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Human Kinetics
ORIGINAL RESEARCH

Astaxanthin Supplementation Increases Glutathione Concentrations but Does Not Impact Fat Oxidation During Exercise in Active Young Men

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This study investigated the effects of 6 mg/day of astaxanthin supplementation on markers of oxidative stress and substrate metabolism during a graded exercise test in active young men. A double-blind, randomized, counterbalanced, cross-over design was used. Fourteen men (age = 23 ± 2 years) supplemented with 6 mg/day of astaxanthin and a placebo for 4 weeks, with a 1 week washout period between treatments. Following each supplementation period, a fasting blood sample was obtained to measure markers of oxidative stress: glutathione, hydrogen peroxide, advanced oxidation protein products, and malondialdehyde. Participants also completed a graded exercise test after each treatment to determine substrate utilization during exercise at increasing levels of intensity. Glutathione was ~7% higher following astaxanthin compared with placebo (1,233 ± 133 vs. 1,156 ± 185 μM, respectively; $p = .02$, $d = 0.48$). Plasma hydrogen peroxide and malondialdehyde were not different between treatments ($p > .05$). Although not statistically significant ($p = .45$), advanced oxidation protein products were reduced by ~28%. During the graded exercise test, mean fat oxidation rates were not different between treatments ($p > .05$); however, fat oxidation decreased from 50 to 120 W ($p < .001$) and from 85 to 120 W ($p = .004$) in both conditions. Astaxanthin supplementation of 6 mg/day for 4 weeks increased whole blood levels of the antioxidant glutathione in active young men but did not affect oxidative stress markers or substrate utilization during exercise. Astaxanthin appears to be an effective agent to increase endogenous antioxidant status.

Keywords: antioxidants, oxidative stress, dietary supplements

Dietary supplements are of interest to individuals seeking an ergogenic effect or improvement in aspects of cardiometabolic health (Brown et al., 2018; Sotler et al., 2019). While antioxidant supplements have frequently failed to demonstrate a direct ergogenic effect (Draeger et al., 2014), some have been found to enhance fat oxidation during exercise and improve markers of cardiometabolic health (Brown et al., 2021; Soory, 2012). The relationship between substrate oxidation during exercise and cardiometabolic health has been recently documented such that individuals with increased risk for developing cardiometabolic disease have impaired ability to oxidize fats during exercise compared with healthy, recreationally active and highly trained individuals (San-Millán & Brooks, 2018). As such, dietary strategies that can increase fat oxidation during exercise and improve blood markers of cardiometabolic health are of interest to active and inactive individuals alike.

Astaxanthin (ASTA) is a naturally occurring, lipid soluble carotenoid which can be found in microalgae, as well as some fish and birds (Brown et al., 2018; Guerin et al., 2003; Hussein et al., 2006). ASTA provides a red–pinkish pigment which can be noted in salmon and flamingos (Ambati et al., 2014). Since ASTA cannot be synthesized endogenously, it is commonly consumed by humans from marine sources, such as salmon, lobster, crab, and shrimp (Visioli & Artaria, 2017). Dietary supplementation of ASTA is an effective method to increase endogenous ASTA concentrations, even following acute ingestion (Brown et al., 2018). Endogenous antioxidants, such as vitamin E and vitamin C, primarily function on only the inner side or outer side of the cells (Sztretye et al., 2019). However, the structure of ASTA is unique as it demonstrates polar ends and a nonpolar middle, which allows it to permeate through phospholipid bilayers of cellular structures and act as a direct scavenger to reactive oxygen species on both inner and outer cellular aspects via both polar and nonpolar zones (Hussein et al., 2006; Kidd, 2011). ASTA also acts as an indirect antioxidant, enhancing endogenous glutathione (GSH) concentrations in rats (Andriani et al., 2019). Dietary ASTA supplementation has been shown to produce improvements to numerous aspects of cardiometabolic and overall health through its antioxidative, anti-inflammatory, anticancer, and antiaging properties (Sztretye et al., 2019). However, additional trials in relation to blood markers of oxidative stress (OS) in human participants are needed due to the majority of supporting evidence being from rodent models and a lack of human trials.

