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# Effect of Astaxanthin on Cycling Time Trial Performance

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## Key words

- cycling
- nutrition
- ergogenic aid
- performance
- supplement

## Abstract

▼ We examined the effect of Astaxanthin (AST) on substrate metabolism and cycling time trial (TT) performance by randomly assigning 21 competitive cyclists to 28d of encapsulated AST (4 mg/d) or placebo (PLA) supplementation. Testing included a  $VO_{2max}$  test and on a separate day a 2 h constant intensity pre-exhaustion ride, after a 10h fast, at 5% below  $VO_{2max}$  stimulated onset of 4 mmol/L lactic acid followed 5 min later by a 20 km TT. Analysis included ANOVA and post-hoc testing. Data are Mean (SD) and (95% CI) when expressed as change (pre vs. post). Fourteen participants successfully completed the

trial. Overall, we observed significant improvements in 20 km TT performance in the AST group ( $n=7$ ;  $-121$  s; 95%CI,  $-185$ ,  $-53$ ), but not the PLA ( $n=7$ ;  $-19$  s; 95%CI,  $-84$ ,  $45$ ). The AST group was significantly different vs. PLA ( $P<0.05$ ). The AST group significantly increased power output (20W; 95%CI, 1, 38), while the PLA group did not (1.6W; 95%CI,  $-17$ , 20). The mechanism of action for these improvements remains unclear, as we observed no treatment effects for carbohydrate and fat oxidation, or blood indices indicative of fuel mobilization. While AST significantly improved TT performance the mechanism of action explaining this effect remains obscure.

## Introduction

▼ Astaxanthin (AST) is a carotenoid belonging to a larger class of phytochemicals known as terpenes that can be found in microalgae, yeast, salmon, trout, krill, shrimp, crayfish, crustaceans, and the feathers of some birds [5,7,9,10,16]. Currently, the FDA permits the use of AST as a food coloring, as well as an additive for animal and fish foods. Perhaps AST's most notable use is as a feed additive for farm raised salmon, giving the salmon its reddish tissue color. While AST is a natural nutritional component found in food it can also be purchased over-the-counter as a dietary supplement. Astaxanthin has recently received attention due to its ability to scavenge free radicals, decrease inflammation, improve indices of lipid metabolism and attenuate lipid accretion, and increase exercise time to exhaustion in mice [1,2,7,12,15].

To date, only one study has examined the efficacy of AST supplementation on endurance exercise performance in humans, whereby 4 weeks of AST supplementation was shown to reduced lactic acid build up following 1 200 m of running [14].

However, if enhanced lipid metabolism is a proposed benefit of AST supplementation, it seems more intuitive to examine exercise bouts of longer duration. Accordingly we are unaware of any published studies examining the effect of AST supplementation for prolonged periods of exercise endurance performance in humans. The primary aim of our current study was to examine whether 28 days of AST supplementation would improve exercise following a 2 h pre-exhaustion ride designed to minimize carbohydrate contribution during exercise performance. Our secondary aim was to determine whether 28 days of AST supplementation modulates indices of carbohydrate and lipid metabolism.

## Material and Methods

### ▼ Participants

We recruited amateur endurance-trained males from 18 to 39 years of age who had a  $VO_{2max}$   $\geq 50$  ml/kg/min, were actively participating in competitive cycling activities such as competitive road cycling or triathlons, and were accumu-

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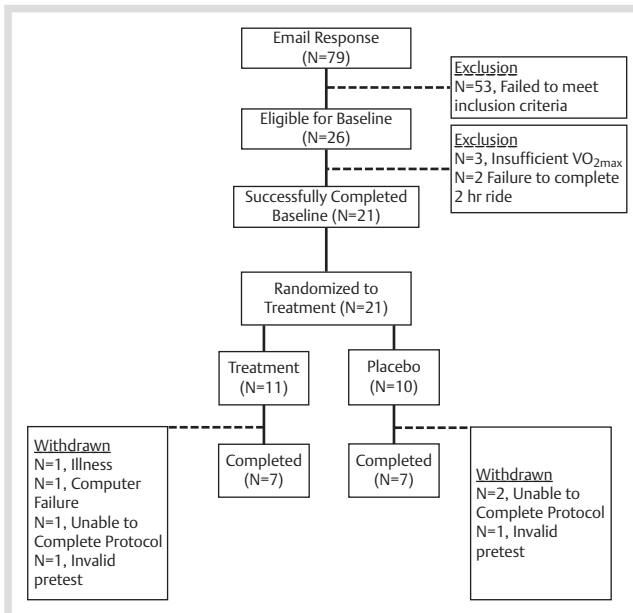
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	All (N=14)	Astaxanthin (n=7)	Placebo (n=7)
VO <sub>2max</sub> (L/min)	3.88 (0.4)	3.98 (0.3)	3.79 (0.4)
VO <sub>2max</sub> (ml/kg/min)	52.84 (3.5)	54.14 (4.1)	51.53 (2.4)
Maximal PO (W)	330 (26)	335 (17)	325 (34)
HR <sub>max</sub> (b/min)	185 (9)	183 (11)	187 (7)
PO (W) @ 2 mmol/L BLa	141 (57)	144 (62)	138 (57)
percent of VO <sub>2max</sub> @ 2 mmol/L BLA	50.90 (12.9)	49.08 (14.7)	52.45 (12.0)
PO (W) @ 4 mmol/L BLA	228 (47)	227 (43)	229 (55)
percent of VO <sub>2max</sub> @ 4 mmol/L BLA	73.65 (9.7)	71.66 (8.9)	75.63 (10.0)

