IMPACT OF ASTAXANTHIN SUPPLEMENTATION ON METABOLIC RESPONSES TO EXERCISE AND POSTPRANDIAL OXIDATIVE STRESS

by

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LIST OF ABBREVIATIONS

Abbreviation	Description
ATP	Adenosine Triphosphate
BP	Blood Pressure
CAT	Catalase
CPT 1	Carnitine Palmitoyltranferase 1
CV	Cardiovascular
FDA	Food and Drug Administration
GSH	Glutathione
HR	Heart Rate
MDA	Malondialdehyde
NO	Nitric Oxide
PDH	Pyruvate Dehydrogenase
PFK	Phosphofructokinase
PGC-1α	Peroxisome Proliferator-Activated Receptor-γ Co-Activator 1α
RER	Respiratory Exchange Ratio
ROS	Reactive Oxygen and Species
RPE	Rating of Perceived Exertion
SOD	Superoxide Anion Dismutase
T2D	Type II Diabetes

TAG	Triacylglycerol
$\dot{V}CO_2$	Volume of Carbon Dioxide Expelled
$\dot{V}O_2$	Volume of Oxygen Inhaled

I. INTRODUCTION

Oxidative stress contributes to development of metabolic and cardiovascular (CV) diseases (Huang et al., 2015; Victor et al., 2009; Wei et al., 2009). The mitochondria are the largest contributors to oxidative stress through the production of reactive oxygen species (ROS), specifically when the mitochondria are not functioning properly (Zorov, Juhaszova, & Sollott, 2014), as is the case with individuals with metabolic syndrome (San-Millán & Brooks, 2018), which causes mitochondrial damage and even greater ROS production (Ansari, Khan, Ahmad, & Haqqi, 2018; Kim & Kim, 2018). Due to this inverse relationship, improvements in mitochondrial function could result in decreased oxidative stress. Thus, ROS production is considered an indicator of mitochondrial functionality. As an alternative, mitochondrial functionality is also measured by observing the intensity in which the mitochondria transition from using primarily fats for adenosine triphosphate (ATP) production to using primarily carbohydrates for ATP production, also known as the crossover effect. Researchers often use graded exercise tests to determine the intensity at which the crossover effect occurs. If the crossover effect occurs at an appropriate intensity—typically around moderate intensity or higher—the individual has metabolic flexibility. When the crossover occurs below moderate intensity, the individual has metabolic *in*flexibility. Metabolic inflexibility is prevalent in individuals with metabolic syndrome, type two diabetes mellitus, and obesity (San-Millán & Brooks, 2018). Individuals that exhibited metabolic inflexibility also performed worse on the graded exercise test than those that exhibited metabolic flexibility. These relationships suggest improvements in the mitochondria will cause improvements in metabolic health and exercise performance. Because of the effect oxidative stress and

mitochondrial function have on basic health, improving both components would lead to better CV health, metabolic health, and exercise performance.

The supplement astaxanthin has exhibited promising results in these areas. Astaxanthin is a carotenoid found in microalgae, salmon, shrimp, and other animals Guerin, Huntley, & Olaizola, 2003).Research supports astaxanthin can act as an antioxidant itself, as well as increase the activity of other antioxidants in the body, which causes decreases in oxidative stress (Guerin, Huntley, & Olaizola, 2003; Seabra & Pedrosa, 2010). Astaxanthin also facilitates increases in lipid metabolism, which is a key aspect of improving metabolic flexibility (Aoi et al., 2008; Mashhadi et al., 2018). This review will elaborate on the role the mitochondrion plays in metabolism, thus the direct effect it has on metabolic health, indirect effect on CV health, and both direct and indirect effects on exercise performance. Furthermore, oxidative stress will be defined, mechanisms contributing to oxidative stress will be examined, in addition to potential therapy with endogenous and exogenous antioxidants. Lastly, the supplement astaxanthin will be discussed in regard to contesting oxidative stress, as well as improving mitochondrial function—in turn leading to improvements in CV health, metabolic health, and exercise performance.

II. REVIEW OF LITERATURE

Metabolic Flexibility

Metabolism is a process in which chemical energy is transferred into usable energy in living organisms—specifically into adenosine triphosphate (ATP) (Brooks, Fahey, & Baldwin, 2005). The human body typically metabolizes three macronutrients for ATP production: (1) carbohydrates, (2) lipids, and (3) proteins (Brooks et al., 2005). The metabolism of carbohydrates begins in the cytoplasm and—under normal physiologic and aerobic conditions—ends in the mitochondria, while both proteins and fatty acids rely solely on the mitochondria to be metabolized (Brooks et al., 2005). The metabolism of these three macronutrients with the use of the mitochondria is known as aerobic metabolism (Brooks et al., 2005).

The mitochondria play a key role in metabolism as well as mediating oxidation in the cell (Brooks et al., 2005). When mitochondria are functioning under typical physiological circumstances, they oxidize substrates through β-oxidation and the citric acid cycle. These processes result in production of ATP through the electron transport chain via oxidative phosphorylation (Brooks et al., 2005). Typically, at lower intensities, the mitochondrion oxidizes more lipids, and at higher intensities the mitochondrion shifts to greater carbohydrate oxidation (Bloomer, Butawan, Farney, & McAllister, 2019). This transition from a heightened reliance on lipids to carbohydrates is known as the crossover effect. When individuals have a more pronounced crossover and greater ability to appropriately transition between substrates, they have what is known as metabolic flexibility (Storlien, Oakes, & Kelley, 2018).

Metabolic flexibility reflects the mitochondria's ability to transition between, or crossover from, fat oxidation to carbohydrate oxidation when the rate of demand for ATP increases and vice versa (Storlien et al., 2018). The inappropriate transition from lipid oxidation to carbohydrate oxidation is known as metabolic inflexibility (San-Millán & Brooks, 2018; van Dijk et al., 2012). Typically, those with metabolic syndrome, have metabolic inflexibility (San-Millán & Brooks, 2018). The study led by San-Millán and Brooks (2018) found individuals diagnosed with metabolic syndrome transition from lipid to carbohydrate oxidation almost immediately during graded exercise testing on a cycle ergometer (San-Millán & Brooks, 2018). In the same study, active individuals experienced the crossover from fat oxidation to carbohydrate around moderate intensity, while elite endurance athletes transitioned from lipid to carbohydrate oxidation at significantly higher intensities. This suggests as individuals become more aerobically trained, they become more efficient at utilizing lipids for energy at higher intensities and rely less heavily on carbohydrate usage for ATP production. In other words, transitioning to primary reliance on carbohydrates is shifted to higher intensities. The researchers also measured lactate levels throughout the exercise testing and found an inverse relationship between fat oxidation and lactate production. This is reflected in the study by elite athletes having lower levels of lactate at the same workloads as less fit participants due to a decreased reliance on carbohydrate oxidation and elite endurance athletes having high functioning mitochondria (Scott K Powers, 2017), which causes them to have greater metabolic flexibility than those with dysfunctional mitochondria—such as those with metabolic syndrome (San-Millán & Brooks, 2018). More specifically, mitochondria become higher functioning due to increases in mitochondrial biogenesis (Budiono et al.,

2012), improvements in mitochondrial proteome (Kavazis, Alvarez, Talbert, Lee, & Powers, 2009; Sun et al., 2008), increases in aerobic enzymes (Padrão et al., 2012), and increases in antioxidant activity (S. K. Powers et al., 1993). Improving mitochondrial function improves metabolic flexibility, which would lead to better overall health (Kelley, He, Menshikova, & Ritov, 2002).

Dietary interventions and an increase in exercise frequency can improve mitochondrial function facilitating an improved metabolic flexibility. Research demonstrates chronic consumption of high sucrose or high fat meals can cause metabolic inflexibility (Jørgensen et al., 2017). This suggests decreasing the frequency of consuming high fat and high sucrose meals can improve metabolic flexibility and allow for a prominent crossover effect. Consuming a high protein diet with low carbohydrate diet can also improve metabolic flexibility (Baum et al., 2006; Trexler et al., 2017). Furthermore, supplementation of nicotinamide riboside, a precursor of nicotinamide adenine dinucleotide, also causes improvements in appropriate substrate utilization in rats (Shi et al., 2017). This study found the most likely cause of the improvement is due to the increase in adiponectin and activity of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) from nicotinamide riboside supplementation (Shi et al., 2017). Adiponectin directly causes increases in lipid metabolism (Asayama et al., 2003; Ibrahim, 2010) while PGC-1α indirectly causes increases in lipid metabolism due to the induction of mitochondrial biogenesis (Brooks et al., 2005; Canto & Auwerx, 2009; Home, 2011). Peroxisome proliferator activated receptor gamma co-activator 1alpha also causes increases in peak oxygen consumption ($\dot{V}O_2$) (Tadaishi et al., 2011). With both an increase in number of mitochondria as well as an increase in reliance on lipid oxidation

from both adiponectin and PGC-1α, reliance on carbohydrates for energy decreases, causing improvements in metabolic flexibility. Additionally, studies have reported chronic aerobic training (Scott K Powers, 2017), resistance training (Porter, Reidy, Bhattarai, Sidossis, & Rasmussen, 2016), and chronic high intensity interval training (De Strijcker et al., 2018) can also cause increases in mitochondrial function, number, and size. Some of these studies report improvements not only in the mitochondrion itself, but enzyme activity as well. This increase in mitochondrial enzyme activity allows for heightened β-oxidation activity, which then inhibits both pyruvate dehydrogenase (PDH) as well as phosphofructokinase (PFK), which decreases the metabolism of carbohydrates (Brooks et al., 2005)—suggesting an improvement again in metabolic flexibility.

One technique to measure peaks in fat oxidation is through a FatMax exercise test (Achten, Gleeson, & Jeukendrup, 2002; Jeukendrup & Achten, 2001; San-Millán & Brooks, 2018). This test has also been used to evaluate CV disease risk (Jeukendrup & Achten, 2001). The FatMax is a graded exercise test on a cycle ergometer that increases workload in three minute stages by 35 Watts (Achten et al., 2002). The measured pulmonary data is used to calculate fat and carbohydrate oxidation rates (Waldman et al., 2018), giving the workload at which peak fat oxidation occurs. With all of these adaptations of the mitochondria, both through dietary interventions and chronic exercise training, improvements can also be induced in terms of the redox state of the cell (Whitaker, Corum, Beeson, & Schnellmann, 2016) through increases in prevalence in antioxidants which decrease the impact of oxidative stress.

