The Effect of Curcumin on Oxidative Stress and Inflammatory Markers in Recreationally Active Women and Men

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Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
In
Human Nutrition, Foods, and Exercise

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April 30, 2024

Blacksburg, VA

Keywords: Oxidative Stress; Reactive Oxygen Species; Inflammation; Biomarkers; Curcumin Supplementation; Physically Active Individuals

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Academic Abstract

Oxidative stress is a state characterized by an imbalance between the production and elimination of reactive oxygen species (ROS) within cells. ROS, also known as free radicals, are crucial for cellular signaling and are generated through natural processes. The antioxidant defense system typically regulates their concentrations to prevent oxidative stress-related damage. However, when ROS concentrations surpass a certain threshold and overwhelm the antioxidant defense system, it can lead to physiological issues and impairments in athletic performance. Additionally, oxidative stress and inflammation are closely related phenomena that can exacerbate each other, creating a vicious cycle. Both oxidative stress and inflammation play key roles in the pathophysiology of various chronic conditions such as cardiovascular diseases, neurodegenerative diseases, cancer, diabetes mellitus, autoimmune diseases, and accelerated aging. Furthermore, acute oxidative stress and inflammation have been shown to negatively affect performance by reducing skeletal muscle force output and increasing fatigue. Therefore, it is crucial to explore strategies to mitigate uncontrolled elevations of oxidative stress and inflammation. Curcumin, a bioactive compound found in turmeric, has been linked to antioxidant and anti-inflammatory properties.

While cell line and animal studies have demonstrated the antioxidant and anti-inflammatory potential of curcumin, its effects in humans remain inconclusive. This dissertation project aimed to evaluate the effect of a four-week turmeric supplementation intervention on biomarkers associated with exercise-induced oxidative stress and inflammation in recreationally active individuals, 18 to 45 years of age. The study investigated curcumin's potential as an antioxidant and anti-inflammatory agent, while contributing to the existing literature on strategies for managing oxidative stress and inflammation. The findings from this research may offer valuable insights for promoting health, well-being, and athletic performance.

The Effect of Curcumin on Oxidative Stress and Inflammatory Markers in Recreationally Active Women and Men

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General Audience Abstract

Oxidative stress arises from an imbalance between the production and elimination of reactive oxygen species (ROS) within cells. ROS, or free radicals, serve essential roles in cell signaling and are naturally generated. While our bodies possess a defense mechanism that typically regulates ROS concentrations to prevent oxidative stress-related damage, an excess of ROS can overwhelm this system, leading to physiological complications and impairing athletic performance. The interplay between oxidative stress and inflammation exacerbates their effects, initiating a detrimental cycle. Both processes are implicated in chronic ailments such as cardiovascular diseases, Alzheimer's disease, cancer, diabetes mellitus, autoimmune disorders, and accelerated aging. Moreover, elevated concentrations of oxidative stress and inflammation can diminish muscle strength and increase fatigue during exercise. Curcumin, a compound found in turmeric, renowned for its antioxidant and anti-inflammatory properties, presents a potential avenue for managing oxidative stress and inflammation. While some studies have demonstrated these benefits in cellular and animal models, the efficacy of curcumin in humans remains uncertain. This study assessed whether a four-week regimen of turmeric supplementation can attenuate markers of oxidative stress

and inflammation in physically active individuals, 18 to 45 years of age. By investigating the potential antioxidant and anti-inflammatory properties of curcumin, this research aimed to contribute novel insights into strategies for mitigating oxidative stress and inflammation, thereby promoting health, well-being, and athletic performance.

Dedication

This dissertation work is dedicated to my Amumma (Grandmother), Rajalakshmi, whose unwavering strength and boundless determination have been a guiding light throughout my journey. Her relentless determination, strength of will and commitment to our well-being have laid the foundation for our dreams to flourish. To Amumma, my source of inspiration—this dissertation is a tribute to your extraordinary legacy and an expression of profound gratitude for the profound impact you have had on my life.

~Rohit

Acknowledgements

I am filled with profound gratitude and appreciation for the individuals whose unwavering support, guidance, and encouragement have been instrumental in the completion of this manuscript.

First and foremost, I extend my deepest gratitude to my advisor, Dr. Stella Volpe, whose mentorship has been invaluable throughout this journey. Your wisdom, expertise, and unwavering support have not only shaped my academic and scientific endeavors but have also fostered personal growth. Thank you for challenging me, believing in me, and inspiring me to strive for excellence. I am forever grateful for your guidance and mentorship.

I would like to express my sincere appreciation to my committee members, Dr. Enette Larson-Meyer, Dr. Michelle Rockwell, and Dr. Michael Bruneau, for their invaluable contributions to this dissertation. Your insightful feedback, words of wisdom, and commitment to my research have been instrumental in shaping its direction and enhancing its quality. I am truly grateful for your guidance and support throughout this process.

To my parents, whose unwavering love, encouragement, and sacrifices have been the cornerstone of my journey, I am eternally indebted. Your boundless support, belief in

my abilities, and unwavering encouragement have been a constant source of strength and motivation.

Finally, to my partner, Elena, whose unwavering love, support, and understanding have been the wind in my sails and my anchor throughout this endeavor. Your steadfast belief in me, encouragement, and support have been the driving force behind my success. I am profoundly grateful for your love, patience, and belief in me.

To all those who have supported and encouraged me along this journey, whether through words of encouragement, acts of kindness, or unwavering belief in my abilities, I extend my deepest gratitude. Your contributions have made a profound impact on my life and have helped shape the person I am today.

Thank you, from the bottom of my heart.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CK	Creatine kinase
CRP	C-reactive protein
ELISA	Enzyme linked immunosorbent assay
GSG	Glutathione
GSSG	Glutathione disulfide
GXT	Graded exercise test
Hb	Hemoglobin
Hct	Hematocrit
IL-6	Interleukin-6
NF-κB	Nuclear factor-kappa B
NRF2	Nuclear factor erythroid 2-related factor 2
PC	Protein carbonyl
TAC	Total antioxidant capacity
TNF-α	Tumor necrosis factor-alpha
VO_2max	Maximal oxygen consumption

CHAPTER 1 - INTRODUCTION

1.1 Introduction

Turmeric, scientifically known as Curcuma longa, belongs to the *Zingiberaceae* family and is an herbaceous perennial plant. The primary active metabolite found in turmeric is curcumin, which has been extensively studied for its various beneficial properties including antioxidant, anti-inflammatory, anti-mutagenic, anti-microbial, and anti-cancer activities.[1,2] Curcumin is a flavonoid compound that exhibits the ability to improve systemic markers of oxidative stress. Structurally, curcumin is a diferuloylmethane compound with two O-methoxy-hydroxyphenolic groups attached to an α, β-unsaturated β-diketone (heptadienedione) moiety.[3] Its antioxidant properties are derived from the hydroxyl (OH) and methoxy (CH2) groups present in its structure. The hydroxyl group possesses strong oxidative capabilities due to its electron-donating nature.[3] Consequently, it forms complexes with free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), which act as electron acceptors because of the presence of an unpaired electron in their outer orbital.

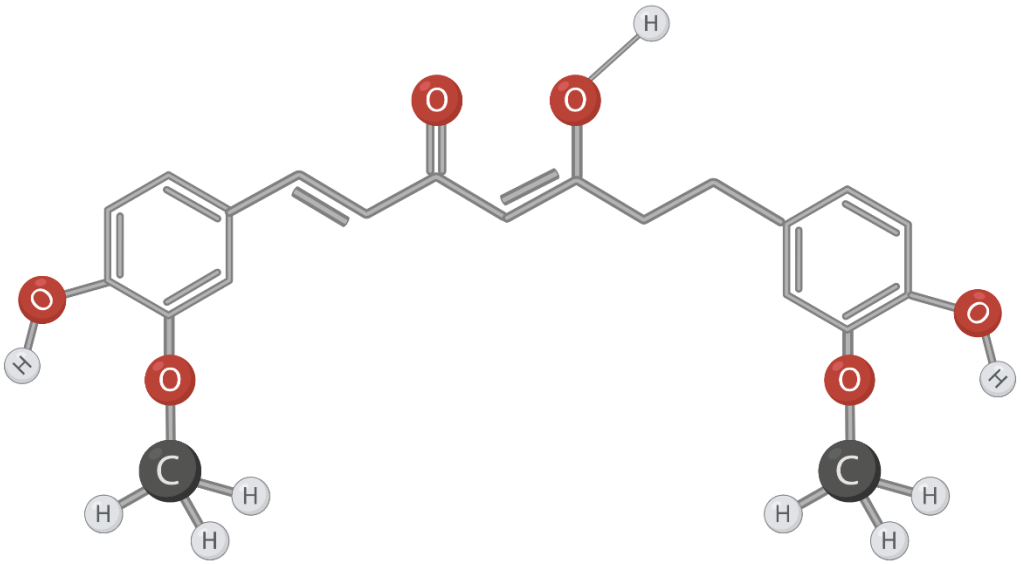

Figure 1.1. Structure of a curcumin molecule. (Created with BioRender.com)

Numerous biological processes, such as cellular respiration and digestion, give rise to the generation of detrimental compounds known as free radicals.[4] In low-to-moderate quantities, these free radicals are advantageous because they fulfill crucial roles in maintaining cellular functions and homeostasis.[5] The observation of biological cells producing free radicals, defined as atoms or molecules with one or more unpaired electrons, dates back to 1954. The term "oxidative stress" was initially coined by Helmut Sies to describe an imbalance in the pro-oxidant/antioxidant equilibrium favoring the former.[6] Over time, this phenomenon has attracted significant attention from researchers. While Sies'[6] initial definition provides a satisfactory starting point, it fails to

capture the intricacies of cellular redox imbalance. Consequently, Sies and Jones[7] proposed a more comprehensive definition of oxidative stress, characterizing it as "an imbalance between oxidants and antioxidants that favors oxidants, resulting in disruption of redox signaling and control, as well as molecular damage". Oxidative stress arises from heightened concentrations of reactive oxygen species (ROS) and reactive nitrogen species (RNS).[5,8] Reactive oxygen species are reactive byproducts of oxygen, while RNS are reactive derivatives of nitrogen. A comprehensive breakdown of the molecules constituting ROS and RNS can be found later in this dissertation. The regulation of redox balance is of utmost importance for maintaining cellular health. Reactive radicals play a crucial role in various biological processes and cellular signaling pathways that are vital for the survival of organisms. It is necessary to have a certain concentration of ROS and RNS to fulfill these functions. However, problems arise when the concentration of these reactive radicals exceed the capacity of the biological system to effectively reduce them. Due to their chemical nature, reactive radicals tend to bond with molecules indiscriminately, leading to cellular damage.[9] To counteract this, biological systems possess antioxidant defense mechanisms that act as a protective barrier against an excess of pro-oxidants, thereby preventing ROS-mediated cellular damage. The antioxidant defense system employs three primary strategies to safeguard cells from ROS-induced harm.[5] First, there are ROS scavenging molecules present both in intracellular and extracellular spaces that neutralize free radicals

through chelation, thereby eliminating their potential threat. Secondly, enzymatic antioxidants react with free radicals and convert them into less reactive molecules, thus reducing their ability to cause further cellular damage in a chain reaction. Some antioxidant enzymes also contain metal binding proteins that sequester pro-oxidant transition metals like iron and copper, preventing the participation of metal ions in ROS formation. Lastly, nonenzymatic antioxidants such as glutathione (GSH), uric acid, bilirubin, vitamin E, and vitamin C contribute to the defense against oxidative stress within cells. These nonenzymatic antioxidants primarily protect against oxidative damage by donating hydrogen atoms to free radicals, resulting in the formation of less reactive and less harmful species.[8,9]

This research study evaluated the role of curcumin in affecting biomarkers of oxidative stress and inflammation in healthy recreationally active individuals using a novel curcumin supplement known as Theracurmin®.

1.2 Specific Aims and Hypotheses

Specific Aim 1: To determine the effect of consuming 600 mg of Theracurmin® per day compared to 300 mg of Theracurmin® per day, compared to a placebo, for four weeks in recreationally active individuals, 18 to 45 years of age, on the following markers of exercise-induced oxidative stress: serum protein carbonyl concentrations, glutathione (GSH), glutathione disulfide (GSSG), the ratio of reduced glutathione to oxidized

glutathione (GSH:GSSG), and total antioxidant capacity (TAC) after a maximal graded exercise test.

Hypothesis 1a: It is hypothesized that four weeks of Theracurmin® supplementation will decrease the formation of serum protein carbonyl concentrations in a dose-dependent manner compared to a placebo in recreationally active individuals, 18 to 45 years of age, after a maximal graded exercise test.

Hypothesis 1b: It is hypothesized that four weeks of Theracurmin® supplementation will lead to increased serum glutathione concentrations in a dose-dependent manner compared to a placebo in recreationally active individuals, 18 to 45 years of age, after a maximal graded exercise test.

Hypothesis 1c: It is hypothesized that four weeks of Theracurmin® supplementation will lead to a lower ratio of reduced glutathione to oxidized glutathione in a dose-dependent manner compared to a placebo in recreationally active individuals, 18 to 45 years of age, after a maximal graded exercise test.

Hypothesis 1d: It is hypothesized that four weeks of Theracurmin® supplementation will lead to a lower depletion of total antioxidant capacity in a dose-dependent manner compared to a placebo in recreationally active individuals, 18 to 45 years of age, after a maximal graded exercise test.

Specific Aim 2: To determine the effect of consuming 600 mg of Theracurmin® per day compared to 300 mg of Theracurmin® per day, compared to a placebo, for four weeks in recreationally active individuals, 18 to 45 years of age, on the following markers of inflammation: interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP) concentrations after a maximal graded exercise test.

Hypothesis 2a: It is hypothesized that four weeks of Theracurmin® supplementation will lead to a diminished increase in serum Interleukin-6 concentrations in a dose-dependent manner compared to a placebo in recreationally active individuals, 18 to 45 years of age, after a maximal graded exercise test.

Hypothesis 2b: It is hypothesized that four weeks of Theracurmin® supplementation will lead a diminished increase in serum tumor necrosis factor-alpha concentrations in a dose-dependent manner compared to a placebo in recreationally active individuals, 18 to 45 years of age, after a maximal graded exercise test.

Hypothesis 2c: It is hypothesized that four weeks of Theracurmin® supplementation will lead to a diminished increase in serum C-reactive protein concentrations in a dose-dependent manner compared to a placebo in recreationally active individuals, 18 to 45 years of age, after a maximal graded exercise test.

Specific Aim 3: To determine the effect of consuming 600 mg of Theracurmin® per day, compared to 300 mg of Theracurmin per day, compared to a placebo, for four weeks in

recreationally active individuals, 18 to 45 years of age, on a marker of muscle damage (creatine kinase [CK] concentrations), after a maximal graded exercise test.

Hypothesis 3: It is hypothesized that four weeks of Theracurmin® supplementation will lead to diminished serum creatine kinase concentrations in a dose-dependent manner compared to a placebo in recreationally active individuals, 18 to 45 years of age, after a maximal graded exercise test.

Specific Aim 4: To determine the moderating effect of aerobic fitness (maximal oxygen consumption [VO_2max]) on the efficacy of 600 mg of Theracurmin® per day compared to 300 mg of Theracurmin®, for four weeks in recreationally active individuals, 18 to 45 years of age, on biomarkers of oxidative stress and inflammation.

Hypothesis 4: It is hypothesized that the effect of Theracurmin® supplementation on biomarkers of oxidative stress and inflammation will be inversely proportional to maximal oxygen consumption in recreationally active individuals, 18 to 45 years of age.

Specific Aim 5: To determine the moderating effect of percent body fat on the effect of 600 mg of Theracurmin® per day compared to 300 mg of Theracurmin®, for four weeks in recreationally active individuals, 18 to 45 years of age, on biomarkers of oxidative stress and inflammation.

Hypothesis 5: It is hypothesized that the efficacy of Theracurmin® supplementation on biomarkers of oxidative stress and inflammation will be directly proportional to percent body fat in recreationally active individuals, 18 to 45 years of age.

1.3 Significance

Free radicals play a crucial role in various biological processes within the body, including molecular cell signaling and defense against invasive antigens. However, excessive concentrations of free radicals can lead to harmful effects if left unattended. Oxidative stress, which is characterized by an imbalance between the production of free radicals and the body's antioxidant defenses, plays a significant role in accelerating the development of chronic non-communicable diseases such as cardiovascular diseases, diabetes mellitus, neurodegenerative diseases, and cancer.[6] Prolonged exposure to high concentrations of free radicals can cause structural defects at the mitochondrial level, dysfunction of enzymes, and defects in cellular structures.[8] These abnormalities can result in aberrations in gene expression. Additionally, elevated concentrations of reactive oxygen species (ROS), can cause damage to lipids, proteins, and deoxyribonucleic acid (DNA).[9] Reactive oxygen species reactions disrupt lipid membranes, increasing their fluidity and permeability.[10] Protein damage caused by high ROS concentrations involves modifications to specific amino acids, fragmentation of peptide chains, aggregation of reaction products, alteration of electric charge, enzymatic

inactivation, and increased susceptibility to proteolysis. Intracellular DNA damage caused by ROS may occur through oxidation of deoxyribose (a component of DNA), strand breakage, removal or modification of nucleotides (building blocks of DNA), and cross-linkage between DNA and proteins.[4,6,10]

While oxidative stress is necessary for normal physiological processes, excessive amounts can have severe negative effects on health. The induction of oxidative stress is further exacerbated by factors such as increased consumption of processed foods and exposure to various chemicals from sources like pesticides, heavy metals, food additives, and environmental pollution. A sedentary lifestyle also contributes to oxidative stress by increasing vascular nicotinamide adenine dinucleotide phosphate (NADPH) oxidase expression, which, in turn, enhances ROS production.[11] These factors are prevalent in modern lifestyles and contribute to the growing burden of chronic diseases. Therefore, it is essential to understand how oxidative stress develops and find ways to mitigate its elevated concentrations to achieve a proper redox balance. Addressing the problem of excessive oxidative stress may have implications for reducing the economic and health burden associated with chronic diseases such as stroke, cancer, myocardial infarction, diabetes mellitus, and other conditions. There is speculation that interactions between free radicals and DNA, leading to mutations that affect the cell cycle and promote neoplasia, may be responsible for the development of many types of cancer.[12] Currently, two leading hypotheses explain cellular aging: the

mitochondrial theory and the free radical theory. The free radical theory proposes that increased concentrations of intracellular free radicals adversely affect mitochondria, resulting in impaired function and reduced cellular regenerative capacity. The mitochondrial theory suggests that free radicals produced by mitochondrial activity damage cellular components.

1.3.1 Acute effects of oxidative stress

Regular physical exercise is widely recognized as an effective method for reducing the risk of all-cause mortality, prolonging longevity and health span.[5] It also leads to physiological adaptations that lower the likelihood of developing cardiovascular diseases, cancer, and diabetes mellitus. However, intense and prolonged exercise can result in oxidative damage caused by free radicals generated by contracting skeletal muscles.[5,13] This oxidative damage can impair cellular function through various means, such as damaging lipids, proteins, and DNA. The paradoxical nature of free radicals is the heart of cellular redox balance and is crucial for maintaining homeostasis in living organisms. It is now understood that excessive oxidative stress damages cellular components, while low to moderate concentrations of oxidants play important roles in gene expression control, cell signaling pathway regulation, and modulation of skeletal muscle force production.[6,8] Researchers have shown that an imbalance in redox status within skeletal muscles can significantly reduce force production.[5] As the production of ROS increases during muscle contractions, there comes a point where cellular redox

balance shifts towards a pro-oxidative state during prolonged or intense exercise. This phenomenon was first demonstrated in an in-situ diaphragm muscle by using an ROS scavenger called N-acetylcysteine, which delayed muscle fatigue.[14] Since then, several researchers have reported a delay in muscular fatigue during submaximal contractions by utilizing enzymatic and non-enzymatic antioxidants to scavenge ROS.[15–17] However, there are conflicting results regarding the effectiveness of antioxidants in delaying fatigue during maximal muscular contractions.

1.3.2 Chronic effects of oxidative stress

Chronic oxidative stress has been found to have a significant effect on the pathophysiology of various chronic diseases. This is primarily due to its detrimental effects on cellular function, including DNA damage, inflammation, lipid peroxidation, and mitochondrial dysfunction. Prolonged exposure to an excessive pro-oxidant state can lead to vascular damage and contribute to the progression of atherosclerosis, hypertension, and cardiovascular disease. Furthermore, oxidative stress has been implicated in impairing insulin signaling and predisposing individuals to the development of type 2 diabetes mellitus. The DNA damage caused by oxidative stress increases the risk of genetic mutations that can initiate cancer formation. Additionally, oxidative stress has been associated with the development of neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease. Lastly, oxidative stress has been shown to be a contributing factor in accelerating the aging process.[4,6]

Oxidative stress and inflammation are widely recognized as key contributors to various chronic diseases, and are particularly relevant in the context of physical activity and exercise. Active individuals often experience heightened oxidative stress due to increased metabolic activity, which can result in inflammation and subsequent tissue damage if not effectively managed. Therefore, interventions aimed at mitigating oxidative stress and inflammation are of significant interest in promoting overall health and well-being among physically active populations. Theracurmin®, a highly bioavailable form of curcumin, has attracted attention for its potential antioxidative and anti-inflammatory properties. Prior research indicates that curcumin supplementation may help reduce oxidative stress and inflammation markers in diverse populations, including healthy individuals and those with chronic diseases.[2,3,18,19] However, limited evidence exists regarding the effects of Theracurmin® supplementation, specifically on biomarkers of oxidative stress and inflammation in active individuals. The aim of this study was to address this gap by examining the effect of four weeks of Theracurmin® supplementation on biomarkers of oxidative stress and inflammation in active individuals using validated indicators. The objective was to provide valuable insights into the potential role of Theracurmin® in modulating oxidative stress and inflammation in physically active individuals. The results of this study have the potential to inform clinical practice and public health initiatives aimed at optimizing the health and performance of active individuals. If Theracurmin® supplementation is found to

effectively reduce oxidative stress and inflammation markers in this population, it could serve as a promising adjunctive strategy for enhancing overall health and reducing the risk of chronic diseases associated with excessive oxidative stress and inflammation. Therefore, this study carries significant implications for advancing knowledge of the effects of Theracurmin® supplementation on oxidative stress and inflammation in active individuals, with potential implications for improving health outcomes and optimizing performance within this population.

1.4 Innovation

Although curcumin has been associated with multiple positive effects when consumed, it faces challenges in its utilization by the human body. The primary limitation of natural turmeric is its low bioavailability.[20] This is primarily due to the hydrophobic nature of curcumin, which leads to rapid degradation in the gastrointestinal tract and reduces absorption into the bloodstream. Turmeric is quickly metabolized by the liver and excreted within a short period of time. Once absorbed, curcumin undergoes a complex metabolic process in the body.[20] The initial step takes place in the liver, where enzymes, such as cytochrome P450 (CYP), modify curcumin's chemical structure, allowing for further modification through conjugation. In the second stage of metabolism, glucuronidation and sulfation occur, wherein curcumin is conjugated with molecules such as glucuronic acid and sulfate to form curcumin glucuronides and

curcumin sulfates, respectively.[20] These metabolites of curcumin have increased solubility in water, facilitating their elimination from the body.

To overcome turmeric's limitations in its natural form, a novel formulation called Theracurmin® was utilized in the present study. Theracurmin® is an innovative form of curcumin with enhanced bioavailability achieved by reducing curcumin to nanoscale particles encapsulated within micelles, which act as a protective layer.[21] The first step involves breaking down curcumin into nanoparticles through techniques like wet milling or high-pressure homogenization. This is followed by micellar encapsulation.[21] Micelles possess both hydrophilic and hydrophobic properties, with the hydrophobic core housing the curcumin nanoparticles, protecting them from degradation and maintaining stability. This process significantly increases curcumin's solubility in water while improving its stability during digestion. The reduced particle size of Theracurmin® enhances absorption by increasing surface area, while micellar encapsulation prevents clumping and premature metabolism. Consequently, Theracurmin's® reduced particle size enhances its ability to traverse the gut lining and enter the bloodstream, while micellar encapsulation prevents interactions with other molecules in the gut.[21]

In recent years, several researchers have demonstrated Theracurmin's® enhanced bioavailability.[22] For instance, Nakagawa et al.[23] demonstrated a 27-fold increase in blood concentration-time curve of Theracurmin® compared to curcumin powder. Oral

consumption of Theracurmin® has been shown to result in significantly higher serum concentrations compared to other curcumin forms. However, the effect of Theracurmin® supplementation for a duration of four weeks on systemic markers of oxidative stress and inflammation in a healthy active population remains unexplored.

The utilization of two distinct dosages of Theracurmin® (300 mg and 600 mg) facilitates comprehensive analyses of the dose-response relationship. This novel approach allows for the exploration of potential dose-dependent effects on biomarkers related to oxidative stress and inflammation, thereby elucidating the optimal dosage for maximizing the therapeutic effect of Theracurmin® supplementation. This research study incorporated the assessment of multiple biomarkers associated with oxidative stress and inflammation, offering a thorough examination of the physiological responses to Theracurmin® supplementation. Through the evaluation of markers such as protein carbonyl, glutathione, glutathione disulfide, total antioxidant capacity, interleukin-6, tumor necrosis factor-alpha, C-reactive protein, and creatine kinase, this study provides a comprehensive insight into the acute biochemical responses underlying the potential effects of Theracurmin® on oxidative stress and inflammation processes. The outcomes of this investigation carry significant implications for general wellbeing and disease prevention. By elucidating the effect of Theracurmin® supplementation on oxidative stress and inflammation biomarkers within this cohort, this study may guide evidence-based interventions aimed at reducing the incidence of

chronic diseases linked to dysregulated oxidative stress and inflammation, thereby fostering enhancements in overall health and well-being.

1.5 Rationale

1.5.1 Rationale for exploring curcumin as an antioxidant

Although curcumin consumption has been associated with several health benefits, its effect as a potent antioxidant and anti-inflammatory agent has not been well studied or understood. Within the scope of dietary antioxidants, vitamin C, vitamin E and carotenoids contribute to the bulk of existing literature on dietary antioxidants. A driving factor in this discrepancy is the problem of bioavailability faced by curcumin, whereas vitamins C and E are readily absorbed by the body. As such, there is a need to expand the existing literature on curcumin's role as an antioxidant and anti-inflammatory agent.

1.5.2 Rationale for using two intervention doses of curcumin

The rationale for employing two varying doses of Theracurmin® supplementation in this study was grounded in investigating dose-response relationships, assessing efficacy, and considering inter-individual variability. Specifically, this study utilized two distinct interventional doses, 600 mg and 300 mg of Theracurmin®. The exploration of the dose-response relationship is essential in determining the optimal dosage for a therapeutic intervention. By incorporating two different doses, this study aimed to

evaluate whether there is a dose-dependent effect on the outcome variables of interest in the study. This methodology enabled the identification of potential threshold effects or saturation points, offering valuable insights into the pharmacological characteristics and optimal dosing schedule of Theracurmin®. Furthermore, the inclusion of two doses facilitated comparative analyses to determine the effect of each dose. Additionally, variations in pharmacokinetics and pharmacodynamics among individuals can affect their response to therapeutic interventions. Therefore, by including two doses, this study sought to address potential inter-individual variability in response. Moreover, having two distinct doses provided the opportunity to discern potential false positive outcomes within the study's findings.

1.5.3 Rationale for incorporating an incline-based graded treadmill test

To produce an acute state of elevated oxidative stress via exercise, there is a need to increase metabolism by increasing exercise intensity. A number of researchers have provided evidence that utilizing a speed-based protocol without incorporating gradient will not effectively increase the metabolic cost at the desired rate. For example, Taylor et al.[24] demonstrated that utilizing a constant speed while gradually increasing the gradient by 2.5% with each increment is more advantageous than maintaining a constant grade and increasing the speed. Additionally, it was observed that the increase in oxygen intake was less significant when speed was increased compared to gradient.[25] This disparity is likely due to engaging more muscle groups during uphill running

compared to running on a flat surface. The additional metabolic cost of working musculature during uphill running amplifies the oxygen uptake to a greater extent than increasing speed on a consistent gradient. Consequently, maximal oxygen consumption (VO_2max) values derived from speed-based protocols have been shown to be lower than those obtained from incline-based protocols.[25] Moreover, it is essential to consider the risk of injury in any exercise testing. Uphill running has been found to result in a decreased vertical loading rate.[26] Vertical loading rate represents the rate of change of force application after the effect of the feet hitting the ground and serves as an indicator of ground effect force and injury prevalence. Lemire et al.[25] investigated differences in vertical loading rate based on gradient angle in 29 healthy individuals. The authors discovered that, although there were no significant variations in vertical loading rate between different uphill gradients (5% vs 10% vs 15% vs 20%), there were meaningful differences compared to running on level ground. Specifically, vertical loading rate decreased by 27% at a 5% gradient and by 54% at a 10% gradient compared to running on level ground with equivalent metabolic cost.[26] Thus, it would be prudent to employ an incline-centric VO_2max protocol that reduces injury risk by diminishing vertical loading rate during the test.[26] Another factor that must be considered when selecting a treadmill protocol is the skill element required to complete the test. Researchers investigating Olympic weightlifters have demonstrated that the optimal activation of fast twitch muscle fibers is a learned skill that accumulates through targeted training.[27]

With running, fast twitch muscle fibers are more active at higher relative speeds compared to lower speeds. Therefore, for the purpose of the proposed study, the most ideal protocol would be an incline-based protocol consisting of gradual increments in gradient with a consistent speed.

1.5.4 Rationale for including both sexes

The existence of a fundamental difference in the physiological response to oxidative stress between women and men requires the need to evaluate both sexes. Several studies have been conducted to assess this difference, and researchers have found that oxidative stress concentrations are generally higher in healthy young men compared to pre-menopausal healthy women. Men exhibit significantly higher baseline (at rest) measures for oxidative stress.[28] This higher concentration of oxidative stress in men may be attributed to a combination of increased production of ROS and reduced activity of the antioxidant defense system.[29] Moreover, because oxidative stress and inflammation are intricately linked, women also demonstrate lower susceptibility to inflammatory conditions than men.[30] Hence, the presence of intersex variation in oxidative stress and inflammation necessitates the inclusion of both sexes in research studies and analyses of their divergent responses to antioxidant supplementation.

1.5.5 Rationale for the selection of biomarkers of interest

Protein Carbonyl. Protein carbonyl is frequently utilized as a biomarker for oxidative stress due to its chemical stability. The transient nature of reactive oxygen species (ROS)

in the blood poses challenges in their quantification, making protein carbonyl a valuable proxy marker for oxidative stress. Additionally, the concentration of protein carbonyls is reported to be elevated in the immediate aftermath of aerobic exercise. Protein carbonyls are generated through protein oxidation via a process known as protein carbonylation, which are facilitated by ROS.[31,32]

Glutathione (GSH): Glutathione is a tripeptide consisting of glutamate, cysteine, and glycine, which is primarily synthesized in the hepatic cells.[33] Glutathione plays a pivotal role in antioxidant defense. Reduced GSH is oxidized by ROS and subsequently reduced again by glutathione reductase. Any condition associated with an increase in ROS will decrease GSH concentrations and decrease the glutathione to glutathione disulfide (GSH:GSSG) ratio. As such, the redox balance of GSH has been used as a marker of antioxidant status in various conditions.

Glutathione Disulfide (GSSG): Glutathione disulfide is the oxidized form of glutathione, where two molecules of glutathione are linked together by a disulfide bond. GSSG is then reduced back to GSH through enzymatic reactions involving glutathione reductase and the cofactor NADPH, which ensures the maintenance of the cellular pool of GSH for antioxidant defense. As such, elevated concentrations of GSSG suggest a decrease in the availability of GSH and are inversely correlated with the antioxidant capacity of cells. Assessment of GSSG concentrations provides valuable insights on the antioxidant status of biological systems.

Ratio of Reduced Glutathione to Oxidized Glutathione (GSH:GSSG): The GSH:GSSG ratio reflects the redox balance between reducing and oxidizing conditions within cells, and serves as a critical indicator of cellular redox status. Measurement of GSH:GSSG ratio is used to assess cellular redox balance, evaluate oxidative stress, and examine the efficacy of the antioxidant system.

Total Antioxidant Capacity (TAC): Total antioxidant capacity is a biomarker that measures the cumulative concentration of both antioxidant enzymes (such as catalase, glutathione reductase, glutathione peroxidase, and superoxide dismutase), as well as nonenzymatic systems.[34] Researchers have shown that both maximal and submaximal bouts of acute exercise lead to an increase in the concentrations of antioxidant enzymes. As such, TAC is used as a general marker of antioxidant defense.

Interleukin-6 (IL-6): Interleukin-6 is a pro-inflammatory cytokine that is transiently produced as a response to tissues damage and infections.[35] Because high-intensity exercise leads to the damage of muscle tissues, damage-associated molecular patterns (DAMPs) are released from the damaged cells, which promote inflammation. Researchers have demonstrated a significant increase in serum IL-6 concentrations after a marathon.[36]

Tumor necrosis factor-alpha (TNF-α): It is widely accepted that a local increase of TNF-α leads to symptoms of inflammation. Several researchers have shown a significant

increase in serum TNF-α concentrations after strenuous exercise.[36–38] It is believed that this cytokine is produced by the contracting muscle cells during strenuous exercise. Furthermore, TNF-α is a downstream byproduct from the activation of nuclear factor-kappa B (NF-κB), which has been shown to be the master regular of the inflammatory response.[39]

C-reactive protein (CRP): C-reactive protein is a cytokine associated with inflammation. Although CRP is usually low in healthy populations, there appears to be a transient increase in serum CRP following a bout of strenuous exercise. This increase is produced by an exercise-induced acute phase response, which is mediated by the inflammatory cytokine system.[38]

Creatine Kinase (CK): Serum creatine kinase concentrations have been shown to increase after strenuous exercise and can be used as a marker for muscle tissue damage. Although CK concentrations peak after around four days following exercise, a meaningful increase in serum CK concentrations can be observed immediately following strenuous exercise.

1.6 Summary

The proposed research project aimed to determine the potential antioxidant and anti-inflammatory properties of curcumin by providing oral supplementation of Theracurmin® to a healthy, active population for four weeks and inducing a state of

acute oxidative stress by means of a maximal graded treadmill exercise test to exhaustion. The proposed study will broaden the scope of curcumin's role as an antioxidant and anti-inflammatory agent, and contribute to the growing literature on ways to combat oxidative stress and inflammation, potentially providing valuable insights for promoting health and well-being.

CHAPTER 2 - LITERATURE REVIEW

PART I

2.1 From Oxygen to Oxidative Stress: A Journey into the Complexities of Life's Fundamental Element

There are a total of 118 elements in the periodic table, of which a small proportion serve as the fundamental components of organic life. Oxygen is one such element that plays a crucial role in the survival of most living systems. At the cellular level, oxygen is essential for energy synthesis, which fuels various physiological processes necessary for life. During cellular respiration, oxygen acts as the final electron acceptor in the electron transport chain within the mitochondrial matrix. It facilitates the movement of electrons along this chain, leading to the production of adenosine triphosphate (ATP). However, this process also results in the formation of reactive oxygen species (ROS) as natural by-products.[40] Scientists have shown that approximately 3% to 5% of oxygen is converted into free radicals.[5,6] Additionally, ROS can be generated by enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (NOXs), xanthine oxidase (XO), nitric oxide synthase (NOS), and peroxisomal constituents.[40,41] Furthermore, ROS can be produced by ionization and ultraviolet (UV) radiation.[40] When living systems are unable to regulate the production of these molecules, it leads to a condition known as oxidative stress.

Before delving into oxidative stress, it is important to understand free radicals and establish a foundation for comprehending this phenomenon. Free radicals are chemical species that possess one or more unpaired electrons. They are highly reactive molecules and readily interact with oxygen due to oxygen's ability to accept electrons, thus resulting in chemically unstable species like superoxide anion ($^{\circ}O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), and singlet oxygen (O_2^-).[40,42] Free radicals primarily originate from oxygen consumption, but can also be produced from nitrogen to form reactive nitrogen species (RNS). Collectively, these species are referred to as reactive oxygen/nitrogen species (RONS). While free radicals play functional roles in living systems by contributing to cellular signaling and homeostasis, excessive production beyond their functional requirements becomes detrimental. This is when oxidative stress occurs, leading to damage mediated by oxidative stress.[40,42]

Oxidative stress can be defined as the disruption of the balance between pro-oxidant (free radicals) production and their subsequent neutralization by the antioxidant defense system, resulting in an excess of free radical expression.[6,42] Excessive production of RONS can arise from various stressors such as exposure to environmental pollutants, excessive intakes of nutrients, or physical exercise.[43–45] To prevent the accumulation of excessive concentration of RONS, living systems employ antioxidant defense systems to maintain homeostasis. The primary purpose of these defense systems is to protect the body from the harmful effects of excessive free radicals.[42] The antioxidant defense

system consists of both endogenous and exogenous compounds. Endogenous compounds include bilirubin, uric acid, superoxide dismutase, catalase, glutathione peroxidase, among others. Exogenous compounds encompass carotenoids, tocopherols, ascorbate, bioflavonoids, and more.[5,42]

Free radicals are crucial for the survival of living systems because they play vital roles in various physiological processes and cellular signaling. Mammalian cells possess signaling pathways that are responsive to changes in intracellular redox states. Alterations in redox state can activate or deactivate numerous pathways designed to respond accordingly.[42,46] Hence, free radicals function as signaling molecules that act as molecular switches initiating various processes based on their presence. Consequently, a basal concentration of RONS is constantly being produced and eliminated by antioxidant defense systems, maintaining a delicate balance that represents the intercellular redox state.[47] Some examples of the intercellular redox state facilitating various physiological processes include: (i) the regulation of vascular tone, which can be achieved through the activation of guanylate cyclase or by modulating the transcriptional/post-transcriptional activity of nitric oxide synthase (NOS) via the activation of nuclear factor kB (NF-κB) or mitogen-activated protein kinases (MAPK), (ii) amplification of immune responses and apoptosis via the activation of activator protein 1 (AP-1) and NF-κB transcription factors in human T cells, (iii) regulation of insulin kinase activity via increased activity of protein tyrosine phosphatases, and (iv)

increased expression of antioxidant enzymes and glutathione in response to MAPK and NF-κB activation in an effort to restore redox balance, which is specifically relevant in the context of physical exercise. An elevation in reactive oxygen and nitrogen species (RONS) during and after acute exercise is hypothesized to act as the crucial "stimulus" for the up-regulation of antioxidant defense mechanisms that are commonly observed with prolonged exercise training.[48–51]

Although changes in redox states are necessary to initiate signaling pathways, restoring homeostatic concentrations is crucial for counteracting the negative effects of excessive oxidative stress.[52] Conditions that promote accelerated or chronic production of reactive oxygen and nitrogen species (RONS) can overwhelm the capacity of the antioxidant defense system, leading to disruptions in normal redox-sensitive signaling and a permanent alteration in redox balance. This permanent shift in the redox environment can result in detrimental effects through direct RONS-mediated oxidative damage to nucleic acids, lipids, and proteins, as well as alterations in gene expression that promote apoptosis in healthy cells and systemic inflammation.[53] Persistent oxidative stress leads to aberrant changes in redox states, leading to a deterioration in physiological functioning. Oxidative stress is a prominent contributor to the early pathophysiology of multiple chronic diseases and aging. This phenomenon serves as an underlying mechanism for the onset of various chronic diseases over prolonged periods, including diabetes mellitus, hypertension, cardiovascular diseases, neurodegenerative diseases,

alcoholic liver diseases, chronic kidney disease, cancer and aging. The interplay between oxidative stress and inflammation, wherein each factor exacerbates the other, adds further complications. The persistence of low-grade inflammation results in the production of cytokines, which subsequently activate nuclear factor-kappa B (NF-κB) transcription, further promoting inflammation. Inflammation is characterized by a chronic state of low-grade inflammation and continuous release of pro-inflammatory cytokines that modulate cellular function, even in the absence of any infection. Chronic inflammation also promotes the up-regulation of inflammatory cells such as neutrophils, monocytes, macrophages, and C-reactive protein (CRP).[42,54]

2.2 The Interplay Between Oxidative Stress and Inflammation

Oxidative stress and inflammation are closely interconnected and bidirectional phenomena. Inflammatory processes have the ability to induce oxidative stress, while conversely, oxidative stress can also elicit inflammation by activating several pathways.[54] During the initiation of inflammatory processes, macrophages and neutrophils generate substantial quantities of reactive oxygen and nitrogen species (RONS) to counteract invading antigens. Nevertheless, a portion of these RONS can disperse and infiltrate different locations, leading to the development of localized oxidative stress and subsequent oxidative stress-mediated tissue damage.[54] The increase in oxidative stress within cells triggers the activation of transcription factors, which then

enhance the expression of genes that encode inflammatory cytokines. One significant transcription factor involved in the signaling cascade of inflammation is nuclear factor-kappa B (NF-κB). Nuclear factor-kappa B controls the pathways responsible for innate and adaptive immune functions, as well as the inflammatory responses observed in chronic and acute inflammatory diseases and various cancers. As a transcription factor, NF-κB regulates the synthesis of multiple biomolecules involved in inflammation, including cyclo-oxygenase-2 (COX-2), cyclin D1, adhesion molecules, matrix metalloproteinases (MMPs), inducible nitric oxide synthase (iNOS), B-cell lymphoma-2 (Bcl-2), B-cell lymphoma-extra large (BcL-XL), and tumor necrosis factor-alpha (TNF-α). In its inactive state, NF-κB relies on pro-inflammatory cytokines like TNF-α, phorbol ester, and free radicals, such as hydrogen peroxide, for its activation. These activators are known to produce reactive oxygen intermediates (ROI), which subsequently activate NF-κB.[39,55,56] In cancer cells, it has been observed that the pro-inflammatory cytokine interleukin-6 (IL-6) can increase the gene expression of NADPH oxidase (NOX4).[57] Conversely, overexpression of NOX4 directly enhances the synthesis of IL-6. As a result, a positive feedback loop is established between these two factors responsible for oxidative stress and inflammation, namely NOX4 and IL-6, respectively. Hence, it is evident that there exists a distinct interplay between oxidative stress and inflammation, necessitating the simultaneous consideration of both conditions when discussing either of them. As such, understanding the molecular mechanism underlying

this interplay is crucial for the development of targeted therapeutic interventions aimed at modulating both oxidative stress and inflammation

2.3 Molecular Master Regulators of Redox Balance

The purpose of the master redox switches is two-fold. The first is to control the activation or deactivation cycles during redox signaling, and the second is to modulate or integrate activity of systems in redox sensing.[6] These redox switches are transcription factors that are sensitive to changes in redox balance. The two primary master switches are the Nrf2/Keap1 and NF-κB transcription factors, which play a role in a broad range of biological functions.

2.3.1 Nuclear factor-E2-related factor 2 (Nrf2)/ Kelch-like ECH-associated protein 1 (Keap1)

Nuclear factor-E2-related factor 2 (Nrf2) belongs to a family of transcription factors that play a role in the regulation of antioxidant and detoxification enzymes. Under normal (unstressed) conditions, Keap1 (Kelch-like ECH-associated protein 1) suppresses the transcriptional activity of Nrf2 by subjecting Nrf2 to rapid ubiquitination and degradation. However, under conditions of oxidative or electrophilic stress, specific cysteinyl residues of Keap1 are modified, which, in turn, results in the loss of Keap1's ability to ubiquitinate Nrf2. As a result, Nrf2 accumulates in the nucleus and activates the expression of its target genes, leading to an up-regulation of antioxidant systems.[58]

Although the activation of the Nrf2/Keap1 is generally considered protective, the overactivation of this system can be counterproductive.[59]

2.3.2 Nuclear Factor Kappa-light-chain-enhancer of Activated B Cells (NF-κB)

Nuclear factor κB (NF-κB) is a heterodimeric transcription factor consisting of multiple subunits, which play a role in inducing gene-expression that regulate inflammatory, immune, and acute phase responses. Like Nrf2, NF-κB contains redox-sensitive cysteine residues that inhibit their activity upon oxidation.[6] The pro-inflammatory function of NF-κB has been widely studied in macrophages, which are innate immune cells existing in numerous tissues. Macrophages are activated in response to various stressors, such as an infection or exercise. Upon activation, they secrete a large array of cytokines and chemokines that promote inflammation. Nuclear factor kB has been shown to be a key transcription factor of macrophages, and is therefore required for the induction of a large number of inflammatory genes, including those that encode TNF-α, interleukin-1β (IL-1β), IL-6, interleukin-12p40 (IL-12p40) and cyclooxygenase-2.[39]

2.4 Exercise-Induced Oxidative Stress

Exercise-induced oxidative stress (EIOS) is an acute state of the intercellular redox balance shifting to a pro-oxidant state due to the production of RONS during exercise. In 1978, researchers first identified that prolonged endurance exercise in humans leads to an increase in biomarkers of oxidative stress.[60] Subsequently, it was observed that

reactive oxygen species (ROS) are produced by contracting skeletal muscles.[61,62] These findings have been replicated by the scientific community over the past few decades, with reports of elevated concentrations of oxidative stress biomarkers resulting from various types of exercise, including prolonged endurance exercise, resistance exercise, high-intensity anaerobic exercise, and eccentric exercise. These effects have been observed in both skeletal muscle tissue and blood.[63–65] The growing interest in oxidative stress is driven by several factors, such as the increased understanding of the role of reactive oxygen and nitrogen species (RONS) in human disease, and efforts to promote exercise for health improvement or maintenance. Additionally, the development and availability of various antioxidant agents have led to their frequent testing using exercise as a stimulus for the production of RONS.[42]

In 1990, it was discovered that reactive oxygen species (ROS) production contributes to skeletal muscle fatigue.[14,66] During the same decade, researchers also found evidence that both endurance exercise training and high-intensity exercise training enhance the antioxidant capacity of cardiac and skeletal muscle myocytes.[5,67] In the 1980s, ROS produced in skeletal muscles during exercise were primarily considered harmful by-products believed to damage muscle fibers without any beneficial role. However, this perspective changed with the discovery of nitric oxide (NO) as a crucial biological signaling molecule. Subsequent research during the 2000s highlighted ROS' critical role as signaling molecules that increase gene expression in myotubes. Numerous

researchers since then have confirmed this finding, not only in skeletal muscle, but also in other tissues.[52,68,69]

2.4.1 Sources of Oxidative Stress

The generation of reactive oxygen and nitrogen species (RONS) in response to acute exercise can occur through various mechanisms. These include mitochondrial respiration, prostanoid metabolism, auto-oxidation of catecholamines, and oxidase enzymatic activity (NADPH oxidase, xanthine oxidase).[67,70] The specific pathways for RONS generation during exercise are influenced by the type (aerobic or anaerobic), intensity, and duration of the exercise. Different types of exercise have varying energy requirements, levels of oxygen consumption, and mechanical stresses on tissues. Additionally, the initial increase in ROS during exercise, as well as after the cessation of physical exertion, can lead to secondary generation of pro-oxidants through phagocytic respiratory burst, disruption of calcium homeostasis, and destruction of iron-containing proteins.[67,70] During exercise, skeletal muscle seems to be the primary site for the production of exercise-induced ROS. The origin of reactive oxygen species (ROS) in working muscle remains a subject of ongoing investigation and debate in the scientific community. Current evidence suggests that mitochondria, phospholipase A2 (PLA2), and NADPH oxidases (NOX2 and NOX4) are the primary sources of ROS production.[67] However, the specific contribution from each source has yet to be fully elucidated, necessitating the development of novel methodologies to achieve conclusive results.

Previously, it was believed that mitochondria were the main generators of ROS. However, numerous researchers have contradicted this claim by demonstrating that mitochondria actually produce less ROS during active state 3 respiration compared to basal state 4 respiration.[71,72] Conversely, some researchers have observed NOX2 as the primary source of ROS during muscular contractions. NADPH Oxidase 2 (NOX2) is localized within the sarcolemma and T-tubule and is activated by specific agonists such as angiotensin II, contractile stress, and cytokines.[73]

2.4.2 Acute Effect of Exercise-induced Oxidative Stress (EIOS) in Skeletal Muscle Fibers

Reactive oxygen species (ROS) play a crucial role in regulating various physiological processes related to exercise. These ROS are constantly produced both at rest and during physical activity. They contribute to the regulation of blood flow, skeletal muscle force production, and the physiological adaptations resulting from regular exercise training. During exercise, the production of ROS is necessary to enhance the antioxidant defense system and increase its activity in trained skeletal muscle.[67] This is important to counteract the oxidative stress caused by repeated muscle contractions during physical exertion. Additionally, it has been suggested that ROS production contributes to the up-regulation of DNA repair mechanisms induced by exercise training.

The effect of ROS on skeletal muscle force production is observed to be biphasic and dependent on the concentration of ROS within the muscle fibers.[13,74] Researchers have

indicated that both superoxide radicals and hydrogen peroxide influence muscle contractile function.[5] The amount of these free radicals produced depends on various factors such as exercise duration, intensity, and muscle temperature. While an optimal concentration of ROS is necessary for muscle fibers to generate force effectively, exceeding a certain threshold leads to a significant decrease in maximal force production. The exact mechanism behind this relationship is not yet fully understood. However, researchers speculate that changes in free calcium concentrations within the muscle and alterations in myofibrillar sensitivity to calcium may contribute to the influence of redox signaling on muscle force production.[70] Another hypothesis suggests that reduced activity of the sodium/potassium (Na+/K+) pump mediated by ROS production may play a role. This is because exercise results in intracellular potassium loss and increased intracellular sodium concentrations despite reduced Na+/K+ pump activity.[75] As a result, there is a decreased trans-sarcolemmal sodium gradient that impairs membrane excitability. Therefore, understanding the balance of the intercellular redox state can provide valuable insights into optimizing exercise training and improving exercise performance. [75]

2.5 Limitations and Caveats of Exercise-induced Oxidative Stress (EIOS) Research

Because the production of RONS resulting in oxidative damage to specific biomolecules is dependent on exercise stimulus exceeding the antioxidant defense system, there is

substantial variability in the induction of acute oxidative stress. Differences in exercise protocols may induce varying concentrations of free radicals because oxidative damage has been shown to be both intensity and duration dependent. [31,76] Furthermore, factors such as age, sex, training status, and dietary intake can affect the degree of free radical production.[77–79] If oxidative stress is indeed successfully induced, the detection of oxidative stress is then dependent on tissue sampled, timing of sampling, and the sensitivity and specificity of the chosen biomarker. The presence of significant or null findings in research studies may be attributed to several factors that affect the specificity of the chosen biomarker.[53,80] It could also be due to inadequate sampling protocols, including insufficient measures or a short time course, or using improper tissue samples for site specific biomarkers, like blood or urine instead of skeletal muscle. Therefore, the inconsistency in reports regarding oxidative stress observed in the literature can be attributed to various factors influencing both the onset and detection of oxidative stress in living organisms.

