant that maintained the greatest speed and reaction time across each of the three cognitive tasks during POST testing, as compared to their respective PRE testing. Participants then had 5 uL of capillary whole blood sampled for βHB and glucose (Precision Xtra; Abbott Diabetes Care, Alameda, CA), as well as lactate (Lactate Plus, Nova Biomedical, Waltham, MA). Blood was sampled and recorded by an investigator otherwise not involved with experimental testing to preserve the blinding of investigators involved in the data collection for the cognitive tests. Participants then completed the battery of cognitive tests as previously described (PRE).

Following PRE cognitive testing, a 350-mL bolus was provided in opaque bottles to each participant with either the KME (~ 188 mg·kg^{-1} body mass and ~ 150 kcal per bolus; target blood βHB range ≥ 1.5 mM) or PLA. The KME is a commercially available R-βHB (R)1,3-butanediol (KE4, Fruit Punch, KetoneAid, Falls Church, VA, USA) and was mixed with a bitter blocker (TrueBlock, Danbury, CT, USA) and ~ 300-mL purified water. The PLA consisted of a low-caloric (5 kcal) and caffeine-free fruit punch additive (Crystal Light, Fruit Punch, USA), along with arrowroot powder (~ 30 kcals) to match the mouth-feel consistency of the KME beverage. Supplements were mixed by an outside investigator otherwise not involved with data collection to preserve blinding. Upon completion of the final experimental trial, participants completed an exit interview and were asked to identify the trial in which they believed they received the KME or PLA. Following ingestion of the supplement, participants passively sat for 15 min before proceeding with either the CON or MF protocol.

During trial 2 (CON), all participants watched a 30-min emotionally neutral documentary (Pegasus-Eagle Rock Entertainment, 2004) which has been used as a control task in prior experiments (Marcora et al., 2009; Martin et al., 2015) and found to maintain a stable physiological response during viewing (Silvestrini & Gendolla, 2007). However, during trials 3 and 4, participants completed a 30-min inverted Stroop protocol, which requires response inhibition and working memory and has been used previously to induce MF (Badin et al., 2016; Daub et al., 2022; Smith et al., 2016; Smith et al., 2015). During the inverted Stroop task, participants were presented with a series of colored words (e.g., yellow, green, blue, and red) in font colors different from the word's meaning and which were individually displayed on the monitor. Participants were instructed to verbally select the font color of the word as quickly as possible by touching the correct font color located at the bottom of the screen. The word was presented until a response was given. When incorrect responses occurred, an audible adverse buzzer would emit from the device.

Immediately following completion of the CON or MF protocol, participants completed a second series of questionnaires to examine perceived workload (i.e., NASA-Task Load Index; Rubio et al., 2004), perceived mental fatigue (VAS-F, 0 mm = "no fatigue" to 150 mm = "extreme fatigue") and exertion via visual analog scale (VAS-E, 0 mm = "no exertion" to 150 mm = "extreme exertion"), and gastrointestinal symptoms. These instruments were chosen based on best practices for assessing MF and mental load according to a recent systematic review (Díaz-García et al., 2021). Participants then had a second capillary sample assessed for blood βHB, glucose, and lactate prior to completing cognitive testing (POST).

Because the primary purpose of the study was to assess the effects of KME versus PLA on markers of cognitive performance, and not if MF itself affected cognitive performance, only subjective measures outcomes (i.e., NASA-TLX, motivation, and VAS-F/E) from CON were statistically compared to KME and PLA.

Statistical Analysis

Results are expressed as mean ± standard deviation (SD). Significance was taken at $P \leq 0.05$. Normality was tested using a Shapiro-Wilk test prior to proceeding with the parametric analysis as described below, and when sphericity was not met, a Greenhouse-Geisser correction was applied. A one-way repeated measures analysis of variance (RM-ANOVA) was used to determine differences between visits 2, 3, and 4 in subjective markers of motivation, perceived task load (i.e., NASA-TLX), and VAS-F/E. A two-way (condition×time) RM-ANOVA was performed to determine differences in blood βHB, glucose, and lactate

concentrations, as well as speed and RCS for each cognitive test (i.e., PVT, task-switch, incongruent flanker), respectively. If significant main effects or interaction effects were observed during repeated measures testing, *post-hoc* testing was performed with Bonferroni's correction, with multiplicity-adjusted *P* values reported when comparing KME to PLA at respective time points. Where significant main effects or interactions occurred, partial eta square (η^2_p: < 0.05 = small effect; .0.05–0.14 = moderate effect; and > 0.14 = large effect) were calculated to provide effect sizes for an interpretation of meaningful differences. All data were analyzed in SPSS, v27 (IBM Corp., Armonk, NY, USA).