Oxidative Stress

Oxidative stress is the condition in which oxidant production cannot be matched with antioxidant activity (Huang et al., 2015). Reactive oxygen species are oxidants the body produces under normal physiological conditions that have an unpaired electron. ROS travel in the cell attempting to remove electrons from other parts of the cell, ultimately oxidizing substances it comes into contact with (Zorov et al., 2014). For example, ROS attempt to remove electrons from DNA, RNA, muscles, and other tissues (Ansari et al., 2018) which can contribute to aging, chronic diseases, and mitochondrial dysfunctionality (Sadowska-Bartosz & Bartosz, 2014). When a mitochondrion is not functioning appropriately, mitophagy (selective death of a cell) or cellular apoptosis (death of a cell) should occur to remove it. Mitophagy, or selective autophagy, of a mitochondrion is achieved by Parkin expression (Ansari et al., 2018). Research states as Parkin expression decreases, ROS production and inappropriate cellular apoptosis increases (Ansari et al., 2018). Not only do ROS cause dysfunctionality of mitochondria, they can also decrease the occurrence of the removal of these malfunctional mitochondria as well.

When there is increased stress on a mitochondrion, (e.g. excessive metabolism) it tends to leak oxygen from complexes I and III of the electron transport chain, causing the production of superoxide anion (O_2^-) when it comes into contact with nicotinamide adenine dinucleotide phosphate oxidase (Kim & Kim, 2018; Zuccarella-Hackl et al., 2016). If there is not an abundance of superoxide anions, the cell can remove these molecules with endogenous antioxidants, specifically superoxide dismutase (SOD), while other ROS are reduced by alternative endogenous antioxidants. Other endogenous

antioxidants include glutathione (GSH) and catalase (CAT) (Alzoubi et al., 2018; Zorov et al., 2014). When the concentration of ROS and reactive nitrogen species—superoxide anion, hydroxyl radicals, hydrogen peroxides, nitric oxides (NO), peroxynitrites, lipid hydroperoxides, alkoxyl radicals, peroxyl radicals, nitrogen centered radicals, sulfate radicals, and singlet oxygens (Zorov et al., 2014)—surpasses what SOD, GSH, and CAT can reduce, the cell is in a state of oxidative stress (Alzoubi et al., 2018; Huang et al., 2015; Urso & Clarkson, 2003).

Moderate amounts of ROS accumulation induce beneficial cellular adaptations from exercise (Busso, 2003; Radak et al., 2017; Radak, Zhao, Koltai, Ohno, & Atalay, 2013). Although high amounts of ROS cause damage, small amounts can generate improvements in normal adaptations to exercise (Radak et al., 2013) including force output (Cheng et al., 2016), organ functionality, prevention of chronic diseases, and improvements in quality of life (Radak et al., 2017). Therefore, to optimize the improvements associated with exercise via signal transduction theory and to minimize significant damage to the cell, ROS production should be elevated but not excessive (Scott K. Powers & Jackson, 2008; Yu et al., 2003). Specifically, NO plays an important role in promoting proper endothelial function which can decrease in hypertension (Korsager Larsen & Matchkov, 2016), and improve other aspects of CV health. Nitric oxide causes relaxation of the endothelial cells of blood vessels, making them dilate (Vanhoutte, Shimokawa, Feletou, & Tang, 2017). This dilation causes decreased pressure on the walls of the blood vessel, thus decreasing blood pressure (BP) and causing a decreased likelihood of having hypertension. However, NO can also be further oxidized into peroxynitrite when it binds with superoxide anion. Peroxynitrite has a greater

oxidative capacity than NO alone, stimulating necrosis, apoptosis, and neuronal death (Ramdial, Franco, & Estevez, 2017). This supports the notion of some ROS being important to CV health, but excess amounts of ROS can be detrimental.

Although oxidative stress is a normal phenomenon that occurs with increased metabolism, there are multiple environmental and physiological aggressors that cause significant chronic increases in ROS production that surpasses the cellular antioxidative capacity. Many of these aggressors are also risk factors of CV disease (Jokinen, 2015), such as smoking (Carnevale et al., 2016; Ellegaard & Poulsen, 2016), sex (Borrás et al., 2003; Tiidus, Bombardier, Hidiroglou, & Madere, 1998; Viña, Sastre, Pallardó, Gambini, & Borrás, 2006), obesity (Huang et al., 2015), type II diabetes (T2D) (Sottero et al., 2015; Wei et al., 2009), and physical inactivity (Pruimboom, Raison, & Muskiet, 2015). Although some of these risk factors are modifiable (e.g. physical inactivity, obesity, T2D, smoking), sex is nonmanipulable.

Research suggests males normally have higher amounts of ROS—thus increased amounts of ROS and susceptibility to oxidative stress—when compared to females (Bloomer, Ferebee, Fisher-Wellman, Quindry, & Schilling, 2009; Bloomer & Lee, 2013). Most evidence supports the reasoning to be the higher amount of estrogen produced naturally in females compared to males (Tiidus, Bombardier, Hidiroglou, & Madere, 1998). Estrogen acts as both a direct and indirect endogenous antioxidant in the human body, and females respond to this antioxidative effect better than males (Tiidus et al., 1998; Viña et al., 2006). Another contributing factor could simply be differences in genetic makeup (Borrás et al., 2003; Viña et al., 2006). Borrás et al. reported males having four times greater cellular damage related to oxidative stress than females,

primarlily due to females having an increased prevalance in antioxidant gene expression (Borrás et al., 2003).

Other contributors to increases in acute oxidative stress are high intensity and long duration exercise (Tiidus, 1998) and high saturated fat meal consumption (Benson et al., 2018) due to the increased strain on the mitochondria causing greater ROS production (Scott K. Powers & Jackson, 2008; Scott K Powers, 2017; Tiidus et al., 1998). High fat meal consumption is displayed as being a greater aggressor when it comes to ROS production than exercise (Bloomer, Kabir, Marshall, Canale, & Farney, 2010; Esser et al., 2013; Gabriel, Ratkevicius, Gray, Frenneaux, & Gray, 2012; McCarthy, Farney, Canale, Dessoulavy, & Bloomer, 2013; Pruimboom, Raison, & Muskiet, 2015; Tsai, Li, Lin, Chao, & Chen, 2004; Yang, Wu, & Chiu, 2018) and is even further exacerbated in cigarette smokers (Bloomer & Fisher-Wellman, 2009). This influx of ROS production induced by high-fat meal consumption is caused by the increased demand on the mitochondria without the increased prevalence of antioxidants—discussed later in this review—that occurs during exercise, which is when oxidative stress is also typically exacerbated (McCarthy et al., 2013).

Literature examining postprandial oxidative stress displays acute increases in lipid peroxidation byproducts represented by malondialdehyde (MDA), hydrogen peroxide (Bloomer et al., 2010), triacylglycerols (TAG) (Farinha et al., 2018), nitrite levels (Fisher-Wellman & Bloomer, 2010), protein oxidation represented by advanced oxidation protein products (AOPP) (Bloomer, Trepanowski, Kabir, Alleman, & Dessoulavy, 2012), while decreasing GSH prevalence (Tsai et al., 2004). An additional aspect of high fat meal induced oxidative stress is the size of the meal. The larger the meal size, the larger

the oxidative stress response. When healthy men consumed differing sizes of a milkshake containing the same percentage of fat, the milkshake that had the greatest caloric density induced the greatest amount of oxidative stress, specifically measured as MDA and hydrogen peroxide. Specifically, in this study, the milkshake with 66 grams of fat had the greatest oxidative stress response (Bloomer et al., 2010). It is important to note that meals containing roughly 60 grams of fat induce greater amounts of oxidative stress when compared to meals that contain roughly 30 grams of fat.

Decreasing Oxidative Stress

To combat excessive ROS production—whether it be induced by exercise or high fat meals—higher concentrations of antioxidants are necessary. There are multiple ways to increase antioxidant production, but the focus will be on exercise and dietary supplementation.

Through an increase in fitness level due to chronic aerobic training, the body adapts and begins to produce more endogenous antioxidants (Bhuvaneswari, Yogalakshmi, Sreeja, & Anuradha, 2014; Frasier, Moore, & Brown, 2011; Kavazis, 2009; Scott K. Powers, Quindry, & Kavazis, 2008). More specifically, because endurance exercise increases mitochondrial mass (Brooks et al., 2005; Meinild Lundby et al., 2018), and since SOD, CAT, and GSH are all primarily found in the mitochondria, this suggests as mitochondrial mass increases, concentrations of SOD, CAT, and GSH levels also increase (Brooks et al., 2005; Lee et al., 2012; Scott K. Powers & Jackson, 2008; Scott K Powers, 2017). Some studies even found exercise to be beneficial in directly decreasing postprandial oxidative stress by acutely increasing antioxidant activity as well. More specifically, aerobic exercise at moderate intensity (Takahashi, Miyashita, Park, Sakamoto, & Suzuki, 2015), combined and circuit training (Farinha et al., 2018), and high intensity interval training (Gabriel et al., 2012) establishes decreases in postprandial oxidative stress, and increases in antioxidant activity.

Alternative means to increase exogenous, or supplemented, antioxidant concentrations are through nutrition. Exogenous antioxidants such as quercetin (Duarte et al., 2002), resveratrol (Sueishi et al., 2012; Venturini et al., 2010), Coenzyme Q-10 (Forsberg et al., 2015; Prangthip, Kettawan, Posuwan, Okuno, & Okamoto, 2016; Zhang

et al., 2013), vitamin A & E (Urso & Clarkson, 2003), and even certain components of coffee (Bloomer, Trepanowski, & Farney, 2013; Chen & Kotani, 2015) may reduce oxidative stress and increase endogenous antioxidant status. Antioxidant supplementation consistently results in decreases in *postprandial* oxidative stress (Bloomer et al., 2013), which has been validated to occur with supplementation of vitamin E in as little as eight days with similar markers as stated previously (Neri et al., 2010). Because of the potential for exogenous antioxidants to increase endogenous antioxidants, such as GSG, this suggests some supplements function as indirect antioxidants as well (Sadowska-Bartosz & Bartosz, 2014). Alternative dietary modifications such as macronutrient interventions, or fasting, exhibited decreased MDA, hydrogen peroxide, AOPP, and TAG in regard to postprandial oxidative stress (Bloomer et al., 2012). Whether it is direct or indirect mechanisms, dietary interventions can cause decreases in oxidative stress through increasing antioxidant concentrations.

