THE EFFECTS OF WHOLE PUREED MANGO INTAKE ON SERUM BIOMARKERS OF MUSCLE DAMAGE IN RESPONSE TO MODERATE INTENSITY AEROBIC EXERCISE IN SEDENTARY ADULTS

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ABSTRACT

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Objective: The aim of this study was to examine the effects of whole pureed mango on cycling performance and biomarkers of muscle damage following exhaustive exercise in healthy sedentary adults.

Methods: A 10 week randomized, placebo-controlled cross-over study was conducted. Participants consumed mango puree or placebo daily for 4 weeks each. An exhaustive exercise trial was performed following each treatment. Differences in biomarkers of muscle damage and performance were analyzed.

Results: Twenty-five participants completed each treatment. There was a significant difference in Lactate Dehydrogenase (LDH) within the placebo group and in Myoglobin (MG) and cycling time at given wattage within both groups. There were no significant differences between treatment groups. There was a non-significant trend of reduced muscle damage biomarkers in the mango group.

Conclusions: Findings from this study do not support the hypothesis that consuming whole mango improves performance and reduces post-exercise biomarkers of muscle damage in sedentary adults.

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CHAPTER I

INTRODUCTION

The World Health Organization (WHO) recommends that adults engage in at least 150-300 minutes of moderate intensity aerobic physical activity each week (WHO, 2020). As of 2020, nearly one in three adults globally did not meet these recommendations (Park et al., 2020). According to the CDC, at least 15% of adults in each of the US states and territories were not physically active in 2020 (CDC, 2020). The detriments of inadequate physical activity have been well researched and include greater risk for all-cause mortality, cardiovascular disease (CVD), type 2 diabetes mellitus (DM), hypertension (HTN), and certain cancers (breast, colon, colorectal, endometrial, and epithelial ovarian) (Park et al., 2020). In contrast, physical activity is associated with a decreased risk of all-cause mortality and incidence of CVD, HTN, and DM (WHO, 2020). This includes low intensities, indicating decreased risk of several chronic conditions with even the slightest increase in physical activity (WHO, 2020).

Despite the health-promoting factors of physical activity, single bouts of exhaustive exercise can result in muscle damage, which may promote the production of reactive oxygen species (Tanabe et al., 2021). Such damage is indicated by elevated serum levels of creatine kinase and lactate dehydrogenase (Callegari et al., 2017). At moderate intensities, acute aerobic exercise can alter cytokine production and promote a pro-inflammatory state (Tanabe et al., 2021). In addition, exercise-induced muscle damage (EIMD) promotes the loss of muscle function and increases soreness (Tanabe et al., 2021). It is, therefore, of interest to investigate interventions to reduce the rise in EIMD as a means to attenuate muscle damage, improve subsequent exercise performance, and shorten recovery time following aerobic exercise.

Current dietary and non-dietary strategies to mitigate the effects of EIMD are of interest for competitive athletes and the general health-pursuing public. Strategies with the most supportive evidence include massage, compression garments, cold water immersion, and polyphenol intake (Martin-Rincon et al., 2020). Polyphenols are plant compounds found in many fruits and vegetables. They have been shown to have anti-inflammatory and free-radical scavenging properties (Martin-Rincon et al., 2020). Current research supports the consumption of cherries, blueberries, black currant, pomegranate, and cocoa for improving biomarkers of oxidative damage and inflammation (Bowtell & Kelly, 2019; Martin-Rincon et al., 2020). However, the dose, duration, mode and exercise intensity at which they are beneficial is still to be determined. Additionally, evidence on the role of polyphenols on exercise performance and biomarkers of muscle damage following exercise is limited. Current research is also limited to the use of fruit derived polyphenol rich extracts and juices, rather than whole fruit.

Mangoes are a plentiful fruit, with high polyphenol concentrations, specifically flavonoids. Several flavonoids are present in mangoes in significant quantities, including gallic acid, quercetin, catechins, and anthocyanins (Lauricella, et al., 2017). These are most known for possessing antioxidant, anti-inflammatory, and anticarcinogenic properties. Beyond the flavonoid content, mangoes have many health promoting components, including both micro- and macronutrients (Lauricella, et al., 2017). In combination with their sweet taste and refreshing sensation, mangoes may provide similar benefits of polyphenol extracts while remaining accessible, affordable, and versatile.

Due to their large nutritional value, this study will examine the effects of whole pureed mangoes on exercise performance and post-exercise biomarkers of muscle damage in sedentary, healthy participants. The exercise trial consisted of a 1-hour cycle at moderate-high intensity to

provide appropriate stimulus for inducing muscle damage. To our knowledge, no published research has been conducted on the impact of whole pureed mango in this setting and population.

Hypothesis and Specific Aims

Hypothesis: The main hypothesis for this study is that 250 grams of whole mango puree drink daily over a 28-day feeding duration will reduce the elevated levels of muscle damage biomarkers following exhaustive exercise in sedentary, normal weight young adults.

Specific Aim 1: To observe the effects of 28-days of whole mango puree (250 mg/day) in comparison to an isoenergetic placebo drink on biomarkers of exercise-induced muscle damage in sedentary adults.

Specific Aim 2: To observe the effects of 28-days of whole mango puree (250 mg/day) in comparison to an isoenergetic placebo drink on ability to maintain 65% of peak power on a bike for a 1-hour period. If unable to maintain 65% peak power, then to observe the timing and number of decreases in wattage needed to complete the 1-hour bike trial.

CHAPTER II

REVIEW OF THE LITERATURE

Physical Activity Guidelines and Prevalence

The World Health Organization (WHO) recommends that adults, ages 18 to 64 years, engage in at least 150-300 minutes of moderate-intensity or 75-150 minutes of vigorous-intensity aerobic physical activity, in addition to two or more days of strength training weekly (WHO, 2020). Physical activity is defined as any bodily movement that requires energy produced by the skeletal muscles. Aerobic physical activity, also called endurance activity, is "activity in which the body's large muscles move in a rhythmic manner for a sustained period of time" (WHO, 2020). Examples of predominantly aerobic physical activity include walking, jogging, swimming, cycling, and rowing, among others. The intensity with which one performs physical activity can be identified by the rate of perceived exertion (RPE) on a scale of 0-10; this determines how long is needed to meet the recommendations. Moderate-intensity physical activity falls between a 5 or 6 RPE, while vigorous physical activity falls between a 7 or 8 RPE. Anaerobic physical activity is characterized by brief intense bursts of movement where oxygen demand exceeds expenditure (WHO, 2020). Examples of predominantly anaerobic activities include weightlifting, sprinting, jumping, and high-intensity interval training (HIIT).

As of 2020, nearly one in three adults globally did not meet these recommendations for physical, totaling over 1.4 billion individuals (Park et al., 2020; WHO, 2022). According to CDC maps, combined data from 2017 to 2020 for 49 of the 50 United States found that the prevalence of physical inactivity outside of the workplace was 25.3% (CDC, 2022a). The highest rate of inactivity by region was the South (27.5%), while the lowest rate was the West (21.0%) (CDC,

2022a). Rates by race were highest among Hispanic adults and lowest among non-Hispanic Asian adults (CDC, 2022a).

Physical Activity and Chronic Disease

The WHO recommendations for physical activity are based on high quality evidence supporting its role in both mental and physical health (Kraus et al, 2019; Teychenne et al, 2020). Today, chronic diseases are among the top causes of death and disability. Such chronic diseases include but are not limited to heart disease, cancer, chronic lung disease, stroke, Alzheimer's disease, type 2 diabetes, and chronic kidney disease. In the US, 60% of adults have a chronic disease, while 40% have two or more (CDC, 2022b). Due to the high prevalence and lasting effects, chronic diseases pose a huge economic burden, equating to over $4 trillion in health care costs annually (CDC, 2022b). There are several modifiable risk factors for chronic disease, including tobacco use, poor nutrition, physical inactivity, and excessive alcohol, all of which can be improved through lifestyle changes.

Engagement in physical activity is associated with a decreased risk of all-cause mortality and incidence of CVD, HTN, and DM (Kraus et al., 2019; WHO, 2020). In fact, numerous studies consistently show that meeting the current physical activity guidelines is associated with a 30-40% reduction in risk of all-cause and CVD mortality (Kraus et al., 2019; Tucker et al., 2022). Physical activity levels meeting the minimal recommendation provides all-cause mortality risk reduction in at least 70% of adults (Kraus et al., 2019). There is also no increased all-cause mortality risk up to 10 times the current guidelines (Kraus et al., 2019). Additionally, aerobic and resistance exercise reduce hemoglobin A1C by 0.6-0.8% and reduce risk of diabetes-related mortality by 42% patients with T2DM (Tucker et al., 2022). Such exercise leads to blood pressure reduction of 5 to 7 mm in hypertensive adults, equating to a 10% decrease in risk of

cardiovascular events (Tucker et al., 2022). The benefits of aerobic physical activity differ from resistance or strength-based activity. The emphasis on aerobic physical activity has long been the focus of guidelines, largely due to its role in cardiovascular health and weight management. Even in the absence of weight loss, adults who partake in aerobic activity are at lower risk of CVD (Tucker et al, 2022). Risk factors of interest include dyslipidemia, metabolic syndrome, T2DM, chronic inflammation, and hypertension.

Cardiorespiratory function (CRF) is a measure of the "ability to transport oxygen from the atmosphere to the mitochondria to perform physical work" and is a reflection of total body health (Ross et al., 2016). CRF is integrated into several functions, including pulmonary, ventricular, vasculature, and capillary action. A large portion of the health benefits associated with exercise can be directly attributed to improvements in CRF (Lavie et al., 2019). Indeed, research shows that low CRF is an independent risk factor for CVD and all-cause mortality (Kodama et al., 2009). Additionally, an inverse relationship exists between CRF and risk for developing pre-diabetes, T2DM, and metabolic syndrome (Ross et al., 2016). Endurance-type physical activity produces significant increases in CRF, with higher intensity physical activity having a greater effect on CRF than low to moderate-intensity activity with longer durations (Ross et al., 2016).

Beyond its direct health benefits, aerobic physical activity is commonly prescribed in the prevention and management of obesity, a major risk factor for numerous chronic diseases. Long term aerobic exercise leads to modest weight loss of 1.6-1.7 kg over 6 to 12 months. However, it results in significant reductions in visceral and hepatic fat (Tucker et al., 2022). This fat is located within and surrounding the major organs and liver and is linked to increased risk of CVD and T2DM (Tucker et al., 2022). Such decreases in visceral fat may be a driving factor in the

reduction of CVD risk associated with exercise, despite little overall weight loss (Tucker et al., 2022)

Resistance training or muscle strengthening physical activity alone also has health benefits. A 2017 meta-analysis that evaluated the effects of resistance training on blood pressure found that progressive intensity resistance training led to significant reductions in systolic and diastolic blood pressure in adults with pre and hypertension (De Sousa et al., 2017). Systolic BP reduced by an average of 8.2 mmHg and diastolic BP reduced by an average of 4.1mmHg (De Sousa et al., 2017). The mechanisms for post-exercise hypotension are unclear but are likely due to improved biosynthesis and activity of endothelial nitric oxide, greater neuromuscular adaptations, and most notably, reduced peripheral vascular resistance (De Sousa et al., 2017; Halliwill, 2001; Tucker et al, 2022). During exercise, peripheral blood vessels dilate to improve blood flow to the active limbs. The vessels remain dilated several hours following exercise, despite a drop to normal cardiac output, resulting in lower blood pressure (Halliwill, 2001). A large prospective cohort study examined the relationship between differing amounts and frequencies of resistance exercise and incidence of obesity. Researchers found that participants who performed resistance exercise at least 2 days per week had a 20-30% reduced risk of obesity, with the greatest improvements on percent body fat (Brellenthin et al., 2021).

While aerobic and resistance exercise each have independent health benefits, when combined, aerobic and muscle-strengthening physical activity work synergistically to provide the greatest protective effects. A 2020 prospective cohort of nearly 500,000 participants in the US found that adults who engaged in only aerobic activity had a 29% reduced risk of all-cause mortality, while those who engaged in only muscle-strengthening activity had an 11% reduction in risk (Zhao et al., 2020). Yet, those who met the recommendation for both aerobic and muscle-

strengthening activities had the greatest reduction in risk for all causes at 40% (Zhao et al., 2020). Similar results were seen in cause specific mortality including from cardiovascular disease, cancer, and chronic lower respiratory tract diseases (Zhao et al., 2020). A 2019 randomized controlled trial reported increased CRF in both the aerobic and combination groups, while lower body muscular strength increased in the resistance and combination groups (Schroeder et al., 2019). When CVD risk factors were combined in a composite risk score, the combination training group had the greatest reduction in risk score (Schroeder et al., 2019). A large cross-sectional study of 1.7 million US adults pooled from 2011-2017 found that those who met both aerobic and muscle-strengthening exercise guidelines had a lower prevalence and severity of obesity (Bennie et al., 2020).

Not only does physical activity lead to desirable health status, but a lack of physical activity causes undesirable health outcomes. Insufficient physical activity to meet the current recommendations is termed physical inactivity (WHO, 2020). The detriments of inadequate physical activity have been well researched and include greater risk for chronic diseases including CVD, T2DM, HTN, and certain cancers (breast, colon, colorectal, endometrial, epithelial), in addition to all-cause mortality (Park et al., 2020).

Physical Activity and Mental Health

Not only is physical activity a significant factor in chronic disease risk, but there is also evidence to support its role in mental health conditions, including depression, anxiety, and post-traumatic stress disorder (PTSD) (Teychenne, et al., 2020). The results of a 2018 meta-analysis indicate that 150 minutes per week of moderate-vigorous physical activity reduced risk of depression development by roughly 22%. (Schuch et al., 2018). According to a 2017 study, those with or at highest risk for mental health concerns have a greater likelihood of being physically

inactive (Hiles et al., 2017). Additionally, mental health symptoms severity was negatively associated with sports participation and general physical activity (Hiles et al., 2017). These chronic disease and mental health benefits follow a dose response curve, indicating that even small increases in physical activity, including those at low intensities, yield positive outcomes (Hiles et al., 2017; WHO, 2020). This is reflected in the general recommendation that "any activity is better than nothing" (Teychenne et al., 2020).

Both aerobic and muscle-strengthening activities alone produce beneficial reduction in depressive symptoms and risk of increasing severity, with no difference between them. However, when combined, adults engaging in both moderate-vigorous aerobic and muscle-strengthening activities yielded the greatest improvements in risk and severity of depression symptoms in a recent study (Bennie et al., 2019). While the impact of physical activity on chronic disease prevention and management is not likely influenced by the location or context of the activity, the same may not be true for mental health. A 2017 meta-analysis found that leisure-time and transport-related physical activity are associated with positive mental health, while work-related physical activity is associated with negative mental health (White et al., 2017).

Barriers to Physical Activity

With such a high rate of physical inactivity, despite the many health benefits, determining why is of utmost importance. Today, less than 20% of jobs in the US require at least moderate intensity physical activity (Church et al., 2011). This has declined dramatically since the early 1960's, at which point nearly 50% of jobs involved this level of activity (Church et al., 2011). Energy expenditure in the workplace has decreased by 100-150 calories over the past 60 years (Church et al., 2011). Considering a significant amount of time is spent at the workplace, it is all the more important to be physically active outside of the workplace.

