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Nutritional ketone salts increase fat oxidation but impair high-intensity exercise performance in healthy adult males

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Abstract

This study investigated the impact of raising plasma beta-hydroxybutyrate (β-OHB) through ingestion of ketone salts on substrate oxidation and performance during cycling exercise. Ten healthy adult males (23 ± 3 years, BMI 25 ± 3 kg/m^2, VO_{2peak} 45 ± 10 ml/kg/min) were recruited to complete two experimental trials. Before enrollment in the experimental conditions, baseline anthropometrics and cardiorespiratory fitness (VO_{2peak}) were assessed and familiarization to the study protocol was provided. On experimental days, participants reported to the laboratory in the fasted state and consumed either 0.3 g/kg β-OHB ketone salts or a flavor-matched placebo 30 minutes prior to engaging in cycling exercise. Subjects completed steady-state exercise at 30%, 60%, and 90% ventilatory threshold (VT) followed by a 150 kJ cycling time trial. Respiratory exchange ratio (RER) and total substrate oxidation were derived from indirect calorimetry. Plasma glucose, lactate, and ketones were measured at baseline, 30 minutes post-supplement, post-steady-state exercise, and immediately following the time trial. Plasma β-OHB was elevated from baseline and throughout the entire protocol in the ketone condition ($p<0.05$). RER was lower at 30% and 60% VT in the ketone compared to control condition. Total fat oxidation was greater in the ketone versus control ($p=0.05$). Average time trial power output was ~7% lower (-16 Watts, $p=0.029$) in the ketone condition. Ingestion of ketone salts prior to exercise increases fat oxidation during steady state exercise but impairs high-intensity exercise performance.

Keywords: ketones, beta-hydroxybutyrate, ketosis, metabolism, substrate oxidation

Introduction

The recent development of exogenous ketone supplements that can raise blood beta-hydroxybutyrate (β-OHB) concentration has garnered considerable attention as an ergogenic aid (Cox et al. 2016; Egan and D'agostino, 2016; Pinckaers et al. 2016). β-OHB is the most abundant circulating ketone body that can serve, among other things, as a source of energy for skeletal muscle (Cox and Clarke 2014). Recently, Cox and colleagues (2016) described the novel effects of exogenous ketone supplements on oxidative metabolism during cycling exercise. Specifically, acute elevation of plasma β-OHB after ingesting nutritional ketone ester supplements led to apparent ketone oxidation and enhanced intramuscular triglyceride utilization, while preserving endogenous glycogen stores during exercise. These results demonstrate the potential metabolic benefit of nutritional ketones to provide an alternative oxidative fuel source, while simultaneously improving lipid utilization during exercise in humans. Mechanistically, the suppression of glycolysis during aerobic exercise after ingestion of nutritional ketones was attributed to an increase in acetyl-CoA concentrations, a higher NADH:NAD^+ ratio from mitochondrial ketone oxidation, and subsequent inhibition of pyruvate dehydrogenase (Cox et al. 2016). This was evidenced by elevations of skeletal muscle β-OHB and increases in glycolytic intermediates seen in skeletal muscle biopsy samples, suggesting that exogenous ketones may have promoted a "Randle cycle-like" effect to impair carbohydrate (CHO) oxidation and/or glycolysis during exercise (Randle et al. 1963).

Given the importance of glycolysis for maintaining skeletal muscle ATP production during high-intensity exercise, the group also cautioned about using ketones in certain athletic events: "ketosis may not be advantageous in physiological conditions that rely almost solely on anaerobic glycolysis or high glycolytic flux for ATP production, such as sprint or short-duration exercise" (Cox et al. 2016). The potential for acute nutritional ketosis to inhibit CHO oxidation and affect performance during high workloads is currently unclear, although "forcing" muscle towards greater lipid oxidation could theoretically reduce performance during high-intensity exercise.