The antioxidative properties of ASTA have also been shown to protect enzymes, such as carnitine palmitoyltransferase 1 (CPT1) and adenosine monophosphate activated protein kinase from oxidative damage (Aoi et al., 2008, 2014). This can result in improvements to fat oxidation during exercise and improved endurance exercise performance in rodent models using ~3- to 5-week supplementation periods (Aoi et al., 2008, 2018; Ikeuchi et al., 2006). Furthermore, the antioxidative properties of ASTA have been used to facilitate recovery from strenuous exercise (Sztretye et al., 2019). These findings led to increasing interest in dietary supplementation with ASTA in relation to exercise

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metabolism, performance, and exercise recovery (Brown et al., 2018). Effective supplementation with exogenous antioxidants can increase endogenous antioxidant protection against OS and provide increased protection against chronic cardiometabolic diseases that are associated with OS, such as Type II diabetes, metabolic syndrome, dyslipidemia, hypertension, insulin resistance, and obesity (Dhalla et al., 2000; Kris-Etherton et al., 2004; Marseglia et al., 2015).

In relation to human trials, the ability of ASTA to enhance fat oxidation during exercise is equivocal. For example, Brown et al. (2021) recently reported improvements in fat oxidation (+0.09 ± 0.13 g/min) associated with improvements in time to completion during a 40-km time trial in male cyclists following 7 days of supplementing with 12 mg/day of ASTA. However, these improvements in fat oxidation or performance were not supported by a past study incorporating supplementation with 20 mg/day for 4 weeks in trained cyclists or triathletes (Res et al., 2013). The discrepancy in findings may be related to the parallel group design in which each group may have started treatment at different baseline levels, possibly confounding the data. Therefore, the purpose of this study was to investigate whether 6 mg/day of ASTA supplementation for 4 weeks can impact markers of OS and substrate oxidation rates during exercise in active young men using a cross-over design. The cross-over design has been shown to reduce interindividual differences, with each participant serving as their own control (Mills et al., 2009). Furthermore, it should be noted that the dosage of ASTA supplementation has varied in these human trials from 4 to 20 mg/day. Therefore, this study involved supplementation of 6 mg/day of ASTA and a placebo for 4 weeks each in a random order among active young men. Due to noted antioxidative and potential lipolytic properties of ASTA, we aimed to study oxidation rates during exercise, as well as blood markers of OS and cardiometabolic health.

Methods

Participants

Participants were recruited via word of mouth from Texas State University campus. All experimental procedures were approved by Texas State University's Institutional Review Board and ethical procedures were in line with the Declaration of Helsinki. Participants provided written informed consent and completed a health history and lifestyle evaluation questionnaire. Inclusion criteria required participants to be: (a) men aged 18–39 years, (b) apparently healthy and at low risk for coronary heart disease by American College of Sports Medicine standards, (c) regularly active by participating in >150 min/week of physical activity (Pescatello et al., 2014), and (d) free from prescription medication. Participants were excluded if they demonstrated any of the following: (a) known blood disorder (e.g., anemia, hemophilia), (b) any known cardiovascular or metabolic disorder, or (c) current smoker or nicotine user. Antioxidant dietary supplements were not allowed throughout the duration of the study and in any instance where a participant was ingesting such upon screening, supplementation was discontinued 2 weeks prior to initiation of the study. Participants were also asked to limit red wine and coffee consumption to <16 oz/day (for each) and to maintain current exercise/physical activity patterns. Height and body mass were collected via a physician's scale and stadiometer (Detecto, Webb City, MO). Power analysis using SAS (version 9.4, SAS Institute, Cary, NC) using our past data (currently in press) showed a sample size of 15 would be required to reach a desired power of at least 0.8 for achieving a mean difference of 73 in blood advanced oxidation protein products (AOPP) concentrations, with a *SD* of 85 and alpha level of .05. Thus, 21 particpants were recruited for participation in the study (see Figure 1).

Experimental Design

This study utilized a double-blinded randomized counterbalanced, cross-over design for which participants were randomly assigned the order in which they supplemented with an ASTA supplement or placebo, with each supplementation period lasting 4 weeks. After the end of each 4-week supplementation period, a fasting blood sample was collected and participants completed a graded exercise test (GXT) to determine rates of fat and carbohydrate oxidation. After the testing sessions, participants completed a 1-week washout period followed by a 4-week supplementation period with the opposite treatment (ASTA/placebo). Upon completion of the second supplementation period, participants returned to the laboratory for a fasting blood sample and GXT to be completed a second time. Prior to experimental testing, participants were asked to refrain from strenuous exercise and alcohol consumption for 48 hr. Moreover, a 24-hr dietary recall was collected during the first session and participants were provided this recall prior to the second experimental testing round and were asked to eat similar foods for 24 hr prior to returning for the second round of experimental testing.