PART II

Curcumin, a polyphenol present in turmeric, has garnered significant attention from the scientific community due to its potential positive effects on human health. However, the efficacy of curcumin on human health remains uncertain and requires further investigation through empirical approaches. Experiments exist that lend support to these claims, while others contradict such assertions. The therapeutic benefits of curcumin supplementation are primarily attributed to its antioxidant and anti-inflammatory properties.[81]

2.6 Investigating the Effect of Curcumin Supplementation on Individuals with Optimal Health

Curcumin has been associated with a multitude of positive health effects in populations with pre-existing health conditions.[81] However, there is limited scientific literature available on the effect of curcumin supplementation on individuals who are in good health. This disparity can be attributed to various factors. For example, studying a healthy population can be challenging because these individuals typically have normative concentrations of baseline biomarkers. Consequently, the potential benefits of curcumin may be difficult to observe within a narrow timeframe of a research study due to limited room for improvement of these biomarkers. This section will explore the

existing scientific literature pertaining to the effects of curcumin on individuals who are in good health.

DiSilvestro et al.[82] investigated the effects of 80 mg of a lipidated form of curcumin in healthy adults, 40 and 60 years of age. They were divided into two groups: one group receiving curcumin (n=19) and the other group receiving a placebo (n=19) for a duration of four weeks. Plasma and saliva samples were collected from each participant before and after the supplementation phase. The researchers reported that curcumin had a significant effect on lowering triglyceride concentrations ($p<0.05$), but did not have any effect on total cholesterol, low-density lipoprotein cholesterol (LDL-C), or high-density lipoprotein cholesterol (HDL-C) concentrations. However, there was a notable significant increase ($p<0.05$) in nitrous oxide (NO) concentration, as well as in soluble intercellular adhesion molecule 1 (sICAM), which is known to be associated with the development of atherosclerosis. Furthermore, DiSilvestro et al.[82] observed a significant increase in inflammation-related neutrophil function, due to elevated concentration of myeloperoxidase ($p<0.05$). Nevertheless, there were no significant changes observed in C-reactive protein or ceruloplasmin concentrations. Interestingly, there was a significant decrease in salivary amylase activity ($p<0.05$), which is considered to be a marker of stress. Additionally, there was a non-significant improvement in salivary radical scavenging capacities and plasma antioxidant enzyme catalase concentrations ($p>0.05$). However, no significant changes were noted in superoxide dismutase or glutathione

peroxidase concentrations. DiSilvestro et al.[82] also found a significant decrease in beta amyloid plaque formation ($p<0.05$) using positron emission tomography (PET) brain imaging, which is indicative of brain aging. Additionally, there was a significant reduction in plasma alanine amino transferase activities ($p<0.05$), which is an indicator of liver damage. The results from this trial indicate that curcumin can provide health benefits for individuals who do not have diagnosed health conditions.

Cox et al.[83] investigated the effect of 80 mg of a solid lipid curcumin (Longvida®) formulation on cognitive function, mood, and blood biomarkers in a group of 60 healthy adults, 60 and 85 years of age. The researchers performed a randomized, double-blind, placebo-controlled trial design, with assessments conducted at various time points including acute (1 hour and 3 hours after a single dose), chronic (four weeks), and acute-on-chronic (1 hour and 3 hours after a single dose following chronic treatment) phases. The researchers observed that after one hour of administration, curcumin demonstrated a significant enhancement in performance on sustained attention and working memory tasks, compared to the placebo group ($p<0.05$). Additionally, chronic treatment with curcumin resulted in significantly improved working memory and mood, specifically reducing general fatigue and stress-induced fatigue, while increasing feelings of calmness and contentedness ($p<0.05$). The researchers also reported a notable acute-on-chronic non-significant treatment effect on alertness and contentedness ($p>0.05$). Furthermore, curcumin was found to be

associated with significantly decreased serum total cholesterol ($p=0.03$) and LDL-C concentrations ($p=0.01$).

In addition to physiological changes, curcumin has also been explored as a therapeutic agent on anxiety and depression. Esmaily et al.[84] conducted a randomized, double-blind, crossover study in 30 adults with obesity (body mass index [BMI] > 30 $kg.m^{-2}$), who were randomly assigned to receive either curcuminoids (1 g/day) or a placebo for a period of 30 days. After a two-week washout period, the participants then switched to the opposite treatment for another 30 days. Participants completed the Beck Anxiety Inventory (BAI) and Beck Depression Inventory (BDI) scales at the beginning of the study and again after four, six, and 10 weeks of supplementation. The researchers reported that curcumin supplementation resulted in a significantly lower BAI score ($p<0.05$) but did not significantly affect BDI scores ($p>0.05$). These findings suggest that curcumin may have potential anti-anxiety effects in healthy individuals who are obese.

2.7 Effect of Curcumin on Oxidative Stress and Inflammation

Numerous researchers have investigated the effect of curcumin on oxidative stress and inflammation, primarily by inducing acute oxidative stress through exercise. This approach was adopted because exercise elevates the production of reactive oxygen and nitrogen species (RONS), which subsequently interact with various biomolecules *in vitro*, leading to the generation of metabolites associated with oxidative stress-induced

damage. Direct measurement of RONS poses considerable challenges due to their transient nature and reactivity. Consequently, the extent of oxidative stress is frequently assessed using metabolites produced as a result of oxidative stress. This section explores the literature pertaining to curcumin's effect on exercise-induced oxidative stress and inflammation.

Basham et al.[85] conducted a randomized, double-blind, placebo-controlled between-subjects trial in 19 healthy males to investigate the effect of curcumin (CurcuFresh, NOW Foods, Bloomingdale, IL, USA) supplementation on various physiological markers including oxidative stress, inflammation, muscle damage, and muscle soreness. The researchers discovered that the administration of 1.5 grams per day of curcumin (CurcuFresh) for a duration of 28 days exhibited potential benefits in reducing muscle damage and muscle soreness in physically active and healthy male participants. However, no significant differences were observed in terms of inflammatory and oxidative stress markers between the curcumin group and the placebo group. Each participant completed two separate trials with a 25-day interval of supplementation, one with a placebo and the other with curcumin. During the testing trials, participants engaged in eccentric single leg sitting exercises using an aerobic step bench for 15 minutes while utilizing their body mass as resistance. The researcher reported that curcumin supplementation resulted in notably lower plasma concentrations of creatine kinase and perceived muscle soreness following the eccentric muscle damage protocol.

The absence of significant changes in inflammatory and oxidative stress markers could potentially be attributed to the nature of the exercise protocol employed, which may not have elicited RONS production to a sufficient extent for noticeable alterations to occur.

In a randomized, double-blind, placebo-controlled parallel design study, Jager et al.[86] investigated the effect of two doses of curcumin supplementation on performance decrements following eccentric downhill running. The researchers included 63 physically active women and men, 19 to 29 years of age. The participants were randomly assigned to one of three groups: a placebo group, a group receiving 250 mg of CurcuWIN (containing 50 mg of curcuminoids), and a group receiving 1,000 mg of CurcuWIN (containing 200 mg of curcuminoids) for a duration of eight weeks. At the end of the supplementation period, participants completed a downhill running protocol. Muscle function and perceived soreness were assessed using isokinetic dynamometry and a visual analog scale, respectively, before the run and at timed intervals of 1-hour, 24-hours, 48-hours, and 72-hours post-run. The researchers reported no significant changes in isokinetic peak extension torque in the group receiving 200 mg of curcuminoids ($p>0.05$). However, significant reductions in isokinetic peak extension torque were observed in both the group receiving 50 mg of curcuminoids and those receiving the placebo during the initial 24 hours of recovery ($p<0.05$). Isokinetic peak flexion torque and power decreased significantly ($p<0.05$) in the group receiving the 50 mg of curcuminoids, while no significant changes ($p>0.05$) were observed in either the

group receiving 200 mg or curcuminoids or the placebo group. No significant changes ($p>0.05$) were detected in isokinetic extension power or isometric average peak torque across all groups. Furthermore, perceived soreness was significantly increased ($p>0.05$) in all groups compared to baseline. These findings suggest that supplementation with a dose of 200 mg of curcuminoids attenuated some, but not all, changes in performance and soreness following eccentric downhill running. However, the non-significant findings outweighed the positive results in this study.

Delecroix et al.[87] conducted a randomized crossover design study to investigate the effects of combined oral consumption of curcumin (MGD Nature, Brandérion, France) and piperine on recovery after exercise-induced muscle damage in elite male rugby players. A total of 10 participants were included in the study, and they were randomly assigned to receive either a placebo or 2 g of curcumin plus 20 mg of piperine supplementation The supplementation was administered 48 hours before and after the exercise-induced muscle damage protocol. The exercise protocol consisted of 25 repetitions of one-leg jumps over a distance of 25 meters on an 8% downhill slope. Various measurements were taken to assess muscle recovery, including concentric and isometric peak torque for the knee extensors, one leg 6-second sprint performance, plasma creatine kinase concentrations, and muscle soreness. These measurements were taken immediately after exercise, as well as at 24, 48, and 72 hours post-exercise. The researchers reported moderate to large effects of the exercise protocol on concentric

peak torque for the knee extensors, one leg 6-second sprint performance, and counter movement jump performance at the 48-hour mark following exercise. Additionally, there was a large effect size of the exercise protocol on creatine kinase concentration at the 72-hour mark in the control group (Effect size = 3.61; 90% Confidence Interval [CI]: 0.24 to 6.98). This decrease in muscle function and increase in creatine kinase concentrations indicate that the exercise protocol successfully induced muscle damage. At the 24-hour mark post-exercise, there was a moderately lower reduction in sprint mean power output in the experimental condition compared to the placebo condition (Effect size = -1.12; 90% CI: -1.86 to -0.86). Based on these findings, the researchers concluded that curcumin plus piperine supplementation before and after exercise can partially attenuate some, but not all, aspects of muscle damage.

McFarlin et al.[37] studied the effects of 400 mg per day of oral curcumin supplementation (Longvida) versus a placebo on muscle soreness, creatine kinase concentrations, and inflammatory cytokine concentrations using a randomized, double-blind, placebo-controlled study design. The researchers included 28 healthy women and men who underwent an exercise protocol consisting of eccentric dual leg press exercise. The participants were given curcumin supplements two days before and up to four days after the exercise protocol. Plasma samples were collected 1 day before the exercise protocol, and 1, 2, 3, and 4 days after the exercise protocol. The researchers reported that there was a significant decrease ($p<0.05$) in concentrations of creatine kinase (-48%),

TNF-α (-25%), and IL-8 (-21%) following the exercise protocol in the curcumin supplementation group compared to the placebo group. However, there were no significant changes observed in concentrations of IL-6 and IL-10, or quadriceps muscle soreness between the two groups. Based on these findings, the authors concluded that consumption of curcumin reduced biological inflammation during the recovery phase after exercise-induced muscle damage but did not alleviate quadricep muscle soreness.

In a series of experiments, Tanabe et al.[88,89] conducted investigations on the efficacy of curcumin in mitigating exercise-induced muscle damage. Tanabe et al.[89] examined the effect of curcumin supplementation on reducing muscle damage following eccentric exercise. Fourteen healthy young male participants, 24±1 years of age, without prior training, performed 50 maximal isokinetic eccentric contractions of their elbow flexors using an isokinetic dynamometer. This exercise was repeated after four weeks with the other arm. The participants were administered either 150 mg of Theracurmin® or (45 mg of curcuminiods)a placebo before and 12 hours after each eccentric exercise bout in a randomized crossover design. Measurements of maximal voluntary contraction (MVC) torque of the elbow flexors, range of motion of the elbow joint, upper-arm circumferences, muscle soreness, serum creatine kinase activity, plasma IL-6 and TNF-α concentrations were obtained before, immediately after, as well as at 24, 48, 72, and 96 hours following each eccentric exercise session. The authors observed that MVC torque, serum creatine kinase activity, and recovery significantly differed between the

curcumin group and the placebo condition ($p<0.05$). However, no other significant differences were found between the conditions. The authors concluded that Theracurmin® ingestion attenuated certain aspects of muscle damage such as MVC loss and creatine kinase activity.

In another study, Tanabe et al.[90] aimed to investigate the timing of curcumin supplementation on markers of exercise-induced muscle damage. In a randomized, single-blind, parallel design study involving 24 healthy young men, 30±3 years of age, participants performed 30 maximal isokinetic eccentric contractions of their elbow flexors using an isokinetic dynamometer. Participants were randomly assigned to consume either a placebo or oral curcumin (Theracurmin®) at a dosage of 180 mg/day either seven days before or four days after the exercise protocol. Measurements of maximal voluntary contraction (MVC) torque of the elbow flexors, range of motion (ROM) of the elbow joint, muscle soreness, serum IL-8 concentrations, and serum creatine kinase activity were obtained before exercise, immediately after exercise, and one to four days after exercise. Changes in these variables were compared over time. The researchers reported that post-curcumin supplementation, ROM and MVC were higher at three to four days post-exercise, and muscle soreness was lower at the three-day mark compared to the placebo group. In the supplementation group who was supplemented with curcumin prior to the exercise protocol, no significant differences were observed in changes in ROM and muscle soreness compared to the placebo group.

However, 12 hours after exercise, there was a significant reduction in serum IL-8 concentrations ($p<0.05$). Additionally, no significant differences were found among the groups regarding changes in MVC torque and serum creatine kinase activity. The authors concluded that curcumin consumption after exercise compared to before exercise, had a more beneficial effect in attenuating muscle soreness and certain markers of exercise-induced muscle damage.

Nicol et al.[91] conducted a randomized, double-blind, controlled crossover trial to investigate the effects of 2.5 g of curcumin supplementation (Eurofins Scientific Inc, Petaluma, CA) compared to a placebo on muscle damage, inflammation, and delayed onset muscle soreness (DOMS) in a sample of 17 male participants, 18 to 39 years of age, who had a background in various sports such as football and basketball. The researchers assessed single-leg jump performance and DOMS following an eccentric single-leg press exercise. Curcumin or placebo was administered starting two days before and continuing up to three days after the eccentric exercise protocol. A 14-day washout period was implemented between the placebo and intervention conditions. Measurements were taken at baseline, as well as at 0, 24, and 48 hours post-exercise. These measurements included limb pain assessed using a visual analogue scale (VAS), muscle swelling, single-leg jump height, and serum markers of muscle damage and inflammation such as creatine kinase, interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) concentrations. The researchers reported that curcumin supplementation

led to moderate to large reductions in pain experienced during activities such as single-leg squat, gluteal stretch, and squat jump. Additionally, there was a small decrease in creatine kinase activity observed at both the 24-hour and 48-hour time points. Furthermore, there was a slight improvement in single-leg jump performance observed when the participants received curcumin compared to when they received the placebo. Moreover, IL-6 concentrations were lower at the 24-hour mark relative to baseline in the when the participants received curcumin compared to when the received the placebo. Based on these findings, the authors concluded that oral curcumin supplementation likely contributes to alleviating pain associated with DOMS and enhances recovery of muscle performance.

Sciberras et al.[92] conducted a double-blind, crossover design study to investigate the effect of curcumin supplementation on cytokine and stress responses following a two-hour cycling session. The researchers recruited 11 male recreational athletes, 36±6 years of age, and assigned them to three double-blind trials involving 500 mg curcumin supplementation (Meriva), placebo supplementation, and no supplementation for a period of three days prior to the exercise test. Additionally, a one-week washout period was implemented among each condition. The researchers measured serum concentrations of interleukin-1 receptor antagonist (IL1-RA), interleukin-6 (IL-6), interleukin-10 (IL-10), cortisol, C-reactive protein (CRP), and subjective assessment of training stress. To standardize the exercise protocol, all athletes were required to

perform the exercise at 95% of their lactate threshold. The authors reported that, while there were noticeable trends, no significant differences ($p>0.05$) were observed due to the curcumin supplementation in any of the outcome measures except for the subjective assessment of psychological stress, which resulted in a significant improvement ($p<0.05$).

Drobnic et al.[93] conducted a randomized, single-blind, placebo-controlled design pilot study to evaluate the potential effect of curcumin in attenuating oxidative stress and inflammation associated with acute muscle injury induced by eccentric continuous exercise. The researchers included 20 healthy male individuals with moderate activity levels, who were randomly assigned to receive either 200 mg of curcumin (Meriva) or a placebo. Subsequently, all participants underwent a downhill running test. The supplementation regimen consisted of three days prior to the exercise protocol and one day after, for a total of four days of supplementation. The researchers assessed muscle damage through magnetic resonance imaging and biochemical analyses on muscle tissue obtained 48 hours after the exercise test. Drobnic et al.[93] reported that the group supplemented with curcumin exhibited significantly fewer instances of muscle injury in both the posterior and medial compartments of their quadriceps compared to the placebo group. Additionally, markers of oxidative stress and inflammation were lower in the group supplemented with curcumin; however, statistically significant differences were only observed in interleukin-8 (IL-8) concentrations two hours post-exercise.

Based on these findings, the authors concluded that curcumin shows promise in preventing delayed onset muscle soreness (DOMS), evidenced by its effects on pain intensity and muscle injury.

Chilelli et al.[94] investigated the potential effect of curcumin and Boswellia serrata (BSE) gum resin supplementation on plasma concentrations of markers related to oxidative stress, inflammation, and glycation in a group of 47 male healthy master cyclists, 45±9 years of age. All participants were instructed to adhere to the Mediterranean diet throughout the study. Out of these individuals, 22 received a placebo, while the remaining 25 were administered a dosage containing 50 mg of turmeric (equivalent to 10 mg of curcumin) and 140 mg of Boswellia extract (equivalent to 105 mg of Boswellia acid) for a duration of 12 weeks. They reported a significant positive reduction compared to the placebo group on glycoxidation (475.7±141.6 to 328.5±164.5 pg/mL in the supplementation group, and 430.6±123.6 to 312.3±156.3 pg/mL in the placebo group; $p<0.01$), and lipid peroxidation (0.16±0.09 to 0.05±0.05 μmol/L in the supplementation group, and 0.10±0.03 to 0.03±0.01 μmol/L in the placebo group; $p<0.05$) among these physically healthy male athletes. The researchers revealed a promising avenue for combining curcumin with other pharmacologically active compounds such as Boswellia serrata in master athletes.

Hewlings et al.[81] conducted a randomized controlled trial in 28 male individuals, 19±3 years of age, who were not engaged in resistance training and were in good health.

They were randomly assigned to one of two groups: a curcumin supplementation group or a placebo group. The curcumin group received a daily dosage of 400 mg of curcumin for two days prior to and four days after participating in an eccentric exercise protocol specifically designed to induce muscle soreness. The researchers reported that the individuals who received curcumin experienced significantly smaller increases ($p<0.05$) in certain biomarkers of inflammation and muscle damage compared to the placebo group. Namely, the increase in creatine kinase (CK) concentration was 48% less in the supplementation group, tumor necrosis factor-alpha (TNF-α) concentration increased 25% less in the supplementation group, and interleukin-8 (IL-8) concentrations increased 21% less in the supplementation group following the exercise protocol. However, there were no significant differences ($p>0.05$) observed between the two groups in terms of interleukin-6 (IL-6), interleukin-10 (IL-10), or subjective assessment of quadriceps muscle soreness. These findings suggest that curcumin supplementation can effectively reduce biological inflammation during the recovery period following exercise. However, this protocol did not have a significant effect on subjective muscle soreness experienced by the participants. Hewlings et al.[81] propose that incorporating curcumin supplementation may potentially shorten recovery time and enhance performance in subsequent exercise sessions.[81]

2.8 Research on the Mechanism of Action of Curcumin

Although the mechanism of action of curcumin is complex, numerous researchers who have conducted *in vitro* studies have shown that curcumin primarily acts as an antioxidant by directly scavenging free radicals and forming complexes with ions.[55,95–100] Curcumin acts as an antioxidant by either forming complexes with free radicals or interrupting the chain reactions that can lead to DNA damage, protein oxidation, or lipid peroxidation. The antioxidant and anti-inflammatory properties of curcumin have been shown to be effective in many studies in humans. However, to better understand the molecular mechanisms underlying these effects, the realm of cell line and animal model studies need to be discussed. The purpose of this section is to elucidate the specific mechanisms through which curcumin exerts its antioxidant and anti-inflammatory actions.

2.8.1 Nuclear Factor Kappa-light-chain-enhancer of Activated B Cells (NF-κB)

The transcription factor, nuclear factor-kappa B (NF-κB), plays a crucial role in various physiological processes, including the transcriptional regulation of pro-inflammatory gene expression in different cell types.[101] Reactive oxygen species (ROS) act as mediators in the NF-κB signaling pathway by activating kinases through the oxidation of kinase-interacting molecules.[101] The specific mechanism of activation depends on the type of ROS interacting with a kinase protein. For instance, ROS can activate tyrosine kinases by inhibiting protein tyrosine phosphatases via oxidation of a highly reactive cysteine

residue at their catalytic site. Jobin et al.[97] demonstrated the ability of curcumin to inhibit NF-κB activity in intestinal epithelial cells (IEC) of mice and humans. They reported that pre-treatment with 20 mM of curcumin resulted in a significant inhibition of cytokine-mediated NF-κB activation ($p<0.05$), accompanied by a significant decrease in ICAM-1 and IL-8 gene expression ($p<0.05$).

2.8.2 KELCH ECH associating protein 1 (Keap1) Nuclear Factor Erythroid 2-related Factor 2 (Nrf2) Antioxidant Response Elements (AREs) (Keap1-Nrf2-ARE)

The KELCH ECH associating protein 1 (Keap1) Nuclear Factor Erythroid 2-related Factor 2 (Nrf2) Antioxidant Response Elements (AREs) (Keap1-Nrf2-ARE) pathway is widely recognized as the principal regulator of oxidative and electrophilic stress responses. Under normal conditions, nuclear factor erythroid 2-related factor 2 (Nrf2), a transcription factor, is bound to KELCH ECH associating protein 1 (Keap1), preventing its translocation into the nucleus and subsequent binding to specific gene regions responsible for up-regulating antioxidant enzymes.[46] However, during instances of oxidative stress, Keap1 undergoes cysteine residue modifications that impair its ability to interact with Nrf2. This leads to the release of Nrf2, allowing it to translocate into the nucleus. Once inside the nucleus, Nrf2 binds to antioxidant response elements (AREs) in regulatory regions of target genes, in conjunction with small Maf proteins, ultimately resulting in the up-regulation of antioxidant enzymes.[102] Researchers[100] investigating the effects of curcumin supplementation in mice found that

appropriate doses of curcumin (50 mg/kg body weight) significantly increased Nrf2 protein concentrations ($p<0.05$). This led to up-regulation of genes involved in antioxidative processes such as haemoxygenase-1 (HO-1) and glutamate-cysteine ligase catalytic subunit (GCLC) in the nucleus. Haemoxygenase-1 (HO-1) plays a crucial role in regulating vascular inflammation and oxidative damage, while GCLC participates in the synthesis of glutathione, an important antioxidant molecule.[9]

Lin et al.[103] used RAW 264.7 cells (a macrophage cell line derived from a male mouse tumor) to investigate the influence of curcumin on the Nrf2-Keap1 pathway. The researchers exposed RAW264.7 cells to various concentrations of curcumin (0, 5, 10, and 20 μM) for a duration of 20 hours. Subsequently, hydrogen peroxide was administered for up to eight hours to induce oxidative stress in the cells exposed to various doses of curcumin, as well as a control group that did not receive curcumin treatment. The researchers assessed the activity concentrations of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-PX), and malondialdehyde (MDA). They reported that cell viability decreased as hydrogen peroxide concentration increased in the control group. Conversely, both the 5μM and 10 μM curcumin-treated groups exhibited higher cell viability compared to the control group. To ascertain the effect of curcumin on the Nrf2-Keap1 pathway, Western blot and qRT-PCR techniques were conducted to measure total and nuclear protein concentrations of Nrf2, and gene expression concentrations related to the Nrf2-Keap1 axis. Lin et al.[103] concluded that curcumin

effectively reduced reactive oxygen species (ROS) by increasing Nrf2 protein concentrations and facilitating its translocation into the nucleus, where it could up-regulate gene expression of antioxidant enzymes such as SOD, CAT, and GSH-PX.

He et al.[95] reported that curcumin treatment in mice fed with a high-fat diet mitigated the anticipated rise in muscular malondialdehyde (MDA) and ROS concentrations, while reversing the decreased concentrations of Nrf2 and hemeoxygenase-1 (HO-1), a stress response protein. Furthermore, Xie et al.[99] demonstrated that curcumin treatment enhanced the expression of Keap1 protein and significantly increased nuclear accumulation of Nrf2 in rats with diabetes mellitus ($p<0.05$). This subsequently inhibited oxidative stress through elevated expression of catalase (CAT) and glutathione peroxidase (GSH-Px), while reducing superoxide dismutase 1 (SOD1) expression. Wicha et al.[104] demonstrated that treating rats with hexahydrocurcumin significantly reduced oxidative stress, malondialdehyde , and nitric oxide concentrations while enhancing Nrf2 and HO-1 expression, as well as antioxidative enzyme activity including SOD1 activity.

2.8.3 Sirtuins 1,2, and 3

The sirtuins (SIRT) refer to a class of intracellular regulatory proteins that play significant roles in various cellular processes such as aging, stress resistance, metabolic regulation, and transcription. Researchers have demonstrated the capacity of SIRT1 and

SIRT3 to suppress oxidative stress within cells, in contrast to the action of SIRT2, which promotes oxidative stress.[98,105]

2.8.3.1 Sirtuin 1 (SIRT1)

Sirtuin 1 (SIRT1) is predominantly localized in the nucleus and operates by decreasing the acetylation of Forkhead box O (FOXO) 3a protein. This process increases the binding of FOXO to DNA and activates the FOXO transcription factors, which, in turn, regulate antioxidant genes such as SOD and CAT, with the goal of reducing cellular concentrations of ROS.[46,106] The effect of curcumin on SIRT1 was demonstrated by Miao et al.[98] The researchers induced inflammation in murine brains using middle cerebral artery occlusion (MCAO), leading to an increase in TNF-α and IL-6 concentrations. However, when curcumin was administered, TNF-α and IL-6 production were found to be significantly attenuated compared to the untreated MCAO group ($p<0.05$). Interestingly, this effect was significantly diminished when sirtinol, a specific inhibitor of SIRT1 and SIRT2, was administered. This suggests that the protective effects of curcumin are also mediated through SIRT1 activation.[98] Furthermore, it has been demonstrated that SIRT1 activates peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1α), which enhances mitochondrial expression of antioxidant genes, such as glutathione peroxidase (GPX), catalase (CAT), and superoxide dismutase SOD.[107] Additionally, SIRT1 may reduce cellular ROS concentrations by inhibiting the expression and production of inducible nitric oxide synthase (iNOS) and nitrous oxide .

This is achieved through deacetylation of p65, ultimately leading to suppression of the NF-kB signaling pathway.[108]

2.8.3.2 Sirtuin 2 (SIRT2)

Sirtuin 2 (SIRT2), a member of the sirtuin protein family predominantly located in the cytoplasm, has been demonstrated to exhibit increased expression in cells under conditions of oxidative stress, leading to elevated concentrations of MDA.[106] Nie et al.[105] reported that oxidative stress amplifies SIRT2 concentrations within cells, with a subsequent decrease in SIRT2 resulting in reduced production of hydrogen peroxide (H_2O_2)-induced ROS. Keskin-Aktal et al.[109] observed that 30 mg/kg/day of curcumin treatment effectively diminished both MDA (2.9±1.1 vs 5.5±2.9, curcumin vs control groups, respectively; $p<0.05$) and SIRT2 expression (0.32±0.07 vs 0.64±0.07, curcumin vs control groups, respectively; $p<0.05$) in the hippocampus of rats. Furthermore, a positive significant correlation between SIRT2 expression and MDA was identified ($p<0.05$).

2.8.3.3 Sirtuin 3 (SIRT3) and Peroxisome Proliferator-activated Receptor-gamma Coactivator-1alpha (PGC-1α)

SIRT3, a member of the sirtuins family, is predominantly localized in the mitochondrial matrix and governs mitochondrial fatty acid oxidation.[110] Overexpression of SIRT3 enhances the expression of PGC-1α and reduces the production of reactive oxygen species (ROS). Zhang et al.[96] demonstrated that, in mice with chronic obstructive

pulmonary disease (COPD), administration of 100 mg/kg of curcumin significantly elevated mRNA and protein expression concentrations of SIRT3 and PGC-1α in skeletal muscle tissue ($p<0.05$), leading to significantly decreased concentrations of MDA (1.26±0.28 vs 4.80±0.16 nmol/mg, curcumin vs control groups, respectively; $p<0.05$), manganese superoxide dismutase ($p<0.05$), glutathione peroxidase ($p<0.05$), catalase ($p<0.05$), IL-6 ($p<0.05$), and TNF-α ($p<0.05$). These findings suggest that curcumin treatment up-regulates the SIRT3/ PGC-1a signaling pathway, which contributes to the attenuation of oxidative stress.

2.8.4 Wingless/Integrated (Wnt)/β-Catenin

The Wingless/Integrated (Wnt)/β-Catenin pathway is activated when a Wnt protein ligand binds to both a Frizzled family receptor and a lipoprotein receptor related protein 6/5 (LRP6 or LRP5). This activation leads to the accumulation of β-catenin in the nucleus, where it forms complexes with T-cells bound to DNA. Lima et al.[111] indicated that the Wnt signaling pathway plays a role in reducing oxidative stress and enhancing antioxidant activity. Similarly, Wang et al.[112] demonstrated that treatment with curcumin increases the mRNA expression of Wnt3a and β-catenin, which translated to a significant increase in protein concentrations, as well as increasing the mRNA expression of c-myc and cyclinD1 ($p<0.05$). Additionally, curcumin treatment led to significantly elevated concentrations of SOD ($p<0.05$) and GSH-Px ($p<0.05$), while significantly reducing ($p<0.05$) the concentration of MDA in rat models of Parkinson's

disease. Based on these findings, curcumin's protective effect against oxidative stress may be linked to the activation of the Wnt/β-catenin signaling pathway.

2.9 Summary

In summary, curcumin supplementation has been shown by some researchers to attenuate oxidative stress and inflammatory biomarkers by primarily influencing the activity of key regulatory transcription factors, NF-κB and Nrf2-Keap1. Some, but not all, research conducted in humans have demonstrated curcumin's capacity to diminish markers of oxidative stress and tissue damage induced by exercise. Investigations using cell lines and animal models have provided insight into the mechanisms underlying these effects. Additional research is necessary to definitively establish curcumin's efficacy as an antioxidant and anti-inflammatory agent.

CHAPTER 3 – METHODOLOGY

3.1 Institutional Review Board Approval

Approval for this study (IRB #22-646) was obtained from the Virginia Polytechnic Institute and State University (Virginia Tech) Institutional Review Board (IRB). The Approval Letter was received on February 10, 2023 (Appendix A).

3.2 Study Design

A randomized, double-blind, placebo-controlled study design was used for this investigation.

The following are the aims for this study:

Specific Aim 1: To determine the effect of consuming 600 mg of Theracurmin® per day compared to 300 mg of Theracurmin® per day, compared to a placebo, for four weeks in recreationally active individuals, 18 to 45 years of age, on the following markers of exercise-induced oxidative stress: serum protein carbonyl concentrations, glutathione (GSH), glutathione disulfide (GSSG), the ratio of reduced glutathione to oxidized glutathione (GSH:GSSG), and total antioxidant capacity (TAC) after a maximal graded exercise test.

Specific Aim 2: To determine the effect of consuming 600 mg of Theracurmin® per day compared to 300 mg of Theracurmin® per day, compared to a placebo, for four weeks in

recreationally active individuals, 18 to 45 years of age, on the following markers of inflammation: interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP) concentrations after a maximal graded exercise test.

Specific Aim 3: To determine the effect of consuming 600 mg of Theracurmin® per day, compared to 300 mg of Theracurmin per day, compared to a placebo, for four weeks in recreationally active individuals, 18 to 45 years of age, on a marker of muscle damage (creatine kinase [CK] concentrations) after a maximal graded exercise test.

Specific Aim 4: To determine the moderating effect of aerobic fitness (maximal oxygen consumption [VO_2max]) on the efficacy of 600 mg of Theracurmin® per day compared to 300 mg of Theracurmin®, for four weeks in recreationally active individuals, 18 to 45 years of age, on biomarkers of oxidative stress and inflammation.

Specific Aim 5: To determine the moderating effect of percent body fat on the effect of 600 mg of Theracurmin® per day compared to 300 mg of Theracurmin®, for four weeks in recreationally active individuals, 18 to 45 years of age, on biomarkers of oxidative stress and inflammation.

3.3 Sample Size and Attrition Rate

The primary objective of this study was to examine the effect of an intervention on oxidative stress concentrations, measured by protein carbonyl concentrations. The power calculations for this study were based on previous studies, where the researchers

investigated changes in protein carbonyl concentrations among healthy adults following aerobic exercise.[113–115]To make meaningful comparisons between groups, a sample size of 14 healthy adults per group (42 participants in total) was determined to achieve 80% statistical power to detect mean differences at a medium effect size (Cohen's d = 0.50). This calculation was conducted using the G*Power software and a one-way repeated measure multivariate analysis of variance (MANOVA) with a significance level set at *a priori* at p=0.05. To account for potential participants dropping from the study, the goal was to recruit 17 participants per group, assuming a possible attrition rate of 20%. This attrition rate was chosen based on the average attrition rate observed in Dr. Volpe's Laboratory for more than 25 years.

3.4 Inclusion and Exclusion Criteria

3.4.1 Inclusion Criteria

Individuals (female and male) between 18 and 45 years of age, who engaged in regular physical activity at least three days per week, were non-smokers, free from chronic illnesses, did not use supplements or medications that may affect the study outcomes, and were generally in good health were recruited for this study. Additionally, participants must have had a history of exercising at least twice per week for the past six months, and continued to remain active throughout the duration of this study.

3.4.2 Exclusion Criteria

The study's exclusion criteria were as follows: individuals who smoked, those who engaged in less than two days of exercise per week (sedentary individuals), those with orthopedic limitations, those diagnosed with an uncontrolled chronic disease without a physician's note, individuals who took medication without the ability to provide a physician's note, individuals taking blood thinners (curcumin can act as a blood thinner, and thus, individuals taking blood thinners were excluded), individuals with systolic blood pressure greater than 140 mmHg or diastolic pressure greater than 90 mmHg, individuals with a body mass index (BMI) exceeding 35 kg/m^2, and pregnant women.

3.5 Methodology

3.5.1 Participant Screening

3.5.1.1 Screening Survey

Recruitment flyers (Appendix B) for the study were posted around the Virginia Polytechnic Institute and State University (Virginia Tech) campus and other strategic locations. Individuals who were interested in participating in the study could email or scan the QR code on the flyer. Either way, prospective participants were directed to complete a QuestionPro survey (Appendix C) aimed at evaluating their suitability for inclusion in the study. Upon completion, eligible candidates received an informed

consent form (Appendix F) via email. A Health Insurance Portability and Accountability Act (HIPAA)-compliant Zoom meeting was arranged within the next few days, based on scheduling availability. In the event that individuals did not meet the pre-determined inclusion criteria, they were informed via email that they did not qualify for the study and were thanked for their time and interest.

3.5.1.2 Introductory Virtual Meeting

An email containing a consent form was sent to eligible participants in advance, providing detailed information regarding the study's objectives, rationale, methodologies, potential advantages, and risks involved. Potential participants who met the established inclusion criteria were then scheduled for a HIPAA-compliant Zoom meeting, during which they received a comprehensive explanation of the study. Participants were actively encouraged to seek clarifications for any uncertainties prior to making an informed decision regarding their participation. Upon obtaining verbal confirmation, individuals were scheduled for their baseline laboratory visit at the Volpe Laboratory (339 Wallace Hall, Virginia Tech).

3.5.1.3 Medical History

Participants were requested to complete a comprehensive medical history questionnaire (Appendix D), which served to assess for potential health issues such as unstable cardiovascular conditions, diabetes mellitus, hypertension, etc., as well as other factors

that may have hindered their participation (e.g., physical limitations caused by osteoarthritis, and sustained injuries).

3.5.1.4 Physical Activity Readiness Questionnaire

Participants were required to complete a Physical Activity Readiness Questionnaire (PAR-Q) (Appendix E) to determine their physical suitability for participation in the study. This questionnaire identifies any underlying medical conditions or limitations that may have impeded their ability to fully engage in the research study.

3.5.1.5 Randomization Procedure

After participants were scheduled for their first testing session, they were randomly assigned into one of the three groups: 1) a placebo group who received 50 mg of microcrystalline cellulose, 2) a group who received 300 mg of Theracurmin® (containing 90 mg of active curcuminoids), and 3) a group who received 600 mg of Theracurmin® (containing 180 mg of active curcuminoids). The allocation of participants into one of these three groups was determined through the utilization of a software-generated randomization plan, employing the randomization tool available at http://www.graphpad.com/quickcalcs/index.cfm. Each group consisted of a total of 17 participants.

3.5.2 Baseline Visit (Session 1)

3.5.2.1 Arrival and Informed Consent

Participants were instructed to arrive at the Volpe Laboratory in 339 Wallace Hall (Virginia Tech) at their designated appointment time. Prior to their arrival, participants were informed of their requirement to adhere to specific guidelines, including maintaining a fasting period of at least four hours, abstaining from caffeine consumption for a duration of 12 hours, and refraining from engaging in vigorous exercise or alcohol consumption for a period of 24 hours. Upon arrival, participants were queried regarding their adherence to these aforementioned conditions. Subsequently, they were guided through the informed consent (Appendix F) process in a comprehensive manner. This procedure aimed to ensure that participants had a complete understanding of the study's procedures, their individual responsibilities, potential benefits, and associated risks. Once the study protocol had been thoroughly explained and all participant inquiries addressed, physical signatures on the consent form were obtained and witnessed by the research personnel.

3.5.2.2 Pregnancy Test

If a participant identified as female and stated that they were pre-menopausal, peri-menopausal, or non-post-menopausal for a period of at least one year, it was necessary for them to undergo a pregnancy test due to the X-ray exposure from dual energy X-ray absorptiometry (DXA). The participant was provided with a small plastic container and

instructed to collect a minimum of three to four tablespoons of urine. Subsequently, the lid was securely placed back on the container before returning it to our laboratory. Upon receiving the urine sample, the absorbent end of the pregnancy test strip was immersed into the urine for a duration of 20 seconds before evaluating the result. Following this assessment, the urine was properly disposed of by flushing it down a toilet, while the container itself was discarded in a designated biohazard container.

3.5.2.3 Blood Pressure

Participants were instructed to assume a relaxed upright posture and remain seated for a duration of 10 minutes. An automated blood pressure monitor (SunTech Tango M2 Blood Pressure Monitor, Morrisville, NC) was utilized to measure resting blood pressure and heart rate. Three readings were acquired, and subsequently, an average of the readings was calculated.

3.5.2.4 Anthropometry

Body weight and height were measured on a digital physician's scale (Detecto 439, Webb City, Missouri, USA) and stadiometer (SECA 777, GMBH & CO., Germany), respectively. Body weight was measured during every lab visit; however, height was measured only at the first visit. Both body weight and height values were obtained in duplicate to ensure the accuracy of the readings obtained. Body weight was measured to the nearest 0.5 pounds and converted to kilograms (kg). Height was measured to the nearest 0.5 inches and converted to centimeters (cm).

3.5.2.5 Dual Energy X-ray Absorptiometry Scan

Prior to instructing participants to assume a supine position on the dual energy X-ray absorptiometer (DXA), the research personnel requested that participants remove any metallic objects they may have had on their person. Subsequently, participants were positioned in the supine position, ensuring that their head and limbs were within the designated scanning area. The technician informed participants of the approximate duration of the scan (ranging from 8 to 12 minutes depending on body size) and emphasized the importance of remaining as still as possible throughout the duration of the scan. Additionally, the technician explained how the DXA scanning arm would gradually pass over their body to ensure that participants were made comfortable to the movement of the scanner arm. The DXA (iDXA, GE Healthcare, Madison, WI) was used to measure lean body mass, percent body fat, and total body bone mineral density W0 only. Following completion of the scan, the results of the scan were briefly described by the research personnel and each participant was assured that they would receive a copy of their scan at the conclusion of the study protocol. All DXA scans were conducted by a Certified Bone Densitometry Technologist (CBDT®) through the International Society for Clinical Densitometry (ISCD).

3.5.2.6 Baseline Blood Draw 1 (B1)

Participants were seated in a designated phlebotomy chair and instructed to rest their arm on a provided arm rest. A trained phlebotomist proceeded to identify a suitable

vein for blood collection. The area was thoroughly cleansed using an alcohol pad to ensure proper hygiene. To enhance visibility and facilitate the extraction process, a rubber tourniquet was applied around the upper arm, effectively engorging the targeted vein. Subsequently, a fine-gauge needle (0.21 mm in diameter) was employed to puncture an appropriate arm vein located on the inner side of the elbow crease. This procedure aimed to draw approximately two tablespoons of blood into a specialized vacutainer tube (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Once the desired sample was obtained, the needle was carefully withdrawn, and gentle pressure with sterile gauze was applied to minimize any potential bleeding. To prevent infection, either a band aid or gauze with an elastic wrap was securely placed over the puncture site. Participants received instructions to keep this dressing intact for at least 10 minutes following the procedure. Some of the collected blood sample was used for the measurement of hemoglobin and hematocrit concentrations.

3.5.2.7a Hemoglobin Analyses (B1)

Utilizing some of the collected blood sample, a quantitative measurement of hemoglobin concentration was acquired using the Hemocue system (HemoCue Hb 201+ Analyzer, Brea, CA, USA). Roughly 100 microliters (μL) of the blood sample was aspirated into the testing strip through capillary action. Following this, the testing strip was carefully inserted into the designated slot in the Hemocue system for analyses.

Following a one-minute waiting period, the value was determined by the device and recorded.

3.5.2.7b Hematocrit Analyses (B1)

Some of the blood sample collected was placed into two capillary tubes, with each tube filled to approximately two-thirds of its capacity. A wax seal was then carefully applied to one end of each capillary tube. The tubes subsequently underwent centrifugation at a speed of 5,000 revolutions per minute (rpm) for a duration of 10 minutes. Following centrifugation, the blood sample was naturally separated into three distinct layers: the supernatant, which consists of plasma and occupies the uppermost portion; the buffy coat, located in the middle and composed of white blood cells and platelets; and finally, the pellet at the bottom, which is comprised mainly of red blood cells. Subsequently, the capillary tubes were positioned on a Micro-Hematocrit Tube reader chart (Critocaps), which enabled the measurement of hematocrit based on the location of the separation between the pellet and the supernatant. This placement involved aligning the base of the sample with the lowermost line on the reader's chart. The positioning of the tube was adjusted until the upper meniscus of the plasma coincided precisely with the upper line on the hematocrit tube reader chart. By examining this alignment, we determined the hematocrit value by visually identifying where the red blood cells (pellet) separated from the plasma (supernatant).

The hemoglobin (Hb) and hematocrit (Hct) concentrations were determined to calculate the difference in plasm volume (ΔPV) after exercise. The Dill and Costill[116] equations (below) were utilized to account for the hemoconcentration effect.

$$\Delta PV = \frac{PV_{post} - PV_{pre}}{PV_{pre}} = \frac{Hb_{pre}\ X\ \left(1 - Hct_{post}\right)}{Hb_{post}\ X\ (1 - Hct_{pre})} - 1$$

Concentration of biomarkers measured in the plasma or serum after the exercise test were corrected using the following equation.[117]

$$PM_{post,c} = PM_{post,u}\ X\ (1 + \Delta PV)$$

where $PM_{post,c}$ and $PM_{post,u}$ indicate corrected and uncorrected serum or plasma biomarker after the exercise test, respectively.

3.5.2.8 Maximal Oxygen Consumption Test

A graded exercise test was conducted on a treadmill to evaluate maximal oxygen consumption (VO_2max) and induce a state of acute oxidative stress. The exercise protocol was clearly communicated to all participants prior to the commencement of the test. Additionally, participants were informed about the hand signals used to increase intensity and indicate volitional exhaustion. Subsequently, participants were fitted with a mask (Hans Rudolph 7450, Shawnee, KS, USA) that ensures a secure seal around the nose and mouth. A 3-lead electrocardiogram (ECG) system (Vyntx CPX, Vyaire Medical, Mettawa, IL) was utilized to monitor heart rate (HR) during the test. This ECG

system consisted of four electrodes, specifically the Right Arm (RA), Left Arm (LA), Right Leg (RL), and Left Leg (LL) electrodes. The RA electrode was positioned on the right interclavicular space aligned with the anterior axillary line, while the LA electrode was placed on the left interclavicular space in line with the anterior axillary line. The RL electrode was positioned beneath the last rib aligned with RA, and the LL electrode was placed beneath the last rib aligned with LA. Prior to electrode placement, participants were instructed to cleanse these areas of their skin using an alcohol pad containing 70% isopropyl alcohol provided by the researcher. The exercise test protocol comprised of three distinct phases: warm-up phase, testing phase, and recovery phase. The warm-up phase involved walking at a speed of 3 miles per hour (mph) for a duration of two minutes. Subsequently, participants transitioned to their self-selected running pace of either 5.5 mph of 7 mph, per their preference, specified before commencing the test. The testing phase required participants to run at a constant pace while the gradient was increased by 1% every minute until they reached volitional exhaustion. The duration of this phase was determined by the participant and typically lasted between 5 to 15 minutes. Once participants were unable to continue running, they utilized a pre-determined hand signal to indicate their point of exhaustion. Following the completion of the test, a cool-down phase occurred, during which participants walked on the treadmill at a slow pace of 2 mph for two minutes. At the end of the test, participants

were assisted with removing the mask (Hans Rudolph 7450 , Shawnee, KS, USA) and detaching the electrodes.

Although for ethical and safety reasons, volitional exhaustion was used to end each test, there are four ways to determine if VO_2max has been reached. They are as follows: 1) an increase in carbon dioxide expiration with a flattening of oxygen consumption with an increase in workload, 2) a flattening of oxygen consumption with an increase in workload (these first two are the most definitive ways to ascertain if someone reached their VO_2max), 3) a respiratory exchange ratio greater than 1.0, and 4) sheer exhaustion. All the aforementioned determinations of ascertaining VO_2max are incorporated in the Volpe Laboratory; however, sheer exhaustion is often used as the first measure, due to ethical and safety reasons.

3.5.2.9 Baseline Blood Draw 2 (B2)

Following the completion of the exercise test, participants remained seated for 20 minutes, after which they were directed to sit in a dedicated phlebotomy chair for the second blood draw. A trained phlebotomist proceeded to identify a suitable vein for blood collection. The area was thoroughly cleansed using an alcohol pad to ensure proper hygiene. To enhance visibility and facilitate the extraction process, a rubber tourniquet was applied around the upper arm, effectively engorging the targeted vein. Subsequently, a fine-gauge (0.21 mm in diameter) needle was employed to puncture an appropriate arm vein located on the inner side of the elbow crease. This procedure

aimed to draw approximately two tablespoons of blood into a specialized vacutainer tube (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Once the desired sample was obtained, the needle was carefully withdrawn, and gentle pressure with sterile gauze was applied to minimize any potential bleeding. To prevent infection, either a band aid or gauze with an elastic wrap was securely placed over the puncture site. Participants received instructions to keep this dressing intact for at least 10 minutes following the procedure. Some of the collected blood sample was used for the measurement of hemoglobin and hematocrit concentrations.

3.5.2.10a Hemoglobin Analyses (B2)

Utilizing some of the collected blood sample, a quantitative measurement of hemoglobin concentration was acquired using the Hemocue system (HemoCue Hb 201+ Analyzer, Brea, CA, USA). Roughly 100 microliters (μL) of the blood sample was aspirated into the testing strip through capillary action. Following this, the testing strip was carefully inserted into the designated slot in the Hemocue system for analyses. Following a one-minute waiting period, the hemoglobin value was determined by the device and recorded.

3.5.2.10b Hematocrit Analyses (B2)

Some of the blood sample collected was placed into two capillary tubes, with each tube filled to approximately two-thirds of its capacity. A wax seal was then carefully applied to one end of each capillary tube. The tubes subsequently underwent centrifugation at a

speed of 5,000 rpm for a duration of 10 minutes. Following centrifugation, the blood sample was naturally separated into three distinct layers: the supernatant, which consists of plasma and occupies the uppermost portion; the buffy coat, located in the middle and composed of white blood cells and platelets; and finally, the pellet at the bottom, which is comprised mainly of red blood cells. Subsequently, the capillary tubes were positioned on a Micro-Hematocrit Tube reader chart (Critocaps), which enabled the measurement of hematocrit based on the location of the separation between the pellet and the supernatant. This placement involved aligning the base of the sample with the lowermost line on the reader's chart. The positioning of the tube was adjusted until the upper meniscus of the plasma coincided precisely with the upper line on the hematocrit tube reader chart. By examining this alignment, we determined the hematocrit value by visually identifying where the red blood cells (pellet) separated from the plasma (supernatant).

The hemoglobin (Hb) and hematocrit (Hct) concentrations were determined to calculate the difference in plasm volume (ΔPV) after exercise. The Dill and Costill[116] equations (below) were utilized to account for the hemoconcentration effect.

$$\Delta PV = \frac{PV_{post} - PV_{pre}}{PV_{pre}} = \frac{Hb_{pre}\ X\ \left(1 - Hct_{post}\right)}{Hb_{post}\ X\ (1 - Hct_{pre})} - 1$$

Concentration of biomarkers measured in the plasma or serum after the exercise test were corrected using the following equation.[117]

$$PM_{post,c} = PM_{post,u}\, X\, (1 + \Delta PV)$$

where $PM_{post,c}$ and $PM_{post,u}$ indicate corrected and uncorrected serum or plasma biomarker after the exercise test, respectively.

3.5.2.11 Dietary Assessment

Participants were asked to complete the 2015 Block Food Frequency Questionnaire (Appendix G) to assess habitual food intake. This questionnaire is self-guided, and participants were provided with a computer to complete the questionnaire.

3.5.2.12 Theracurmin® Supplement/Placebo

Participants were given the required number of capsules (28 capsules) and asked to consume the required dosage (2 capsules) every day for two weeks. Because this was a double-blind study, neither the participants nor the research team were cognizant to which group the participant had been allocated. Both the placebo and intervention capsules were modified to weight the same and look identical to each other.