According to the CDC, common barriers to physical activity include lack of time, social support, energy, motivation, skill, fear of injury, and high costs (2022b). These barriers to physical activity are reflected in the literature in studies that consider various factors such as demographics, cognition, behaviors, social environment, and physical environment. A study was conducted among college students to evaluate both the perceived benefits of and the perceived barriers to physical activity using the standardized Exercise Benefits / Barriers Scale (EBBS) (Brown, 2005). Researchers found that 73% of the perceived benefits of exercise were identified, while 40% of the perceived barriers were identified by the participants (Brown, 2005). A negative correlation was found between the benefits and barriers. The primary perceived benefits of exercise were related to physical performance, feeling better, task improvement, pleasure, and preventative health. The primary barriers to exercise included fatigue (tiring, hard work) and facility obstacles (location, schedule, lack of access) (Brown, 2005).

Another study looked at how perceived barriers to physical activity impact physical activity and sedentary behavior in adults living in Singapore (Koh, et al., 2022). Researchers found the two most common perceived barriers after adjusting for sex, age, and race were lack of time and feeling tired/fatigue. Other barriers included lack of footpaths and parks, weather, pollution, age, and safety concerns. Additionally, participants who cited work and limited access to exercise facilities as barriers to physical activity were more likely to be sedentary compared to those who cited safety concerns (Koh, et al., 2022).

Physiological Responses and Adaptations to Physical Activity

The body's response to physical activity is coordinated across multiple physiological systems including cardiovascular, respiratory, and musculoskeletal. Over time repeated bouts of physical activity leads to adaptations that promote greater exercise capacity and muscular

efficiency (Tanabe et al., 2021). The adaptations of each physiological system to exercise are largely dependent on the type, duration, and intensity of exercise. The physiological responses to aerobic and resistance physical activity result in several key differences. The process involves a sequence of exercise resulting in muscle damage followed by muscle healing and adaptation (Markus et al., 2021, Tanabe et al., 2021).

When engaging in a bout of physical activity, skeletal muscle adapts to extract oxygen, change energy sources, and get rid of waste (CDC, 1999). There are two main types of muscle fibers: slow twitch and fast twitch. Slow twitch, or type I, muscle fibers have a large quantity of mitochondria, high oxidative capacity, high blood flow and capillary density, and high fatigue resistance (CDC, 1999). Fast twitch, or type II, muscle fibers have moderate to low quantities of mitochondria, blood flow and capillary density, oxidative capacity, and fatigue resistance, but high glycolytic capacity and fast contractile speed (CDC, 1999). The type of exercise or sport one engages in regularly correlates with the predominant muscle fiber type. Long distance runners will have almost exclusively slow-twitch, oxidative, muscle fibers in their leg muscles, while the legs of sprinters will have a greater percent of fast-twitch muscle fibers (CDC, 1999). The response of skeletal muscle to endurance exercise is through increases in the percent of type I muscle fibers, mitochondrial content and function, and capillary density. These adaptations result in increased oxygen delivery, uptake, and utilization in the active muscle. Skeletal muscle adaptations in response to resistance exercise involve both an increase in muscle size and increase in recruitment of type I and II muscle fibers.

Consequences of Excessive Physical Activity

Despite the health-promoting factors of physical activity, excessive moderate to vigorous intensity physical activity can result in undesirable side effects. Bouts of exhaustive aerobic and

resistance exercise can result in mechanical damage to muscle tissue, which triggers the production of reactive oxygen species (Tanabe et al., 2021). Such damage is indicated by elevated serum levels of creatine kinase and lactate dehydrogenase (Callegari et al., 2017). At moderate-high intensities, acute aerobic exercise can alter cytokine production and promote a pro-inflammatory state (Tanabe et al., 2021). In addition, exercise-induced muscle damage promotes the loss of muscle function and increases soreness (Tanabe et al., 2021). It is, therefore, of interest to investigate dietary interventions to reduce the rise in oxidative damage during exercise and symptoms of exercise-induced muscle damage as means to improve exercise performance and accelerate recovery rate following exercise. Additionally, high intensity, long duration aerobic exercise may have adverse effects on the immune system. A single bout of such exercise results in reduced function of major immune cells, decrease antibody synthesis and plasma immunoglobulins, as well as impair macrophage phagocytosis (CDC, 1999).

Both aerobic and resistance physical activity result in muscle changes that cause soreness. Soreness that occurs 24 to 48 hours beyond the bout of exercise is termed delayed onset muscle soreness (DOMS) and is the result of structural damage to the muscle tissue (CDC, 1999). This type of damage can be achieved through aerobic or anaerobic exercise but has proven the most severe following bouts of eccentric muscle action. The significance of muscle action and differences between modes of exercise are discussed further in the next section.

Prolonged bouts of high intensity physical activity will result in the breakdown of glycogen for energy (Ivy, 2004). Glycogen stores should be replenished via ingestion of carbohydrates immediately following strenuous activity. This recommendation is based on the increase in insulin sensitivity and membrane permeability of muscle to glucose, which provides an immediate source of energy to the muscle and allows for maximum muscle glycogen storage

(Ivy, 2004). Protein should be consumed alongside the carbohydrates post exercise, as it appears to enhance these effects and increase the rate of glycogen synthesis (Ivy, 2004). However, highly muscle-damaging exercise can not only lead to greater glycogen depletion but may reduce muscle's ability to replenish glycogen stores (Ivy, 2004).

These consequences of high levels of vigorous physical activity are particularly a concern for individuals competing in sports. The inflammation, soreness, immune suppressing, muscle damage, and glycogen depleting effects all contribute to longer recovery times (Cheung et al., 2003; Harty et al., 2019; Ivy, 2004). Additionally, training sessions following the initial exercise bout may be less productive, resulting in fewer improvements. Persistent soreness may lead to overcompensation by other muscles and increase risk of injury (Cheung et al., 2003). Injuries require more time off, which has the potential to be unenjoyable and demotivating. Ultimately, strenuous exercise may play a major role in decreasing performance and hindering progress in the sport.

Exercise-Induced Muscle Damage

Exercise-induced muscle damage (EIMD) is the term used to describe a variety of symptoms that present immediately and up to 14 days following a single bout of exercise (Owens et al., 2019). Such symptoms may include loss of skeletal muscle function, soreness, and reduced exercise capacity (Owens et al., 2019). In addition to resistance exercise, long periods of endurance exercise can cause microstructure damage to muscle tissue. To regenerate and heal the damaged muscle fibers, the body starts an inflammatory process (Markus et al., 2021). There are two phases of the inflammatory response to exercise (Markus et al., 2021; Tanabe et al., 2021). The primary response is the result of mechanical stress to the muscle, including overstretching of the sarcomeres. This disruption triggers a secondary inflammatory response, characterized by the

production of reactive oxygen species and the activation of proinflammatory cytokines (Tanabe et al., 2021). This inflammatory response is normal following exercise and essential for tissue repair and muscle adaptation (Markus et al., 2021; Tanabe et al., 2021). However, if poorly controlled, it can cause excessive damage to the tissues (Peake et al., 2017; Tanabe et al., 2021).

The recruitment and accumulation of immune cells at the site of damage leads to edema and increases membrane permeability. This permeability causes proteins, such as creatine kinase and myoglobin, to leak into the bloodstream (Markus et al., 2021). The inflammatory response also accounts for loss of muscle force, reduced range of motion, and delayed-onset muscle soreness following exercise (Markus et al., 2021; Tanabe et al., 2021). Ultimately, the degree of EIMD determines the required recovery time and impairment to exercise performance (Peake et al., 2017; Tanabe et al., 2021).

EIMD Based on Mode of Exercise

The degree of EIMD depends on several factors, notably, the mode of exercise. Both resistance and aerobic exercise bouts have the potential to induce muscle damage at sufficient intensity and durations (Stožer et al, 2020). However, current literature suggests that EIMD is greater following exercise with an emphasis on the eccentric phase of muscle contraction (Stožer et al, 2020). Eccentric muscle action occurs during the lengthening phase, while concentric muscle action occurs during the shortening phase (CDC, 1999; Stožer et al, 2020). Generally, resistance activity involves a greater eccentric load than aerobic exercise (Stožer et al, 2020). This is proposed to be, in part, because eccentric muscle actions require less motor unit activation and less oxygen than the concentric phase (Stožer et al, 2020). The majority of aerobic based exercise does not involve a large force or long duration of the eccentric phase of muscle contraction (Stožer et al, 2020). An exception is downhill running or walking, which requires

significant eccentric loading as the leg muscles work against gravity to control each step (Stožer et al, 2020). Therefore, it is likely to produce greater EIMD compared to other forms of aerobic exercise. Training level also influences EIMD, with untrained individuals having worse and prolonged symptoms of EIMD compared to trained individuals who underwent the same testing protocols (Ertel et al, 2020; Spanidis et al, 2018).

Biomarkers of Muscle Damage

Creatine kinase (CK) and lactate dehydrogenase (LD) are proteins found in the myosin chain of muscle fibers (Callegari et al., 2017). EIMD compromises the integrity of the muscle, causing CK and LD to leak into circulation. Increases in CK in the blood following exercise positively correlate to the amount of muscle damage (Markus et al., 2021).

Myoglobin is a protein found in oxidative skeletal muscle fibers. Its function is to bind oxygen and store it or deliver it to muscle cells during periods of low oxygen or increased activity. Myoglobin is released from muscle tissue following damage to the muscle cells and impaired muscle cell membrane permeability. An activation of calcium-dependent enzymes further metabolize and destroy muscle cell membrane, allowing for the release of intracellular myoglobin **(**Zafar Gondal, et al., 2023). Myoglobin has shown to increase in circulation in resistance and long-distance endurance exercise (Martin-Rincon, et al., 2020).

CK, LD, and myoglobin are established indirect biomarkers for muscle damage. (Bessa et al., 2016; Callegari et al., 2017). CK is the most commonly used as it is more widely studied and provides the greatest response (Bessa et al., 2016; Markus et al., 2021). A randomized control trial of sedentary males indicated that the level of intensity of aerobic exercise was associated with serum CK levels following a single exhaustive exercise session (Moflehi et al., 2012). At moderate (60% VO_{2max}) and high intensities (80% VO_{2max}), the CK levels significantly increased

compared to the low intensity (40% VO_{2max}); however, a limitation of this study is that it involved running and only looked at CK concentration immediately before and after exercise (Moflehi et al., 2012). Another study found that creatine kinase doubled 3 hours following a resistance test plus a 1 hour cycling test, reaching peak levels at 12 hours post (3x baseline) and returning to baseline by 48 hours (Bessa et al., 2016). The same study also found that serum LDH was elevated by 25% at 3 hours, reaching peak levels at 6 hours, and returning to baseline at 12 hours (Bessa et al., 2016).

Factors That Influence Exercise Performance

There are several systems and functions within the body that impact performance, especially in the context of aerobic activity. Most notable are the cardiorespiratory system, acid-base balance, and mitochondrial biogenesis. When these factors are not optimized, skeletal muscle action becomes compromised, resulting in reduced ability to perform.

The pulmonary and cardiovascular systems are major factors in exercise performance. Together, they are termed the cardiorespiratory system. Oxygen is necessary for the production of energy in the muscle. Therefore, when the muscle does not receive adequate oxygen, its capacity for work decreases dramatically. The lungs are responsible for bringing oxygen into the blood, while the heart is responsible for pumping the oxygen rich blood to the tissues (Bassett & Howley, 2000). The tissues then extract the oxygen from the blood, while dumping carbon dioxide into the blood for removal. Studies have shown that the limiting factor in oxygen uptake during exercise is not oxygen extraction by the skeletal muscle, but rather the cardiorespiratory system failing to transport sufficient oxygen to the tissue site (Bassett & Howley, 2000).

The disruption of acid-base balance also plays a large role in performance. Exercise at any intensity will result in the production of lactate, a by-product of anaerobic glycolysis (CDC,

1999). Lactate can be used for energy when oxygen is available, such as during low-intensity exercise. However, prolonged bouts of exercise at a moderate to high intensity, results in accumulation of lactate, as the body is no longer able to clear lactate from the blood at the rate it is being produced. This results in the accumulation of hydrogen ions causing blood pH to drop, forcing the body to stop to return to homeostasis. Lactate threshold is the point during incremental exercise where lactate accumulates in the blood at a faster rate than it can be cleared, which leads to an exponential rise in blood lactate. This is predicted to be the cause of the burning sensation felt in the working muscles and a major player in performance of moderate to high intensity aerobic exercise. The intensity at which lactate threshold is reached is between 50 and 60% of VO_{2max} in untrained individuals, 65-80% in recreational athletes, and 85-95% in elite endurance athletes (University of Virginia, n.d.) Lactate threshold can be improved with training, hence the greater intensity required to reach it and greater work capacity prior to exhaustion as training level increases (CDC, 1999).

Another highly significant factor in exercise performance is mitochondrial biogenesis in the muscle, which is directly tied to muscular efficiency (Craig et al, 2015). Mitochondria are the site of oxygen dependent energy generation in the form of ATP. Continuous production of ATP is necessary to maintain energy output during exercise. Greater biosynthesis of skeletal muscle mitochondria enhances oxidative capacity. This allows for more fatty acids to be utilized for energy during submaximal exercise, which yields more ATP at a more efficient rate (Craig et al, 2015). Improving oxidative energy production is especially beneficial for delaying muscle time to fatigue and improving performance of aerobic exercise. Training adaptations within the muscle, including increased mitochondrial size, content, number, and function, can result in

greater muscular efficiency, with subsequent improvements in exercise performance (Margolis & Pasiakos, 2013; Gaesser & Brooks, 1975).

Power Output as an Indicator of Exercise Performance

Endurance exercise performance is most commonly evaluated during running or cycling. For cycling, endurance exercise performance is typically evaluated using a time trial or time to exhaustion trial. However, another measure of endurance exercise performance is average power output (wattage) during an acute bout of cycling. Wattage is a measure of power that can help indicate the intensity of exercise. Observing changes to wattage over a bout of exercise helps illustrate the body's response to the exercise, by quantifying how much power is being transferred to the bike which ultimately represents improved or diminished performance.

There are several factors that impact the ability to maintain power output for extended periods of time. Such factors can be physiological, environmental, or biomechanical (Abbiss & Laursen, 2005; Twist & Eston, 2009). The primary limitation to maintaining power output during an exercise bout is the ability to resist fatigue. Fatigue itself is influenced by many systems, both internal and external. Physiological factors shown to contribute to fatigue include muscle fiber type, oxygen availability, pain receptor activation, and neurological alterations, among others (Abbiss & Laursen, 2005; Twist & Eston, 2009). Additionally, the ability to maintain power output and resist fatigue is highly influenced by exercise intensity, determined by cadence and wattage in the case of cycling. Functional threshold power (FTP) quantifies an individual's metabolic steady state by using data on power output (Sorensen et al, 2019). It is the estimated maximum average power sustained over a 1-hour period, estimated as 90-95% of the power output during an 8-20 minute-test. Although not the same, FTP correlates with lactate threshold and offers a less expensive, more accessible way to measure performance (Sitko et al, 2022).

