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Anaerobic, aerobic, and cognitive outcomes are similar after supplementing with exogenous ketone salts and a sports drink

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Abstract

Background: Ketosis may improve exercise performance, thus serving as an effective ergogenic aid based on its capacity to contribute ketones as an alternative oxidative fuel source.

Objective: The effects of a single dose of exogenous ketone salt supplementation on anaerobic and cardiorespiratory fitness, cognitive performance, and substrate levels were investigated in 19 healthy male and female subjects.

Methods: In this triple-blinded, randomized, cross-over designed study, participants received one serving of exogenous ketone salts (KS) and one isocaloric serving of Gatorade G2 sports drink (SD) with a one-week washout period between supplements. Anaerobic performance was determined by a 30-second Wingate test and cardiorespiratory fitness was determined by a VO_{2peak} test. In a time-sensitive order, blood measures to assess glucose, ketone, and lactate levels were taken at four time points including baseline, 30-minutes post-supplement consumption, post-Wingate test, and post- VO_{2peak} test. A cognitive performance battery was administered at the same time points.

Results: Paired-samples *t*-tests showed no significant difference ($p = .25$) in relative VO_{2peak} between KS (40.91 ± 8.14 ml*kg-1*min-1) and SD (40.07 ± 7.01 ml*kg-1*min-1). There were no significant differences ($p > .05$ for all) between KS and SD for Wingate test variables peak power (671.58 ± 210.01 vs 674.68 ± 202.94 W), mean power (490.21 ± 139.02 vs 500.74 ± 146.00 W), or fatigue index (12.00 ± 5.35 vs 11.47 ± 5.20 W*s-1). Although cognitive assessment values varied between time points, no significant interaction effect between supplement and time was observed for cognitive performance indices ($p > .05$ for all). Blood glucose and ketone levels both demonstrated a significant time by condi-

tion interaction ($p < 0.00$). KS attenuated glucose increase and elevated ketones compared to SD.

Conclusions: An acute dose of exogenous ketones had a similar effect on anaerobic performance and cardiorespiratory fitness as the sports drink Gatorade G2.

Keywords: beta-hydroxybutyrate, VO_{2peak}, Wingate, ergogenic aid, blood glucose, sports drink

1.INTRODUCTION

Ketone bodies, which include the compounds beta-hydroxybutyrate (BHB), acetoacetate (AcAc), and acetone, are organic, lipid-derived molecules that serve as a primary fuel source when carbohydrate availability is limited.[1] Because AcAc and BHB are short-chained acids, they possess the ability to independently diffuse across cellular membranes. Therefore, they can serve as fuel sources for organs such as the brain, heart, kidney, and skeletal muscle.[2] The resulting transient physiological condition of ketosis is a metabolic state in which fat oxidation is increased due to an absence of carbohydrates as a primary source of fuel, thus increasing the rate of ketogenesis. Blood ketone concentrations may be elevated through prolonged fasting, a ketogenic diet (KD), exogenous ketone supplementation, and/or prolonged exercise.[3,4] Existing literature suggests that prompting a state of ketosis, through either a KD and/or ketone supplementation may improve exercise performance, thus serving as an effective ergogenic aid based on its capacity to contribute ketones as an alternative oxidative fuel source.[5,6] Exogenous ketone supplements have since become a practical approach to inducing rapid ketosis.

Cox et al. indicated that a state of acute ketosis, achieved via dietary and nutritional alterations, creates a physiological condition in which circulating blood ketone levels are elevated. This may improve muscular endurance by altering substrate competition for respiration, ideally in a glycolytic condition.[5] Likewise, Clarke & Cox highlighted similar improvements in exercise performance in elite rowers. Consumption of exogenous ketones resulted in a moderate improvement in a 30-minute bout of rowing exercise.[4] However, as primary metabolic pathways shift during varying intensities of exercise, it is difficult to extrapolate the results of existing literature on all types of exercise. Contrary to most of the literature that implicates a beneficial impact of ketones, O'Malley and colleagues relay the opposite effect. Their research with ketone salts indicated that whereas increased ketone levels via beta-hydroxybutyrate concentration increased fat oxidation during a high intensity, steady state cycling exercise, it impaired exercise performance.[7] More current studies have found no performance effects on an incremental bicycle exercise test to exhaustion or a 5k running time trial when supplementing with exogenous ketones compared to a placebo.[8,9]

Current literature also indicates that ketones may enhance cognitive functioning as well. Therefore, ketones have been proposed as an operative agent to combat several neurodegenerative diseases such as Parkinson's, Alzheimer's, and epilepsy.[10] Though implications through human trials are limited, research on rodents has demonstrated that ketosis improves cognitive functioning in rats that may be translated to human cognitive functioning.[11]

This concept of ketosis as a means for enhancing performance was initially explored in 1983 with the understanding that chronic ketosis could improve exercise performance by sparing glycogen and carbohydrate stores while simultaneously oxidizing fat stores.[12] Kesl et al. more recently re-

vealed that an acute dose of ketone salts elevated blood beta-hydroxybutyrate and lowered blood glucose concentrations at rest in rodents.[3] Limited research demonstrates the shifts in blood substrate concentrations after performing anaerobic and aerobic exercise when supplementing with ketone salts.

Therefore, the primary purpose of this study was to determine if one dose of exogenous ketone salts (KS) in non-keto-adapted individuals alters maximal physical fitness and/or cognitive performance outcomes. The secondary purpose of this study was to determine how one dose of KS affects blood substrate levels in humans during maximal aerobic and anaerobic tests compared to a typical sports drink (SD).

2.METHODS

Participants

Twenty participants started the study and 19 completed the study (n = 10 males and 9 females). Participants were recruited via advertisements distributed around a local college and word of mouth. Inclusionary criteria required individuals to be between the ages of 18-35, recreationally active (participates in aerobic and anaerobic activities on a weekly basis and is not an elite athlete) and apparently healthy (BMI between 18.5-29.9), non-smoking, and not consuming any medications that may affect the cardiovascular system or brain.

Study Design

This randomized, triple-blinded, cross-over study consisted of three laboratory visits: one familiarization visit and two data collection visits separated by a one–week washout period. During familiarization, participants completed a health history questionnaire, physical activity readiness questionnaire, and informed consent. In addition, height and weight were recorded, testing protocols were explained in detail, and equipment familiarization was encouraged. Participants confirmed that they were not supplementing with ketones or adhering to a ketogenic diet.