3.5.3. Mid-Point Visit (Session 2)

3.5.3.1 Anthropometry

Body weight was measured on a digital physician's scale (Detecto 439, Webb City, Missouri, USA). Body weight was measured during every lab visit; however, height was measured only at the first visit. Both body weight was obtained in duplicate to

ensure the accuracy of the readings obtained. Body weight was measured to the nearest 0.5 pounds and converted to kilograms (kg).

3.5.3.2 Theracurmin® Supplement/Placebo

Participants were requested to return any missed supplements to collect data on their adherence. Participants received an adequate supply of capsules at each visit and were instructed to take the required dosage of their respective pill on a daily basis for the following two weeks.

3.5.4 Final Visit (Session 3)

3.5.4.1 Pre-Arrival Correspondence

Prior to participant arrival for their final visit, participants were informed via email of their requirement to adhere to specific guidelines, including maintaining a fasting period of at least four hours, abstaining from caffeine consumption for a duration of 12 hours, and refraining from engaging in vigorous exercise or alcohol consumption for a period of 24 hours. Participants were asked if they adhered to these conditions on the day of their visit.

3.5.4.2 Blood Pressure

Participants were instructed to assume a relaxed upright posture and remain seated for a duration of 10 minutes. An automated blood pressure monitor (SunTech Tango M2 Blood Pressure Monitor, Morrisville, NC) was utilized to measure resting blood

pressure and heart rate. Three readings were acquired, and subsequently, an average of the readings was calculated.

3.5.4.3 Anthropometry

Body weight was measured on a digital physician's scale (Detecto 439, Webb City, Missouri, USA). Body weight was measured during every lab visit; however, height was measured only at the first visit. Both body weight was obtained in duplicate to ensure the accuracy of the readings obtained. Body weight was measured to the nearest 0.5 pounds and converted to kilograms (kg).

3.5.4.4 Final Blood Draw 1 (F1)

Participants were seated in a designated phlebotomy chair and instructed to rest their arm on a provided arm rest. A trained phlebotomist proceeded to identify a suitable vein for blood collection. The area was thoroughly cleansed using an alcohol pad to ensure proper hygiene. To enhance visibility and facilitate the extraction process, a rubber tourniquet was applied around the upper arm, effectively engorging the targeted vein. Subsequently, a fine-gauge (0.21 mm in diameter) needle was employed to puncture an appropriate arm vein located on the inner side of the elbow crease. This procedure aimed to draw approximately two tablespoons of blood into a specialized vacutainer tube (Becton, Dickingson and Company, Franklin Lakes, NJ, USA). Once the desired sample was obtained, the needle was carefully withdrawn, and gentle pressure with sterile gauze was applied to minimize any potential bleeding. To prevent infection,

either a band aid or gauze with an elastic wrap was securely placed over the puncture site. Participants received instructions to keep this dressing intact for at least 10 minutes following the procedure. Some of the collected blood sample was used for the measurement of hemoglobin and hematocrit concentrations.

3.5.4.5a Hemoglobin Analyses (F1)

Utilizing some of the collected blood sample, a quantitative measurement of hemoglobin concentration was acquired using the Hemocue system (HemoCue Hb 201+ Analyzer, Brea, CA, USA). Roughly 100 microliters (μL) of the blood sample was aspirated into the testing strip through capillary action. Following this, the testing strip was carefully inserted into the designated slot in the Hemocue system for analyses. Following a one-minute waiting period, the hemoglobin value was determined by the device and recorded.

3.5.4.5b Hematocrit Analyses (F1)

Some of the blood sample collected was placed into two capillary tubes, with each tube filled to approximately two-thirds of its capacity. A wax seal was then carefully applied to one end of each capillary tube. The tubes subsequently underwent centrifugation at a speed of 5,000 rpm for a duration of 10 minutes. Following centrifugation, the blood sample was naturally separated into three distinct layers: the supernatant, which consists of plasma and occupies the uppermost portion; the buffy coat, located in the middle and composed of white blood cells and platelets; and finally, the pellet at the

bottom, which is comprised mainly of red blood cells. Subsequently, the capillary tubes were positioned on a Micro-Hematocrit Tube reader chart (Critocaps), which enabled the measurement of hematocrit based on the location of the separation between the pellet and the supernatant. This placement involved aligning the base of the sample with the lowermost line on the reader's chart. The positioning of the tube was adjusted until the upper meniscus of the plasma coincided precisely with the upper line on the hematocrit tube reader chart. By examining this alignment, we determined the hematocrit value by visually identifying where the red blood cells (pellet) separated from the plasma (supernatant).

The hemoglobin (Hb) and hematocrit (Hct) concentrations were determined to calculate the difference in plasm volume (ΔPV) after exercise. The Dill and Costill[116] equations (below) were utilized to account for the hemoconcentration effect.

$$\Delta PV = \frac{PV_{post} - PV_{pre}}{PV_{pre}} = \frac{Hb_{pre}\ X\ \left(1 - Hct_{post}\right)}{Hb_{post}\ X\ (1 - Hct_{pre})} - 1$$

Concentration of biomarkers measured in the plasma or serum after the exercise test were corrected using the following equation.[117]

$$PM_{post,c} = PM_{post,u}\ X\ (1 + \Delta PV)$$

where $PM_{post,c}$ and $PM_{post,u}$ indicate corrected and uncorrected serum or plasma biomarker after the exercise test, respectively.

3.5.4.6 Maximal Oxygen Consumption Test

A graded exercise test was conducted on a treadmill to evaluate maximal oxygen consumption (VO_2max) and induce a state of acute oxidative stress. The exercise protocol was clearly communicated to all participants prior to the commencement of the test. Additionally, participants were informed about the hand signals used to increase intensity and indicate volitional exhaustion. Subsequently, participants were fitted with a mask (Hans Rudolph 7450, Shawnee, KS, USA) that ensures a secure seal around the nose and mouth. A 3-lead electrocardiogram (ECG) system (Vyntx CPX, Vyaire Medical, Mettawa, IL) was utilized to monitor heart rate (HR) during the test. This ECG system consisted of four electrodes, specifically the Right Arm (RA), Left Arm (LA), Right Leg (RL), and Left Leg (LL) electrodes. The RA electrode was positioned on the right interclavicular space aligned with the anterior axillary line, while the LA electrode was placed on the left interclavicular space in line with the anterior axillary line. The RL electrode was positioned beneath the last rib aligned with RA, and the LL electrode was placed beneath the last rib aligned with LA. Prior to electrode placement, participants were instructed to cleanse these areas of their skin using an alcohol pad containing 70% isopropyl alcohol provided by the researcher. The exercise test protocol comprised of three distinct phases: warm-up phase, testing phase, and recovery phase. The warm-up phase involved walking at a speed of 3 miles per hour (mph) for a duration of two minutes. Subsequently, participants transitioned to their self-selected running pace of

either 5.5 mph of 7 mph, per their preference, specified before commencing the test. The testing phase required participants to run at a constant pace while the gradient was increased by 1% every minute until they reached volitional exhaustion. The duration of this phase was determined by the participant and typically lasted between 5 to 15 minutes. Once participants were unable to continue running, they utilized a pre-determined hand signal to indicate their point of exhaustion. Following the completion of the test, a cool-down phase occurred, during which participants walked on the treadmill at a slow pace of 2 mph for two minutes. At the end of the test, participants were assisted with removing the mask (Hans Rudolph 7450 , Shawnee, KS, USA) and detaching the electrodes.

Although for ethical and safety reasons, volitional exhaustion was used to end each test, there are four ways to determine if VO_2max has been reached. They are as follows: 1) an increase in carbon dioxide expiration with a flattening of oxygen consumption with an increase in workload, 2) a flattening of oxygen consumption with an increase in workload (these first two are the most definitive ways to ascertain if someone reached their VO_2max), 3) a respiratory exchange ratio greater than 1.0, and 4) sheer exhaustion. All the aforementioned determinations of ascertaining VO_2max are incorporated in the Volpe Laboratory; however, sheer exhaustion is often used as the first measure, due to ethical and safety reasons.

3.5.4.7 Final Blood Draw 2 (F2)

Following the completion of the exercise test, participants remained seated for 20 minutes, after which they were directed to sit in a dedicated phlebotomy chair for the second blood draw. A trained phlebotomist proceeded to identify a suitable vein for blood collection. The area was thoroughly cleansed using an alcohol pad to ensure proper hygiene. To enhance visibility and facilitate the extraction process, a rubber tourniquet was applied around the upper arm, effectively engorging the targeted vein. Subsequently, a fine-gauge (0.21 mm in diameter) needle was employed to puncture an appropriate arm vein located on the inner side of the elbow crease. This procedure aimed to draw approximately two tablespoons of blood into a specialized vacutainer tube (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Once the desired sample was obtained, the needle was carefully withdrawn, and gentle pressure with sterile gauze was applied to minimize any potential bleeding. To prevent infection, either a band aid or gauze with an elastic wrap was securely placed over the puncture site. Participants received instructions to keep this dressing intact for at least 10 minutes following the procedure. Some of the collected blood sample was used for the measurement of hemoglobin and hematocrit concentrations.

3.5.4.8a Hemoglobin Analyses (F2)

Utilizing some of the collected blood sample, a quantitative measurement of hemoglobin concentration was acquired using the Hemocue system (HemoCue Hb 201+

Analyzer, Brea, CA, USA). Roughly 100 microliters (μL) of the blood sample was aspirated into the testing strip through capillary action. Following this, the testing strip was carefully inserted into the designated slot in the Hemocue system for analyses. Following a one-minute waiting period, the hemoglobin value was determined by the device and recorded.

3.5.4.8b Hematocrit Analyses (F2)

Some of the blood sample collected was placed into two capillary tubes, with each tube filled to approximately two-thirds of its capacity. A wax seal was then carefully applied to one end of each capillary tube. The tubes subsequently underwent centrifugation at a speed of 5,000 rpm for a duration of 10 minutes. Following centrifugation, the blood sample was naturally separated into three distinct layers: the supernatant, which consists of plasma and occupies the uppermost portion; the buffy coat, located in the middle and composed of white blood cells and platelets; and finally, the pellet at the bottom, which is comprised mainly of red blood cells. Subsequently, the capillary tubes were positioned on a Micro-Hematocrit Tube reader chart (Critocaps), which enabled the measurement of hematocrit based on the location of the separation between the pellet and the supernatant. This placement involved aligning the base of the sample with the lowermost line on the reader's chart. The positioning of the tube was adjusted until the upper meniscus of the plasma coincided precisely with the upper line on the hematocrit tube reader chart. By examining this alignment, we determined the

hematocrit value by visually identifying where the red blood cells (pellet) separated from the plasma (supernatant).

The hemoglobin (Hb) and hematocrit (Hct) concentrations were determined to calculate the difference in plasm volume (ΔPV) after exercise. The Dill and Costill[116] equations (below) were utilized to account for the hemoconcentration effect.

$$\Delta PV = \frac{PV_{post} - PV_{pre}}{PV_{pre}} = \frac{Hb_{pre}\ X\ \left(1 - Hct_{post}\right)}{Hb_{post}\ X\ (1 - Hct_{pre})} - 1$$

Concentration of biomarkers measured in the plasma or serum after the exercise test were corrected using the following equation.[117]

$$PM_{post,c} = PM_{post,u}\ X\ (1 + \Delta PV)$$

where $PM_{post,c}$ and $PM_{post,u}$ indicate corrected and uncorrected serum or plasma biomarker after the exercise test, respectively.

3.5.4.9 Physical Activity Assessment

Participants were asked to complete the 2015 Block Adult Physical Activity Questionnaire (Appendix H) to assess habitual physical activity patterns. This questionnaire is self-guided, and participants were provided with a computer to complete the questionnaire.

3.5.4.10 Blood Sample Storage and Analyses

The blood samples from every blood draw (B1, B2, F1, and F2) were collected and stored in a freezer at a temperature of -80°C until analyses. Prior to storage, the samples underwent centrifugation at a speed of 3,500 rpm and a temperature of 4°C for a duration of 15 minutes. Following centrifugation, the plasma and serum samples were aliquoted into individual cryovials (Corning Incorporated, Corning, NY, USA) and preserved at -80°C. Analyses of the serum samples were conducted after all blood samples had been collected and the study was completed. The following biomarkers were evaluated for this study: serum protein carbonyl (PC) concentrations, serum glutathione (GSH) concentrations, serum glutathione disulfide (GSSG) concentrations, the ratio of GSH:GSSG, serum total antioxidant capacity (TAC), serum C-reactive protein (CRP) concentrations, serum Interleukin-6 (IL-6) concentrations, serum tumor necrosis factor-alpha (TNF-α) concentrations, and serum creatine kinase (CK) concentrations. These biomarkers were assessed using commercially available enzyme-linked immunosorbent assay (ELISA) kits purchased from Abcam for PC (kit# ab238536), GSH (kit# 205811), GSSG (kit# 205811), GSH:GSSG ratio (kit# 205811), and TAC (kit# ab65329); ALPCO for CRP (kit# 30-9710S) and IL-6 (kit# 04-BI-IL6); R&D systems for TNF-α (kit #QK210); and RayBiotech for CK (kit# ELH-CKMB). A BioTek Synergy H1 Hybrid Multi-Mode Monochromator Fluorescence Microplate reader (Fisher Scientific, Hanover Place, IL) was used for analyses. These analyses included

samples obtained during both the baseline (B1 and B2) and final visits (F1 and F2). All blood analyses were conducted during the same week, in duplicate, at the Virginia Tech Metabolism Core by Dr. Ryan McMillan at the end of the study to minimize differences among ELISA kits.

3.6 Statistical Design

The proposed experimental study was a randomized, double-blind, placebo-controlled study that was conducted in a cohort of recreationally active individuals, 18 to 45 years of age. Participants were randomly assigned to one of three groups: 1) a placebo group who received 50 mg of microcrystalline cellulose, 2) a group who received 300 mg of Theracurmin® (containing 90 mg of active curcuminoids), and 3) a group who received 600 mg of Theracurmin® (containing 180 mg of active curcuminoids). The allocation of participants into one of these three groups was determined through the utilization of a software-generated randomization plan, employing the randomization tool available at http://www.graphpad.com/quickcalcs/index.cfm. Each group consisted of a total of 17 participants.

3.6.1 Preliminary Analyses

Descriptive statistics, including measures of central tendency (mean, median) and variation (standard deviation, interquartile range, range) for continuous measures and frequencies and percentages for dichotomous and categorical measures, were used to characterize the sample. If deviations from normality emerged, transformations were

applied, or non-parametric tests were implemented and compared to parametric tests. Of note, while it is expected that the groups were balanced on baseline characteristics due to randomization, imbalances that occurred by chance were adjusted in all analyses.

3.6.2. Statistical Analyses of Aims

The primary aim of this study was to examine the effects of consuming different doses of curcumin for four weeks on biomarkers of oxidative stress and inflammation. A repeated measure multivariate analysis of variance (MANOVA) was used to calculate the differences in markers of oxidative stress and inflammation at baseline and post-intervention. The secondary aim of this study was to determine if fitness levels moderated the effect of curcumin supplementation on biomarkers of oxidative stress and inflammation. A within-group multiple regression was used to test the moderation effect of fitness (VO_2max and percent body fat) by testing the regression coefficient of interaction.

3.7 Timeline

This study was conducted over a 10-month period, from June 2023 to March 2024. The duration of an individual's participation in this study was approximately 40 days, with a four-week intervention phase (40 days denotes the time participants were recruited and entered the study, until they completed the study). This protocol was approved by

the Institutional Review Board (IRB) at Virginia Polytechnic Institute and State University (Virginia Tech) (IRB# 22-646).

CHAPTER 4: MANUSCRIPT 1

To be submitted to: ***International Journal of Sports Medicine***

Effect of Curcumin Supplementation on Biomarkers of Oxidative Stress in Active Individuals

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Abstract

Objective: The aim of our research was to assess the effect four weeks of oral supplementation of 300 mg/day vs 600 mg/day of Theracurmin® compared to a placebo on markers associated with exercise-induced oxidative stress in physically active women and men, 18 to 45 years of age.

Methods: We conducted a randomized, double-blind, placebo-controlled trial in which participants underwent two incline-based maximal graded exercise tests (GXT) to exhaustion on a treadmill. The GXTs were separated by a four-week supplementation period. Blood samples were collected before and 20 minutes after the GXT protocol to assess the concentration of protein carbonyl (PC), glutathione (GSH), glutathione disulfide (GSSG), ratio of glutathione to oxidized glutathione (GSH:GSSG), and total antioxidant capacity (TAC). Participants' anthropometry, body composition, bone mineral density, and dietary intake were also assessed.

Results: We found significant reductions in PC concentrations post-GXT in both the placebo ($p=0.01$) and 300 mg Theracurmin® ($p=0.019$) groups at baseline (Week 0), although no significant differences were observed after the supplementation period. GSH concentrations were significantly different at baseline in the placebo ($p=0.01$) and 300 mg Theracurmin® ($p=0.04$) groups, with no significant effects observed at Week 4. GSSG concentrations were significantly elevated in all groups post-GXT at Week 0, but at Week 4, only the placebo ($p=0.02$) and 600 mg Theracurmin® ($p=0.01$) groups

exhibited differences between the two GXT conditions. TAC remained unaffected at both timepoints. Linear Mixed Effect Model analyses revealed non-significant effects of curcumin dosage on biomarkers associated with oxidative stress.

Conclusion: Our results indicate that an incline-based treadmill test to exhaustion can potentially induce acute oxidative stress in physically active individuals. Nevertheless, there were no statistically significant effects observed on the markers of exercise-induced oxidative stress following curcumin supplementation in either of the intervention groups. Our results suggest that four weeks of curcumin supplementation does not influence markers of oxidative stress in recreationally active women and men, 18 and 45 years of age. Additional studies are warranted to elucidate the mechanisms involved in exercise-induced oxidative stress and the potential effect of curcumin on these physiological processes.

4.1 Introduction

Oxidative stress is characterized by the dysregulation between pro-oxidants and antioxidant status, with the former surpassing the latter.[6] This imbalance can result in physiological effects by enhancing the susceptibility of cells and cellular components (such as membranes, lipids, proteins, deoxyribonucleic acid [DNA], and lipoproteins) to attacks from reactive oxygen species (ROS). If not effectively regulated and countered, oxidative stress can lead to acute pathologies (such as trauma and stroke) and contribute to the development of various chronic and degenerative diseases.[6,44,118] To adequately shield cells against the detrimental effects of free radicals, the human body employs a series of defense mechanisms encompassing preventive, reparative, and scavenging processes, as well as enhancement of antioxidant activities.[6,115,119]

Physical activity in the form of aerobic or anerobic exercise can result in exercise-induced oxidative stress. The redox homeostasis is offset primarily through the generation of ROS including hydroxyl ions, superoxide, hydroperoxyl, and lipid peroxyl radicals due to increased phospholipase A2 (PLA2), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and xanthine oxidase (XO) activities.[115,120,120,121] During activities involving strength-based exercises, short-term maximal sprints, and exercise near the anaerobic threshold, there is a notable increase in lipid peroxidation concentrations immediately post-exercise and up to 48 hours thereafter.[64,122–124] This rise in oxidative stress is attributed to various factors including

the mitochondrial electron transport chain complex, ischemia-reperfusion injury, and local inflammation which contribute to the production of free radicals during physical exertion.[122,123,125] The principal adverse effects of prolonged oxidative metabolism during endurance exercise are the accumulation of hydrogen ions, resulting in lactic acidosis, and an increase in body temperature, known as hyperthermia.[88] To sustain muscle redox homeostasis, the antioxidant defense system initiates a series of protective responses to mitigate the accumulation of ROS. Failure of the antioxidant defense system to neutralize ROS accumulation can lead to oxidative stress, causing potential muscle damage. Consequently, this can result in fatigue, muscle soreness, initiation of muscle damage, reduced muscle strength and range of motion (ROM), and elevated creatine kinase (CK) concentrations in blood samples.[88,89]

To counteract oxidative stress, the consumption of antioxidants has been shown to enhance the antioxidant defense system and inhibit the accumulation of ROS, by either augmenting the effectiveness of the antioxidant defense system, directly combating ROS, or both. While some researchers have indicated that anti-inflammatory agents are effective in mitigating exercise-induced oxidative stress, others have not observed such effects.[89,125–127] The heterogeneity in findings can be attributed to variations in the type of substances utilized, experimental methodologies, dosages administered, duration of interventions, and the nature of exercise protocols used to induce oxidative stress in a laboratory setting. Antioxidants such as tart cherry juice, pomegranate extract,

quercetin, among others, have demonstrated potential in mitigating oxidative stress.[128–130]

Curcumin, a flavonoid compound and naturally occurring polyphenol derived from turmeric (Curcuma longa), an herbaceous perennial plant of the Zingiberaceae family, has garnered increased attention in scientific research due to its therapeutic potential in treating a variety of conditions. As the primary metabolite found in turmeric, curcumin possesses numerous beneficial properties such as antioxidant, anti-inflammatory, anti-mutagenic, anti-microbial, and anti-cancer activities.[56,131]

It has been demonstrated in *in vivo* studies that curcumin exhibits antioxidant and anti-inflammatory properties by modulating the nuclear factor-kappa B (NF-κB) pathway, leading to the downregulation of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) expression concentrations.[98,99,102,132,133] Moreover, the antioxidant and anti-inflammatory effects of curcumin have been utilized in the management of various medical conditions including diabetes mellitus, rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis.[56] Supplementation with curcumin has been shown to attenuate increases in IL-6 and TNF-α concentrations in murine muscles following downhill running.[134,135] Additionally, recent findings by Kawanishi et al.[136] suggest that oral administration of curcumin at a dosage of 3 mg can mitigate oxidative stress by reducing hydrogen peroxide concentration in skeletal muscle post downhill running-induced muscle damage in mice. In human studies, the effect of curcumin

supplementation on oxidative stress markers remains inconclusive. While some researchers have reported positive effects, others have failed to observe any significant changes.[37,85,89–91,93,137–139]

To date, there are few human studies that have examined the effect of varying dosages of curcumin ingestion on oxidative stress markers induced by a maximal graded exercise test. As such, the primary aim of this research was to explore the antioxidative potential of curcumin in a recreationally active adult population. We evaluated the effect of oral supplementation with 300 mg and 600 mg of Theracurmin® compared to a placebo over a four-week period on oxidative stress markers elicited by a maximal graded exercise test in physically active female and male participants.

4.2 Materials and Methods

4.2.1 Ethical Approval

The research protocols outlined in this study were approved by the Institutional Review Board (IRB) of Virginia Polytechnic Institute and State University (Virginia Tech). Prior to participating, all individuals provided their verbal and written informed consent. The procedures adhered to the guidelines established in the most recent version of the Declaration of Helsinki and were conducted in compliance with the regulations set forth by the Virginia Tech IRB.

4.2.2 Study Design

We used a randomized, double-blind, placebo-controlled study design to investigate the effect of curcumin supplementation (Theracurmin®) in modulating biomarkers associated with oxidative stress. The study consisted of three scheduled visits to the laboratory: baseline (Week 0 [W0]), midpoint (Week 2 [W2]), and final (Week 4 [W4]). Each visit was spaced two weeks apart. The supplementation phase spanned four weeks, commencing from W0 to W4. Physiological measurements were taken during the W0 and W4 visits. Lean body mass, percent body fat, and total body bone mineral density were measured at the W0 timepoint to retrospectively analyze the mediating effect of these parameters on the efficacy of the intervention. The graded exercise test (GXT) protocol was administered at both W0 and W4. Blood samples were collected twice, before and after the GXT, during the W0 and W4 assessments. Body weight was measured at W0, W2, and W4, whereas height was only measured at W0. W2 assessment also served to monitor adherence to the supplementation regimen and assessed participants' self-reported general well-being (Figure 4.1).

4.2.3 Study Population

A total of 42 physically active female and male individuals who met the criteria of engaging in recreational exercise at least three times a week, were recruited for this research study. During the study period, participants were instructed to adhere to their usual dietary intake and exercise routine while avoiding the consumption of any substances that could potentially affect the study outcomes.

4.2.4 Inclusion and Exclusion Criteria

To ensure the eligibility of participants in this study, stringent inclusion and exclusion criteria were employed. Inclusion criteria necessitated individuals to be 18 to 45 years of age, actively engaged in regular physical activity (at least three days per week) for a minimum of 12 months, non-smokers, free from chronic illnesses, refraining from the intake of dietary supplementation that could affect study outcomes, and in overall good health with the ability to perform treadmill running. Conversely, individuals were excluded if they smoked, engaged in less than two days of exercise per week, had orthopedic restrictions, were outside the specified age range (under 18 or over 45 years of age), suffered from uncontrolled chronic ailments, exhibited systolic blood pressure exceeding 140 mmHg or diastolic blood pressure surpassing 90 mmHg, possessed a body mass index (BMI) exceeding 35 kg/m^2, or were pregnant.

4.2.5 Study Familiarization and Informed Consent

Before commencing the study protocol, all participants engaged in a Health Insurance Portability and Accountability Act (HIPAA)-compliant video call during which the detailed methodology utilized in our study was explained. In this session, all participants were apprised of the complexities of the study protocol, and any queries they had were addressed. Verbal and written informed consent were procured from all

participants in the presence of a witness prior to the initiation of any data collection procedures.

4.2.6 Randomization, Determination of Dosing and Supplementation Protocol

Participants were randomly assigned into one of three groups: 1) a placebo group who received 50 mg of microcrystalline cellulose, 2) a group who received 300 mg of Theracurmin® (containing 90 mg of active curcuminoids), and 3) a group who received 600 mg of Theracurmin® (containing 180 mg of active curcuminoids). Each participant was instructed to consume two capsules daily with breakfast to enhance absorption, because research has shown that curcumin absorption is increased when consumed with dietary fat. [20]The allocation of participants into one of these three groups was determined through the utilization of a software-generated randomization plan, employing the randomization tool available at http://www.graphpad.com/quickcalcs/index.cfm.

4.2.7 Collection of Physiological Measures

Our study had three visits in total, with each visit being two weeks apart (Figure 4.1). During Week 0 (W0) and Week 4 (W4), blood pressure, body weight. Height, lean body mass, percent body fat, and total body bone mineral density were only measured at W0. All measurements (except lean body mass, percent body fat, and bone mineral density) were taken in duplicate for accuracy. Blood pressure was assessed using an automated

blood pressure monitor (SunTech Tango M2 Blood Pressure Monitor, Morrisville, NC). Body weight and height were measured on a digital physician's scale (Detecto 439, Webb City, Missouri, USA) and stadiometer (SECA 777, GMBH & CO., Germany), respectively. Lean body mass, percent body fat, and total body bone mineral density were analyzed using dual x-ray absorptiometry (DXA) (iDXA, GE Healthcare, Madison, WI) by a certified DXA technician only at W0. During the midpoint visit, body weight was measured, adherence to the supplementation was checked, and well-being was assessed to ensure absence of any adverse events related to the supplementation.

4.2.8 Maximal oxygen consumption (VO_2max)

Maximal oxygen consumption (VO_2max) was measured at W0 and W4 using an incline-based graded exercise test (GXT). The VO_2max test was separated by four weeks of supplementation (placebo, 300 mg or 600 mg Theracurmin®). The GXT was conducted using a metabolic cart (Vyntx CPX, Vyaire Medical, Mettawa, IL) and treadmill (Trackmaster TMX428CP Treadmill, Full Vision, Kansas) to assess VO_2max and induce acute oxidative stress by increasing physical exertion until the point of exhaustion. The exercise protocol consisted of three phases: warm-up, testing, and recovery. The warm-up phase involved walking at a speed of 3 mph for two minutes. Subsequently, participants transitioned to their self-selected running pace of either 5.5 or 7 mph during the testing phase. The exercise intensity was incrementally increased by raising the gradient by 1% every minute until volitional exhaustion was reached (modified

Taylor Protocol)[24]. Although for ethical and safety reasons, volitional exhaustion was used to end each test, there are four ways to determine if VO_2max has been reached. They are as follows: 1) an increase in carbon dioxide expiration with a flattening of oxygen consumption with an increase in workload, 2) a flattening of oxygen consumption with an increase in workload (these first two are the most definitive ways to ascertain if someone reached their VO_2max), 3) a respiratory exchange ratio greater than 1.0, and 4) sheer exhaustion. All the aforementioned determinations of ascertaining VO_2max are incorporated in our laboratory; however, sheer exhaustion is often used as the first measure, due to ethical and safety reasons. Following the testing phase, participants walked on the treadmill at a pace of 2 mph for two minutes.

4.2.9 Blood Sample Collection

Plasma and serum blood samples were obtained at four distinct time points, comprising two collections during the baseline visit (W0) and two during the final visit (W4) to assess biomarkers of oxidative stress. A certified phlebotomist conducted venipuncture to collect all blood samples. The first sample was obtained prior to the commencement of the GXT, while the second was drawn 20 minutes post-exercise. Similarly, blood samples were obtained both before and after 20 minutes of the GXT during the final visit. These blood samples were promptly assessed for hemoglobin and hematocrit concentrations to account for the change in plasma volume immediately after exercise.[116] Plasma and serum blood samples were centrifuged at 3,500 revolutions per

minute (rpm) at 4°C for 15 minutes and stored at -80°C for analyses of serum protein carbonyl concentrations, serum glutathione (GSH) concentrations, serum glutathione disulfide (GSSG) concentrations, the GSH:GSSG ratio, and serum total antioxidant capacity. They were then stored in -80 C freezers until further analyses.

4.2.10 Calculation of Change in Plasma Volume

Intense exercise leads to an acute change in plasma volume due to the hemoconcentration effect.[117] The Dill and Costill[40] equations with hemoglobin concentration (Hb) and hematocrit (Hct) measurements were used to calculate the change in plasma volume (ΔPV).[116,117]

$$\Delta PV = \frac{PV_{post} - PV_{pre}}{PV_{pre}} = \frac{Hb_{pre}\ X\ \left(1 - Hct_{post}\right)}{Hb_{post}\ X\ (1 - Hct_{pre})} - 1$$

Concentration of biomarkers measured in the plasma or serum after the GXT were corrected using the following equation.

$$PM_{post,c} = PM_{post,u}\ X\ (1 + \Delta PV)$$

where $PM_{post,c}$ and $PM_{post,u}$ indicate corrected and uncorrected serum or plasma biomarker after the GXT, respectively. Note that we adjusted for plasma volume changes for all biomarkers assessed.

4.2.11 Blood Sample Storage and Analyses

The blood samples from every blood draw (pre- and post-GXT in both W0 and W4) were collected and stored in a freezer at a temperature of -80°C until analyses. Prior to storage, the samples underwent centrifugation at a speed of 3,500 rpm and a temperature of 4°C for a duration of 15 minutes. Following centrifugation, the plasma and serum samples were aliquoted into individual cryovials (Corning Incorporated, Corning, NY, USA) and preserved at -80°C. Analyses of blood samples were conducted after all blood samples had been collected and the study was completed. Serum protein carbonyl (PC) concentrations, serum glutathione (GSH) concentrations, serum glutathione disulfide (GSSG) concentrations, the GSH:GSSG ratio, and serum total antioxidant capacity (TAC) were all analyzed with commercially available enzyme-linked immunosorbent assay (ELISA) kits purchase from Abcam (Cambridge, UK) for PC (kit# ab238536), GSH (kit# 205811) , GSSG (kit# 205811), GSH:GSSG (kit# 205811), and TAC (kit# ab65329). A BioTek Synergy H1 Hybrid Multi-Mode Monochromator Fluorescence Microplate reader (Fisher Scientific, Hanover Place, IL) was used for analyses. These analyses included samples obtained at pre- and post-GXT in both W0 and W4. All blood analyses were conducted during the same week, in duplicate, at the Virginia Tech Metabolism Core at the end of the study to minimize differences among ELISA kits.

4.2.10 Dietary and Physical Activity Records

To evaluate the overall dietary intake and physical activity, participants completed a 2015 Block Food Frequency Questionnaire (FFQ) and the 2015 Block Adult Physical Activity Questionnaire (PAQ), respectively.[140] Detailed verbal instructions were provided to all participants regarding the protocol for documenting their diet and physical activity. The Block FFQ and PAQ evaluate dietary and intake over the past year and habitual physical activity. Participants were instructed to maintain their typical dietary and physical activity routines during the four-week supplementation period.

4.2.12 Statistical Analyses

A Wilcoxon signed-rank test was conducted to evaluate the effect of the GXT on the concentration of the biomarkers of oxidative stress obtained from blood samples before and after the GXT in all groups.

To address the hierarchical nature of the data and effectively capture the correlations within grouped observations, a linear mixed-effects (LME) model was utilized. This method enabled the examination of the fixed effects related to supplementation dosage (placebo, 300 mg and 600 mg of Theracurmin®), timepoint (W0 and W4), and GXT (pre- and post-GXT), while also accounting for random variability attributed to individual participants. The study's dependent variables (oxidative stress markers) were analyzed utilizing a model in which they were treated as a function of fixed effects such as

supplementation dosage, timepoint, and GXT. Participant identification (ID) was incorporated into the model as a random effect to accommodate participant-level variability. The formula for the model was specified as follows:

$$DV = \beta_0 + \beta_1(Supplementation\ Dosage) + \beta_2(Timepoint) + \beta_3(GXT\ Status) + \cup_{subject} + \varepsilon$$

where β_0 is the intercept, β_1, β_2, β_3 are the coefficients for the fixed effects, $\cup_{subject}$ represents the random effect for each participant, and ε is the error term.

All statistical analyses were conducted using the IBM SPSS Statistics (V.27, SPSS Inc., Chicago, IL) software. Significance was set *a priori* at $p<0.05$.

4.3 Results

4.3.1 Baseline characteristics

A cohort of 42 recreationally active individuals, consisting of 17 women and 25 men, was recruited for the study. Participants were randomly assigned to one of three groups: 1) a placebo group who received 50 mg of microcrystalline cellulose, 2) a group who received 300 mg of Theracurmin® (containing 90 mg of active curcuminoids), and 3) a group who received 600 mg of Theracurmin® (containing 180 mg of active curcuminoids), with each group comprising 14 individuals. Baseline characteristics of all participants are detailed in Table 4.1. There were no significant differences observed in baseline characteristics among the three groups, with the exception of resting heart rate. There were expected differences between the female and male participants (Table

4.1). Dietary habits are presented in Table 4.2. The distribution of concentrations for protein carbonyl (PC), glutathione (GSH), glutathione disulfide (GSSG), GSH:GSSG ratio, and total antioxidant capacity (TAC) in the dataset are depicted in Figures 4.2, 4.3, 4.4, 4.5, and 4.6, respectively.

4.3.3. The Effect of Graded Exercise Test (GXT) on Biomarkers of Oxidative Stress

A Wilcoxon signed-rank test was conducted to evaluate the effect of the GXT on the concentration of oxidative biomarkers before and after the GXT. At W0, the mean protein carbonyl concentrations prior to GXT for the placebo, 300 mg and 600 mg Theracurmin® groups were 136.7±19.7, 144.6±49.8, and 144.2±52.1 pmol/mg, respectively. Following GXT, mean protein carbonyl concentrations for the placebo, 300 mg and 600 mg Theracurmin® groups were 110.2±40.5, 105.8±31.3, and 115.7±36.3 pmol/mg respectively. There were significant reductions in protein carbonyl concentrations at W0 from pre-GXT to post-GXT in the placebo group ($p=0.013$) and the 300 mg Theracurmin® group ($p=0.019$). However, no significant changes were observed in the 600 mg Theracurmin® group ($p=0.241$). At W4, the mean protein carbonyl concentrations prior to the GXT for the placebo, 300 mg and 600 mg Theracurmin® groups were 119.4±40.8, 98.3±30.5, and 112.6±20.8 pmol/mg, respectively. Following the GXT, mean protein carbonyl concentrations for the placebo, 300 mg and 600 mg Theracurmin® groups were 120.5±35.7, 118.1±43.4, and 152.0±79.4 pmol/mg, respectively. Furthermore, at W4, no statistically significant differences were observed

between pre-GXT and post-GXT conditions in any of the three groups, suggesting that the GXT did not induce a significant increase in protein carbonyl concentrations (Figure 4.7).

At W0, the average glutathione concentrations prior to the GXT in the placebo, 300 mg and 600 mg Theracurmin® groups were 9.7±1.6, 8.41±0.9, and 9±0.9 μM, respectively. Post-GXT, the mean glutathione concentrations in the placebo, 300 mg, and 600 mg Theracurmin® groups were 9.1±0.8, 8.2±1.1, and 8.7±1.2 μM, respectively. Significant differences in glutathione concentrations were observed in the placebo ($p=0.017$) and 300 mg Theracurmin® ($p=0.04$) groups, but not in the 600 mg Theracurmin® group at W0. At W4, the mean glutathione concentrations in the placebo, 300 mg and 600 mg Theracurmin® groups before and after the GXT were 9.5±1.4, 8.6±1.5, and 8.9±1.2 μM, respectively, with concentrations changing to 8.5±0.9, 8.5±1.1, and 8.6±1.3 μM, respectively. A significant difference was observed only in the placebo group ($p=0.02$), with no significant differences noted in the other two groups receiving different doses of Theracurmin® supplementation (300 mg or 600 mg) (Figure 4.9).

At W0, the average glutathione disulfide (GSSG) concentrations prior to the GXT for the placebo, 300 mg and 600 mg Theracurmin® groups were 4.8±0.8, 4.2±0.5, and 4.3±0.6 μM, respectively. Post-GXT, the mean GSSG concentrations for the placebo, 300 mg and 600 mg Theracurmin® groups were 4.6±0.6, 3.9±0.3, and 4.2±0.5 μM, respectively. There was a statistically significant change ($p<0.05$) in GSSG concentrations at W0 in all

groups following the GXT. At W4, average GSSG concentrations before the GXT for the placebo, 300 mg and 600 mg Theracurmin® groups were 4.7±0.7, 4.2±0.7, and 4.3±0.6 μM, respectively. After the GXT, the mean concentrations were observed as 4.4±0.6 μM for placebo, 4.1±0.6 μM for 300 mg Theracurmin® group and 4.1±0.5 μM for the 600 mg Theracurmin® group. At W4, there were significant differences in both the placebo ($p=0.02$) group and the 600 mg Theracurmin® group ($p=0.01$), but not in the 300 mg Theracurmin® group (Figure 4.11).

In the 600 mg Theracurmin® group, the mean total antioxidant capacity (TAC) at baseline was 109.06±12.51 mM before the GXT and 105.03±8.81 mM post-GXT. At W4, the mean TAC for the 600 mg Theracurmin® group was 107.72±8.59 mM pre-GXT and 108.93±9.09 mM post-GXT. In the 300 mg Theracurmin®group, the mean TAC at baseline was 107.46±12.46 mM pre-GXT and 104.43±13.56 mM post-GXT, with W4 measurements of 107.75±13.19 mM pre-GXT and 107.84±9.15 mM post-GXT. For the placebo group, initial TAC was 108.14±12.78 mM pre-GXT and 106.46±13.08 mM post-GXT, with W4 measurements of 108.28±13.56 mM pre-GXT and 106.30±11.93 mM post-GXT. There was no statistically significant effect of the GXT on TAC in any of the groups at both timepoints, suggesting that the GXT did not lead to notable alterations in TAC among participants receiving different doses of Theracurmin® supplementation (Figure 4.15).

4.3.4 The effect of Theracurmin® supplementation on Biomarkers of Exercise-Induced Oxidative Stress

Linear Mixed Effect Model (LMEM) analyses were conducted to assess the effect of Theracurmin® supplementation, time, influence of the GXT, and sex on oxidative stress markers. The model included fixed effects for Theracurmin® dosage groups, timepoints, and GXT, with interactions among these factors. The random effect was participant ID to control for repeated measures within participants.

With respect to serum protein carbonyl concentrations, the 300 mg Theracurmin® group had a non-significant decrease of 30.9±27.8 pmol/mg compared to the control group ($p=0.266$), while the 600 mg Theracurmin® group showed a non-significant decrease of 5.8±27.7 pmol/mg ($p=0.834$). There was a non-significant increase of 32.7±25.4 pmol/mg in protein carbonyl concentrations ($p=0.198$) from W0 to W4. The pre-GXT was associated with a non-significant increase of 40.2±25.4 pmol/mg in protein carbonyl concentrations compared to post-GXT ($p=0.114$). Male participants had a non-significant increase of 3.8±14.1 pmol/mg in protein carbonyl concentrations compared to females ($p=0.789$). All tested interactions among group, timepoint, and GXT were found to be non-significant, with p-values ranging from $p=0.121$ to $p=0.918$ (Figure 4.4).

For plasma glutathione concentration, no significant effects of Theracurmin® dosage were observed. The 300 mg Theracurmin® group had a non-significant increase of

4.02±40.8 μM compared to the control group (p=0.921), while the 600 mg Theracurmin® group had a non-significant increase of 3.98 ± 40.8 μM (p=0.922). The change from W0 to W4 resulted in a negligible non-significant decrease of 0.07±33.2 uM in GSH concentrations (p=0.998). Pre-GXT was associated with a non-significant increase of 0.53±33.2 μM GSH concentrations compared to post-GXT (p=0.987). Male participants exhibited a non-significant decrease of 32.0±24.1 μM in GSH concentrations compared to females (p=0.184) at both the W0 and W4 timepoints. All tested interactions among group, timepoint, and GXT were found to be non-significant with p-values ranging from p=0.132 to p=0.999 (Figure 4.7).

There were no significant effects of Theracurmin® dosage on plasma glutathione disulfide concentrations. Both the 300 mg and 600 mg of Theracurmin® groups showed decreased GSSG concentrations compared to the control group; however, these changes were not statistically significant (0.30±0.281 μM (p=0.272) and 0.32±0.281 μM (p=0.246), for the 300 mg and 600 mg Theracurmin® groups, respectively). The change from W0 to W4 resulted in a negligible decrease of 0.01±0.1 μM in GSSG concentrations (p=0.866). Pre-GXT was associated with an increase of 0.3±0.1 μM in GSSG concentrations compared to post-GXT, and this difference was statistically significant (p=0.006). Male participants exhibited a slight decrease of 0.08±0.2 μM in GSSG concentrations compared to females, but this difference was not statistically significant (p=0.707). All

tested interactions among group, timepoint, and GXT were found to be non-significant with p-values ranging from p=0.266 to p=0.848 (Figure 4.10).

There were no significant effects of Theracurmin® dosage on TAC. The 300 mg Theracurmin® group showed a decrease of 1.74±4.7 mM compared to the control group, which was not statistically significant (p=0.712). The 600 mg Theracurmin® group had an increase of 2.65±4.7 mM, also not statistically significant (p=0.574). There was a non-significant decrease of 0.895±2.9 mM in TAC from W0 to W4 (p=0.763). Pre-GXT was associated with a non-significant increase of 0.939±2.9 mM in TAC compared to post-GXT, (p=0.752). Male participants showed a significant decrease of 7.154±3.2 mM in TAC compared to females (p=0.030). All tested interactions among group, timepoint, and GXT were found to be non-significant, with p-values ranging from p=0.307 to p=0.925 (Figure 4.16).

4.4 Discussion

We evaluated the effect of a four-week regimen of daily oral supplementation with 300 mg and 600 mg of Theracurmin® on exercise-induced oxidative stress biomarkers in physically active women and men, 18 to 45 years of age, in a randomized, double-blind, placebo-controlled study. We found that supplementation with Theracurmin® did not yield statistically significant effects on oxidative stress biomarkers, including serum protein carbonyl, plasma glutathione (GSH), plasma glutathione disulfide (GSSG), the

GSH:GSSG ratio, and serum total antioxidant capacity (TAC). These biomarkers were selected due to their ability to reflect aspects of oxidative stress and antioxidant defense mechanisms within the body.

4.4.1 Bioavailability of Theracurmin®

Turmeric is well-known for its poor bioavailability and limited absorption due to the rapid metabolism of its active compound, curcumin. Curcumin is quickly metabolized through glucuronidation and sulfation processes, forming curcumin glucuronides and curcumin sulfates that are rapidly excreted from the body.[21,141] To address this issue and improve the efficacy of turmeric supplementation, we used Theracurmin® for our supplement. Theracurmin® is a novel form of curcumin that enhances bioavailability by reducing curcumin to nanoscale particles enclosed within micelles, providing a protective barrier. The decreased particle size of Theracurmin® improves absorption by increasing surface area, while micellar encapsulation prevents aggregation and premature metabolism. Furthermore, the reduced particle size of Theracurmin® enables better passage through the intestinal lining into the bloodstream, while micellar encapsulation inhibits interactions via conjugation with other molecules in the gut.[21,22] A number of researchers have reported on the improved bioavailability of Theracurmin®. For example, Nakagawa et al.[23] showcased a 27-fold rise in the blood concentration-time curve of Theracurmin® in comparison to curcumin powder.[23] Stohs et al.[142] reported that the oral ingestion of Theracurmin® yielded significantly elevated serum concentrations

of curcumin metabolites when compared with other forms of curcumin.[142] However, due to the lack of testing for curcumin metabolites in the bloodstream our study, the efficacy of Theracurmin® in enhancing the bioavailability of curcumin and facilitating its pharmacological actions could not be definitively determined.

4.4.2. Effect of Graded Exercise Test (GXT)

The primary objective of the GXT in our study was to induce an acute state of oxidative stress by systematically increasing exercise intensity until volitional exhaustion was attained by the participants, thereby stimulating both aerobic and anaerobic metabolism to a maximal extent. The escalation in ROS production from contracting skeletal muscles, as well as enzymatic activities of NADPH oxidase and xanthine oxidase during exercise, leads to a transient shift in the intracellular redox balance towards a pro-oxidant state. Researchers have demonstrated the induction of acute oxidative stress through physical activity, whether aerobic or anaerobic in nature.[63,115,127,143] Notably, maximal exhaustive aerobic exercise has been reported to result in higher oxidative stress concentrations compared to nonaerobic isometric exercises.[144] Furthermore, researchers have shown that an incline-based treadmill protocol elicits a more pronounced increase in energy expenditure compared to a speed-based protocol, wherein exercise intensity is modulated by elevating speed.[24] The heightened metabolic demand on musculature during uphill running intensifies oxygen uptake to a greater degree than incremental speed adjustments on a consistent gradient, elevating acute

oxidative stress concentrations. We found significant disparities between pre-GXT and post-GXT conditions in serum protein carbonyl concentrations within all groups, as well as plasma concentrations of reduced GSH and oxidized GSSG across all groups at W0 and W4. These findings indicate that the GXT successfully induced an acute state of oxidative stress to a modest degree.

4.4.3 Effect of GXT and Theracurmin® Supplementation on Protein Carbonyl Concentration

Protein carbonyl is frequently utilized as a biomarker for oxidative stress due to its chemical stability.[32] The transient nature of ROS in the blood poses challenges in their quantification, making protein carbonyl a valuable proxy marker for oxidative stress. Protein carbonyls are generated through protein oxidation via a process known as protein carbonylation, which can be facilitated by ROS.[32] The increased presence of protein carbonyls resulting from the oxidation of proteins presents a significant concern due to the resistance of protein aggregates to cellular degradation, leading to impaired cellular function.[32] Elevated concentrations of protein carbonyl have been documented in various conditions such as aging, neurodegenerative disorders, obesity, diabetes mellitus, age-related macular degeneration, anemia, sickle cell disease, neonatal bronchopulmonary dysplasia, and hepatocellular carcinoma.[145] Additionally, the concentrations of protein carbonyl is reported to be elevated in the immediate aftermath of aerobic exercise.[32,146,147] This elevation gradually returns to baseline concentrations

within a few hours. Bloomer et al.[146] have shown a transient rise in protein carbonyl concentrations following various cycling protocols of differing durations and intensities, in both female and male participants. Additionally, Alessio et al.[144] observed a 67% increase in protein carbonyl concentrations following exhaustive aerobic exercise in male participants. However, despite a number of researchers documenting acute elevations in protein carbonyl concentrations in response to exercise, some researchers have observed reductions in protein carbonyl concentrations following exercise in both trained and untrained individuals.[124,147–150] Wadley et al.[148] demonstrated a significant 10% decrease in plasma protein carbonyl concentrations immediately after a GXT to volitional exhaustion in active young men. The inconsistent trends in protein carbonyl concentrations post-exercise are intriguing and may be attributed to the involvement of the 20S proteasome system in mediating the clearance of plasma protein carbonyl following physical activity. It has been proposed that exercise could enhance the removal of baseline plasma protein carbonyl, while simultaneously increasing the production of ROS that promote the formation of new protein carbonyl groups.[147] The equilibrium between these processes likely accounts for why certain researchers have reported no change or even decreases in plasma protein carbonyl concentrations post-exercise, when other indicators of oxidative stress (such as markers of lipid peroxidation) were elevated. We observed that serum protein carbonyl concentrations exhibited considerable variability among participants both pre- and post-GXT. At W0, a

statistically significant reduction in protein carbonyl concentrations was noted following the GXT across all groups (placebo, 300 mg and 600 mg Theracurmin® groups: 19%, 13%, and 21% reduction, respectively). Conversely, at W4, a statistically significant increase in protein carbonyl concentrations was observed after the GXT in all groups (placebo, 300 mg and 600 mg Theracurmin® groups: increase of 14%, 7%, and 19%, respectively). This unexpected reversal in trends cannot be attributed to Theracurmin® supplementation alone because it was also evident in the placebo group.

4.4.4 Effect of GXT and Theracurmin® Supplementation on Glutathione, Glutathione Disulfide and Ratio of Reduced to Oxidized Glutathione

Glutathione (GSH) is a tripeptide composed of glutamate, cysteine, and glycine, primarily synthesized within hepatic cells.[151] GSH serves as the principal non-enzymatic antioxidant defense system in all cells of the body. As the most abundant low molecular weight thiol, GSH fulfills crucial functions as a reducing agent that shields cells against oxidative harm, hinders lipid peroxidation, preserves redox balance, and facilitates cellular detoxification.[151] It stands as one of the most abundant antioxidants within the organism, with a considerable portion of cellular GSH utilized by three isoforms of glutathione peroxidase (GPx) for enzymatic defensive countermeasures against free radicals. Under conditions of oxidative stress, GSH undergoes oxidation to form glutathione disulfide (GSSG) in response to pro-oxidants such as free radicals, subsequently being reduced back to its original form by the enzyme glutathione

reductase.[151] The ratio of reduced glutathione to oxidized glutathione (GSH:GSSG) holds significant importance as a marker of oxidative stress because it determines intracellular redox potential and serves as a pivotal indicator of cellular well-being. Our findings indicate that factors such as curcumin supplementation (300 and 600 mg), duration of supplementation (4 weeks), and exercise (pre- or post-GXT), do not exert a significant influence on glutathione (GSH) concentration in our study population. We found that the only significant effect on GSH and glutathione disulfide (GSSG) concentrations was observed in relation to exercise, where individuals exhibited higher concentrations before the GXT compared to after the GXT. Theracurmin® supplementation, time duration, and sex were not found to have a significant effect on GSH or GSSG concentrations within our study cohort. Consequently, this lack of effect also extended to the GSH:GSSG ratio, with no significant differences observed. As such, these results indicate that a four-week regimen of Theracurmin® supplementation did not lead to a significant increase in endogenous glutathione concentration. Additionally, when oxidative stress was induced through exercise, all study groups displayed comparable glutathione concentrations at W4 regardless of the Theracurmin® dose administered. In contrast, McAllister et al.[152] observed a significant attenuating effect of curcumin supplementation (1.5 g of curcumin over a three-day period) on the reduction of whole blood GSH concentration 30 minutes after 35 minutes of exercise at 60% of peak oxygen consumption (VO_2peak).