Data on FTP in comparison to maximum power is limited, but one study that included both untrained and trained cyclists found that FTP values ranged from 51.3-80.2% of power max (Denham et al, 2020). Sedentary males reportedly reached the lactate threshold at 62% of VO2 max, while trained males reached blood lactate threshold at 69% (Ghosh, 2004). At this workload (wattage) it is likely that such individuals would tire quickly and need to reduce wattage to continue.

Muscle Damage Influence on Power Output

One model that describes what causes fatigue is the muscle trauma model. This refers to fatigue resulting from EIMD. Damage to the muscle tissue ranges from disruptions to the sarcolemma to complete myofibril tears (Abbiss & Laursen, 2005). Prolonged cycling has the potential to cause significant damage, leading to alterations to intramuscular structural and chemical homeostasis (Abbiss & Laursen, 2005). These imbalances, in addition to the activation of pain receptors, are thought to influence the neuromuscular sensory pathways (Abbiss & Laursen, 2005). Tests of this theory found that increased muscle pain resulted in a reduced activation of muscle agonist by 30% and a reduction in endurance time during sustained submaximal exercise (Abbiss & Laursen, 2005; Ciubotariu et al, 2004). The muscular fatigue was also associated with decreased force production of the pain-induced muscle and reduced activation of surrounding muscles (Abbiss & Laursen, 2005; Ciubotariu et al, 2004). While the mechanism behind this relationship is not fully understood, it may be related to the relationship between pain receptors and the central nervous system (Abbiss & Laursen, 2005). Additionally, reductions in force production may be related to mitochondrial damage by ROS, limiting the ability for the muscle to utilize oxygen (Abbiss & Laursen, 2005). Beyond this, some studies suggest that ROS may reduce activity of Na+-K+-ATPase, which maintains homeostatic ionic

gradients at the sarcolemma (Abbiss & Laursen, 2005). The sum of the muscle trauma model of fatigue indicates prolonged cycling leads to decreased activation and power-producing capacity of active muscles, ultimately decreasing performance (Abbiss & Laursen, 2005).

Non-Dietary and Dietary Strategies for Reduction of Muscle Damage

Pharmacological, physiotherapeutic, and nutritional strategies to reduce the degree of EIMD have been evaluated (Peake et al., 2017). Due to the production of ROS and inflammatory cytokines from exhaustive exercise, protocols that offer antioxidant and anti-inflammatory properties may counteract these effects and reduce the severity and symptoms of EIMD (Bowtell et al., 2019). Post-exercise interventions that have shown promising results include massage, compression garments, cold water immersion. Dietary interventions with potential beneficial effects include pre and post exercise supplementation of cherry juice and other sources rich in polyphenols (Myburgh, 2014; Peake et al., 2017). Strategies that have produced poor or inconsistent findings include nonsteroidal and anti-inflammatory drugs, high protein intake, and fish oil supplementation (Peake et al., 2017).

Polyphenols

Polyphenols are a category of phenolic compounds and large molecules found in most families of plants and in varying concentrations of the leaf, epidermis, bark layer, flowers, and fruits (Abdel-Shafy & Mansour, 2017). The name is derived from the Greek word polus, meaning “many, much” and its chemical structure containing a hydroxyl and an aromatic benzenoid-(phenyl) ring (Abdel-Shafy & Mansour, 2017). Foods generally contain a mixture of polyphenols, with some polyphenols being specific to particular foods. For example, quercetin is found in all plant foods, including fruits, vegetables, tea, wine, cereal, and legumes; while flavanones are specific to citrus fruits and isoflavones are specific to soy (Abdel-Shafy &

Mansour, 2017). While no Dietary Reference Intake (DRI) exists for polyphenols, consuming a wide variety of fruits, vegetables, grains, and legumes can ensure adequate variety of polyphenols. Polyphenols are non-essential, non-energy containing compounds that have been shown to provide numerous health benefits. Much of the literature to date supports their antioxidant, anti-inflammatory, anti-microbial, and anti-carcinogenic properties (Abdel-Shafy & Mansour, 2017).

The phytochemicals with the most health promoting activity belong to the flavonoid and xanthones classes of polyphenols (Maldonado-Celis et al., 2019). These compounds have been shown to play a role in reducing oxidative damage and inflammation (Abdel-Shafy & Mansour, 2017; Bowtell et al., 2019). Current evidence suggests that supplementation of polyphenols derived from cherries, blueberries, blackcurrant, pomegranate, and cocoa may cause changes in biomarkers of oxidative damage and inflammation (Bowtell et al., 2019). These have free radical-scavenging properties that down-regulate the expression of superoxide-producing enzymes (Gelabert-Rebato, Wiebe, et al., 2019). However, the dose of fruit derived polyphenols, as well as the specific duration, mode, and intensity of exercise for which they are appropriate has not yet been identified (Bowtell et al., 2019).

Nutritional Composition of Mango

Mangoes are known as “the king of fruits” due to their popularity in tropical regions. Mangoes are grown around the world, existing in over one thousand varieties (Lauricella et al., 2017). Mangoes are rich in polyphenolic compounds: gallic acid, mangiferin, quercetin, catechins, and anthocyanins (Lauricella et al., 2017). They are also great sources of antioxidant vitamins, including vitamin C and carotenoids (provitamin A) (Lauricella et al., 2017). According to the United States Department of Agriculture (USDA) database, a single mango

(weighing 250-300 grams) contains over 100% of the recommended daily allowance (RDA) for vitamin C and 10-12% of the RDA for retinol (vitamin A). Similar to all fruits, mangoes provide dietary fiber, roughly 2.5 grams per 250 g of mango. Mangoes also provide essential minerals and electrolytes including calcium, sodium, copper, iron, phosphorus, manganese, magnesium, zinc, boron, and selenium.

The mango pulp (mesocarp) is the main and consumable component of mangoes. Flavonoids present in mango include gallic acid, quercetin, catechins, anthocyanins, and tannic acid. Mango pulp is high in the flavonoid quercetin and its glycosides, totaling roughly 46.6 mg per 1 kg of mango (Lebaka et al., 2021). The primary xanthone present in mango is called mangiferin. Mangiferin is found in the bark, fruit, roots, and leaves of mangoes, but is most concentrated in the peel. The content of mangiferin varies from as little as 29 to 32.5 mg per kg of mango pulp based on cultivar and ripeness but is typically on the higher end range (Maldonado-Celis et al., 2019).

Potential Role of Mangoes on Exercise Performance During Exhaustive Exercise

Current research suggests that fruit extracts and polyphenol rich supplements may play a role in improving exercise performance. Outcomes of interest that have been studied include increased VO_{2max}, oxygen extraction, mitochondrial function, oxygen consumption, and overall performance improvements.

Quercetin is the most highly studied polyphenol in mangoes and may play a large role in the benefits of mangoes in relation to exercise performance and reductions in EIMD. A randomized, double blinded, placebo-controlled crossover trial in 12 untrained participants supplemented with 500 mg of quercetin twice a day for seven days indicated improvements in $VO_{2max,}$ and endurance capacity compared to a placebo (Davis et al., 2010). A randomized

crossover study in untrained young males examined the influence of 2 weeks of 1000 mg daily quercetin supplementation resulted in significant improvement in a 12-min treadmill time trial performance (Nieman et al., 2010).

A cross-over study of 40 adults examined the effect of a single dose of Zynamite, a mango leaf extract high in mangiferin, 1 hour prior to exercise on repeated-sprint performance (Gelabert-Rebato, Martin-Rincon, et al., 2019). The primary intervention included 140 mg of Zynamite and 140 mg of quercetin (Gelabert-Rebato, Martin-Rincon, et al., 2019). The researchers concluded that the Zynamite combined with quercetin improved performance, muscle oxygen extraction, and mitochondrial oxygen consumption (Gelabert-Rebato, Martin-Rincon, et al., 2019).

Another study that looked at the combination of mangiferin and luteolin in physically active men taking both high (420 mg/day to equal 300 mg of mangiferin) and low (140 mg/day to equal 100 mg of mangiferin) quantities of mango leaf extract over 48 hours and 15 days had similar results. The luteolin concentrations were 47.5 mg/day in the low dose group and 95 mg/day in the high dose group. There were improved sprint performance, muscle oxygen extraction, and brain oxygenation at both doses and at both time points (Gelabert-Rebato, Wiebe, et al., 2019).

Potential Role of Mangoes on Muscle Damage Following Exhaustive Exercise

Prior studies have looked not only at the direct effects mangoes and other antioxidant supplements may have on performance, but also on post-exercise muscle damage. Studies that analyze serum muscle protein concentrations, such as LDH, CK, and MG, following exercise and supplement protocols offer insight into their effects on post-exercise muscle damage and recovery.

The majority of studies that show favorable results on post exercise markers of muscle damage following polyphenol supplementation include resistance exercise protocols. For example, three crossover studies evaluated lower body and upper body eccentric exercise protocols and polyphenol rich supplements, including TensLess®, curcumin, and tart cherry extract, in recreationally active and untrained adults (Hooper et al., 2021; Romain et al., 2017; Tanabe et al., 2015). Together, the studies resulted in decreased peak values of myoglobin and creatine kinase in the supplement groups compared to the placebo groups (Hooper et al., 2021; Romain et al., 2017; Tanabe et al., 2015).

The effects of polyphenols on markers of muscle damage following aerobic exercise have also been examined; however, the results are less consistent than those that involve resistance exercise. This is likely due to the known ability of eccentric focused resistance exercise to induce greater muscle damage than aerobic exercise. An RCT was conducted in 2016 to observe the effects of green tea and sour tea supplementation in 54 male soccer players (Hadi et al., 2017). The consumption of 450 mg/d of sour tea extract for six weeks resulted in decreased LDH levels ($p = 0.02$), but no significant changes in CK levels (Hadi et al., 2017).

There is some evidence to suggest that mangiferin, in addition to quercetin, is beneficial in mitigating the leakage of muscle proteins into the bloodstream. Several studies look at the combined effect of these compounds on exercise performance. A 2020 RCT tested the effect of Zynamite in combination with quercetin on recovery after EIMD (Martin-Rincon et al., 2020). The 57 participants ran a 10 km race, half of which consumed a placebo and the other half a Zynamite and quercetin supplement at 1 hour before and every 8 hours after the competition for 24 hours (Martin-Rincon et al., 2020). CK and LDH were not analyzed but increases in serum

myoglobin and alanine aminotransferase were attenuated in male subjects taking the Zynamite and quercetin. (Martin-Rincon et al., 2020).

These studies show net favorable results yet did not evaluate the effects of whole mangoes (Hadi et al., 2017; Hooper et al., 2021; Martin-Rincon et al., 2020; Romain et al., 2017; Tanabe et al., 2015). The studies examined the effects of supplementation of fruit extracts containing between 150 mg and 1000 mg of known polyphenols per day. The total phenol content in mango pulp may be up to 236.9 mg/100 gm, with a total flavonoid content of 84.2 to 94.6 mg/100 gm, based on ripeness (Soria-Lara et al., 2020). Based on these findings, as little as 160 grams of mango pulp per day may be able to provide the same flavonoid content. Yet, it is unclear if consumption of whole pureed mango pulp will result in sufficient absorption to reach adequate blood concentrations and produce such beneficial effects.

Potential Use of Mango in Industry

The market for mango and mango-related food products is large and growing. Mango is ranked fifth in production among major fruit crops and is produced in over 90 countries (Matheyambath et al., 2016). The global market size value for mango products in 2020 was USD 18.65 billion, with a CAGR of 6.4% from 2019 to 2025, and an estimated revenue forecast of USD 25.55 billion in 2025 (GVR, n.d.). Fruit, including mangoes, is promoted and encouraged by numerous health organizations globally. Positive results from this and similar studies, will only further support the mounting health benefits of mangoes. Fruit-based products in general are an easy selling point, due to the promotion they receive for their many health benefits, sweet taste, and refreshing qualities. Mango assumes the same fate as it satisfies both sensory and health driven consumers.

Popular processed based fruit products include mango salsas, chutneys, dried mango, mango puree, and mango flour (Matheyambath et al., 2016). Additionally, mango juice, nectar, wine, jellies, pickles, smoothies, and chips are common products (Matheyambath et al., 2016; Owino & Ambuko, 2021). Mango powder is often used to enrich and add flavor to products such as yogurt, ice cream, beverages, and soft drinks (Owino & Ambuko, 2021). The pulp is commonly used as a flavoring agent and in neonatal food products (Lebaka et al., 2021). The seeds can be processed into applicable products, such as oils and flour; while the peel has demonstrated use in bakery products, cereals, pasta products and even cream, cheese, and yogurts (Owino & Ambuko, 2021).

Furthermore, the health and fitness industry continues to grow with the development of new products that offer a performance benefit to their consumers. Mangoes are highly refreshing, hydrating, and a good source of quick releasing carbohydrates, making them a perfect candidate for incorporation into sports drinks. As evidence continues to mount for the use of polyphenol rich mango in the sports and exercise space, economic growth for companies in this industry is bound to follow.

CHAPTER III

METHODOLOGY

Study Design

A randomized, placebo controlled, crossover within subject study design was conducted from February 2022 to September 2022. The study had a 10-week duration, split between two treatment arms: 4 weeks of placebo and 4 weeks of mango in a randomized order. There was a minimum washout period of 2 weeks in between treatment arms. A total of 34 (n=34) self-reported sedentary adults (men and women) with normal BMI were recruited to participate in the study. Participants were recruited from Texas Woman's University (TWU) campus (student, faculty, staff) via email and from surrounding areas through social media platforms, such as Facebook (see Appendix A for Recruitment Flyer). The study was approved by the Texas Woman's University Institutional Review Board (Denton, TX) (FY2021-296) (see Appendix B for Approval Letter).

Inclusion and Exclusion Criteria

Qualification for the study was determined by a phone screening that assessed inclusion and exclusion criteria (see Appendix C for screening questionnaire). The phone screening included detailed questions related to demographics, smoking and drinking history, medical history, medications and supplement intake, dietary history such as food allergies and adherence to a special diet (vegetarian, low fat, low carb, etc.), and physical activity history. The inclusion criteria included the following: 18-40 years of age; men or women; sedentary; normal body weight as defined by body mass index (BMI).; nonsmoker; non-asthmatic. For the purposes of this study, sedentary was defined as engaging in less than 1 hour of recreational or structured

physical activity per week over the last 2 years. A BMI between 18.5 and 25 kg/m^2 is considered normal weight.

Exclusion criteria included pre-existing diseases, such as liver, lung, kidney, and heart diseases, or any musculoskeletal disorder within six months prior to the study. Participants were excluded if they engaged in cigarette smoking or heavy alcohol drinking (>2 drinks per day for men; >1 drink per day for women). Additional exclusion criteria included the use of protein and amino acid supplements and other ergogenic aids, allergies or intolerance to mangoes.