At present, ketone ester supplements are not commercially available. However, β-OHB ketone salts are available and are marketed to increase fat oxidation and enhance high-intensity exercise performance. We are unaware of any published data on the impact of β-OHB ketone salts on exercise metabolism or high-intensity performance. Accordingly, we examined whether β-OHB ketone salts influenced substrate utilization during steady-state exercise and assessed their impact on high-intensity exercise performance.

Methods

Participants

Ten males were recruited and provided written informed consent. All participants were considered healthy, recreationally active (exercising at least three times per week but not specifically training for endurance sports), and reported no contraindications to exercise. Participants were not currently taking medication, following a low-carbohydrate or ketogenic diet, or currently consuming nutritional ketone supplements. Physical characteristics included; age 23 ± 3 years, BMI 25 ± 3 kg/m^2, body mass 83 ± 13 kg, VO_{2peak} 45 ± 10 ml/kg/min, peak power output 299 ± 60 W. The study was conducted in accordance with the Declaration of Helsinki, and was approved by the University of British Columbia Clinical Research Ethics Board (registered at clinicaltrials.gov: NCT02825823).

Study Design

A double-blind placebo-controlled crossover design was employed consisting of baseline testing, familiarization, and two experimental trials. Subjects completed a maximal oxygen uptake test and anthropometrics were measured for baseline characteristics. As not all participants explicitly achieved a plateau in VO2, the highest oxygen uptake value was recorded and considered the VO_{2peak} (Howley et al. 1995). A familiarization trial was completed on a separate visit from baseline prior to completion of the experimental conditions. Familiarization trials were identical to experimental trials except that

participants were not fasted and consumed no supplement drink prior to exercise. After familiarization, participants then completed two experimental conditions (ketone or placebo), the order of which were completed in a randomized and counterbalanced fashion separated by ~7 days (range 4-9).

Baseline Testing

Upon entrance to the laboratory, height and weight were measured to determine body mass index of subject. VO_{2peak} testing was used to assess aerobic capacity and determine ventilator threshold values. Participant engaged in a ramped-protocol on an electronically braked cycle ergometer (Lode Ergometer Manager, Groningen, The Netherlands). The test began at 50 W for 4 minutes, followed by a 1 W increase every 3 seconds (20 W/min) until volitional fatigue. Expired gas was analyzed by a metabolic cart (TrueOne 2400 Metabolic Measurement System, Parvo Medic, Murray, United States of America). Individual ventilatory threshold (VT) was determined using the V-slope method from the baseline VO_{2peak} test.

Supplement

Ketone salts were acquired from a commercially available ketone supplement (KetoForce®, Prototype Nutrition, Urbana, United States of America). The ketone supplement contained 0.3g β-OHB/kg, 0.01g Potassium/kg, and 0.01g Sodium/kg. Placebo drink contained 0.01g Potassium/kg, 0.01g Sodium/kg. Each drink was mixed with 30 ML of lemon juice and 14 mg stevia lead extract in order to maintain blind. Both drinks were consumed in 500 mL of water.

Experimental Trials

Participants reported fasted on experimental testing days and were given either a commercially available ketone supplement or a flavor-matched placebo. Thirty minutes after consumption participants completed a three-stage steady-state incremental exercise protocol comprised of five

minutes of cycle ergometer exercise at each of 30%, 60%, and 90% of individual ventilatory threshold. Metabolic gases were collected throughout each stage using a metabolic cart. Five minutes after completion of steady-state exercise, participants completed a simulated 150 kJ time trial. The ergometer was set in linear mode and the time trial was equated to a distance of 10 km by the associated software program (Lode Ergometer Manager, Groningen, The Netherlands). Participants received visual feedback as "distance covered" on a computer monitor. Time to completion and average power output were recorded after each test. Coefficient of variation (CV) for time trials in our lab is 2.6% based on two tests performed 3-7 days apart (n=6 males). At the end of each trial, participants were asked to guess which condition they completed that day (ketone or placebo). Participants recorded their diet on the day prior to the first experimental trial and were instructed to replicate food intake before the second, which was verified by a research assistant upon arrival at the laboratory.