Data are mean and SD

**Table 1** Baseline fitness characteristics of study participants.



**Fig. 1** CONSORT diagram of study enrollment.

lating a weekly cycling volume of 160 km per week. Initially, we considered using a mixed cohort of men and women, however, decided against it for 2 primary reasons. First, it has been purported that carbohydrate and fat metabolism differ between genders with respect to prolonged exercise [18]. Thus, a mixed cohort would add variance to our study that we would not be able to account for using a relatively small sample size. Second, we, and others in our community (Baton Rouge, LA, USA), have found it difficult to recruit women for more strenuous exercise protocols such as the one undertaken for this study. Hence, we focused our current recruitment efforts on men, whose cardiorespiratory fitness measures are presented in **Table 1**. The ethical review committee at Pennington Biomedical Research Center approved our study and informed consent was obtained from all participants before entering the trial. All study procedures conformed to the Declaration of Helsinki and ethical standards of IJSM [8]. We have provided a CONSORT schematic outlining the overall study timeline in **Fig. 1**.

### Recruitment and baseline testing

We initiated our recruitment efforts by contacting local cycling clubs via email, who therein posted our study details on various online forums and discussion boards. Upon expressing interest in the study, we contacted potential candidates via phone and email. Following a successful screening procedure we invited study candidates to come to the Pennington Biomedical Research Center Exercise Biology Laboratory Testing Core where they were further screened to participate in our study protocol. The

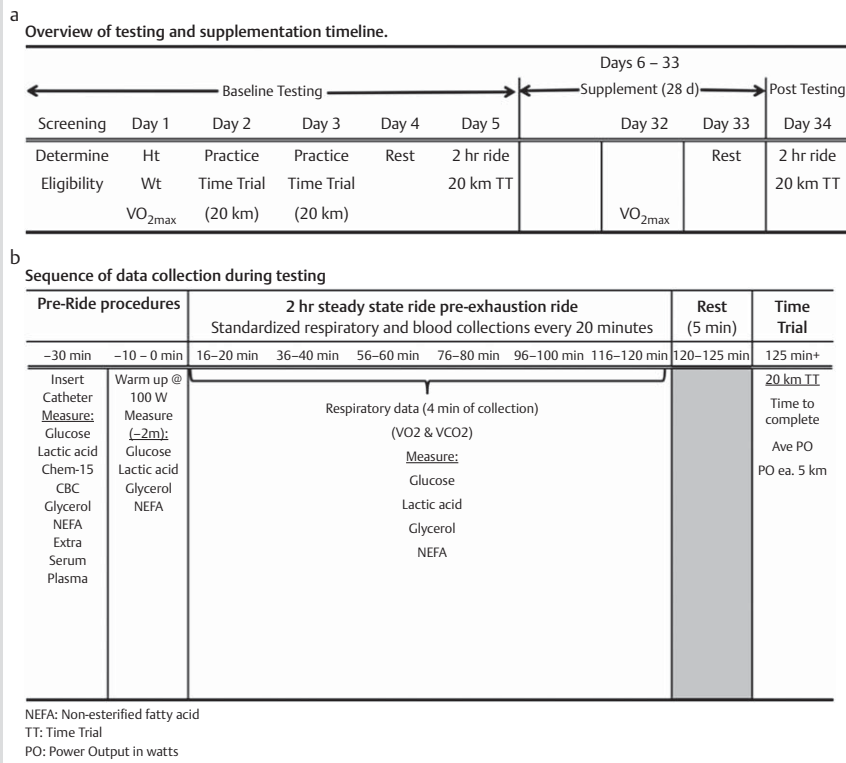
entire length of the study for each participant ranged from 35 to 40 days. Briefly, participants attended a baseline run-in period, inclusive of exercise testing to determine maximal cardiorespiratory capacity (details below), 20 km TT rehearsals, and a fasted 2 h pre-exhaustion ride that was followed immediately by a 20 km TT (details below) on their own bike using a Computrainer ergometer (Seattle, WA). Before each Computrainer test the ergometer was turned on and allowed to warm-up for 30 min. Immediately before each TT we also performed an individualized calibration procedure that accounts for rolling resistance. We have presented a schematic of all testing procedures in **Fig. 2a**.