Baseline and Final Lab Visits

Participants who met all qualifications were invited to participate in the randomized, placebo-controlled, crossover, 10-week study. There was a total of 4 study visits, 2 per treatment arm: one visit at each baseline prior to each treatment intervention, and one visit at each final following each treatment intervention. The first baseline visit indicated the start of the first treatment arm. During this visit, participants were provided a written consent form (see Appendix D for Stamped Consent Form); a signed copy of the consent form was collected prior to the start of the study. All questions and concerns were answered and addressed throughout the study. The first baseline visit was followed 4 weeks later by the first final visit, which marked the end of the first treatment arm. This was followed by a 2-week washout period, during which no treatment intervention was given. Following the washout period, was the second baseline visit at week 6, marking the start of the second treatment arm, which concluded 4 weeks later at the second final visit. Subjects were asked to avoid caffeine and alcohol intake 24 hours preceding and avoid exhaustive exercise or physical activity 48 hours preceding each trial day. All study visits took place at the Institute of Woman's Health in Woodcock Hall at the TWU, Denton Campus.

Anthropometric Assessment

At each study visit, weight was measured to the nearest 0.1 kilograms with a calibrated digital scale. Height was measured on the first visit to the nearest 0.1 mm with a stadiometer. Measures of body composition, including visceral adipose tissue mass and volume, android/gynoid ratio, and total % body fat were determined using dual-energy X-ray absorptiometry (DXA) to observe and compare changes between the first and last visit.

Blood Collection and Analysis

All blood samples were collected by a trained phlebotomist using one 10 ml EDTA-2 Na coated Vacutainer. A single overnight (minimum 8 hours) fasted blood sample was collected at the baseline visit for each treatment arm. On the final visit for each treatment arm, following the 28-day intervention, blood samples were collected after an overnight fast at pre-exercise (prior to treatment consumption), immediately post-exercise, and 120 minutes post-exercise. The pre-exercise blood was collected using a single stick and a temporary venous catheter was placed in the preferred arm of the participants for the remaining three blood draws. All blood samples were centrifuged at 300 rpm for 15 minutes and aliquoted in microcentrifuge tubes within 30 minutes then stored in -80 degrees C until analysis of muscle damage biomarkers.

Muscle damage was evaluated using serum creatine kinase (CK), lactate dehydrogenase (LDH), and myoglobin (MG). Assays at baseline, pre-exercise, and post exercise time points during each treatment were used for biomarker analysis. The Sigma-Aldrich Lactate Dehydrogenase Activity Assay Kit was used to determine LDH activity in the samples by colorimetric assay. An optimization trial was conducted to determine the most appropriate volume of sample per well. The results of the trial indicated that 10 microliters of sample with 40 microliters of sample diluent per well was optimal and therefore used as the sample dilution

factor for the LDH assays. The Invitrogen Human CKMB ELISA Kit was used to detect and quantify CK in the samples. The RayBio Human Myoglobin ELISA Kit was used to detect and quantify MG in the samples. All procedures were followed as indicated in the respective protocol, including the reconstitution of components, dilution of standards and samples, plating in duplicates on 96-well plates, incubation times and plate washes. The Agilent Biotek Cytation 4 was used for all of the biomarker analysis. Absorbance was measured at 450 nm within 15 minutes of adding the stop solution. Average sample concentrations between duplicates were generated by the software based on absorbance against the standard curve.

Treatment Composition

At the baseline visit for each treatment, participants were randomly assigned to consume a pureed mango drink or placebo drink (see Appendix E for randomized treatment sheet). Based on the preference and freezer availability of each participant, either the full 28 days of product was provided at the baseline visit or was split between multiple additional visits. The products were packaged in individual cups and kept frozen to maintain freshness. Each participant consumed a total of either 250 grams of whole pureed mango drink or 186 grams of placebo drink per day for 28 days per treatment, with a two-week washout period in between. The serving of each product for a single day was halved and packaged into individual cups of 125 gm of mango and 92.5 gm of placebo using a gram scale. Participants were instructed to drink 2 cups per day to reach the total 250/185g of daily product.

The products were matched for calorie and sugar quantities. The mango drink provided 34 g of sugar and 141 kcal per day. The placebo provided 33.6 g of sugar and 140 kcal per day (see Appendix F for Mango and Placebo Recipe and Nutrition Facts). The mango product consisted of frozen mango and water. The placebo consistsed of a non-mango containing mango

flavored syrup, ice, xanthan gum, citric acid, and food dye. The products were produced in the Food Science Sensory Laboratory at the TWU Denton campus by trained researchers, then stored in a food-grade freezer to preserve product quality. Participants consumed one cup in the morning and one cup in the evening. Participants were given instructions to store the treatment in the freezer and slightly thaw in the fridge prior to consumption. Participants tracked their consumption on a calendar provided at the initial baseline visit and were asked to return the completed calendar at their final study visit.

Maximal Test for Peak Power

At the first visit, a maximal ramp test for peak power was performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). The researchers adjusted the bike seat and handlebar for each participant and the settings were saved for use at all bike trials during the study. The peak power test began with a 5-minute warm up on the cycle ergometer at 0 watts. A standardized ramp test began immediately following the warmup. The ramp test started at 10 watts and gradually increased in wattage over the course of 15 minutes, ending at a maximum of 300 watts. Participants were instructed to maintain a minimum cadence of 60 rpm for as long as possible. Upon the first incidence of the cadence falling below 60 rpm, participants were encouraged to increase it back to at least 60 rpm. If the participant could not increase the cadence back to 60 rpm within 15 seconds or had a second instance of dropping below 60 rpm, the test was terminated. The maximum wattage achieved was recorded and defined as their peak power. Upon stopping the test, participants were instructed to continue pedaling at a cooldown load of 20-50 watts for at least 2 minutes. Heart rate was monitored prior to, during, and following the test via smartwatch or Polar chest strap provided by the researchers. Researchers recorded the participants' heart rate at rest, every minute of the test, and at maximal

(see Appendix G for Max Exercise Protocol Datasheet). The study utilized two ergometers of the same model in IWH, both of which were pre-programmed with the identical exercise protocol. Each participant used the same bike for all exercise trials. During all tests, a fan was placed in front of the ergometers and an air purification system was placed behind the ergometers.

Exhaustive Exercise Trial

Participants completed two pre-programed exhaustive exercise trials throughout the study. The exercise trial involved a 60-minute moderate intensity aerobic exercise on a cycle ergometer. Each trial was performed on day 29 of each treatment arm. Prior to the exercise trial, half of the amount of daily mango (125 g) or placebo (92.5 g) was consumed.

The exercise trial began with a 5-minute warm-up on the bike at 0 watts. Immediately following the warmup, the workload was increased to 65% of their peak power determined by the peak power test during the first visit. Participants were instructed to cycle at a minimum cadence of 60 rpm for the remaining 55 minutes of the trial. If necessary to complete the full 60-minute duration, researchers reduced the workload by 10% of peak power wattage. Reductions in wattage were based on participant feedback and observed rpm as indications of ability to complete the full trial duration at the appropriate cadence. Encouragement was provided to keep the current wattage, but further reductions in 10% increments down to a minimum of 25% peak power were allowed. Immediately following the 60-minute exercise trial, the workload was decreased to 20-50 watts for a 2-minute cool down. Heart rate was recorded at rest, at 10 min intervals during the exercise, and 5 minutes following the completion of the trial.

Tracking of Wattage

A printed data sheet was used to record wattage and time at which wattage was held or decreased, if needed (see Appendix H for 1-HR Exercise Protocol Datasheet). This data was used

to determine if the participant was able to maintain 65% of peak power for the full 55 minutes of the trial. If unable to maintain 65% for the full trial, the time point at which the wattage was reduced was recorded and then used to calculate the duration of time spent at each percentage of peak power (65%, 55%, 45%, 35%, and 25%). Analysis of this data was used to provide insight into the rate at which the participant required reductions in wattage to maintain the required minimum 60 rpm. Data sheets were collected during the exercise trials at the end of both treatment arms and compared within individuals as means to assess possible differences in exercise performance.

Statistical Analysis

Using g* power, a minimum total sample size of 14 was required to conduct analysis with alpha = 0.05, power = 0.8, and moderate effect size. Twenty-five participants completed each treatment arm (Mango $n = 25$, Placebo $n = 25$). Descriptive statistics were calculated for all continuous variables including demographics of study participants. Independent samples *t*-test was used to test for potential baseline and order differences in parameters of interest. Repeated measures ANOVA was conducted to assess anthropometric, body composition, maintenance or reduction of wattage, and blood analysis of muscle damage markers between groups and within groups, comparing exercise and response immediately post-exercise and 120 minutes post-exercise. Paired samples *t*-test was used to determine between group differences for variables with incomplete data to keep as many cases as possible. Data were reported as mean ± standard deviation (SD). SPSS version 28 was used to perform statistical analysis. Statistical significance was achieved with a p-value ≤ 0.05.

CHAPTER IV

RESULTS

Demographic Data

A total of 169 male and female subjects were screened for participation in the study. Thirty-six subjects met inclusion criteria, were accepted into the study, and were scheduled for an initial visit. Of the 36 participants scheduled, 34 initiated the study, 32 of which were female and 2 were male. During the study, 9 participants withdrew. Seven participants withdrew from treatment arm 1 and three participants withdrew from treatment arm 2. A total of 25 participants completed the mango treatment and 25 participants completed the placebo treatment. Data on participant screening, enrollment, and dropout rate are presented in Table 1 and 2. Participant demographics data in highlighted in Table 3.

Anthropometrics and Body Composition

Anthropometrics and body composition data is presented as mean ± SEM in Table 4. The average BMI at baseline for the mango treatment was 23.30 ± 0.59 kg/m^2. The average BMI at the final visit for the mango treatment was 23.30 ± 0.56 kg/m^2. The average BMI at baseline for the placebo treatment was 23.31 ± 0.52 kg/m^2. The average BMI at the final visit for the placebo treatment was 23.42 ± 0.55 kg/m^2. There were no significant changes in BMI throughout the study within or between treatments. There was also no significant change in visceral adipose tissue (VAT) volume (cm^3) between the initial baseline visit (of 350.52 ± 30.71 cm^3) and the final visit (367.29 ± 32.33 cm^3) of the 10-week study. Likewise, VAT mass (g) did not change significantly from baseline to final visit (324.14 ± 28.38 grams and 339.76 ± 29.89 grams, respectively). Additionally, baseline measurements for android/gynoid ratio did not change significantly from initial baseline (0.826 ± .040) to final visit (0.845 ± .035).

Muscle Damage Biomarkers

An independent *t*-test for equality of mean did not show significant difference based on the order of treatment. Repeated measures ANOVA of LDH values revealed that there was no significant time effect, $F = 2.489$, $p = 0.94$. However, eta squared = 0.151 indicates large magnitude of LDH changes over time. Therefore, the non-significant result on this overall model may be due to the small sample size. Post hoc pairwise comparison indicated that in the placebo group, LDH was significantly increased from baseline to Final 2 ($p = .002$) and Final 4 ($p = .035$). LDH level in the placebo group was also significantly higher in Final 2 as compared to Final 1 ($p = .030$). However, LDH was not significantly changed in the mango group. There was also no significant difference in LDH between placebo and mango group at any time point.

Repeated measures ANOVA of CK values revealed that there was no significant time effect, $F = .497$, $p = .533$. There was a moderate effect size as indicated by eta squared = .066. There were no significant differences in CK within or between placebo and mango groups at any time point (Baseline: $p = .93$, FT-1: $p = .53$, FT-2: $p = .54$, FT-4: $p = 0.31$).

Repeated measures ANOVA of MG values revealed that there was a significant time effect for placebo group and mango groups with large effect sizes ($F = 3.153$, $p = 0.43$, eta squared = .283 and $F = 3.903$, $p = .021$, eta squared = .328, respectively). Post hoc pairwise comparisons revealed no significant change between baseline and other final timepoints in the Placebo group. However, MG was significantly higher in Final timepoint 4 as compared to Final timepoint 1 in the Placebo group ($p = .030$). There was significant increase in MG in the mango group from baseline to Final 2 ($p = .011$). Additionally, MG level in the mango group was significantly higher in Final 2 as compared to Final 1 ($p = .022$). Paired samples *t*-test revealed no significant differences between the placebo and mango group at any timepoint (Baseline: $p =$

.260, FT-1: p = .208, FT-2: p = .526, FT-4: p = 0.140). Data on muscle damage biomarkers are highlighted in Table 5 and 6 and Figure 1, 2, and 3.

Exercise Performance

The mean peak power from the maximal test was 151.81 watts with a maximum of 263 and minimum of 81 watts. Mean maximum heart rate values achieved during the exercise trials were 179.17 for the maximal test at baseline, 170.65 bpm for the placebo final test, and 167.26 bpm for the mango final test (see Figure 4 for Maximum Heart Rate data). Repeated measures ANOVA of values revealed that there was a significant time effect, F= 15.139, p <0.001, with eta squared = 0.151 indicating large magnitude of maximal heart rate changes. Post hoc pairwise comparison indicated that the maximal trial (baseline) maximum heart was significantly higher than the placebo and mango final trials (p = .001 and p <.001, respectively). However, maximum heart rate was not significantly different between the placebo and mango finals (p =.173).

During the exhaustive bike trial, all participants in each group completed the 60-minute protocol (5-minute warmup + 55-minute at 65% with 10% reduction as needed). However, only 8% of the mango group and 4% of the placebo group maintained 65% of their peak power for the full 55-minutes. The remaining 92% and 96%, respectively, required at least one 10% reduction in wattage to maintain a cadence of 60 rpm for the full duration of the trial (Data on wattage values and counts for each percent peak power is presented in Table 7).

Descriptive statistics for the time in minutes participants completed at each percent of peak power prior to need for reduction is provided in Table 8 and Figure 6. The 25% peak power data was excluded from analysis due to missing values. Repeated measures ANOVA of time at peak power values revealed that there was a significant time effect, F= 4.266, p =0.04, with eta squared = 0.416. Post hoc pairwise comparison indicated that time spent cycling was

significantly increased from 65% PP (baseline) to 45% PP (p = .002) in the Mango group. In the placebo group, time spent cycling was significantly increased from 65% PP to 45% PP (p=.028) and 35% PP (p =.001). Time cycling in the placebo group was also significantly higher at 35% PP as compared to 55% (p =.025). Data on within group differences in cycling time are highlighted in Table 9. There was no significant difference in time cycling between placebo and mango group at any percent peak power wattage. Mean times at each percent peak power were also computed individually to allow more cases to be included. The resulting data differs slightly from that provided by the repeated measures ANOVA output. Differences in trends of the data sets can be observed in the Figure 6 and 7, respectively.

CHAPTER V

DISCUSSION

Exercise induced muscle damage is a common response to prolonged exercise of moderate to high intensities (Stozer et al., 2020). However, in excess EIMD can result in increased severity and duration of DOMS, stiffness, swelling, and functional detriments, such as loss of force and proprioception (Callegari et al., 2017; Hody et al., 2019). Tears to the sarcomere of muscle fibers causes the leakage of muscle proteins, such as lactate dehydrogenase, creatine kinase, and myoglobin, in the bloodstream (Callegari et al., 2017; Stozer et al., 2020). Given these risks associated with EIMD, it is of interest to determine effective strategies to mitigate its severity and duration (Hody et al., 2019).