4.4.5. Effect of GXT and Theracurmin® Supplementation on Total Antioxidant Capacity Concentration

The total antioxidant capacity (TAC) represents the combined action of all antioxidants in the serum and is considered a comprehensive indicator of antioxidant effectiveness.[129,153] TAC is a significant marker in assessing redox status and monitoring antioxidant intake. Regular physical activity typically results in an elevation of TAC; however, immediately post-exercise, TAC in the serum tend to decrease due to heightened oxidative stress.[34,153] Although variations in TAC were observed following the GXT protocol, these changes did not reach statistical significance in our study. The findings suggest that Theracurmin® supplementation may potentially exhibit a stabilizing effect on TAC, particularly in the higher dosage group (600 mg). The changes observed in TAC were moderate across all groups, indicating that the antioxidant properties of curcumin may contribute to maintaining antioxidant capacity in conditions of elevated oxidative stress. Furthermore, no significant interaction was found among group, timepoint, and exercise conditions. The lack of substantial changes in TAC following the exercise test could be attributed to several factors. The study participants were all physically active individuals with a proficient antioxidant defense system, suggesting that Theracurmin® did not significantly affect TAC after four weeks of supplementation. While some researchers have reported significant differences in TAC, there are conflicting findings from other researchers, as well.[85,154] Nakhastin-Roohi

et al.[154] reported elevated TAC immediately post-resistance exercise in untrained men supplemented with an acute dose of 150 mg of curcuminoids prior to the exercise protocol. In contrast, Basham et al.[85] reported significant reductions in TAC after a bout of intense exercise in elite athletes who were supplemented with 500 mg of curcuminoids.

4.4.6. Conclusion

Our study represents a novel approach in researching the effect of Theracurmin® supplementation on markers of oxidative stress in physically active individuals. It is the first study of its kind to utilize a four-week supplementation phase within a randomized, double-blind, placebo-controlled design. We also examined the dose-response relationship of curcumin supplementation by including two different dosages of Theracurmin® alongside a placebo group. Additionally, our research incorporated a maximal GXT in conjunction with the supplementation protocol to assess the effect of Theracurmin® on exercise-induced oxidative stress. Importantly, our study included both female and male participants; previous research in this area has predominantly focused on male participants. Moreover, we used the Dill and Costill[40] equations to appropriately account for variations in plasma volume post-exercise by measuring alterations in hemoglobin and hematocrit concentrations. This practice, often overlooked in research studies, enhanced the precision and dependability of our results. However, our study is subject to several limitations. One of these was the inclusion of a

physically active healthy population, which restricts the extent to which oxidative stress can be induced with physical exertion. This limitation arises from the fact that the study participants were physically active individuals who were well accustomed to exercise, resulting in an optimal and robust antioxidant defense system that may limit the potential for detecting changes in oxidative stress. Additionally, the GXT protocol we used may not have induced enough of a stressor to substantially increase the oxidative stress markers across all timepoints and groups, thereby limiting the ability to assess any potential effect of curcumin on exercise-induced oxidative stress. This lack of a robust response may be attributed to insufficient time spent at high intensities during the GXT protocol. Furthermore, we only included one blood draw following the GXT, potentially missing peak concentrations of certain oxidative stress markers in the bloodstream that may have occurred after the initial response to the GXT protocol. Additionally, a limitation of our study was the absence of measurements for curcumin metabolites in blood samples, making it difficult to determine if the supplementation protocol resulted in adequate absorption of curcumin in serum for potential beneficial effects.

In conclusion, the novel discovery of the current investigation indicates that daily oral supplementation with 300 mg and 600 mg of Theracurmin® over a period of four weeks did not yield statistically significant alterations in concentrations of serum protein carbonyl, plasma glutathione (GSH), glutathione disulfide (GSSG), and total antioxidant

capacity (TAC) in physically active female and male participants. Subsequent research endeavors should aim to meticulously investigate the intricate relationship between oral curcumin dosage, concentration of oxidative stress biomarkers, blood curcumin metabolites, and various exercise modalities. Furthermore, future researchers should incorporate the assessment of localized and intracellular oxidative stress within various sites such as the skeletal muscle tissue.

4.5 References for Manuscript 1

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Table 4.1. Baseline Characteristics of All Participants

Variable	Total	Group			Sex	
	n=42	Placebo (n=14)	300 mg (n=14)	600 mg (n=14)	Females (n=17)	Males (n=25)
Age, years	30 ± 8	30 ± 7	32 ± 7	29 ± 8	31.1 ± 8	30 ± 8
Weight, kg	74.3 ± 13.9	70.1 ± 12.2	73.3 ± 12.9	80.7 ± 14.8	64.5 ± 8.9	81.7 ± 12.1*
Height, cm	171.7 ± 8.9	168.7 ± 8.6	171.4 ± 7.6	176.0 ± 8.9	164.2 ± 5.3	177.4 ± 6.2*
BMI, kg/m^2	25.0 ± 3.0	24.4 ± 2.2	24.8 ± 2.7	26.0 ± 3.8	23.9 ± 2.9	25.9 ± 2.8*
SBP, mmHg	126 ± 10	128 ± 12	128 ± 6	123 ± 11	121 ± 9	130 ± 10*
DBP, mmHg	77 ± 9	80 ± 9	79 ± 10	72 ± 7	77 ± 8	77 ± 10
HR, bpm	63 ± 11	67 ± 9	63 ± 14	57 ± 10*	68 ± 12	60 ± 10**
Body Fat, %	24.7 ± 8.4	25 ± 7.4	25 ± 8.9	23.4 ± 9.3	30.5 ± 6.7	20.4 ± 6.9**
FFM, kg	56.8 ± 12.7	53.3 ± 12.9	55.9 ± 12.0	62.3 ± 11.8	45.3 ± 6.7	65.3 ± 8.4*
SAT mass, kg	0.83 ± 0.57	0.73 ± 0.42	0.90 ± 0.56	0.88 ± 0.75	0.86 ± 0.48	0.82 ± 0.65
VAT mass, kg	0.33 ± 0.35	0.28 ± 0.20	0.37 ± 0.33	0.33 ± 0.50	0.16 ± 0.16	0.44 ± 0.41*
RMR, kcal/day	1,531 ± 252	1,468 ± 264	1,512 ± 237	1,638 ± 231	1,303 ± 132	1,700 ± 168*
BMD, g/cm^2	1.28 ± 0.15	1.25 ± 0.10	1.26 ± 0.16	1.34 ± 0.14	1.21 ± 0.11	1.33 ± 0.14*

Data presented as mean ± standard deviation

kg, kilograms; cm, centimeters; BMI, body mass index; m^2, meters squared; SBP, systolic blood pressure; mmHg, millimeters of mercury; DBP, diastolic blood pressure; HR, heart rate; bpm, beats per minute; %, percent; FFM, fat free mass; SAT mass, subcutaneous adipose tissue mass; VAT mass, visceral adipose tissue mass; RMR, resting metabolic rate; kcal, kilocalories; BMD, bone mineral density; g, grams; cm^2, centimeters squared

* Males significantly greater than females ($p<0.05$)

** Males significantly lower than females ($p<0.05$)

Table 4.2. Habitual Dietary Intake of Participants Obtained from Food Frequency Questionnaire at Baseline

	Total	Group		
Variable	**n=39**	**Placebo (n=14)**	**300 mg (n=13)**	**600 mg (n=12)**
Energy Intake, kcal/day	1,962 ± 697	2,067 ± 655	1,852 ± 505	2,002 ± 929
Fat, g/day	82.3 ± 29.5	88.2 ± 26.0	79.6 ± 29.3	78.5 ± 34.7
Saturated Fat, g/day	25.2 ± 10.6	26.5 ± 9.0	26.0 ± 11.7	22.5 ± 11.3
MUFA, g/day	31.1 ± 11.7	33.7 ± 10.2	29.6 ± 12.6	29.8 ± 12.6
PUFA, g/day	19.3 ± 8.1	20.7 ± 8.6	17.7 ± 4.9	19.8 ± 10.5
Protein, g/day	80.3 ± 33.6	88.7 ± 30.5	70.0 ± 18.3	84.3 ± 45.0
Carbohydrates, g/day	224.2 ± 94.5	227.3 ± 93.8	211.7 ± 73.3	242.1 ± 118.6
Cholesterol, mg/day	299.3 ± 196.5	350.3 ± 208.0	264.8 ± 157.0	298.8 ± 225.3
Dietary Fiber, g/day	23.2 ± 12.5	23.8 ± 12.7	22.5 ± 12.8	23.9 ± 13.0
Vitamin A, RAE/day	1,132 ± 594	1,106 ± 406	1,108 ± 583	1,178 ± 806
Beta-carotene, mg/day	5,573 ± 4,499	4,620 ± 2,632	5,796 ± 4,865	6,5423 ± 5,804
Vitamin C, mg/day	110.8 ± 74.9	108.1 ± 63.2	107.6 ± 88.1	124.7 ± 77.1
Vitamin E, mg/day	11.6 ± 5.7	12.0 ± 5.3	10.5 ± 4.5	12.5 ± 7.4
Iron, mg/day	15.5 ± 6.9	15.8 ± 6.2	14.3 ± 5.4	16.8 ± 9.1
Zinc, mg/day	12.9 ± 6.1	12.9 ± 4.3	12.6 ± 8.0	13.2 ± 5.8
Sodium, mg/day	3,456.7 ± 1363.5	3,577 ± 1292	3,166 ± 1003	3,829 ± 1850

Data presented as mean ± standard deviation

kcal, kilocalories; g, grams; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; mg, milligrams; RAE, retinol activity equivalents

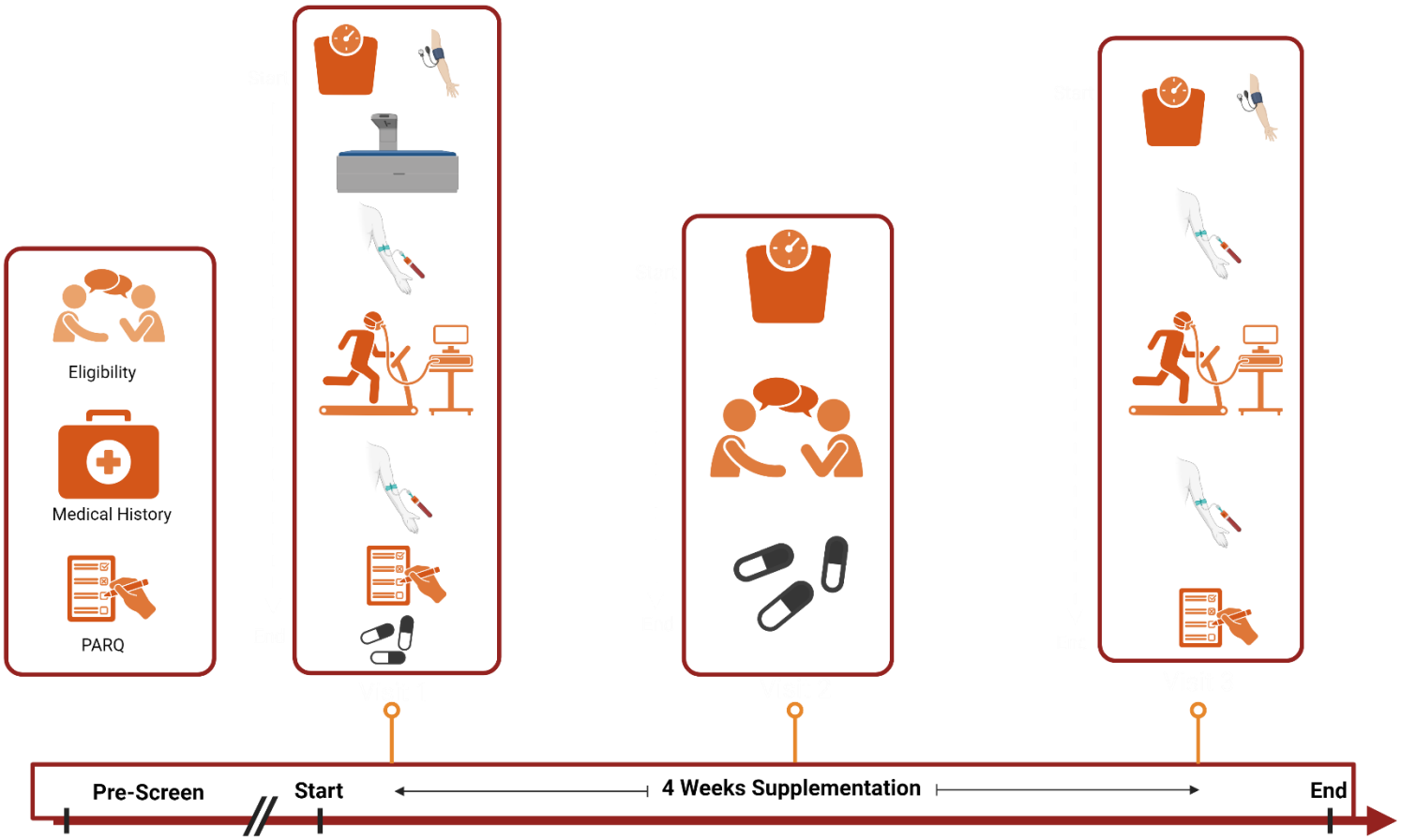

Figure 4.1. Diagram of the protocol employed in the study.

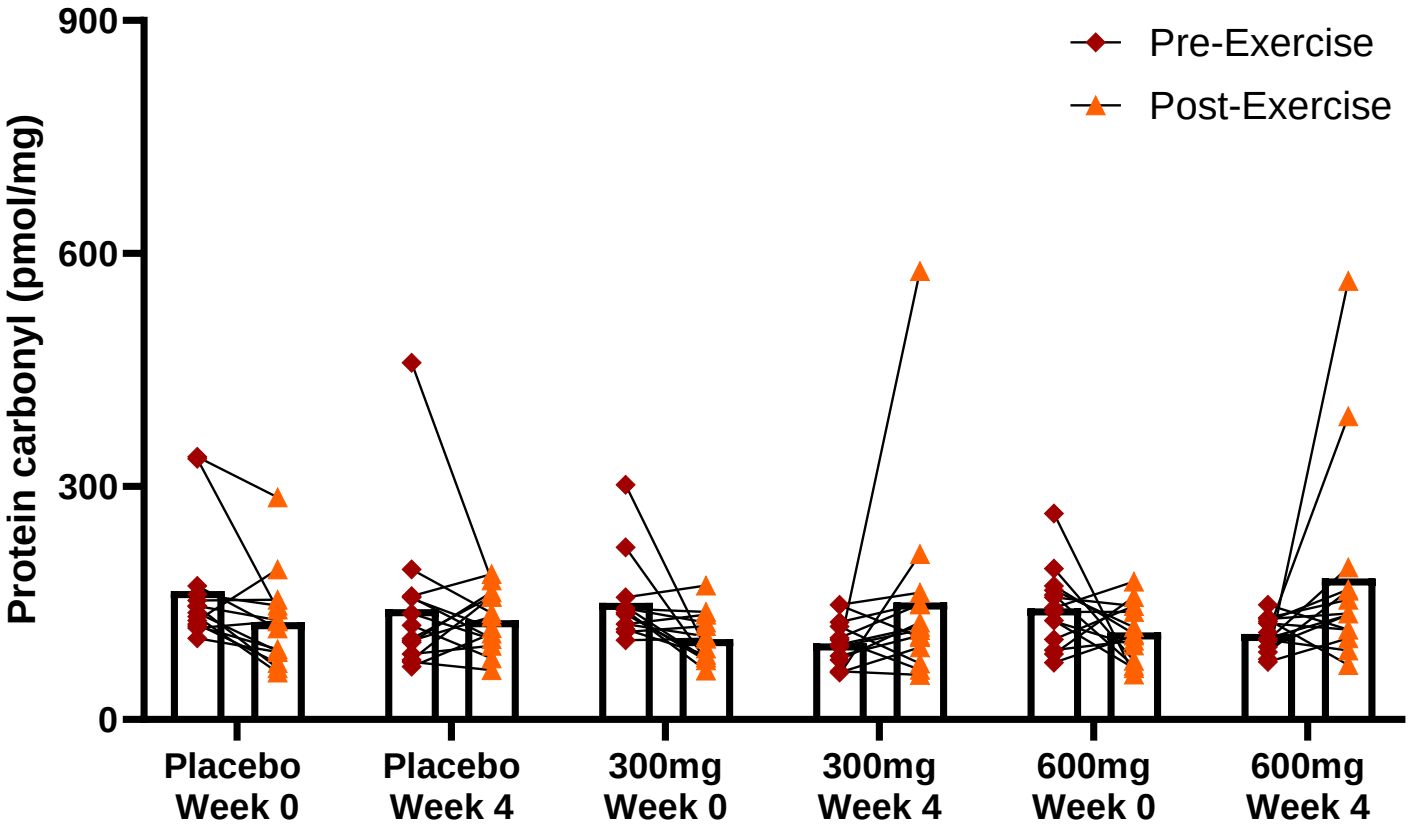

Figure 4.2. Serum protein carbonyl concentrations in placebo, 300 mg and 600 mg Theracurmin® groups during pre- and post-graded exercise testing at Week 0 and Week 4.

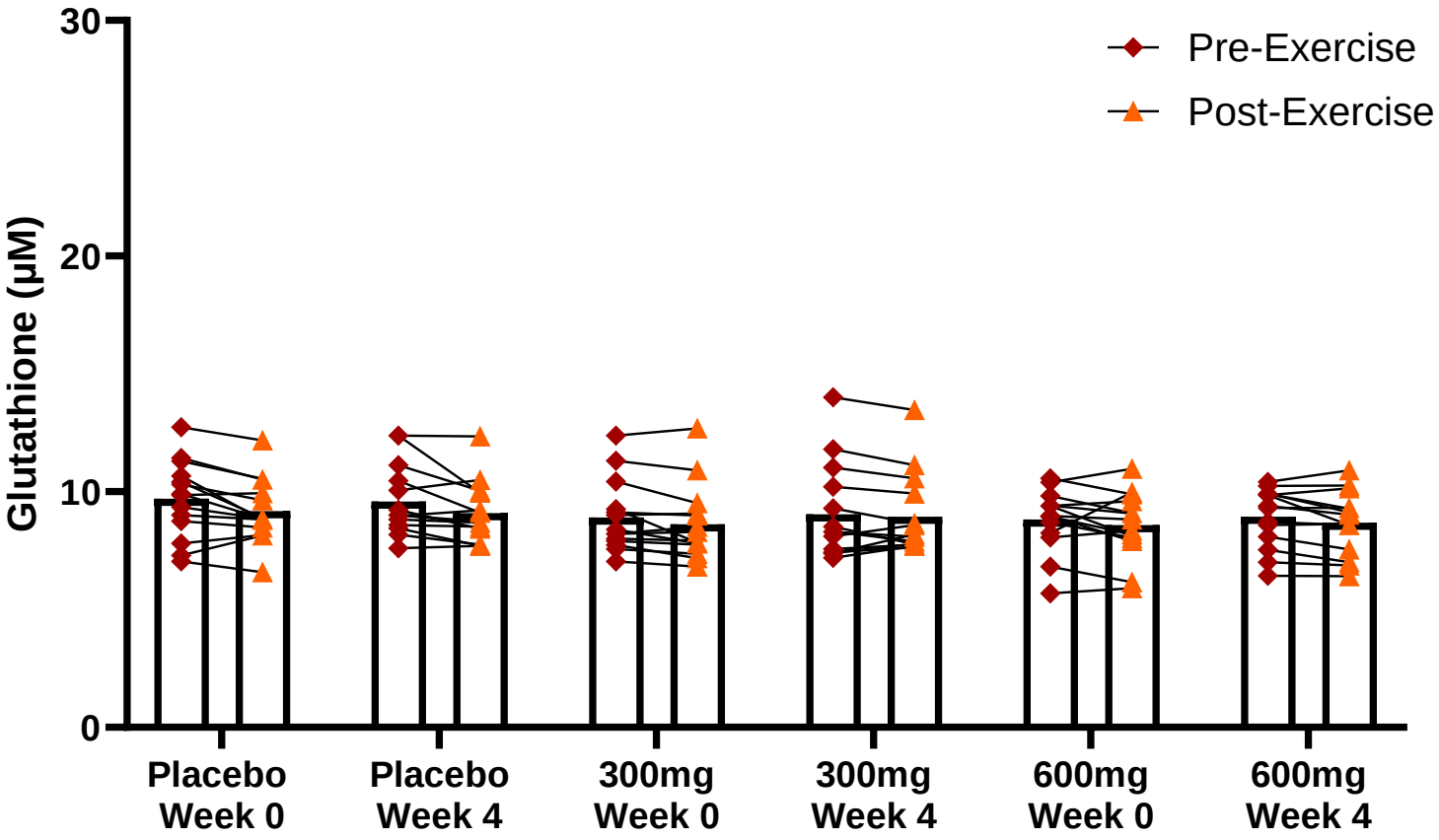

Figure 4.3. Plasma glutathione concentrations in placebo, 300 mg and 600 mg Theracurmin® groups during pre- and post-graded exercise testing at Week 0 and Week 4.

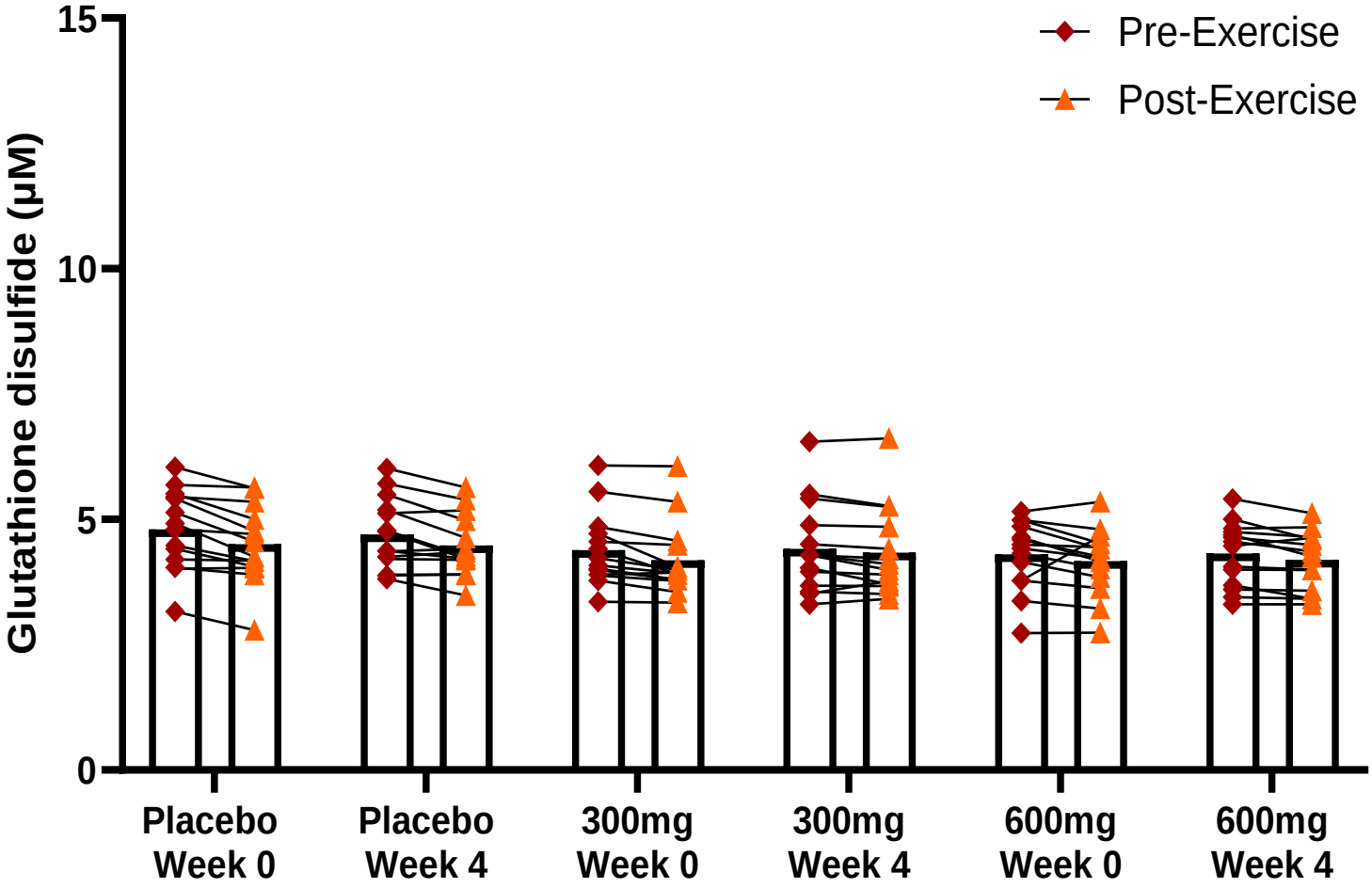

Figure 4.4. Plasma glutathione disulfide concentrations in placebo, 300 mg and 600 mg Theracurmin® groups during pre- and post-graded exercise testing at Week 0 and Week 4.

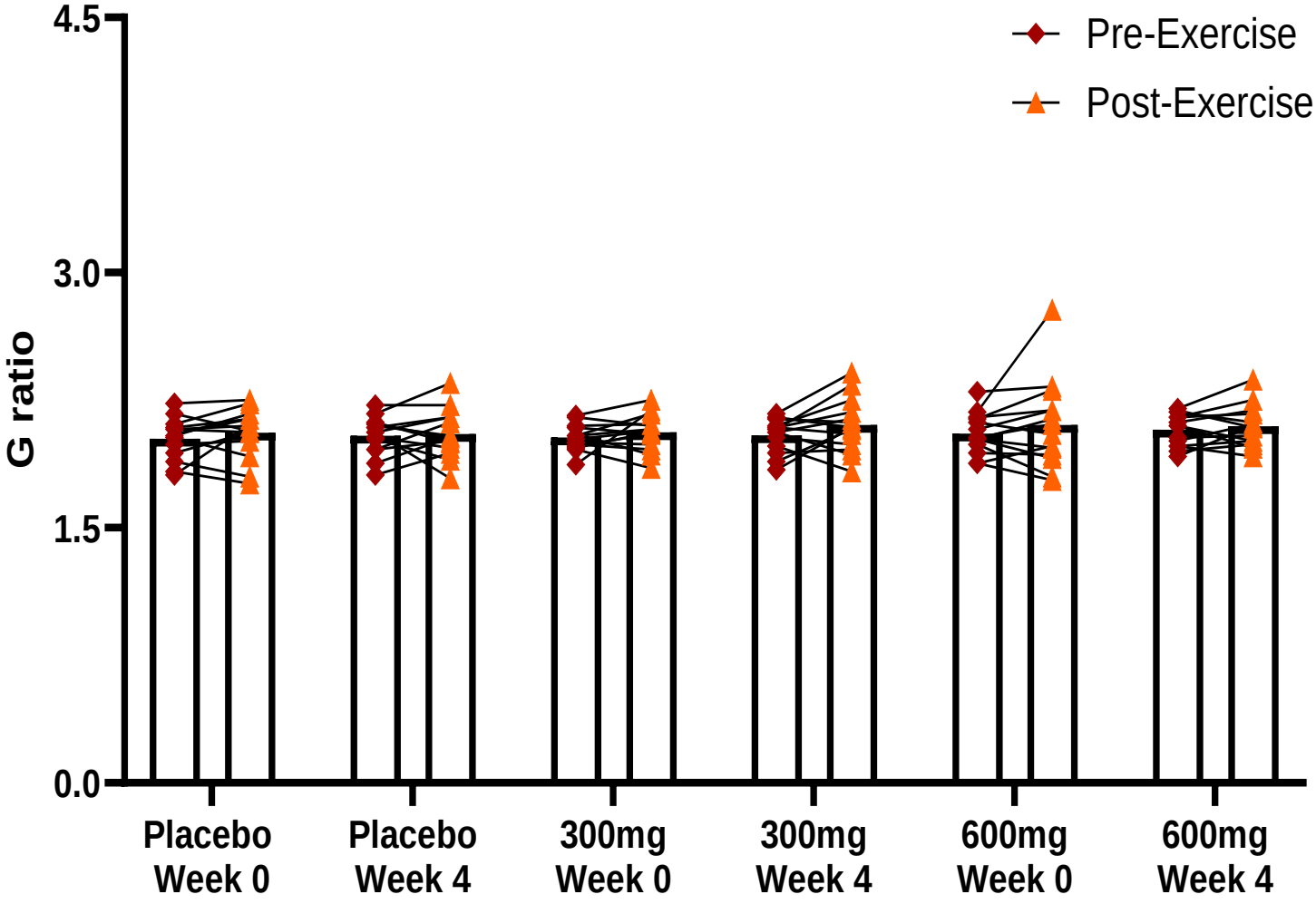

Figure 4.5. The ratio of reduced glutathione to oxidized glutathione (GSH:GSSG) in placebo, 300 mg and 600 mg Theracurmin® groups during pre- and post-graded exercise testing at Week 0 and Week 4.

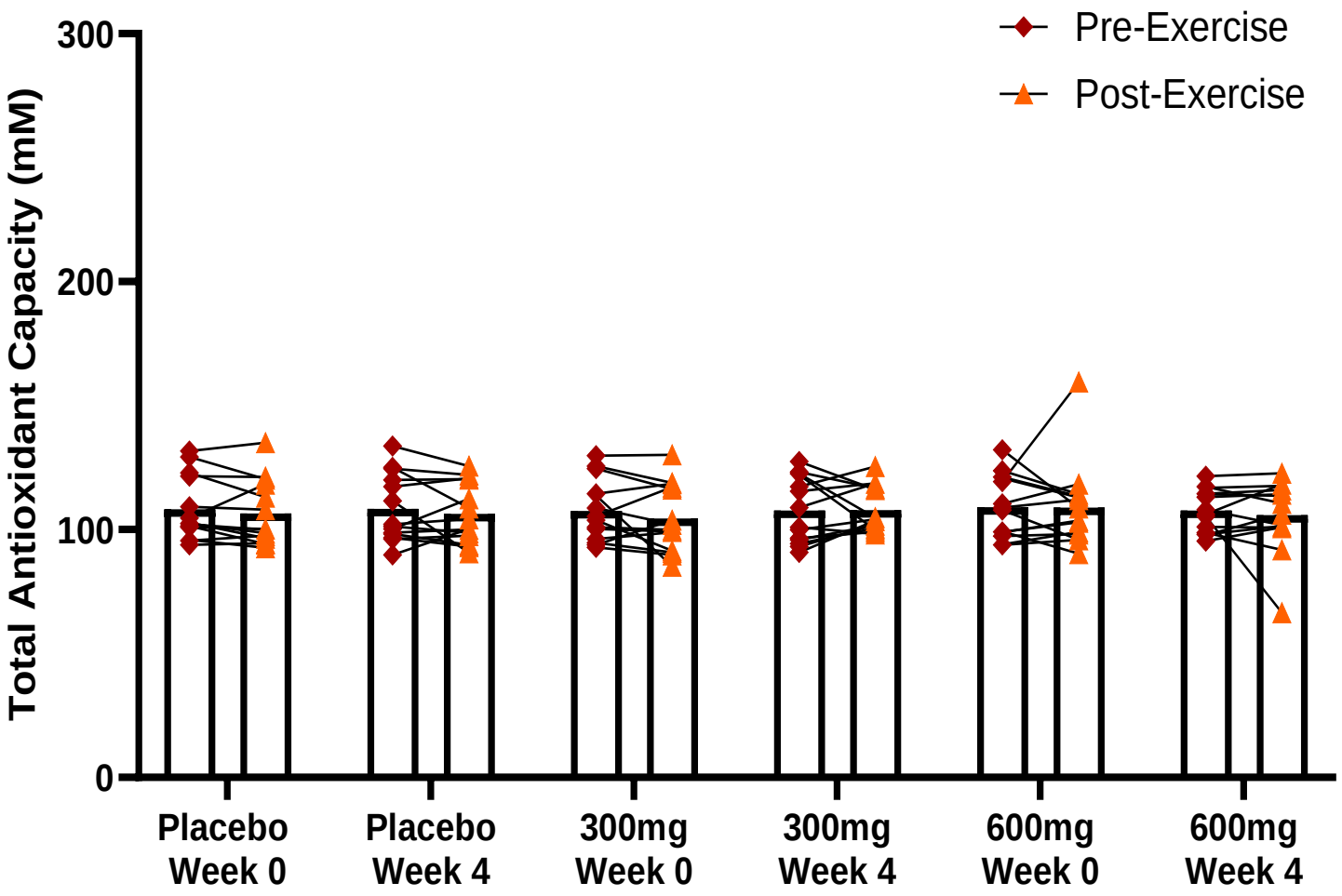

Figure 4.6. Serum total antioxidant capacity in placebo, 300 mg and 600 mg Theracurmin® groups during pre- and post-graded exercise testing at Week 0 and Week 4.

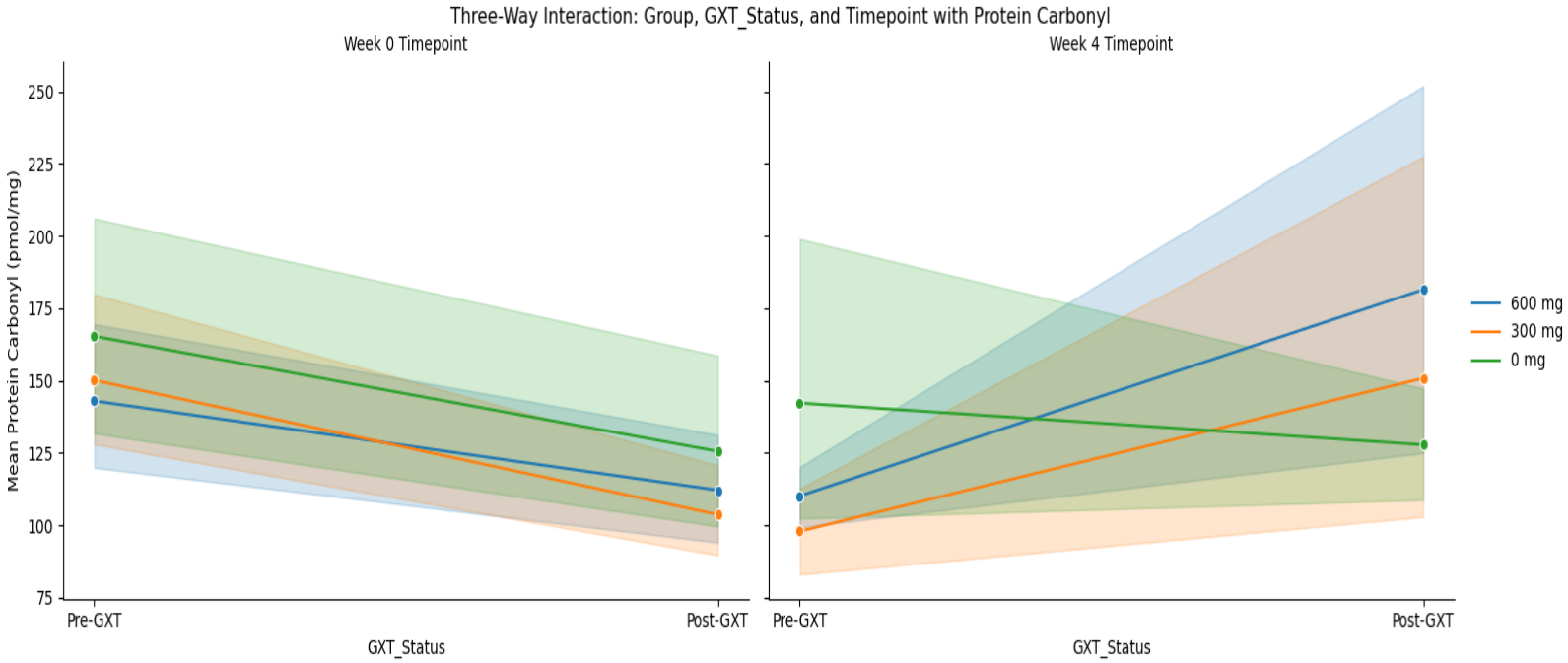

Figure 4.7. The three-way interaction plot provides insights into how supplementation dosage, timepoint, and graded exercise testing (GXT) interact to affect protein carbonyl concentrations.

Facets (Timepoint): Each plot represents a different timepoint (Week 0 vs Week 4).

X-axis: Shows the difference between pre- and post-graded exercise test.

Y-axis: Indicates the average value at each graded exercise test for each group within the specific timepoint.

Lines: Each line within a facet represents a different group (placebo, 300 mg of Theracurmin® and 600 mg of Theracurmin®). The interaction effect within each timepoint is visualized by how these lines differ in slope and position.

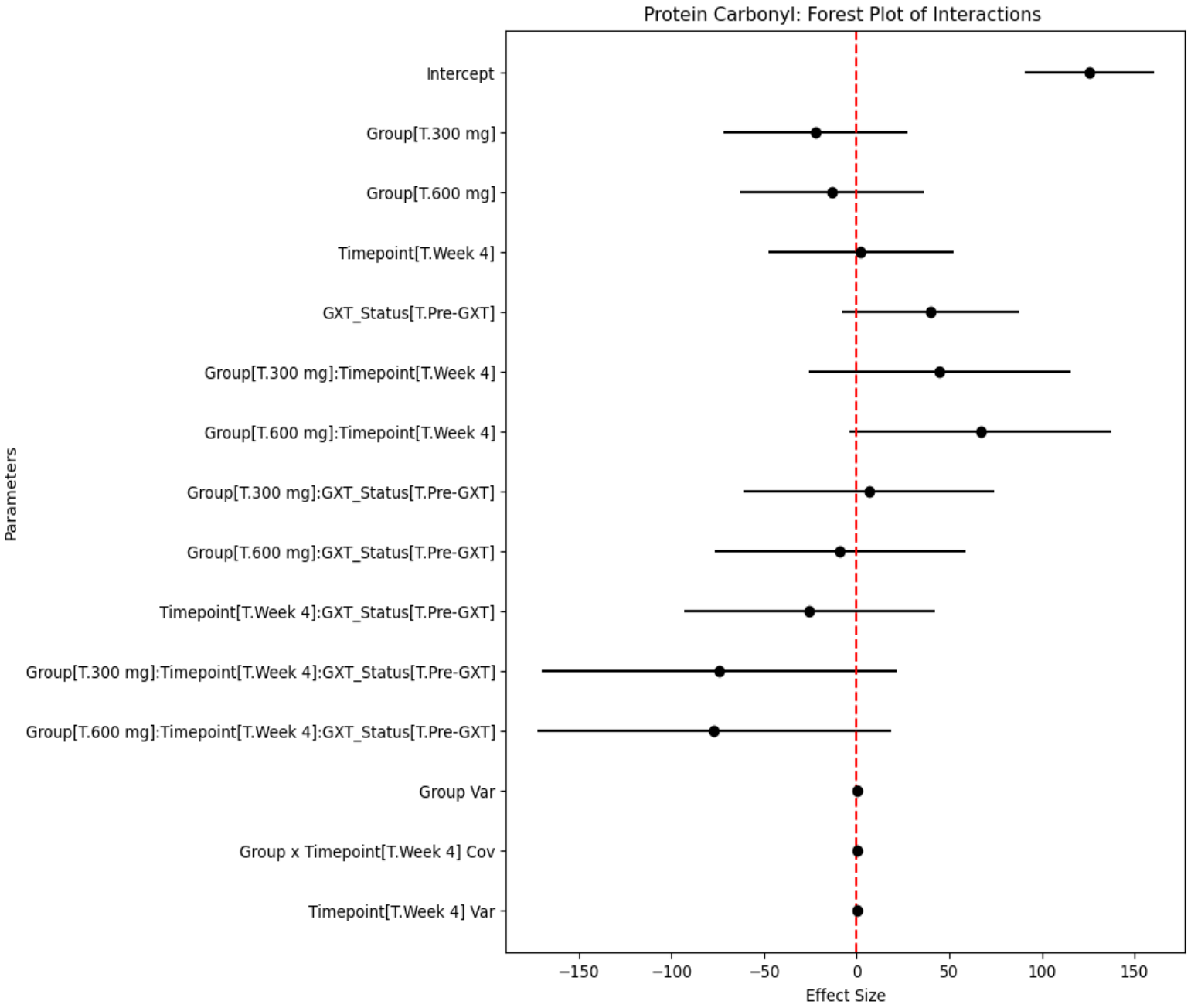

Figure 4.8. The Forest plot displays the interactions and their confidence intervals from the fitted linear mixed model for protein carbonyl concentrations. This plot visualizes the coefficient estimates for each interaction along with their confidence intervals, providing a clear overview of the effects and their statistical significance.

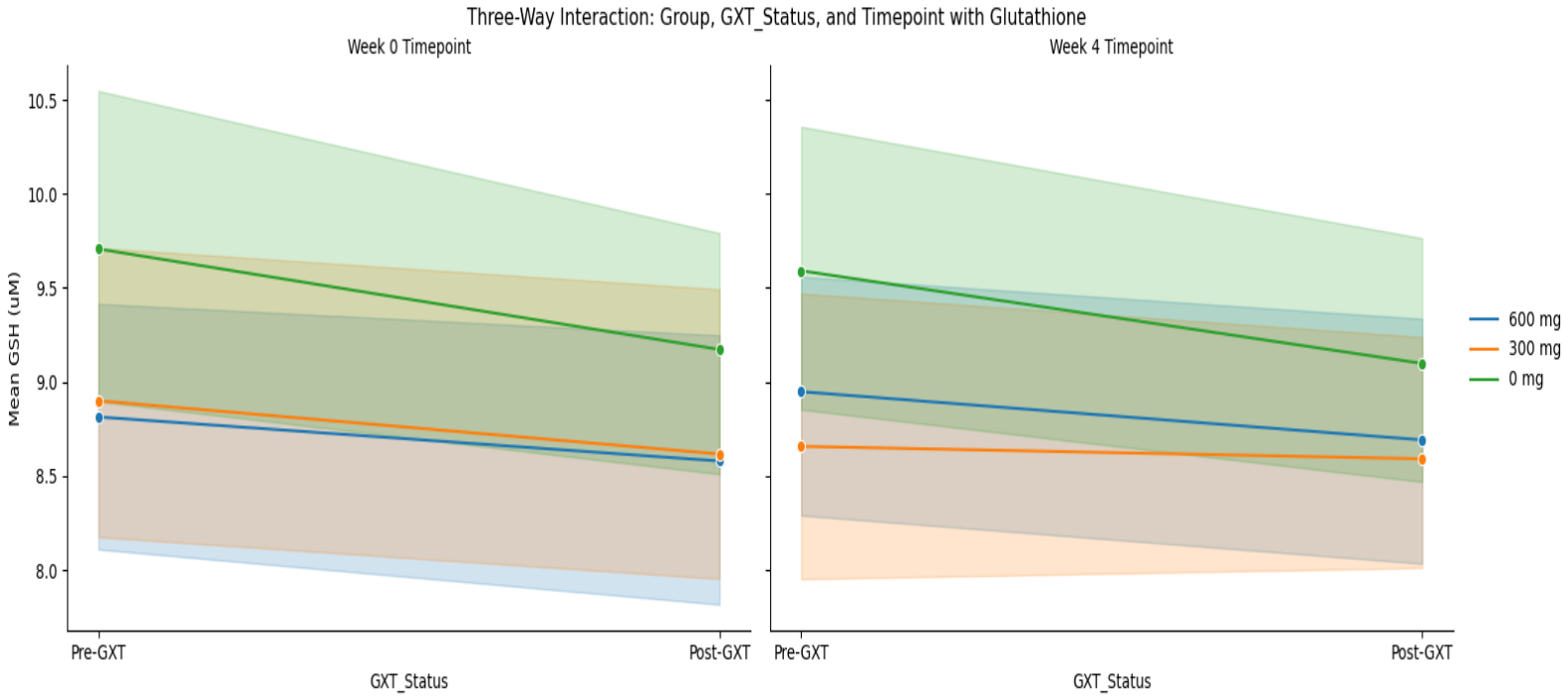

Figure 4.9. The three-way interaction plot provides insights into how supplementation dosage, timepoint, and graded exercise testing (GXT) interact to affect glutathione concentrations.

Facets (Timepoint): Each plot represents a different timepoint (Week 0 vs Week 4).

X-axis: Shows the difference between pre- and post-graded exercise test.

Y-axis: Indicates the average value at each graded exercise test for each group within the specific timepoint.

Lines: Each line within a facet represents a different group (placebo, 300 mg of Theracurmin® and 600 mg of Theracurmin®). The interaction effect within each timepoint is visualized by how these lines differ in slope and position.

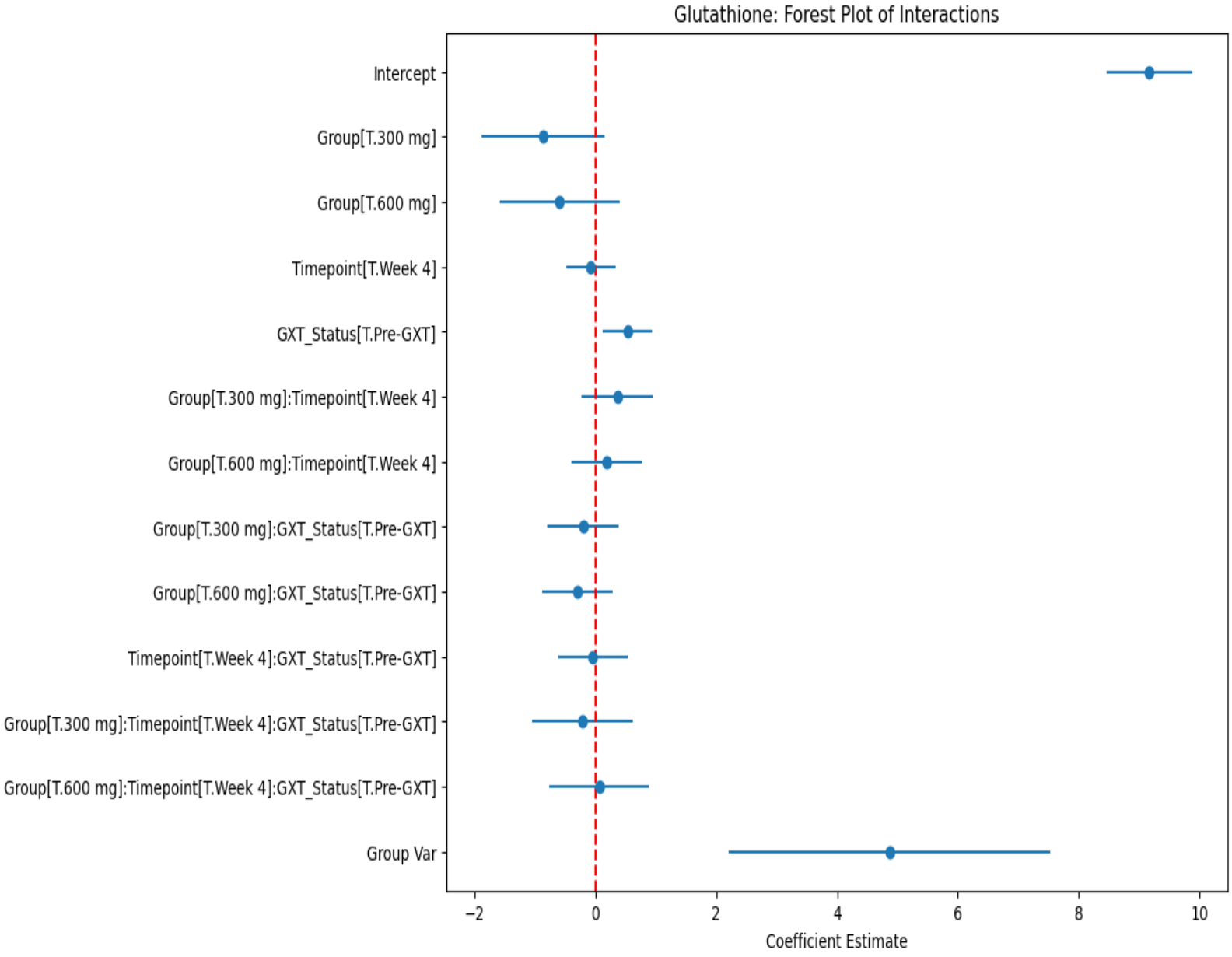

Figure 4.10. The Forest plot displays the interactions and their confidence intervals from the fitted linear mixed model for glutathione concentrations. This plot visualizes the coefficient estimates for each interaction along with their confidence intervals, providing a clear overview of the effects and their statistical significance.