### Maximal cardiorespiratory exercise

We instructed each subject to prepare for his VO<sub>2max</sub> test as if preparing for a race and to abstain from exercise for 24 h before the test. This preparation included not changing their training parameters or dietary patterns for the week preceding each test. We asked participants to record what they ate in a food log 3 days before testing and to duplicate the same food intake on the penultimate day before testing during the follow-up visit. Participants were also instructed to eat a light snack ~3 h before their tests and to abstain from ingesting any medications that would influence heart rate on the day of each test. The one exception was caffeine, which we asked participants not to consume for at least 5 h before testing.

We performed all VO<sub>2max</sub> exercise tests on a Lode Excalibur Sport Ergometer (Groningen, The Netherlands) and analyzed the riders for various cardiorespiratory parameters using a Parvomedics TrueMax Metabolic System (Salt Lake City, UT). Each cycling test began with a warm-up at 50 W (10 min) and then progressed to 75 W (2 min). After the warm-up, the test began by increasing the power output (PO) to 100 W. Once PO was set at 100 W, each stage inclusive of the 100-W stage lasted 3 min and progressed 35 W every 3 min until the rider reached exhaustion or could no longer maintain a pedal cadence of 50 rpm. We allowed each rider to choose their preferred cadence within a range of 70–90 rpm.

We collected gas exchange data continuously using an automated computerized breath-by-breath Parvomedics TrueMax Metabolic System. The TrueMax system uses a pneumotachometer, a paramagnetic O<sub>2</sub> analyzer, and an infrared CO<sub>2</sub> analyzer to analyze respiratory O<sub>2</sub> and CO<sub>2</sub>, respectively. Before each test, we followed a standard calibration procedure for each metabolic cart including a flow calibration using a 3-L calibration syringe and calibration against standardized gases (16% O<sub>2</sub>, 4% CO<sub>2</sub>, and balanced nitrogen) obtained from the manufacturer of the metabolic system. We averaged all gas exchange data in 60 s intervals and only the last minute of each stage was used in our analyses. Blood samples (25 µL) for the measurement of blood lactate (Lactate Pro, Quesnel, BC, Canada) were taken from the riders fingertips at rest and during the last 30 s of each stage starting at 75 W. From these lac-



**Fig. 2** Overview of study timeline (panel a) and testing procedures (panel b).

tate measurements we determined the corresponding PO associated with the accumulation of 2 mmol/L and 4 mmol/L of lactic acid accumulation by drawing a 3<sup>rd</sup> order line of best fit through all data points. For the 2 h ride we asked riders to work at a PO corresponding to 5% lower than the PO associated with the accumulation of 4 mmol/L of lactic acid.

### Two-hour and 20 km TT performance testing

We have presented a schematic outlining the procedures for the 2 h pre-exhaustion and TT ride performed at baseline and follow-up in **Fig. 2b**. For the 2 h pre-exhaustion fasting ride, we asked participants to report to the Pennington Exercise Testing Core on the morning of their test where we placed an indwelling catheter into an antecubital vein 30 min before their ride. We also determined participant hydration status designated as having a urine specific gravity (USG) less than 1.025. During the TT we instructed participants to ride the 20 km distance as fast as possible on his own bicycle. To ensure minimal variability between tests we asked the participants ride the same bike with the same configuration for positioning during the rehearsal, baseline and follow-up testing conditions. Approximately 20 min after the insertion of the catheter, we collected an initial series of blood samples to assess resting blood values in order to monitor the pre- and post-treatment safety corresponding to supplementation with AST. These measures included a standard serum 15-panel blood test to measure creatinine, potassium, uric acid, albumin, calcium, magnesium, creatine phosphokinase (CPK), alanine aminotransferase (ALT), alkaline phosphatase (ALK), iron, cholesterol (LDL & HDL), and triglycerides. We also performed a complete blood count (CBC) with differentials to determine hemoglobin, hematocrit, mean cell volume, platelet count, white blood count, granulocytes, neutrophils, eosinophils, and basophils.