Metabolic Gases

Gas samples were collected throughout the steady-state cycle. The final two minutes of gas collection from each steady-state stage were analyzed. The proportion of CHO and fat fuel oxidation during steady-state cycling was calculated from the respiratory exchange ratio (RER), with the assumption that protein metabolism was negligible. Substrate oxidation rates were calculated from indirect calorimetry measurements (Jeukendrup and Wallis 2005).

Blood Sampling and Analysis

Blood β-OHB and glucose (Precision Neo, Abbott Laboratories, Witney, UK) and lactate (Lactate Pro, Arkray, Kyoto, Japan) were measured in capillary blood. Fingertip capillary samples were collected using a lancet following cleaning with alcohol and allowing to air dry. The first blood droplet was wiped away with a cotton swab and the subsequent droplets were used for analysis. Blood samples

were measured at baseline, 30 minutes post-supplement ingestion, post steady-state exercise, and at the end of the time trial.

Statistical Analysis

All data were analyzed using GraphPad Prism v6. Differences between conditions for mean power output in the time trial were analyzed using a repeated measures ANOVA accounting for the order effect. Mean power output data were log-transformed prior to analysis to express effects as percentage differences between conditions. Effects are presented together with 95% confidence intervals (CI), which indicate a range of effects compatible with the data. A priori, we defined thresholds for small, moderate, large, and extremely large differences in mean power output as 0.3, 0.9, 1.6, 2.5 and 4.0 × the typical within-athlete CV from competition-to-competition, in line with contemporary guidelines for analyses of exercise performance data (Hopkins et al. 2009). For road cycling time trials this CV is approximately 3.5% (Malcata and Hopkins 2014). Differences between conditions in respiratory exchange ratio (RER), β-OHB, glucose, and lactate were analyzed using 2-factor (condition X time) repeated-measures analysis of variance (ANOVA) with significant main effects and/or interactions followed by Sidak's post-hoc test comparing ketone to placebo at each time point. Differences in substrate oxidation were analyzed with a paired t-test. Significance was set at $p<0.05$. Data are reported as mean ± SD unless otherwise noted.

Results

Substrate Utilization

RER was lower during exercise in the ketone compared to the control condition (main effect of condition, $p<0.001$). Post-hoc tests revealed significantly lower RER at 30% and 60% VT in the ketone condition (both $p<0.05$; Fig 1A). Total fat oxidation was greater ($p=0.02$), and total CHO oxidation lower ($p=0.005$) during exercise in the ketone condition versus placebo (Fig 1B). Plasma β-OHB

increased from baseline in the ketone condition (condition X time interaction, $p<0.001$) and levels were elevated throughout the entire protocol versus placebo ($p<0.05$ for all; Fig 2A). Glucose was lowered in the ketone condition (main effect of condition, $p=0.002$) with post-hoc tests indicating that glucose was lower immediately post-exercise and post-time trial in the ketone condition compared to placebo (both $p<0.05$; Fig 2B). Blood lactate rose from baseline during exercise (main effect of time, $p<0.0001$) but there were no significant differences seen between conditions at any time point (Fig 2C). Participants were accurate at correctly predicting the designated condition (ketone or placebo) 50% of the time, indicating that the ketone supplement was adequately masked.

Performance

It took participants longer to complete the time trial in the ketone condition (711±137 seconds) when compared to the control condition (665±120 seconds, $p=0.03$). Average power output during the time trial was 74±8 % power at VO_{2peak} (101±22% VT) in the ketone condition compared to 79±14% (107±21% VT) in the placebo ($p=0.012$). Average power output in the cycling time trial was on average ~7% (-16 Watts, $p=0.029$) lower in the ketone versus placebo condition (95% CI, -12% to -1%, Fig 3). The point estimate of 7% represents a large effect with the confidence interval ranging from a small to very large decrement in performance.