Blood Sampling and Analysis

Fasting blood samples (~21 ml) were collected using a 21 G butterfly needle. Immediately after collection, whole blood was treated with a 5-sulfosalicylic acid solution (SSA) (Sigma Aldrich, St. Louis, MO) for preparation of subsequent analysis of GSH concentrations. Briefly, 700 µl of whole blood were removed from the vacutainer and mixed with 700 µl of a 5% SSA solution. This mixture was stored for 10 min at 5 °C–8 °C and subsequently centrifuged at 10,000*g* for 10 min at 4 °C. The clear supernatant was aliquoted and stored at −80 °C until analysis. Sodium heparin vacutainers containing whole blood were centrifuged at 1,500 revolutions per minute for 15 min at 4 °C. Plasma was stored in multiple aliquots at −80 °C until analysis.

Treated whole blood samples were thawed and analyzed in duplicate for GSH concentrations using a commercially available assay kit (Arbor Assays, Ann Arbor, MI). Plasma samples were thawed and analyzed in duplicate for markers of OS: hydrogen peroxide (H_2O_2), AOPP, and malondialdehyde (MDA). Plasma was also analyzed in duplicate for markers of cardiometabolic health including levels of triglycerides (TAG) and total cholesterol. Plasma H_2O_2 was analyzed using an Amplex Red assay by methods described by the manufacturer (Molecular Probes, Invitrogen Detection Technologies, Eugene, OR). Samples were also assayed for AOPP using a commercially available kit (STA-318 OxiSelect AOPP; Cell Biolabs, Inc., San Diego, CA) using methods described previously (Witko-Sarsat et al., 1996). The MDA was analyzed utilizing a commercial colorimetric kit (NWK-MDA01; Northwest Life Science Specialties, Vancouver, WA) using similar methods as previously described (Jentzsch et al., 1996). Absorbance was determined with a colorimetric plate reader (BioTek, Winooski, VT). Total cholesterol was determined with a chemistry analyzer (Vet Axel chemistry analyzer; Alfa