Results

Blood βHB, Glucose, and Lactate Concentrations

A condition*time interaction effect ($P < 0.001$, $\eta^2_p = 0.88$) was found for blood βHB concentrations (Fig. 1A, B). From PRE (KME, 0.2 ± 0.1 mM; PLA, 0.2 ± 0.1 mM) to POST (KME, 2.0 ± 0.7 mM; PLA, 0.2 ± 0.1 mM), the KME condition resulted in a significant increase in blood βHB compared to PLA ($P < 0.001$), respectively. For blood glucose, a main effect of time ($P < 0.01$, $\eta^2_p = 0.53$) and a condition*time interaction effect ($P < 0.01$, $\eta^2_p = 0.51$) was

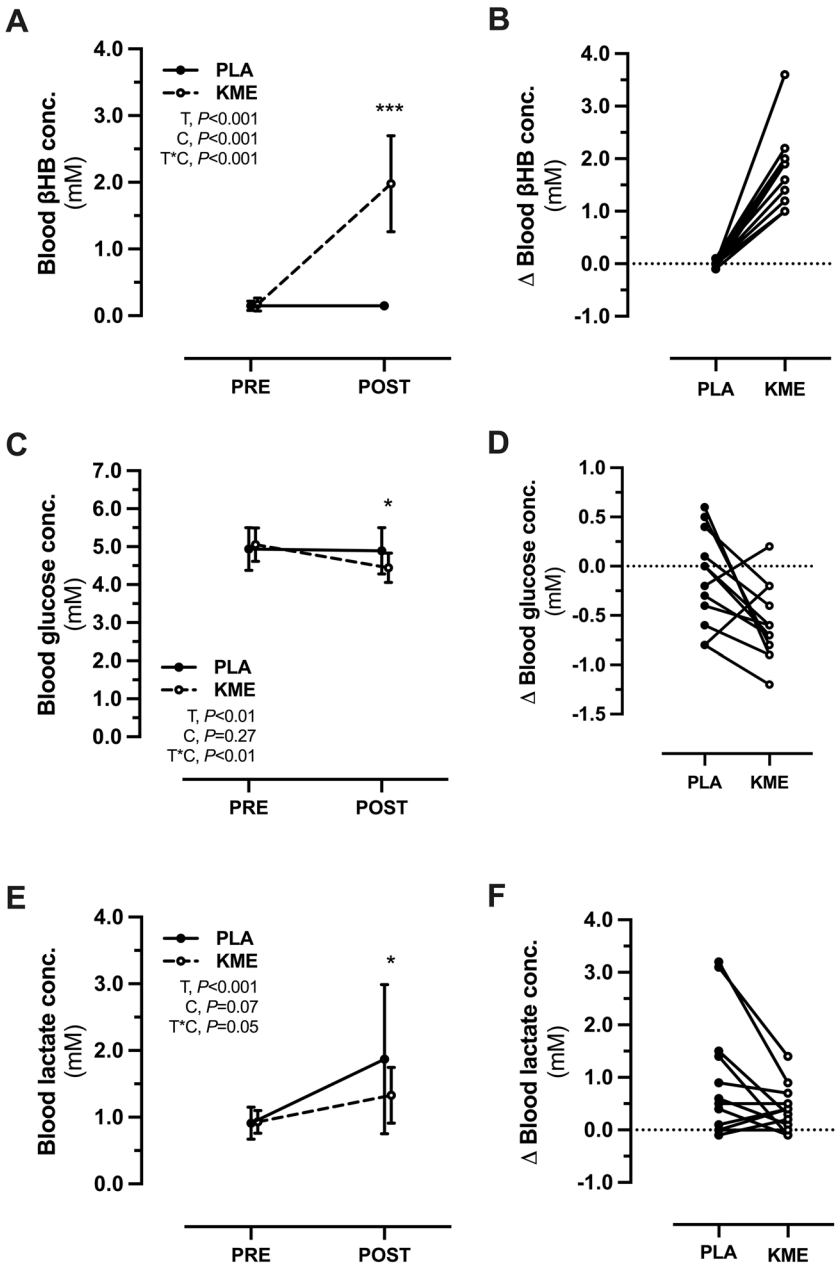

Fig. 1 Pairwise comparisons and delta changes of whole blood βHB (**A**, **B**), glucose (**C**, **D**), and lactate (**E**, **F**) concentrations between KME and PLA. Filled circles represent PLA and clear circles represent KME individual differences with data presented as mean ± SD values. *$P < 0.05$ for KME vs PLA. ***$P < 0.001$ for KME vs PLA

found (Fig. 1C, D). However, no main effect for the condition was observed ($P = 0.28$). For glucose, only the KME group experienced a significant reduction from PRE (KME, 5.1 ± 0.5 mM; PLA, 5.0 ± 0.6 mM) to POST (KME, 4.5 ± 0.4 mM; PLA, 4.9 ± 0.6 mM) ($P < 0.001$), and POST blood glucose was ~ 0.6 mM lower in the KME group compared to PLA. A condition*time interaction effect ($P = 0.05$, $\eta^2_p = 0.31$) was found for blood lactate (Fig. 1E, F). A significant increase from PRE (KME, 0.9 ± 0.2 mM; PLA, 0.9 ± 0.2 mM) to POST (KME, 1.3 ± 0.4 mM; PLA, 1.9 ± 1.1 mM) ($P = 0.01$) was observed in both conditions, although at POST, blood lactate was significantly less in the KME condition compared to PLA ($P = 0.04$).

Subjective Measures of Perceived Workload and Fatigue