Discussion

Our findings provide evidence that ketone salts can alter the metabolic response during steady-state exercise. A lower respiratory ratio and greater total fat oxidation during steady-state cycling in the nutritional ketone condition compared to placebo suggests the role of β-OHB in promoting lipid utilization during aerobic exercise. These findings are in agreement with Cox et al. (2016), who observed an increase in intramuscular triglyceride oxidation and reduction in glycolytic intermediates during one hour of steady-state cycling at 75% maximal power in elite athletes following consumption

of ketone ester supplements. Mechanistically, β-OHB may provide acetyl-CoA groups and thus increase the acetyl-CoA/CoA ratio leading to feedback inhibition of glycolysis (Randle et al. 1963). Inhibition of glycolysis necessitates a greater reliance of lipid oxidation to support ATP demand at a given exercise intensity. Previous reports using ketone esters in elite athletes provide support for an increase in lipid oxidation during sub-maximal cycling exercise (Cox et al. 2016).

We were unable to directly measure β-OHB oxidation in the present study but the reduction in RER at 30% VT (from ~0.85 in the placebo condition to ~0.83 in the ketone condition, Fig 1A) support the notion that lipid utilization was increased when plasma β-OHB was elevated. The theoretical RER for oxidizing β-OHB is 0.89 (Frayn 1983); it would therefore be expected that the RER would be increased ("pulled up") closer to 0.89 at 30% VT in the ketone condition if the increase in circulating β-OHB was simply leading to an increase in β-OHB oxidation. However it is currently unknown how provision of exogenous ketones might impact substrate oxidation equations, which are theoretically based on CHO and lipid substrates.

In line with the speculation that high-intensity exercise performance could be impaired with elevated ketones (Cox et al. 2016; Pinckaers et al. 2017), we observed that average power output during the time trial was reduced in the ketone versus placebo condition. A high degree of CHO oxidation to support ATP demand is necessary to maintain the workload during the time trial as supported by blood lactate values of ~10 mmol/l at the end of the time trial (Fig 1E). Attenuation of CHO metabolism during high-intensity exercise by ketone salts is likely to reduce power output during heavy workloads as seen here.

Blood glucose was significantly lower in the ketone condition post steady-state exercise and following the time-trial (albeit by a small magnitude of ~0.3-0.7 mmol/l lower than the placebo condition). These findings are consistent with reports from Cox et al. (2016) where blood glucose was lowered during steady-state cycling exercise after ingestion of ketone ester supplements compared to consumption of CHO or fat. Interestingly, these authors did not report an elevation in insulin following

ketone ester supplement ingestion, which is in contrast to some previous experiments showing that infused ketones lead to lowered glucose primarily through insulinotropic effects (Biden and Taylor 1983; Miles et al. 1981). It is possible that there are differences in responses to ingested ketone supplements as opposed to infused ketones. As we did not measure insulin in this study we cannot speculate as to the mechanism(s) underlying the reductions in glucose seen following exercise in the ketone condition. Nonetheless, reductions in plasma glucose may have influenced performance during the time trial as low blood glucose is known to impair exercise performance through limiting availability of CHO (Coyle 1999).

Relatively similar blood lactate values were found in both conditions despite lower CHO oxidation in the ketone condition. However, since we did not obtain muscle biopsy samples we cannot confirm if ketone salts altered the rate of glycolysis or lactate production directly in muscle, as shown by Cox et al. (2016) following ingestion of ketone ester supplements. It is possible that glycolysis was impaired, yet blood lactate levels were similar, because skeletal muscle and blood lactate are not perfectly correlated (Saltin et al. 1974). Indeed, interpretation of the blood lactate data is limited to the overall concentration of circulating lactate, and does not provide any information on the rate of lactate production or clearance by skeletal muscle. Additionally, the measurements do not account for hepatic lactate uptake, or the utilization of lactate by other organs (Okorie and Dellinger 2011). Future studies employing muscle biopsies will be needed to confirm the impairment in glycolysis seen by Cox et al. (2016) following ingestion of exogenous ketone supplements.