Considering the relationship between improved mitochondrial function and decreased oxidative stress susceptibility and the link they have to CV health, metabolic health, and exercise performance, finding a supplement that can simultaneously achieve these improvements is of great interest.

Astaxanthin

Astaxanthin is a lipid soluble carotenoid produced typically from a microalgae named Haematococcus Pluvialis (Boussiba, Bing, Yuan, Zarka, & Chen, 1999; Pérez-López et al., 2014; Zgheib, Saade, Khallouf, & Takache, 2018). It can be found in other organisms—such as salmon, krill, lobster, and shrimp (Guerin, Huntley, & Olaizola, 2003)—but the concentrations of astaxanthin found in Haematococcus Pluvialis are significantly higher than in other organisms (Ambati, Moi, Ravi, & Aswathanarayana, 2014; Guerin et al., 2003; Kidd, 2011). Research has indicated astaxanthin is safe for consumption in humans up to 20 mg/day for four consecutive weeks without any adverse effects (Satoh et al., 2009), while the Food and Drug Administration (FDA) approves 12-24 mg/day for human consumption for 30 consecutive days when it is derived from Haematococcus Pluvialis (Zuluaga, Gueguen, Pavon-Djavid, & Letourneur, 2017).

Studies have established that carotenoids in general work well as antioxidants, as well as promote antioxidant activity (Brown, Gough, Deb, Sparks, & McNaughton, 2018; Johnson & Schroeder, 1996; Martin et al., 1999; Palozza et al., 2009). In comparison to other carotenoids, astaxanthin has greater polarity (Hussein, Sankawa, Goto, Matsumoto, & Watanabe, 2006), more conjugated double bonds (Guerin et al., 2003b), and consists of oxygen (Kidd, 2011) making it a stronger antioxidant. Also, because it is polar on both ends but nonpolar in the middle, astaxanthin spans across the entire phospholipid bilayer of the cytoplasm, allowing a single astaxanthin molecule to cover a greater area than other carotenoids (Hussein et al., 2006; Kidd, 2011). Research supports these assumptions based off of molecular structure—specifically when comparing it to β-carotene and canthaxanthin—in decreasing prevalence of CV disease and cancer due to

its tumor suppression ability in conjunction to its antioxidative effects (Chew & Park, 2004; Palozza, Barone, Mancuso, & Picci, 2008; Palozza et al., 2009). Specifically, even with only 3 weeks of supplementation, literature determines astaxanthin has decreased MDA while causing increased SOD and total antioxidant capacity (Choi, Kim, Chang, Kyu-Youn, & Shin, 2011) while simultaneously improving blood lipid profile (Choi, Youn, & Shin, 2011) in obese and overweight adults. These results suggest not only does astaxanthin act as an antioxidant itself, but it also causes increases in antioxidant activity (Brown et al., 2018; Kidd, 2011; Martin et al., 1999; Wolf et al., 2010). With the direct and indirect antioxidative properties of astaxanthin, it is likely to decrease oxidative stress efficiently.

Research indicates astaxanthin can increase fat metabolism through inhibition of oxidative damage to carnitine palmitoyltranferase 1 (CPT 1) in skeletal muscle (Aoi et al., 2008). CPT 1 is the enzyme that carries fatty acids to a mitochondrion to be oxidized by binding fatty acyl-CoA to carnitine (Brooks et al., 2005; Choi, Youn, et al., 2011). More importantly, it is the rate-limiting aspect of fat oxidation during exercise (Roepstorff et al., 2005; van Loon, Greenhaff, Constantin-Teodosiu, Saris, & Wagenmakers, 2001). When CPT I is damaged, the functionality of it is altered, leading to decreased fat oxidation, especially during exercise (Aoi et al., 2008). In addition, when CPT 1 is altered, it can cause alterations to carnitine—which also acts as an antioxidant in skeletal muscle (Bloomer et al., 2019). Not only does astaxanthin decrease oxidative effects on CPT 1, but it can also cause increases in expression of PGC-1α (Liu et al., 2014) as well as increases in adiponectin concentrations (Mashhadi et al., 2018).

Alternatively, Res et al. found no changes in antioxidative effects in astaxanthin when measuring lipid peroxide MDA during exercise (Res et al., 2013). This suggests astaxanthin does not increase antioxidant activity or decrease the damage to CPT 1. It is however, important to note that the researchers used participants that were well-trained endurance athletes with a $\dot{V}O_2$ peak over 60 mlO_2/kg*min (Res et al., 2013). Due to their high aerobic fitness level, the participants are less susceptible to oxidative stress and have better metabolic flexibility (Gregorio-Arenas et al., 2016; San-Millán & Brooks, 2018). In a contrasting study with overweight participants supplementing with astaxanthin—rather than highly trained triathletes as in the previous study—MDA decreases (Choi et al., 2011). In an analogous study with healthy untrained males, there were also decreases in MDA with astaxanthin supplementation (Karppi et al., 2007). These two studies suggest a contributing factor to the measurement of lipid peroxidation through concentrations of MDA is dependent on the health and fitness level of the population being tested.

Statement of Purpose: Considering the findings in the literature regarding astaxanthin's potential to favorably improve lipid metabolism and antioxidative effects, the purpose of this study is to evaluate astaxanthin's ability to improve metabolic flexibility and decrease oxidative stress.

Hypothesis: Four weeks of supplementation with 6 mg of astaxanthin daily will result in greater metabolic flexibility and decreased oxidative stress compared to placebo.

Significance: If astaxanthin can improve metabolic flexibility along with decreased oxidative stress, this gives the general public an alternative therapy to improve

CV health, metabolic health, and exercise performance, ultimately leading to better quality of life.

III. METHODS

Participants

Participants were healthy males recruited verbally and by flyer from Texas State University classes and recreational facilities. Participants were required to be males between the ages of 18-39, overall healthy, and participate in at least 150 minutes of physical moderate activity every week. Exclusion criteria included lactose intolerance, participate in veganism or have been smokers for the past 6 months in addition to no lower extremity injuries. Participants that were allergic to astaxanthin or related substances—such as salmon and algae—were not allowed to participate in the study. Participants were also excluded if they have any of the following: (1) cardiorespiratory and/or metabolic disorders, (2) any known blood disorders (e.g., anemia, hemophilia), (3) any use of prescription medication, tobacco products or dietary supplements that would impact the outcome measures (e.g. vitamins, antioxidant supplements) for at least 6 weeks prior to the beginning of the study. Throughout the study, the participants were asked to minimize each red wine and coffee consumption to <16 oz per day. Participants were screened using a health history questionnaire and a lifestyle questionnaire to ensure they met inclusion criteria. Participants' height and weight were collected using a physician's scale and stadiometer (Detecto, Webb City, MO, USA).

Experimental Procedures

This study was a randomized, counterbalanced crossover, placebo controlled double blind design. The PI had the supplement key but was unaware whether participants were taking supplement 'A' or 'B' during data collection. Other researchers

did not know placebo from astaxanthin but did know whether a participant was taking supplement 'A' or 'B' at the time of data collection. Participants completed two-four week supplementation periods in which each concluded with two testing sessions conducted at the Metabolic and Applied Physiology laboratory at Texas State University. Session one consisted of high fat milkshake ingestion for the purpose of evaluating postprandial oxidative stress. Session two consisted of a graded exercise test, the FatMax (Jeukendrup et al., 2001), in which carbohydrate and fat oxidation rates were measured. Participants completed a 24-hour dietary recall after the first supplementation period prior to both testing sessions, then were asked to follow the same dietary recall at the end of the second supplementation period. The study overview can be seen in Figure 1.

Supplementation

Participants were randomized into one of two groups: placebo first group or astaxanthin first group. A random number generator (random.org) was used with a minimum number of 1 and a maximum number of 100 to randomize the participants. When the number generator produced an odd number, the participant underwent the astaxanthin supplementation period first and placebo supplementation period second. The following participant was given the opposite order—in this case placebo supplementation first then astaxanthin supplementation second. A sealed-unlabeled bottle containing either the placebo or 6mg AstaReal® was given to the respective participants.

The participants consumed either the astaxanthin supplement or the placebo once a day for four weeks. The astaxanthin capsules contained 6 mg of astaxanthin in addition to sunflower oil, while the placebo was color and odor matched with only sunflower oil

inside. Participants were asked to take the supplement once a day for a 4 week period. Participants were reminded periodically throughout the supplementation period to take their supplement. A one week washout period between the treatments was allowed, in which the participants did not take any supplements for a week, to decrease likelihood of the opposing treatment impacting the measures taken. This time period was recommended by the company that provided the supplement, AstaReal.

High Fat Milkshake Ingestion (Test Session One)

The following pre-test instructions were given to the participant: (1) no strenuous exercise 48 hours prior, (2) avoid alcohol ingestion 48 hours prior, (3) avoid caffeine ingestion at least 8 hours prior, (4) and be within eight to 10 hours fasted before testing begins. All testing began between the hours of 6-7 am while participants were still fasting. Session one consisted of an initial blood sample from the participant's antecubital vein. After the first blood sample, the participant consumed the high-fat milkshake within a 15-minute time period. The milkshake contained whole milk, Breyers® "All Natural" vanilla ice cream, and heavy whipping cream. The milkshake consisted of approximately 1.0 g/kg of fat, 1.0 g/kg of carbohydrate, and 0.25 g/kg of protein, making the milkshake total macronutrient count relative to the participant. Following the ingestion of the milkshake, a blood sample was taken at two and four hours postprandial. During this time participants were asked to remain in the ingestion room and work on school work or watch television.