All data for subjective measures are reported in Table 1. For motivation, no significant differences were found between treatments ($P = 0.59$) and participants responded as "moderately motivated" across all trials. For the NASA-TLX, no significant differences were found for physical demand ($P = 0.22$), but participants did report a significantly greater mental demand ($P < 0.001$, $\eta^2_p = 0.42$), temporal demand ($P < 0.001$, $\eta^2_p = 0.32$), effort ($P = 0.001$, $\eta^2_p = 0.26$), and frustration ($P < 0.001$, $\eta^2_p = 0.28$) during the experimental trials PLA and KME compared to CON. For KME only, participants reported a greater perceived level of performance during the MF protocol in comparison to CON ($P = 0.05$, $\eta^2_p = 0.16$). For VAS-F and VAS-E, participants reported a significantly greater perception of fatigue ($P < 0.01$, $\eta^2_p = 0.25$) and exertion ($P < 0.01$, $\eta^2_p = 0.26$) during the experimental trials PLA and KME compared to CON.

Battery of Cognitive Tests

All data for speed for each respective cognitive variable tested are presented in Fig. 2A–F. For PVT, no significant interaction effect was found for speed ($P = 0.09$) or RCS (PRE: KME, 2.21 ± 0.13 vs. PLA, 2.01 ± 0.53; POST: KME, 2.16 ± 0.10 vs. PLA, 2.15 ± 0.12; P=0.12). For task-switching, no significant interaction effects were found for speed (Fig. 2C, D) or RCS (PRE: KME, 1.21 ± 0.37 vs. PLA, 1.05 ± 0.26; POST: KME, 1.21 ± 0.34 vs. PLA, 1.09 ± 0.24; $P = 0.18$). For the incongruent flanker, no significant interaction effects were found for speed (Fig. 2E, F) or RCS (PRE: KME, 1.73 ± 0.24 vs. PLA, 1.78 ± 0.16; POST: KME, 1.75 ± 0.30 vs. PLA, 1.78 ± 0.20; $P = 0.75$).

Gastrointestinal Symptoms

Among the twelve participants, four (33%) reported symptoms during the KME trial and comprised of 1 (8%), 1 (8%), and 2 (16%) incidences of nausea, reflux, and headache, respectively. During the PLA trial, two participants (16%) reported symptoms of headache.