Transport of β-OHB into skeletal muscle is mediated through several monocarboxylic transporter (MCT) systems (Newman and Verdin 2014). Previous reports have indicated that β-OHB uptake saturation in skeletal muscle occurs at 1-2 mmol/L in the resting state (Mikkelsen et al. 2014). In addition, maximal clearance rates of β-OHB begin to occur during exercise at concentrations of ~2 mmol/L (Fery and Balasse 1986). These findings suggest that a plateau in β-OHB uptake into the tissues occurs at relatively low plasma concentrations of β-OHB, similar to those achieved by ingestion

of ketone salts in this study. Given that uptake of plasma lactate is also dependent on MCT1 (McCullagh et al. 1996), it is possible that elevated circulating β-OHB may influence lactate transport. It is therefore plausible that, during exercise, the rate of lactate transport into skeletal muscle is reduced in states of ketosis due to competition for MCTs. This may partially explain why circulating lactate was not different in the ketone condition despite evidence of altered CHO metabolism.

Previous studies have examined the impact of ketosis on exercise metabolism and performance by having participants follow a high-fat, low-carbohydrate ketogenic diet (e.g., Fleming et al. 2003; Phinney et al. 1983). While such diets are successful in elevating plasma ketones through hepatic production, ketogenesis occurs at the expense of depleted intramuscular and liver glycogen. The performance effects of this dietary strategy are hotly debated (Burke et al. 2016) as reductions in high-intensity exercise performance with low CHO availability are well documented (Fleming et al. 2003; Langfort et al. 1997). It is important to note that exogenous supplements, such as the β-OHB ketone salts used in the present study, increase circulating ketones without a significant reduction in CHO storage and therefore creates a metabolic environment in which both CHO and ketone substrates are readily available. Accordingly, such an elevation in blood β-OHB induced from exogenous sources as in this study may represent a markedly different response to exercise then a state of endogenous ketosis brought about by ketogenic diets (Evans et al. 2017).

Our investigations were performed in the fasted state and results may be different if CHO were consumed prior to or during exercise. Previous dosing strategies using both ketone esters and CHO have shown performance benefits during longer duration time trials in elite cyclists (Cox et al. 2016). In addition, preliminary data suggests the combination of ketones and CHO may provide a strategy for restoring of intramuscular glycogen concentrations (Holdsworth et al. 2017). We utilized ketone salts, which provide a racemic mixture of β-OHB and appear to result in lower concentrations of blood β-OHB (~1-1.2 mM, Fig 1C) than the ketone ester supplements (~3 mM) (Cox et al. 2016).

In conclusion, our data complement the recent study by Cox et al. (2016) and support the hypothesis that elevating blood ketones through consumption of oral ketone supplements alters lipid and CHO metabolism during exercise in humans. Our findings provide the first evidence that raising β-OHB through provision of ketone salts may impair high-intensity exercise performance. The underlying mechanisms for the performance impairment following ketone salt ingestion is not clear but based on lower CHO oxidation during exercise may be relating to inhibition of glycolysis, as postulated by others (Cox et al. 2016; Pinckaers et al. 2017). Future research is necessary to directly assess the effects of nutritional ketones on metabolism during high-intensity exercise and other situations that demand high rates of glycolysis to provide ATP.

Conflict of Interest

All authors report no conflicts of interest associated with this manuscript.

Author Contributions

TO, EMC, and JPL designed the study. TO, EMC, CD, and JPL performed the experiments. TO and JPL analyzed the data and drafted the initial manuscript. EMC and CD edited the manuscript.