FatMax Testing (Test Session Two)

The second experimental session took place the day after session one. Before the session two, participants were given the same pre-test instructions as the day before, which are the following: (1) refrain from strenuous exercise 48 hours prior, (2) avoid alcohol ingestion 48 hours prior, (3) avoid caffeine ingestion at least 8 hours prior, (4) and be within eight to 10 hours fasted before testing begins. The exercise testing consisted of the participant riding a cycle ergometer (LODE Excalibur Sport, Groningen, Netherlands) to complete a FatMax test (Achten et al., 2002; Jeukendrup & Achten, 2001; Jeukendrup, Saris, Brouns, & Kester, 1996). The test included multiple stages lasting three minutes each. The test began with the participant riding the cycle ergometer at a workload of 50 Watts for one stage. At the end of each stage, the workload increased by 35 Watts until four stages have been completed. After the fourth stage (12_{th} minute of the exercise test), the workload increased by 50 Watts at the end of each stage until volitional exhaustion, in which the participant could no longer pedal. Immediately after the test, the workload of the ergometer was decreased back down to 50 Watts for the participant to complete a cool down. Throughout the test, the participant was connected to a TrueOne 2400 (Parvo Medics, Sandy, UT, USA) metabolic cart with headgear to collect pulmonary data—specifically volume of oxygen consumption ($\dot{V}O_2$), volume of carbon dioxide expulsion ($\dot{V}CO_2$), and respiratory exchange ratio (RER). The participant also wore a chest H10 HR monitor (Polar Electro Inc., Bethpage, NY, USA) throughout the exercise testing to monitor HR. Participants also reported their rate of perceived exertion (RPE) at the end of every minute. At minute three of the first four stages, $\dot{V}O_2$, $\dot{V}CO_2$, and RER were documented. During the cool down period, blood samples were collected via finger prick at two, four, six, and eight minutes after the onset of the

cooldown period for lactate assessment. A lactate analyzer was utilized to acquire the measurements of lactate levels (Nova Biomedical, Waltham, MA, USA). FatMax exercise protocol overview is represented in Figure 2.

Calculations

The $\dot{V}O_2$ and $\dot{V}CO_2$ at the end of the first four stages of the graded exercise test were used to calculate oxidation rates. These equations assume protein oxidation was minimal and did not make significant contributions to energy production during the test. The equations are as follows: ([1.718 x $\dot{V}O_2$] – [1.718 x $\dot{V}CO_2$]) for fat oxidation and ([4.170 x $\dot{V}CO_2$] – [2.965 x $\dot{V}O_2$]) for carbohydrate oxidation (Waldman et al., 2018).

Supplementation Compliance

Compliance was calculated with the following equation: ([soft gels ingested]/28 x 100). The same equation was be used for each supplemental period. Participants must have maintained a compliance rate of 80%, meaning they could only miss 3 days of soft gel ingestion per supplementation period. Any participant that did not have 80% compliance was removed from the study.

At the end of the second four-week period, the participant came back in to complete second milkshake session then exercise session, mimicking the protocols at the end of the first four-week period. Participants were also asked to follow the 24-hour dietary recall that they previously completed during the first round of testing. Compliance was measured by counting the amount of placebo or astaxanthin soft gels left in the bottle at the end of the four-week supplementation period.

Blood Sampling & Treatment

A total of 21 mL was collected at each blood sample on the milkshake consumption day. Blood samples were collected using a 21G butterfly needle and blood was collected into three, sealed 7mL sodium heparin vacutainers. Whole blood was treated by taking 700 uL of blood and mixing it with 700 uL of 5% 5-sulfosalicylic acid solution (Sigma Aldrich, St. Louis, MO, USA) which was allowed to sit in 5-8 ºC for 10 minutes. The mixture was then centrifuged at 10,000 x g at 4 ºC for 10 minutes. The aliquot was stored at -80 ºC until ready for analysis. Whole blood samples were separately centrifuged for 15 minutes at 1,500 rpm. Plasma was aliquoted and stored at -80 ºC until analysis.

For finger prick sampling procedures, a self-retracting safety lancet was used to puncture the lateral aspect of an index or middle finger after cleaning the area with an alcohol swab. A small sample of ~ 25uL was collected to analyze lactate levels (Lactate Plus, Nova Biomedical, Waltham, MA, USA). Gauze was provided to apply pressure to the puncture site. After all samples were collected, a bandage was placed on the puncture site(s).

Blood Analysis

The following measures were analyzed utilizing plasma samples: hydrogen peroxide (H_2O_2) (Invitrogen, Carlsbad, CA, USA), total triacylglycerols (TAG) (Pointe, Canton, MI, USA), and advanced oxidation protein products (AOPP) (Cell BioLabs, San Diego, CA, USA) per the assay instructions. GSH was analyzed utilizing the whole blood

aliquot that had been treated with 5% 5-sulfosalicylic acid solution ((Sigma-Aldrich, St. Louis, MO, USA). An EPOCH 2 microplate reader (BioTech, Winooski, VT, USA) was utilized to read absorbance.

Statistical Analysis

Lactate, CHO and fat oxidation rates, and RER were analyzed using a 2 x 4 (treatment x stage) repeated measures ANOVA (SAS, Cary, NC, USA). Measures of H_2O_2, GSH, AOPP, MDA, and TAG 2 x 3 (treatment x timepoint) repeated measures ANOVA. When a main effect was significant ($p < 0.05$) Fisher's Least Significant Difference post hoc test was conducted to compare means.

IV. RESULTS

Participant Compliance

All data are reported as mean ±SD unless otherwise noted. Macronutrient distribution associated with milkshake ingestion is shown in Table 1. Participant demographics and compliance are represented in Table 2. Participant compliance for the postprandial oxidative stress portion of the study (n = 13) was 95% ± 6.5, while for the FatMax portion (n = 13) the compliance was 92% ± 6.5. Compliance differs between the sessions due to one participant from the postprandial oxidative stress testing not participating in the FatMax test and vice versa. One subject was removed from the study due to 77% compliance during the placebo supplementation period and is not included in any statistical analysis.

Postprandial Blood Markers

There was no treatment x timepoint interaction for TAG ($F = 0.02$, $p = 0.98$) or a main effect for treatment ($F = 0.0$, $p = 0.95$), while there was a significant increase in TAG levels over time noted by a main effect ($F = 15.80$, $p < 0.01$) with 2 and 4 hours post ingestion being significantly higher than pre ingestion TAG levels ($p < 0.01$). Mean TAG levels are shown in Figure 3.

In terms of GSH, while there was no treatment x timepoint interaction ($F = 0.66$, $p = 0.52$) or main effect for time ($F = 2.10$, $p = 0.13$); however,, the main effect for treatment did approach significance ($F = 3.67$, $p = 0.06$). Mean GSH levels are represented in Figure 4.

Furthermore, in relation to H_2O_2, a treatment x timepoint interaction ($F = 5.94$, $p < 0.01$) was noted. H_2O_2 levels were significantly lower ($p < 0.05$) 2 and 4 hours post

ingestion following astaxanthin supplementation when compared to the placebo treatment as displayed in Figure 5.

Regarding AOPP, there was no treatment x timepoint interaction ($F = 0.97$, $p = 0.39$) or a significant main effect for treatment ($F = 0.15$, $p = 0.70$), while there was a main effect for time ($F = 17.14$, $p < 0.01$) with significantly higher levels of AOPP at two and four hours postprandial compared to pre ingestion ($p < 0.01$) as represented in Figure 6.

In regard to MDA, there was no treatment x timepoint interaction ($F = 0.05$, $p = 0.94$) or a significant main effect for treatment ($F = 2.80$, $p = 0.10$), but a main effect for time was noted ($F= 24.66$, $p < 0.01$) with significantly higher ($p < 0.01$) MDA levels 2 and 4 hours post ingestion when compared to pre ingestion levels as displayed in Figure 7.

FatMax Test

There was no treatment x stage interaction ($F = 0.51$, $p = 0.67$) or significant main effect for treatment ($F = 0.84$, $p = 0.36$) noted for CHO oxidation rates, but there was a main effect for stage ($F = 202.43$, $p < 0.01$) where a significant increase occurred at each stage ($p < 0.01$) as represented in Figure 8.

In terms of mean fat oxidation rates, there was no treatment x stage interaction ($F = 0.34$, $p = 0.80$) or a significant main effect for treatment ($F = 0.24$, $p = 0.63$), while there was a main effect noted for stages ($F = 23.41$, $p < 0.01$). Fat oxidation rates significantly decreased ($p < 0.01$) at each stage as shown in Figure 9.

In terms of RER, there was no treatment x stage interaction ($F = 1.29$, $p = 0.28$) or a main effect for treatment ($F = 1.11$, $p = 0.29$) observed, but there was a main effect

between stages ($F = 96.09$, $p < 0.01$) noted with each stage being significantly higher than any of the previous stages. RER is represented in Figure 10.

Regarding lactate, there was no treatment x stage interaction ($F = 0.43$, $p = 0.74$) noted nor a significant main effect for treatment ($F = 1.11$, $p = 0.30$), while there was a main effect for time ($F = 8.63$, $p < 0.01$). Lactate levels 2 and 4 minutes post exercise were not significantly different. Lactate levels 6 and 8 minutes post exercise were not significantly different. Lactate levels 6 and 8 minutes post exercise were significantly lower than 2 and 4 minutes post exercise. Lactate means are displayed in Figure 11.

V. DISCUSSION

Four weeks of astaxanthin supplementation demonstrated a decrease in postprandial oxidant levels in healthy males. These findings suggest astaxanthin can assist in the mitigation of oxidative stress in multiple scenarios—whether it be chronic in the case of obese and sedentary individuals, or acute in the case of high fat meal ingestion. The reduction of oxidative stress would contribute to a decreased risk of the development of cardiometabolic diseases for even healthy populations.

It should be noted although the participants in the present study have a body mass index (BMI) that would put them in the 'overweight' category, BMI is not a measurement of body composition and can cause false classification of individuals being overweight due to higher fat free mass. The lack of body composition measurement in this study could be seen as a limitation.

Postprandial Oxidative Stress

The findings from the current study suggest astaxanthin can significantly contribute to a decrease in postprandial hyperlipidemia induced pro-oxidant levels. TAG levels were consistent between the astaxanthin and placebo treatments following the high fat meal ingestion, suggesting the milkshake induced similar hyperlipidemia after each treatment. The current study also demonstrated an increase in biomarkers of ROS and oxidative stress—AOPP, MDA, H_2O_2 in both treatments. High fat meals are commonly used to promote oxidative stress (Bloomer et al., 2010; Esser et al., 2013; Gabriel et al., 2012; McCarthy et al., 2013; Pruimboom et al., 2015; Tsai et al., 2004; Yang et al., 2018). Additionally, research indicates postprandial lipemia induces greater levels of oxidative stress than strenuous exercise (Bloomer et al., 2010; Esser et al., 2013; Gabriel

et al., 2012; McCarthy et al., 2013; Pruimboom et al., 2015; Tsai et al., 2004; Yang et al., 2018). It is important to note the current study generated high levels of postprandial oxidative stress without impacting antioxidant levels, which allowed for the proper redox environment to assess astaxanthin's effects on oxidative stress. Additionally, the current study is in line with previous work regarding meals with greater than 60g of fat cause significant increases in oxidative stress and TAG levels, in even healthy individuals (Bloomer et. al, 2010).