After we collected the first set of blood samples we asked each rider to perform a 10 min warm up at a low intensity (50 W) before initiating the 2 h ride. During the 2 h pre-load ride we collected blood samples every 20 min to determine several indices indicative of carbohydrate and lipid metabolism including glucose, lactic acid, glycerol, and non-esterified fatty acids (NEFA). At these same time intervals we also measured cardiorespiratory parameters ( $VO_2$  and  $VCO_2$ , L/min) to determine the relative contributions of carbohydrate and fat oxidation during testing using standard stoichiometric equations [6]. Riders consumed 250 ml of water every 15 min throughout the test. For the blood measurements we collected each blood sample during the last 30 s of the 20 min period. For  $VO_2$  and  $VCO_2$  we began data collection 4 min before each 20 min demarcation in order to obtain steady state values. However, we only used the last minute of this collection period for our analysis. All blood samples were spun on a centrifuge and stored at  $-80^\circ\text{C}$  until we could analyze them "in batch" (i.e., pre/post test sample together) using a Beckman Coulter DXC600 (Brea, CA) analyzer.

We analyzed glucose and lactic acid using an oxygen electrode and enzymatic endpoint method of analysis, respectively. For glucose, all samples were spun on a centrifuge at 3500 rpm for 15 min at room temperature. For lactic acid, each sample was spun at the same speed at  $4^\circ\text{C}$ . For glycerol and NEFA we analyzed each sample using enzymatic colorimetric detection analysis, respectively. For glycerol, centrifugation took place at 3500 rpm for 15 min at room temperature, while for NEFA the centrifuge speed was 3000 rpm for 15 min at  $4^\circ\text{C}$ .

### Randomization and treatment

After completing the entire baseline testing procedures, we randomized each participant, in a double-blind, placebo controlled, parallel group designed manner to receive either 4 mg/d of encapsulated AST or a matched placebo for 4 weeks. We chose

4 weeks as studies in mice have shown that AST improves lipid metabolism and exercise performance within a 4–5 week period [2, 11]. All supplements were provided by Fuji Health Science, Inc. (Burlington, NJ). Each treatment was delivered in a black soft gel capsule and each AST soft gel contained 80 mg of 5% natural astaxanthin extract from *Haematococcus pluvialis* algae, yielding 4 mg of active astaxanthin per soft gel, and 120 mg of medium chain triglyceride as filler. The placebo soft gel was comprised of 200 mg medium chain triglyceride plus a small amount of caramel food coloring to make the capsules identical in appearance to the active soft gels. We asked all participants to consume their respective treatments with a meal and provided each person with enough supplement for 5 extra days in case they required an extended period to complete the study protocol. This latter aspect of our study also better enabled us to perform a pill count at the end of the study to determine the degree of compliance to the study protocol. Lastly, people independent of the study dispersed all treatments using a unique, individualized 4-digit number sequence. We chose a randomized number sequence in case we observed any side effects during the course of the study. This procedure allows for the breaking of a single treatment code without sacrificing the integrity of the entire treatment cohort.

### Statistical analysis

Our primary outcome analysis was aimed at determining whether AST supplementation improved various indices of cycling performance following a 2 h pre-exhaustion bout of exercise intended to minimize carbohydrate contribution. The primary outcomes for our investigation were the rider's ability to complete a 20 km TT immediately following a 2 h pre-exhaustion ride. Accordingly, we examined TT performance in terms of the time it took to complete the 20 km TT and the average PO generated by the rider. As a secondary analysis we examined several laboratory indices of physical performance such as  $VO_{2max}$ , maximal PO, and the PO observed at lactate threshold (2 mmol/L) and onset of blood lactate accumulation (4 mmol/L) during the  $VO_{2max}$  test. As a tertiary analysis we assessed several metabolic parameters traditionally used as a means of providing a mechanism of action should performance changes be observed. These indices included the analysis of blood variables indicative of improved carbohydrate and fat usage including: plasma lactate, glucose, non-esterified fatty acids, and glycerol. We approached our analysis of these variables in 2 ways. First, we analyzed the overall effectiveness of AST on these variables by using an integrated area-under-the-curve (AUC) assessment for all time intervals associated with our data collection beginning with the resting measurement as "time 0". Our second strategy was to examine potential differences in these variables at each 20 min time point during the 2 h ride. These specific time points included resting blood values obtained after a 10 h fast, the end of warm-up, and every 20 min therein during the 2 h ride. During these same time intervals we also examined the respiratory derived measurements for the stoichiometric assessment of carbohydrate and fat oxidation. For our primary analysis we examined each variable as change from baseline. Determinations for within group significance were based on the mean change and accompanying 95% confidence interval (95% CI) for each respective performance parameter. We used a general linear model to examine between group differences. Effect sizes were calculated for time to complete and average power output during the 20 km TT using Cohen's *d* (ES) [4]. For the AUC analysis we analyzed our