During each of the two data collection visits, subjects consumed one of two supplements and completed a Wingate anaerobic test to assess anaerobic performance, followed by a VO_{2peak} test to assess cardiorespiratory fitness (CRF). The first data collection occurred one week after familiarization. Immediately prior to the visit, participants were asked to fast eight hours, refrain from exercise, caffeine, and nicotine for 12 hours,

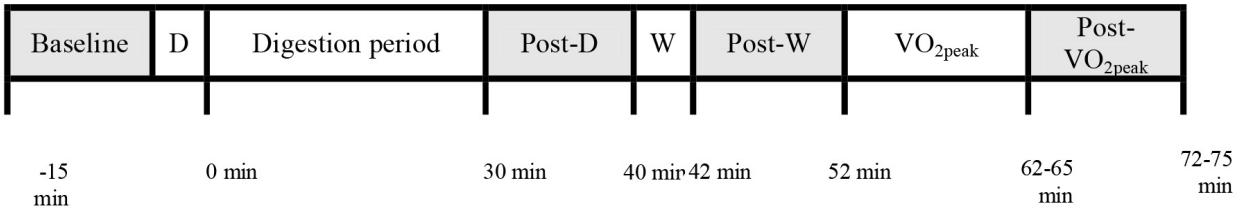
Table 1. *Participant characteristics*

Characteristic	Overall (*n* = 19)	Males (*n* = 10)	Females (*n* = 9)
Age (years)	21.9 ± 3.1	22.8 ± 3.9	20.9 ± 1.5
Height (cm)	174.4 ± 8.7	180.2 ± 5.5	168 ± 6.9
Weight (kg)	72.1 ± 11.8	78.35 ± 8.9	65.2 ± 10.9

Data are presented as mean ± SD.

and refrain from alcohol 24 hours. Upon arrival, a heart rate monitor (Polar T31C Heart Rate Sensor; POLAR, Kempele, Finland) was strapped around the participant's chest and after resting in a seated position without external stimuli for five minutes, resting heart rate was recorded. The heart rate monitor remained in position for the duration of the laboratory visit. Physiological and cognitive measures were then taken at four segments throughout the study including baseline, 30-minutes post-drink consumption, immediately post-Wingate, and immediately post-VO_{2peak}. Each measurement segment was 10 minutes in length to ensure similar recovery times between participants, as indicated in Figure 1. The second data collection visit occurred one week after the first, at the same time of day, and with the same procedures. This study was approved by Augusta University's Institutional Review Board, and all procedures performed were in compliance with the institutional guidelines.

Figure 1. *Data Collection Timeline*



….= blood measurements (ketones, glucose, lactate) followed by cognitive assessments
D = drink consumed (either SD or KS)
W = Wingate test including 30 sec warm-up and cool-down
VO_{2peak} = Bruce Protocol $VO2_{peak\ test}$

Supplementation

In a previously determined randomized order, participants ingested either a ketone salt (KS) supplement or a sports drink (SD) during the first testing lab visit and ingested the alternate supplement during the second testing lab visit. The supplements were randomized prior to participant recruitment and prepared by being placed in brown paper bags that were stapled shut to keep the researchers blinded. A serving of KS (KETO//OS® MAX; Prüvit Ventures, Inc., Melissa, TX, USA) consisted of 7 grams of beta-hydroxybutyrate, Maui punch flavor, and 40 calories. A serving of the SD consisted of Gatorade G2® (Gatorade, Chicago, IL, USA), fruit punch flavor, and 40 calories. See Table 2 for the nutritional facts and ingredients of both drinks. Both supplements were in powder form and mixed in and consumed with 16.9 ounces of room temperature bottled water. Study personnel and subjects were blinded to the drink condition of each session, and subjects were randomly allocated to the order of drink condition as indicated in Table 3. After consumption of the supplement, there was a 30-minute digesting

period; the participants rested for the first 15-minute period and warmed up lightly for the second 15-minute period as the testing protocol was explained.

Blood substrate measurements

Venous blood samples were collected, consisting of blood glucose (mg/dL) and ketone (mmol) levels (Precision Glucose Ketone Meter; Abbott Laboratories, Abbott Park, IL), blood lactate (mmol) levels (Lactate Plus; Nova Biomedical, Waltham, MA, USA) via finger pricks on the non-dominant hand. A total of 8 time-sensitive blood measurements were collected; at baseline, 30-minutes post-supplement consumption, immediately post-Wingate, and immediately post-VO_{2peak} in each of the two data collection visits.

Cognitive Assessment

Cognitive assessment was measured using five tests from the American Neuropsychological Assessment Metrics (Vista LifeSciences, Parker, CO, USA) on a Windows desktop computer.[13, 14] These five tests included Simple Reaction Time (SRT), Memory Search (MS), and three difficulty levels of the Stroop test (Stroop 1, 2, and 3). The SRT test assesses visuo-motor processing speed, simple motor speed, and attention. It presents

Table 2. *Supplemental drink nutrition facts and ingredients*

Drink Information	Sports Drink (SD): Gatorade G2®	Ketone Salt (KS): KETO//OS® MAX
Nutrition Facts	Calories: 40 Fat (g): 0 Sodium (mg): 200 Carbohydrate (g): 10.7 Sugar (g): 9.5 Sugar Alcohol (g): 0 Protein (g): 0 Caffeine (mg): 0	Calories: 40 Fat (g): 0 Sodium (mg): 910 Carbohydrate (g): 5 Sugar (g): 0 Sugar Alcohol (g): 4 Protein (g): 0 Caffeine (mg): 0
Ingredients	Sugar, citric acid, salt, sodium citrate, natural and artificial flavor, monopotassium phosphate, modified food starch, calcium silicate, sucralose, red 40, acesulfame potassium	Beta-hydroxybutyrate, erythritol, L-taurine, fermented L-leucine, natural flavor, malic acid, citric acid, stevia, xanthan gum

Table 3. *Blinding procedures*

Group or individual blinded	Information withheld	Method of blinding	Blinding compromised
Person assigning subjects to condition order	Drink condition	Order of drink condition was randomized by a third party not involved in the research project. The supplements were put in brown paper bags and sealed by this person, and labeled with an "A" or "B". The supplement organizer did not communicate with investigators about supplement identity. Hence, allocation (A or B) schedule was not concealed, but group identification was concealed from investigators.	No
Participants	Drink condition	Similar tasting supplements (sports drink or ketone salts) with the same color and volume of water used for each drink condition	No
Data collectors and managers	Drink condition	Supplements were put in a sealed zip plastic bag in powder form, and then stored in a brown paper bag and until ready for consumption by the subjects; the supplement was mixed in water by the subject without any investigators present; subjects were instructed not to discuss the taste and texture of any of the supplements	No
Statistician	Drink condition, subject identities	Participants and groups given alpha-numeric identifiers	No

a series of "*" symbols and the participant is instructed to respond as quickly as possible by pressing the mouse button each time the symbol appears. The MS test assesses short term memory, immediate recognition, and attention. Participants are to memorize a string of six letters and respond once memorized. The letters disappear and then individual letters appear one at a time. The participant decides if the letter presented was in the original string of letters. The Stroop 1, 2, and 3 tests assess processing speed, selective attention, interference, and executive functioning. In Stroop 1, the words RED, GREEN, and BLUE appear in black font and the participant is instructed to read each word aloud and press a corresponding key for each word. In Stroop 2, a series of XXXX's appear in one of three colors and the participant is instructed to say the color aloud and press the corresponding key based on the color. In Stroop 3, a series of words appear in a color that does not match the name of the color depicted by the word and the participant is instructed to press the response key assigned to that color. Thus, the Stroop tests increase in difficulty from 1 to 3, with level 1 and 2 being congruent and level 3 being incongruent. The participants were instructed to fully concentrate on the tasks and perform their best on each trial. Silence in the lab was ensured during cognitive testing to prevent environmental distractions. This assessment was taken a total of four times on each of the testing sessions; baseline, 30-minutes post-supplement consumption, post-Wingate, and post- VO_{2peak}.