Previous research suggests the reasoning behind astaxanthin decreasing oxidative stress is primarily due to attenuation of lipid peroxidation, which is associated with lower MDA levels (Goto et al, 2001; Choi et al. 2011). The current study, however, did not result in reductions in MDA. These contradicting findings with Goto et al. could be attributed to in vitro methodology (Goto et al., 2001). Although Choi et al. did measure MDA levels in humans, the population observed is categorized as overweight and/or obese (Choi et al., 2011). Choi et al. found MDA levels decrease over 3 weeks with astaxanthin supplementation, and that MDA levels in the obese and overweight group are higher than healthy adults (Choi et al., 2001). Because the current study involved testing in healthy adults, it is possible there is not much room for improvement in MDA as healthy individuals typically demonstrate lower levels of oxidative markers compared to overweight/obese individuals (Choi et al., 2001). To illustrate, MDA in the obese population after 3 weeks of supplementation decreased to ~1.7 μM (Choi et al., 2001) while the current study's participants resting-fasted levels were closer to 1μM with both treatments. Furthermore, Choi et al. measured levels of 15‐Isoprostane F_{2t} (ISP), which is a biomarker of degree of lipid peroxidation (Michel et al. 2008). The study found ISP

levels to not only decrease over three weeks of supplementation, but to also reach levels lower than the healthy population. It is therefore possible that even though MDA did not decrease, other markers of lipid peroxidation could have decreased. Because of this, the lack of measurement of ISP can be viewed as a limitation in the current study. The inconsistencies in methodologies likely explain the lack of reduction in postprandial MDA. Furthermore, the present study measured compliance by counting the supplement returned to researchers by the participants. It is possible participants threw away some of their supplement versus having taken it on certain days, giving the impression of compliance.

Additionally, the current study did not display decreases in AOPP levels. This could be a result of the current study's methodology. The present study collected biological samples over a 4-hour postprandial period, as most postprandial oxidative stress research does, some previous studies measured changes in oxidative stress markers 6 hours postprandial (Melton et al, 2009). Melton et al. observed postprandial oxidative stress in pre-diabetic women, and some markers were significantly different at 4 hours while others only became significantly different at 6 hours (Melton et al. 2009). Because of a decrease in AOPP with the astaxanthin treatment 2 to 4 hours postprandial, it is possible it would continue to decrease from 4 to 6 hours postprandial and become significant. Additionally, it is possible this study did not have these findings with AOPP due to again the population that underwent testing were healthy males that do not have unhealthy baseline levels of AOPP present. Choi et al. found healthy individuals have lower fasted-resting oxidative stress markers than obese individuals, and even with three weeks of astaxanthin supplementation causing decreases in their fasted-resting oxidative

stress markers, the decreases did not always result in lower levels than the healthy population. Despite healthy individuals getting potentially less benefits than unhealthy individuals, any chance of mitigating oxidative stress should be investigated due to its contribution to the development of many chronic diseases.

Although astaxanthin did not cause decreases in oxidative stress markers which as reported in previous research both in vitro and in vivo with obese, overweight, and normal weight individuals (Choi et al., 2001; Goto et al., 2001), the current study did exhibit decreased postprandial H_2O_2 levels. This could potentially be due to the ability of astaxanthin to span across the phospholipid bilayer of the cell, decreasing the ability for H_2O_2 to enter the plasma and cause oxidation of surrounding tissues (Hussein et al., 2006; Kidd, 2011). Hydrogen peroxide, though, is not the only ROS that causes oxidation of surrounding tissues (Heussein et al., 2006). The current study did not measure superoxide anion, peroxynitrite anion, or hydroxyl radicals. The non-measured analytes could potentially be the contributing factors of increased presence of oxidative stress, as measured with MDA and AOPP. There are a number of oxidative stress markers that were not measured, such as 8-oxo-2' deoxyguanosine, 8-hydroxydeoxyguanosine, acrolein, ascorbic acid, and ISP, which may have demonstrated changes due to potential antioxidant activity of astaxanthin (Lowe, 2014). However, the biomarkers analyzed are typically what are measured in research observing postprandial oxidative stress (Bloomer et al., 2009; Bloomer et al., 2012; Bloomer et al., 2013) while the others are typically measured in studies that are more interested in aging.

Although a previous study found astaxanthin to cause increases in endogenous antioxidants the findings for this study did not reflect those same results (Choi et al,

2011). There are a number of endogenous antioxidants that were unmeasured in the current study, such as SOD, CAT, and glutathione S-transferases, which could be seen as a limitation. It is also important to note the variation in results between Choi et al. and the present study could be attributed to the population differences, as Choi et al. studied obese and overweight individuals while the current study looked at healthy young males. The current study produced a change in GSH, but the change of ~ 6% did not reach statistical significance. Additionally, the lack of standardized dietary protocol prior to sample collection in Choi et al. could have also contributed to differing results from the current study. According to Arablou et al., the meals you eat the day prior to measuring antioxidants can impact the measured biomarkers (Arablou et al., 2019). Further, it is unclear whether or not Choi et al. collected fasted or non-fasted blood samples. Participants in the current study were at least 8 hours fasted, which could also contribute to having different findings than previous research. Because of these findings, the meals participants consumed in Choi et al. could have impacted the amount of antioxidants present (Arablou et al, 2019), whereas the present study took this into account and asked participants to follow the same diet 24 hours prior to testing days. This increase in endogenous antioxidants would lead to greater defense against oxidative stress. This would be meaningful in populations in which oxidative stress is consistently high or induced regularly such as smokers (Carnevale et al., 2016; Ellegaard et al., 2016), males (Borrás et al., 2003; Tiidus et al., 1998; Viña et al., 2006), obese individuals (Huang et al., 2015), individuals with T2D (Sottero et al., 2015; Wei et al., 2009), and sedentary people (Pruimboom et al., 2015). Further research is warranted to observe the effect of astaxanthin on postprandial oxidative stress in obese and less fit populations. It is worth

mentioning the current study observed favorable improvements in a ROS biomarker in an already healthy population. Research on individuals at a higher risk for chronic oxidative stress, such as those with cardiometabolic diseases, is needed examine the antioxidative properties of astaxanthin.

Oxidation Rates

The present results found no change in oxidation rates, which contradicts previous research (Aoi et al., 2008; Ikeuchi et al., 2006). However, most of the previous research was conducted in rodent models (Aio et al., 2008; Ikeuchi et al., 2006). Regarding human trials, Earnest et al. studied substrate utilization rates in a population of amateur endurance athletes that were exercising at a single intensity (Earnest et al., 2011). Even though the methods and participants differed from the current study, the findings were consistent with the present study. However, the current study attempted to expand upon these findings by examining oxidation rates across a range of intensities as completed in other studies (Jeukendrup et al., 2001; San Millan et al, 2016). Previous research by San Millan et al. reports different training levels typically result in different oxidation rates, and even with these differences between populations (San Millan et al., 2016), neither Earnest et al. or the present study found changes in oxidation rates (Earnest et al., 2011). Although the current study's participants were less physically fit than in Earnest et al., it is possible changes could have been observed if the population was sedentary. San-Millan et al. explains unhealthy individuals have metabolic inflexibility, which leaves more room for improvements in oxidation rates (San-Millan, 2016). Additionally, Özgünen et al. reveals during a FatMax test, it is preferable to take data from earlier during each stage, rather than later (Özgünen, Özdemir, Korkmaz-Eryılmaz, Kılcı,

Günaştı, & Kurdak, 2019). Özgünen et al. found when the later portion of the FatMax stages are utilized for oxidation rates, it is significantly lower than what the true fat oxidation rate(Özgünen et al., 2019). This study also used different oxidation rate equations than the current study (Özgünen et al., 2019). Although, this data were published after data collection was completed in addition to being inconsistent with established FatMax protocols used in previous research in the past (Jeukendrup et al., 2001). Furthermore, the ParvoMedics TrueOne 2400 metabolic analyzer used in the current study is not as sensitive as other equipment, such as the MOXUS or Cosmed (Jeukendrup et al., 2001; Özgünen et al., 2019), which makes it more difficult to measure minute changes in oxidation rates.

Furthermore, the lack of change in oxidation rates were also associated with no change in lactate levels post FatMax testing (Meltzer, 2011). A review by Melzer examines the relationship between lactate production and macronutrient utilization, stating as reliance on carbohydrates increase, production of lactate increases along with it (Meltzer, 2011). Further studies need to be conducted with different metabolic analyzers that may be more sensitive to the changes in oxidation rates to validate this theory.

VI. CONCLUSION

Four weeks of astaxanthin supplementation was able to cause improvements in postprandial ROS levels without changes in endogenous antioxidant levels. However, no change in oxidative stress markers were demonstrated. This could be in part due to only observing 4 hours postprandial versus 6 hours postprandial, in addition to only looking at two biomarkers of oxidative stress. Further, astaxanthin supplementation did not cause any changes in oxidation rates during a FatMax test or lactate levels post exercise testing. This could be due to the metabolic analyzers not being ideal to calculate oxidation rates. The findings from the current study could potentially benefit populations at high risk for oxidative stress, such as cigarette smokers, obese populations, males, and sedentary individuals, due to its demonstrated ability to cause decreases in ROS postprandial. Further research is needed to assess astaxanthin's impact on antioxidative capacity and oxidation rates with larger population sizes, less active populations, differing biomarkers of oxidative stress, and a greater postprandial window.