data using a 2×2 [treatment (placebo/treatment) × time (pre/post)] ANOVA. For the time parameter analysis we used a repeated measures ANOVA. Lastly, we also examined the mean change in outcomes between the pre-test and post-test condition using a one-way ANOVA.

We examined blood chemistry values (i.e., Chem-15/CBC) and food frequency evaluations using the same statistical techniques as described for the primary outcome data. The reason for the blood chemistry analysis was to determine whether AST supplementation might adversely affect hepatorenal function, in order to ensure that supplementation with AST was safe. Lastly, we examined by pill count the number of assigned treatment capsules vs. the number of supplement days during the study period. We excluded any outliers in the studies who fell outside 3 SD for time changes from pre to post treatment. All individual time point data in our paper are presented as mean ± SD. Statistical significance was set at  $P \leq 0.05$ .

### Results

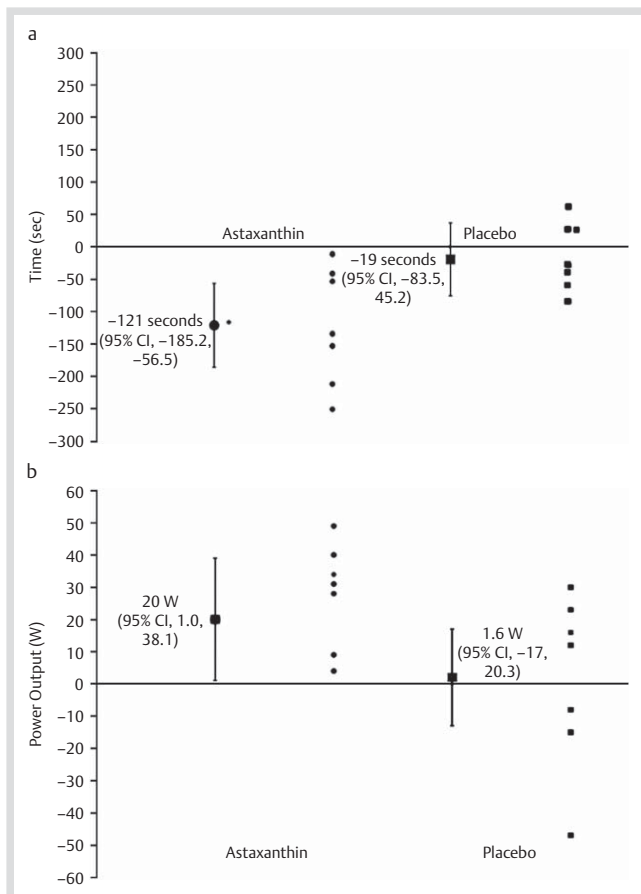


We randomized 21 participants into our study (AST=11, PLA=10). However, only 7 participants from each treatment group completed the entire testing protocol. The reasons for this completion rate are presented in ◉ Fig. 1, but generally fall under the categories of illness, equipment failure or inability to complete the 2 h pre-exhaustion ride or the subsequent TT. We did however remove 2 outlier performances from our analysis. In one instance, we removed a rider from the AST group for demonstrating a 7 min time improvement from the pre to post test condition. In the other instance we removed one rider from the PLA group for an abnormal performance loss of 5 min. Though we could have kept these 2 riders in our analysis, their relative changes in performance given their respective treatment groups, only served to separate our hypothesis further from the null. In essence, leaving these 2 riders in the analysis increased the treatment effect for the AST group, while simultaneously making the PLA group appear slower.