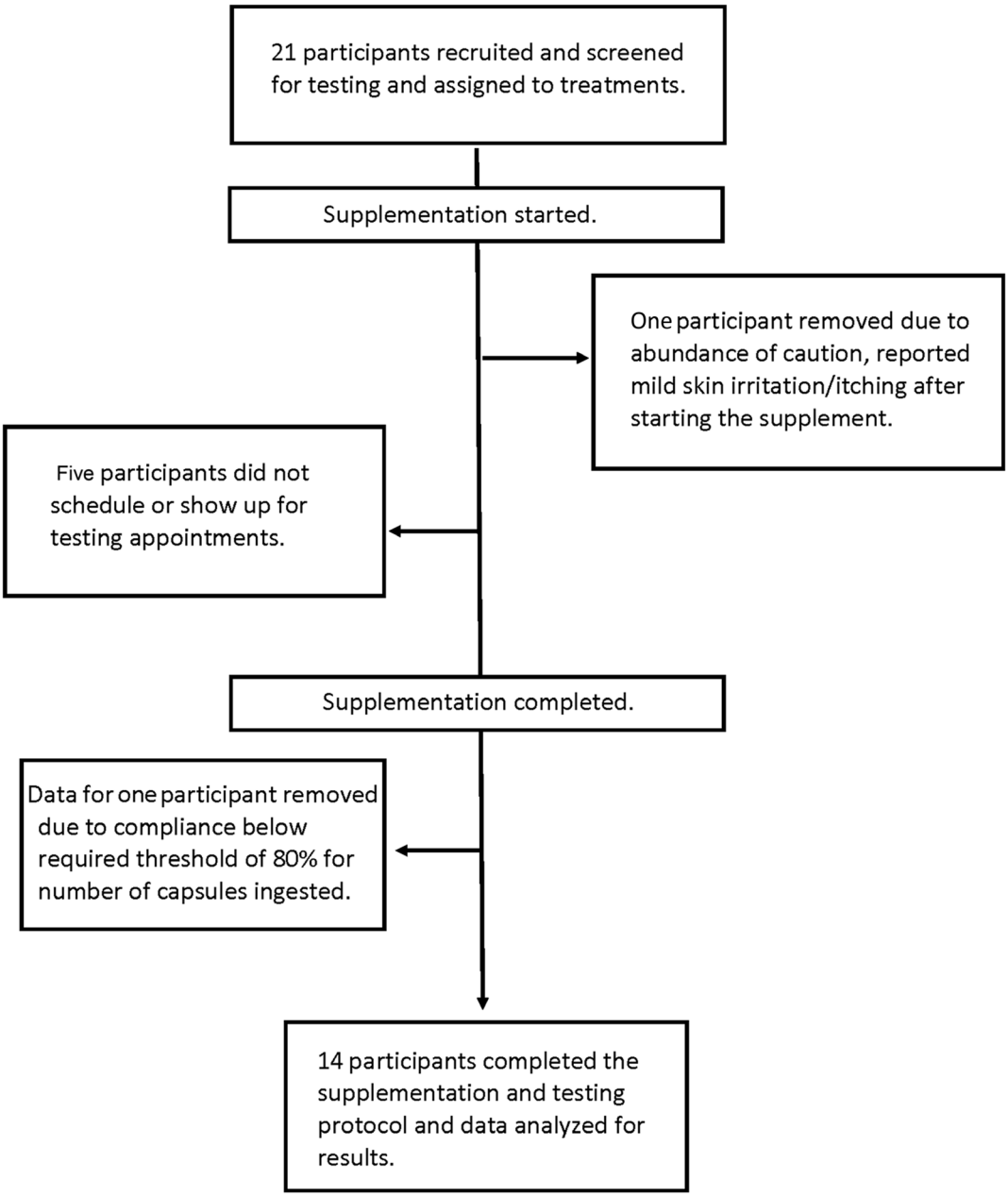

Figure 1 — Overview of participant recruitment and drop out rate of the study.

Wassermann, West Caldwell, NJ). Plasma concentrations of TAG were analyzed using an enzyme immunoassay (Pointe Scientific, Canton, MI) and a Pointe 180QT spectrophotometer (Pointe Scientific).

Exercise Testing

Participants returned to the laboratory 24 hr after the fasting blood sample was collected to complete the GXT. Participants were asked to arrive in a fasted state (>8 hr). The GXT was completed on an electronically braked cycle ergometer (LODE Excalibur Sport, Groningen, The Netherlands). Participants were fitted with a heart rate monitor (Polar Electro Inc., Bethpage, NY) which was fixated across the chest at the xiphoid process. The GXT started at 50 W and workload was increased by 35 W every 3 min. Exercise continued until volitional exhaustion; however, only data obtained during the first three stages (i.e., 50, 85, and 120 W) were used for analysis of oxidation rates. Participants were asked to pedal at a comfortable cadence between 70 and 90 revolutions per minute. The test was followed by a cooldown at 50 W.

During the GXT, breath-by-breath pulmonary data were collected using a metabolic cart (Parvo Medics, Sandy, UT) with the participants wearing a headset and mouthpiece. Data were collected for volume of oxygen consumption ($\dot{V}O_2$), volume of carbon dioxide expenditure ($\dot{V}CO_2$), and respiratory exchange ratio (RER) throughout the test. One minute averages were calculated for $\dot{V}O_2$, $\dot{V}CO_2$, and RER values during the last minute of each stage and rates of fat and carbohydrate oxidation were calculated with

Stoichiometric equations: $([1.718 \times \dot{V}O_2] - [1.718 \times \dot{V}CO_2])$ for fat oxidation and $([4.170 \times \dot{V}CO_2] - [2.965 \times \dot{V}O_2])$ for carbohydrate oxidation (Randell et al., 2017).

Supplementation

Using a double-blinded cross-over design, participants were randomly assigned the order in which they supplemented with either ASTA or a color, odor matched placebo for a period of 4 weeks each. Prior to the start of the study, capsules containing ASTA and placebo were counted and dispersed into bottles labeled "A" or "B." Randomization was done using a random number generator and assigning an even or odd number to treatment "A" or "B," which was a code for the ASTA and placebo conditions. One researcher was responsible for randomization and assigning treatments to the participants but was not aware of which was ASTA or placebo until completion of the study. None of the active researchers were aware of the order in which the participants were taking ASTA or placebo until the study completion. The supplemental treatment consisted of ingesting one capsule per day. For the ASTA treatment, each softgel contained 6 mg of ASTA with sunflower oil (AstaReal, Nacka, Sweden). The placebo softgel (AstaReal) was a color and odor matched softgel which contained sunflower oil only. This dosage was chosen in an attempt to fill a gap in the literature since supplementation with 4–20 mg/day has been commonly incorporated in research trials and 12 mg/day for 7 days (Brown et al., 2021) has been shown to enhance fat oxidation. While a higher dose of 20 mg/day failed to show improvements in fat oxidation (Res et al., 2013), the present study was the first (to our knowledge) to investigate the impact of 4 weeks of supplementation with 6 mg/day using a cross-over design. Participants were provided with an undisclosed but previously counted number of capsules in a bottle and asked to return the remaining capsules upon completion of the 4-week supplementation period. Upon completion of the 4-week supplementation period, experimental testing was completed, followed by a 7-day washout period. Subsequently, the participants then completed another 4-week supplementation period with the opposite treatment. Based on a calculation done by Brown et al. (2021), it was estimated that a 7-day washout was sufficient to eliminate ~99% of ASTA based on half-life predictions. Text message reminders were sent weekly to participants to facilitate compliance. Compliance was calculated as ([capsules ingested]/28) × 100. Compliance less than 80% was defined as not acceptable and resulted in one participant being removed from the study and data excluded from analysis.