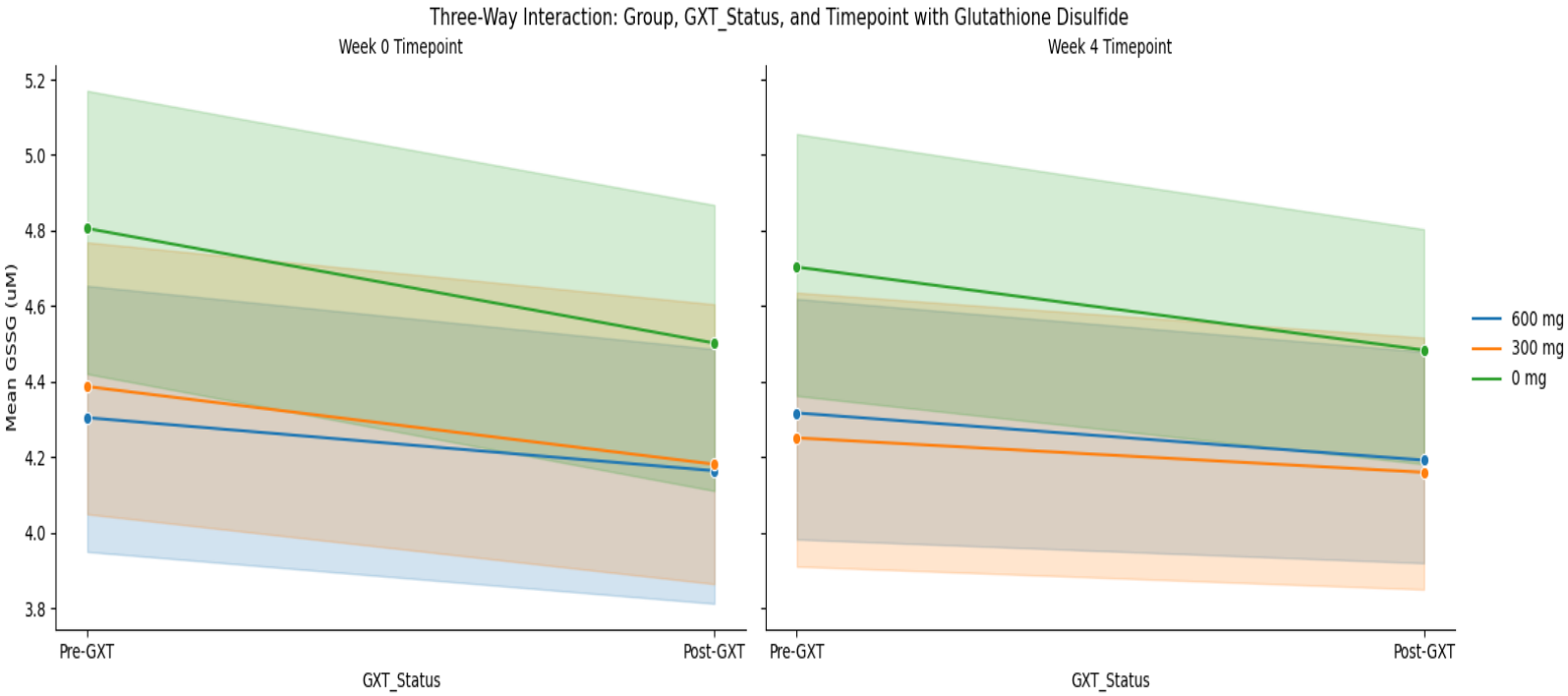

Figure 4.11. The three-way interaction plot provides insights into how supplementation dosage, timepoint, and graded exercise testing (GXT) interact to affect glutathione disulfide concentrations.

Facets (Timepoint): Each plot represents a different timepoint (Week 0 vs Week 4).

X-axis: Shows the difference between pre- and post-graded exercise test.

Y-axis: Indicates the average value at each graded exercise test for each group within the specific timepoint.

Lines: Each line within a facet represents a different group (placebo, 300 mg of Theracurmin® and 600 mg of Theracurmin®). The interaction effect within each timepoint is visualized by how these lines differ in slope and position.

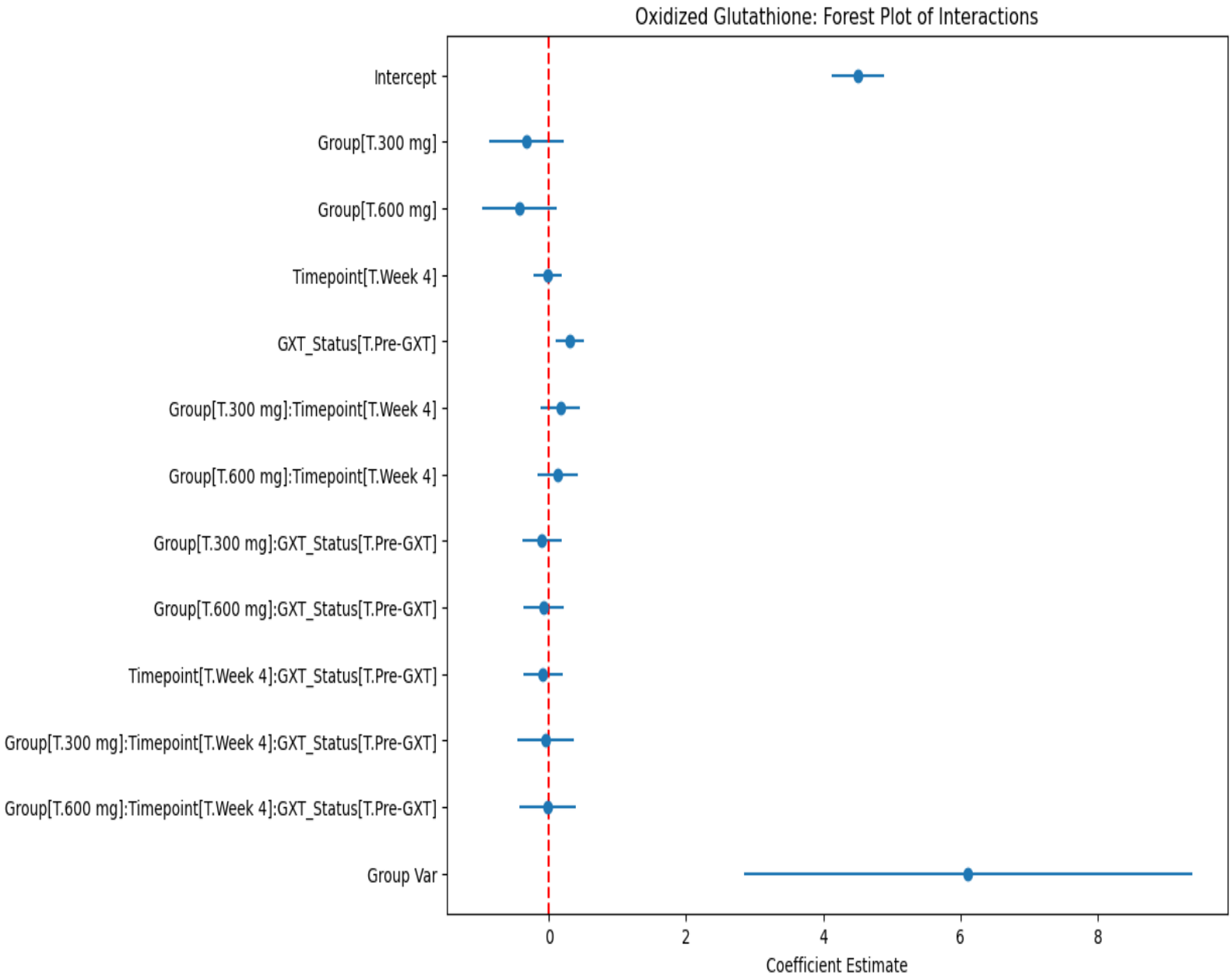

Figure 4.12. The Forest plot displays the interactions and their confidence intervals from the fitted linear mixed model for glutathione disulfide concentrations. This plot visualizes the coefficient estimates for each interaction along with their confidence intervals, providing a clear overview of the effects and their statistical significance.

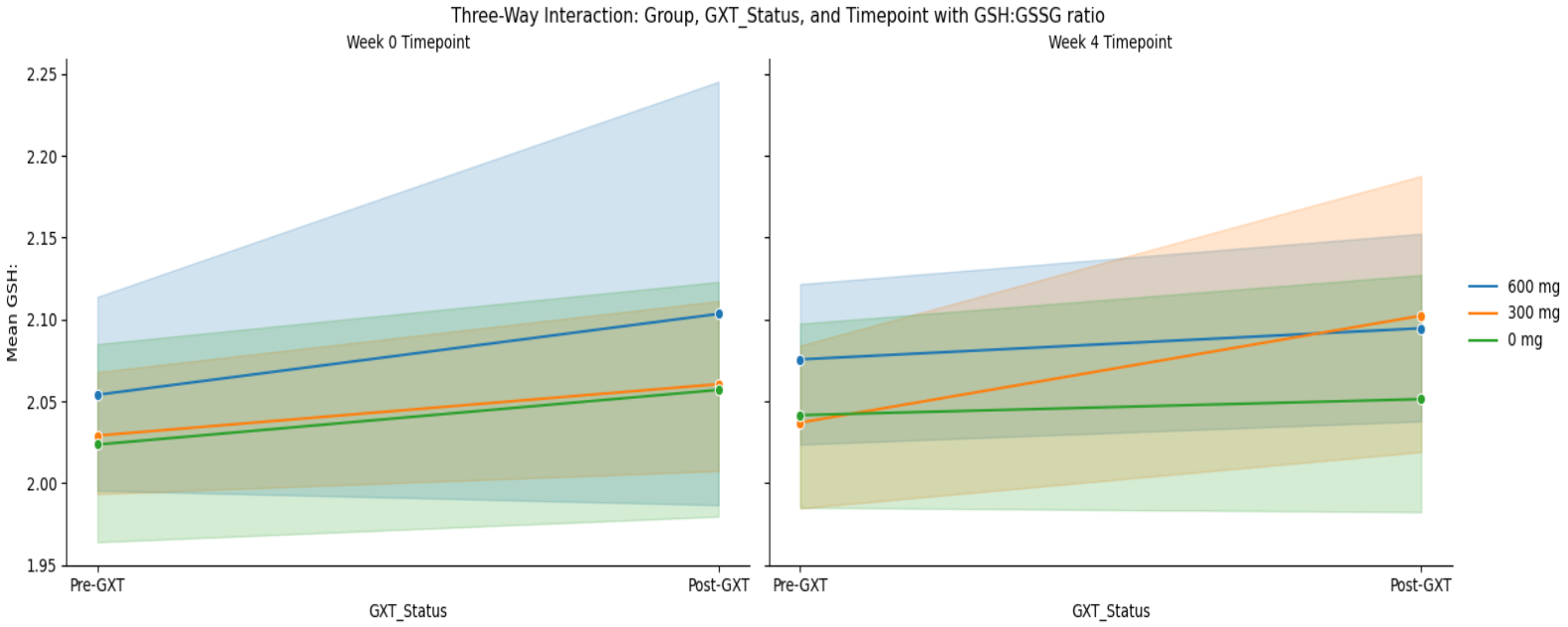

Figure 4.13. The three-way interaction plot provides insights into how supplementation dosage, timepoint, and graded exercise testing (GXT) interact to affect the ratio of reduced glutathione to oxidized glutathione (GSH:GSSG).

Facets (Timepoint): Each plot represents a different timepoint (Week 0 vs Week 4).

X-axis: Shows the difference between pre- and post-graded exercise test.

Y-axis: Indicates the average value at each graded exercise test for each group within the specific timepoint.

Lines: Each line within a facet represents a different group (placebo, 300 mg of Theracurmin® and 600 mg of Theracurmin®). The interaction effect within each timepoint is visualized by how these lines differ in slope and position.

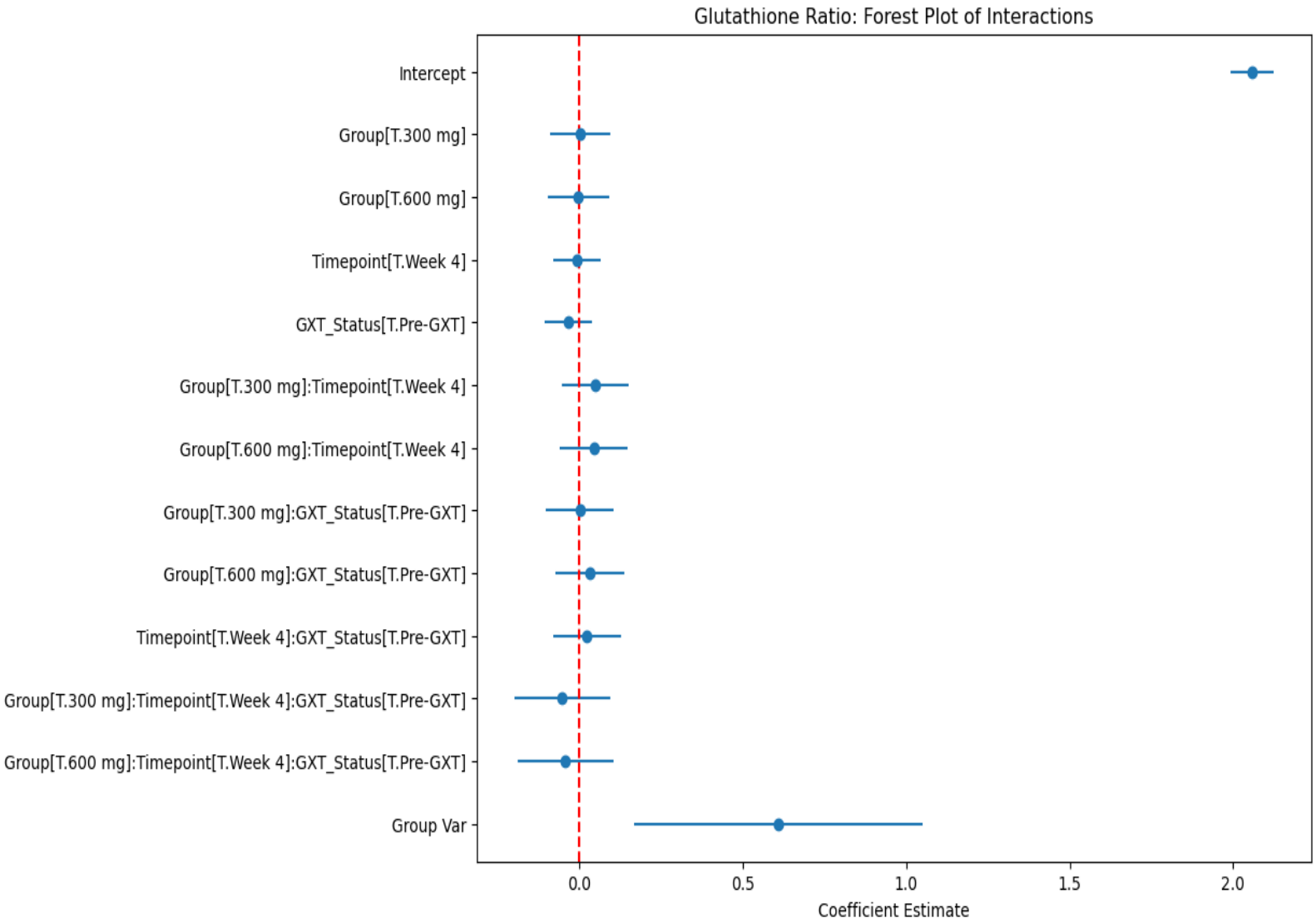

Figure 4.14. The Forest plot displays the interactions and their confidence intervals from the fitted linear mixed model for the ratio of reduced glutathione to oxidized glutathione (GSH:GSSG). This plot visualizes the coefficient estimates for each interaction along with their confidence intervals, providing a clear overview of the effects and their statistical significance.

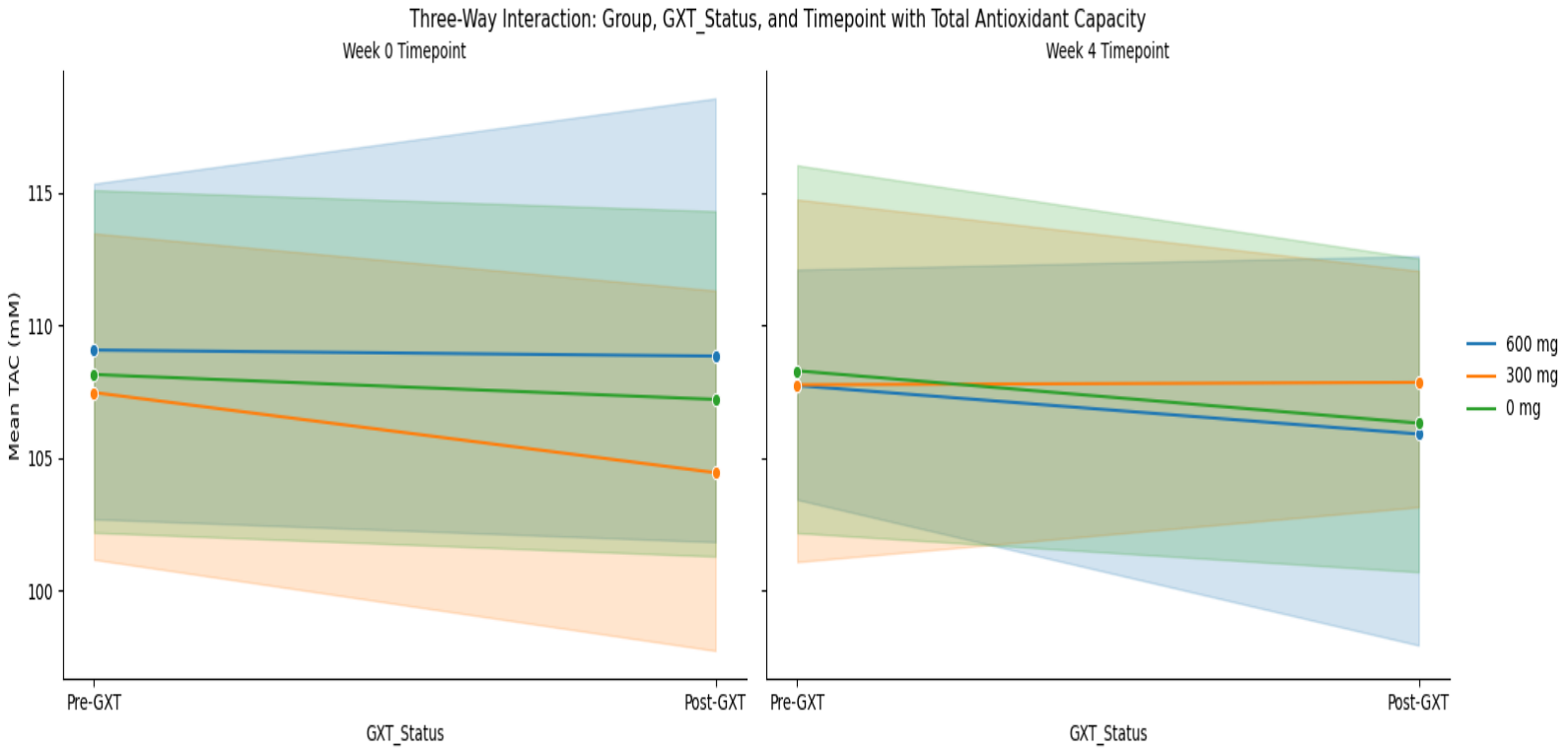

Figure 4.15. The three-way interaction plot provides insights into how supplementation dosage, timepoint, and graded exercise testing (GXT) interact to affect total antioxidant capacity.

Facets (Timepoint): Each plot represents a different timepoint (Week 0 vs Week 4).

X-axis: Shows the difference between pre- and post-graded exercise test.

Y-axis: Indicates the average value at each graded exercise test for each group within the specific timepoint.

Lines: Each line within a facet represents a different group (placebo, 300 mg of Theracurmin® and 600 mg of Theracurmin®). The interaction effect within each timepoint is visualized by how these lines differ in slope and position.

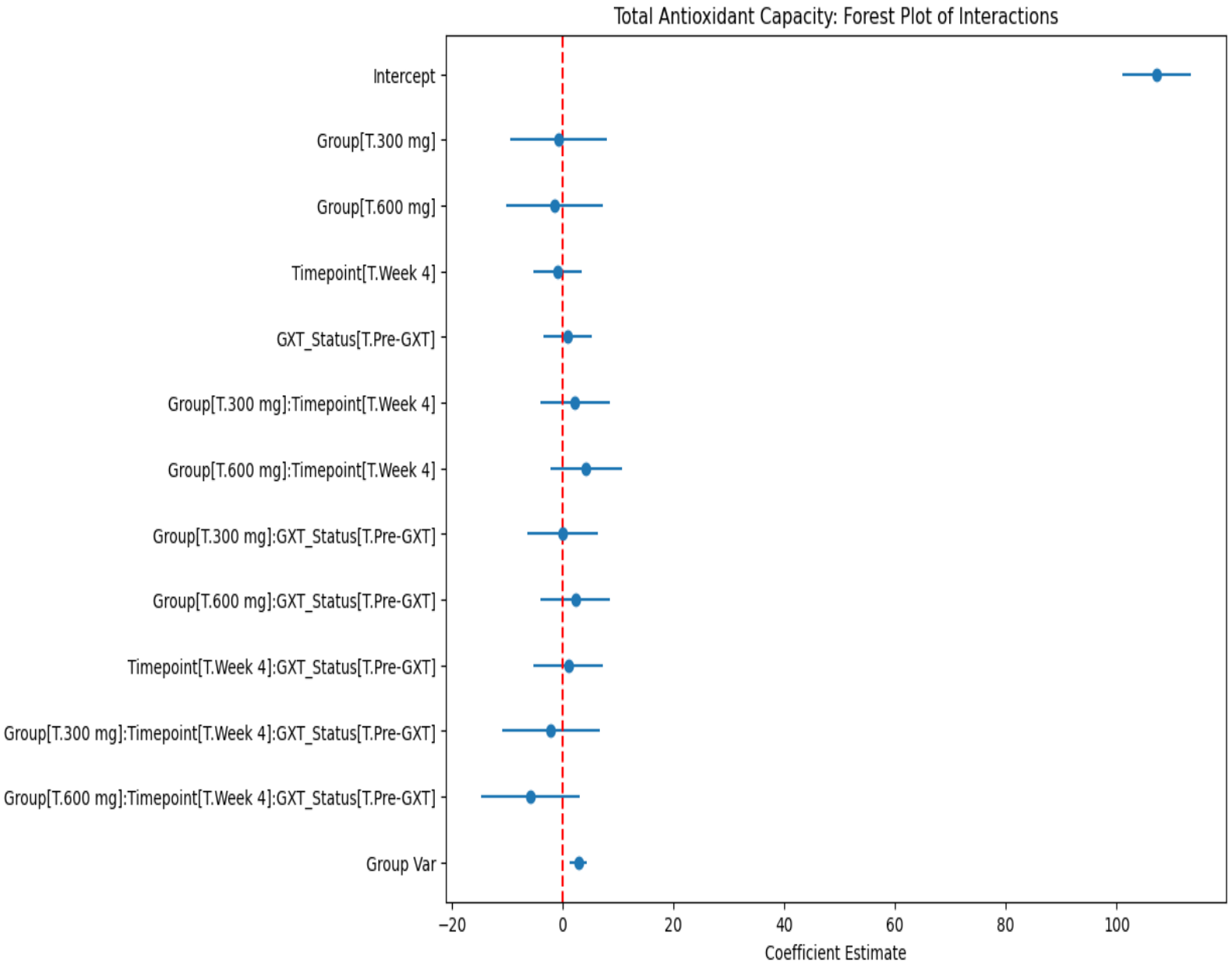

Figure 4.16. The Forest plot displays the interactions and their confidence intervals from the fitted linear mixed model for total antioxidant capacity. This plot visualizes the coefficient estimates for each interaction along with their confidence intervals, providing a clear overview of the effects and their statistical significance.

CHAPTER 5: MANUSCRIPT 2

To be submitted to: *International Journal of Sport Nutrition and Exercise Metabolism*

Effect of Graded Exercise Test and Curcumin Supplementation on Biomarkers of Inflammation and Muscle Damage in Active Individuals

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Abstract

Objective: To investigate a four-week daily oral supplementation regimen with 300 mg and 600 mg of Theracurmin® compared to a placebo on biomarkers of inflammation and muscle damage in physically active individuals, 18 to 45 years of age.

Methods: We used a randomized, double-blind, placebo-controlled study design in which participants underwent two incline-based maximal graded exercise tests (GXT) to exhaustion on a treadmill. The GXTs were separated by a four-week supplementation period. Blood samples were collected before and 20 minutes after the GXT protocol to assess the concentration of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP), and creatine kinase (CK) concentrations after a maximal GXT. Anthropometry, body composition, bone mineral density, and dietary intake were also assessed.

Results: There were no significant changes in IL-6, TNF-α, CRP, and CK concentrations following GXT at Week 0 and Week 4 across all groups. Analysis of covariance (ANCOVA) indicated no significant differences in inflammatory biomarkers among groups at Week 0 and Week 4. These findings suggest that Theracurmin® supplementation did not significantly affect biomarkers of inflammation and muscle damage in physically active individuals, 18 to 45 years of age.

Conclusion: We reported no statistically significant effects on biomarkers of inflammation and muscle damage following Theracurmin® supplementation in either intervention group following a GXT. Further research is needed to elucidate the underlying mechanisms of inflammation and the potential role of curcumin in modulating these physiological processes.

5.1 Introduction

Inflammation is a biological response elicited by the immune system in response to various stimuli, such as pathogens, cellular damage, and toxic substances.[155,156] It serves as a crucial component of the body's defense mechanisms, facilitating the identification and elimination of harmful foreign agents, while initiating the healing process. Inflammation can manifest as either acute or chronic. Acute inflammation arises from tissue damage caused by trauma, microbial invasion, or exposure to harmful substances.[155] On the other hand, chronic inflammation is characterized by a persistent, long-term inflammatory state lasting for extended periods ranging from months to years. The severity and consequences of chronic inflammation are contingent upon the underlying cause of injury and the body's capacity to repair and overcome the resulting damage.[155]

Chronic inflammation is a significant contributor to a variety of diseases affecting multiple organ systems, including the respiratory (such as asthma and chronic obstructive pulmonary disease), cardiovascular (such as atherosclerosis, myocardial infarction, and stroke), neurological (including dementia and Alzheimer’s disease), musculoskeletal (such as systematic lupus, rheumatoid arthritis, and psoriasis), gastrointestinal (including Crohn’s and Celiac diseases, ulcerative colitis), dental (such as Oral Lichen Planus), and endocrine (including diabetes mellitus and hypothyroidism) systems.[12,155,157] Additionally, chronic inflammation has been shown to

increase the risk of cancer.[19] Treatment of chronic inflammation with anti-inflammatory drugs has demonstrated efficacy in slowing disease progression in many of these organ systems.[19,158,159] However, the long-term use of traditional anti-inflammatory drugs is associated with serious side effects.[160,161] Furthermore, newer biological agents used for treatment are often prohibitively expensive.[162,163] As a result, individuals are increasingly turning to lifestyle modifications and naturopathic remedies as alternative approaches to managing chronic inflammation. In contrast, acute inflammation represents the organism's immediate reaction to noxious stimuli, typically enduring for a brief period ranging from days to weeks. Conversely, chronic inflammation persists over extended durations, spanning months to years. The progression from acute to chronic inflammation occurs when the body fails to eliminate the offending agent responsible for initiating the inflammatory response. Specifically, in cases where the organism is unable to expel an irritant, toxin, or pathogenic microorganism, the immune system maintains the inflammatory cascade.[155]

Exercise-induced inflammation is a well-documented phenomenon in the field of exercise physiology. It is widely recognized that intense exercise can lead to muscle damage and inflammation, with the extent of these effects being influenced by factors such as exercise mode, intensity, and duration.[127,164] Research has shown that exercise can trigger an inflammatory response in the body, characterized by an increase in inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor

necrosis factor-alpha (TNF-α).[19,165] While it was initially believed that these cytokines were solely expressed in immune cells, it is now understood that they are also produced in various tissues throughout the body. The regulation of inflammatory cytokines involves complex pathways involving molecules such as nuclear factor-kappa B (NF-κB), activator protein-1 (AP-1), and cyclooxygenase-2 (COX-2).[35,39] Muscle damage caused by exercise-induced free radicals can activate these pathways, leading to an up-regulation of inflammatory cytokine production.[127] This cascade of events can result in pain and performance deficits in muscle function. The understanding of these mechanisms is crucial for developing strategies to mitigate the negative effects of exercise-induced inflammation on overall health and well-being.

Researchers have provided compelling evidence indicating that turmeric (Curcuma longa rhizomes) exhibits potent anti-inflammatory properties across various inflammation models.[166] The principal bioactive polyphenolic-flavonoid present in turmeric, curcumin, has been identified as the main contributor to its anti-inflammatory effects. Researches have shown that in certain experimental conditions, curcumin demonstrates comparable anti-inflammatory efficacy to commonly used nonsteroidal anti-inflammatory drugs (NSAIDs), while mitigating many of the associated side effects such as gastrointestinal disturbances and cardiovascular complications.[167] The mechanistic basis of curcumin's anti-inflammatory activity is attributed to its modulation of multiple targets including transcription factors, growth regulators, and

cellular signaling molecules. Specifically, curcumin directly effects various inflammatory regulators by reducing NF-κB activation, inhibiting AP-1 binding to deoxyribonucleic acid (DNA), and suppressing the expression of COX-2 enzyme, all pivotal players in the inflammatory cascade.[39,95] Furthermore, several researchers have indicated that curcumin indirectly hampers these inflammatory mediators by scavenging free radicals.[81] Despite evidence supporting the anti-inflammatory properties of curcumin, there remains a scarcity of literature on its potential benefits in alleviating inflammation and enhancing recovery post-exercise-induced muscle damage.[165]

There are few human studies where the effect of varying dosages of curcumin ingestion over a four-week period on inflammatory markers induced by a maximal graded exercise test (GXT) is used to induce oxidative stress. As such, the primary aim of our research was to explore the anti-inflammatory potential of curcumin. We evaluated the effect of oral supplementation with 300 mg or 600 mg of Theracurmin® compared to a placebo over a four-week period on biomarkers of inflammation and muscle damage at baseline and when a state of acute inflammation was elicited by a maximal GXT in recreationally active female and male participants.

5.2 Materials and Methods

5.2.1 Ethical Approval

The research protocol outlined in our study was approved by the Institutional Review Board (IRB) of Virginia Polytechnic Institute and State University (Virginia Tech). Prior to participating, all individuals provided their verbal and written informed consent. The procedures adhered to the guidelines established in the most recent version of the Declaration of Helsinki and were conducted in compliance with the regulations set forth by the Virginia Tech IRB.

5.2.2 Study Design

We utilized a randomized, double-blind, placebo-controlled study design to investigate the effect of four weeks of Theracurmin® supplementation in modulating biomarkers associated with inflammation. Our study consisted of three scheduled visits to the laboratory: baseline (Week 0 [W0]), midpoint (Week 2 [W2]), and final (Week 4 [W4]). Each visit was spaced two weeks apart. Physiological measurements of participants were taken during the W0 and W4 visits. Lean body mass, percent body fat, and total body bone mineral density were measured at W0. The graded exercise test (GXT) protocol was administered at both W0 and W4. Blood samples were collected twice, before and after the GXT, during W0 and W4. At W2, we assessed body weight, monitored adherence to the supplementation regimen, and evaluated participants' self-reported general well-being (Figure 5.1).

5.2.3 Study Population

A total of 42 physically active female and male recreationally active adults, who met the criteria of engaging in recreational exercise at least three times a week, were recruited for our study. During the study period, participants were instructed to adhere to their usual dietary intake and exercise routine, while avoiding the consumption of any substances that could potentially affect the study outcomes.

5.2.4 Inclusion and Exclusion Criteria

To ensure the eligibility of participants in this study, stringent inclusion and exclusion criteria were employed. Inclusion criteria required individuals to be 18 to 45 years of age, actively engaged in regular physical activity (at least three days a week) for a minimum of 12 months, non-smokers, free from chronic illnesses, refraining from the intake of dietary supplementation that could affect study outcomes, and in overall good health with the ability to perform treadmill running. Conversely, individuals were excluded if they smoked, engaged in less than two days of exercise per week, had orthopedic restrictions, were outside the specified age range (under 18 or over 45 years), suffered from uncontrolled chronic ailments, exhibited systolic blood pressure exceeding 140 mmHg or diastolic blood pressure surpassing 90 mmHg, possessed a body mass index (BMI) exceeding 35 kg/m^2, or were pregnant.

5.2.5 Study Familiarization and Informed Consent

Before commencing the study protocol, all participants engaged in a Health Insurance Portability and Accountability Act (HIPAA)-compliant video call during which the detailed methodology utilized in our study was explained. In this session, all participants were apprised of the complexities of the study protocol, and any queries they had were addressed. Verbal and written informed consent were procured from all participants in the presence of a witness prior to the initiation of any data collection procedures.

5.2.6. Randomization, Determination of Dosing and Supplementation Protocol

Participants were randomly assigned into one of three groups: 1) a placebo group who received 50 mg of microcrystalline cellulose, 2) a group who received 300 mg of Theracurmin® (containing 90 mg of active curcuminoids), and 3) a group who received 600 mg of Theracurmin® (containing 180 mg of active curcuminoids). Each participant was instructed to consume two capsules daily with breakfast to enhance absorption, because research has shown that curcumin absorption is increased when consumed with dietary fat.[20] The allocation of participants into one of these three groups was determined through the utilization of a software-generated randomization plan, employing the randomization tool available at http://www.graphpad.com/quickcalcs/index.cfm.

5.2.7. Collection of Physiological Measures

Our study comprised of three visits in total, with each visit being two weeks apart (Figure 5.1). During Week 0 (W0) and Week 4 (W4), blood pressure, body weight, height, lean body mass, percent body fat, and total body bone mineral density were collected. Height, lean body mass, percent body fat, and total body bone mineral density were only measured at W0. All measurements (except lean body mass, percent body fat, and bone mineral density) were taken in duplicate for accuracy. Blood pressure was assessed using an automated blood pressure monitor (SunTech Tango M2 Blood Pressure Monitor, Morrisville, NC). Body weight and height were measured on a digital physician's scale (Detecto 439, Webb City, Missouri, USA) and stadiometer (SECA 777, GMBH & CO., Germany), respectively. Lean body mass, percent body fat, and total body bone mineral density were analyzed using dual x-ray absorptiometry (DXA) (iDXA, GE Healthcare, Madison, WI) by a certified DXA technician only at W0. At W2, we assessed body weight, monitored adherence to the supplementation regimen, and evaluated participants' self-reported general well-being (Figure 5.1).

5.2.8 Maximal oxygen consumption (VO_2max)

Maximal oxygen consumption (VO_2max) was measured at W0 and W4 using an incline-based graded exercise test (GXT). The VO_2max test was separated by four weeks of supplementation (placebo, 300 mg or 600 mg Theracurmin®). The GXT was conducted using a metabolic cart (Vyntx CPX, Vyaire Medical, Mettawa, IL) and treadmill (Trackmaster TMX428CP Treadmill, Full Vision, Kansas) to assess VO_2max and induce

acute oxidative stress by increasing physical exertion until the point of exhaustion. The exercise protocol consisted of three phases: warm-up, testing, and recovery. The warm-up phase involved walking at a speed of 3 mph for two minutes. Subsequently, participants transitioned to their self-selected running pace of either 5.5 or 7 mph during the testing phase. The exercise intensity was incrementally increased by raising the gradient by 1% every minute until volitional exhaustion was reached (modified Taylor Protocol)[24]. Although for ethical and safety reasons, volitional exhaustion was used to end each test, there are four ways to determine if VO_2max has been reached. They are as follows: 1) an increase in carbon dioxide expiration with a flattening of oxygen consumption with an increase in workload, 2) a flattening of oxygen consumption with an increase in workload (these first two are the most definitive ways to ascertain if someone reached their VO_2max), 3) a respiratory exchange ratio greater than 1.0, and 4) sheer exhaustion. All the aforementioned determinations of ascertaining VO_2max are incorporated in our laboratory; however, sheer exhaustion is often used as the first measure, due to ethical and safety reasons. Following the testing phase, participants walked on the treadmill at a pace of 2 mph for two minutes.

5.2.9 Blood Sample Collection

Blood samples were obtained at four distinct time points, comprising two collections during the baseline visit (W0) and two during the final visit (W4) to assess biomarkers of inflammation and muscle damage. A certified phlebotomist conducted venipuncture

to collect all blood samples. The first sample was obtained prior to the commencement of the GXT, while the second was drawn 20 minutes post-exercise. Similarly, blood samples were obtained both before and after 20 minutes of the GXT during the final visit. These blood samples were promptly assessed for hemoglobin and hematocrit concentrations to account for the change in plasma volume immediately after exercise.[116] Plasma and serum blood samples were centrifuged at 3,500 revolutions per minute (rpm) at 4°C for 15 minutes and stored at -80°C for analyses of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP), and creatine kinase (CK) concentrations.

5.2.10. Calculation of Change in Plasma Volume

Intense exercise leads to an acute change in plasma volume due to the hemoconcentration effect.[117] The Dill and Costill[24] equations with hemoglobin concentration (Hb) and hematocrit (Hct) measurements were used to calculate the change in plasma volume (ΔPV).[116,117]

$$\Delta PV = \frac{PV_{post} - PV_{pre}}{PV_{pre}} = \frac{Hb_{pre}\ X\ \left(1 - Hct_{post}\right)}{Hb_{post}\ X\ (1 - Hct_{pre})} - 1$$

Concentration of biomarkers measured in the plasma or serum after the GXT were corrected using the following equation.

$$PM_{post,c} = PM_{post,u}\ X\ (1 + \Delta PV)$$

where $PM_{post,c}$ and $PM_{post,u}$ indicate corrected and uncorrected serum or plasma biomarker after the GXT, respectively. Note that we adjusted for plasma volume changes for all biomarkers assessed.

5.2.11 Blood Sample Storage and Analyses

The blood samples from every blood draw (pre- and post-GXT in both W0 and W4) were collected and stored in a freezer at a temperature of -80°C until analyses. Prior to storage, the samples underwent centrifugation at a speed of 3,500 rpm and a temperature of 4°C for a duration of 15 minutes. Following centrifugation, the plasma and serum samples were aliquoted into individual cryovials (Corning Incorporated, Corning, NY, USA) and preserved at -80°C. Analyses of blood samples were conducted after all blood samples had been collected and the study was completed. To assess concentrations of biomarkers of inflammation and muscle damage (interleukin-6 [IL-6], tumor necrosis factor-alpha [TNF-α], C-reactive protein [CRP], and creatine kinase [CK] concentrations) commercially available enzyme-linked immunosorbent assay (ELISA) were purchased from ALPCO (Westlake, OH, USA) for IL-6 (kit# 04-BI-IL6) and CRP (kit# 30-9710S), R&D systems (Minneapolis, MN,USA) for TNF-α (kit #QK210), and RayBiotech (Norcross, GA, USA) for CK (kit# ELH-CKMB). A BioTek Synergy H1 Hybrid Multi-Mode Monochromator Fluorescence Microplate reader (Fisher Scientific, Hanover Place, IL) was used for analyses. These analyses included samples obtained at pre- and post-GXT in both W0 and W4. All blood analyses were conducted during the

same week, in duplicate, at the Virginia Tech Metabolism Core at the end of the study to minimize differences among ELISA kits.

5.2.12 Dietary and Physical Activity Records

To evaluate the overall dietary intake and physical activity, participants completed a 2015 Block Food Frequency Questionnaire (FFQ) and the 2015 Block Adult Physical Activity Questionnaire (PAQ), respectively.[140] Detailed verbal instructions were provided to all participants regarding the protocol for documenting their diet and physical activity. The Block FFQ and PAQ evaluate dietary and intake over the past year and habitual physical activity. Participants were instructed to maintain their typical dietary and physical activity routines during the four-week supplementation period.

5.2.13 Statistical Analyses

To evaluate the effects of four weeks of supplementation of Theracurmin® on biomarkers of inflammation and muscle damage, an Analysis of Covariance (ANCOVA) was utilized. The primary hypothesis tested was whether there would be a significant difference in the W4 biomarker and muscle damage concentrations among supplementation groups, after adjusting for sex and baseline biomarker concentrations.

A paired t-test was conducted to evaluate the effect of the GXT on biomarkers of inflammation and muscle damage obtained from blood samples before and after the GXT in all groups.

This statistical methodology was selected to account for potential confounding factors, particularly sex differences, that may affect the primary outcomes. The dependent variables in the analyses were the concentrations of inflammatory and muscle damage biomarkers measured at two time points prior to the administration of GXT: baseline (Week 0 [W0]) and post-intervention (Week 4 [W4]), while the independent variable was the supplementation group (placebo, 300 mg and 600 mg of Theracurmin®). The covariate included in the model was sex, incorporated to control for any sex-specific influences on the biomarker concentrations. The model was structured as follows:

$$Biomarker_{post} = \beta_0 + \beta_1(Group) + \beta_2(Sex) + \beta_3\,(Biomarker_{pre}) + \varepsilon$$

Where $Biomarker_{post}$ is the post-intervention concentration of the biomarker, group indicates dosage of Theracurmin® supplementation (placebo, 300 mg, 600 mg), sex is a binary variable indicating female or male, $Biomarker_{pre}$ is the baseline concentration of the biomarker, $\beta_0, \beta_1, \beta_2, \beta_3$ are the parameters estimated by the model and ε is the error term. All statistical procedures were conducted using IBM SPSS Statistics (V.27, SPSS Inc., Chicago, IL) software. Significance level was set *a priori* at $p<0.05$.

5.3 Results

5.3.1 Baseline characteristics

A cohort of 42 recreationally active individuals, consisting of 17 females and 25 males, was recruited for the study. Participants were randomly assigned to one of three groups: 1) a placebo group who received 50 mg of microcrystalline cellulose, 2) a group who received 300 mg of Theracurmin® (containing 90 mg of active curcuminoids), and 3) a group who received 600 mg of Theracurmin® (containing 180 mg of active curcuminoids) at the start of the study protocol, with each group comprised of 14 individuals. The baseline characteristics of all participants are detailed in Table 5.1. There were no significant differences observed in baseline characteristics among the three groups, with the exception of resting heart rate. There were expected differences between the female and male participants (Table 5.1). Dietary intake from the food frequency questionnaire are presented in Table 5.2. The distribution of concentrations of IL-6, TNF-α, CRP, and CK in the dataset are depicted in Figures 5.2, 5.3, 5.4, and 5.5, respectively.

5.3.2. The Effect of Theracurmin® Supplementation on Inflammation

An analysis of covariance (ANCOVA) was performed to assess the inter-group differences in the concentration of inflammatory biomarkers and muscle damage at W0 and W4 while controlling for sex as a covariate.

Interleukin-6 (IL-6)

The mean difference in serum IL-6 concentrations across the two timepoints for the placebo, 300 mg and 600 mg Theracurmin® groups were -4.41±13.35, 0.99±5.54, and -2.33±16.90 pg/mL, respectively. The negative values observed indicate a higher serum IL-6 concentrations at W0, while the positive values indicate higher serum IL-6 concentrations at W4. There were no significant differences among the groups in terms of the change in IL-6 concentrations across the two timepoints when adjusting for sex. The p-values for group and sex were non-significant (p=0.565 and p=0.821, respectively), indicating that neither group nor sex significantly influenced IL-6 concentrations across the two timepoints (Figure 5.7).

Tumor Necrosis Factor-alpha (TNF-α)

The difference in serum TNF-α concentrations between W0 and W4 for the placebo, 300 mg and 600 mg Theracurmin® groups were -223.95±1153.4, -382.2±509.6, and -381.25±1492.7 pg/mL, respectively. The negative values indicate higher serum TNF-α concentrations at W0, while positive values indicate higher serum TNF-α concentrations at W4. There were no significant differences among the groups in TNF-α concentrations across the two timepoints when adjusting for sex, as evidenced by non-significant p-values for both group (p=0.945) and sex (p=0.547) (Figure 5.9).

C-Reactive Protein (CRP)

The mean difference in serum CRP concentrations between W0 and W4 for the placebo, 300 mg and 600 mg Theracurmin® groups were -1.08±3.73, 0.1±5.01, and 1.28±4.23 μg/mL, respectively. The negative values indicate higher serum CRP concentrations at W0, while the positive values indicate higher serum CRP concentrations at W4. No significant differences were observed among the groups in terms of changes in CRP concentrations when adjusting for sex (p=0.449 for group and p=0.172 for sex). This suggests that neither group nor sex had a significant effect on CRP concentrations (Figure 5.11)

Creatine Kinase (CK)

We found no statistically significant differences in creatine kinase (CK) concentrations among the placebo, 300 mg and 600 mg Theracurmin® groups at two different timepoints. The mean differences in serum CK concentrations were -0.44±3.98, -1.17±4.0, and -1.15±7.73 ng/mL for the placebo, 300 mg and 600 mg Theracurmin® groups, respectively. When adjusting for sex, both group and sex did not show statistically significant differences in serum CK concentrations across the two timepoints (p=0.841 and p=0.174, respectively) (Figure 5.2).

5.3.3 The Effect of Graded Exercise Test on the Biomarkers of Inflammation

The main purpose of this study was to assess the effects of Theracurmin® supplementation on biomarkers of inflammation and muscle damage. We also evaluated changes in biomarkers of inflammation and muscle damage following the GXT.

Interleukin-6 (IL-6)

In the group receiving 600 mg of Theracurmin®, the serum IL-6 concentrations were 30.13±59.33 pg/mL pre-GXT and 8.89±8.97 pg/mL post-GXT. At W4, the mean serum IL-6 concentrations were 27.80±47.74 pg/mL pre-GXT and 8.78±6.21 pg/mL post-GXT. In the group receiving 300 mg of Theracurmin®, mean serum IL-6 concentrations at W0 were 12.66±16.64 pg/mL pre-GXT and 21.84±37.91 pg/mL post-GXT. At W4, mean serum IL-6 concentrations measurements were 13.64±15.56 pg/mL pre-GXT and 23.15±36.5 pg/mL post-GXT. For the placebo group, initial IL-6 concentrations were 39.98±80.19 pg/mL pre-GXT and 56.45±90.58 pg/mL post-GXT, and at W4 IL-6 concentrations for the placebo group were 35.6±67.9 pg/mL pre-GXT and 47.2±73.7 pg/mL post-GXT. There were no significant effects of the GXT on serum IL-6 concentrations in any of the groups at both timepoints, indicating that the GXT did not result in significant alterations in IL-6 concentrations among any of the groups (Figure 5.6).

Tumor Necrosis Factor-alpha (TNF-α)

The mean concentrations of tumor necrosis factor-alpha (TNF-α) for the placebo, 300 mg and 600 mg Theracurmin® groups pre-GXT were 3166.4±3412.4 pg/mL, 4214.29±3170.3 pg/mL, and 4752±3533.8 pg/mL, respectively. Subsequent to the GXT administration, the mean concentrations within these groups were 3064±3197.74 pg/mL, 4132±3250.6 pg/mL, and 4991.7±3648.3 pg/mL, respectively. There were no significant differences in serum TNF-α concentrations pre- and post-GXT at W0 ($p=0.374$, $p=0.570$, and $p=0.126$ for the placebo, 300 mg and 600 mg Theracurmin® groups, respectively). After four weeks of supplementation, the mean TNF-α concentrations for the placebo, 300 mg and 600 mg Theracurmin® groups before GXT were 2942.4±3354.23 pg/mL, 3832.2±3239.78 pg/mL, and 4370.7±3538.2 pg/mL, respectively. Post-GXT, serum TNF-α concentrations were: 3065.4±3333.7 pg/mL (placebo), 4094.9±3137.26 pg/mL (300 mg), and 4420.95±3434.24 pg/mL (600 mg). Upon analyses it was determined that there was no significant differences in serum TNF-α concentrations pre-GXT and post-GXT in the placebo group ($p=0.52$) and in the group receiving 600 mg of Theracurmin® ($p=0.72$). However, in the group receiving 300 mg of Theracurmin®, there was a significant difference in serum TNF-α concentrations pre-GXT and post-GXT ($p=0.015$) (Figure 5.8).

C-Reactive Protein (CRP)

In the 600 mg Theracurmin® group, C-reactive protein (CRP) concentrations were 10.25±5.16 μg/mL pre-GXT and 10.10±5.63 μg/mL post-GXT at W0. At W4, the mean serum CRP concentrations were 11.53±5.2 μg/mL pre-GXT and 11.74±5.9 μg/mL post-

GXT. In the 300 mg Theracurmin® group, the mean CRP concentrations at W0 were 12.33±6.57 μg/mL pre-GXT and 11.98±6.45 μg/mL post-GXT. At W4, CRP concentrations were 12.44±6.25 μg/mL pre-GXT and 12.85±7.14 μg/mL post-GXT. For participants in the placebo group, initial CRP concentrations were 13.14±5.87 μg/mL pre-GXT and 13.43±6.9 μg/mL post-GXT. W4 CRP concentrations for the placebo group were 12.06±5.44 μg/mL pre-GXT and 12.05±5.45 μg/mL post-GXT. There were no significant differences in CRP concentrations after the GXT. There were no significant effects of the GXT on CRP concentrations in any of the groups at both timepoints, suggesting that the GXT did not elicit significant changes in serum CRP concentrations among any of the groups (Figure 5.10).

Creatine Kinase (CK)

Mean serum creatine kinase (CK) concentrations in the 600 mg Theracurmin® group at W0 were 9.64±16.41 ng/mL prior to the GXT and 10.24±13.18 ng/mL post-GXT. At W4, the mean serum CK concentrations were 8.49±8.98 pg/mL pre-GXT and 10.86±16.25 ng/mL post-GXT in the 600 mg Theracurmin® group. In the 300 mg Theracurmin® group, the mean serum CK concentrations were 11.23±13.8 ng/mL pre-GXT and 11.33±11.5 ng/mL post-GXT at W0, and 10.1±11.58 ng/mL pre-GXT and 10.87±14.33 ng/mL post-GXT at W4. For the placebo group, CK concentrations were 9.64±16.41 ng/mL pre-GXT and 10.24±13.18 ng/mL post-GXT at W0, and 8.49±8.98 ng/mL pre-GXT and 10.86±16.25 ng/mL post-GXT at W4. The results from the paired t-test indicated that

there were no significant effects of the GXT on serum CK concentrations in any of the groups at both timepoints, suggesting that the GXT did not result in significant changes in serum CK concentrations among participants in all groups (Figure 5.12).

5.4 Discussion

The purpose of our randomized, double-blind, placebo-controlled study design was to evaluate the effect of daily oral supplementation with 300 mg or 600 mg of Theracurmin® versus a placebo, over a four-week period on biomarkers of inflammation and muscle damage in physically active individuals, 18 and 45 years of age. We also evaluated the effect of a GXT on these biomarkers. We found that neither Theracurmin® supplementation nor GXT had any significant effect on the concentrations of inflammatory biomarkers and muscle damage. These biomarkers were selected for their ability to provide insights into the inflammatory status and muscle damage in the body.

5.4.1 Bioavailability of Theracurmin®

Turmeric is well-known for its poor bioavailability and limited absorption due to the rapid metabolism of its active compound, curcumin. Curcumin is quickly metabolized through glucuronidation and sulfation processes, forming curcumin glucuronides and curcumin sulfates that are rapidly excreted from the body.[21,21,22] To address this issue and improve the efficacy of turmeric supplementation, we used Theracurmin®.

Theracurmin® is a novel form of curcumin that enhances bioavailability by reducing curcumin to nanoscale particles enclosed within micelles, providing a protective barrier. The decreased particle size of Theracurmin® improves absorption by increasing surface area, while micellar encapsulation prevents aggregation and premature metabolism. Furthermore, the reduced particle size of Theracurmin® enables better passage through the intestinal lining into the bloodstream, while micellar encapsulation inhibits interactions via conjugation with other molecules in the gut.[21,22] A number of researchers have illustrated the improved bioavailability of Theracurmin®. For example, Nakagawa et al.[23] showcased a 27-fold rise in the blood concentration-time curve of Theracurmin® in comparison to curcumin powder. Stohs et al.[142] evaluated the oral ingestion of Theracurmin® and reported significantly elevated serum concentrations of curcumin metabolites when compared with other forms of curcumin. However, due to the lack of testing for curcumin metabolites in the bloodstream in our study, the efficacy of Theracurmin® in enhancing the bioavailability of curcumin and facilitating its pharmacological actions could not be definitively determined.