For those who completed the study, we observed no statistical difference at baseline between treatment groups for any of our measurements associated with the study including age ( $28 \pm 6$  years), body mass ( $74.6 \pm 8.8$  kg), and height ( $175.6 \pm 8.5$  cm). For our post-test analysis, we observed a significant within group improvement for the time it took to complete the 20 km TT for the AST supplemented riders ( $2387 \pm 206$  s vs.  $2266 \pm 190$  s; range, -02, -251 s) and the average power output measured during the TT ride ( $162 \pm 37$  W vs.  $186 \pm 34$  W; range, 05–40 W,  $P < 0.05$ ). For our analysis of time to complete the 20 km TT, we observed that the AST group's time improvement was significantly different from the PLA group ( $P < 0.02$ ). Though some improvement in time was observed for the PLA group, neither TT time ( $2251 \pm 260$  s vs.  $2233 \pm 283$  s; range, -101, 62) or PO ( $186 \pm 57$  W vs.  $187 \pm 75$  W) was statistically significant following the 28 d supplementation period. The ES for the difference between treatment groups in their time to complete the time trial was 1.25, whilst the ES for the between group difference in PO was 0.95. According to Cohen, these ES would be considered large [4]. We have presented the mean and 95% CI change for time and PO along with accompanying individual responses for each treatment group in ◉ Fig. 3.

For our analysis of fuel utilization we were unable to detect a significant treatment difference for any blood marker indicative



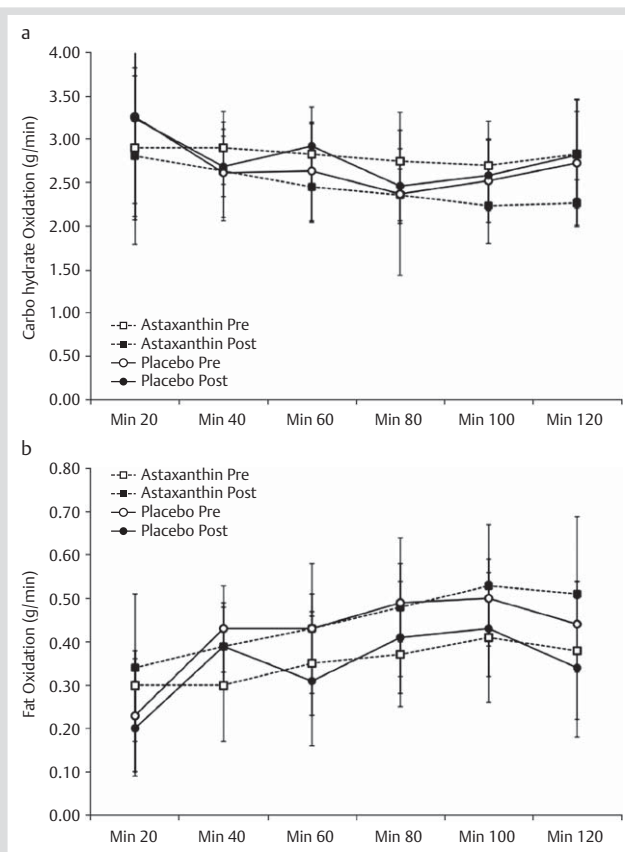


**Fig. 3** Data represent the mean and 95% CI within group changes in time performance (panel **a**) and power output (panel **b**) for cyclists receiving 28 d of AST (left side) or PLA (right side) supplementation. \* $P < 0.05$  for between group differences.

of enhanced carbohydrate or fat oxidation (○ Fig. 4 a, b), neither were we able to detect a change in any blood parameter suggestive of a shift in fuel metabolism (○ Fig. 5 a–d) for any given 20 min time interval. While we did observe a significant increase in plasma concentrations of NEFA with increasing exercise time, these increases were similar within each group but not different between groups or the pre/post treatment condition. When we further analyzed our blood work data as an AUC for the entire 2 h pre-exhaustion ride we were also unable to detect a significant difference for treatment group. Lastly, we did not observe any differences in either the blood chemistries suggesting the treatment group significantly altered hepatorenal function during the treatment period (data not shown).

## Discussion

The primary aim of our study was to examine the effect of AST supplementation on 20 km TT performance following a 2 h pre-exhaustion ride undertaken after a 10 h fast. The goal in using this type of protocol was to minimize the contributions of carbohydrate for energy provision; hence, creating an increased reliance on fat oxidation. This decision was based on observations from animal studies showing an increased reliance on fat utilization while simultaneously increasing time to exhaustion during exercise [1,2,11,12]. We observed an approximate 2 min mean improvement (5%) in the time necessary to complete the 20 km