Wingate Test (Anaerobic Assessment)

To test anaerobic performance, each participant completed a 30-second maximal Wingate test during data collection visits. The Velotron™ cycle ergometer and the Racermate One™ software (SRAM, Chicago, IL, USA) was used to analyze the results of the participants. Prior to each test, the cycle ergometer and software were calibrated and personalized with the age, height, and weight of each participant. During the familiarization session, the participants were given an opportunity to get acclimated to the cycle ergometer after using it briefly. Before the testing period, the researcher verbally explained the test procedure and guidelines described by Vandewalle, Péerès, & Monod (1987).[15] During the Wingate test, there was no external motivation provided by the researchers. The participants warmed up on the cycle ergometer for 60 seconds with no loaded resistance. At the conclusion of the 60-second warm-up period, the test was initiated. At the initiation of the test, there was a 15-second time period with no resistance during which the participants were instructed to build up power and speed. At the conclusion of the 15-second period, a resistance of 7.5% of total bodyweight (kg) was automatically loaded on the Velotron™ cycle ergometer as the participants continued pedaling at maximal effort. The only instruction given during the test by the researcher was the amount of time remaining until the conclusion of the test. At the conclusion of the 30 seconds, the researcher(s) ensured that the participants were feeling well based on verbal confirmation. They were then instructed to cool down by continuing to pedal lightly with no resistance for 60 seconds. Mean power and peak power, each

measured in Watts (W), along with fatigue index, measured in $W*sec^{-1}$, were the three dependent variables measured.

VO_{2peak} Test (Aerobic Assessment)

To test CRF, each participant completed a peak oxygen uptake (VO_{2peak}) assessment during data collection visits. The TrueOne® 2400 metabolic cart (PARVO Medics, Salt Lake City, UT, USA) was used for VO_{2peak} testing. Prior to each test, the metabolic cart was calibrated and adjusted according to environmental conditions. The metabolic cart software was personalized with the age, gender, height, and weight of each participant. Participants were previously familiarized with the motorized treadmill and the progression according to the Bruce Treadmill Protocol. The Bruce Treadmill Protocol was used for VO_{2peak} testing trials, in which the speed and grade of the motorized treadmill was increased manually every 3 minutes until the participant voluntarily terminated the test.[16] Verbal instructions regarding the termination of the test were provided prior to testing. The participants were also familiarized to Borg Rating of Perceived Exertion (RPE) and told to indicate RPE at the conclusion of each stage by pointing to a physical scale placed in front of them during the test. The participants were instructed to refrain from using the treadmill handrails during the entirety of the test. Heart rate was monitored and participants were periodically asked to confirm conditional status throughout the test. Verbal encouragement by a researcher was provided to ensure maximal effort. One investigator provided all verbal check-ins and end of test encouragement to ensure similarity in external motivation between participants. When the participants felt they could no longer continue the test, they straddled the handrails on the sides of the treadmill indicating to the researcher(s) to end the test, at which point the test was terminated. At the conclusion of the test, the researcher(s) removed the headgear, as the participants cooled down for 3-5 minutes at 2 mph. The two dependent variables for the VO_{2peak} test were VO_{2peak}(the highest 10-sec average VO2 recorded during the test) and the respiratory exchange ratio (RER) recorded during the final stage of exercise.

Statistical Analysis

Power analyses using effect size reports from published research and unpublished observations from our lab were conducted using G*Power software, version 3.1. All minimum sample size estimations used an alpha level of .05 and power of .80. A minimum sample size of 16 subjects was estimated to detect a significant difference between the two drink conditions for any of the exercise performance and fitness variables, assuming a medium-large paired samples effect size of $d = .75$. A minimum sample size of 14 subjects was estimated to detect a significant interaction effect between drink condition and time point for any of the substrate or cognitive test ANOVAs, assuming a medium-large effect size of $\eta^2 = .10$.

Outcome measures from the Wingate and VO_{2peak} tests were analyzed using paired samples t-tests to compare the two drink conditions. Cognitive performance variables and substrate variables were analyzed using a series of 2 x 4 (drink condition x time point) repeated-measures ANOVAs.

SPSS version 26 was used to complete all

statistical analyses with a predetermined alpha level of .05. For all analyses, the assumption of normality in each cell was tested by analyzing the studentized residuals with the Shapiro-Wilk test of normality. No adjustments were made to correct for any normality violations since the ANOVA is robust to non-normal data.[17] In analyses for which outliers (scores with studentized residual > 3 SD from cell mean) were identified, the statistical analysis and all simple main effects (if applicable) were performed with identified outliers (a) included and (b) removed to verify that the results of these tests were unchanged.

Two outliers were identified for the ketone measurements. One of these outliers was determined to be uncommon, but not abnormally different than what would be expected (a baseline blood ketone level of 0.50 mmol/L). The other ketone outlier was judged to be a measurement error and was removed for the analysis. Two outliers were identified for the blood lactate measurements. Both extreme values were deemed measurement device errors (resting lactate levels > 10 mmol/L) and were removed from the analysis of blood lactate.

For the ANOVA tests, if a violation of sphericity was found using Mauchley's test of sphericity ($p < .05$), the Greenhouse-Geisser corrected statistical values were reported. Effect size is available for exercise performance and fitness variables (Cohen's *d*) and for interaction and main effects analyses for cognitive performance variables (partial eta squared, η_p^2).

All assumptions were met unless otherwise noted. In the event of a statistically significant interaction effect, simple main effects for drink condition were performed by comparing the two conditions at each of the four time points. Simple main effects for time point were also performed by comparing values at all time points in each drink condition. A Bonferroni-adjusted level of significance based on the number of comparisons was manually applied to determine if differences between drink conditions at the four time points were statistically significant. Main effects of time point or drink condition are not reported for analyses with a significant interaction effect.