5.4.2 Acute Inflammatory Response to Exercise

Regular exercise has been shown to provide numerous benefits across various physiological systems, including metabolic, cardiac, and psychological aspects.[168] Engaging in consistent physical training through aerobic or anaerobic exercises can enhance the body's resilience to inflammation and increase antioxidant capacity by

promoting both pro- and anti-inflammatory responses. However, it is important to acknowledge that exercise can also pose risks, particularly when taken to an excessive level.[5,168,169] Exercise acts as a stressor during and post-activity, leading to the potential induction of inflammation.[170] Following exercise, there is a rapid release of cytokines, which typically return to baseline concentrations within 5 to 24 hours post-exercise.[170]

The temporal cascade of cytokine release in response to exercise begins with an initial elevation in plasma IL-6 concentrations, followed by subsequent increases in IL-1Ra, IL-10, and soluble TNF-receptors (TNF-R). This pattern of pro-inflammatory cytokine release followed by anti-inflammatory cytokines mirrors that observed in sepsis and acute inflammatory states. Notably, unlike sepsis, moderate acute exercise does not induce a concurrent up-regulation of TNF-α.

Multiple pre-clinical studies have demonstrated the potential of curcumin in modulating key redox regulatory pathways, such as nuclear factor-kappa B (NF-κB) and Keap1-Nrf2-ARE (KELCH ECH associating protein 1-nuclear factor erythroid 2-related factor 2-antioxidant response element).[39,95] It is hypothesized that curcumin exerts its anti-inflammatory effects by directly interacting with these transcription factors, which are crucial in regulating the inflammatory response and oxidative stress by initiating the expression of various pro-inflammatory cytokines.[97] These cytokines subsequently trigger a series of biochemical reactions that drive the inflammatory response.

5.4.3 Effect of GXT and Theracurmin® Supplementation on Interleukin-6 Concentrations

Interleukin-6 (IL-6) is a multifunctional cytokine that plays a crucial role in immunoregulation, hematopoiesis, and inflammation. It has been extensively studied in the context of inflammation. Prolonged elevation of IL-6 has been shown to inhibit the expression of proteins involved in the mitochondrial electron transport chain, while enhancing the oxidative capacity of neutrophils, leading to increased production of reactive oxygen species (ROS). These ROS can cause damage to contractile protein filaments within myofibrils, ultimately impairing muscle function.[35,43] Therefore, it is essential to explore potential strategies for modulating IL-6 and inflammation in general.

Moreover, extensive research has been conducted within the scientific community on the response of IL-6 to exercise, which is considered a crucial cytokine in exercise physiology.[171,172] It has been observed that IL-6 concentrations significantly increase (up to 100-fold) in response to exercise, with a subsequent rapid decrease post-exercise.[171] The extent of IL-6 elevation is influenced by various factors including exercise intensity, duration, and individual exercise capacity.[168,173] De Gonzalo-Calvo et al.[174] investigated the effect of moderate and intense exercise on circulating concentrations of interleukin-6 (IL-6), interleukin-8 (IL-8), and interleukin-10 (IL-10). They observed a significant increase in all evaluated cytokines following exercise, with this elevation persisting for more than 24 hours before returning to baseline concentrations. Similarly, Wadley et

al.[148] examined the cytokine profile in response to both moderate and intense cycling. They reported a significant increase in IL-6 concentrations at 30 minutes post-exercise regardless of exercise intensity ($p<0.05$). In contrast, Brenner et al.[175] only observed a significant increase in IL-6 concentrations following moderate exercise during the recovery period ($p<0.05$). Overall, it is suggested that the increase in IL-6 and IL-8 concentrations is more pronounced with higher intensity compared to moderate intensity exercise.[170] While many researchers have reported an elevation in IL-6 concentrations following exercise, these findings are certainly not homogenous. Indeed, various researchers exploring the effect of exercise on IL-6 concentrations have reported no significant increase in IL-6 concentrations following either moderate-intensity or high-intensity exercise. This suggests that acute exercise may not have a substantial effect on IL-6 concentrations.[36,119,175,176]

Although there was an increase in mean IL-6 concentrations after the GXT in our study, this increase was not statistically significant. Moreover, the increase in IL-6 concentrations were not replicated in all the groups across both the timelines. The 600 mg Theracurmin® group had lower IL-6 concentrations after the GXT at both W0 and W4. Our study suggests that neither Theracurmin® supplementation nor a GXT affect IL-6 concentrations in recreationally active female and males, 18 to 45 years of age.

5.4.4 Effect of GXT and Theracurmin® Supplementation on Tumor Necrosis Factor-alpha Concentrations

Tumor necrosis factor-alpha (TNF-α) is another crucial cytokine involved in the inflammatory response. Along with interleukin-1β (IL-1β), TNF-α is considered a classic pro-inflammatory cytokine released in response to cellular damage.[36,173] *In vivo*, these cytokines stimulate a pro-inflammatory response by activating immune cells and increasing systemic prostaglandins. Moderate exercise does not typically lead to an increase in TNF-α concentrations, but prolonged or strenuous exercise has been shown to elevate TNF-α concentrations. For instance, after a marathon race, TNF-α concentrations have been reported to double.[36] In contrast, the anti-inflammatory cytokine IL-6 shows a 50-fold increase in concentrations following strenuous exercise.[36] These findings suggest that, while intense physical activity may induce an elevation of pro-inflammatory cytokines like TNF-α, the overall response is mitigated by the induction of anti-inflammatory cytokines, resulting in an anti-inflammatory effect.

The effect of various types of exercise on TNF-α concentrations were assessed by several researchers, including both moderate exercise interventions and intense exercise interventions. Cerqueira et al.[32] and Azizbeigi et al.[43] found no significant alterations in TNF-α concentrations in their studies using moderate exercise interventions. In contrast, among the intense exercise interventions, Brenner et al.[40] and Bernecker et al.[41] reported no changes in TNF-α concentrations post-exercise, while Ulven et al.[44] and Ostrowski et al.[45] reported an increase in TNF-α concentrations immediately after exercise.[177,178] All researchers who reported changes in TNF-α concentrations involved

exercise durations exceeding one hour. Furthermore, only Ostrowski et al.[45] included repetitive measurements in their study, and they reported a gradual decrease in TNF-α values over time, with concentrations not returning to baseline even after four hours.[178] In our study, neither four weeks of Theracurmin® supplementation nor a GXT had an effect on the serum concentrations of TNF-α. Our results suggest that a brief bout of high-intensity treadmill exercise may not be enough to produce significant changes in TNF-α concentrations in active individuals who are accustomed to regular physical training. Researchers who have shown significant changes in TNF-α concentrations typically involving exercise interventions lasting longer than the duration we used in our study. Moreover, four weeks of Theracurmin® supplementation did not lead to significant changes in TNF-α concentrations. This observation could potentially be attributed to the effective functioning of antioxidant defense mechanisms within the already active, healthy population in our study.

5.4.5. Effect of GXT and Theracurmin® Supplementation on C-reactive Protein Concentrations

Increases in C-reactive protein (CRP) concentrations have been observed post-exercise by some researchers. However, the exact relationship between increases in CRP concentrations and exercise intensity is unclear, and the results from studies are equivocal. For example, Draganidis et al.[46] reported a significant increase in CRP concentrations persisting for one to two days after exercise ($p<0.05$), before returning to

baseline. However, CRP concentrations significantly increase immediately following exercise. Conversely, Fatouros et al.[47] observed significant increases in CRP concentrations ($p<0.05$) immediately post-exercise.[179] Marklund et al.[48] reported a significant increase in CRP concentrations ($p<0.05$) 30 minutes after moderate exercise, with further significant increases in CRP concentrations 28 hours later ($p<0.05$). De Gonzalo-Calvo et al.[38] reported significant increases in CRP concentrations 24 hours post-exercise ($p<0.05$), without any alterations noted prior to that time point. Overall, several researchers have demonstrated an increase in CRP concentrations post-exercise, with peak concentrations typically observed at 24 hours post-exercise.[174]

Regarding exercise intensities, it has been reported that greater exercise intensities induced higher increases in CRP concentrations.[170] However, research on changes in CRP concentrations after moderate-intensity exercise are generally limited[144] We did not find significant differences in CRP concentrations following a GXT. This lack of significance may be attributed to the possibility that the GXT protocol utilized may not have provided a sufficient stimulus to induce inflammatory responses, or that the study participants were habituated to regular physical training. Furthermore, it is plausible that the timing of our blood sample collection, which occurred 20 minutes post-GXT, may have missed the peak serum CRP concentrations following exercise. Conversely, researchers who reported significant increases in CRP concentrations post-exercise delayed their blood draws by at least 30 minutes post-exercise.

To date, four meta-analyses have been published on the effect of curcumin on inflammatory markers.[180–183] Panahi et al.[180] observed a significant correlation between curcumin intake and CRP concentrations. Additionally, meta-analyses by Derosa et al.[50] and Sahebkar et al.[52] demonstrated reductions in CRP concentrations with curcumin compared to a control group. Nonetheless, Samadian et al.[53] and Wahono et al.[54] reported no significant differences in inflammatory markers with curcumin supplementation compared to a control group. Additionally, it is worth noting that the outcomes of meta-analyses supporting the anti-inflammatory effects of curcumin are primarily influenced by a study conducted by Belcaro et al. [184], which involved the combination of curcumin and soy phosphatidylcholine. Because phosphatidylcholine itself possesses anti-inflammatory properties, the results of their study do not conclusively establish the anti-inflammatory effects of curcumin in isolation. In our investigation, no significant disparities were observed in inflammatory markers among participants who received Theracurmin®. These results indicate that curcumin supplementation may not exert a substantial effect on inflammatory markers or muscle damage. However, the lack of discernible differences may be attributed to the observation that many of our participants maintained normal inflammatory concentrations throughout the study period. While some researchers have highlighted the effect of curcumin supplementation in mitigating inflammation, others have reported inconclusive findings regarding its efficacy in clinical cohorts.[154,166,167,180–182]

5.4.6. Effect of GXT and Theracurmin® Supplementation on Creatine Kinase Concentrations

Creatine kinase (CK) is generally well regarded as a marker that signifies muscle damage. Creatine kinase concentrations have been found to increase following intense and moderate exercises, while no significant changes were observed by some researchers.[164,174,179,185] Draganidis et al.[185] reported that CK concentrations peaked at 24 hours post-exercise in a moderate-intensity exercise group, and at 48 hours in a high-intensity exercise group. Conversely, De Gonzalo-Calvo et al.[174] found that CK concentrations peaked at 24 hours after intense exercise, and Marklund et al.[48] reported significant peak CK concentrations ($p<0.05$) at 28 hours after moderate exercise compared to baseline measures. With respect to curcumin supplementation, Kusnanik et al.[186] documented a significant reduction ($p<0.05$) in CK concentrations 24 hours post-vigorous physical activity in a curcumin supplementated group compared to a placebo, despite the absence of increased malondialdehyde concentrations. When comparing intense and moderate exercise, it has been noted that the increase in CK concentrations was greater following moderate exercise, although only two groups of researchers examined this intensity compared to four groups of researchers who researched intense exercise. In our study, neither four weeks of Theracurmin® supplementation nor a GXT had any significant effect on serum CK concentrations. The lack of a discernible effect on CK concentrations following the GXT is somewhat perplexing, considering previous

researchers have shown a significant increase in CK concentrations immediately after high-intensity exercise. It is possible that the relatively brief duration of the GXT did not induce substantial muscle damage. Additionally, the incline-based nature of the GXT protocol, as opposed to a decline test, which typically results in greater muscle damage due to prolonged eccentric contractions, may not have elicited enough damage in a population accustomed to running.

5.4.7. Conclusion

Our study represents a novel approach in investigating the efficacy of Theracurmin® supplementation on markers of inflammation in physically active individuals. It is the first study of its kind to utilize a four-week supplementation phase within a randomized, double-blind, placebo-controlled design. We also examined the dose-response relationship of curcumin supplementation by including two different dosages of Theracurmin® (300 mg and 600 mg) alongside a placebo group. Additionally, our research incorporated a maximal graded exercise test in conjunction with the supplementation protocol to assess the effect of Theracurmin® on inflammation and muscle damage. Importantly, we included both female and male participants, which is significant, because previous research in this area has predominantly focused on male participants. Moreover, we included the Dill and Costill[24] equations to appropriately account for variations in plasma volume post-exercise by measuring alterations in

hemoglobin and hematocrit concentrations. This practice, often overlooked in research studies, enhances the precision and dependability of our results.

However, our study had several limitations. One of these was the inclusion of a physically active healthy population, which may have resulted in a lower inflammatory response post-exercise. This limitation arises from the fact that the study participants were young and physically active, resulting in optimal concentrations of inflammation and a robust antioxidant defense system that may limit the potential for detecting changes in inflammation. Additionally, the GXT protocol we used may not have induced an increase in inflammatory markers and muscle damage, thereby limiting the ability to assess any potential effects of Theracurmin® on exercise-induced inflammation and muscle damage. This lack of response may be attributed to insufficient time spent at high intensities during the GXT protocol. Furthermore, we only included one blood draw following the GXT, potentially missing peak concentrations of certain inflammatory markers that may have occurred after the initial hour post-GXT. Additionally, a limitation of our study was the absence of measurements for curcumin metabolites in blood samples, making it difficult to determine if the supplementation protocol resulted in adequate absorption of curcumin in serum for potential beneficial effects.

In conclusion, the novel discovery of the current investigation indicates that daily oral supplementation with 300 mg or 600 mg of Theracurmin® over a period of four weeks

did not yield statistically significant alterations in serum concentrations of IL-6, TNF-α, CRP, and CK in physically active female and male participants. Additionally, the incline-based graded exercise test did not trigger an inflammatory response or elicit substantial muscle damage. Subsequent research endeavors should aim to meticulously investigate the intricate relationship between oral curcumin dosage, blood inflammatory cytokine concentration, blood curcumin metabolites, and various exercise modalities. Furthermore, future studies should incorporate the assessment of localized and intracellular inflammation within various sites such as the skeletal muscle tissue.

5.5 References for Manuscript 2

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Table 5.1. Baseline Characteristics of All Participants

	Total	Group			Sex	
Variable	**n=42**	**Placebo (n=14)**	**300 mg (n=14)**	**600 mg (n=14)**	**Females (n=17)**	**Males (n=25)**
Age, years	30 ± 8	30 ± 7	32 ± 7	29 ± 8	31.1 ± 8	30 ± 8
Weight, kg	74.3 ± 13.9	70.1 ± 12.2	73.3 ± 12.9	80.7 ± 14.8	64.5 ± 8.9	81.7 ± 12.1*
Height, cm	171.7 ± 8.9	168.7 ± 8.6	171.4 ± 7.6	176.0 ± 8.9	164.2 ± 5.3	177.4 ± 6.2*
BMI, kg/m^2	25.0 ± 3.0	24.4 ± 2.2	24.8 ± 2.7	26.0 ± 3.8	23.9 ± 2.9	25.9 ± 2.8*
SBP, mmHg	126 ± 10	128 ± 12	128 ± 6	123 ± 11	121 ± 9	130 ± 10*
DBP, mmHg	77 ± 9	80 ± 9	79 ± 10	72 ± 7	77 ± 8	77 ± 10
HR, bpm	63 ± 11	67 ± 9	63 ± 14	57 ± 10*	68 ± 12	60 ± 10**
Body Fat, %	24.7 ± 8.4	25 ± 7.4	25 ± 8.9	23.4 ± 9.3	30.5 ± 6.7	20.4 ± 6.9**
FFM, kg	56.8 ± 12.7	53.3 ± 12.9	55.9 ± 12.0	62.3 ± 11.8	45.3 ± 6.7	65.3 ± 8.4*
SAT mass, kg	0.83 ± 0.57	0.73 ± 0.42	0.90 ± 0.56	0.88 ± 0.75	0.86 ± 0.48	0.82 ± 0.65
VAT mass, kg	0.33 ± 0.35	0.28 ± 0.20	0.37 ± 0.33	0.33 ± 0.50	0.16 ± 0.16	0.44 ± 0.41*
RMR, kcal/day	1,531 ± 252	1,468 ± 264	1,512 ± 237	1,638 ± 231	1,303 ± 132	1,700 ± 168*
BMD, g/cm^2	1.28 ± 0.15	1.25 ± 0.10	1.26 ± 0.16	1.34 ± 0.14	1.21 ± 0.11	1.33 ± 0.14*

Data presented as mean ± standard deviation

kg, kilograms; cm, centimeters; BMI, body mass index; m^2, meters squared; SBP, systolic blood pressure; mmHg, millimeters of mercury; DBP, diastolic blood pressure; HR, heart rate; bpm, beats per minute; %, percent; FFM, fat free mass; SAT mass, subcutaneous adipose tissue mass; VAT mass, visceral adipose tissue mass; RMR, resting metabolic rate; kcal, kilocalories; BMD, bone mineral density; g, grams; cm^2, centimeters squared

* Males significantly greater than females ($p<0.05$)

** Males significantly lower than females ($p<0.05$)

Table 5.2. Habitual Dietary Intake of Participants Obtained from Food Frequency Questionnaire at Baseline

	Total	Group		
Variable	**n=39**	**Placebo (n=14)**	**300 mg (n=13)**	**600 mg (n=12)**
Energy Intake, kcal/day	1,962 ± 697	2,067 ± 655	1,852 ± 505	2,002 ± 929
Fat, g/day	82.3 ± 29.5	88.2 ± 26.0	79.6 ± 29.3	78.5 ± 34.7
Saturated Fat, g/day	25.2 ± 10.6	26.5 ± 9.0	26.0 ± 11.7	22.5 ± 11.3
MUFA, g/day	31.1 ± 11.7	33.7 ± 10.2	29.6 ± 12.6	29.8 ± 12.6
PUFA, g/day	19.3 ± 8.1	20.7 ± 8.6	17.7 ± 4.9	19.8 ± 10.5
Protein, g/day	80.3 ± 33.6	88.7 ± 30.5	70.0 ± 18.3	84.3 ± 45.0
Carbohydrates, g/day	224.2 ± 94.5	227.3 ± 93.8	211.7 ± 73.3	242.1 ± 118.6
Cholesterol, mg/day	299.3 ± 196.5	350.3 ± 208.0	264.8 ± 157.0	298.8 ± 225.3
Dietary Fiber, g/day	23.2 ± 12.5	23.8 ± 12.7	22.5 ± 12.8	23.9 ± 13.0
Vitamin A, RAE/day	1,132 ± 594	1,106 ± 406	1,108 ± 583	1,178 ± 806
Beta-carotene, mg/day	5,573 ± 4,499	4,620 ± 2,632	5,796 ± 4,865	6,5423 ± 5,804
Vitamin C, mg/day	110.8 ± 74.9	108.1 ± 63.2	107.6 ± 88.1	124.7 ± 77.1
Vitamin E, mg/day	11.6 ± 5.7	12.0 ± 5.3	10.5 ± 4.5	12.5 ± 7.4
Iron, mg/day	15.5 ± 6.9	15.8 ± 6.2	14.3 ± 5.4	16.8 ± 9.1
Zinc, mg/day	12.9 ± 6.1	12.9 ± 4.3	12.6 ± 8.0	13.2 ± 5.8
Sodium, mg/day	3,456.7 ± 1363.5	3,577 ± 1292	3,166 ± 1003	3,829 ± 1850

Data presented as mean ± standard deviation

kcal, kilocalories; g, grams; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; mg, milligrams; RAE, retinol activity equivalents

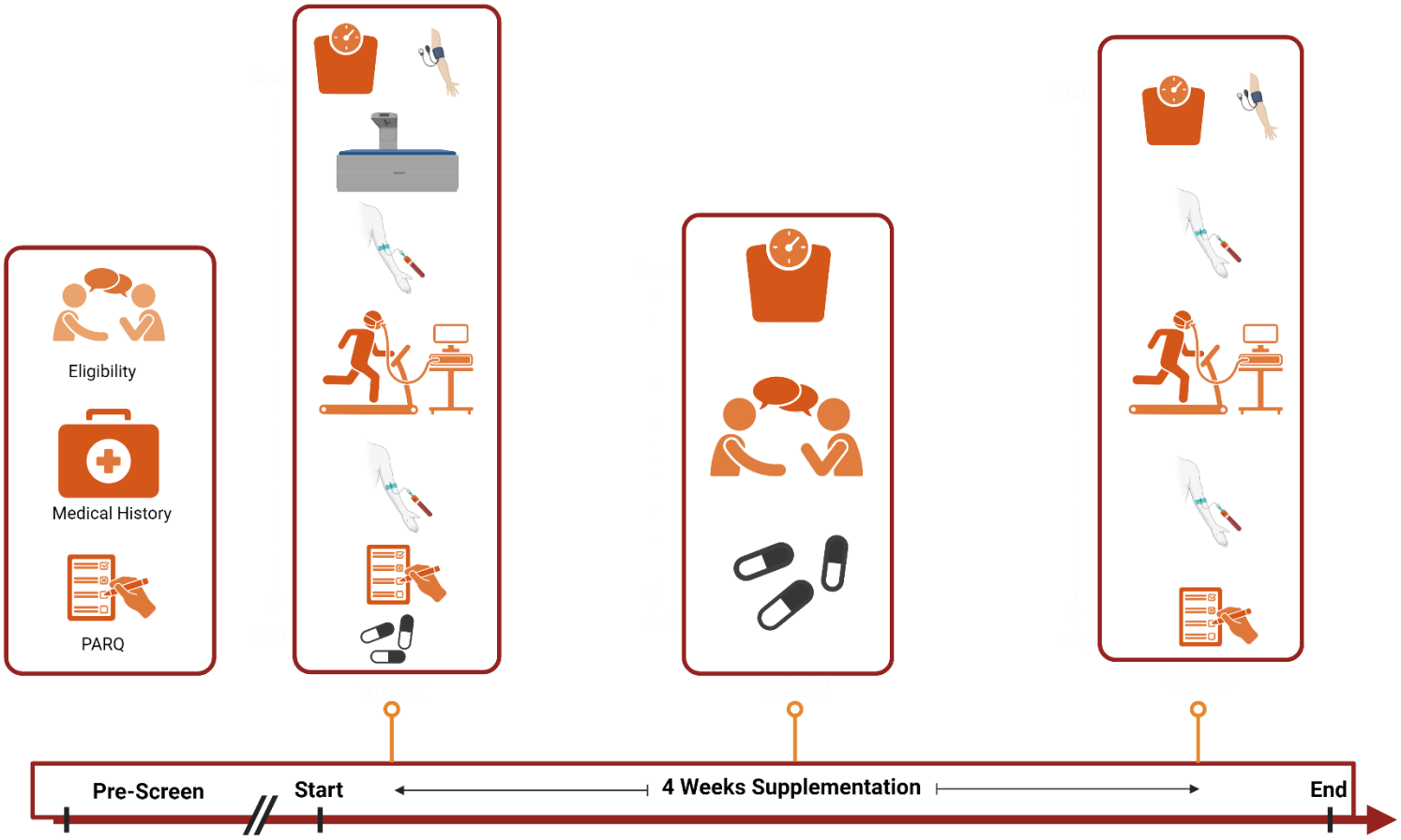

Figure 5.1. Diagram of the protocol employed in the study.

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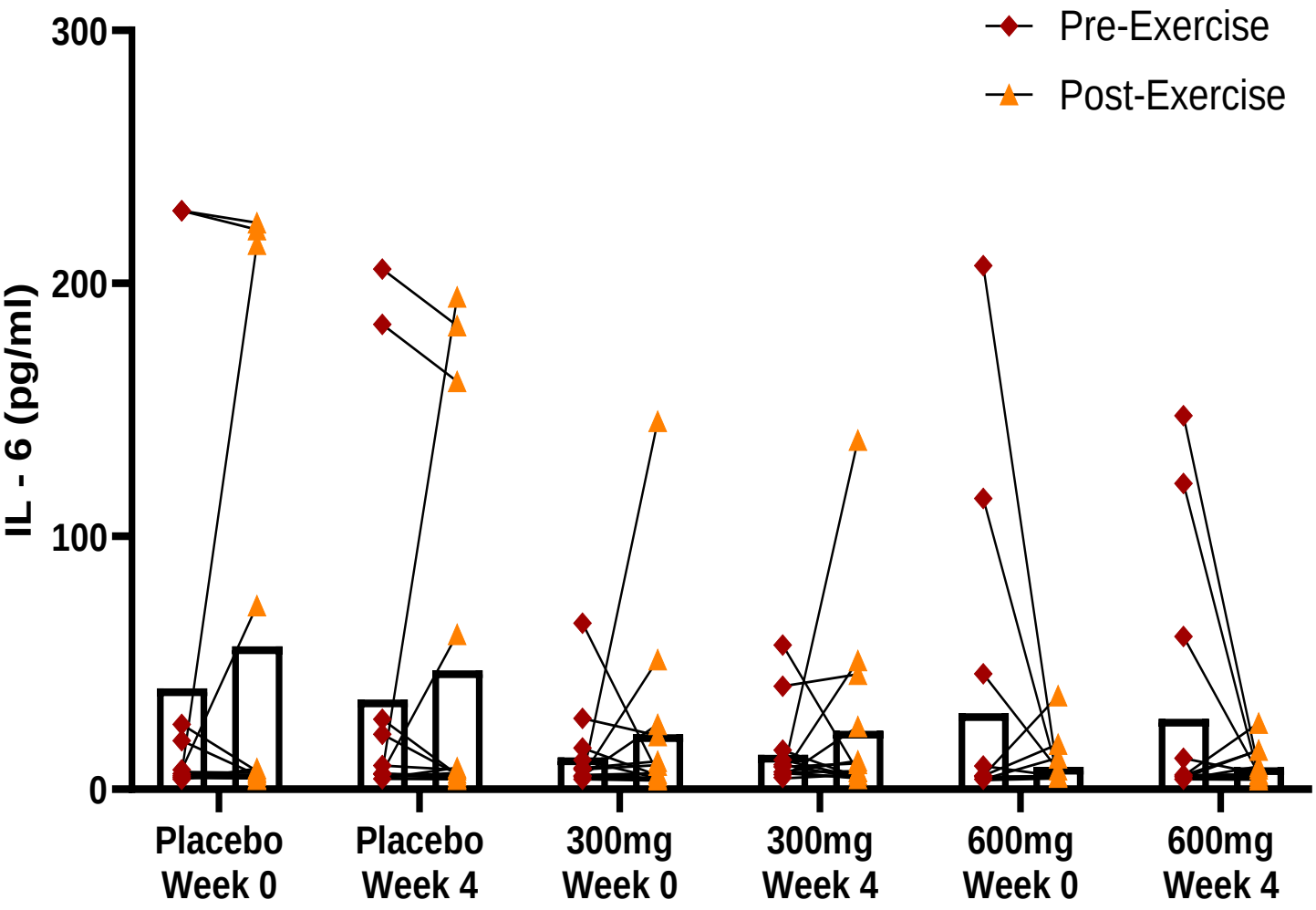


Figure 5.2. Serum interleukin-6 concentrations in placebo, 300 mg and 600 mg Theracurmin® groups during pre- and post-graded exercise testing at Week 0 and Week 4.

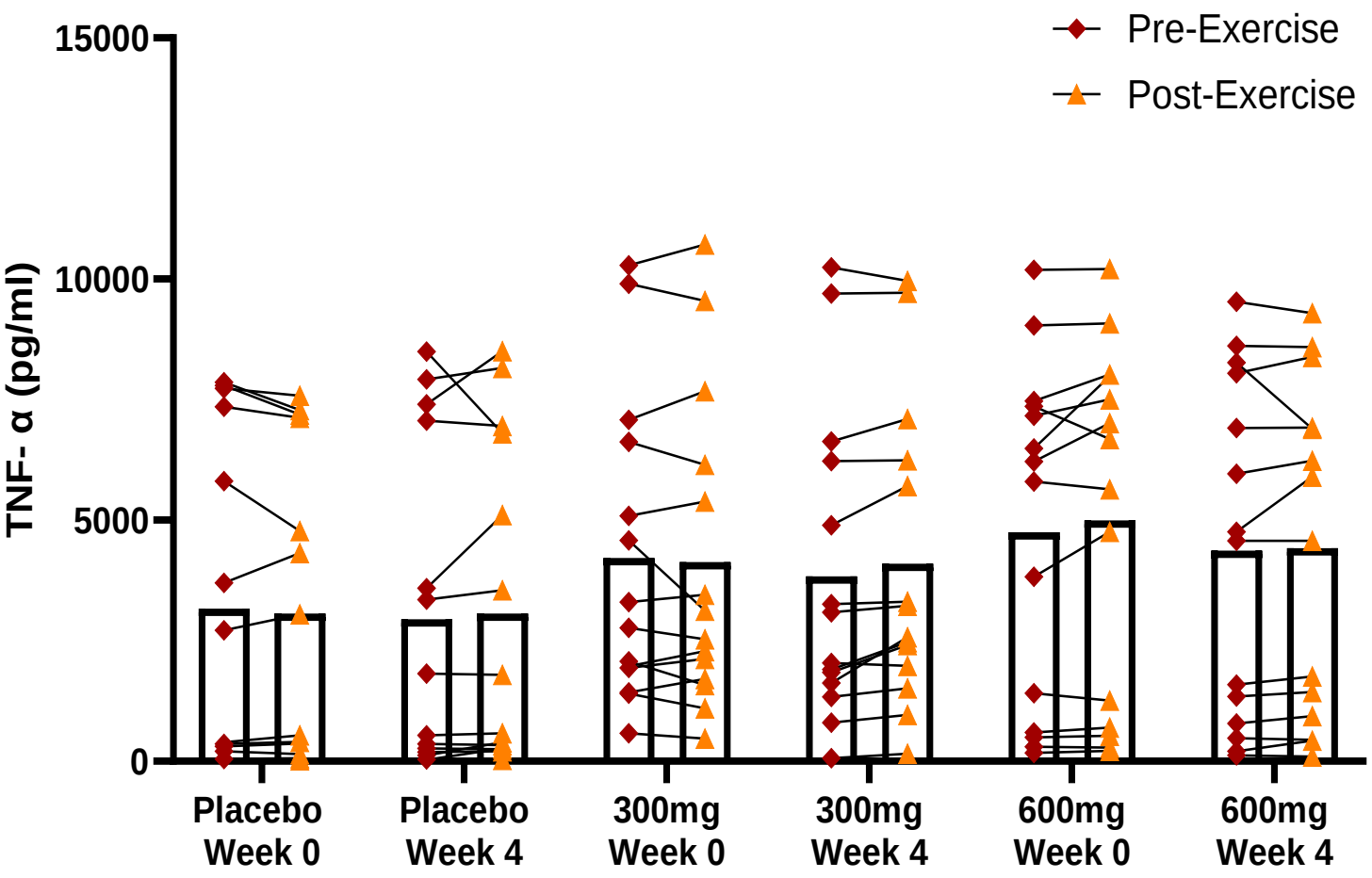


Figure 5.3. Serum tumor necrosis factor-alpha concentrations in placebo, 300 mg and 600 mg Theracurmin® groups during pre- and post-graded exercise testing at Week 0 and Week 4.

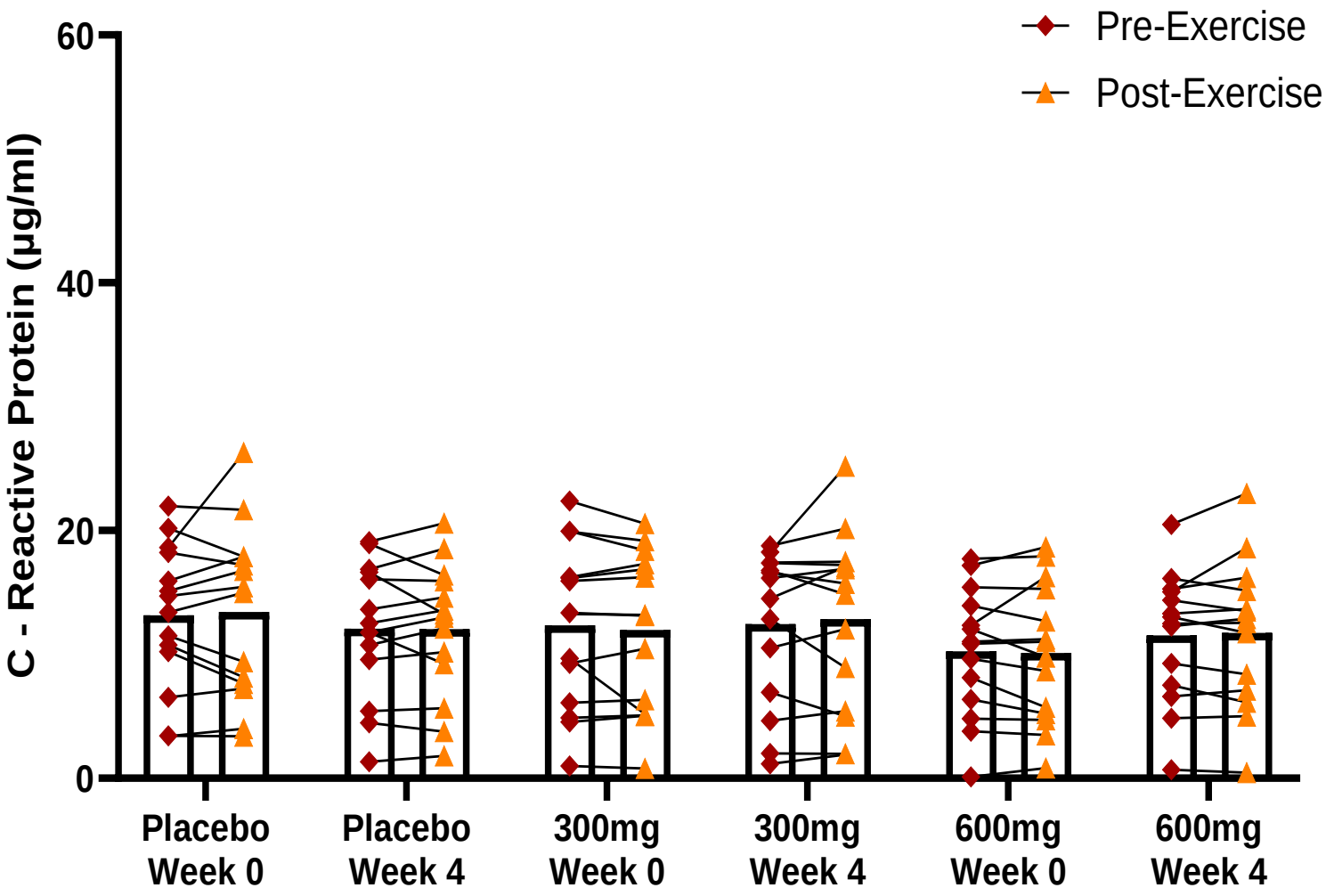


Figure 5.4. Serum C-reactive protein concentrations in placebo, 300 mg and 600 mg Theracurmin® groups during pre- and post-graded exercise testing at Week 0 and Week 4.

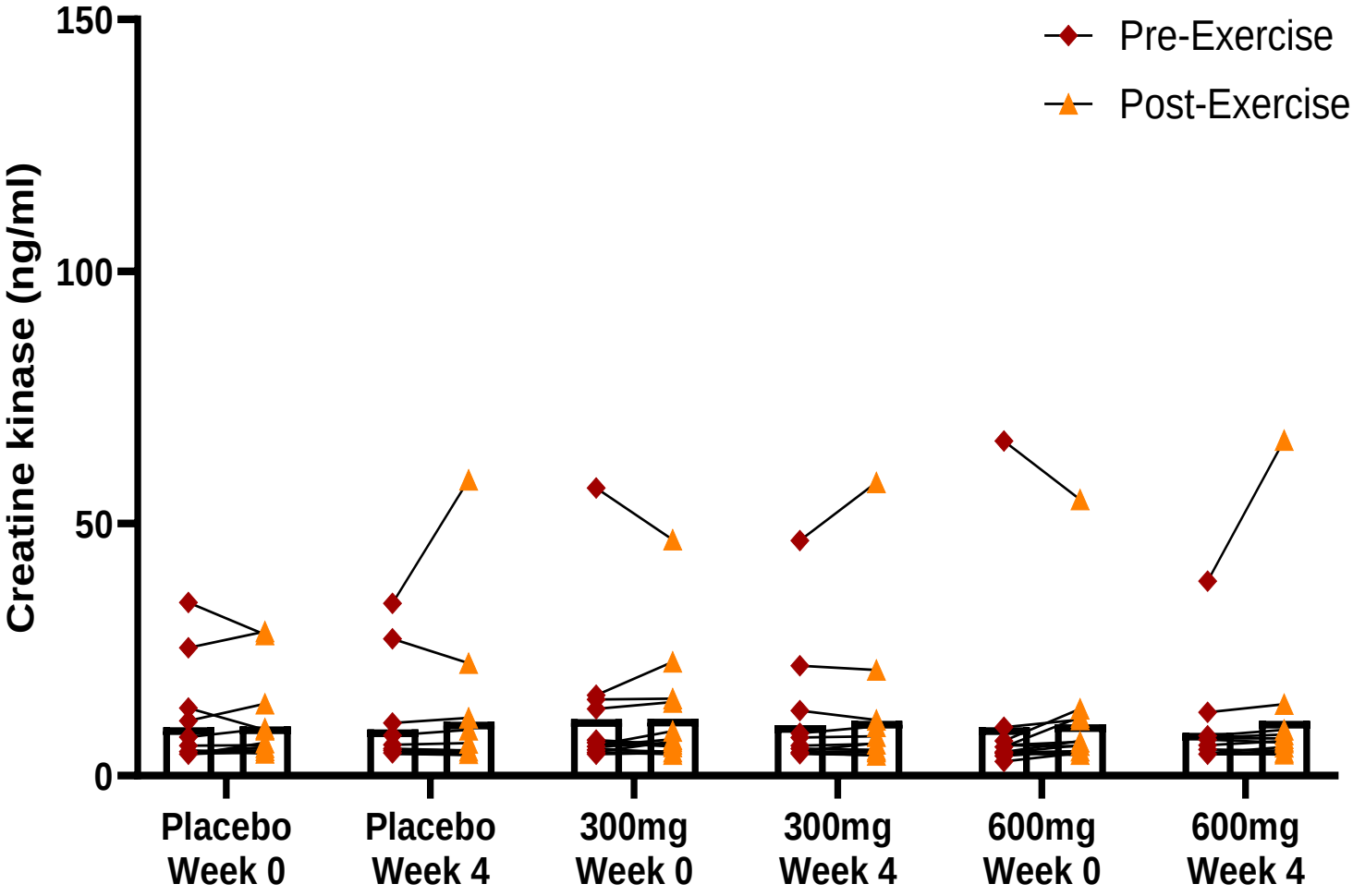


Figure 5.5. Serum creatine kinase concentrations in placebo, 300 mg and 600 mg Theracurmin® groups during pre- and post-graded exercise testing at Week 0 and Week 4.

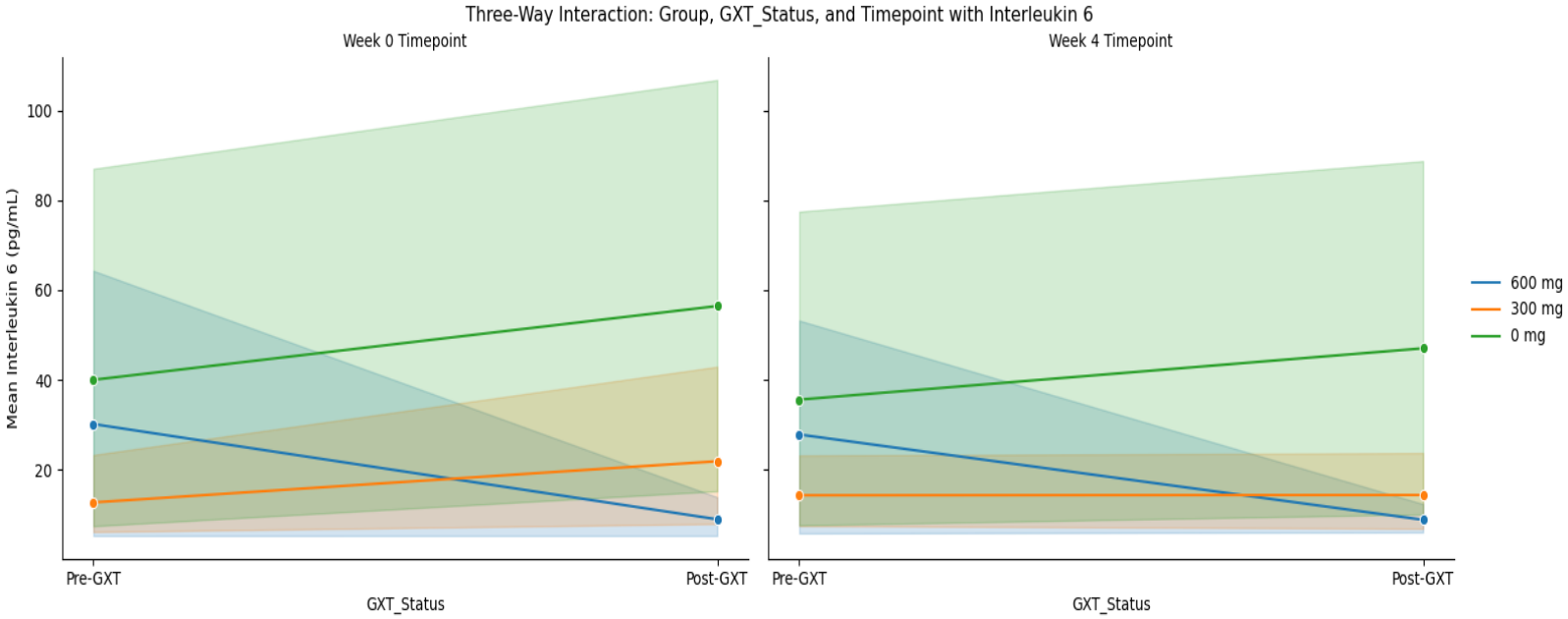


Figure 5.6. The three-way interaction plot provides insights into how supplementation dosage, timepoint, and graded exercise testing (GXT) interact to affect interleukin-6 concentrations.

Facets (Timepoint): Each plot represents a different timepoint (Week 0 vs Week 4).

X-axis: Shows the difference between pre- and post-graded exercise test.

Y-axis: Indicates the average value at each graded exercise test for each group within the specific timepoint.

Lines: Each line within a facet represents a different group (placebo, 300 mg of Theracurmin® and 600 mg of Theracurmin®). The interaction effect within each timepoint is visualized by how these lines differ in slope and position.

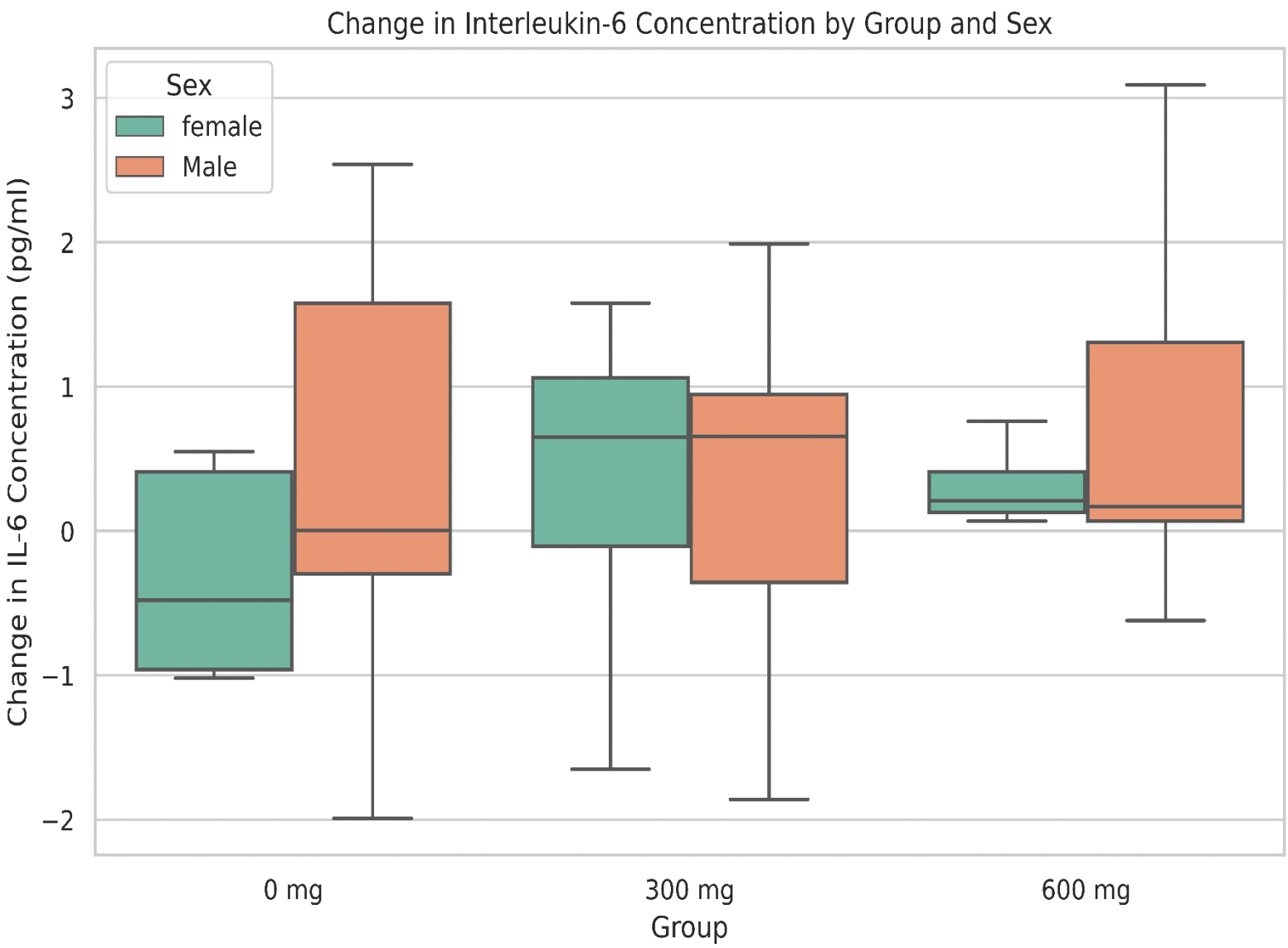


Figure 5.7. This box plot illustrates the changes in interleukin-6 (IL-6) concentrations across three different groups (placebo [0 mg], 300 mg and 600 mg of Theracurmin®) after four weeks of supplementation, with the data segregated by sex. The y-axis represents the change in IL-6 concentrations in picograms per milliliter (pg/mL), calculated as the difference between the post-intervention (week 4) and baseline (week 0) concentrations. Each box represents the interquartile range (IQR) of the change in IL-6 concentration, with the median change indicated by the line within the box. The whiskers extend to the furthest points that are considered not to be outliers.

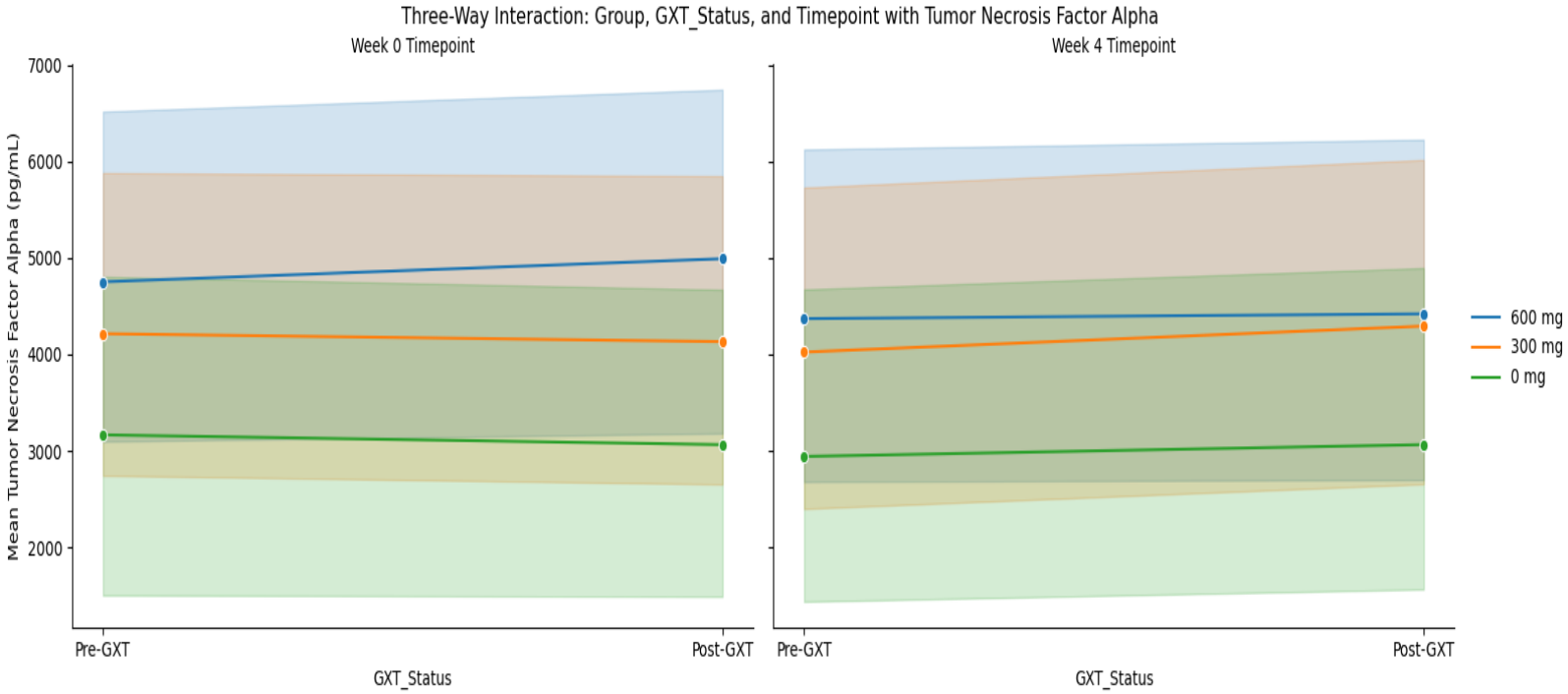


Figure 5.8. The three-way interaction plot provides insights into how supplementation dosage, timepoint, and graded exercise testing (GXT) interact to affect tumor necrosis factor-alpha concentrations.