VII. FIGURES

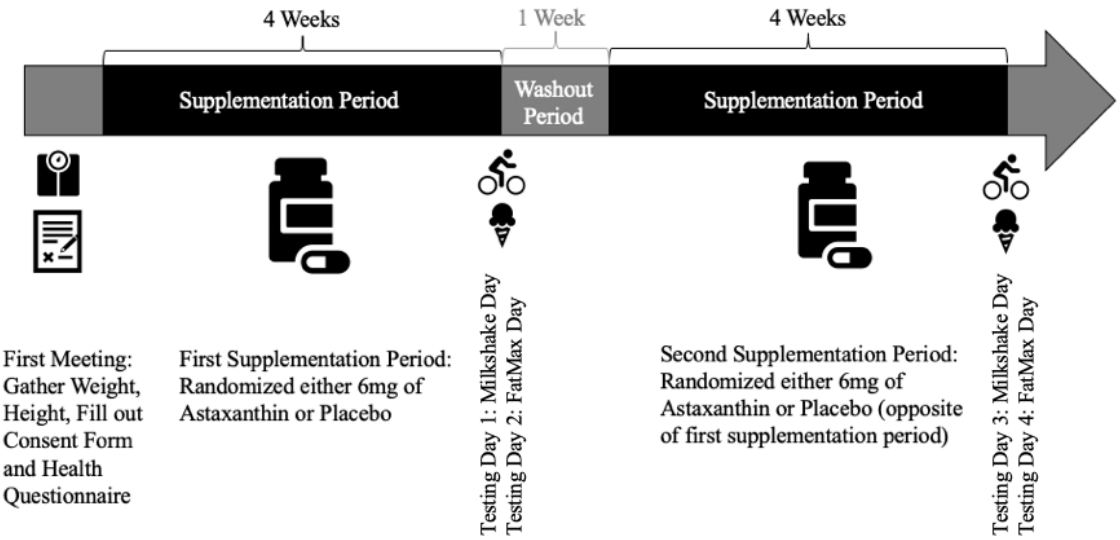

Figure 1. Study Timing

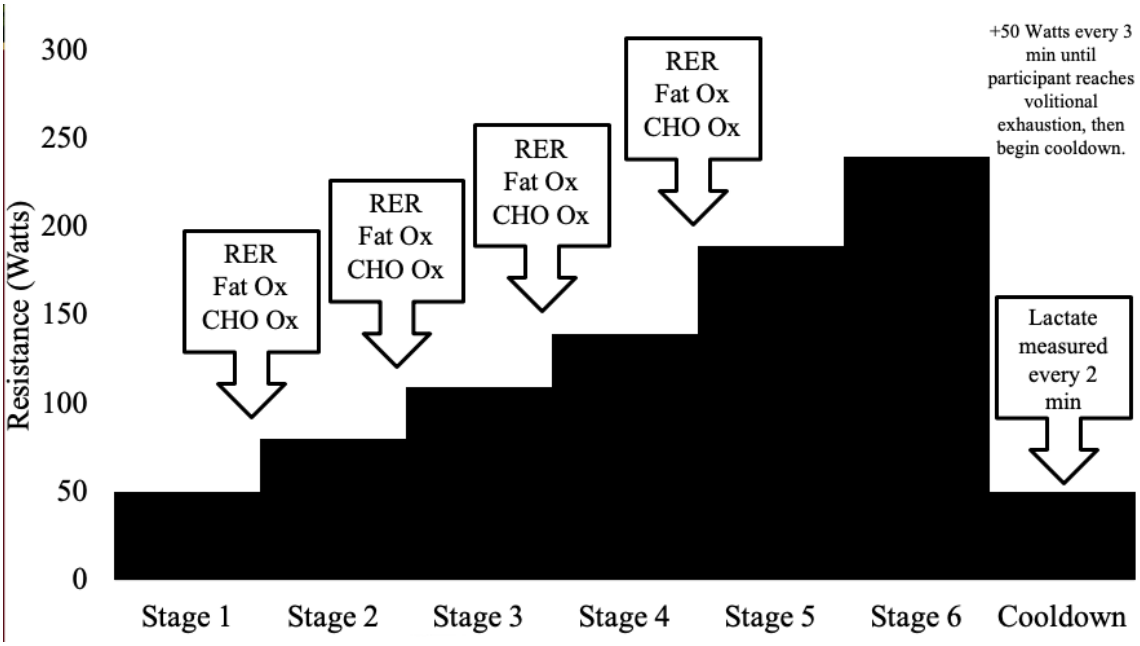

Figure 2: FatMax Protocol

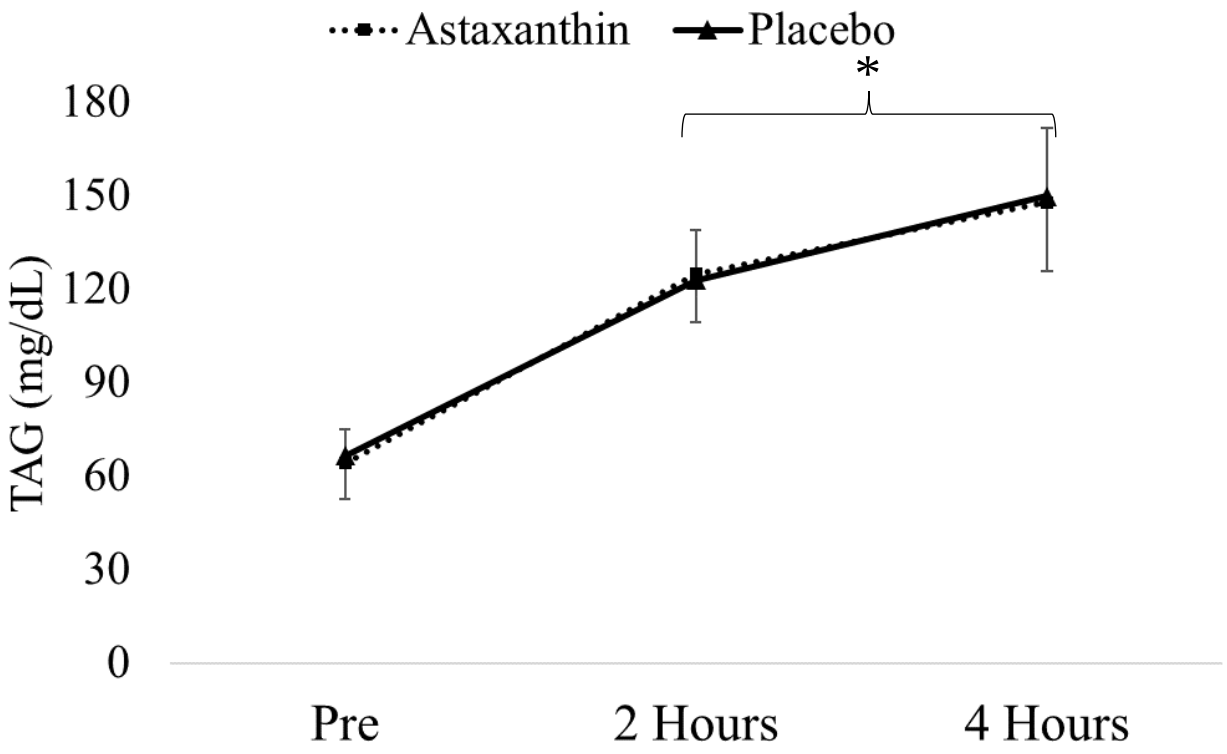

Figure 3: TAG Levels. TAG = Triacylglycerol. Plasma TAG levels prior to as well as two and four hours postprandial. * Denotes a significant increase ($p < 0.01$) from pre-levels. Data are reported as mean ± SE.

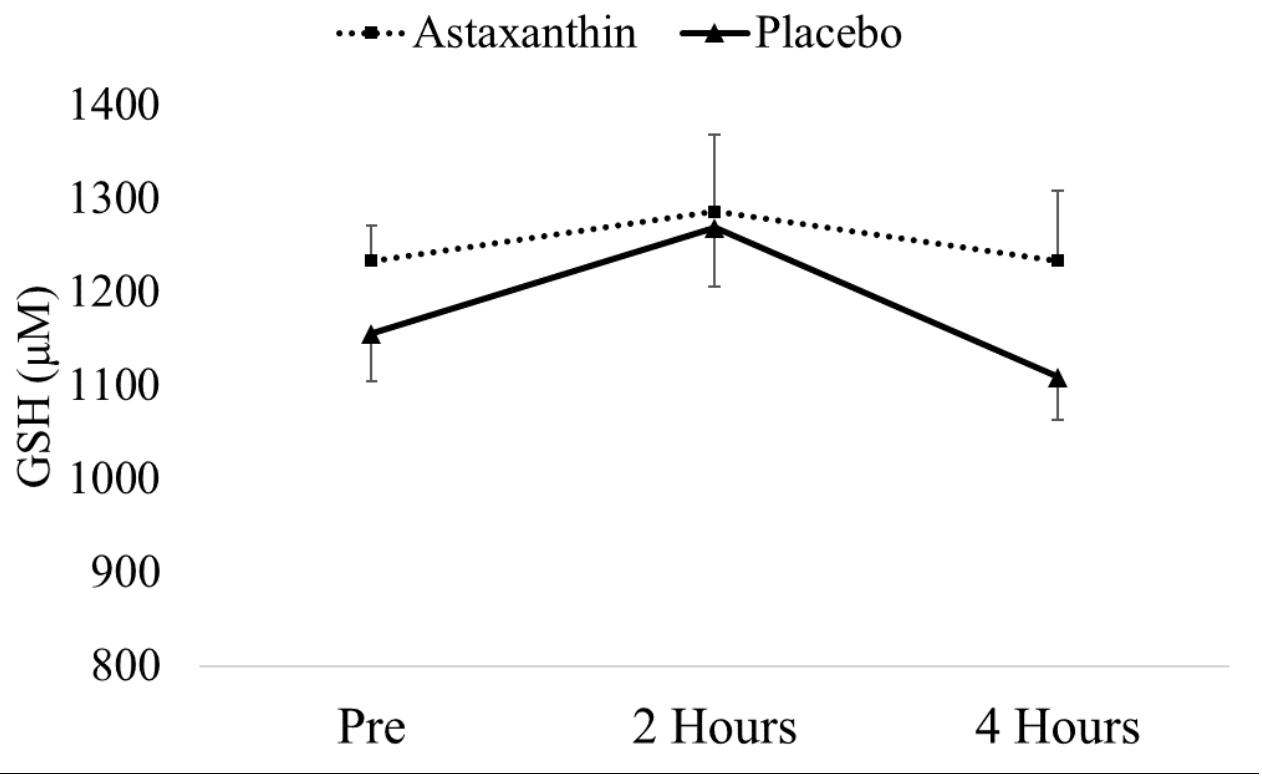

Figure 4: GSH Levels. GSH = Glutathione. Whole blood GSH levels prior to as well as two and four hours postprandial. Data are reported as mean ± SE.