3.RESULTS

Exercise Test Variables

Wingate test

No differences between drink conditions (SD vs KS) were observed for mean power (500.74 ± 145.96 vs. 490.21 ± 139.02; $t = 1.041$, $p = .312$, $d = .239$), peak power (674.68 ± 202.95 vs. 671.58 ± 210.01; $t = .110$, $p = .914$, $d = .025$), or fatigue index (11.47 ± 5.15 vs. 12.00 ± 5.34; $t = -.528$, $p = .604$, $d = .121$).

VO_{2peak} test

No differences between drink conditions (SD and KS) were observed for VO_{2peak} (40.07 ± 7.01 vs. 40.91 ± 8.04; $t = -1.179$, $p = .254$, $d = .270$). There was a significant difference observed in final-stage RER between SD and KS, $t = -3.107$, $p = .006$, $d = .713$. RER at the end of the VO_{2peak} test was significantly higher for KS (1.133 ± .079) than for SD (1.101 ± .072).

Cognitive Test Variables

A total of five violations of normality were observed using a significant ($p < .05$) test result

for the Shapiro-Wilk test.

SRT

There was no significant interaction effect of drink and time on SRT (p = .309, η_p^2 = .064), nor were there main effects of drink (p = .063, η_p^2 = .179) or time (p = .190, η_p^2 = .089).

MS

The assumption of sphericity was violated for the drink condition and the interaction term. There was no significant interaction effect of drink and time on MS (p = .898, η_p^2 = .006) or main effect of drink condition (p = .255, η_p^2 = .071). There was a significant main effect of time on MS, F(2.01, 36.11) = 57.85, p <.0005, η_p^2 = .763. MS increased over time from baseline (84.151 ± 11.176) to post-drink consumption (96.789 ± 9.838) to post-Wingate (102.653 ± 10.361) to post-VO_{2peak} (108.027 ± 11.137), and the value at each time point was significantly greater than all others before it ($p \leq .016$ in all cases).

Stroop 1

The assumption of sphericity was violated for the interaction term There was no significant interaction effect of drink and time on Stroop 1 (p = .353, η_p^2 = .054) or main effect of drink (p = .585, η_p^2 = .017). There was a significant main effect of time on Stroop 1, F(3, 54) = 47.16, p < .0005, η_p^2 = .724. Stroop 1 increased over time from baseline (84.014 ± 13.238) to post-drink consumption (95.212 ± 12.205; significantly greater than baseline, p < .0005) to post-Wingate (97.169 ± 12.950; significantly greater than baseline, p < .0005) to post-VO_{2peak} (99.527 ± 14.585; significantly greater than baseline, p < .0005, and post-drink consumption, p = .028).

Stroop 2

There was no significant interaction effect of drink and time on Stroop 2 (p = .137, η_p^2 = .096) or main effect of drink (p = .145, η_p^2 = .114). There was a significant main effect of time on Stroop 2, F(3, 54) = 14.25, p <.0005, η_p^2 = .442. Stroop 2 increased over time from baseline (91.844 ± 12.854) to post-drink consumption (94.652 ± 12.275) to post-Wingate (98.457 ± 12.100; significantly greater than baseline, p < .0005, and post-drink consumption, p = .012) to post-VO_{2peak} (99.496 ± 15.613; significantly greater than baseline, p = .001, and post-drink consumption, p = .011).

Stroop 3

The assumption of sphericity was violated for drink condition and the interaction term. There was no significant interaction effect of drink and time on Stroop 3 (p = .983, η_p^2 = .003) or main effect of drink (p = .769, η_p^2 = .005). There was a significant main effect of time on Stroop 3, F(2.02, 36.32) = 19.86, p <.0005, η_p^2 = .525. Stroop 3 increased over time from baseline (70.819 ± 12.523) to post-drink consumption (76.042 ± 14.040; significantly greater than baseline, p = .036) to post-Wingate (80.877 ± 14.642; significantly greater than baseline, p =.001, and post-drink consumption, p = .012) to post-VO_{2peak} (83.157 ± 15.749; significantly greater than baseline, p < .0005, and post-drink consumption, p = .001).

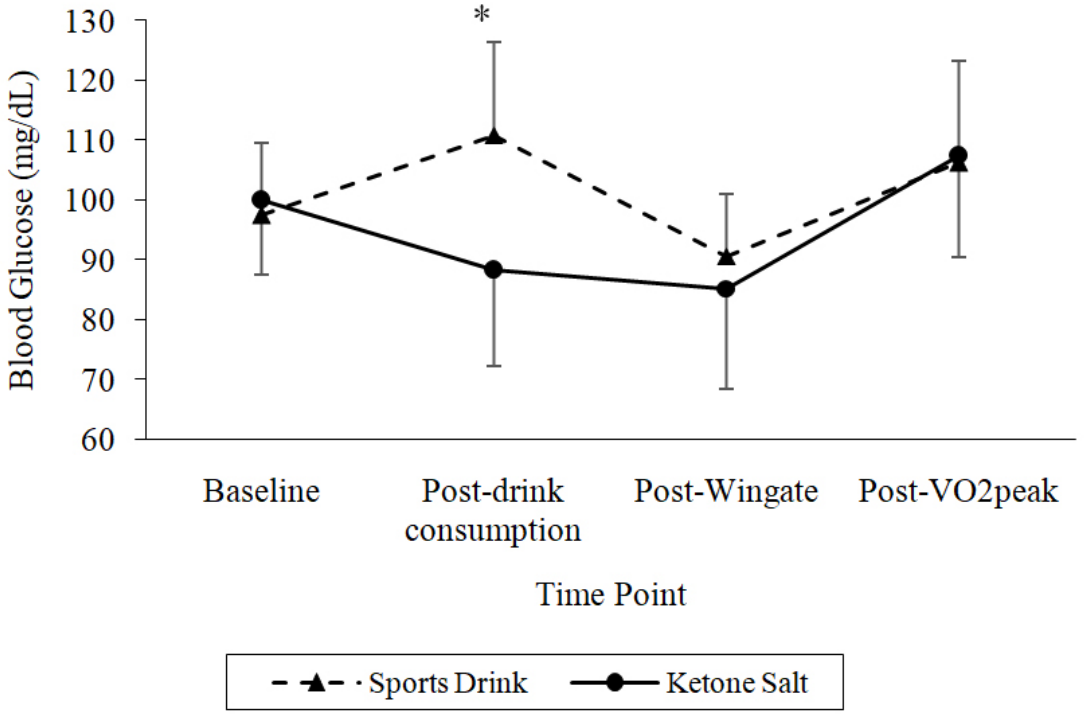
Substrate

Descriptive values (means and standard deviations) and significant results of simple main effects and relevant main effects tests are displayed graphically in Figures 2-4 for glucose, ketones, and lactate, respectively. Complete statistical results for substrate and cognitive performance measures are available through the linked supplemental resource: DOI 10.17605/OSF.IO/VGRCN.