Facets (Timepoint): Each plot represents a different timepoint (Week 0 vs Week 4).

X-axis: Shows the difference between pre- and post-graded exercise test.

Y-axis: Indicates the average value at each graded exercise test for each group within the specific timepoint.

Lines: Each line within a facet represents a different group (placebo, 300 mg of Theracurmin® and 600 mg of Theracurmin®). The interaction effect within each timepoint is visualized by how these lines differ in slope and position.

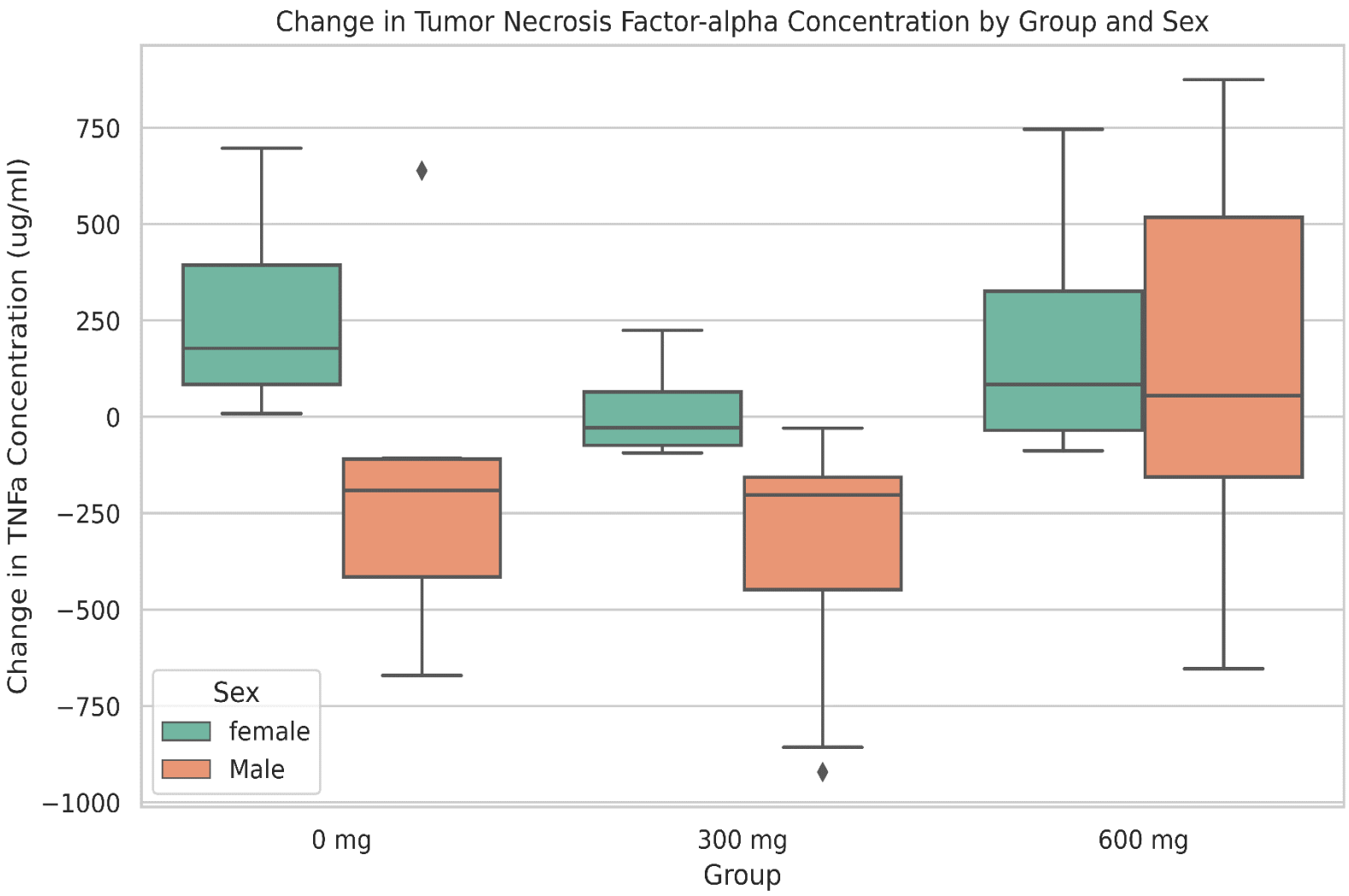


Figure 5.9. This box plot illustrates the changes in tumor necrosis factor-alpha (TNF-α) concentrations across three different groups (placebo [0 mg], 300 mg and 600 mg of Theracurmin®) after four weeks of supplementation, with the data segregated by sex. The y-axis represents the change in TNF-α concentrations in micrograms per milliliter (µg/mL), calculated as the difference between the post-intervention (week 4) and baseline (week 0) concentrations. Each box represents the interquartile range (IQR) of the change in TNF-α concentration, with the median change indicated by the line within the box. The whiskers extend to the furthest points that are considered not to be outliers.

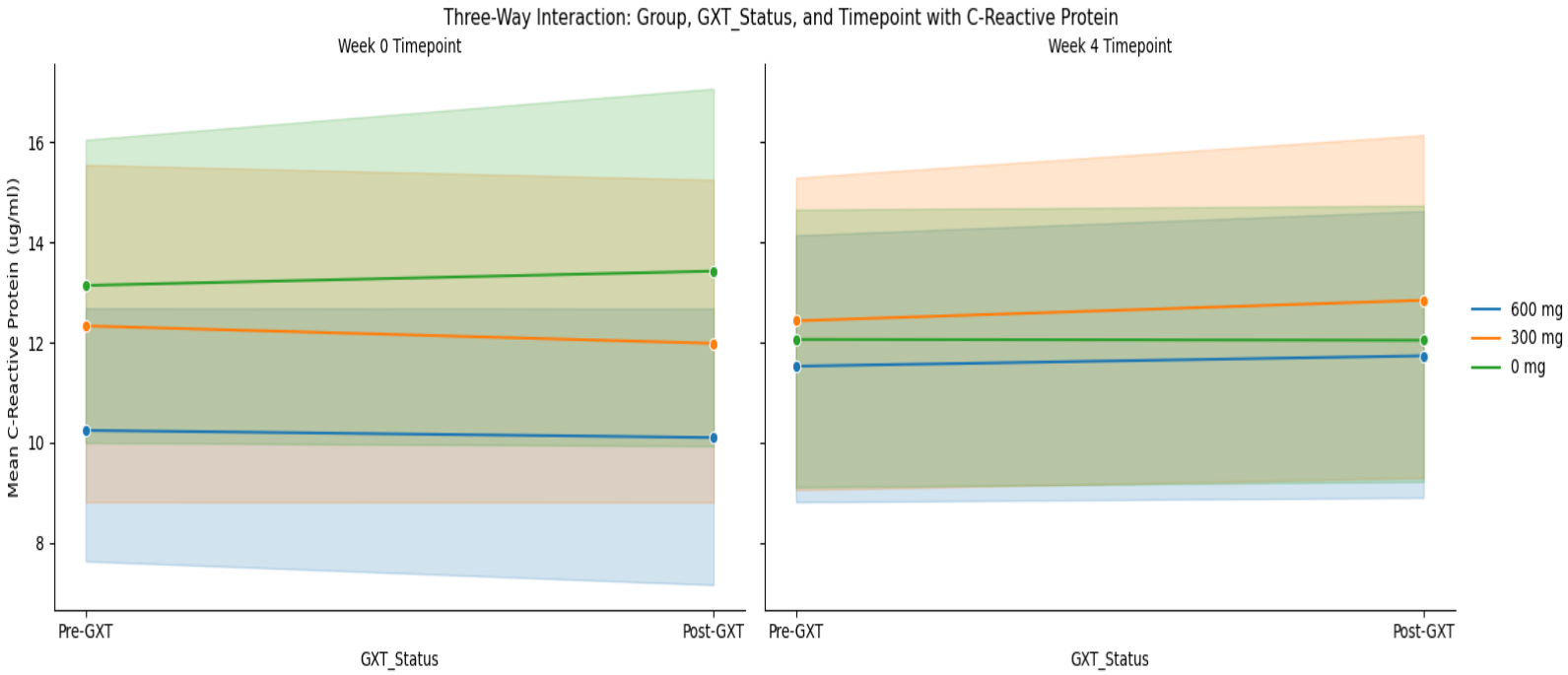


Figure 5.10. The three-way interaction plot provides insights into how supplementation dosage, timepoint, and graded exercise testing (GXT) interact to affect C-reactive protein concentrations.

Facets (Timepoint): Each plot represents a different timepoint (Week 0 vs Week 4).

X-axis: Shows the difference between pre- and post-graded exercise test.

Y-axis: Indicates the average value at each graded exercise test for each group within the specific timepoint.

Lines: Each line within a facet represents a different group (placebo, 300 mg of Theracurmin® and 600 mg of Theracurmin®). The interaction effect within each timepoint is visualized by how these lines differ in slope and position.

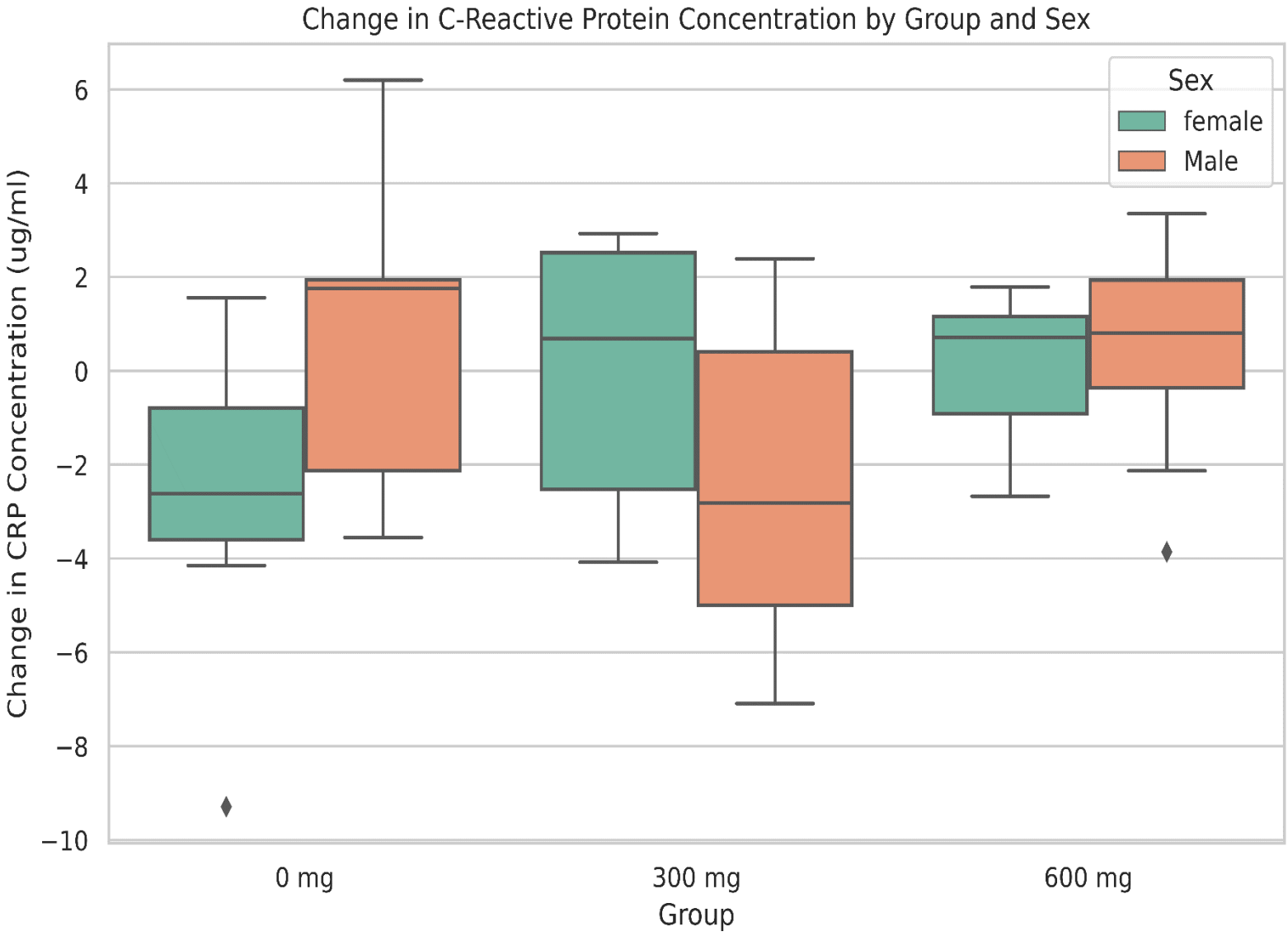


Figure 5.11. This box plot illustrates the changes in C-reactive protein (CRP) concentrations across three different groups (placebo [0 mg], 300 mg and 600 mg of Theracurmin®) after four weeks of supplementation, with the data segregated by sex. The y-axis represents the change in CRP concentrations in micrograms per milliliter (µg/mL), calculated as the difference between the post-intervention (week 4) and baseline (week 0) concentrations. Each box represents the interquartile range (IQR) of the change in CRP concentration, with the median change indicated by the line within

the box. The whiskers extend to the furthest points that are considered not to be outliers.

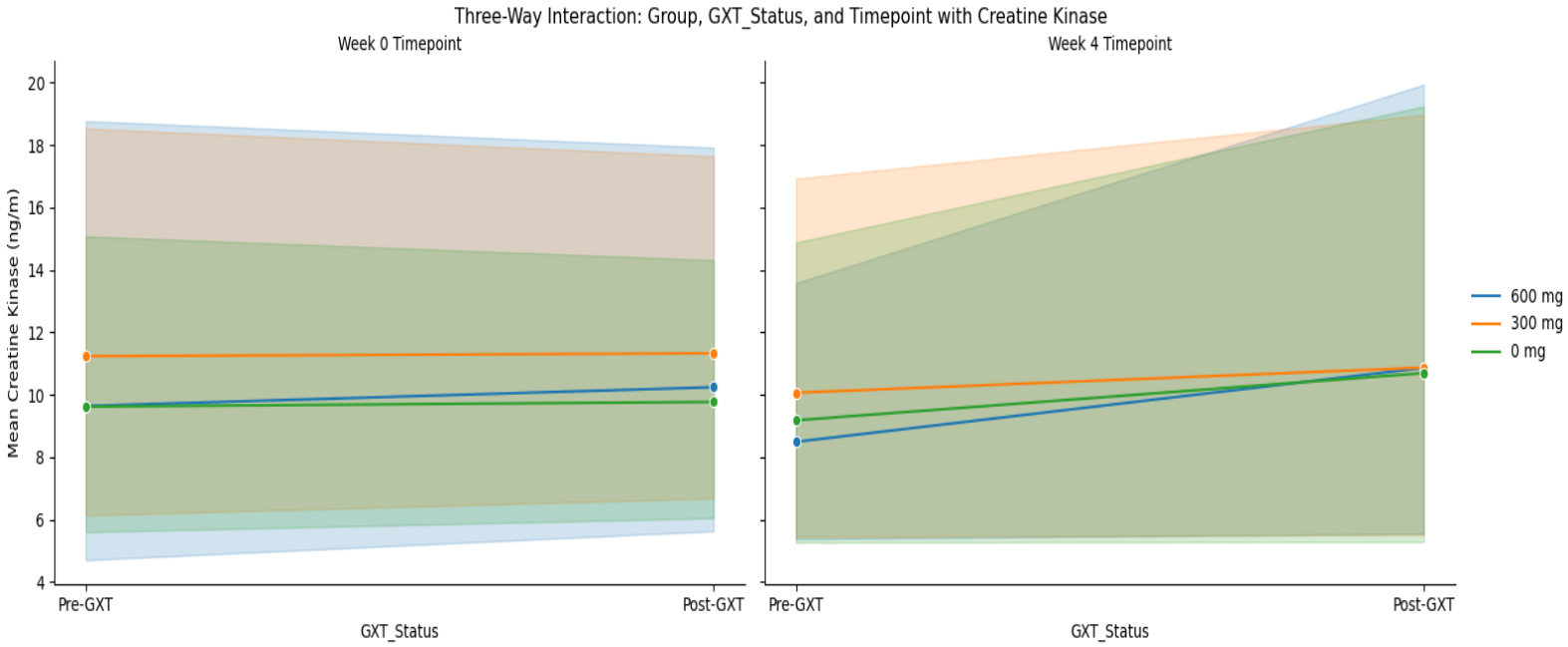


Figure 5.12. The three-way interaction plot provides insights into how supplementation dosage, timepoint, and graded exercise testing (GXT) interact to affect creatine kinase concentrations.

Facets (Timepoint): Each plot represents a different timepoint (Week 0 vs Week 4).

X-axis: Shows the difference between pre- and post-graded exercise test.

Y-axis: Indicates the average value at each graded exercise test for each group within the specific timepoint.

Lines: Each line within a facet represents a different group (placebo, 300 mg of Theracurmin® and 600 mg of Theracurmin®). The interaction effect within each timepoint is visualized by how these lines differ in slope and position.

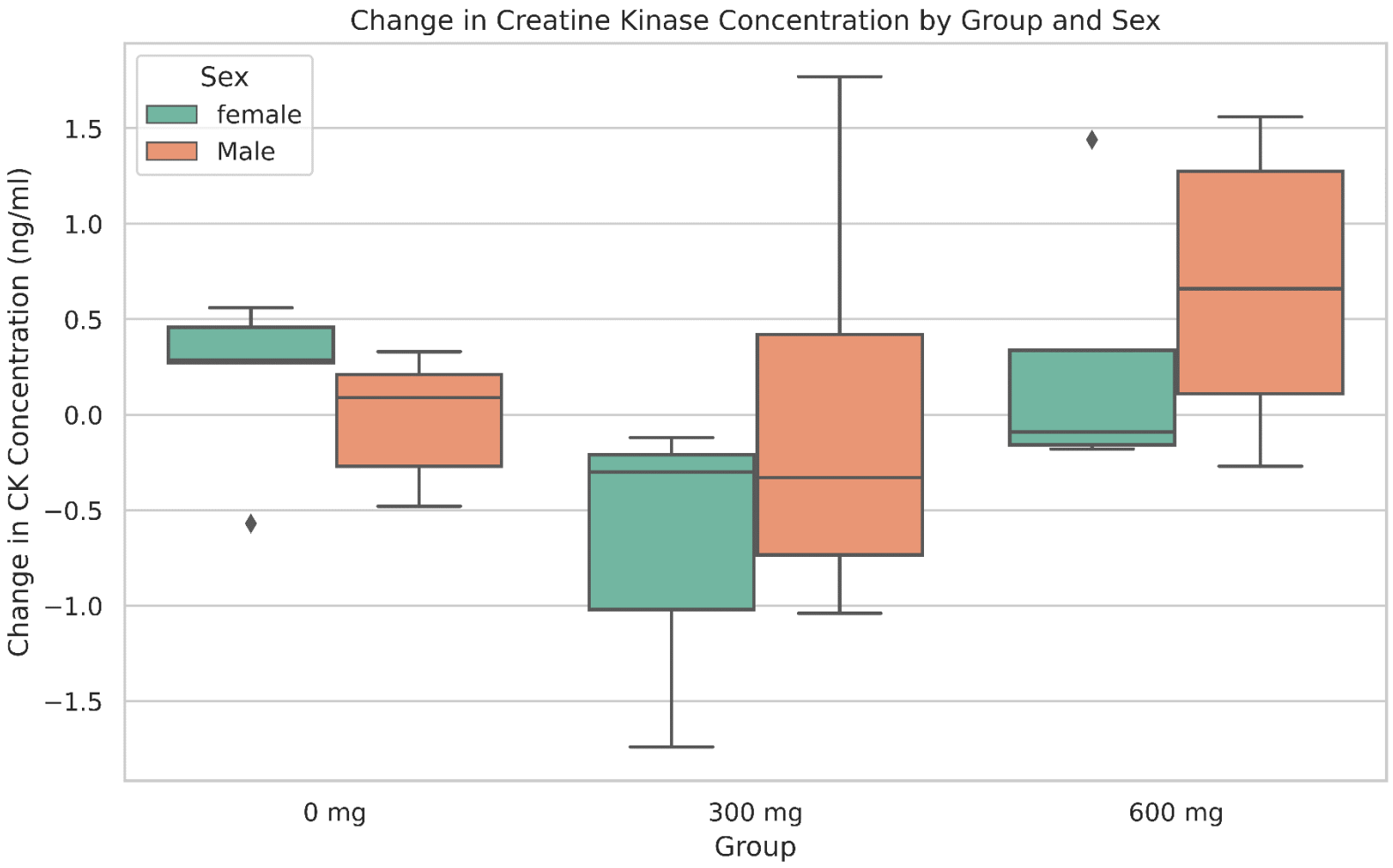


Figure 5.13. This box plot illustrates the changes in creatine kinase (CK) concentrations across three different groups (placebo [0 mg], 300 mg and 600 mg of Theracurmin®) after four weeks of supplementation, with the data segregated by sex. The y-axis represents the change in CK concentrations in nanograms per milliliter (ng/mL), calculated as the difference between the post-intervention (week 4) and baseline (week 0) concentrations. Each box represents the interquartile range (IQR) of the change in CK concentration, with the median change indicated by the line within the box. The whiskers extend to the furthest points that are considered not to be outliers.

CHAPTER 6: CONCLUSIONS AND FUTURE DIRECTIONS

The primary objective of this research was to assess the effect of a four-week supplementation regimen with varying doses of Theracurmin® (300 mg and 600 mg) compared to a placebo in modulating biomarkers associated with oxidative stress, inflammation, and muscle damage induced by exercise. Specifically, the study focused on evaluating concentrations of protein carbonyl (PC), glutathione (GSH), glutathione disulfide (GSSG), total antioxidant capacity (TAC), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP), and creatine kinase (CK) in physically active individuals, 18 to 45 years of age.

The study included 42 recreationally active participants, consisting of 17 females and 25 males, who were randomly assigned to one of three groups: 1) a placebo group who received 50 mg of microcrystalline cellulose, 2) a group who received 300 mg of Theracurmin® (containing 90 mg of active curcuminoids), and 3) a group who received 600 mg of Theracurmin® (containing 180 mg of active curcuminoids).

Statistical analyses were conducted using a Wilcoxon signed rank test or paired t-tests to assess the effects of the graded exercise test (GXT) on markers of oxidative stress, inflammation and muscle damage. Furthermore, an analysis of covariance (ANCOVA) was employed to investigate whether four weeks of supplementation resulted in significant differences in biomarkers of inflammation and muscle damage.

Additionally, a linear mixed-effects (LME) model was utilized to examine the three-way interaction in oxidative stress markers among group, GXT, and timepoint.

The novel findings of this investigation revealed that a four-week regimen of daily oral Theracurmin® supplementation did not yield significant variations in oxidative stress, inflammatory markers and muscle damage among any of the groups, in both female and male participants. Furthermore, the implementation of an incline-based test to exhaustion was not associated with an acute inflammatory response, but did induce acute oxidative stress. These findings underscore the necessity for extended supplementation durations, implementation of multiple dosages, and broader analytical procedures for evaluating markers of oxidative stress, inflammation, and muscle damage with higher sensitivity.

While pre-clinical evidence from cell- and murine-model studies suggests that curcumin may possess antioxidant and anti-inflammatory properties, the translation of these findings to human trials has been inconsistent. It is imperative for future research to employ rigorous methodologies in investigating the antioxidant and anti-inflammatory effects of oral curcumin supplementation to enhance the quality of data in this field.

Future research should aim to incorporate randomized, double-blind, placebo-controlled or crossover design studies to enhance robustness. Additionally, in oral

supplementation trials, it is imperative to consider evaluating the veracity of the data by assessing the concentration of curcumin metabolites in the bloodstream during the supplementation period. This necessitates the development of practical methods to facilitate the quantification of curcumin metabolites in serum or plasma, because current methodologies are time-consuming and unfeasible. Furthermore, investigations in this field should encompass diverse populations of both sexes, including active individuals, those with chronic diseases, and untrained individuals.

In summary, the ultimate determination regarding the efficacy of oral curcumin supplementation in influencing oxidative stress, inflammation and muscle damage remains inconclusive, predominantly due to variations in research methodologies and inadequate comprehensive assessment of relevant outcome measures. Consequently, further investigations are imperative to advance understanding in this area and unveil new perspectives that could offer pivotal approaches in regulating oxidative stress, inflammation and muscle damage across diverse populations.

In the current literature, most studies test for markers in the blood stream or perform scans to quantify changes in physiological outcome measures. However, data regarding intracellular investigations are severely lacking. Consequently, future studies should explore various sites of oxidative stress, inflammation and muscle damage with the goal of bridging the significant gap existing in the literature regarding the effects of different exercise and supplementation regimens on oxidative stress, inflammation markers and

muscle damage in intracellular muscle tissue. Although analyzing muscle tissue poses challenges due to data collection complexities, it offers a novel approach to understanding exercise-induced oxidative stress and inflammation. Intracellular concentrations of oxidative stress by-products and antioxidant enzymes provide a more accurate depiction of oxidative stress and antioxidant defense status compared to whole blood samples because these are the primary sites wherein oxidative stress first develops. Furthermore, future research should integrate biomarker assessments with functional outcomes to establish links between optimal biomarker concentrations and physiological measures such as muscular fatigue, isometric force generation, and recovery.

In the context of exercise protocols, future research should prioritize investigating the effects of higher-intensity and whole-body movements compared to single-joint exercises on the induction of acute oxidative stress. These types of exercises have shown to be more effective at elevating markers of oxidative stress, inflammation and muscle damage. Additionally, there is a need for further investigation into the optimal timing for supplementation of curcumin, because existing studies have reported variability in its effectiveness based on timing of intake. As such, future research should aim to elucidate the relationship between oral curcumin supplementation and time of consumption. Furthermore, in studies examining the effect of antioxidant agents such as curcumin, there is a lack of research addressing the potential confounding effects of

dietary intake of antioxidants naturally present in food. However, it is important for research studies to accurately capture dietary intake data through methods such as food records or food frequency questionnaires because they are strong confounding factors that may affect the results of supplementation trials. Quantification of dietary intake and habitual physical activity will help bolster the reliability of findings in studies investigating the effects of antioxidant supplementation.

APPENDIX A:
INSTITUTIONAL REVIEW BOARD LETTER OF APPROVAL

Division of Scholarly Integrity and Research Compliance
Institutional Review Board
North End Center, Suite 4120 (MC 0497)
300 Turner Street NW
Blacksburg, Virginia 24061
540/231-3732
irb@vt.edu
http://www.research.vt.edu/sirc/hrpp

MEMORANDUM

DATE: August 3, 2023

TO: Stella Lucia Volpe, Pankti Patel, Sean Finley, Eleni Laskaridou, Yaokun Lai, Guillermo Zorrilla-Revilla, Elaina Lynn Marinik, Janet T Rinehart, Rohit kumar Ramadoss, Fardib Mahbub, et. al.

FROM: Virginia Tech Institutional Review Board (FWA00000572)

PROTOCOL TITLE: The Effect of Curcumin on Oxidative Stress and Inflammatory Markers in the Corps of Cadets and Recreationally Active Women and Men

IRB NUMBER: **22-646**

Effective August 3, 2023, the Virginia Tech Institution Review Board (IRB) approved the Amendment request for the above-mentioned research protocol.

This approval provides permission to begin the human subject activities outlined in the IRB-approved protocol and supporting documents.

Plans to deviate from the approved protocol and/or supporting documents must be submitted to the IRB as an amendment request and approved by the IRB prior to the implementation of any changes, regardless of how minor, except where necessary to eliminate apparent immediate hazards to the subjects. Report within 5 business days to the IRB any injuries or other unanticipated or adverse events involving risks or harms to human research subjects or others.

All investigators (listed above) are required to comply with the researcher requirements outlined at:

https://secure.research.vt.edu/external/irb/responsibilities.htm

(Please review responsibilities before beginning your research.)

If the status of your study has changed with this amendment log into the ClinicalTrials.gov account associated with this protocol and update the study status.

PROTOCOL INFORMATION:

Approved As:	**Full Review**
Protocol Approval Date:	**February 10, 2023**
Protocol Expiration Date:	**January 8, 2024**
Continuing Review Due Date*:	**December 11, 2023**

*Date a Continuing Review application is due to the IRB office if human subject activities covered under this protocol, including data analysis, are to continue beyond the Protocol Expiration Date.

APPENDIX B: RECRUITMENT FLYER

HUMAN NUTRITION FOODS & EXERCISE
hnfe.vt.edu
VIRGINIA TECH

Participants Needed for Research Study Investigating Turmeric's Effect on Physical Well-Being

IRB #22-646

Our research team wants to determine if four weeks of turmeric supplementation affects recovery, inflammation, and physical well-being in active women and men.

Participants must:

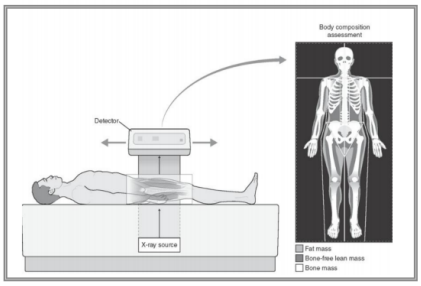
- Be 18 years of age or older
- Exercise two or more days per week
- Be able to run on a treadmill
- Not be taking medications that effect the study
- Not have hypertension
- Be non-smokers
- Not be pregnant
- Be without any major medical problems

This study involves:

- 3 visits to the lab in Wallace Hall
- 1 measure of your bone mineral density and body composition
- 2 exercise tests on a treadmill
- 4 weeks of supplementation with turmeric or a placebo
- 6 hours of time commitment

By participating:

- You will receive data on your aerobic fitness, body composition, and bone mineral density
- You will not receive monetary compensation for participation



If you are interested in participating in this research study, please contact the Volpe Laboratory at Virginia Tech by email: **volpelabvt@gmail.com**.

Or you can scan me to enroll!

APPENDIX C: SCREENING SURVEY

Please enter your contact information

First Name

Last Name

Phone

Email Address

What is your date of birth?

MM/DD/YYYY

What is your sex?

- ○ Male
- ○ Female
- ○ Non-Binary
- ○ Prefer not to answer

Are you currently or possibly pregnant?

- ◯ Yes
- ◯ No
- ◯ Prefer not to answer

Do you smoke?

- ◯ Yes
- ◯ No
- ◯ Former

How long has it been since you last smoked?

- ◯ Less than four months
- ◯ Less than six months
- ◯ Between six months and a year
- ◯ One year
- ◯ More than one Year

On average, how many days per week do you exercise or does your team/program practice?

- ◯ 0 days
- ◯ 1 day
- ◯ 2 days
- ◯ 3 days
- ◯ 4 days
- ◯ 5 days

- ◯ 6 days
- ◯ 7 days

Describe the intensity of your cardiovascular workouts.

- ◯ Easy
- ◯ Easy-Moderate
- ◯ Moderate
- ◯ Moderate-High
- ◯ High

Describe the intensity of your strength training workouts.

- ◯ Easy
- ◯ Easy-moderate
- ◯ Moderate
- ◯ Moderate-high
- ◯ High

Are you in the Corps of Cadets?

- ◯ Yes
- ◯ No

Do you exercise in addition to the Corps of Cadets workouts?

- ◯ Yes

- [ ] No

Please describe those other activities here. If you are also part of another team/program, please describe, as well

Have you been diagnosed, or are you aware of any pre-existing cardiac or other medical conditions that would affect your ability to participate in this study? (i.e. hypertension, cardiovascular disease, pregnancy, asthma, etc.) If yes, please describe.

Are you currently taking any medications or oral supplements?

- [ ] Yes
- [ ] No

Please list your medications and oral supplements

Would you be willing to take oral supplements daily for 4 weeks?

- [ ] Yes
- [ ] No

APPENDIX D: MEDICAL HISTORY QUESTIONNAIRE

Virginia Tech
Department of Human Nutrition, Foods, and Exercise

HEALTH HISTORY QUESTIONNAIRE

STUDY_____ **DATE**_____

SUBJECT ID #_____

PLEASE PRINT

1. Address:_____

   City:_____ State:_____ Zip Code_____

   Home Phone: _____ Work Phone:_____

   E-mail address:_____

   Emergency Contact:_____ Phone:_____

   Relation to you:_____

2. Employer:_____ Occupation:_____

3. Age:_____ Sex:___

   Race and/or Ethnic Origin

   ☐ American Indian or Alaskan Native ☐ Asian or Pacific Islander ☐ Black, not of Hispanic Origin

   ☐ Hispanic ☐ White, not of Hispanic Origin

   ☐ Other

4. **GENERAL MEDICAL HISTORY**

Do you have any food allergies? YES ☐ NO ☐ If Yes, please explain:

Have taken antibiotics in the three months? YES ☐ NO ☐ If Yes, please explain:

Do you have any current medical conditions? YES ☐ NO ☐ If Yes, please explain:

Are you allergic to any medications?	YES ☐	NO ☐	If Yes, please explain:
Have you had any major illnesses in the past?	YES ☐	NO ☐	If Yes, please explain:
Have you ever been hospitalized or had surgery? (include date and type of surgery, if possible)	YES ☐	NO ☐	If Yes, please explain:
Have you ever had an EKG?	YES ☐	NO ☐	If Yes, please explain:
Have you been diagnosed with diabetes?	YES ☐	NO ☐	If Yes, please explain:

Age at diagnosis_____

Are you currently taking any medications or supplements, including aspirin, hormone replacement therapy, or other over-the-counter products?

YES ☐ NO ☐ If Yes, please explain:

Medication/Supplement	**Reason**	**Times taken per Day**	**Taken for how long?**

FAMILY HISTORY

	Age (if alive)	Age of Death	Cause of Death
Father	_____	_____	_____
Mother	_____	_____	_____
Brothers/Sisters	_____	_____	_____
	_____	_____	_____
	_____	_____	_____
	_____	_____	_____

Do you have a family history of any of the following: (Blood relatives only, please give age at diagnosis if possible)

	YES	NO	Relation	Age at Diagnosis
a. High blood pressure	☐	☐		
b. Heart Attack	☐	☐		
c. Coronary bypass surgery	☐	☐		
d. Stroke	☐	☐		
e. Diabetes	☐	☐		
f. Obesity	☐	☐		

6. **TOBACCO/ALCOHOL HISTORY** (check one)

None ☐
Quit ☐ (when)_____
Cigarette ☐
Cigar ☐
Pipe ☐
Chew Tobacco ☐
Snuff ☐

CURRENT TOBACCO USE
(if applicable)

	# per day
Cigarette	_____
Cigar	_____
Pipe	_____
Chew Tobacco	_____
Snuff	_____

Total years of tobacco use_____

Do you consume alcohol? Drinks per day ____ Drinks per week ____

5. CARDIORESPIRATORY/METABOLIC HISTORY

	YES	NO
Are you presently diagnosed with heart disease?	☐	☐
Do you have any history of heart disease?	☐	☐
Do you have a heart murmur?	☐	☐
Occasional chest pain or pressure?	☐	☐
Chest pain or pressure on exertion?	☐	☐
Episodes of fainting?	☐	☐
Daily coughing?	☐	☐
High blood pressure?	☐	☐
Shortness of breath?		
At rest?	☐	☐
lying down?	☐	☐
After 2 flights of stairs?	☐	☐
Do you have asthma?	☐	☐
Do you have a history of bleeding disorders?	☐	☐
Do you have a history of problems with blood clotting?	☐	☐
Do you have high cholesterol? Or, low good (HDL) cholesterol?	☐	☐
Do you have thyroid problems?	☐	☐

If you checked YES to any of the above, you will be asked to clarify your response by an investigator so we can be sure to safely determine your ability to participate.

6. **NUTRITIONAL HABITS**

Have you ever dieted? YES ☐ NO ☐

If YES, have you dieted within the past 12 months or are you currently on a diet?

YES ☐ NO ☐

If YES, please describe the diet:

a). Name (if applicable):_____

b). Prescribed by a Physician/nutritionist? YES ☐ NO ☐

c). Have you lost weight? YES ☐ NO ☐

d). Duration of diet_____

Do you have regular bowel movements? YES ☐ NO ☐

☐ 1 or more times per day ☐ 4-6 times per week ☐ 3 or fewer times per week

You may be asked to complete a more detailed diet survey if you are volunteering for a research study.

7. **PHYSICAL ACTIVITY SURVEY**

Compared to a year ago, how much regular physical activity do you get? (Check one)

Much less ☐

Somewhat less ☐

About the same ☐

Somewhat more ☐

Much more ☐

Have you been exercising regularly for the past three months? YES ☐ NO ☐

If YES, what type of exercise do you regularly participate in? (check those that apply)

		Days per week	**Minutes per session**	**Intensity** (1=easy, 10=very hard)
Walking	☐	_____	_____	_____
Running	☐	_____	_____	_____
Cycling	☐	_____	_____	_____
Swimming	☐	_____	_____	_____
Aerobics	☐	_____	_____	_____
Weight Training	☐	_____	_____	_____
Martial Arts	☐	_____	_____	_____
Other (describe)		_____	_____	_____

8. OBSTETRIC/GYNECOLOGICAL HISTORY

	YES	**NO**
Are you post-menopausal?	☐	☐

If yes, how long has it been since your last menstrual cycle? _____

How many full term pregnancies have you had? ____ How long ago was your more recent pregnancy?_____

Have long since you have last breast fed?____

9. EDUCATION

Please check the highest degree obtained:

Grade School ☐
Junior High ☐
High School ☐
College Degree ☐
Master's Degree ☐
Doctorate ☐

10. FAMILY PHYSICIAN

Name:_____

Address:_____

_____

Phone: _____

Should it be necessary, may we send a copy of your results to your physician? YES☐ NO☐

Signature:_____ Date:_____

Witness:_____ Date:_____
Print Name Signature

Reviewer:_____ Date:_____
Print Name Signature

APPENDIX E:
PHYSICAL ACTIVITY READINESS QUESTIONNAIRE (PAR-Q)

Modified Physical Activity Readiness Questionnaire

Date: _____

Has your physician ever said that you have a heart condition and that you should only do physical activity recommended by a doctor? Circle one: YES NO

Do you feel pain in your chest when you do physical activity?
Circle one: YES NO

Have you ever had chest pain when not doing physical activity?
Circle one: YES NO

Do you lose balance because of dizziness or have you ever lost consciousness?
Circle one: YES NO

Do you have a bone or joint problem (e.g. back, knee, hip) that could be made worse by strenuous physical activity or impact? Circle one: YES NO

Is your physician currently prescribing drugs for your blood pressure or heart condition? Circle one: YES NO

Do you suffer from exercise induced asthma or any other form of asthma?
Circle one: YES NO

If you answered YES to any of the above questions, please explain below and consult with your physician.

"I have read, understood, and completed this questionnaire to the best of my knowledge."

Name (print): _____

Signature: _____

Date: _____

APPENDIX F: INFORMED CONSENT FORM

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Consent to Take Part in a Research Study

Title of Research Study: The Effect of Curcumin on Oxidative Stress and Inflammatory Markers in the Corps of Cadets and Recreationally Active Women and Men

Principal Investigator: Stella L. Volpe, PhD, stellalv@vt.edu

Other study contact(s): Elaina Marinik, PhD, emarinik@vt.edu, Janet Rinehart, rinehart@vt.edu, Rohit Ramadoss, MS, rr627@vt.edu

Key Information: The following is a short summary of this study to help you decide whether or not to be a part of this study. More detailed information is listed later on in this form.

This study aims to examine the effect of curcumin supplementation on oxidative stress and inflammation in Corps of Cadets and recreationally active women and men. Oxidative stress is a phenomenon caused by an imbalance between the production and elimination of potentially toxic reactive oxygen species (ROS) in cells and tissues, while inflammation is the immune system's response to an irritant. Over the course of the study, we will ask you to participate in two graded exercise tests on a treadmill, four blood draws, and the consumption of oral capsules once per day for four weeks.

Why am I being invited to take part in a research study?

We invite you to take part in a research study because you are part of the Corps of Cadets or a recreationally active individual who is 18 years of age or older, a non-smoker, and free from chronic disease.

What should I know about being in a research study?

- Someone will explain this research study to you
- Whether or not you take part is up to you
- You can choose not to take part
- You can agree to take part and later change your mind
- Your decision will not be held against you
- You can ask all the questions you want before you decide

Why is this research being done?

Curcumin, the active compound in turmeric, has been studied as a potential supplement for a vast number of physiological ailments. However, curcumin's effect in decreasing exercise-induced oxidative capacity and inflammation has not been thoroughly examined. Exercise-induced oxidative stress is a phenomenon where intense physical activity leads to an elevation in the production of reactive molecules in the body called as free radicals, such as reactive oxygen species (ROS). These

free radicals may induce harmful effects if their concentration in the body exceeds the capacity of the antioxidant defense system. As such, the proposed research aims to study the efficacy of curcumin supplementation in reducing the extent of oxidative stress accumulated after a maximal aerobic capacity graded exercise test. Moreover, because curcumin also exhibits anti-inflammatory properties, we also seek to study curcumin's role in reducing inflammation for the duration of the research protocol. Unchecked chronic inflammation can contribute to reduced exercise performance and impair recovery.

The results from this study could potentially benefit those who are physically active by providing insight into a means by which adverse accumulation of oxidative stress and inflammation can be circumvented. Oxidative stress and inflammation are normal occurrences post-exercise; however, we want to test if curcumin will help to decrease these blood markers post-exercise, and help with overall recovery post-exercise.

How long will the research last and what will I need to do?

We expect that your participation in this research study will last approximately 6 hours over four to five weeks. This will include one virtual meeting and three in-person visits to our lab. The actual time and frequency may depend on your schedule and the research staff's schedules.

Your first visit will be online. We will determine whether you would like to participate in the study by reviewing what is involved in the study and any risks or benefits of doing so, as well as ask you to complete some questions about your health history.

During your second visit, your body composition will be measured using a dual-energy X-ray absorptiometry (DXA) scan, we will then invite you to participate in a graded exercise test lasting up to 20 minutes. Two blood draws will be performed before and after the exercise test. Finally, you will be asked to complete a food frequency questionnaire to record your dietary pattern. At the end of the session, you will be provided with an accelerometer and a supply of oral capsules to be consumed every day for the following two weeks. An accelerometer is a small electronic device worn on the wrist that will estimate the duration and intensity of physical activities.

During your third visit, we will ask you to visit the lab for a short check-in visit, wherein you will return the accelerometer and receive another supply of oral capsules to be consumed every day for the next two weeks.

During your fourth and final visit, you will be asked to participate in a graded exercise test lasting up to 20 minutes (same as the second visit). Two blood draws will be performed before and after the exercise test.

More detailed information about the study procedures can be found under, **"What happens if I say yes, I want to be in this research?"**

Is there any way being in this study could be bad for me?

We will ask you to perform two graded exercise tests, where you may feel out of breath and have a fast heart rate and sore muscles for a short time after the test. This goes away on its own after a few minutes.

We will ask you to participate in a DXA scan, which will expose you to a minimal amount of radiation. The radiation exposure will be less than the amount of exposure received during a cross-country flight.

More detailed information about the risks of this study can be found under **"Is there any way being in this study could be bad for me? (Detailed Risks)".**

Will being in this study help me in any way?

We cannot promise any health benefits to you or others from your taking part in this research. However, possible benefits may include positive changes in oxidative stress and inflammation, and receiving information about your body composition and aerobic capacity. You should not consider this a wellness or medical exam, and there will be no direct medical benefit to you. You should discuss any concerns about your health information with your physician.

What happens if I do not want to be in this research?

Participation in research is completely up to you. You can decide to participate or not to participate.

If you are a student, the decision whether to participate or not participate will have no effect on your grades or relationship with Virginia Tech.

Detailed Information: The following is more detailed information about this study in addition to the information listed above.

Who can I talk to?

If you have questions, concerns, or complaints, or think the research has hurt you, talk to the principal investigator, Dr. Stella Volpe at: stellalv@vt.edu (phone: 540-231-3805) or contact Rohit Ramadoss, the doctoral student working on this research at: rr627@vt.edu

This research has been reviewed and approved by the Virginia Tech Institutional Review Board (IRB). You may communicate with them at irb@vt.edu or 540-231-3732 if:

- You have questions about your rights as a research participant
- Your questions, concerns, or complaints are not being answered by the research team
- You cannot reach the research team
- You want to talk to someone besides the research team to provide feedback about this research

How many people will be studied?

We plan to include about 51 people in this research study.

What happens if I say, yes, I want to be in this research?

Virtual Session: You will first meet with someone from the research team. This will be a screening to see if you qualify and/or want to participate in the research study. This will be a Health Insurance Portability and Accountability Act (HIPAA)-approved Zoom call (approximately 1 hour).

Medical History: You will be asked to complete a medical history questionnaire, which will be used to screen for health problems (e.g., unstable heart disease), or other reasons that would preclude your participation (e.g., osteoarthritis that limits physical activity).

Physical Activity Assessment: You will be asked to complete a questionnaire regarding habitual physical activity and exercise with a physical activity readiness questionnaire (PAR-Q).

Session 1: 339 Wallace Hall (approximately 2 hours)

Pregnancy Test: If you are a female who is either pre-menopausal, peri-menopausal, or not post-menopausal for at least 1 year, you will be required to undergo a pregnancy test. You will be provided a small plastic cup and asked to provide at least three to four tablespoons of urine, put the lid back on the cup, and return it to us in the lab.

Blood Pressure: You will be asked to sit for 10 minutes in a relaxed posture. The researcher will measure your resting blood pressure and heart rate using an automated blood pressure monitor.

Anthropometry: Your body weight and height will be measured on a digital physician's scale. Your body weight will be measured during every lab visit. We will measure your height on your first visit only, using a stadiometer. You will then be asked to remove any jewelry or belts, and then be asked to lie down on the DXA table. Once on the table the researcher will ask you to move your head and limbs to within the scanning area. Next, the researcher will explain how the DXA scanning arm will pass slowly over your body (but it will not touch your body). Finally, the researcher will ask you to remain as still as possible throughout the scan, which typically takes 8 to 10 minutes, depending on your body size.

Blood Draw 1, Session 1: You will sit in a phlebotomy chair and be asked to rest your arm on an armrest. The phlebotomist will identify a suitable vein, clean the area with alcohol, and place a rubber tourniquet around your upper arm. A small needle will be inserted into one of your arm veins to draw a blood sample (approximately two tablespoons). The blood sample will be used to measure markers of oxidative stress and inflammation.

Maximal Aerobic Capacity Test: This procedure involves having you run on a treadmill for the graded exercise test. We will have you wear a face mask and nose clip (or a face mask that covers your nose and mouth) during this test to measure the air you are breathing in and the carbon dioxide you are breathing out. You will also be connected to a 3-lead electrocardiogram (ECG) to record your heart rate during the test. Before and after the test, you will have the chance to warm-up and

cool down for two minutes while walking on the treadmill at a slow pace. During the test, you will run at a self-selected pace of either 5.5 mph or 7 mph, but the grade on the treadmill will be increased every minute. The test will last for 15 to 20 minutes.

Blood Draw 2, Session 2: Within 20 minutes after the completion of the graded exercise test, you will sit in a phlebotomy chair and be asked to rest your arm on an armrest. The phlebotomist will identify a suitable vein, clean the area with alcohol, and place a rubber tourniquet around your upper arm. A small needle will be inserted into one of your arm veins to draw a blood sample (approximately two tablespoons). The blood sample will be used to measure markers of oxidative stress and inflammation.

Dietary Assessment: You will be asked to complete a food frequency questionnaire to assess overall food intake. This will take you approximately 20 minutes to complete.

Turmeric Supplement/Placebo: You will be given oral capsules and asked to consume the required dosage every day for two weeks. You will be randomly assigned to one of three groups: 300 mg/day turmeric group, or 600 mg/day turmeric group, or a placebo group by using a software generated randomization plan. You will have a 66.6% chance of receiving one of the turmeric supplements, and 33.3% chance of receiving a placebo. You will not have a choice as to which group you will be assigned, because this will be a random assignment. Because this is a double blinded study, neither you nor the research team will know to which group you are randomly assigned.

Accelerometer: You will be instructed to wear a device called an accelerometer on your non-dominant arm to assess your habitual physical activity for the next 14 days. Accelerometers measure how much physical activity you perform each day. You will wear this accelerometer all day, every day for two weeks. You can wear it in the shower, and if you swim, while swimming.

Session 2: 339 Wallace Hall (approximately 30 minutes)

Body Weight: Your body weight will be measured using a digital physician's scale.

Turmeric Supplement/Placebo: You will be given oral capsules and asked to consume the required dosage every day for the next 14 days.

Accelerometer: You will be asked to return the accelerometer during this visit.

Session 3: 339 Wallace Hall (approximately 2 hours)

Blood Pressure: You will be asked to sit for 10 minutes in a relaxed posture. The researcher will measure your resting blood pressure and heart rate using an automated blood pressure monitor.

Blood Draw 1, Session 3: You will sit in a phlebotomy chair and be asked to rest your arm on an armrest. The phlebotomist will identify a suitable vein, clean the area with alcohol, and place a rubber tourniquet around your upper arm. A small needle will be inserted into one of your arm veins to draw a blood sample (approximately two tablespoons). The blood sample will be used to measure markers of oxidative stress and inflammation.

Maximal Aerobic Capacity Test: This procedure involves having you run on a treadmill for the graded exercise test. We will have you wear a face mask and nose clip (or a face mask that covers your nose and mouth) during this test to measure the air you are breathing in and the carbon dioxide you are breathing out. You will also be connected to a 3-lead electrocardiogram (ECG) to record your heart rate during the test. Before and after the test, you will have the chance to warm-up and cool down for two minutes while walking on the treadmill at a slow pace. During the test, you will run at a self-selected pace of either 5.5 mph or 7 mph, but the grade on the treadmill will be increased every minute. The test will last for 15 to 20 minutes.

Blood Draw 2, Session 3: Within 20 minutes after the completion of the graded exercise test, you will sit in a phlebotomy chair and be asked to rest your arm on an armrest. The phlebotomist will identify a suitable vein, clean the area with alcohol, and place a rubber tourniquet around your upper arm. A small needle will be inserted into one of your arm veins to draw a blood sample (approximately two tablespoons). The blood sample will be used to measure markers of oxidative stress and inflammation.

Physical Activity Questionnaire: You will be asked to complete a physical activity questionnaire to determine your physical activity patterns.

In some cases, the Maximal Aerobic Capacity Test may be scheduled in Wallace Hall Room 228, depending on the availability of the metabolic cart, the instrument used to measure Maximal Aerobic Capacity. We will plan the time and location of this with you in advance. You will be notified by phone or email 48 hours in advance in the unlikely event that a change of location is needed to accommodate the scheduling of all participants. Every effort will be made to avoid such changes.