This study examined the effects of consuming whole pureed mango on biomarkers of muscle damage following, and cycling performance during, a 1-hour exercise trial in sedentary individuals. There were no significant differences between the mango and placebo group in this study; however, there were significant changes within groups. LDH increased significantly from baseline to FT-2 and FT-4, and from FT-1 to FT-2 in the placebo group. Myoglobin increased significantly from FT-1 to FT-4 in the placebo group, and from Baseline to FT-1 and FT-1 to FT-2 in the Mango group. Additionally, despite lacking statistical significance, trends within and between groups were observed. LDH values in the mango group had a smaller increase than those in the placebo group following the 1-hr exercise trial. There was a clear decline in CK in the mango group following the exercise trial compared to an increased in the placebo group.

Although not all statistically significant changes, LDH and MG increased in both the mango and placebo groups between the baseline blood draw pre-exercise and the final timepoint blood draw at 120 minutes post exercise. CK increased marginally in the placebo group, but not

the mango group. LDH, CK, and MG are all important proteins found primary in skeletal muscle. Recent studies suggest that these are valuable indicators of skeletal muscle damage. Their leakage into the bloodstream is prompted by increased permeability of the sarcolemma as a result of sarcomere overstretch (Stozer et al., 2020). Not only did these biomarkers in the mango group have a lesser increase compared to the placebo group, but they also decreased at a faster rate. This increased rate of muscle protein clearance from the blood, if paired with other indicators, may indicate facilitated recovery and alleviation of symptoms (Baird et al., 2012).

There are several reasons for why there were not more significant changes in biomarkers of muscle damage. Such reasons include the mode, intensity, duration of exercise, in addition to the timing of blood draws. A review that evaluated studies looking at CK values following exercise found that changes differed based on type of exercise and timing of blood collection (Totsuka et al., 2002). Peak CK was observed at 24-48 hours after isometric muscle contraction exercise and 3-7 days after eccentric muscle contraction exercise (Totsuka et al., 2002). CK was significantly elevated 3 hours after endurance exercise and gradually increased to its peak on day 3 (Totsuka et al., 2002). Additionally, the researchers concluded that CK reaches its break point at two to three times higher than at rest (Totsuka et al., 2002). A 2005 clinical trial in recreational runners indicated a peak CK activity at 24-hour post exercise with a 15-fold increase (Kobayashi et al., 2005). A RTC on post-game performance markers in soccer players found that both CK and LDH increased post-game but did not peak until 48 hours post (Ispirlidis et al., 2008). Our study only examined concentrations up to 2 hours post-exercise. Serum myoglobin peaks sooner and clears more quickly, usually within 1 to 3 hours and returns to baseline within 6-24 hours (O'Connor et al., 2020). It is likely that all three serum markers of muscle damage, MG, CK and

LDH did not have sufficient time in between the exercise bout and the final blood draw to increase to significant levels above baseline.

The validity of this data depends greatly on if the exercise protocol was sufficient to induce muscle damage. The max trial aimed to determine an individualized wattage for participants to maintain over a 55-minute cycle. The intent of the prolonged exercise trial at 65% of the peak power was to induced muscle damage. A few studies indicate muscle damage using similar protocols (Bessa et al., 2016, Moflehi et al., 2012, Yfanti et al., 2012). Based on the rise in most of the indirect markers of muscle damage following the exhaustive exercise trial, it is likely that this need was satisfied. However, only 8% of the Mango group and 4% of the Placebo group was able to successfully complete the full exercise trial at 65% of peak power. Not only did 84% of each group require at least two 10% reductions down to 45% of peak power, but time spent at 45% peak power was significantly greater than at 65% peak power. Therefore, the degree of muscle damage may have been insufficient to produce a clear understanding of whether the mango product had a greater benefit in comparison to the placebo.

The literature indicates that including a form of eccentric focused muscular contraction would have provided a more appropriate stimulus to assess changes in muscle damage (Hody et al., 2019; Stožer et al., 2020). One exception to this was found in a study on professional male athletes. The researchers compared markers of muscle damage and performance following training (lower intensity) and competition (higher intensity) periods in volleyball, basketball, and cycling athletes. The results showed that despite having a lesser eccentric emphasis, the cycling group had a greater increase in muscle damage following the competition period (Cordova-Martinez et al., 2022). The researchers hypothesized that this contradiction may be associated with high cortisol levels as a result of the high demand, short recovery, and stress associated with

competition (Cordova-Martinez et al., 2022). As such, it is possible that these factors played a role in the results of our study results.

The current study did not result in any significant changes in cycling performance. This adds to the inconsistent findings in the literature on polyphenol rich compounds and exercise performance. It does not support the results of several recent studies which suggest polyphenols from a variety of fruits induce beneficial effects on aerobic exercise performance (Deley et al., 2017; Roberts et al., 2023). Nor does it support the findings from studies that show performance benefits with the consumption of mango specific polyphenol extracts (Davis et al., 2010; Gelabert-Rebato, Martin-Rincon, et al., 2019; Gelabert-Rebato, Wiebe, et al., 2019; Nieman et al., 2010). Our study was the one of the first studies to utilize whole mango puree and the resulting impact on cycling performance was unremarkable.

The ability to recovery and perform in the days following exercise was not examined in the study but may be an important consideration for future studies. A 2021 systematic review concluded that consuming polyphenol-rich foods, juice and concentrates increased recovery rate of muscle function and reduced muscle soreness, with maximal benefit at 48 hours post-exercise (Rickards et al., 2021). Similarly, a 2020 study found that Zynamite in combination with quercetin did not improve running performance but did reduce muscle pain and loss of jumping performance, and mechanical impulse at 24 hours following (Martin-Rincon et al., 2020). These studies suggest that the polyphenol rich supplementation may not be of benefit during an initial exercise bout but may help shorten recovery length and improve subsequent exercise performance. Additionally, a study conducted by Cordova-Martinez et al. found that despite increases in markers of muscle damage, performance was not limited in any of the groups

(2022). This suggests that performance may not be impacted, and intervention may not be beneficial until a certain level of muscle damage has been surpassed.

The present study had several limitations. The participant demographics largely reflected that of the university at which it was conducted with mostly female participants under the age of 30 years. Additionally, the dropout rate was moderately high which resulted in smaller sample sizes than intended (n = 23 v. n = 25). Limitations with the study design include a lack of dietary intake and exercise control. Participants were asked to avoid dietary changes and abstain from physical activity throughout the duration of the trial. Additionally, participants were sent reminders via text messages and emails prior to visits with instruction to arrive fasted and avoid of caffeine and alcohol prior to their visit. However, there were no further protocols in place to determine if participants were compliant with these instructions. Participants were given a calendar to track the consumption of treatment twice daily; however, there were numerous participants that failed to return their tracking calendars at the end of the study. Another potential limitation is the extension of washout periods in some cases due to scheduling conflicts. Beyond these, other limitations included minor technical difficulties with the heart rate monitors, poor hydration status which resulted in blood collection difficulties, and variability in competitiveness amongst participants which likely impacted effort, intensity, and ultimately muscle damage.

Despite these limitations, the study had several strengths. One key strength of the study was the crossover design. This study design allowed each subject to act as their own control between treatment arms. Not only did this allow the sample size to be acceptably smaller, but it also resulted in less baseline variance in the data compared to if other studies methods were used. Another strength was the thorough development of treatments. The placebo product was methodically tested and compared to the mango in sensory analyses performed by TWU faculty

members. Both products were assessed for taste, texture, appearance, and smell. Feedback from these tests was used to adjust the placebo recipe to create a similar product to the mango. The isocaloric value of the treatments is another strength of the study, as differing carbohydrate intake values would have been potential confounding factors for performance improvement. Additionally, the products were prepared and packaged systematically. A gram food scale was used to weigh ingredients for preparing and for distributing into individual portion cups. Product cups were stored in a food-grade freezer and packed into cooler bags with ice packs to maintain product texture and quality during transport.

In conclusion, this study is the first to examine the effects of whole pureed mango on biomarkers of muscle damage following and exercise performance during a prolonged, moderate-high intensity aerobic exercise bout in healthy, sedentary, normal weight young adults. There were significant increases in LDH and MG following the exhaustive exercise trial. The mango group appears to have had mitigated muscle damage as indicated by CK and MG compared to the placebo group, yet not significantly. Cycling performance was not affected significantly, nor did any clear trends present. The evidence presented indicates a potential beneficial effect of whole pureed mango on markers of muscle damage, yet further research is needed to evaluate the connection between biomarker values, EIMD symptoms, and performance outcomes.

Future studies are needed to further explore the effects on biomarkers of muscle damage in a larger timeframe, including 12 hours, 24 hours, and 48 hours post exercise. Additionally, data related the symptoms of EIMD should be collected to determine the physical impact. Expanding the treatment duration before and including treatment following the exercise bout may produce more significant findings. Future studies may also consider including normal BMI

and overweight BMI participants, as recruiting for individuals with normal BMI alone was a challenge faced in this study. Also, including highly- trained and recreationally trained athlete groups could contribute to further understanding of what populations may benefit from the effects of whole mango.

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Table 1

Participant Screening

Interested Individual	Screened Participants	Qualified Participants	Initiated Study	Completed Treatment Arm 1	Completed Treatment Arm 2
205	169	36	34	27	23

Note. Participants were randomly assigned a treatment group, n = 25 mango, n = 25 placebo

Table 2

Participant Dropout Rate

	Qualified and Initiated Treatment Arm	Completed Treatment Arm	Participants Dropout	Dropout Rate
Treatment Arm 1	34	27	7	20.5%
Treatment Arm 2	26	23	3	11.5%

Note. Dropout from treatment arm 2 was mostly due to availability issues, issues with blood draw procedure, or unexpected health issues.

Table 3

Demographics of Study Participants

Qualified and Initiated the Study	Female	Male	Age Range	Average Age
34	32*	2	18-37	24.25

* Most participants were female, a reflection of the university's demographics.

Table 4

Effect of Mango and Placebo on Body Composition

Body composition	Mango		Placebo	
	Baseline	*Final*	*Baseline*	*Final*
Weight (kg)	63.4 ± 1.9	63.8 ± 1.9	63.0 ± 1.8	63.5 ± 1.9
Body Mass Index kg/m^2	23.3 ± 0.6	23.3 ± 0.6	23.3 ± 0.5	23.4 ± 0.6
	*Baseline**		*Final**	
Total Body % Fat	34.5 ± 1.2		34.9 ± 1.1	
Android/Gynoid Fat Ratio	0.83 ± .04		0.85 ± .04	
Visceral Adipose Tissue (VAT) Mass (g)	324.1 ± 28.4		339.8 ± 29.9	
Visceral Adipose Tissue (VAT)Volume (cm^3)	350.5 ± 30.7		367.3 ± 32.3	

Note. This table represents body composition of participants. Values represent Mean ± SEM. Weight n = 22, BMI n = 22, Total Body % Fat n = 23, Android/Gynoid Fat Ratio n = 21, VAT g n = 21, VAT cm^3 n = 21.
* Measurement collected using DXA on the first (initial visit) and last lab visit (final visit of treatment arm 2).

Table 5

Effects of Whole Pureed Mango compared to Placebo on Biomarkers of Muscle Damage in Normal Weight, Healthy Adults

		Baseline	FT-1	FT-2	FT-4
LDH	Mango	254.11 (91.35)	273.50 (82.18)	326.17 (159.05)	265.67 (75.33)
	Placebo	247.65 (46.63)	284.68 (79.44)	362.32 (113.52)*	310.31 (92.41)*
CK	Mango	5.59 (2.79)	5.28 (2.96)	5.35 (3.19)	5.08 (2.83)
	Placebo	5.64 (3.52)	5.91 (3.95)	5.87 (3.87)	5.76 (3.44)
MG	Mango	11.99 (8.47)	13.22 (4.80)	22.69 (11.04)*	18.53 (6.16)
	Placebo	18.81 (10.39)	14.22 (8.58)	20.25 (13.75)	26.44 (14.85)

Note. This table represents the values of biomarkers of muscle damage at the given timepoints in the mango and placebo groups. Values represent Mean (Standard Deviation).
LDH: Lactate Dehydrogenase, CK: Creatine Kinase, MG: Myoglobin.
LDH $n = 15$, CK $n = 8$, MG $n = 9$
Baseline: initial visit for each treatment arm-fasted blood draw, FT1: final visit-fasted blood draw pre-exercise, F2: final visit-blood draw at 0-minute post-exercise, F4: final visit-blood draw at 2 hr post-exercise.
*Significance as compared to baseline within treatment arm ($p \leq 0.05$).

Table 6

Muscle Damage Biomarkers Within Group Significant Change in the Mango and the Placebo

(I) Time	(J) Time	Mean Difference (I-J)	Std. Error	Sig.[b]
LDH - Placebo- Baseline	FT-2	-114.67*	30.87	0.002
	FT-4	-62.65*	26.79	0.035
LDH - Placebo - FT-1	FT-2	-77.64*	32.038	0.030
MG - Placebo - FT-1	FT-4	-12.22*	4.65	0.030
MG - Mango - Baseline	FT-2	-10.69*	3.23	0.011
MG - Mango - FT-1	FT-2	-9.46*	3.32	0.022

Note. This table represents the biomarkers of muscle damage with significant changes within groups.
LDH: Lactate Dehydrogenase, CK: Creatine Kinase, MG: Myoglobin.
LDH n = 15, CK n = 8, MG n = 9
Baseline: initial visit for each treatment arm-fasted blood draw, FT1: final visit-fasted blood draw pre-exercise, F2: final visit-blood draw at 0-minute post-exercise, F4: final visit-blood draw at 2 hr post-exercise
* The mean difference is significant at $p \leq 0.05$
b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Table 7

Effects of Mango and Placebo on Wattage Reductions Needed to Complete Exhaustive Exercise Trial

	65%	55%	45%	35%	25%
Wattage	98.78 (25.45)	85.04 (20.45)	67.32 (14.19)	53.67 (9.90)	34.00 (5.39)
Mango	2 (8%)	2 (8%)	11 (44%)	8 (32%)	2 (8%)
Placebo	1 (4%)	3 (12%)	12 (48%)	5 (20%)	4 (16%)

Note. This table represents the number of participants that completed the exercise protocol at each percent peak power.
Values for wattage are represented as Mean (Standard Deviation). Values for number of participants who finished the trial at each percent peak power represent Count (Percent of Total).
Wattage: 65% *n*=27, 55% *n*=26, 45% *n*=2, 35% = 12, 25% *n*=5
Count/Percent of participants: Placebo *n*=25, Mango *n*=25

Table 8

Effects of Mango and Placebo on Cycling Time at Each Percent of Peak Power

	65%	55%	45%	35%
Mango	6.49 (2.83)	9.43 (5.91)	14.64 (5.07)*	19.64 (7.08)
Placebo	6.59 (2.99)	6.31 (6.84)	14.53 (8.08)*	22.13 (15.61)*

Note: This table represents the amount of time (minutes) spent at each percent of peak power in the mango group compared to the placebo group, using the ANOVA descriptive Statistics output
Values represent Mean time in minutes (Standard Deviation)
65%, 55%, 45%, 35% *n*=7
*Significance as compared to time at 65% within treatment arm ($p \leq 0.05$).