Glucose

There was a statistically significant interaction effect between drink condition and time, $F(3, 54) = 15.31$, $p < .001$, $\eta_p^2 = .460$. Therefore, simple main effects were run for each factor. There was a significant difference in glucose levels at post-drink consumption between SD (110.79 ± 15.56 mg/dL) and KS (88.32 ± 16.14), $F(1, 18) = 25.13$, $p < .001$, $\eta_p^2 = .583$, a mean difference of 22.47 mg/dL, 95% CI [13.05, 31.89]. Comparisons at all other time points were not significantly different.

Figure 2. *Graph representing the change in blood glucose over time for both drink conditions. Results are displayed as mean values with a standard deviation error bar.*



* = significant difference between conditions at matched time points ($p < .0125$)

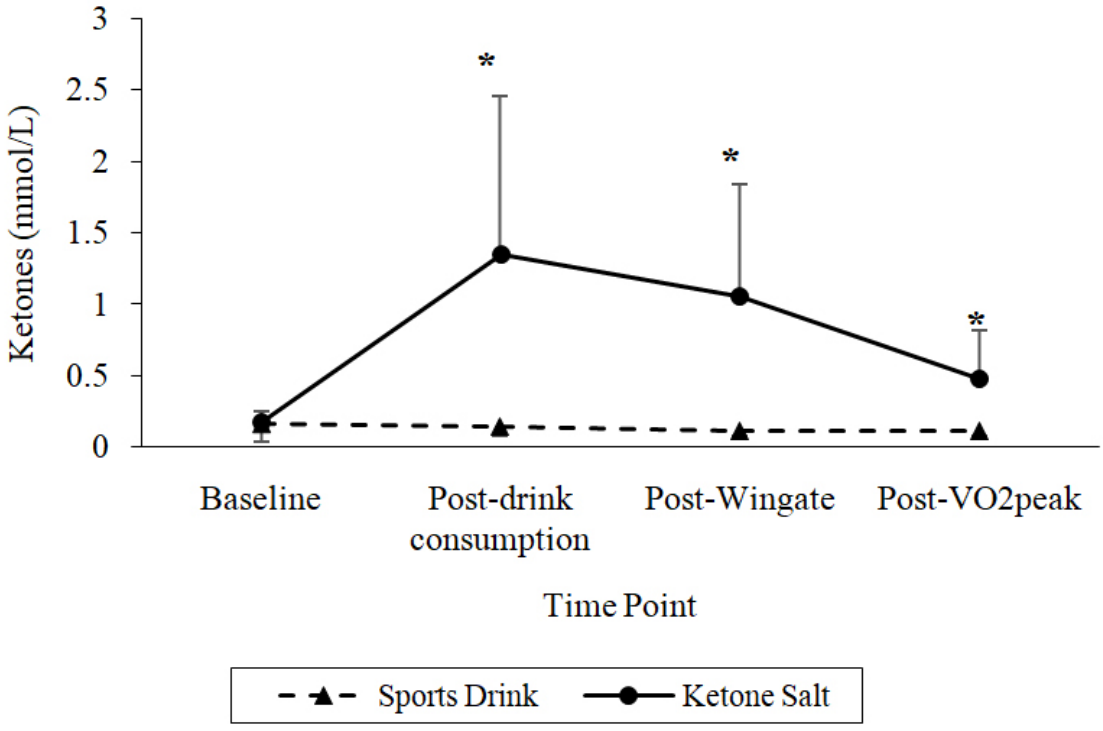
[a] = significantly different than previous time point for SD condition ($p < .025$)

[b] = significantly different than post-drink consumption and post-Wingate values for KS condition ($p < .025$)

Ketones

The assumption of normality was violated in all cells as assessed by the Shapiro-Wilk test of normality.The assumption of sphericity was violated for the interaction term. There was a statistically significant interaction effect between drink condition and time, $F(3, 51) = 15.66$, $p < .001$, $\eta_p^2 = .479$. Therefore, simple main effects were run for each factor. Blood ketone levels were significantly ($p < .001$) higher in the KS condition than in SD at post-drink (mean difference = 1.32, 95% CI [0.74 – 1.90]), post-Wingate (mean difference = 0.95, 95% CI [0.56 – 1.34]), and Post-VO_{2peak} (mean difference = 0.39, 95% CI [0.21 – 5.23]). Baseline ketone levels were not different between groups ($p = .630$).

Figure 3. *Graph representing the change in ketone levels over time for both drink conditions. Mean values are displayed with a standard deviation error bar.*



* = significant difference between conditions at matched time points ($p < .0125$)

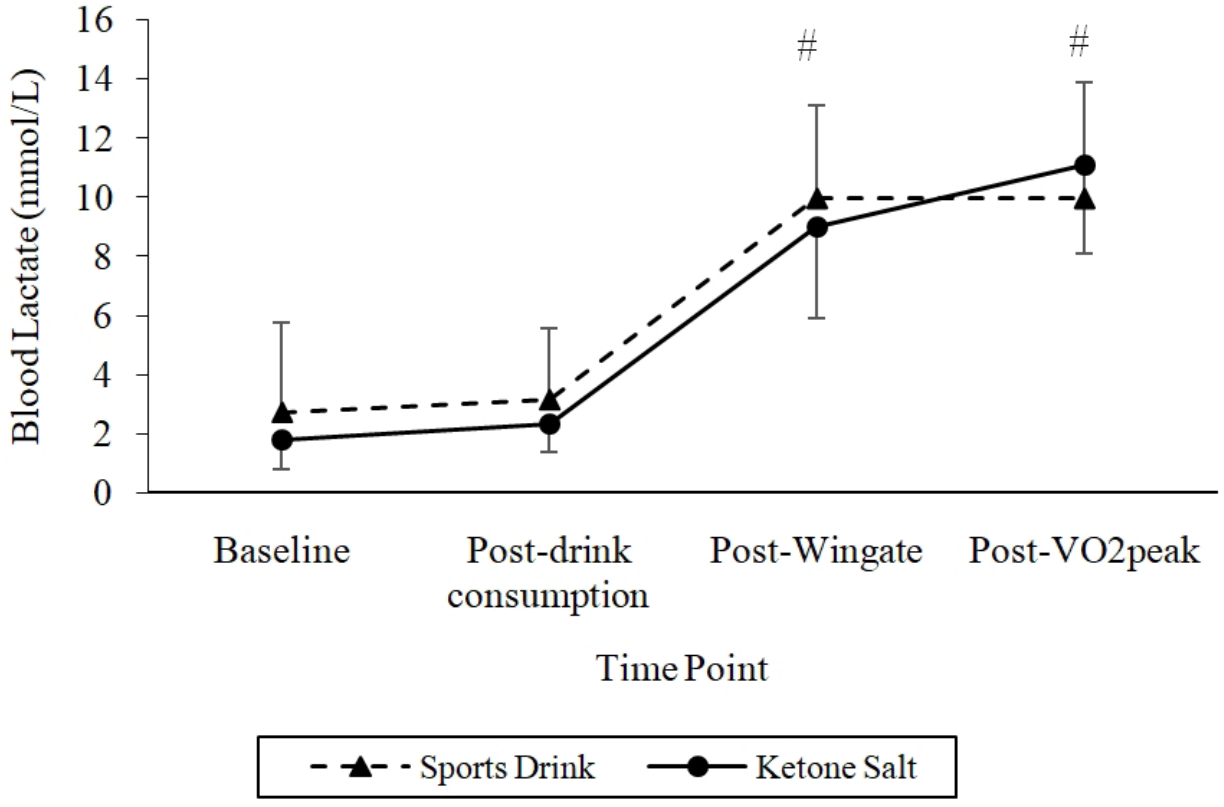
[a] = significantly lower than all other time points in KS condition ($p < .025$)