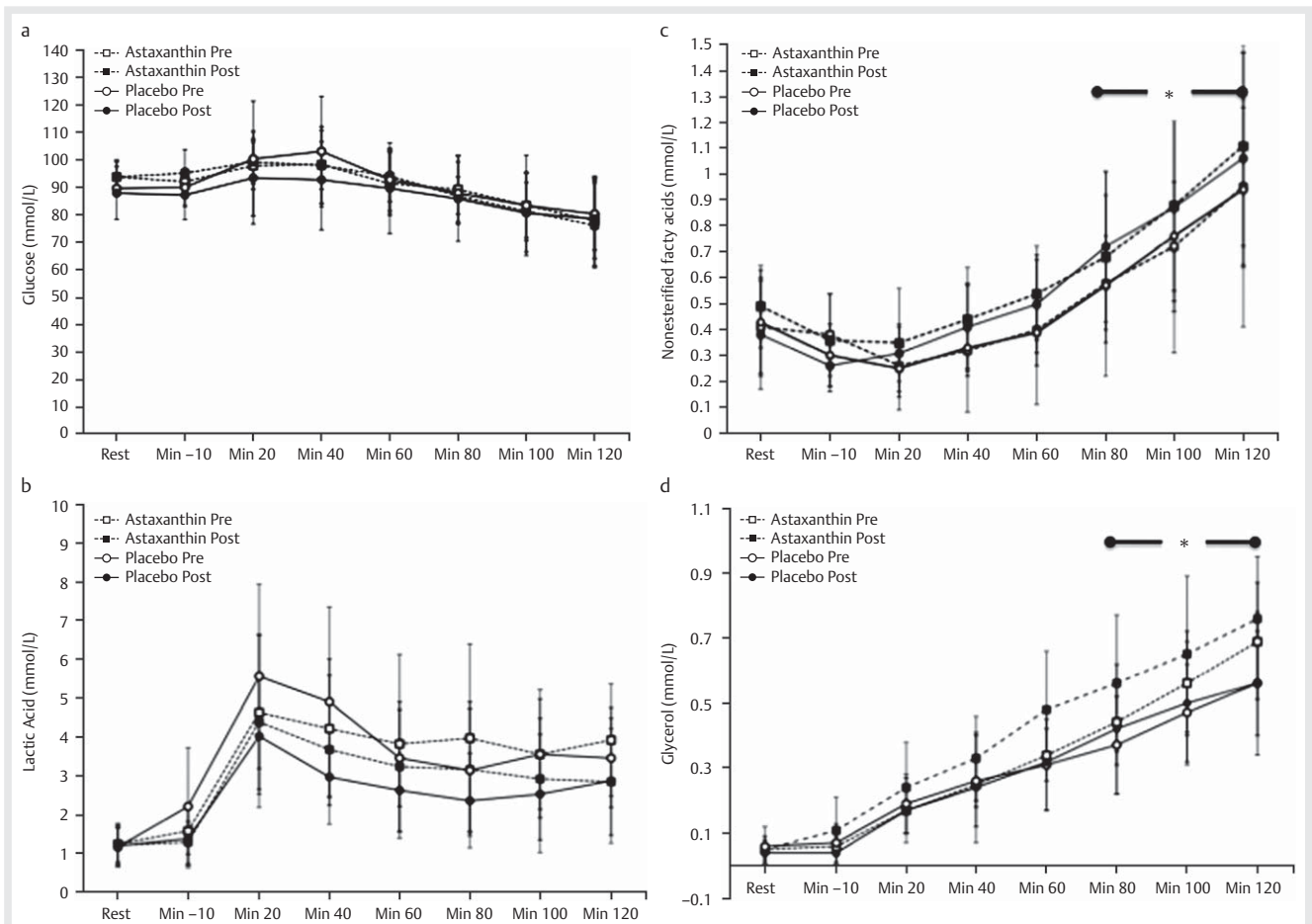


**Fig. 4** Data represent mean and standard deviations for carbohydrate (panel **a**) and fat oxidation (panel **b**) observed during the 2 h pre-exhaustion ride.

TT that was accompanied by a 20 W increase (15%) in the average power output generated by riders during the TT condition. Changes in time and power output for the PLA group were 0.8% and 0.5%, respectively. An examination of ○ Fig. 3 also shows that each of the riders in the AST group improved 20 km performance time and power output, while the PLA group showed minimal improvements and were fairly evenly split between faster and slower performances. Despite these improvements in cycling performance the mechanism of action to explain our findings remain enigmatic.

Most of the research conducted on AST to date has used murine models to examine exercise performance and energy distribution patterns. For example, Aoi et al. [2] examined mice divided into 4 groups of mice that were either (a) sedentary, (b) sedentary, yet, treated with AST, or assigned to run on treadmill (c) without or (d) with AST supplementation [2]. In their study, the authors reported that each exercise group was able to run longer on the treadmill before exhaustion; however, those mice treated with AST also increased fat utilization during exercise compared to mice on a normal diet. At a cellular level these findings were supported by the observation that AST fed mice increased the localization of fatty acid translocase (FAT/CD36) and carnitine palmitoyltransferase I (CPT I) in skeletal muscle. In essence, AST improved various mechanisms associated with transporting long chain fatty acids into the mitochondria. In our current study, however, we see no evidence for the preferential use of fat.