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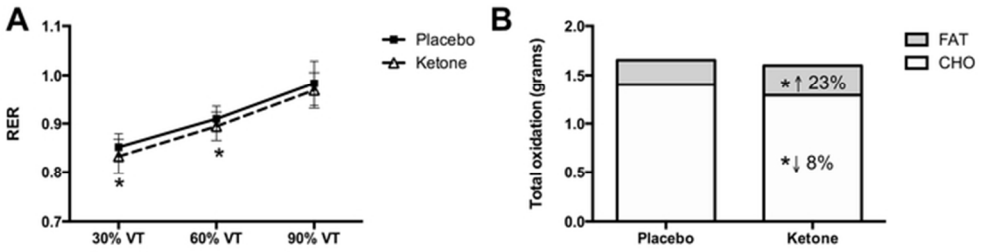
Figure Captions

Figure 1. Nutritional ketone salt supplementation increases fat oxidation and decreases carbohydrate oxidation during exercise. Recreationally active males (N=10) participated in two experimental trials involving pre-exercise consumption of nutritional ketone salts (0.3 g/kg β-hydroxybutyrate [OHB]; Ketone) or Placebo. Supplements were consumed in the fasted state 30 minutes prior to cycling exercise consisting of three 5-minute stages at 30%, 60%, and 90% ventilatory threshold (VT). Expired gas was collected throughout and the last two minutes of data were analyzed. **A**) Respiratory exchange ratio (RER) was lower at 30% VT and 60% VT in the Ketone condition compared to Placebo. **B**) Total grams of fat oxidized were higher and total grams of carbohydrate oxidized were lower in the Ketone condition compared to Placebo. Values are means±SD. *Significant difference between Ketone and Placebo conditions ($p<0.05$).

Figure 2. Impact of nutritional ketone salt supplementation on blood β-hydroxybutyrate, glucose, and lactate concentration. Recreationally active males (N=10) participated in two experimental trials involving pre-exercise consumption of nutritional ketone salts (0.3 g/kg β-hydroxybutyrate [OHB]; Ketone) or Placebo. Supplements were consumed in the fasted state 30 minutes prior to cycling exercise consisting of three 5-minute stages at 30%, 60%, and 90% ventilatory threshold (VT) followed by a 150 kJ cycling time trial. Finger stick blood samples were collected in the fasting state, 30 minutes post-supplementation, following the three 5-minute stages, and at the end of the time trial. **A)** Blood β-OHB was higher 30 minutes post-supplement and remained elevated throughout exercise in the oral ketone condition. **B**) Blood glucose was lower at the end of steady state cycling exercise and 150 kJ time trial in the oral ketone supplement condition versus placebo. **C**) Blood lactate concentration rose during exercise but was not affected by oral ketone supplementation. Values are means±SD. *Significant difference between Ketone and Placebo conditions ($p<0.05$).

Figure 3. Nutritional ketone salt supplementation impairs performance during a 150 kilojoule cycling time trial. Recreationally active males (N=10) participated in two experimental trials involving pre-exercise consumption of nutritional ketone salts (0.3 g/kg β-hydroxybutyrate [OHB]; Ketone) or Placebo. Supplements were consumed in the fasted state 30 minutes prior to cycling exercise consisting of three 5-minute stages at 30%, 60%, and 90% ventilatory threshold (VT). Five minutes following steady state cycling, participants completed by a 150 kJ cycling time trial. Mean power output was significantly lower following consumption of oral ketones versus placebo (individual values are connected by lines with means for each conditions shown by bars). Values are means±SD. *Significant difference between Ketone and Placebo conditions ($p<0.05$).