[b] = significantly greater than post-$VO2_{max}$ value for KS condition ($p < .025$)

Lactate

The assumption of normality was violated in two of the cells as assessed by the Shapiro-Wilk test of normality ($p < .05$). The assumption of sphericity was violated for the interaction term and for the time factor. There was no significant interaction effect between drink condition and time ($p = .09$). There was a significant main effect for time, $F(2.15, 34.40) = 94.39$, $p < .001$, $\eta_p^2 = .855$. Lactate was significantly higher at post-Wingate and post-VO_{2peak} time points than at both baseline and post-drink consumption time points. Lactate significantly increased from post-drink to post-Wingate (mean difference = 7.17 mmol/L, 95% CI [5.12, 9.21], and was not different between baseline and post-drink (mean-difference = 0.71, 95% CI [-.10, 1.52] or post-Wingate and post-VO_{2peak} (mean difference = 1.07, 95% CI [-1.40, 3.53].

Figure 4. *Graph representing the change in blood lactate over time for both drink conditions. Mean values are displayed with a standard deviation error bar.*



= main effect of time; significantly different than baseline and post-drink consumption values ($p < .05$)

4.DISCUSSION

The primary objective of this study was to determine if non-keto-adapted healthy subjects would experience an improvement in anaerobic performance, CRF, and/or cognitive performance after taking a single dose of KS compared to a serving of a conventional SD. The main findings are that an acute dose of KS had a similar effect on anaerobic and aerobic maximal effort exercise outcomes as the isocaloric SD. Cognitive performance was also similar between supplemental conditions. These results occurred despite differences in substrate availability (ketones and glucose) between conditions.

Anaerobic Exercise Performance

Anaerobic performance for the 30-s Wingate test was no different between the KS and SD conditions. Evan and Egan reported that no performance improvement was observed for intermittent 15-m sprint test to exhaustion in team sport athletes when a ketone monoester (KE) supplement was ingested compared to sports drink. There were favorable metabolic changes in the KE trial, specifically attenuated increase in lactate, yet no changes in HR, RPE, sprint time, or sprint exercise to exhaustion were noted.[18] Our results are similar to those reported by Waldman et al. that repeated 15-s sprint cycling performance was not different after consuming KS compared with a placebo beverage.[19] A suboptimal concentration of blood ketones could explain the null results reported by Waldman et al., yet the average ketone peak concentration reached by subjects in our study (1.35 ± 1.11 mmol/L) was substantially higher than those in the Waldman et al. study (0.53 ± 0.19 mmol/L) and a similar lack of performance improvement resulted. Given the meager and non-significant change in ketone levels between the post-drink consumption and post-Wingate time points in this study, it is unlikely that ketones were a main substrate or that ketone concentration in the blood limited anaerobic performance.

Glucose is typically the preferred substrate for activities completed at greater than 85% VO_{2max}[20] and attenuated glycolysis following exogenous ketone ingestion has the potential to limit maximal-intensity sprint performance.[21] Ketone supplements may be best suited to endurance exercise during which lipids and ketones can make a more substantial contribution to exercise fuel, yet the fact that a single, maximal effort anaerobic performance test was not impaired by KS ingestion is promising for activities during which high-intensity bursts may be necessary.

Aerobic Exercise CRF

Similar to anaerobic exercise, aerobic capacity did not differ between KS and SD conditions. Our results are similar to those reported by other researchers who found no improvement in maximal aerobic exercise. Rodger et al. found that in a group of elite cyclists, 4-min maximal cycling performance test outcome (power output) was no different following BHB salt ingestion than when a sports drink alone was consumed. Some metabolic advantages, such as greater RER and VO_2 levels during the 4-min performance test were reported, yet these did not translate to improved performance.[22]

KE supplementation has been shown to be more effective at increasing blood ketone levels than KS, however, they are not as common due to the higher cost and bitter flavor.[23] Some researchers have reported that KE ingestion prior to exercise actually impaired performance. Leckey et al. (2018) observed an average 2% slower finishing time for a 31-km simulated race in elite cyclists when KE was consumed beforehand, compared to a control condition. Lactate increase was attenuated, yet all subjects reported some degree of gastrointestinal distress following KE consumption, with a range of severity. RPE was also reported to be similar between the two conditions even though power output was lower in the slower KE trial, suggesting that digestive side effects contributed to the perceived difficulty of the trial and resulting slower time.[24]

Conversely, Cox et al. conducted a series of experiments and found that after 1 hour of fixed intensity cycling at 75% of maximal power output, trained cyclists performed better on a 30-min time trial when KE and carbohydrate solution was consumed compared to carbohydrate solution alone. The exercise protocol used by Cox et al. and ensuing differences in substrate requirement likely explain the differences in results that we report here. Additionally, the training status of subjects differed substantially and may have contributed to the lack of significant improvement in aerobic capacity we observed.[5]

The glucose sparing effect of exogenous ketone consumption is commonly reported in which increases in glucose with exercise are blunted until later in the exercise session[18].This effect was present in our findings as well. Glucose significantly increased from the post-Wingate time to the post-VO_{2peak} time point in the KS trial. This increase in glucose level coincided with an increased RER value at exercise test termination which was significantly greater in the KS condition than in the SD condition. A greater RER indicates that a larger proportion of energy is derived from glucose, so it may be be that the increased "spared" blood glucose from KS contributed to in part to the higher RER at the end of the test compared to SD. However, we did not directly assess glucose uptake and utilization at the site of the muscle, and therefore cannot conclude that this was the case. Importantly, the blunting of glucose levels following exogenous ketone ingestion may be interpreted as a detrimental effect resulting from impaired hepatic glycogenolysis and glycolysis.[25] Regardless, sufficient glucose was available in the blood in the KS condition to match that in the SD condition, and was likely available to use in the muscle cells to achieve an RER value greater than 1.0. These metabolic differences were insufficient to yield CRF advantages to subjects during the KS trial, yet no detrimental effects were observed.