Blinding

Five (42%) participants correctly identified the trial in which they received the KME, which four attributed to taste alone. One participant correctly identified the KME based on a feeling of "light-headedness" following ingestion. This participant also experienced the single greatest increase in blood βHB (0.2 to 3.8 mM) and a decrease in blood glucose (5.3 to 4.4 mM), compared to the other participants. The remaining seven participants either incorrectly identified the supplement that they received during visits 3 and 4, or could not identify the supplement that they received (marked as "I am not sure") during these visits.

Discussion

The present study investigated whether the acute ingestion of a commercially available KME supplement affected cognitive performance following a MF protocol, and notably is the first to do so in a female cohort. Compared with PLA, ingestion of a KME elevated βHB to 2.0 ± 0.7 mM prior to cognitive testing at POST, but no benefits were observed to any measures of cognitive performance. Secondary findings included a reduction in circulating glucose (~ 13%) and a diminished rise in blood lactate (~ 34%) following KME ingestion. Moreover, KME ingestion positively influenced

Table 1 Measures of subjective responses assessed between each trial consisting of a mental fatigue protocol, or control (CON), during which a ketone monoester (KME) or placebo (PLA) was ingested (mean ± SD; $n = 12$)

	NASA							VAS	
	Motivation	*Mental demand*	*Physical demand*	*Temporal demand*	*Performance*	*Effort*	*Frustration*	*Fatigue*	*Exertion*
CON	4.57 ± 0.72	5.0 ± 4.2	1.4 ± 2.9	3.3 ± 4.0	3.3 ± 2.6	5.3 ± 5.8	2.8 ± 3.6	38 ± 27	34 ± 33
PLA	4.38 ± 0.27	12.9 ± 5.9[A]	2.8 ± 3.6	9.1 ± 4.8[A]	5.5 ± 4.6	12.2 ± 5.2[A]	9.8 ± 5.6[A]	79 ± 38[A]	78 ± 33[A]
KME	4.37 ± 0.80	13.8 ± 4.3[A]	4.3 ± 5.1	8.9 ± 3.6[A]	7.8 ± 5.3[A]	11.3 ± 5.2[A]	8.2 ± 5.4[A]	80 ± 41[A]	74 ± 39[A]