Statistical Analysis

All statistical procedures were conducted with SAS. Fasting blood concentrations of GSH, H_2O_2, AOPP, MDA, TAG, and total cholesterol were compared between treatments with paired *t* tests. Cohen's *d* (<0.2 = trivial, 0.2–0.5 = small, 0.5–0.8 = moderate, >0.8 = large effect) was calculated to provide effect sizes (Cohen, 1988).

Regarding changes in substrate oxidation rates and RER data, differences between treatments and stages were compared via 2 × 3 repeated-measures analysis of variance. In the instance of a significant interaction or main effect ($p < .05$), Fisher's least significant difference test was used as post hoc analysis to further compare means. In addition, effect sizes were calculated and provided as partial eta squared (η_p^2) values ($\eta_p^2 < .01$, small effect; $\eta_p^2 = .09 - .25$, moderate effect; $\eta_p^2 > .25$, large effect). All data are reported as mean ± *SD* unless otherwise noted.

Results

A total of 14 participants ($n = 14$) completed experimental testing and reached adequate compliance with the ASTA capsules. However, one participant was not able to provide a fasting blood sample and one participant was not able to complete the GXT. Thus, 13 participants completed the exercise testing and 13 participants were available for analysis of blood markers. Note the demographics differ slightly between the GXT and blood marker data and are displayed in Table 1. An overview of total participant recruitment and drop out rate for the study is shown in Figure 1. The body mass index for the participants were calculated as 26 ± 4 kg/m^2. However, it should be noted that these were active men; therefore, this may not be an accurate reflection of body composition or health status.

OS Markers

Data were not available for one participant for MDA, AOPP, and total cholesterol; thus, data for 12 participants ($n = 12$) were analyzed for these measures. Data for 13 participants ($n = 13$) were analyzed for GSH, TAG, and H_2O_2. The GSH concentrations following ASTA supplementation were significantly (~7%) higher compared with placebo ($p = .02$, $d = 0.48$, Figure 2). There was no significant difference in fasting blood concentrations for AOPP, H_2O_2, MDA, TAG, or total cholesterol between treatments ($p > .05$; Table 2).

Table 1 Subject Demographics

	OS markers	GXT
Age (years)	23 ± 2	23 ± 2
Height (cm)	173 ± 5	172 ± 5
Mass (kg)	80 ± 12	79 ± 12

Note. Values are presented as mean ± *SD*. OS = oxidative stress; GXT = graded exercise test.

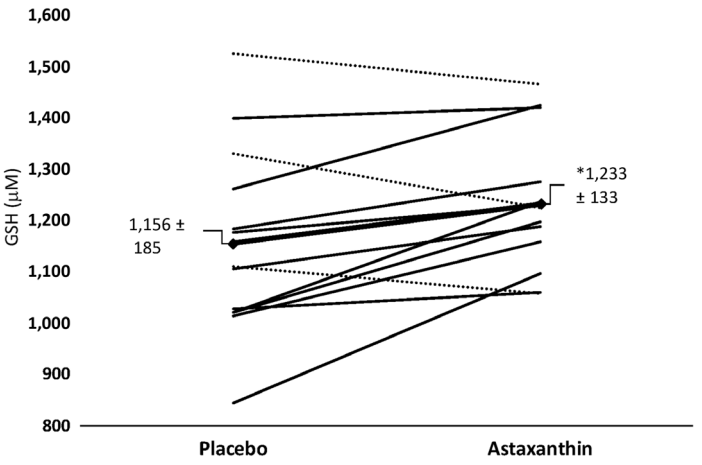

Figure 2 — The GSH concentrations following each treatment condition. *Note*. Three individuals experienced a decrease in GSH (dotted lines). Ten individuals experienced an increase in GSH concentrations (solid lines). *Significantly higher ($p < .05$) concentrations following astaxanthin supplementation compared with placebo. GSH = glutathione.