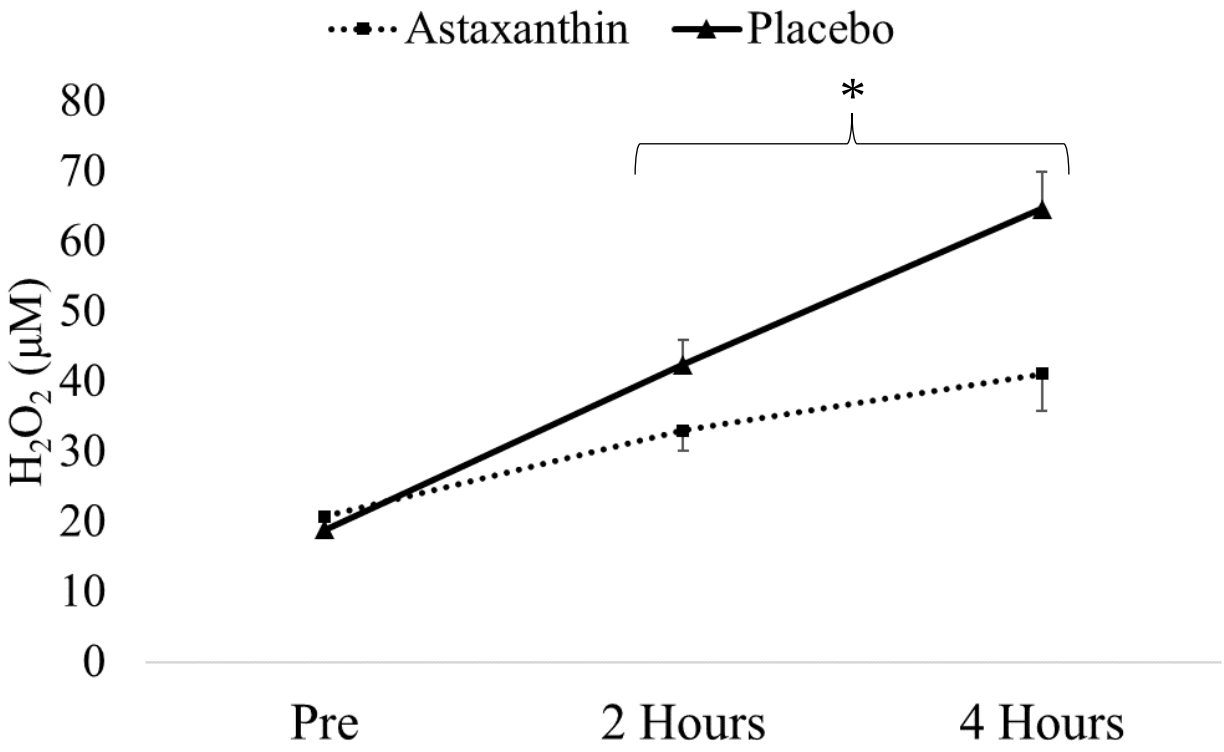

Figure 5: H_2O_2 Levels. H_2O_2 = Hydrogen Peroxide. H_2O_2 levels prior to as well as two and four hours postprandial. *Denotes significantly lower ($p < 0.05$) H_2O_2 levels with the astaxanthin treatment. Data are reported as mean ±SE.

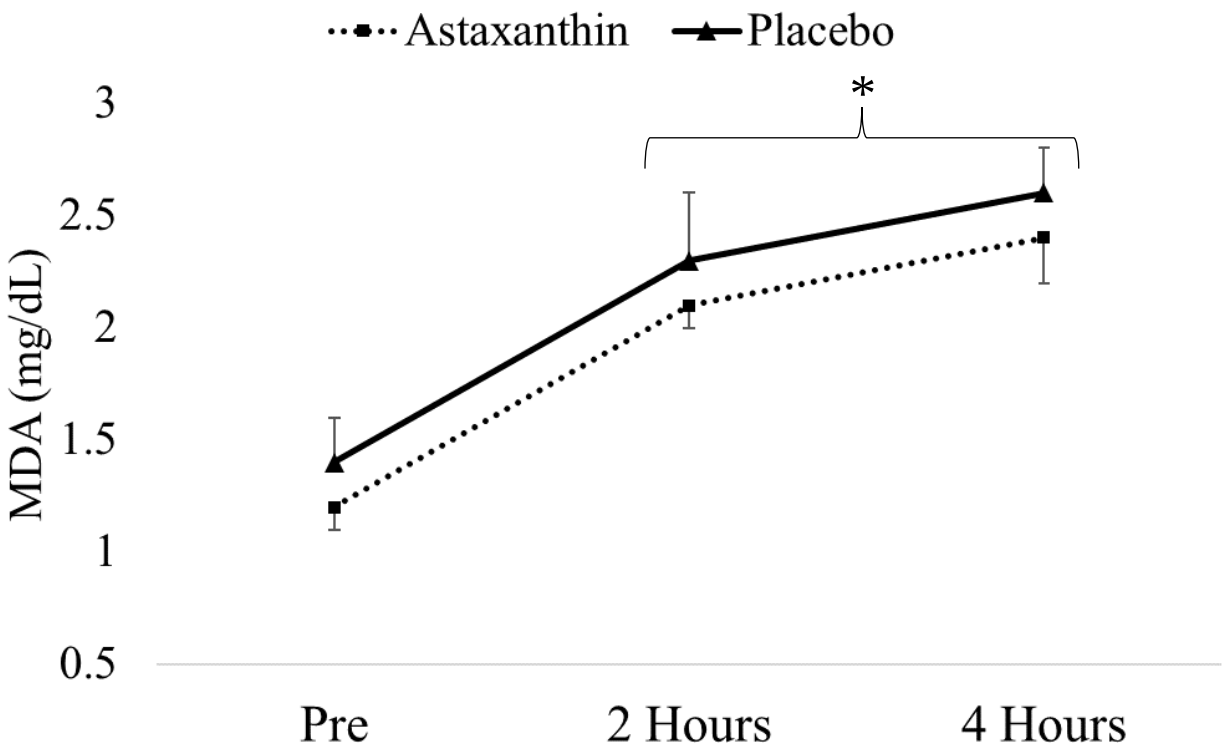

Figure 6: MDA Levels. MDA = Malondialdehyde. MDA levels prior to as well as two and four hours postprandial. *Denotes a significant increase ($p < 0.01$) from pre-levels. Data are reported as mean ±SE.

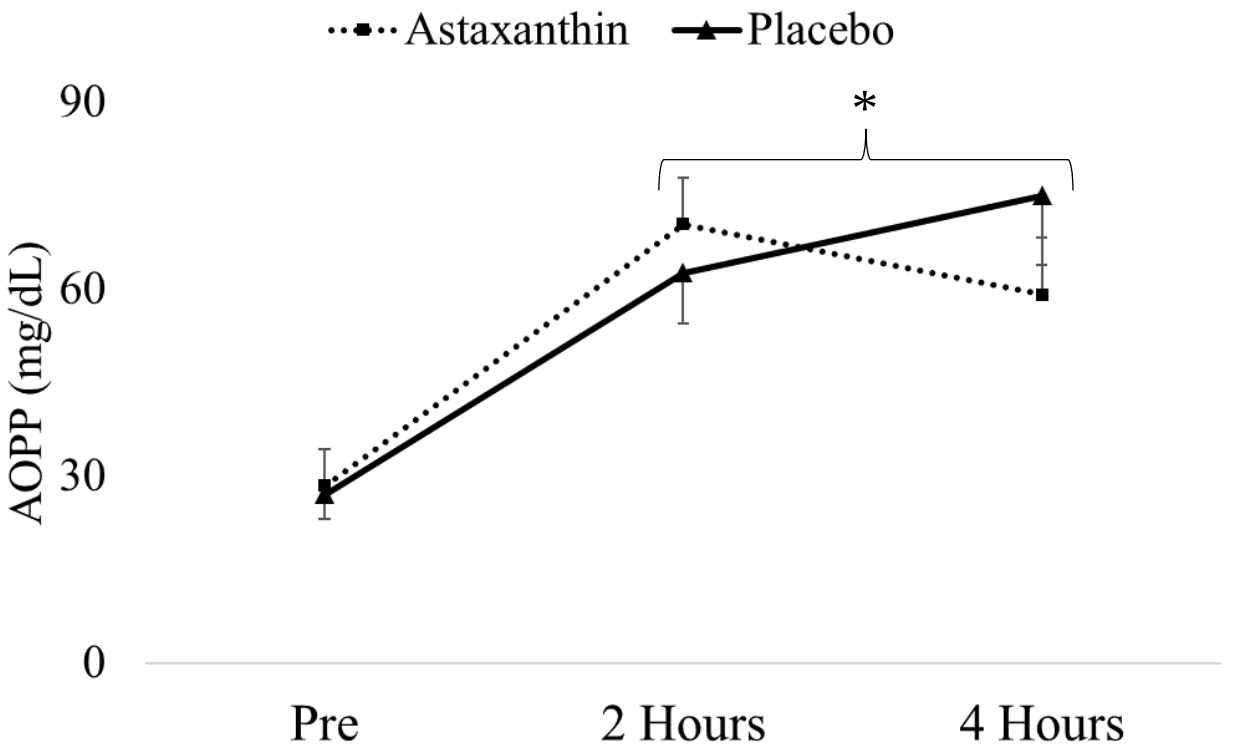

Figure 7: AOPP Levels. AOPP = Advanced Oxidative Protein Products. AOPP levels prior to as well as two and four hours postprandial. *Denotes a significant increase ($p < 0.01$) from pre-levels. Data are reported as mean ±SE.