Cognitive Performance

There were no statistically significant differences in any of the indices of cognitive performance at matched time points between the two supplemental conditions. This same general finding was reported by Waldman et al. who found that KS supplementation prior to a dual task exercise challenge did not alter cognitive performance. Subjects completed a mental arithmetic task and modified Stroop Color Word task while

they cycled at 60% of VO_{2peak} for 20 min under experimental (0.38 g/kg body mass of KS) and control (solution of matched volume, taste, and mineral content) conditions, with no difference in cognitive performance between trials.[16] In an earlier study, Waldman et al. also observed no difference in cognitive performance during concurrent cycling activity between KS and control conditions. In both instances, exercise was not exhaustive and blood ketone levels did not exceed 1.0 mmol/L, which was acknowledged to have potentially limited the results.[26]

In contrast, Evans and Egan reported a cognitive benefit from taking KE prior to exhaustive sprint exercise. A multitasking test was completed before and after exercise, with a greater decline in performance observed in the sports drink (control) condition compared to the KE condition. Notably, subjects reached ketone levels of 1.5-2.6 mmol/L during exercise which was substantially higher than in the current study (1.35 ± 1.11 mmol/L).[27] It is possible that we would have observed differences in cognitive performance on some of the indices used, had a larger dose of KS been administered.

One potential reason for the lack of performance improvement from KS ingestion compared to SD in this study was the concentration of blood ketones achieved by subjects. Nutritional ketosis is often denoted by a concentration of >0.5 mmol/L of ketones in the blood, yet Evans et al. propose that an optimal level of ketone concentration is between 1 and 3 mmol/L to yield performance benefits.[18] On average, subjects in this study attained peak ketone concentration of 1.35 mmol/L prior to any exercise test, though there was substantial variability between subjects (standard deviation of 1.11 mmol/L). Only 7 of the 19 subjects exceeded the proposed 1.0 mmol/L threshold level for performance benefits, thus limiting the ability to observe maximal exercise benefits or cognitive performance improvements. Excessive amounts of KS have been linked to gastrointestinal issues, dehydration, and pH imbalance,[28, 29] which makes the use of KE more attractive (though more expensive and less feasible) when seeking to achieve necessary levels of ketones for cognitive and physical performance improvement.

Another possible reason for the lack of improvement in maximal aerobic CRF and anaerobic exercise performance, and cognitive performance, is the fact that subjects in this study were not keto-adapted. Adaptation to a ketogenic diet and limited carbohydrate availability takes several weeks[30] and is distinctly different from the temporary state of nutritional ketosis achieved in this study with KS.[28] A key feature of a keto-adapted individual is the increase in expression of the protein monocarboxylate transporter-1 (MCT-1) which regulates the transportation of ketones into the cells of the body.[31] More transport proteins enable a greater uptake and utilization of ketone bodies by the muscles and brain (tissues that were the focus of the tests in this study). Increased MCT-1 expression is the result of chronically elevated ketone concentration in the blood, which is a feature of prolonged KD adherence but not NK. Non-keto-adapted individuals like the subjects in this study may have a limited ability to utilize exogenous ketone bodies as a substrate compared with keto-adapted individuals. Few studies have examined the effect of KS on exercise performance and CRF

in keto-adapted people.

Limitations

There were several limitations to this study that may warrant consideration in future research. First, our sample size consisted of healthy, college aged participants. Therefore, the results may only be applicable to this specific population. It would be highly informational for future related research to study similar effects with a sample from an alternate population (elderly, cognitively impaired, athletic, obese, sedentary, keto-adapted, or KD individuals, etc.). Second, we were only able to conduct our study on a relatively small sample size. It would be beneficial for future research to conduct studies of similar nature on a larger sample size given the variability of ketone metabolism rate in different people. Third, the supplement used for comparison in our experimentation was not a placebo, as Gatorade G2 is truly a sport performance enhancing supplement as well. This may in part explain the partial rejection of the hypothesis. The impact that SD and KS had on physical and cognitive performance, if any, was similar. Since SD and KS do not appear to have opposing effects on these outcomes, athletes and other individuals who must rely on enduring physical activity and demanding cognitive challenges may feel free to substitute the supplements without experiencing detrimental effects.

Relevance of Results

Substrate availability (glucose and ketones) was modulated as a result of acute KS ingestion, yet these changes did not result in improvement of exercise or cognitive performance. However, decrements in performanceand fitness were also not observed. Given the potential for increased exogenous ketone utilization in keto-adapted individuals who possess the metabolic machinery to take up and process ketones as fuel, these findings may be relevant to people in the initial phases of KD adherence. The low risk of KS supplementation to performance, coupled with the potential performance increases upon achieving keto-adaptation, could prove beneficial for people interested in KS supplementation who are transitioning to a KD and seek athletic improvement. The effects of exogenous ketones in keto-adapted individuals are largely unexplored, and further investigation into this area is warranted in future studies.

Conclusion

Even though anaerobic exercise, aerobic capacity, and cognitive performance did not improve following KS ingestion, performance in these domains was maintained comparable to a SD. After a single acute dose of KS in a fasted state, the participants displayed similar performance and fitness results as with the leading isocaloric sport performance supplement, Gatorade G2.

Previous studies have evaluated the effects of ketones on exercise outcomes and cognitive functioning distinctly, with limited human trials, indicating that both may be improved due to KS or KD. We evaluated the results of the acute KS on both maximal exercise outcomes and cognitive performance simultaneously with applicable results.