Table 9

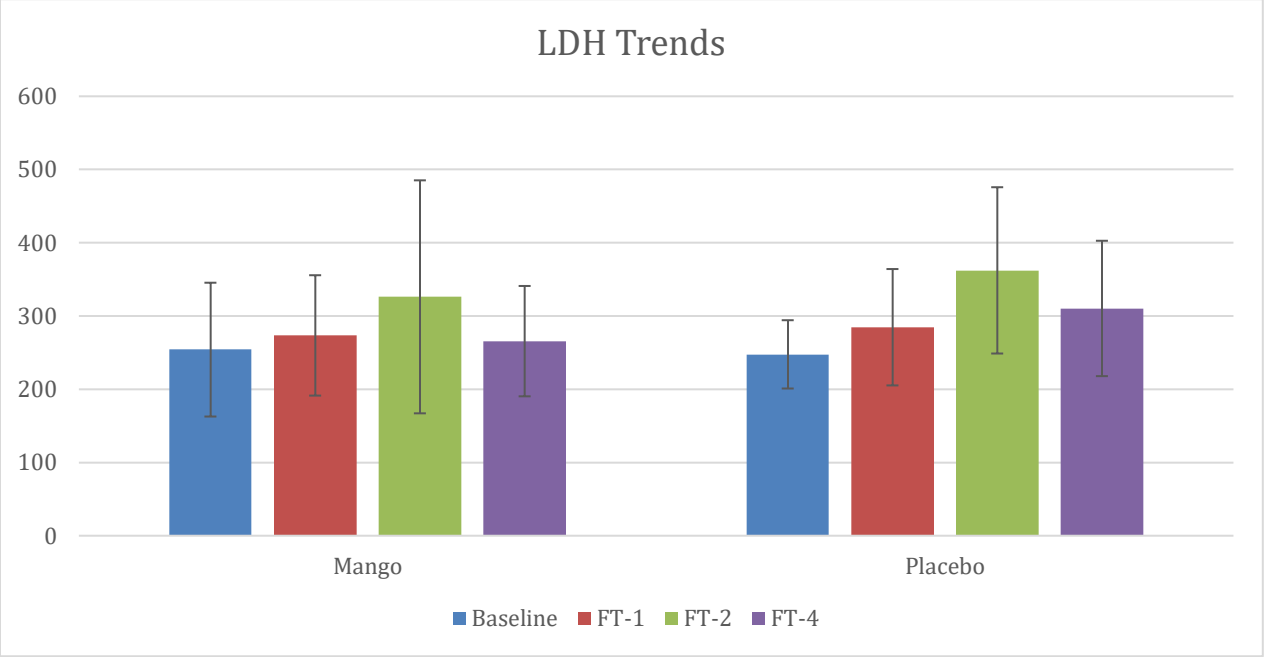
Time at Each Percent Peak Power Within Group Significant Change

(I) Time	(J) %PP	Mean Difference (I-J)	Std. Error	Sig.[b]
Mango – 65%	45%	-7.94*	2.62	0.023
Placebo – 65%	45%	-8.15*	2.82	0.028
	35%	-13.16*	2.34	0.001
Placebo – 55%	35%	-10.21*	3.44	0.025

Note. This table represents the percent peak power at which there was a significant difference in time within groups.
Values represent Mean time in minutes.
* The mean difference is significant at $p \leq 0.05$
b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Figure 1

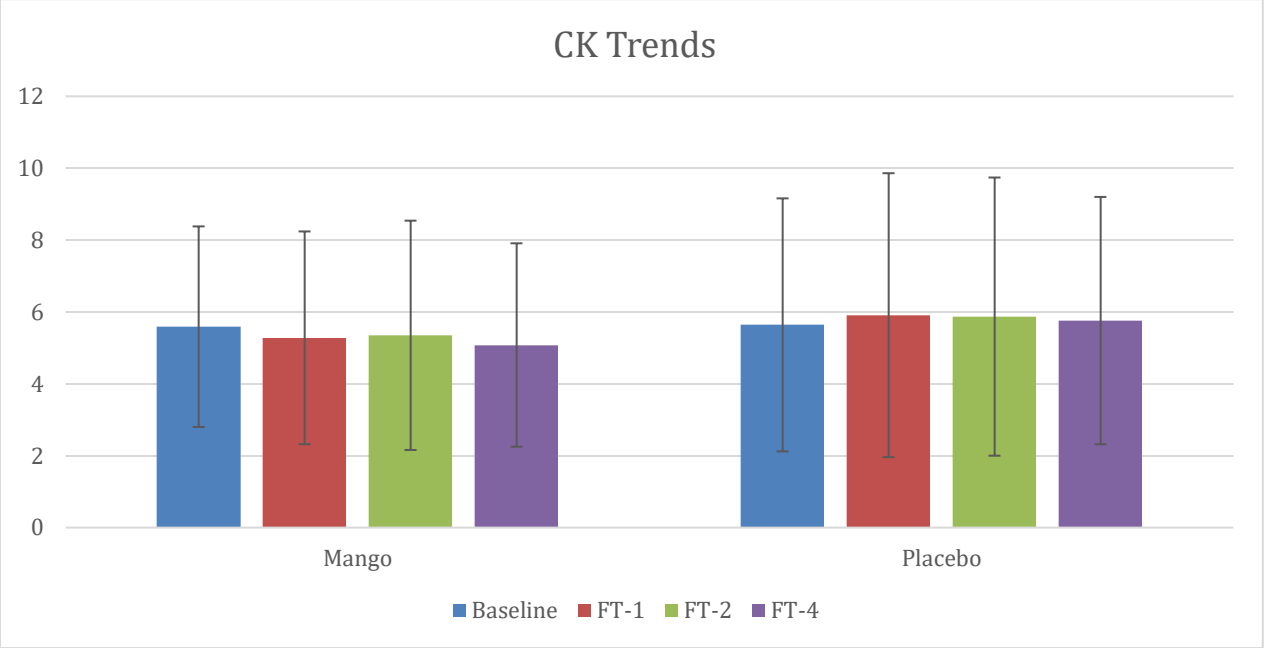
Lactate Dehydrogenase Trends Within and Between Groups



Note. This figure represents trends in the values of lactate dehydrogenase at the given timepoints in the mango and placebo groups. Values represent Mean (Standard Deviation).
LDH: Lactate Dehydrogenase. LDH *n* = 15 Units: milliunits/ml
Baseline: initial visit for each treatment arm-fasted blood draw, FT1: final visit-fasted blood draw pre-exercise, F2: final visit-blood draw at 0-minute post-exercise, F4: final visit-blood draw at 2 hr post-exercise.

Figure 2

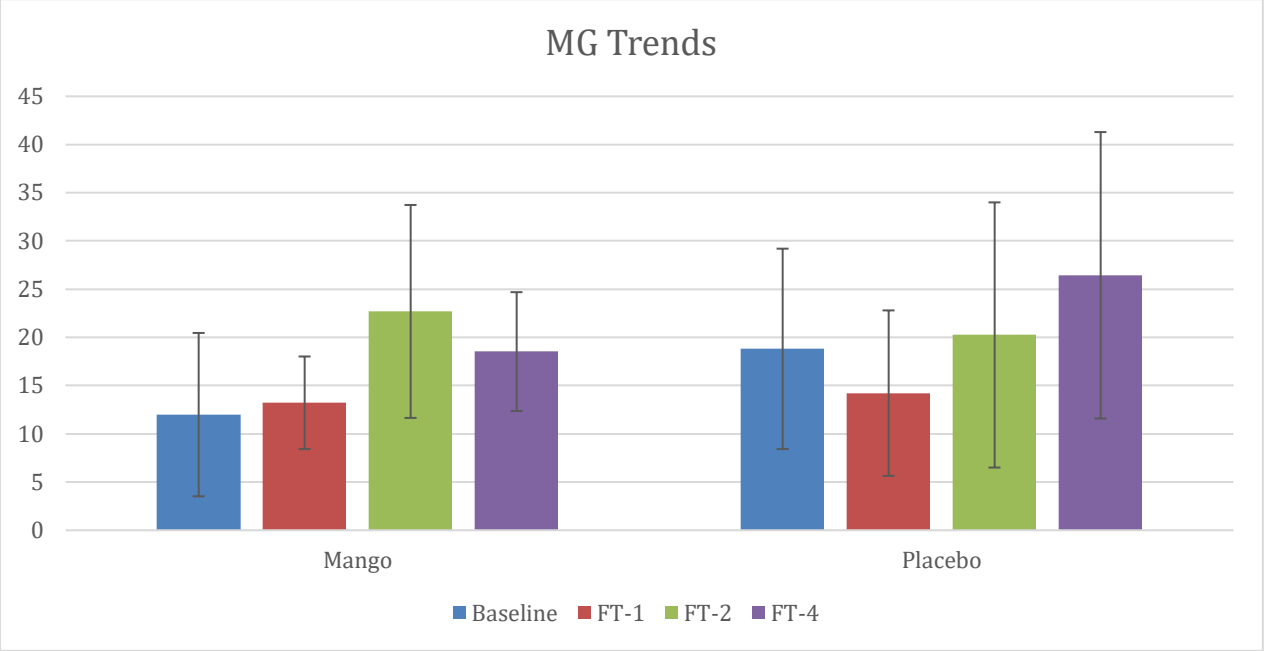
Creatine Kinase Trends Within and Between Groups



Note. This figure represents trends in the values of creatine kinase at the given timepoints in the mango and placebo groups. Values represent Mean (Standard Deviation).
CK: Creatine Kinase. CK *n* = 8. Units: ng/ml
Baseline: initial visit for each treatment arm-fasted blood draw, FT1: final visit-fasted blood draw pre-exercise, F2: final visit-blood draw at 0-minute post-exercise, F4: final visit-blood draw at 2 hr post-exercise

Figure 3

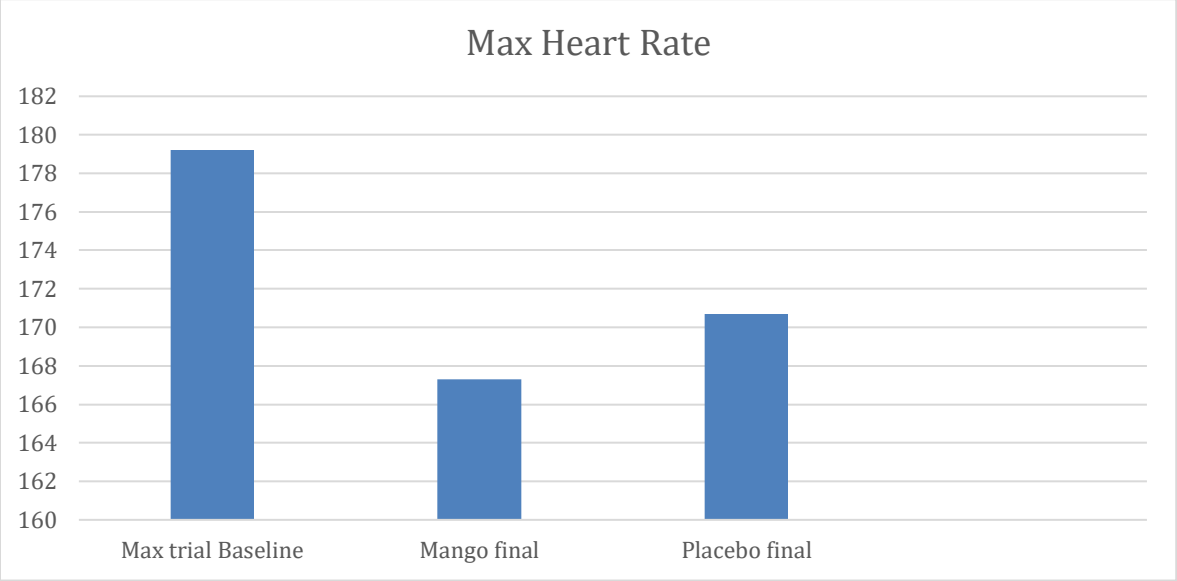
Myoglobin Trends Within and Between Groups



Note. This figure represents trends in the values of myoglobin at the given timepoints in the mango and placebo groups. Values represent Mean (Standard Deviation).
MG: Myoglobin. MG *n* = 9. Units: ng/ml
Baseline: initial visit for each treatment arm-fasted blood draw, FT1: final visit-fasted blood draw pre-exercise, F2: final visit-blood draw at 0-minute post-exercise, F4: final visit-blood draw at 2 hr post-exercise

Figure 4

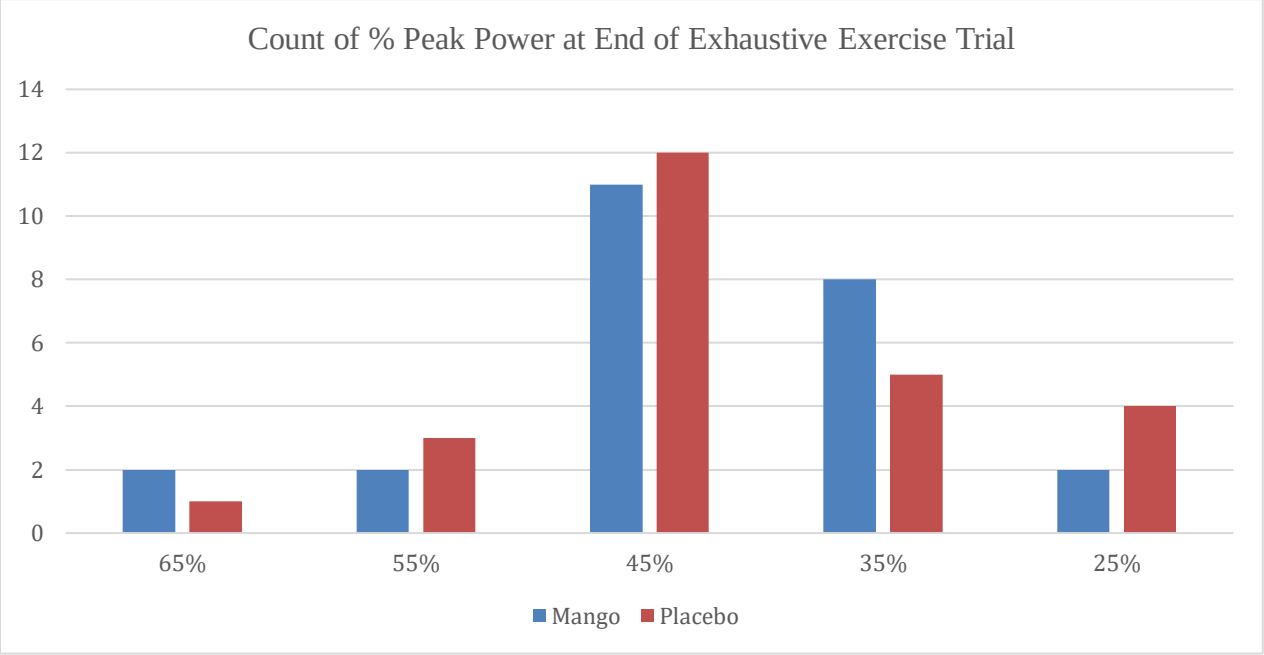
Average Maximum Heart Rate Achieved During Exercise Trials



Note. The figure represents maximum recorded heart rate values during each exercise trial. Values from repeated measures ANOVA descriptive Statistics. Max trial- peak power ramp test at baseline visit, Mango Final- 60-minute exhaustive trial, Placebo Final- 60-minute exhaustive trial. *N* = 23. Units: beats per minute