Table 2 Fasting Blood Markers

	Astaxanthin	Placebo	Cohen's *d*	*p* value
AOPP (μM)	26 ± 22	36 ± 35	0.34	.45
MDA (μM)	1.2 ± 0.4	1.2 ± 0.5	0.00	.69
H_2O_2 (μM)	20 ± 9	23 ± 19	0.20	.70
Chol (mg/dl)	179 ± 28	187 ± 37	0.24	.31
TAG (mg/dl)	64 ± 41	66 ± 30	0.05	.86

Note. TAG, Chol, and H_2O_2, $n = 13$; MDA and AOPP, $n = 12$. Values are presented as mean ± *SD*. TAG = plasma triglycerides; AOPP = plasma levels of advanced oxidation protein products; MDA = plasma malondialdehyde; H_2O_2 = plasma hydrogen peroxide; Chol = total cholesterol (plasma).

Fat and Carbohydrate Oxidation Rates

There was no Treatment × Stage interaction or main effect for treatment in terms of mean fat oxidation ($p > .05$). However, a main effect for stage was noted, $F = 9.65$, $p < .001$, $\eta_p^2 = .24$. Post hoc analysis demonstrated a significant reduction in mean fat oxidation from Stages 1 (50 W) to 3 (120 W) ($p < .001$) and from Stages 2 (85 W) to 3 (120 W) ($p = .004$; Figure 3a).

With respect to mean carbohydrate oxidation, no Treatment × Stage interaction was noted ($p > .05$). There was no main effect for treatment ($p > .05$); however, there was a main effect for stage, $F = 110$, $p < .001$, $\eta_p^2 = .78$. Post hoc analysis demonstrated a significant increase in carbohydrate oxidation at each stage during the GXT ($p < .001$; Figure 3b).

In relation to mean RER values during exercise, there was no Treatment × Stage interaction or main effect for treatment ($p > .05$). There was a main effect for stage, $F = 68.3$, $p < .001$, $\eta_p^2 = .69$. Post hoc analysis demonstrated a significant increase in mean RER values during each stage during the GXT ($p < .001$). Mean RER data were similar between the two treatments and were: 0.83 ± 0.06 at 50 W, 0.88 ± 0.08 at 85 W, and 0.96 ± 0.88 at 120 W.

Discussion

The primary findings of this study suggest 4 weeks of ASTA supplementation with 6 mg/day does not impact substrate oxidation during exercise, but increases whole blood GSH concentrations (~7%). Past work has shown improvements in fat oxidation but these increases were associated with a higher dose of ASTA (12 mg/day) (Brown et al., 2021). The present study was, to our knowledge, the first to investigate the impact of 4 weeks of supplementation with 6 mg/day in physically active noncompetitive athletes. These findings show increased GSH concentrations with a lower dose of ASTA, and the improvement may be attributed to the potential ability of ASTA to act as a direct and/or indirect antioxidant (Andriani et al., 2019; Hussein et al., 2006). The lack of change in oxidation rates during exercise may be attributed to the measurement technique used or the dose of ASTA, which was lower than past work involving 12 mg/day (Brown et al., 2021).

The increase in GSH concentrations may be attributed to the ability of ASTA to act as a direct or indirect antioxidant (Kohandel et al., 2021; Sztretye et al., 2019). Past work in rats demonstrated ASTA increased tissue-specific levels of GSH when exposed to oxidative damage induced by oral formaldehyde (Andriani et al., 2019). The authors are currently unaware of any human trials that have studied the effect of oral ASTA supplementation on GSH concentrations; however, the present findings are in line with work by Andriani et al. (2019) performed in rodents suggesting ASTA can favorably impact endogenous antioxidant status. In terms of the potential mechanism, it has been recently suggested that ASTA may activate nuclear factor erythroid 2-related factor 2 (Nrf2) (Kohandel et al., 2021). The Nrf2 is a transcription factor primarily involved in endogenous regulation of proper redox and inflammatory status. The Nrf2 is involved in the activation of several endogenous antioxidants, such as GSH, as well as catalase and superoxide dismutase (Kohandel et al., 2021; Ma, 2013). It should be noted that GSH concentrations can be maintained or increased by either a de novo pathway or a resynthesis pathway (Andriani et al., 2019). De novo synthesis of GSH involves the production of GSH using endogenous amino acids glutamate, cysteine, and glycine, while the resynthesis pathway recycles GSH from oxidized GSH via enzyme GSH reductase and nicotinamide adenine dinucleotide phosphate (NADPH) (Townsend et al., 2003). Since ASTA may activate the Nrf2 pathway, it is possible that ASTA supplementation increases GSH by increasing de novo synthesis through the activation of γ-glutamyl cystine ligase (Kohandel et al., 2021; Ma, 2013). It is also possible that the antioxidative activity of ASTA can neutralize reactive oxygen species and therefore reduce GSH oxidation (Andriani et al., 2019). The potential for ASTA to impact Nrf2 activity and endogenous antioxidant status can have an impact on a variety of cardiometabolic diseases that are facilitated by chronic OS and inflammation and thus, additional trials involving ASTA supplementation in clinical populations are warranted (Kohandel et al., 2021; Ma, 2013; Sztretye et al., 2019).