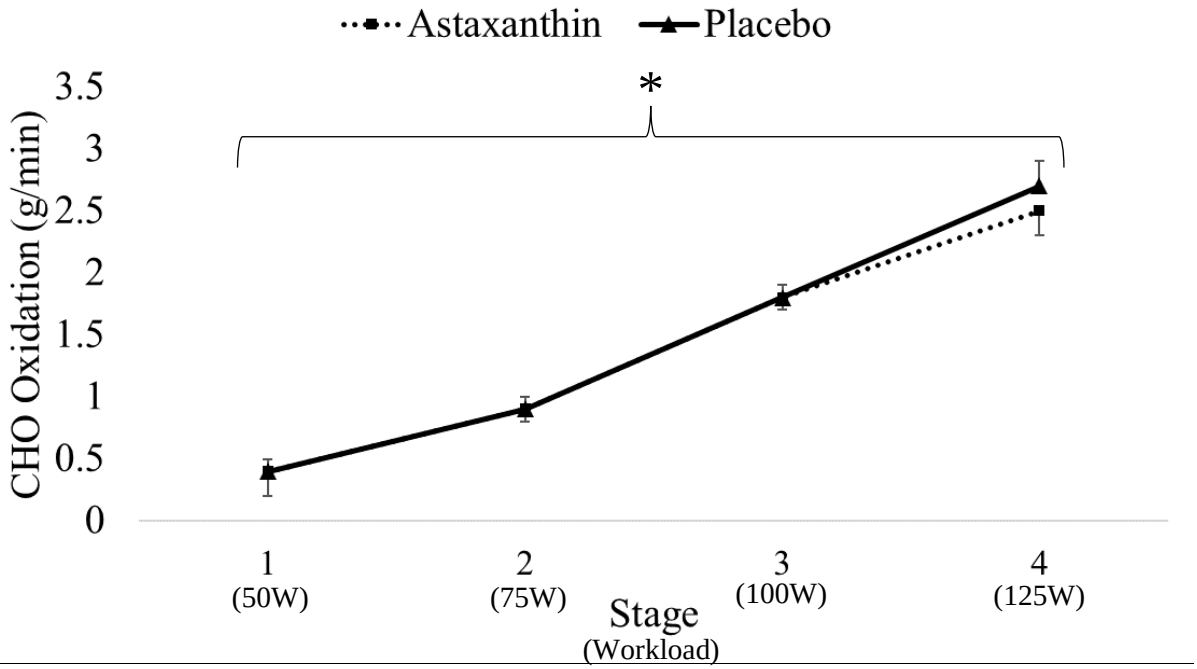

Figure 8: CHO Oxidation. CHO = carbohydrate. CHO oxidation rates at stages 1-4. * Denotes a significant increase ($p < 0.01$) at each progressive stage. Data are reported as mean ±SE

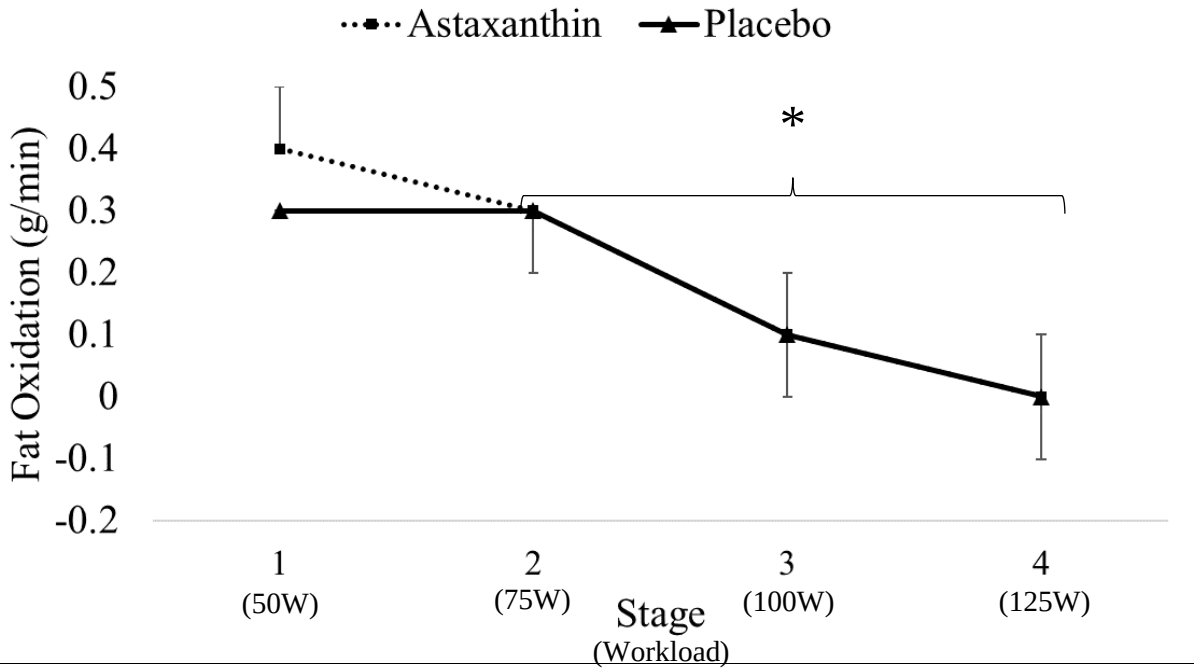

Figure 9: Fat Oxidation. Fat oxidation rates at stages 1-4. * Denotes a significant decrease ($p < 0.01$) from stage one. Data are reported as mean ±SE.

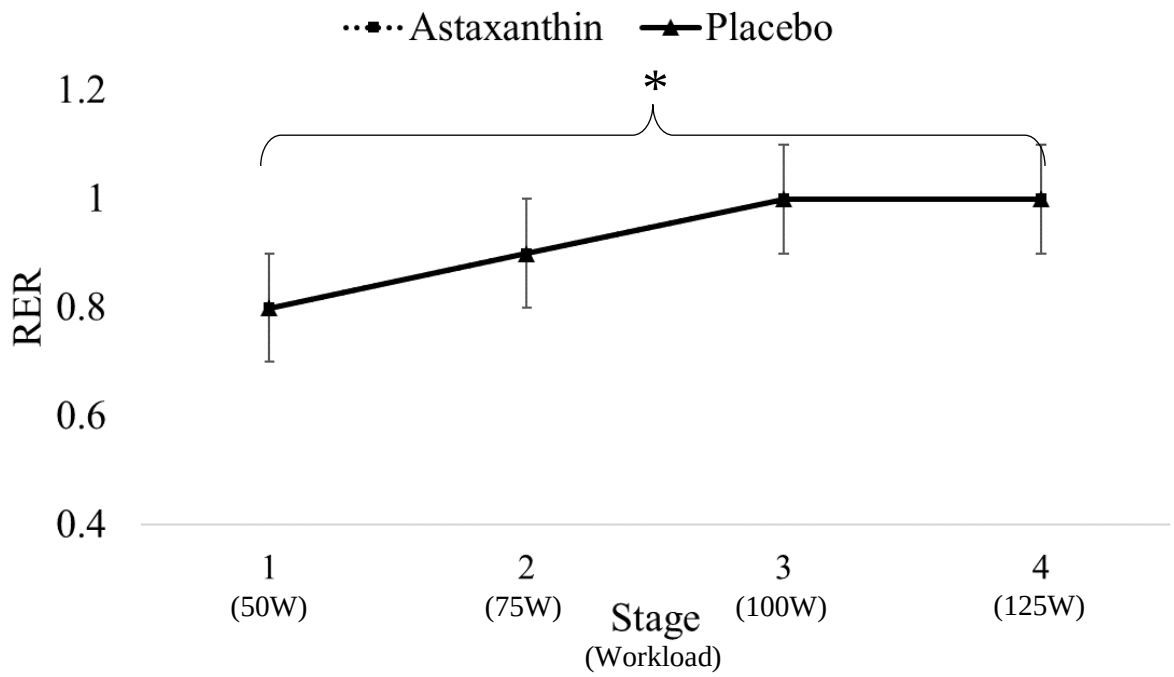

Figure 10: RER. RER = Respiratory Exchange Ratio. RER stages 1-4 during the FatMax test. * Denotes a significant increase ($p < 0.01$) at each stage. Data are reported as mean ±SE.

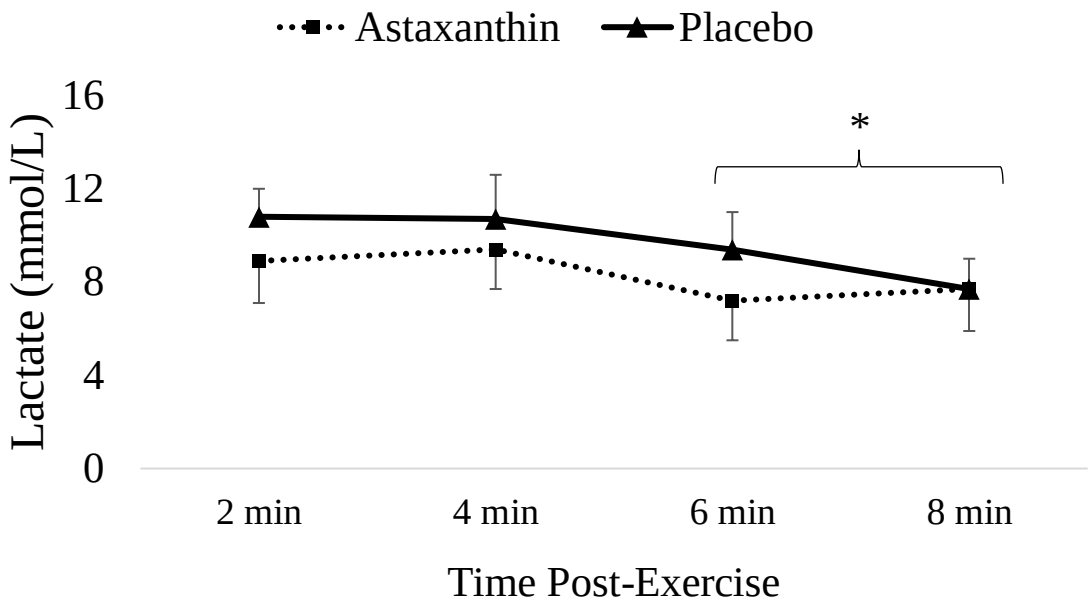

Figure 11: Lactate Levels. Lactate levels two, four, six, and eight min post FatMax test at 50 Watts on the cycle ergometer. * Denotes a significant decrease ($p < 0.05$) from timepoints 2 and 4 minutes post-exercise. Note there was no difference between min 6 and 8, and min 2 and 4 are not different ($p > 0.05$). Data are as mean ±SE.

VIII. TABLES

Table 1: Milkshake Macronutrient Distribution.

Macronutrients	Mean Content (g)
Carbohydrate	84 ± 14.5
Fat	81 ± 13.3
Protein	26 ± 4.8

Distribution of macronutrients in the high fat milkshakes given to participants. All values are reported as mean ±SD**.**

Table 2: Participant Demographics.

	Postprandial Oxidative Stress, (N = 13)	**FatMax, (N = 13)**
Age (years)	23.7 ± 2.7	23.7 ± 2.7
Height (cm)	171.7 ± 5.9	172.2 ± 4.9
Weight (kg)	79.8 ± 12.6	80.8 ± 12.7
BMI (kg/m2)	27.1	27.2
Compliance (%)	95 ± 6.5	92 ± 6.5

Participant demographics and compliance calculated in accordance to the session in which they were involved. All values are reported as mean ± SD**.** Note mean ± SD differs between sessions despite no change in N.

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