Figure 5

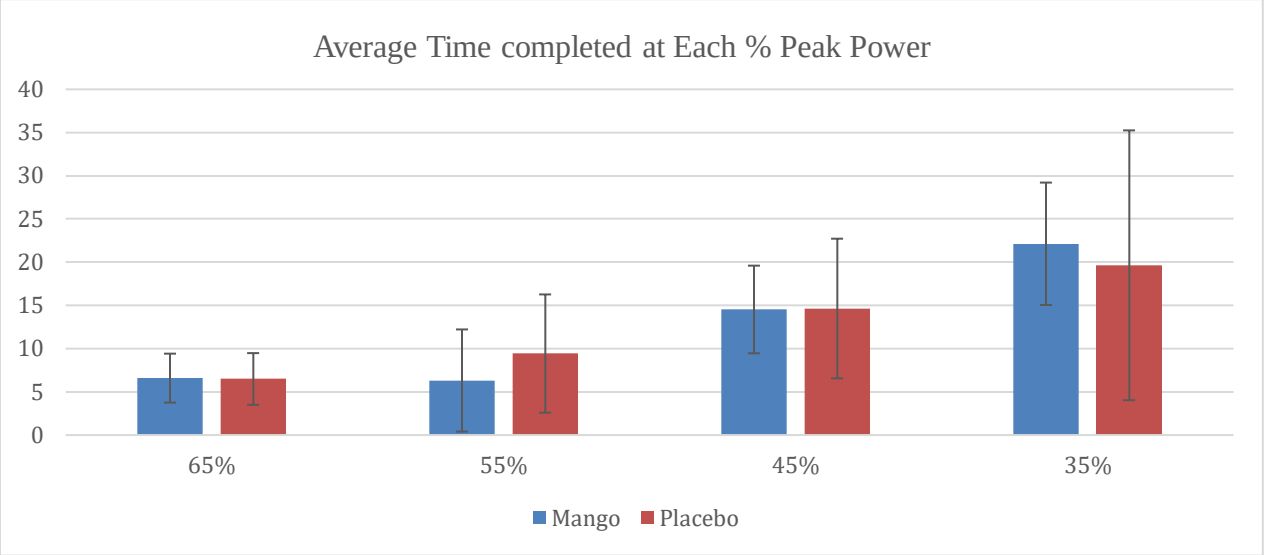
Count of Participants at each Percent Peak Power at Completion of Exhaustive Exercise Trial



Note. This figure represents the distribution in the number of participants at each percent peak power at completion of the exercise protocol in the mango and placebo groups. Mango, Placebo $n = 25$

Figure 6

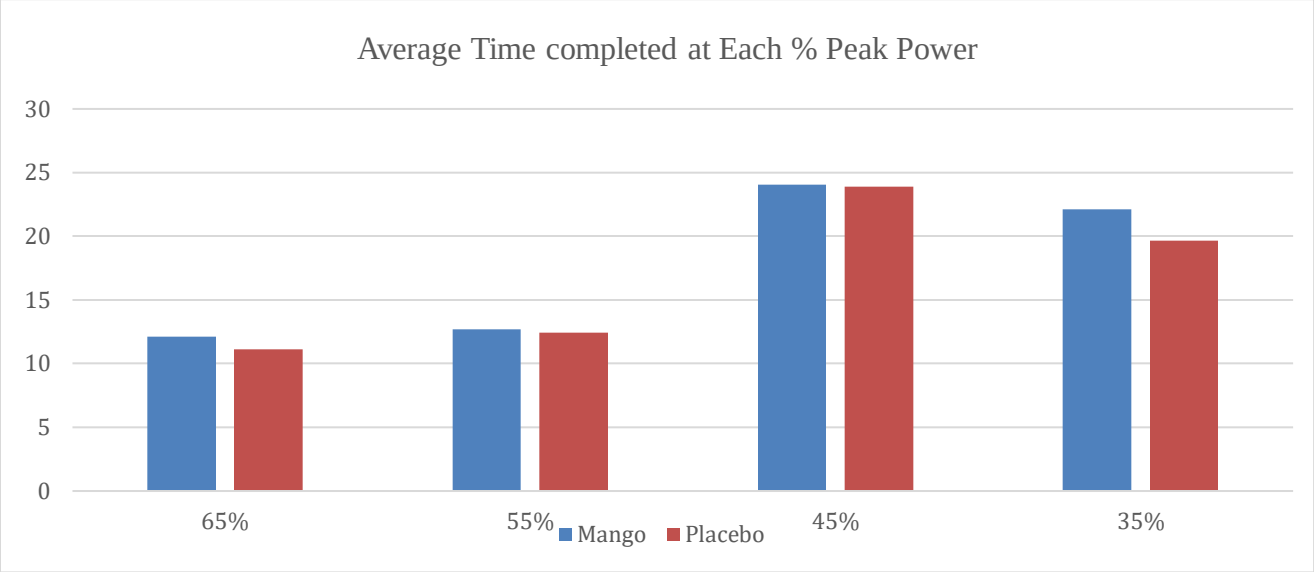
Average Time Completed at Each Percent Peak Power - ANOVA



Note: This figure represents the mean time completed at each percent of peak power during the exhaustive exercise test for the mango and placebo groups using the repeated measures ANOVA Descriptive Statistics output. Data on 25% Peak Power was excluded due to limited data. Data presented as Mean (Standard Deviation).
65%, 55%, 45%, 35% n = 7. Units: minutes

Figure 7

Average Time Completed at Each Percent Peak Power – Paired T-test



Note: This figure represents the mean time completed at each percent of peak power during the exhaustive exercise test for the mango and placebo groups using Paired T-test output. Data on 25% Peak Power was excluded due to limited data.
Data presented as Mean (Standard Deviation).
65% n = 23, 55% n = 21, 45% n = 17, 35% n = 7 Units: minutes

APPENDIX A

EXERCISE AND MANGO STUDY RECRUITMENT FLYER

TEXAS WOMAN'S UNIVERSITY

Do you like mangoes?

RESEARCH PARTICIPANTS NEEDED

Who?

- Healthy adult
- Ages 18 - 40
- Not physically active
- Willing to consume 1 cup of mango and placebo smoothie (56 days total)
- Able to complete 1 hour exercise testing (2 times)

Benefits

Compensation of up to $150 divided into two partial payments of $75

Assessments of full body composition and blood markers of metabolism

Additional

There will be:

Blood draws

Body composition assessments

A total of 4 in-person visits

Interested?

Please Contact: Shanil Juma, PhD

Department of Nutrition & Food Sciences

nutritionresearch@twu.edu

940-898-2704

STUDY PARTICIPATION IS VOLUNTARY

There is a potential risk of loss of confidentiality in all email, downloading, and internet transactions. Current Guidelines and Policies on COVID-19 will be strictly followed based on CDC, State, Locale, and Texas Woman's University.

APPENDIX B

IRB APPROVAL LETTER

Texas Woman's University
Institutional Review Board (IRB)
irb@twu.edu
https://www.twu.edu/institutional-review-board-irb/

June 14, 2021

Shanil Juma
Nutrition and Food Sciences

Re: Initial - IRB-FY2021-296 The Impact of Whole Mango Feeding on Post Exercise Response in Biomarkers of Inflammation, Immune Function, and Circulating MicroRNA in Healthy Sedentary Young Adults

Dear Shanil Juma,

The above referenced study has been reviewed at a fully convened meeting by the TWU IRB - Denton operating under FWA00000178 and approved on June 11, 2021. If you are using a signed informed consent form, the approved form has been stamped by the IRB and uploaded to the Attachments tab under the Study Details section. This stamped version of the consent must be used when enrolling subjects in your study.

Note that any modifications to this study must be submitted for IRB review prior to their implementation, including the submission of any agency approval letters, changes in research personnel, and any changes in study procedures or instruments. Additionally, the IRB must be notified immediately of any adverse events or unanticipated problems. All modification requests, incident reports, and requests to close the file must be submitted through Cayuse.

Approval for this study will expire on June 10, 2022. A reminder of the study expiration will be sent 45 days prior to the expiration. If the study is ongoing, you will be required to submit a renewal request. When the study is complete, a close request may be submitted to close the study file.

If you have any questions or need additional information, please email your IRB analyst at irb@twu.edu or refer to the IRB website.

Sincerely,

TWU IRB - Denton

APPENDIX C

SCREENING QUESTIONNAIRE

Appendix B **Screening Questionnaire**

ID Number:		
Sex:	**Age:**	
Weight:	**Height:**	**Calculated BMI:**
Do you smoke:	Cigarettes per day:	
Do you drink:	Alcohol per day:	
Any medical conditions? Heart Diseases_____ Hypertension_____ Hyperlipidemia _____ (including high cholesterol) Diabetes _____ Kidney Diseases _____ Lung Diseases _____ Liver Diseases _____ Allergies _____		
Any medications, drugs, prescription drugs, over the counter drugs that you are taking? List the amount (mg) and times taken (daily, weekly etc.)		
Any vitamins or dietary/food supplements you are taking? List the amount (mg) and times taken (daily, weekly etc.)		
Dietary information: Are you allergic to mangoes? Yes or No Are you ok consuming mango puree and placebo juice each for a 4 weeks period? Yes or No Are you on a special diet? Yes or No If yes, what type of special diet? Vegetarian_____ Low fat diet _____ Low carb diet _____ Weight loss diet _____ Low salt diet _____ Others (specify) _____		
Do you have any food allergies? Yes or No If yes, specify foods that you are allergic to_____		
Physical Activity: What is your occupation? _____ How much physical activity is involved in your daily work? _____ Outside of your job, how much exercise do you do weekly? Times per week _____ Duration each time _____ Type of activity_____ Additional notes about physical activities _____		
Here is the list of items (drugs/foods) you, as the participant, will be exposed to during the study: mangoes		

APPENDIX D

STAMPED CONSENT FORM

Texas Woman's University

Appendix C **Consent to Participate in Research**

Title: ***IMPACT OF WHOLE MANGO FEEDING ON POST EXERCISE RESPONSE IN BIOMARKERS OF INFLAMMATION, IMMUNE FUNCTION, AND CIRCULATING MICRORNA IN HEALTHY SEDENTARY YOUNG ADULTS***

Investigators: Shanil Juma, PhD…… 940-898-2704 sjuma@twu.edu

Summary and Key Information about the Study

- You are being asked to participate in a research study conducted by Shanil Juma, PhD at Texas Woman's University.
- The purpose of the study is to find out if eating whole mango can have an impact on post exercise response in biomarkers of inflammation, immune function, and circulating microRNA.
- You have been invited to the study because you meet the inclusion criteria of being a sedentary healthy adult with a normal weight.
- As a participant, you will be asked to consume 250g total of whole pureed mango drink for 28 days or 250 g of placebo drink without mango for 28 days. After that, you will not consume any of these products for 2 weeks. Then you will be asked to consume 250g total of whole pureed mango drink for 28 days or 250 g of placebo drink without mango for another 28 days, during which period, the product you consumed will be different from what you consumed during the first 28 days. If you received the whole puree mango drink during the first 28 days, you will then receive the placebo drink without mango for the second 28 days of the study product consumption period.
- During this study, you will be asked to come to the TWU Institute of Women's Health Clinics in Woodcock Hall on the Denton Campus for 4 visits, at baseline and at the end of each treatment arm (baseline, 4-week, 6-week, 10-week time point). During each study visit, we will measure your weight, height, waist and hip measurements, body fat and muscle percentage measurements, draw blood, and answer Refreshing Perception Questionnaire. You will be asked to refrain from caffeine and alcohol 24 hours before each exercise trial.
- A 5-6 digit code (letters and numbers) will be assigned to you at the beginning of the study. Your personal information and blood specimen will be de-identified using this code to protect your confidentiality.
- The total time commitment for this study is approximately 10 hours.

Participant Initials

- Following the completion of the study, you will receive $150 in cash, within which $75 will be paid at the completion of the first 28 days, and another $75 will be paid at the completion of the whole study.
- The greatest risks of this study include loss of confidentiality, exposure to low level radiation associated with body composition assessment, allergic reactions to study products, discomfort from blood drawings, and coercion.

Explanation and Purpose of Research

We are asking you to participate in a research study at Texas Woman's University. The purpose of the study is to investigate the anti-inflammatory, immune responsive, and muscle protective role of whole pureed mango in an exhaustive exercise condition. Find out if Mangoes are rich in antioxidant vitamins (vitamin C, carotenoids) as well as polyphenolic compounds such as mangiferin, catechins, quercetin, anthocyanins, and gallic acid. The anti-oxidative and anti-inflammatory properties of mango polyphenols have been demonstrated in various in vitro and in vivo studies investigating ageassociated chronic diseases. In relation to exercise, a recent study with mangiferin supplementation over short duration has indicated improvement in exercise performance and muscle oxygen utilization. In the research, we will ask the following objectives:

1) To compare the effects of 250 grams of whole pureed mango in comparison to placebo drink without mango (matched in amount of carbohydrates (sugar) and calories) on post exercise changes in biomarkers of inflammation and muscle damage in sedentary men and women.
2) To examine whether 250 grams of whole pureed mango in comparison to placebo drink without mango favorably alters immune response and recovery after exercise as assessed by peripheral blood mononuclear cells.
3) To evaluate whether 250 grams of whole pureed mango in comparison to placebo drink reverses the exercise induced downregulation of microRNA as induced by inflammation and oxidative stress.

Inclusion and Exclusion Criteria

Inclusion criteria: We are recruiting adult men and women, regardless of ethnicity, between age 18-40 years with a BMI between 18.5 to 25 kg/m2, and who are otherwise healthy. You will need to have a history of physical activity < 1 hr/week, < 20 minutes/session, and have a sedentary occupation for two years prior to enrollment in this study.

Exclusion criteria: If you smoke or have any form of pre-existing of any musculoskeletal disorder within six months before the study and any consumption of particular use of supplements within the last year (protein, ergogenic, and other amino acids). You also must not be consuming other mango or mango products or have allergies to mango.

Participant Initials

Research Procedures

For this study, we will ask you to consume either 250g total of whole pureed mango drink or 250 g of placebo drink without mango for 28 days. Then, you will stop consuming either product for 2 weeks (wash-out period).

After the 2 weeks, we will ask you to consume the different product as compared to what you consumed in the 1st 28 days, whole pureed mango drink or placebo drink without mango daily for another 28 days.

Each product (whole mango puree drink and placebo drink without mango) is packaged individually in a 125 g portion cup size and will be kept frozen to maintain freshness. A 125 g portion of the product will be approximately half a cup. You will be asked to consume the product twice daily for a total of 250 grams per day, one portion at the first meal of the day and the second portion at the last meal of the day. You will receive a two week supply of product at the baseline visit. We will ask you to come to TWU campus to pick up supplies every two weeks.

During the study, you will be asked to come to the Institute for Women's Health Clinics at the TWU Denton Campus located in Woodcock Hall for 4 visits, at baseline and at the end of each treatment course (baseline, 4-week, 6-week, 10-week time point). Prior to each study visit, we will ask you not to eat any food after 10 PM and to appear the next day at the clinic located in Woodcock Hall.