It is important to mention that in the present study, GSH concentrations were increased (~7%) without a change in markers of OS, such as MDA and H_2O_2. However, it should be noted that AOPP concentrations decreased by ~28% (although not statistically significant [$p = .45$]). There are several explanations for these findings. First, it should be noted that a sample size of 15 was preliminarily calculated as being required for a desired power of 0.8 and the present study included analysis from 13 participants and thus, it is likely that a larger sample size may provide different findings. Furthermore, participants involved in the present study were apparently healthy and did not suffer from any known cardiovascular, metabolic, or neurological diseases that are typically associated with increased inflammation and OS. Therefore, it is expected that their fasting concentrations of the aforementioned OS markers is lower than what is commonly noted in overweight, obese, and clinical populations (Mahjoub & Roudsari, 2012). In addition, the participants were not exposed to an environmental factor that can cause OS, such as intense physical exertion, heat, or smoke exposure (Huang et al., 2013). Therefore, future work should study the impact of ASTA supplementation when exposed to sources of OS such as these. Finally, the current study was limited by not including additional markers of OS, such as nitric oxide or peroxynitrate, which may also be impacted by a change in GSH concentrations (Aquilano et al., 2014).

Past work has suggested that the antioxidative properties of ASTA can have a favorable effect on exercise metabolism and performance by increasing fat oxidation and facilitating exercise recovery (Brown et al., 2018). Improvements in fat oxidation may also result in improvements to aspects of cardiometabolic health, especially since individuals with metabolic syndrome lack the ability to oxidize fats during exercise compared with more active counterparts (San-Millán & Brooks, 2018). With respect to substrate utilization during exercise, the antioxidative properties of ASTA have been suggested to support fat metabolism by reducing oxidation of CPT1 (Aoi et al., 2008). The CPT1 is an enzyme that

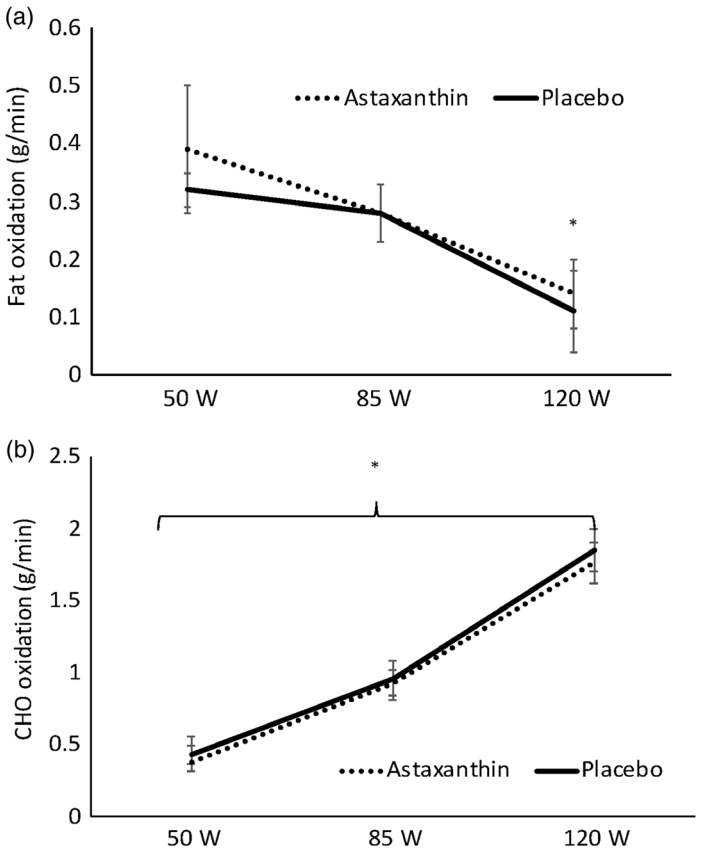

Figure 3 — (a) Changes in fat oxidation across stages during exercise between the two treatments. *Significant decrease compared to Stages 1 (50 W) and 2 (85 W) ($p < .05$). (b) Changes in CHO oxidation across stages during exercise between the two treatments. *Significant increase at each stage ($p < .05$). *Note*. Data are shown as mean ± *SE*. Stage 1 = 50 W, Stage 2 = 85 W, Stage 3 = 120 W. CHO = carbohydrate.