A question that may be raised pertains to dosing equivalents between murine and human studies. In one such study, Ikeuchi



**Fig. 5** Data represent mean and standard deviations for plasma glucose (panel **a**), lactic acid (panel **b**), non-esterified fatty acid (panel **c**) and glycerol concentrations observed during the 2 h pre-exhaustion ride. \* $P < 0.05$  for within group time point differences from rest through 40 min (all groups).

et al. [11] examined the effects of AST supplementation on exercise-induced fatigue in mice by administering AST (1.2, 6, or 30 mg/kg body weight) for 5 weeks via stomach intubation. Post-test analysis showed an overall dose dependent increase in exercise time to exhaustion in mice receiving 6 and 30 mg/kg of AST vs. control. This would equate to approximately 420 mg and 2.1 g of AST in humans, respectively, assuming the AST was given to a 70 kg “reference male”. Thus, the dosage given to the mice was significantly higher than what we administered in our study. The authors of this study also observed a significant reduction in lactic acid and higher concentration of non-esterified fatty acids and plasma glucose concentrations throughout exercise in the AST treated groups [11]. Though this larger dosage pattern may ultimately affect energy substrate utilization, it does not reconcile the improvements in performance that we observed in our trial.

Only two studies have examined the efficacy of AST for improving exercise performance in humans, while only one of those studies examined some type of endurance performance. In 2002, Keisuke et al. examined the effectiveness of AST on the build up of lactic acid following 1 200 m of running before and after 4 weeks of supplementation and found that the 2 min post-running lactic acid concentration was significantly lower in the AST supplemented runners vs. control [14]. In a second study, Bloomer et al. supplemented resistance trained men with AST (4 mg/d) for 3 weeks and found no supplementation related effects on plasma levels of creatine

kinase, lactate dehydrogenase, delayed onset muscle soreness or exercise performance [3].

### Strengths and Limitations

▼ A strength of our current investigation is that we examined the effectiveness of AST supplementation under conditions aimed at decreasing the reliance on carbohydrate metabolism by use of an overnight fast without the presence of CHO ingestion during the 2 h pre-exhaustion ride. Though we were successful in demonstrating that AST appears to have an ergogenic effect, we acknowledge that the true effectiveness of AST supplementation relative to competitive exercise performance is not yet known given that our feeding schema does not adequately represent dietary practices associated with competition. Therefore, to test the true ergogenic effect of a supplement would necessitate doing so under conditions that best approximate those conditions involved in competition. We further acknowledge that our results are currently predicated on a relatively small sample size. One of the strengths of our findings is the observation that all of the participants in the AST group improved their time in completing the 20 km TT via an improvement in power output. Though an examination of the range of improvement varies from 2 to 251 s, the 95% confidence intervals suggest that the likely range for the true value of the AST treatment is 56–185 s. Our calculation of effect sizes for both performance indices

would be characterized as large by Cohen [4]. It should also be noted that our trial using AST accompanied by fasting and without carbohydrate ingestion during the cyclists ride produced similar results to those of Smith et al., who recently reported similar findings to ours using carbohydrate supplementation during a 20 km TT under a similar feeding schema [17].

These findings are particularly intriguing as Jeukendrup and Martin [13] estimate that carbohydrate and caffeine will produce similar performance benefits in 40 km TT [13]. For example, they project that carbohydrates will improve 40 km TT performance by 00:42 s, 0:36 s, and 0:32 s for novice, well-trained, and elite cyclists, respectively. In their model, it has also been suggested that caffeine improves 40 km TT performance by 1:24 (min:s), 1:03 (min:s), and 0:55 s, respectively. Thus, if one is to place value on the effectiveness of an ergogenic aid during cycling performance, the results of our study suggest that supplementation with AST may promote similar time gains as to those noted above using other nutritionally based ergogenic aids. However, a greater body of conformational research findings needs to be accumulated before making such a conclusion. Despite our inability to identify a mechanism of action for the observed changes in exercise performance our study does suggest that AST supplementation may be an effective supplement for improving exercise performance. If fat metabolism is of future research interest, those investigators undertaking that task may wish to consider a lower intensity exercise regime more closely identified with the purported range of maximal fat oxidation. If performance is an area of interest, we suggest that these issues be examined under conditions more closely related to feeding strategies of those athletes engaged in the sport of interest.

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