[A]Significantly different ($P < 0.05$) from CON

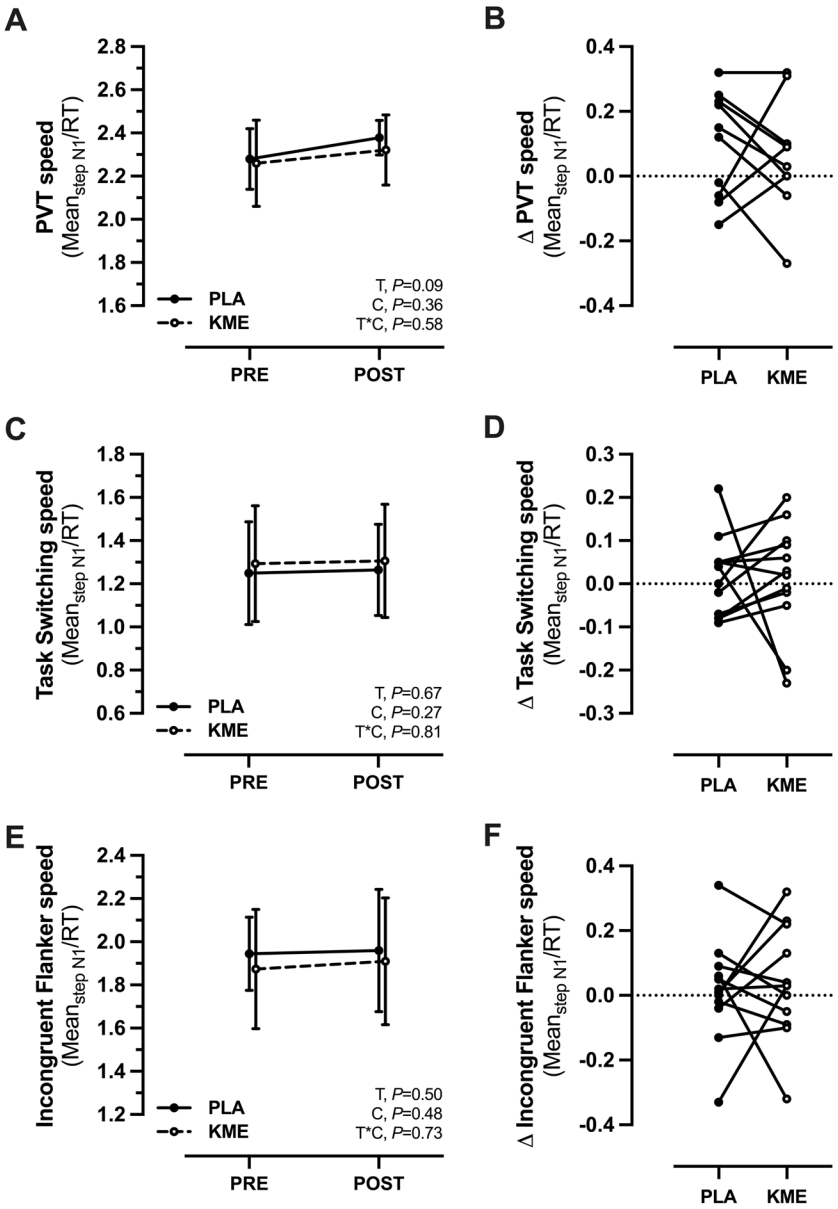

Fig. 2 Pairwise comparisons and delta changes of individual differences between KME compared to PLA for PVT speed (**A**, **B**), task-switching speed (**C**, **D**), and incongruent flanker speed (**E**, **F**) with data presented as mean ± SD values

perceived performance collected from the NASA-TLX compared with CON, even though cognitive outcomes did not reflect these perceptions. These findings add to a growing body of literature regarding exogenous ketone supplementation and measures of cognitive performance in healthy cohorts (Evans & Egan, 2018; Evans et al., 2019; Prins et al., 2020; Poffé et al., 2023; Quinones & Lemon, 2022; Waldman et al., 2018; Waldman et al., 2020).

Since their appearance almost a decade ago, researchers, coaches, and athletes alike have taken an interest in the potential for exogenous ketone supplements to impact measures of cognitive performance (Evans et al., 2022). However, only recently have human studies demonstrated a potential role for exogenous ketones to preserve or improve cognitive function (Evans & Egan, 2018; Poffé et al., 2023; Quinones & Lemon, 2022; Walsh et al., 2021). Earlier investigations incorporating ketone salts found potential applications for ketone salt administration and cognitive performance prior to exercise (Prins et al., 2020), with other findings demonstrating no effect during exercise (Waldman et al., 2018; Waldman et al., 2020). It is important to note though, that ingestion of the ketone salt often carries with it a greater gastrointestinal symptom load burden in comparison to KME ingestion (Stubbs et al., 2019), and will only modestly elevate blood βHB (< 1.0 mM) when consumed in tolerable doses (Evans et al., 2022). In contrast, recent work has demonstrated that ingesting >500 mg·kg of KME prior to or during exercise may preserve executive function (Evans & Egan, 2018) or completely negate cognitive decrements from exhaustive exercise (Poffé et al., 2023). Likewise, Quinones

and Lemon (2022) showed similar findings when their male soccer athletes ingested ~330 mg·kg^{-1} of a KME and subsequently, mitigated a decrement to cognitive function (i.e., % of correct answers) following a MF protocol and simulated soccer match. Collectively, these findings suggest a possible role for athletes supplementing with exogenous ketones that extend beyond merely exercise performance. Provided that athletes are often presented with a multitude of decisions throughout the competition, interventions that may positively impact markers of cognitive performance could benefit sporting outcomes.

However, these cognitive findings are not always observed following KME ingestion (Evans et al., 2019), and we speculate that in order for the KME to exert a cognitive effect within the context of sport, prior physical or MF is likely required to overcome a "ceiling effect" wherein cognitive effects are not observed because cognitive performance is either not impaired or already at close to optimal levels. Indeed, cognitive operations of the higher order such as executive function, are not static but dynamic and tightly regulated (Botvinick et al., 2001). Thus, high-level domains of cognition that require problem solving, task switching and resisting distractions, or interacting with the environment should theoretically be robust to systemic changes to protect the coherence of the organism. For these reasons, we implemented a 30-min inverted Stroop protocol from previous investigations that successfully induced MF (Badin et al., 2016; Smith et al., 2016; Smith et al., 2015). However, whereas decrements in subjective markers of MF such as mental demand, fatigue, effort, and frustration were observed in the present study, objective markers of MF were not observed at POST. The absence of a decline is an important methodological consideration given that previous work has demonstrated that KME ingestion can attenuate declines in cognitive performance, rather than produce an improvement in absolute terms (Evans & Egan, 2018; Quinones & Lemon, 2022). Therefore, the absence of differences in KME compared to PLA in measures of cognitive performance in the present study may be partially explained by MF not inducing a decrement in cognitive performance as intended.