facilitates the attachment and transport of long-chain fatty acids into the mitochondria via the carnitine transport mechanism. When individuals are exposed to OS, such as during intense or prolonged exercise, this transport mechanism is at risk for oxidative damage, which may limit fat oxidation potential (Brown et al., 2018). However, past work has shown conflicting results in terms of whether or not ASTA can produce meaningful changes in fat oxidation during exercise. Data from rodent models support ASTA in relation to increased fat oxidation during exercise; specifically, 4 weeks of ASTA supplementation has been shown to enhance CPT1 function, facilitating enhanced fatty acid transport and a glycogen sparing effect (Aoi et al., 2008; Ikeuchi et al., 2006).

However, these findings are not consistently documented in humans. For example, two studies noted ergogenic effects in trained and/or recreationally trained cyclists supplementing with ASTA (Brown et al., 2021; Earnest et al., 2011). Findings from Earnest et al. (2011) suggested an ergogenic effect in trained cyclists without a significant effect on substrate utilization during exercise. In addition, findings from Res et al. (2013) failed to suggest an ergogenic effect or any significant effect on fat oxidation during exercise. However, both of these studies utilized a between-subjects, parallel design, which may explain the lack of significant treatment effects due to between-group differences at baseline (Earnest et al., 2011; Res et al., 2013). It is also worth noting that these studies involved supplementation with 4 and 20 mg/day for 4 weeks (Earnest et al., 2011; Res et al., 2013). However, recent findings from Brown et al. (2021) demonstrated an ergogenic effect in trained cyclists ,which was associated with an increase in fat oxidation during exercise following 7 days of supplementation with 12 mg/day. As such, the findings from the present study are in line with Earnest et al. (2011) and Res et al. (2013), who reported no significant effect on substrate utilization during exercise.

The present study is limited by a number of factors. The present findings suggest ASTA is effective at increasing endogenous GSH concentrations; however, oxidized GSH was not measured so we are not able to determine if GSH recycling from oxidized GSH was enhanced, or if the increase in GSH was attributed to de novo amino acid synthesis. Past work showing improvements in fat oxidation during exercise involved a higher dose of ASTA as well as participants who were recreationally trained cyclists (Brown et al., 2021). Moreover, the exercise protocol used previously (Brown et al., 2021) involved a 40 km time trial (which typically involves exercising at a self-selected pace) as opposed to a steady state GXT, which may explain a discrepancy in the results. While the present study involved testing of apparently healthy and regularly active young men, physical activity was not monitored during the study. It should be noted that the participants were instructed to attempt to maintain consistent physical activity levels throughout the study. However, variations in activity levels or physical conditioning may have impacted the results. It should also be mentioned that the present study measured fat oxidation at absolute intensities (i.e., 50 W, 85 W, and 120 W) as opposed to relative intensities, which should be viewed as a limiting factor since individuals may vary in their aerobic conditioning status and/or because conditioning levels may change over the duration of the study. There was also no familiarization done prior to the first GXT and therefore that could be viewed as a limitation.

The current findings suggest 4 weeks of 6 mg/day of ASTA supplementation in active young men results in a significant increase in GSH concentrations, but does not impact substrate utilization during exercise. These findings may have implications for individuals chronically exposed to high levels of OS and inflammation (Kris-Etherton et al., 2004). It is possible that the dosage of 6 mg/day is not effective at producing changes in oxidation rates during exercise, as improvements in exercise performance and fat oxidation during exercise were noted following a 12 mg/day supplementation period (Brown et al., 2021). Increased GSH concentrations are likely to be associated with reduced susceptibility to OS (Mahjoub & Roudsari, 2012). Moreover, individuals with metabolic syndrome lack the ability to oxidize fats during exercise compared with more active counterparts (San-Millán & Brooks, 2018); therefore, future trials should implement ASTA supplementation (with a larger sample size) in individuals with metabolic syndrome. In addition, since findings appear to be dose dependent, future trials should consider implementing 6 mg/day or higher.

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