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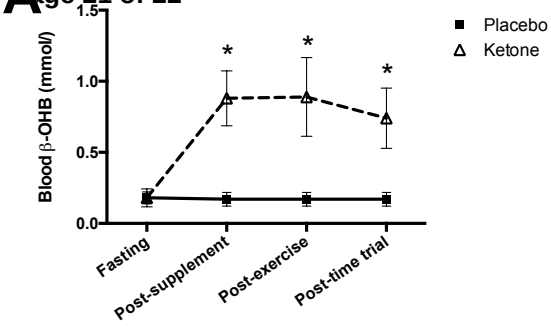

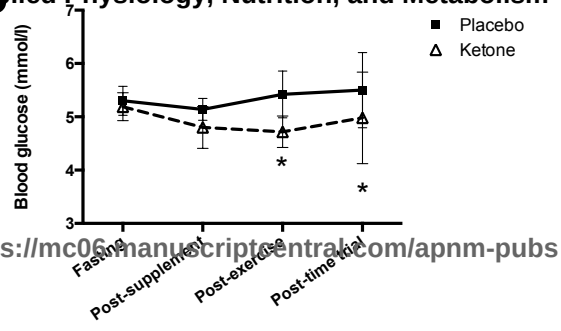

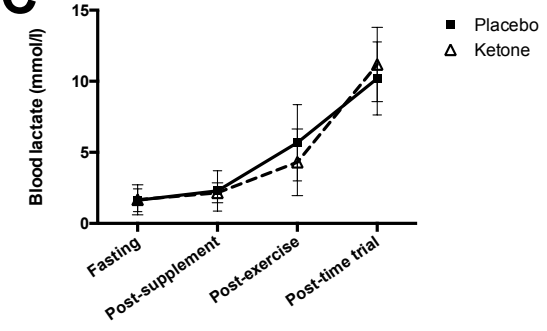

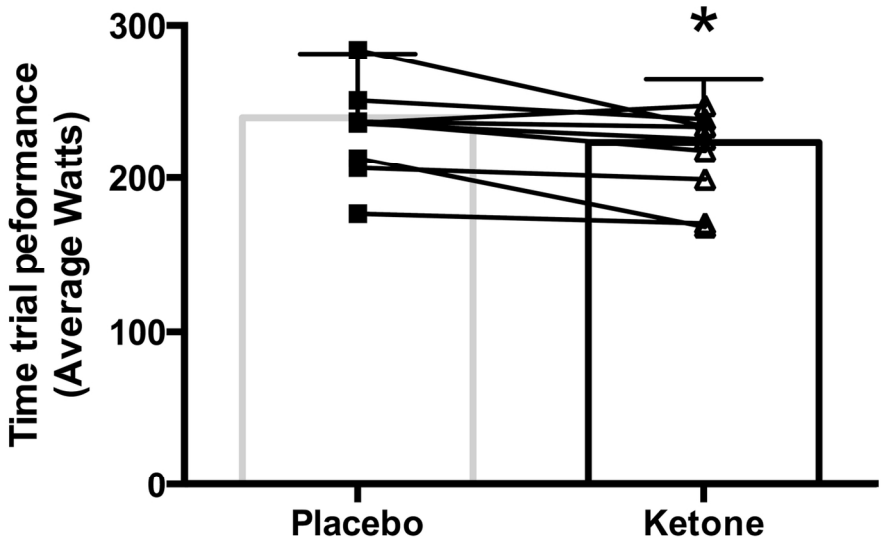

Figure 3. Nutritional ketone salt supplementation impairs performance during a 150 kilojoule cycling time trial. Recreationally active males (N=10) participated in two experimental trials involving pre-exercise consumption of nutritional ketone salts (0.3 g/kg β-hydroxybutyrate [OHB]; Ketone) or Placebo. Supplements were consumed in the fasted state 30 minutes prior to cycling exercise consisting of three 5-minute stages at 30%, 60%, and 90% ventilatory threshold (VT). Five minutes following steady state cycling, participants completed by a 150 kJ cycling time trial. Mean power output was significantly lower following consumption of oral ketones versus placebo (individual values are connected by lines with means for each conditions shown by bars). Values are means±SD. *Significant difference between Ketone and Placebo conditions ($p<0.05$).

139x87mm (300 x 300 DPI)