On the day of a study visit (total of 4 study visits, which are at baseline, and final point for each treatment course), a female researcher (for female participants) or a male researcher (for male participants) will take your weight, height, waist and hip circumference measured to the nearest 0.1 centimeter using a Lufkin steel tape using techniques developed by Institutional Standards for Anthropometric Assessment. Weight will be measured to the nearest 0.1 kilogram with a calibrated digital scale. Height will be assessed to the nearest 0.1 mm with a stadiometer. Body composition will be determined using dual energy X-ray absorptiometry or DXA, an FDA-approved device (Hologic Horizon, Hologic Inc, Marlborough, MA). The instrument is located within the IWH clinics (Woodcock Hall 017). A registered and certified technician will perform all body composition assessment which includes fat and lean mass, visceral fat composition, android/gynoid ratio (above versus below waist percentage). For the scan, you will be asked to lie face up, wearing loose fitting clothing with no metals in clothing and no jewelry on a padded instrument table. The technician will provide instruction for this assessment to assure accuracy and safety in completing this measurement. The estimated time to complete the body composition assessment including analysis of the result may require 20 minutes.

A phlebotomist (person taking the blood) will place a temporary catheter in the vein in one arm (the arm will be based on personal preference of you), and draw 3 tablespoons of your blood, at baseline, after the first treatment pre-exercise, at 0 minutes after exercise

trial, then at 60 minutes and again at 120 minutes post exercise during recovery time. Exercise trials will occur at the end of each of the treatments. Refreshing Perception Questionnaire will be collected during each of the final visits, visit 2 and visit 4. The questionnaires will be answered 4 times during each visit right after the blood draw (at baseline, 0 minutes after exercise, 60 minutes, and again at 120 minutes after the exercise).

Maximal test for peak power: A maximal ramp test for peak power will be performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). Briefly, participants will be adjusted to the bike seat and handlebar and these settings will be used for the duration of the study. Participants will then perform a warm up on the bike at 0 watts for 5 minutes. Immediately following the warmup, participants will perform a standardized ramp test that will start at 10 watts and increase in wattage over the course of 15 minutes, ending at a maximum of 300 watts, but much less than this for sedentary populations.

Participants will maintain a cadence of 60 rpm minimum and will be encouraged to increase their rpm, if it falls below 60. Once a participant cannot maintain 60rpm for 15 seconds, the test will be terminated and peak power for the test will be recorded. Resting and maximal heart rates will be recorded.

Exercise trial: Before each exercise trial, you will be asked consume half the amount of the mango or placebo smoothie for that day (125 g). The participants will be asked to complete twoexercise trials at study visit 2 and 4 during the study. A trial will be performed on day 29, 28 days after each of the two treatment arms (placebo an mango.

The exercise trial will consist of moderate intensity aerobic exercise performed on a cycle ergometer. More specifically, for each exercise trial, participants will cycle for 60 min at a minimum cadence of 60 rpm and at a workload that correlates to 65% of peak power. This exercise protocol has been previously shown to elicit a robust circulating miRNA response (Nielsen et al., 2014). If necessary to complete the 60 min trial, workload can be reduced by 10% based on participant feedback on their current ability to continue the trial. Encouragement will be provided to keep exercising, but further reductions in workload will be allowed. Heart rate will be recorded at 10 min intervals using a heart rate monitor.

You will remain at the Clinic for about 1 hour for laboratory visits 1 and 3, and for 4.5 hours for laboratory visits 2 and 4. During that time you cannot leave.

Time Commitment

The study will include two treatment courses, each treatment course is 4 weeks. In between the two treatment courses, there is a 2-week wash-out period (no study products need to be consumed during this time). Total of 10 weeks (2.5 months) will be involved in this study. Your time commitment includes initial phone screening (~ 15 minutes), and consent form (~ 15 minutes), max test (15-20 minutes), exercise trial of cycling (~ 60 minutes), post exercise monitor (~ 120 minutes), blood draws (~ 30 minutes), body composition assessment (~ 20 minutes). Each of the other three visits will involve similar duration without phone screening and consent form.

Total time commitment for you is approximately 10 hours.

Study Schedule Table

Time Points	Baseline of treatment arm 1	4 weeks	Final of treatment arm 1	2 weeks washout period	Baseline of treatment arm 2	4 weeks	Final of treatment arm 2
Research Activities	Lab Visit - Anthropometric measurements - -Blood draw -Consume mango/placebo product -Max test -BXA	Consume assigned feeding products on your own (125g/each) with first and last meals of the day	Lab Visit - Anthropometric measurements - Blood draw - Consume mango/placebo product - Refreshing Perception Questionnaire -Exercise Trial -Blood draw at 0, 60, and 120 minutes - Refreshing Perception Questionnaire at 0, 60, and 120 minutes	No products need to be consumed	Lab Visit*	treatment course**	Lab Visit ***

*same procedure as the first initial visit except the Max test
** same procedure as the first 4 Weeks
*** same procedure as the final of treatment of arm 1

Participant Initials

Potential Risks

A potential risk to you as a participant in this study is release of confidential information. Confidentiality will be protected to the extent that is allowed by law. To protect confidentiality, you will be given a code number which will be used in all records. A master list containing your name and match code name will be used and will be stored away from other data. Only Shanil Juma, PhD and the study coordinator Mirna Murad will know your identity and have access to the master list. All records will be stored in a locked filing cabinet in Shanil Juma, PhD's office. Blood samples will be stored in a locked -80 0C freezer in Scientific Research Commons (SRC). Paper data (except the copies of the consent form which will be returned to the IRB office) will be shredded within 5 years of completion of the study. And the original consent forms will be stored as required by IRB for at least 3 years. The blood samples will be sterilized in biological safety bags and disposed in February 2026. Your name or any other identifying information will not be included in any publication that may result from the study. A DEXA-trained person will know not to enter your names into the DEXA in order to protect confidentiality. A code name/number will be used instead. There is a potential risk of loss of confidentiality in email, downloading, and internet transactions. Identifiers will be removed from the identifiable private information or identifiable biospecimens and that, after such removal, the information or biospecimens could be used for future research studies or distributed to another investigator for future research studies without additional informed consent from you or the legally authorized representative.

If you allow your de-identified data to be used in future studies, please initial here_____.

Or, if you would like to participate in the current study but not allow your de-identified data to be used for future research, please initial here _____.

Coronavirus (COVID-19) Exposure: The potential risk of COVID-19 will be minimized based on the current CDC plan, state, local, and TWU guidelines. These are as follows:
a) Prior to coming to research lab, the individuals conducting the evaluations and you will self-screen for any of the following new or worsening signs or symptoms of COVID19: cough, shortness of breath or difficulty breathing, chills, repeated shaking with chills, muscle pain, headache, sore throat, loss of taste or smell, diarrhea, or feeling feverish. b) A non-contact thermometer will be used to monitor all key personnel and your temperature upon entry into the clinic. Individuals with temperature greater than or equal to 100 degrees Fahrenheit will be requested to refrain from testing that day and isolate for 14 days before returning to campus.
c) You and the investigators will be discouraged from gathering in any common areas in all spaces. A 6 feet distance will always be kept between individuals at all possible times.

_____
Participant Initials

d) All researcher personnel will use adequate personal protective equipment (PPE) including gloves, facemasks, and lab coats while conducting test procedures.
e) All equipment/ testing devices will be sanitized using disinfectant sprays and wipes after every individual test.
Research personnel will attempt to maintain social distancing while monitoring and running ergometer and muscle fatigue testing, please initial here_____.

A possible risk will be exposed to low-level radiation associated with body composition assessment using the DEXA. Use of DEXA for body composition involves x-ray and exposure to low level radiation. All of us are exposed to unavoidable radiation from the environment. You will be exposed to less than 10 millirem of radiation for each scan, which is equivalent to the natural background radiation from one day on earth. You should not be pregnant, lactating, nor at risk of becoming pregnant at the time of the study. You may consider not participating in the study due to the DEXA scan and withdraw at any time. Trained personnel who have the necessary credentials will be running the DEXA machine.

A possible risk is that you may not like the food (intervention products) or are allergic to mango. If you do not like the food, there is no penalty for not eating it. You are free to quit the study at any time. Before committing to the study, you will be informed that the study products contain mango. You will also be informed of common symptoms of allergic reactions. If you reveal you are allergic to mangos, you will not be considered for participation. If you become allergic to the study products used in the study, you will be asked to stop the study. You will also be advised to consult a physician if you experience any allergic reactions.

Another possible risk is peripheral venous blood draw infection, bleeding, and/or bruising. There is a small risk of the needle going through the vein or not going into a blood vessel. Also, you may experience discomfort, bleeding, and/or bruising. On a rare occasion, you may feel dizzy or faint. The likelihood of these complications is very remote (about 1 in 10,000), when the procedure is carried out by trained personnel and proper equipment is used. Precautions will be used during all blood draw procedures. Sites for blood draws will be cleaned with alcohol immediately before each blood draw. Each new needle that is opened will be disposed of in proper containers after use. A trained individual will obtain these blood samples to minimize these risks. The amount of blood taken over the course of the study will not affect normal daily activities. A typical donation of blood is about one pint (1 pint = 450mL, American Red Cross). Each blood draw in this study is about 3 tablespoons (~ 45 mL).

You will receive time to relax before and after the blood draw. A light snack and water will be available to you. This will reduce the possibility of your fainting. If you faint during the blood draw, we will lay you down and make you comfortable. We will

carefully watch you until you regain consciousness and will not make another attempt to draw your blood again that day.

You may be allergic to the latex gloves the phlebotomist wears for blood draw. In that case, the phlebotomist will use a different type of gloves.

In addition to the risks above, you may experience anxiety or embarrassment related to weight, height, waist and hip circumference, and body composition measurements. In order to minimize this risk, you will be assured of complete confidentiality before taking these measurements. All measurements will be taken only by an experienced/trained male or female investigator in a private room.

Another possible risk to you is fatigue. You can watch videos, relax, and take breaks as needed. You can withdraw from the study at any time without any penalty.

There is also a possibility of the risk of cardiac or cerebrovascular event during high intensity exercise. The overall risk of a cardiac or cerebrovascular event has been estimated at 6 in 10,000 during high-intensity exercise among healthy individuals and individuals with a known cardiovascular disease (Gibbons et al., 1980). All technicians present during testing and training are certified in CPR and AED techniques.

There are risks associated with submaximal exercise. According to the American College of Sports Medicine, there is little to no risk of an adverse event during submaximal exercise. However, risks include injury to muscles, joints, or organs, a sudden cardiac event or even death. You may rest as needed and are encouraged to be properly hydrated before beginning the exercise trial. All of the physiologic risks inherent with exercise testing and training will be minimized through preliminary screening, adherence to standards of practice for exercise testing published by the American College of Sports Medicine, and personal monitoring of each test by trained personnel.

Coercion may be another potential risk. Before committing to the study, you will be informed that participating in the study is completely voluntary, and you can withdraw from the study at any time. The decision of whether to participate will have no impact on the availability of care during the study.

The researchers will try to prevent any problem that could happen because of this research. You should let the researchers know at once if there is a problem and they will help you. However, TWU does not provide medical services or financial assistance for injuries that might happen because you are taking part in this research.

Participation Benefits

Your participation in this research study is completely voluntary, and you may discontinue your participation in the study at any time without penalty. As a participant in the study, you will receive the study food for a total of 8 weeks**.** You will also receive a cash incentive up to $150 in total by completing the study. A partial payment of $75 will be provided upon completion of one treatment course, and upon completion of the second treatment course, you will receive another $75 in cash. In addition, at completion of the study a summary of results, as well as the results of your blood analysis, will be mailed to you upon request.*

Questions Regarding the Study

You will be given a copy of this signed and dated consent form to keep. If you have any questions about the research study you may ask the researchers; their phone numbers are at the top of this form. If you have questions about your rights as a participant in this research or the way this study has been conducted, you may contact the Texas Woman's University Office of Research and Sponsored Programs at 940-898-3378 or via e-mail at IRB@twu.edu.

_____ _____

Signature of Participant Date

This page will be detached and filled separately.

* If you would like to receive a summary of the results of this study, please provide an address to which this summary should be sent:

_____
_____
_____

Participant Initials

APPENDIX E

RANDOMIZED TREATMENT SHEET

Randomized Treatment Sheet

A=mango, B=Placebo

1	B	19	B
2	A	20	B
3	A	21	A
4	A	22	A
5	A	23	A
6	B	24	A
7	B	25	A
8	B	26	A
9	A	27	B
10	A	28	A
11	B	29	A
12	A	30	B
13	B	31	B
14	B	32	B
15	B	33	B
16	B	34	A
17	A	35	A
18	B		

APPENDIX F

MANGO AND PLACEBO RECIPES AND NUTRITION FACTS

Mango and Placebo Recipes and Nutrition Facts

Placebo Recipe: Full blender batch
-994g Ice
-294g Monin mango syrup
-7g citric acid (1.5 tsp)
-3.5g xanthan gum (heaped 3⁄4 tsp)
-5 drops of yellow food coloring
-4 drops of orange food coloring
Per day: 185g Per serving: 92.5g
Mango Recipe: Full blender batch
-1,000g mango
-400g water (1 2⁄3 cup)
Per day: 350g Per serving: 175g

	Mango	**Monin**
Serving Size	250 g	1.4 fl oz
Sugar	34 g	33.6 g
Kcal	141 kcal	140 kcal

APPENDIX G

MAX EXERCISE PROTOCOL DATASHEET

2021 Mango Study – Max Exercise Protocol Datasheet

Participant ID: _____ Researcher:_____ Date:_____ Time:_____

Resting Heart Rate:_____ Height (cm):_____ Weight (kg): _____

Bike Settings (**A or B**):_____

Seat (Horizontal/Vertical):_____/_____Handlebar (Horizontal/Vertical): _____/_____

Warmup = 5min @ 0 Watts **HR@ 4:30min**:_____

Peak Power Max Test: (Select Protocol->Bicycle->Predefined->Ramp 10-300 Watt)

Record Heart Rates @

1:00:_____ 9:00:_____

2:00:_____ 10:00:_____

3:00:_____ 11:00:_____

4:00:_____ 12:00:_____ Stop Time: _____

5:00:_____ 13:00:_____ Peak Power: _____

6:00:_____ 14:00:_____ 65% of Peak Power:_____

7:00:_____ 15:00:_____

8:00:_____ **Highest HR Achieved**:_____

APPENDIX H

1-HR EXERCISE PROTOCOL DATASHEET

2021 Mango Study – 1HR Exercise Protocol Data

Participant ID: _____ Researcher:_____ Date:_____

1HR Trial Number (circle one) **1 2 3 4**

PRE Blood Time _____ Mango Drink Time _____ Resting Heart Rate _____

Bike (**A or B**)_____ (Use settings from Max trial)

Record Heart Rates @

4min_____ 30 min_____ 60 min_____

10min_____ 40 min_____ 5min Resting_____

20 min_____ 50min_____

Watt Settings Start Time_____ End Time (60min total)_____

0W Warmup Time___0min___ to __5:00min_

65%_____ Time___5:00___ to _____

55%_____ Time_____ to _____

45%_____ Time_____ to _____

35%_____ Time_____ to _____

25%_____ Time_____ to _____

*Cooldown @ 25-50W for at least 2min

NOTES