Our pilot work observed doses greater than > 500 mg·kg^{-1} body mass elevated average blood βHB to > 3.0 mM and resulted in several participants reporting dizziness, light-headedness, or nausea which then impacted their POST-cognitive testing. Thus, we aimed to surpass the 1.0 mM βHB threshold by adopting a "low" dose of KME, while also preventing potential adverse responses in our participants. These aims were successful based on our minimal reported symptoms of gastrointestinal distress (four participants total). In a series of well-executed studies, Stubbs et al. (2019) demonstrated that in comparison to a ketone salt supplement, ingestion of a KME had minimal impact on gastrointestinal and systemic scores. In fact, ingestion of a low dose of KME (141 mg·kg^{-1} body mass) was not significantly different from ingestion of a high dose of KME (282 mg·kg^{-1} body mass), even though it was noted that the high KME dose induced more systemic symptoms (e.g., dizziness and headache) than the low dose. Moreover, while Stubbs et al. (2019) did not observe scores that were statistically different between low and high conditions, symptoms were significantly greater when participants ingested the KME in a *fasted* state compared to fed. Because the present study is the first to examine a KME in a female-only cohort, and the primary aim was to examine the effect of KME alone on cognitive performance following its ingestion, examining KME ingestion in a fasted state was the chosen approach. There may be potential though to combine the KME with a carbohydrate supplement and thereby, further mitigating gastrointestinal distress (Díaz-García et al., 2021) while also preserving glucose utilization and elevating blood βHB (Howard et al., 2023).

The inclusion of a female cohort is a key distinction from previous studies of exogenous ketone supplements examining cognitive performance, since female participants may have different mechanisms, magnitudes, and time courses by which fatigue and specifically MF are induced. Regarding MF, the dual regulation system model proposes that a high cognitive demand activates both the mental facilitation and inhibition systems, and thereby causing MF (Ishii et al., 2014). When the neural networks which modulate mental facilitation are activated, participants experience an increased effort to maintain cognitive performance on a given task. Over time, this heightened response in mental facilitation can result in MF. Neuroimaging research has revealed a greater resiliency to MF among females in comparison to their male counterparts (Wylie et al., 2022), and between-sex differences favoring females among various cognitive tasks such as working memory and mitigating cognitive decline during mentally demanding task assessments have been observed (Lejbak et al., 2009; Pliatsikas et al., 2020). Despite the successful implementation of the MF protocol in several previous studies (Badin et al., 2016; Daub et al., 2022; Smith et al., 2016; Smith et al., 2015), the present study did not elicit the intended decline in cognitive performance by MF in either condition (KME or PLA), and this is a limitation of the present study. However, this limitation is important to note for future investigations and methodologies as alternative roles for exogenous ketone supplementation continue to be explored beyond exercise performance settings. Future studies may consider a longer MF protocol (≥ 60 min) or pending technology and software accessibility, incorporating a MF protocol that maintains the participants' heart rate within a defined and elevated range throughout the protocol (e.g., 60–75% of maximal heart rate).

In conclusion, acute ingestion of a commercially available KME demonstrated no benefits to measures of cognitive performance compared to PLA in a female cohort. The MF stimulus increased perceptions of workload and fatigue, and in KME only participants reported subjectively better performance, even though none were objectively evident. Given the well-established between-sex differences (e.g., metabolic, hormonal, perceptual) additional work in females with exogenous ketone supplementation is required, because prior to the present study, all work has been conducted in a male-only or mixed cohort.

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Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Informed Consent Informed consent was obtained from all individual participants included in the study.

Conflict of Interest DD is an inventor of patents on the use of exogenous ketones, advisor for Levels Health, and co-owner of Ketone Technologies LLC, which does consulting and public speaking events. The remaining authors have no conflicts of interest to disclose.

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