REFERENCES

1. Newman JC, Verdin E. Ketone bodies as signaling metabolites. *Trends Endocrinol*. 2014;25(1):42-52. doi:10.1016/j.tem.2013.09.002
2. Pardridge WM. Blood-brain barrier transport of glucose, free fatty acids, and ketone bodies. *Fuel Homeostasis and the Nervous System*. 1991:43-53. doi:10.1007/978-1-4684-5931-9_5
3. Kesl SL, Poff AM, Ward NP, et al. Effects of exogenous ketone supplementation on blood ketone, glucose, triglyceride, and lipoprotein levels in Sprague–Dawley rats. *Nutr. metab.* 2016;13(1):1-5. doi:10.1186/s12986-016-0069-y
4. Cox PJ, Clarke K. Acute nutritional ketosis: implications for exercise performance and metabolism. *Extreme Physiol. Med.* 2014; (1):1-9. doi:10.1186/2046-7648-3-17
5. Cox PJ, Kirk T, Ashmore T, et al. Nutritional ketosis alters fuel preference and thereby endurance performance in athletes. *Cell metab.* 2016;24(2):256-68. doi:10.1016/j.cmet.2016.07.010
6. Pinckaers PJ, Churchward-Venne TA, Bailey D, van Loon LJ. Ketone bodies and exercise performance: the next magic bullet or merely hype?.*Sports Med.* 2017;47(3):383-91. doi:10.1007/s40279-016-0577-y
7. O'Malley T, Myette-Cote E, Durrer C, Little JP. Nutritional ketone salts increase fat oxidation but impair high-intensity exercise performance in healthy adult males. *Appl. Physiol. Nutr. Metab.* 2017;42(10):1031-5. doi:10.1139/apnm-2016-0641
8. Dearlove DJ, Faull OK, Rolls E, Clarke K, Cox PJ. Nutritional ketoacidosis during incremental exercise in healthy athletes. *Front. physiol.* 2019;10:290. doi:10.3389/fphys.2019.00290
9. Prins PJ, Koutnik AP, D'Agostino DP, Rogers CQ, Seibert JF, Breckenridge JA, Jackson DS, Ryan EJ, Buxton JD, Ault DL. Effects of an Exogenous Ketone Supplement on Five-Kilometer Running Performance. *J. hum. kinet.*2020;72:115. doi: 10.2478/hukin-2019-0114
10. Veech RL, Chance B, Kashiwaya Y, Lardy HA, Cahill Jr GF. Ketone bodies, potential therapeutic uses. *IUBMB life.* 2001;51(4):241-7. doi:10.1080/152165401753311780
11. Xu K, Sun X, Eroku BO, Tsipis CP, Puchowicz MA, LaManna JC. Diet-induced ketosis improves cognitive performance in aged rats. *Oxygen Transport to Tissue XXXI.* Springer; 2010:71-75. doi:10.1007/978-1-4419-1241-1_9
12. Scott JM, Deuster PA. Ketones and Human Performance. *J. spec. oper. med.* 2017;17(2):112-6. PMID: 28599043
13. Kabat MH, Kane RL, Jefferson AL, DiPino RK. Construct validity of selected Automated Neuropsychological Assessment Metrics (ANAM) battery measures. *Clin. neuropsychol.* 2001;15(4):498-507.

doi:10.1076/clin.15.4.498.1882

14. Reeves DL, Winter KP, Bleiberg J, Kane RL. ANAM® Genogram: Historical perspectives, description, and current endeavors. *Arch.clin.neuropsychol.* 2007;22(Suppl_1):S15-37. doi:10.1016/j.acn.2006.10.013
15. Vandewalle, H., Péerès, G. Monod, H. Standard Anaerobic Exercise Tests. *Sports med.* 1987;4:268–289. doi:10.2165/00007256-198704040-00004
16. Swain DP, Brawner CA. American College of Sports Medicine. ACSM's resource manual for guidelines for exercise testing and prescription. Wolters Kluwer Health/ Lippincott Williams & Wilkins; 2014.
17. Blanca Mena MJ, AlarcónPostigo R, Arnau Gras J, Bono Cabré R, Bendayan R. Non-normal data: Is ANOVA still a valid option? *Psicothema.* 2017;29(4):552-557. doi: 10.7334/psicothema2016.383
18. Evans M, Cogan KE, Egan B. Metabolism of ketone bodies during exercise and training: physiological basis for exogenous supplementation. *J. physiol.* 2017;595(9):2857-71. doi: 10.1113/JP273185
19. Waldman HS, Shepherd BD, Egan B, McAllister MJ. Exogenous ketone salts do not improve cognitive performance during a dual-stress challenge. *Int. J. Sport Nutr. Exerc. Metab.* 2020;30(2):120-7. doi:10.1123/ijsnem.2019-0122
20. Romijn JA, Coyle EF, Sidossis LS, Gastaldelli A, Horowitz JF, Endert E, Wolfe RR. Regulation of endogenous fat and carbohydrate metabolism in relation to exercise intensity and duration. *Am. j. physiol. endocrinol. metab.* 1993;265(3):E380-91. doi: 10.1152/ajpendo.1993.265.3.E380
21. Dearlove DJ, Faull OK, Clarke K. Context is key: exogenous ketosis and athletic performance.*Curr. opin. physiol.*2019;10:81-9. doi:10.1016/j.cophys.2019.04.010
22. Rodger S, Plews D, Laursen P, Driller M. The effects of an oral β-hydroxybutyrate supplement on exercise metabolism and cycling performance. *J. sci. cycl.* 2017;6(1):26-31.
23. Stubbs BJ, Evans R, Clarke K, Cox PJ, Stubbs BJ, Clarke K, Cox PJ, Evans R. Ketone ester drinks increase blood ketone levels more effectively than ketone salt drinks. *Age.*2016;16:19.
24. Leckey JJ, Ross ML, Quod M, Hawley JA, Burke LM. Ketone diester ingestion impairs time-trial performance in professional cyclists. Frontiers in physiology. 2017 Oct 23;8:806. doi: 10.3389/fphys.2017.00806
25. Mikkelsen KH, Seifert T, Secher NH, Grøndal T, van Hall G. Systemic, cerebral and skeletal muscle ketone body and energy metabolism during acute hyper-D-β-hydroxybutyratemia in post-absorptive healthy males. *J. clin. endocrinol. metab.* 2015;100(2):636-43. doi: 10.1210/jc.2014-2608
26. Waldman HS, Basham SA, Price FG, Smith JW, Chander H, Knight AC, Krings BM, McAllister MJ. Exogenous ketone salts do not improve cognitive responses after a

high-intensity exercise protocol in healthy college-aged males. *Appl. physiol. nutr. metab.* 2018;43(7):711-7. doi: 10.1139/apnm-2017-0724

27. Evans M, Egan B. Intermittent running and cognitive performance after ketone ester ingestion. *Med. sci. sports exerc.* 2018;50(11):2330-8. doi:10.1249/MSS.0000000000001700
28. Shaw DM, Merien F, Braakhuis A, Maunder E, Dulson DK. Exogenous ketone supplementation and keto-adaptation for endurance performance: disentangling the effects of two distinct metabolic states. *Sports med.* 2020;50(4):641-56. doi: 10.1007/s40279-019-01246-y
29. Stubbs BJ, Cox PJ, Kirk T, Evans RD, Clarke K. Gastrointestinal effects of exogenous ketone drinks are infrequent, mild, and vary according to ketone compound and dose. Int *J. sport nutr. exerc. metab.* 2019;29(6):596-603. doi: 10.1123/ijsnem.2019-0014
30. Shaw DM, Merien F, Braakhuis A, Maunder ED, Dulson DK. Effect of a Ketogenic Diet on Submaximal Exercise Capacity and Efficiency in Runners. *Med. sci. sport.* exerc. 2019;51(10):2135-46. doi: 10.1249/MSS.00000 00000002008
31. García-Cáceres C, Fuente-Martín E, Argente J, Chowen JA. Emerging role of glial cells in the control of body weight. *Mol. metab.* 2012;1(1-2):37-46. doi:10.1016/j.molmet.2012.07.001

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AH-W designed the study. AQ and AH-W collected the data. AM analyzed the data. All authors wrote the first draft, reviewed, and commented on subsequent drafts of the manuscript.