What are my responsibilities if I take part in this research?

If you take part in this research, you will be responsible to:

- Give an accurate history of any health problems that you have or medicine that you take before the study begins
- Tell the investigators of any discomfort or unusual feelings before, during or after any of the study sessions
- Be on time and attend all scheduled visits
- Follow all instructions given to you by the researchers for each session

What happens if I say yes, but I change my mind later?

You can leave the research at any time, for any reason, and it will not be held against you. We may use the data collected before you leave the study unless you ask us to destroy it.

Is there any way being in this study could be bad for me? (Detailed risks)

Blood Draw: You might feel pain or discomfort when the needle is placed in the vein, but this should not last long. During the blood draws, you may have pain and/or bruising at the place on your arm where the blood was taken. In about 10% of the cases (1 in 10 cases), a small amount of

bleeding under the skin will cause bruising. The risk of a blood clot forming in the vein is about 1 in 200 cases. The risk of infection or significant blood loss is 1 in 1000 cases. There is a small risk of the vein becoming inflamed and/or painful in the hours or days after the needle is removed. If you feel faint during or after a blood draw, you should notify the researchers immediately and lie down right away to avoid falling down. The research members, who are experienced in performing blood draws, minimizes these risks.

HIV/AIDS Hepatitis B or C Virus: If a researcher or other staff person is exposed to your blood, your blood will be tested for certain viruses (HIV, the Hepatitis B Virus, and the Hepatitis C Virus). There will not be any cost to you for this test. The research team will follow the procedures for testing and reporting any positive tests, as required by Virginia State law. This includes sending a sample of your blood to a certified laboratory. Your results will be sent to the local health department, and you will be informed of your test results. Should your blood test positive for any of these viruses, you will be given the opportunity to receive counseling about the results and next steps in a reasonable timeframe.

Dual-energy X-ray Absorptiometry (DXA) Scan: The amount of radiation that you will receive in the two DXA exams is far less than the amount that the Food and Drug Administration (FDA) allows per year. The amount you will receive for each scan is equal to 1/20th of a chest X-ray (two DXA scans equal 1/10th of a chest X-ray). The more radiation you receive over your lifetime, the more your risk increases in developing certain kinds of cancer. The radiation in this study is not expected to greatly increase these risks. The exact increase in that risk is not known. DXA scans will only be performed by an International Society for Clinical Densitometry (ISCD) Certified Bone Densitometry Technologist to minimize risk.

Maximal Aerobic Capacity Test: There is a small risk of injury (e.g., sprained ankle), complications requiring you to go to the hospital, heart attack, or even death. In studies involving people with heart disease, the risk of hospitalization was 1 in 500 tests (<0.20%). The risk of heart attack was 1 in 2,500 tests (0.04%). The risk of dying was 1 in 10,000 tests (0.01%). The risks are likely to be lower in young, healthy individuals. Only experienced research staff members will conduct these tests, and you will be monitored throughout the test for signs of problems. You will be tired after this test and may have sore muscles for a few days.

Turmeric Supplementation: Turmeric has been clinically shown to be safe for consumption up to 12 grams per day, which is far more than what you will potentially be given to consume. However, potential interactions with medications such as chemotherapy drugs, anticoagulant and antiplatelet drugs, antidiabetic drugs, antitumor antibiotics, and Warfarin (Coumadin) may result in undesirable side effects. Based on your medical history, the research team will ensure that potential interactions with medications do not occur.

In addition to these risks, taking part in this research may harm you in unknown ways. It is not possible to identify all potential risks in an experimental study. However, the research staff will take all possible safeguards to minimize any known and potential risks to your well-being. All the procedures are well established and used routinely in the principal investigator's laboratory.

What happens to the information collected for the research?

We will make every effort to limit the use and disclosure of your personal information and any research study information to people who have a need to review this information. We cannot promise complete confidentiality. Organizations that may inspect and copy your information include the Virginia Tech IRB, Human Research Protection Program, and other authorized representatives of Virginia Tech.

We may publish the results of this research, but we will keep your name and any information that can identify you private.

We protect your information from disclosure to others as the law requires. We cannot promise complete secrecy.

If identifiers are removed from your private information or samples that are collected during this research, that information or those samples could be used for future research studies or distributed to another investigator for future research studies without your additional informed consent.

Can I be removed from the research without my OK?

The principal investigator and research team can remove you from the research study without your approval. Possible reasons for removal include:

- It is in your best interest
- If the researchers are unable to obtain measurements that are necessary for the study
- You have a side effect that requires stopping the research
- You need a treatment not allowed in this research
- You become pregnant
- You are unable to keep your scheduled appointments

We will tell you about any new information that might affect your health, welfare, or choice to stay in the research.

What else do I need to know?

If you need medical care because of taking part in this research study, contact the principal investigator and medical care will be made available. Generally, this care will be billed to you, your insurance, or other third party. Virginia Tech has no program to pay for medical care for research-related injuries.

You will not receive monetary compensation for your participation in the study. However, your measurement data on Maximal Aerobic Capacity and the DXA scan will be shared with you. As such, you will gain data on your aerobic fitness, body composition, and bone mineral density. The

monetary value of the shared data is approximately $100 to $250. The final results of the study will also be shared with you after the completion of the trial; as such, you may benefit from the information shared with you. This research is being funded by the McCormick Science Institute.

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY

Consent to Take Part in a Research Study

Signature Block for Capable Adult

Your signature documents your permission to take part in this research. We will provide you with a signed copy of this form for your records.

Signature of participant	Date
Printed name of participant	
Signature of person obtaining consent	Date
Printed name of person obtaining consent	

APPENDIX G:
2015 BLOCK FOOD FREQUENCY QUESTIONNAIRE

FOOD AND ACTIVITY QUESTIONNAIRE

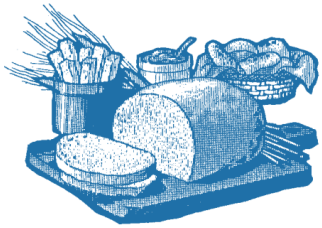
RESPONDENT ID #

TODAY'S DATE

Jan Feb Mar Apr May Jun Jul Aug Sep Oct Nov Dec

DAY

YEAR 2014 2015 2016 2017 2018 2019 2020 2021 2022 2023



ABOUT THIS SURVEY

Please answer each question as best you can. Estimate if you aren't sure.

- **DETACH THE LAST PAGE OF THIS BOOKLET.** These are your portion pictures.
- **USE ONLY A NUMBER 2 PENCIL.**
- **FILL IN THE CIRCLES COMPLETELY** and erase completely if you make any changes.

ABOUT YOU

SEX
Male
Female

If female, are you pregnant or breast feeding?
No
Yes
Not female

AGE

WEIGHT pounds

HEIGHT feet inches

INSTRUCTIONS

This form is about the foods you usually eat. Think about your usual intake over the last year. This includes all meals or snacks, at home, in a restaurant, or carry-out.

Please tell us...

1. **HOW OFTEN,** on average, did you eat the food?
   DO NOT SKIP any foods. Mark "Never" if you didn't eat any of the food.
2. **HOW MUCH** of the food did you usually eat on the days you ate it?
   Sometimes we ask "how much" as A, B, C or D. **LOOK AT THE PORTION PICTURES.**
   Pick the picture that looks the most like the serving size you usually eat.
   (If you don't have pictures: A=1/4 cup, B=1/2 cup, C=1 cup, D=2 cups.)
3. **WHAT TYPE?** For some foods we ask the type (low-fat, low-sugar...) near the end of the survey.

EXAMPLE: This person drank orange juice twice a week, and had one glass each time.
Once a week this person ate a "C"-sized serving of cold cereal (about 1 cup).

	HOW OFTEN in the past year?									HOW MUCH on those days? SEE PORTION SIZE PICTURES FOR A-B-C-D
	NEVER	A FEW TIMES per YEAR	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	
Orange juice	○	○	○	○	○	●	○	○	○	How many glasses: ● 1 ○ 2 ○ 3 ○ 4
Cold cereal	○	○	○	○	●	○	○	○	○	Which bowl: ○ A ○ B ● C ○ D

PLEASE DO NOT WRITE IN THIS AREA

SERIAL #

EGGS and DAIRY FOODS	NEVER	A FEW TIMES per YEAR	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH on those days? SEE PORTION SIZE PICTURES FOR A-B-C-D
Breakfast sandwiches or breakfast burritos with eggs or meat	○	○	○	○	○	○	○	○	○	How many sandwiches in **a day** ○ 1 ○ 2
Other eggs like scrambled or boiled, or quiche (not egg substitutes)	○	○	○	○	○	○	○	○	○	How many eggs **a day** ○ 1 ○ 2 ○ 3 ○ 4
Yogurt (not frozen yogurt)	○	○	○	○	○	○	○	○	○	Which **bowl** ○ B ○ C ○ D
Cottage cheese, ricotta cheese	○	○	○	○	○	○	○	○	○	How **much** ○ A ○ B ○ C ○ D
Cream cheese, sour cream, dips	○	○	○	○	○	○	○	○	○	How many **tablespoons** ○ 1 ○ 2 ○ 3 ○ 4
Cheese, sliced cheese, cheese spread, including in sandwiches and quesadillas	○	○	○	○	○	○	○	○	○	How many **slices** ○ 1 ○ 2 ○ 3 ○ 4
CEREALS, GRAINS, BREADS										
Cold cereals, ANY KIND, like corn flakes, fiber cereals, sweetened cereals	○	○	○	○	○	○	○	○	○	Which **bowl** ○ B ○ C ○ D
Oatmeal, or whole grain cereal like Wheatena or Ralston	○	○	○	○	○	○	○	○	○	Which **bowl** ○ A ○ B ○ C ○ D
Grits, cream of wheat, cornmeal mush	○	○	○	○	○	○	○	○	○	Which **bowl** ○ A ○ B ○ C ○ D
Milk or milk substitutes on cereal	○	○	○	○	○	○	○	○	○	
Brown rice, or dishes made with brown rice	○	○	○	○	○	○	○	○	○	How much **in a day** ○ B ○ C ○ D
White rice, or dishes made with white rice, like rice and beans	○	○	○	○	○	○	○	○	○	How much **in a day** ○ B ○ C ○ D
Pancakes, waffles, French toast, crepes	○	○	○	○	○	○	○	○	○	How **many** ○ 1 ○ 2 ○ 3 ○ 4
Breakfast pastries, like muffins, scones, sweet rolls, Danish, Pop Tarts, pan dulce	○	○	○	○	○	○	○	○	○	How many **pieces** ○ 1 sm ○ 1 med ○ 2 ○ 3
Biscuits, not counting breakfast sandwiches	○	○	○	○	○	○	○	○	○	How **many** ○ 1 sm ○ 1 med ○ 2 ○ 3
Corn bread, corn muffins, hush puppies	○	○	○	○	○	○	○	○	○	How many **pieces in a day** ○ 1/2 ○ 1 ○ 2 ○ 3
Hamburger buns, hotdog buns, submarine or hoagie buns	○	○	○	○	○	○	○	○	○	How many **buns in a day** ○ 1/2 ○ 1 ○ 2 ○ 3
Bagels or English muffins, dinner rolls, pita, naan	○	○	○	○	○	○	○	○	○	How **many** ○ 1/2 ○ 1 ○ 2 ○ 3
Tortillas (not counting in tacos or burritos)	○	○	○	○	○	○	○	○	○	How many **in a day** ○ 1 ○ 2 ○ 3 ○ 4
Any other bread or toast, including white, dark, whole wheat, and what you have in sandwiches	○	○	○	○	○	○	○	○	○	How many **slices in a day** ○ 1 ○ 2 ○ 3 ○ 4
VEGETABLES										
Broccoli, Chinese broccoli, or Brussels sprouts	○	○	○	○	○	○	○	○	○	How **much** ○ A ○ B ○ C ○ D
Carrots and mixed vegetables containing carrots	○	○	○	○	○	○	○	○	○	How **much** ○ A ○ B ○ C ○ D
Corn	○	○	○	○	○	○	○	○	○	How **much** ○ A ○ B ○ C ○ D
Green beans, string beans, green peas	○	○	○	○	○	○	○	○	○	How **much** ○ A ○ B ○ C ○ D
Cooked greens like spinach, collards, turnip greens, kale, mustard greens	○	○	○	○	○	○	○	○	○	How **much** ○ A ○ B ○ C ○ D

	NEVER	A FEW TIMES per YEAR	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH on those days? SEE PORTION SIZE PICTURES FOR A-B-C-D
Cabbage, cole slaw, Chinese cabbage	○	○	○	○	○	○	○	○	○	How much ○ A ○ B ○ C ○ D
Green salad with lettuce or raw spinach	○	○	○	○	○	○	○	○	○	How much ○ 1/2 cup ○ 1 cup ○ 2 cups ○ 3+ cups
Raw tomatoes	○	○	○	○	○	○	○	○	○	How much ○ 1/4 ○ 1/2 ○ 1 ○ 2
Salad dressing	○	○	○	○	○	○	○	○	○	How many tablespoons ○ 1 ○ 2 ○ 3 ○ 4
Avocado, guacamole	○	○	○	○	○	○	○	○	○	How many tablespoons ○ 1 ○ 2 ○ 3 ○ 4
Sweet potatoes, yams	○	○	○	○	○	○	○	○	○	How much ○ A ○ B ○ C ○ D
French fries, home fries, hash browns, tater tots	○	○	○	○	○	○	○	○	○	How much ○ A ○ B ○ C ○ D
Potatoes not fried, like baked, boiled, mashed, or in stew or potato salad	○	○	○	○	○	○	○	○	○	How much ○ A ○ B ○ C ○ D
Any other vegetable, like squash, cauliflower, peppers, okra, nopales	○	○	○	○	○	○	○	○	○	How much ○ A ○ B ○ C ○ D
FRUITS										
How often do you eat the following 2 items, just during the summer months when they are in season?										
Watermelon, cantaloupe, honeydew, other melons, in season	○	○	○	○	○	○	○	○	○	How much ○ A ○ B ○ C ○ D
Strawberries or other berries, in season	○	○	○	○	○	○	○	○	○	How much ○ A ○ B ○ C ○ D
How often do you eat the following fruits all year round? Estimate your average for the whole year. Include fresh or frozen fruits. Only include canned or dried fruit when mentioned.										
Bananas	○	○	○	○	○	○	○	○	○	How many in a day ○ 1/2 ○ 1 ○ 2
Apples or pears	○	○	○	○	○	○	○	○	○	How many in a day ○ 1/2 ○ 1 ○ 2
Oranges, tangerines, grapefruit	○	○	○	○	○	○	○	○	○	How many ○ 1/2 ○ 1 ○ 2
Peaches and nectarines	○	○	○	○	○	○	○	○	○	How many ○ 1/2 ○ 1 ○ 2
Any other fresh fruit, like grapes, plums, mango, fruit salad	○	○	○	○	○	○	○	○	○	How much ○ A ○ B ○ C ○ D
Raisins, dates, other dried fruit	○	○	○	○	○	○	○	○	○	How much ○ A ○ B ○ C
Canned fruit, like applesauce, fruit cocktail, canned peaches or pineapple	○	○	○	○	○	○	○	○	○	How much ○ A ○ B ○ C ○ D
BEANS, TOFU, and MEAT SUBSTITUTES **Include those eaten alone, or in mixed dishes like burritos, chili, stir-fry, salad**										
Refried beans, bean burritos, or hummus	○	○	○	○	○	○	○	○	○	How much ○ A ○ B ○ C ○ D
Pinto beans, black beans, kidney beans, baked beans, lentils	○	○	○	○	○	○	○	○	○	How much ○ A ○ B ○ C ○ D
Tofu or tempeh	○	○	○	○	○	○	○	○	○	How much ○ A ○ B ○ C ○ D
Meat substitutes, like veggie burgers, veggie chicken, vegetarian hot dogs or vegetarian lunch meats	○	○	○	○	○	○	○	○	○	How much ○ A ○ B 1 patty or dog ○ C ○ D

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SERIAL #

SOUPS, MIXED DISHES, and NOODLES	NEVER	A FEW TIMES per YEAR	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH on those days? SEE PORTION SIZE PICTURES FOR A-B-C-D				
Split pea, bean, or lentil soup	○	○	○	○	○	○	○	○	○	Which **bowl**		B	C	D
Vegetable soup, vegetable beef soup, or tomato soup	○	○	○	○	○	○	○	○	○	Which **bowl**		B	C	D
Any other soup, including chicken noodle, cream soups, Cup-A-Soup, ramen	○	○	○	○	○	○	○	○	○	Which **bowl**		B	C	D
Pizza or pizza pockets	○	○	○	○	○	○	○	○	○	How many **slices**	1	2	3	4
Macaroni and cheese	○	○	○	○	○	○	○	○	○	How **much**		B	C	D
Spaghetti, lasagna, other pasta with tomato sauce	○	○	○	○	○	○	○	○	○	How **much**		B	C	D
Other noodles like plain pasta, pasta salad, sopa seca	○	○	○	○	○	○	○	○	○	How **much**		B	C	D
Egg rolls, won tons, samosas, empanadas	○	○	○	○	○	○	○	○	○	How many **pieces**	1	2	3	4
MEAT and CHICKEN														
Hamburgers, cheeseburgers, turkey burger, at home or from a restaurant	○	○	○	○	○	○	○	○	○	How **many**	1 sm	1 lrg	2	3
Hot dogs or dinner sausage like Polish, Italian, chicken apple	○	○	○	○	○	○	○	○	○	How **many**	1	2	3	4
Bacon or breakfast sausage	○	○	○	○	○	○	○	○	○	How many **pieces**	1	2	3	4
Lunch meats like bologna, sliced ham, sliced turkey, salami	○	○	○	○	○	○	○	○	○	How many **slices**	1	2	3	4
Meat loaf, meat balls	○	○	○	○	○	○	○	○	○	How **much**		B	C	D
Steak, roast beef, pot roast, including in frozen dinners or sandwiches	○	○	○	○	○	○	○	○	○	How **much**	A	B	C	D
Tacos, burritos, enchiladas, tamales, tostadas, with meat or chicken	○	○	○	○	○	○	○	○	○	How **much**	A	B	C	D
Ribs, spareribs	○	○	○	○	○	○	○	○	○	How **much**	A	B	C	D
Pork chops, pork roast, cooked ham (including for breakfast)	○	○	○	○	○	○	○	○	○	How **much**	A	B	C	D
Any other beef or pork dish like stew, pot pie, corned beef hash, chili, Hamburger Helper, curry	○	○	○	○	○	○	○	○	○	How **much**		B	C	D
Liver, including chicken livers or liverwurst	○	○	○	○	○	○	○	○	○	How **much**	A	B	C	
Pigs feet, neck bones, oxtails, tongue, chitlins	○	○	○	○	○	○	○	○	○	How **much**	A	B	C	
Veal, lamb, goat, deer meat, other game	○	○	○	○	○	○	○	○	○	How **much**	A	B	C	
Fried chicken, including chicken fingers, chicken nuggets, wings, chicken patty	○	○	○	○	○	○	○	○	○	How many **medium pieces**	1	2 pcs/ 6 nuggets	3	4
Roasted or broiled chicken or turkey	○	○	○	○	○	○	○	○	○	How **much**	A	B medium piece	C	D half chicken
Any other chicken or turkey dish, like chicken stew or curry, chicken salad, stir-fry, Chinese chicken dishes	○	○	○	○	○	○	○	○	○	How **much**		B	C	D

FISH, SEAFOOD	NEVER	A FEW TIMES per YEaR	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH on those days? SEE PORTION SIZE PICTURES FOR A-B-C-D
Oysters	○	○	○	○	○	○	○	○	○	How much: ○ A ○ B ○ C
Shellfish like shrimp, scallops, crab	○	○	○	○	○	○	○	○	○	How much fish: ○ A ○ B ○ C ○ D
Tuna, tuna salad, tuna casserole	○	○	○	○	○	○	○	○	○	How much of the tuna: ○ A ○ B ○ C
Salmon, mackerel, sea bass, trout, sardines, herring, without breading	○	○	○	○	○	○	○	○	○	How much: ○ A ○ B ○ C ○ D
Fried fish, fish sticks, fish sandwich, breaded fillets	○	○	○	○	○	○	○	○	○	How much: ○ A ○ B ○ C ○ D
Any other fish	○	○	○	○	○	○	○	○	○	How much fish: ○ A ○ B ○ C ○ D
NUTS, SEEDS, SNACKS										
Peanut butter or other nut butters	○	○	○	○	○	○	○	○	○	How many tablespoons: ○ 1/2 ○ 1 ○ 2 ○ 3
Walnuts or flax seeds (don't count flaxseed oil)	○	○	○	○	○	○	○	○	○	How much: ○ 1 Tbsp ○ 2 Tbsp ○ 1/4 cup ○ 1/2 cup
Peanuts, sunflower seeds, other nuts or seeds	○	○	○	○	○	○	○	○	○	How much: ○ A ○ B ○ C ○ D
Energy or protein bars, like Power Bar, Clif, Balance, Luna, South Beach, Atkins	○	○	○	○	○	○	○	○	○	How much: ○ Small ○ Medium ○ Large
Breakfast bars, cereal bars, granola bars (not energy or protein bars)	○	○	○	○	○	○	○	○	○	How many: ○ 1 ○ 2 ○ 3
Popcorn	○	○	○	○	○	○	○	○	○	How many cups: ○ 1-2 ○ 3-6 ○ 7-9 ○ 10-12
Whole grain crackers, like Wheat Thins, RyeKrisp, Ryvita, Wasa	○	○	○	○	○	○	○	○	○	How much: ○ A ○ B ○ C ○ D
Any other crackers, like saltines, Ritz, Cheez-Its, cheese-filled pretzels	○	○	○	○	○	○	○	○	○	How much: ○ A ○ B ○ C ○ D
Tortilla chips or corn chips, like Fritos, Doritos, corn nuts	○	○	○	○	○	○	○	○	○	How much: ○ A ○ B ○ C ○ D
Any other snack chips, like potato chips, Cheetos, Chex mix	○	○	○	○	○	○	○	○	○	How much: ○ A ○ B ○ C ○ D
SWEETS AND DESSERTS										
Donuts	○	○	○	○	○	○	○	○	○	How many: ○ 1 mini ○ 1 med ○ 2 ○ 3
Cake or snack cakes like cupcakes, Twinkies, pound cake, banana bread	○	○	○	○	○	○	○	○	○	How many pieces: ○ 1 sm ○ 1 med ○ 2 ○ 3
Cookies, brownies	○	○	○	○	○	○	○	○	○	How many: ○ 1-2 ○ 3-4 ○ 5-6 ○ 7+
Pumpkin pie, sweet potato pie	○	○	○	○	○	○	○	○	○	How many pieces: ○ 1/2 ○ 1 ○ 2 ○ 3
Any other pie or cobbler, including fast food pies, snack pies	○	○	○	○	○	○	○	○	○	How many pieces: ○ 1/2 ○ 1 ○ 2 ○ 3
Ice cream, ice cream bars, frozen yogurt, fast food milkshakes	○	○	○	○	○	○	○	○	○	How much: ○ B ○ C ○ D
Pudding, custard, rice pudding, flan	○	○	○	○	○	○	○	○	○	How much: ○ B ○ C ○ D
Chocolate or other flavored sauces or syrup, on ice cream	○	○	○	○	○	○	○	○	○	How much: ○ 1-2 Tbspn ○ 3-4 Tbspn ○ 1/2 cup

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SERIAL #

	NEVER	A FEW TIMES per YEAR	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH on those days? SEE PORTION SIZE PICTURES FOR A-B-C-D
Popsicles, jello, frozen fruit bars, slushies, sherbet (don't count sugar-free)	○	○	○	○	○	○	○	○	○	How **much** ○ A ○ B ○ C ○ D
Chocolate candy, candy bars like Snickers, Hershey's, M&Ms	○	○	○	○	○	○	○	○	○	How much **in a day** ○ 1 mini ○ 1 med ○ 1 lrg ○ 1 king
Any other candy, not chocolate, like hard candy, Lifesavers, Skittles, Starburst, breath mints, chewing gum (NOT sugar free)	○	○	○	○	○	○	○	○	○	How much **in a day** ○ 1-2 pcs ○ 1/2 pkg ○ 1 pkg ○ 2 pkgs
SPREADS, SAUCES, OTHER FOODS										
Margarine (not butter) on bread, rice, vegetables, or other foods	○	○	○	○	○	○	○	○	○	How many **pats (tsps)** ○ 1 ○ 2 ○ 3 ○ 4
Butter (not margarine) on bread, rice, vegetables, or other foods	○	○	○	○	○	○	○	○	○	How many **pats (tsps)** ○ 1 ○ 2 ○ 3 ○ 4
Mayonnaise, sandwich spreads	○	○	○	○	○	○	○	○	○	How many **tablespoons** ○ 1/2 ○ 1 ○ 2 ○ 3
Ketchup, salsa, chili sauce, chili peppers	○	○	○	○	○	○	○	○	○	How many **tablespoons** ○ 1/2 ○ 1 ○ 2 ○ 3
Mustard, barbecue sauce, soy sauce	○	○	○	○	○	○	○	○	○	How many **tablespoons** ○ 1/2 ○ 1 ○ 2 ○ 3
Gravy, or other rich sauces like Alfredo, white sauce, mole, peanut sauce	○	○	○	○	○	○	○	○	○	How many **cups** ○ 1/4 ○ 1/2 ○ 1
Jam, jelly, marmalade	○	○	○	○	○	○	○	○	○	How many **tablespoons** ○ 1/2 ○ 1 ○ 2 ○ 3
Pickles, pickled vegetables, sauerkraut, kimchi	○	○	○	○	○	○	○	○	○	How **much** ○ A ○ B ○ C ○ D
Salt, added to your food at the table	○	○	○	○	○	○	○	○	○	How many **shakes in a day** ○ 1-3 ○ 4-5 ○ 6-7 ○ 8+
BEVERAGES										
Chocolate milk, cocoa, hot chocolate	○	○	○	○	○	○	○	○	○	How many **12 ounce servings** ○ 1/2 ○ 1 ○ 2 ○ 3
Glasses of milk or soy milk, (not counting on cereal, in coffee, or chocolate milk)	○	○	○	○	○	○	○	○	○	How many **8 ounce servings** ○ 1 ○ 2 ○ 3 ○ 4
Meal replacement drinks like Slim Fast, Ensure, or high protein drinks or powders	○	○	○	○	○	○	○	○	○	How many **cans or glasses** ○ 1 ○ 2 ○ 3 ○ 4
Tomato juice, V-8, other vegetable juice	○	○	○	○	○	○	○	○	○	How many **8 ounce servings** ○ 1/2 ○ 1 ○ 2 ○ 3
Real 100% orange juice or grapefruit juice. Don't count orange soda or Sunny Delight.	○	○	○	○	○	○	○	○	○	How many **8 ounce servings** ○ 1/2 ○ 1 ○ 2 ○ 3
Other 100% juices, like apple, grape, 100% fruit blends, or fruit smoothies	○	○	○	○	○	○	○	○	○	How many **8 ounce servings** ○ 1/2 ○ 1 ○ 2 ○ 3
Hi-C, cranberry juice cocktail, Hawaiian Punch, Tang	○	○	○	○	○	○	○	○	○	How many **12 ounce servings** ○ 1/2 ○ 1 ○ 2 ○ 3
Drinks with some juice like Sunny Delight, Knudsen	○	○	○	○	○	○	○	○	○	How many **12 ounce servings** ○ 1/2 ○ 1 ○ 2 ○ 3
Iced tea, homemade, instant or bottled, like Nestea, Lipton, Snapple, Tazo	○	○	○	○	○	○	○	○	○	How many **16-oz. glasses or bottles** ○ 1/2 ○ 1 ○ 2 ○ 3
Gatorade, Powerade, or other sports drinks	○	○	○	○	○	○	○	○	○	How much **in a day** ○ 1 16-ounce bottle ○ 1 20-ounce bottle ○ 2 16-ounce bottles ○ 2 20-ounce bottles

	NEVER	A FEW TIMES per YEAR	ONCE per MONTH	2-3 TIMES per MONTH	ONCE per WEEK	2 TIMES per WEEK	3-4 TIMES per WEEK	5-6 TIMES per WEEK	EVERY DAY	HOW MUCH on those days? SEE PORTION SIZE PICTURES FOR A-B-C-D
Energy drinks like Red Bull, Rockstar, Monster	○	○	○	○	○	○	○	○	○	How much in a day: ○ 1 8-ounce can ○ 1 12-16 ounce can ○ 1 20-ounce can ○ 24 ounces or more
Kool-Aid, lemonade, fruit flavored drinks, like Crystal Light, atole, horchata (not iced tea)	○	○	○	○	○	○	○	○	○	How much in a day: ○ 1 8-ounce glass ○ 1 12-16-ounce glass or bottle ○ 1 20-ounce bottle ○ 30 ounces or more
Soft drinks, soda, pop, like cola, 7-Up, orange soda, regular or diet	○	○	○	○	○	○	○	○	○	How many in a day: ○ 1 can ○ 1 20-ounce bottle ○ 2 cans ○ Big Gulp or 3 cans
Beer or non-alcoholic beer	○	○	○	○	○	○	○	○	○	How much in a day: ○ 1 can ○ 2 cans ○ 3-4 cans or small pitcher ○ 5+ cans or large pitcher
Wine or wine coolers	○	○	○	○	○	○	○	○	○	How many glasses in a day: ○ 1/2 glass ○ 1 glass ○ 2 glasses, 1/2 bottle ○ 4+ glasses
Liquor or mixed drinks, cocktails	○	○	○	○	○	○	○	○	○	How many drinks: ○ 1 ○ 2 ○ 3 ○ 4
Water, bottled or tap	○	○	○	○	○	○	○	○	○	How many glasses: ○ 1 ○ 2 ○ 3-4 ○ 5+
Milky coffee drinks like latte, mocha, cappuccino, Frappuccino	○	○	○	○	○	○	○	○	○	How much in a day: ○ 12 oz ○ 16 oz ○ 20 oz ○ 24+ oz
Coffee (brewed or instant), regular or decaf	○	○	○	○	○	○	○	○	○	How many in a day: ○ 1 ○ 2 ○ 3 ○ 4+
Hot tea (not including herbal tea)	○	○	○	○	○	○	○	○	○	How many cups in a day: ○ 1 ○ 2 ○ 3 ○ 4+

MILKY COFFEE DRINKS: What kind do you usually drink? MARK ONLY ONE

○ Frappuccino ○ Mocha ○ Latte or cappuccino ○ Café con leche ○ Some of each ○ Don't drink them

What are your milky coffee drinks usually made with? **MARK ONLY ONE**

○ Whole milk ○ Skim milk or non-fat ○ Something else
○ 1 or 2% milk (reduced fat) ○ Soy milk ○ Don't drink

COFFEE: Is your coffee usually regular or decaf? ○ Decaf ○ Regular ○ Both kinds ○ Don't drink coffee

What do you usually add to your regular or decaf coffee? **MARK ONLY ONE**

○ Cream or half-n-half ○ Condensed milk ○ None of these
○ CoffeeMate, non-dairy creamer ○ Any other milk

Do you usually add sugar (or honey) to coffee? ○ No ○ Yes IF YES, how many teaspoons each cup? ○ 1 ○ 2 ○ 3 ○ 4

HOT TEA: Is your hot tea usually regular or decaf? ○ Decaf ○ Regular ○ I drink both kinds ○ Don't drink tea

What do you usually add to your hot tea? **MARK ONLY ONE**

○ Cream or half-n-half ○ Condensed milk ○ None of these
○ CoffeeMate, non-dairy creamer ○ Any other milk

Do you usually add sugar (or honey) to hot tea? ○ No ○ Yes IF YES, how many teaspoons each cup? ○ 1 ○ 2 ○ 3 ○ 4

If you eat the following foods, what type do you usually eat? MARK ONLY ONE ANSWER FOR EACH QUESTION

Milk	○ Whole milk	○ 2% milk	○ 1% milk (low-fat)	○ Skim milk, non-fat	
	○ Soy milk	○ Rice milk	○ Almond milk, other	○ Don't drink	
Slimfast, Ensure, or high protein drinks		○ Slimfast, Ensure, regular	○ Slimfast, Ensure, light or low-carb		
	○ High protein drinks, regular	○ High protein drinks, light or low-carb	○ Don't know/Don't drink		
Real 100% orange or grapefruit juice		○ Calcium-fortified	○ Not calcium-fortified	○ Don't know	○ Don't drink
Iced tea	○ Home-made, no sugar	○ Bottled, no-sugar	○ Don't drink		
	○ Home-made, with sugar	○ Bottled, pre-sweetened			
Drinks like Kool-Aid, lemonade, Crystal Light		○ Low-calorie, sugar-free	○ Regular		○ Don't drink
Energy drinks like Red Bull, Monster		○ Sugar-free	○ Regular		○ Don't drink
Soft drinks, soda, pop	○ Diet, low-calorie	○ Regular	○ Don't drink		
Do they usually have caffeine?		○ Has caffeine	○ No caffeine	○ Don't drink	
Beer	○ Regular	○ Light	○ Non-alcoholic		○ Don't drink
Wine or wine cooler	○ Red wine	○ White wine	○ Both red and white wine		○ Don't drink
Cheese	○ Low-fat	○ Regular-fat	○ Don't eat		
Yogurt	○ Plain (no sugar or fruit)	○ With fruit or other flavors			
Yogurt	○ Low-fat	○ Non-fat	○ Regular (whole milk)		○ Don't eat
Salad dressing	○ Low-fat, lite	○ Fat free	○ Regular	○ Oil & vinegar	○ Don't use
Spaghetti or lasagna	○ Meatless	○ With meat sauce or meatballs			○ Don't eat
Noodles, pasta	○ Rarely whole grain	○ Sometimes whole grain	○ Usually whole grain	○ Don't know/don't eat	
Burgers	○ Hamburger	○ Cheeseburger	○ Turkey burger		○ Don't eat
Beef or pork	○ Avoid eating the fat	○ Sometimes eat the fat	○ Often eat the fat		○ Don't eat
Chicken or turkey	○ Avoid eating the skin	○ Sometimes eat the skin	○ Often eat the skin		○ Don't eat
Hot dogs, dinner sausage	○ Beef or pork	○ Chicken or turkey, low-fat			○ Don't eat
Lunch meats	○ Beef or pork	○ Chicken or turkey, low-fat			○ Don't eat
Cakes, snack cakes, cupcakes	○ Low-sugar, low-carb	○ Low-fat	○ Regular-fat		○ Don't eat
Cookies, brownies	○ Low-sugar, low-carb	○ Low-fat	○ Regular-fat		○ Don't eat
Ice cream, frozen yogurt	○ Low-sugar, low-carb	○ Low-fat or frozen yogurt	○ Regular		○ Don't eat
Energy or protein bars	○ High energy	○ High protein	○ Some of each	○ Don't know	○ Don't eat
Bagels, English muffins, rolls	○ White	○ Multi-grain	○ 100% whole wheat	○ Eat all kinds	○ Don't eat
Burger, hot dog, submarine buns	○ White	○ Multi-grain	○ 100% whole wheat	○ Eat all kinds	○ Don't eat
Bread	○ White (not whole grain)	○ 100% whole wheat			○ Don't eat
	○ Multi-grain, rye, or other brown bread	○ Eat some of each			
Tortillas	○ Corn tortillas	○ Flour tortillas (wheat)	○ Eat all kinds or don't know		○ Don't eat
Popcorn	○ Air popped, fat-free	○ Low-fat or light	○ Regular	○ Caramel corn	○ Don't know / ○ Don't eat
Crackers, pretzels	○ Low-fat, including RyeKrisp, rice cakes, or plain pretzels			○ Don't know	
	○ Regular-fat crackers or filled pretzels			○ Don't eat	
Mayonnaise or sandwich spreads	○ Low-fat, light	○ Regular		○ Don't eat	

If you eat **cold cereals,** what do you usually eat? Choose **ONE or TWO** that you eat most often. If you usually eat just one kind, only choose one.

○ All-Bran Original	○ Cinnamon Toast Crunch	○ Grape Nuts	○ Special K, plain
○ All-Bran Complete, Complete	○ Cocoa Krispies, Pebbles, Puffs	○ Honey Bunches of Oats	○ Special K, flavors
○ Apple Jacks, Cookie Crisp	○ Corn Flakes, Corn Puffs	○ Kashi GOLEAN, Heart to Heart	○ Total
○ Bran Flakes	○ Corn Pops	○ Life	○ Wheaties
○ Cap'n Crunch	○ Fiber-One, Bran Buds	○ Lucky Charms, Fruity Pebbles	○ *Other sweet cereal*
○ Cheerios, plain or Multi-Grain	○ Froot Loops	○ Oatmeal Squares, Oat Bran	○ *Other unsweetened cereal*
○ Cheerios, Honey Nut, flavors	○ Frosted Flakes	○ Raisin Bran	○ *Other whole grain cereal*
○ Chex, Wheat	○ Frosted Mini-Wheats	○ Rice Krispies, puffed rice	○ *Other bran or fiber cereal*
○ Chex, other	○ Granola	○ Shredded Wheat	○ *Don't eat cereal*

Which **fats or oils** are used most often for **cooking or frying** (not baking) in your home? **MARK ONLY ONE OR TWO**

○ Non-stick spray or none	○ Soft tub margarine	○ Corn oil, vegetable oil and blends	○ Other oil
○ Butter or ghee	○ Low-fat margarine	○ Peanut oil	○ Don't know
○ Butter/margarine blend	○ Olive oil	○ Lard, fatback, or bacon fat	
○ Stick margarine	○ Canola oil, safflower oil	○ Vegetable shortening, Crisco	

PLEASE DO NOT WRITE IN THIS AREA

○○○○○○○○○○○○○○○○○○○○○○○○○

SERIAL #

What vitamin supplements do you take fairly regularly?

Multiple Vitamins. Do you take...	HOW OFTEN: DIDN'T TAKE	A FEW DAYS per MONTH	1 DAY per WEEK	2 DAYS per WEEK	3-4 DAYS per WEEK	5-6 DAYS per WEEK	EVERY DAY	FOR HOW MANY YEARS? LESS THAN 1 YEAR	1-4 YEARS	5-9 YEARS	10+ YEARS
Prenatal vitamins	○	○	○	○	○	○	○	○	○	○	○
Regular One-A-Day, Centrum, "senior" vitamins or house brands of multiple vitamins	○	○	○	○	○	○	○	○	○	○	○
Stress-tabs or B-Complex type	○	○	○	○	○	○	○	○	○	○	○
Antioxidant combination, eye formula	○	○	○	○	○	○	○	○	○	○	○
Single Vitamins or Minerals, taken alone or in combination. Do not count what is in your multiple vitamins above.											
Vitamin A (not beta-carotene)	○	○	○	○	○	○	○	○	○	○	○
Vitamin B-6	○	○	○	○	○	○	○	○	○	○	○
Vitamin B-12	○	○	○	○	○	○	○	○	○	○	○
Vitamin C	○	○	○	○	○	○	○	○	○	○	○
Vitamin D	○	○	○	○	○	○	○	○	○	○	○
Vitamin E	○	○	○	○	○	○	○	○	○	○	○
Folic acid, folate	○	○	○	○	○	○	○	○	○	○	○
Calcium or antacids with calcium, like Tums	○	○	○	○	○	○	○	○	○	○	○
Iron	○	○	○	○	○	○	○	○	○	○	○
Zinc	○	○	○	○	○	○	○	○	○	○	○
Cod liver oil, other fish oils, omega-3, flax seed oil, algae	○	○	○	○	○	○	○	○	○	○	○
Fiber supplements like Benefiber, Metamucil	○	○	○	○	○	○	○	○	○	○	○

If you take One-A-Day, Centrum or other types of multiple vitamins, do you usually take types that

○ Contain minerals, iron, zinc, etc. ○ Do not contain minerals ○ Don't know

If you take vitamin C, how many milligrams of **vitamin C** do you usually take, on the days you take it? (Select the closest amount)

○ 100 ○ 250 ○ 500 ○ 750 ○ 1000 ○ 1500 ○ 2000 ○ 3000+ ○ Don't know

If you take vitamin E, how many IUs of **vitamin E** do you usually take, on the days you take it? (Select the closest amount)

○ 100 ○ 200 ○ 300 ○ 400 ○ 600 ○ 800 ○ 1000 ○ 2000+ ○ Don't know

If you take calcium, how many milligrams of **calcium** do you usually take, on the days you take it? (Select the closest amount)

○ 100 ○ 350 ○ 650 ○ 1250+ ○ Don't know

If you take vitamin D, how many IUs of **vitamin D** do you usually take, on the days you take it? (Select the closest amount)

○ 400 ○ 600 ○ 800 ○ 1000 ○ 2000 ○ 3000 ○ 4000 ○ 5000+ ○ Don't know

If you take omega-3 supplements, what type do you usually take? **MARK ALL THAT APPLY**

○ Fish oil ○ Flax oil, hemp oil, other seed oil ○ Krill oil ○ Algae ○ Don't know

SOME LAST QUESTIONS ABOUT YOU

	RARELY	1-2 per WEEK	3-4 per WEEK	5-6 per WEEK	1 per DAY	1 1/2 per DAY	2 per DAY	3 per DAY	4+ per DAY
About how many servings of vegetables do you eat, not counting salad or potatoes? 1 serving = 1/2 cup.	○	○	○	○	○	○	○	○	○
About how many servings of fruit do you eat, not counting juices? 1 serving = 1/2 cup or 1 medium fruit.	○	○	○	○	○	○	○	○	○
How often do you eat foods prepared at home that are cooked or fried in **fat or oil?**	○	○	○	○	○	○	○	○	○

During a regular day, how many **meals and snacks** do you usually eat?

Meals per day	○ 1	○ 2	○ 3	○ 4	○ 5+
Snacks per day	○ 1	○ 2	○ 3	○ 4	○ 5+

PHYSICAL ACTIVITY SURVEY

Think about the last 12 months. How often did you do the activities listed below?

	HOW OFTEN IN THE PAST YEAR						HOW MUCH TIME ON THOSE DAYS			
	RARELY OR NEVER	A FEW TIMES A MONTH	ONCE OR TWICE A WEEK	3-4 TIMES A WEEK	5-6 TIMES A WEEK	ALMOST EVERY DAY	LESS THAN 30 MINUTES	30-60 MINUTES	1-2 HOURS	3 OR MORE HOURS
Cooking, shopping, light cleaning like doing laundry or dusting, or running errands	○	○	○	○	○	○	○	○	○	○
Slow walking like walking the dog, or light work around the house like watering	○	○	○	○	○	○	○	○	○	○
Work on the job involving standing, like store clerk, or work involving driving (like truck driver)	○	○	○	○	○	○	○	○	○	○
Taking care of children (feeding, dressing), or moderate housework like sweeping, mopping, cleaning the tub	○	○	○	○	○	○	○	○	○	○
Weeding, raking, mowing the lawn, or light house repairs	○	○	○	○	○	○	○	○	○	○
Brisk walking, dancing, hunting or fishing, golf (NOT using a golf cart), or 'friendly' outdoor games like softball	○	○	○	○	○	○	○	○	○	○
Factory work, mechanic, restaurant work, or work involving walking, like mail carrier	○	○	○	○	○	○	○	○	○	○
Construction, painting, feeding livestock, or homecare like caring for an adult family member	○	○	○	○	○	○	○	○	○	○
Heavy work like moving boxes, heavy digging or shoveling snow, farm chores like baling hay, or other HARD labor	○	○	○	○	○	○	○	○	○	○
Exercising at the gym or at home, aerobics, weight training, jogging, or vigorous sports like basketball, soccer, tennis	○	○	○	○	○	○	○	○	○	○
Bicycling or swimming for exercise	○	○	○	○	○	○	○	○	○	○

Are you ○ Hispanic or Latino ○ Not Hispanic or Latino ○ Do not wish to provide this information

What race do you consider yourself to be? **MARK ALL THAT APPLY**

- ○ White
- ○ Black or African American
- ○ Asian
- ○ American Indian or Alaska Native
- ○ Native Hawaiian or Other Pacific Islander
- ○ Do not wish to provide this information

Thank you very much for filling out this questionnaire.
Please take a minute to go back and fill in anything you may have skipped.

PLEASE DO NOT WRITE IN THIS AREA

SERIAL #

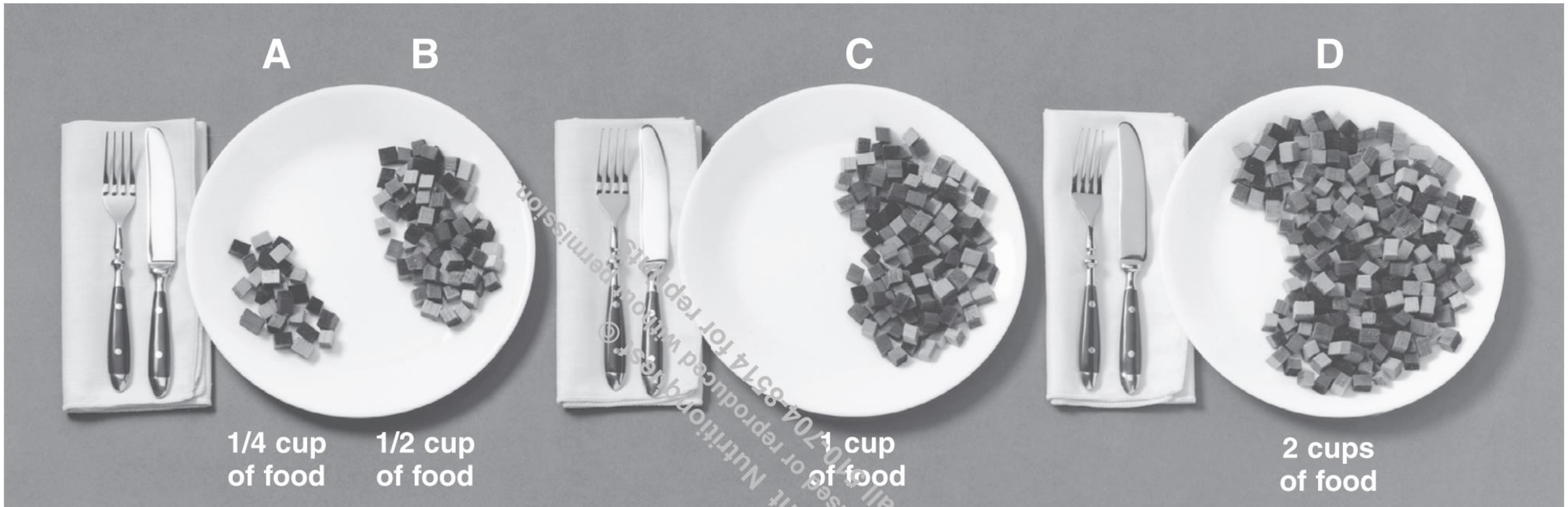
Fold along this line, then carefully detach.

1/4" SPINE PERF

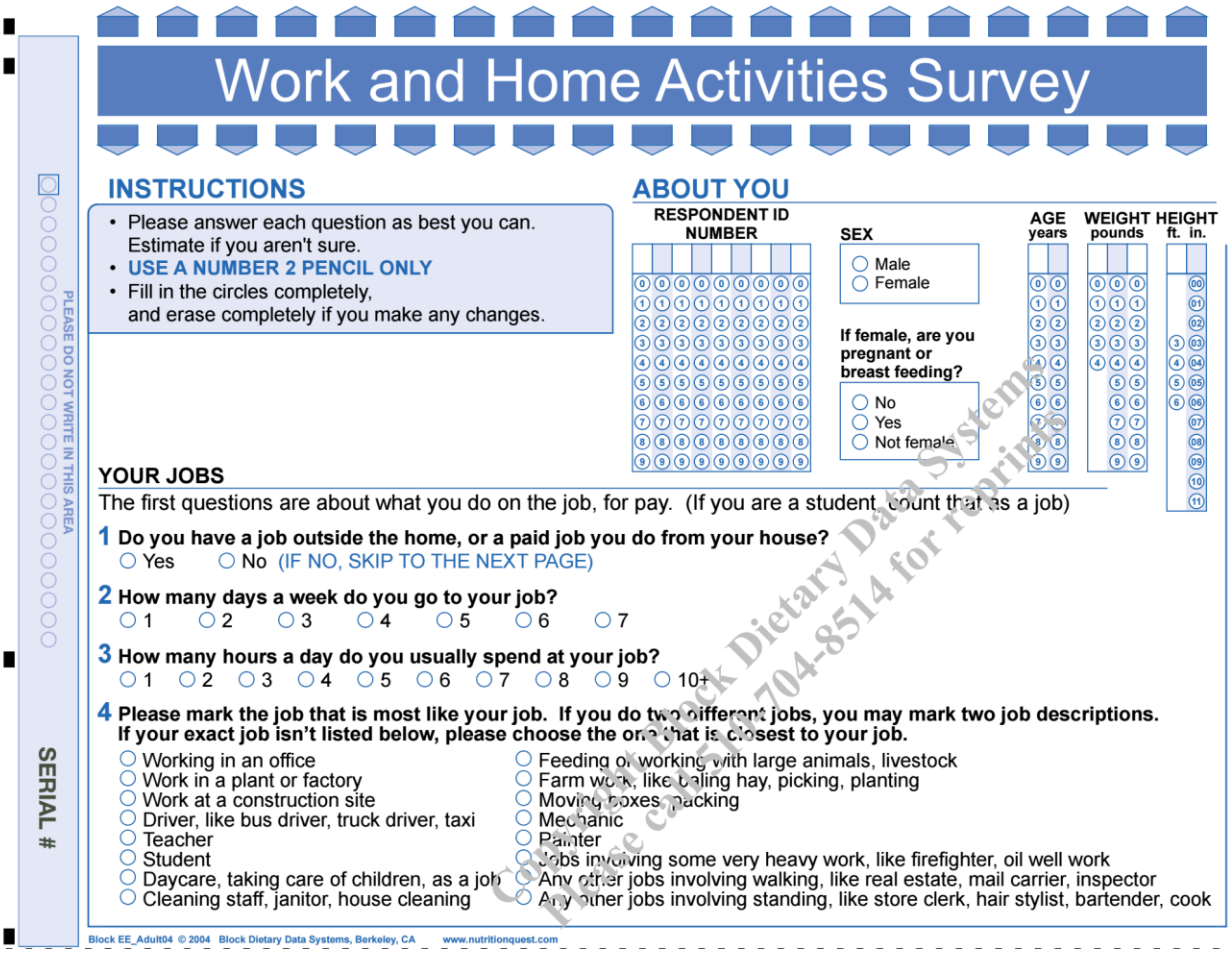
Portion Size Choices

Keep this in front of you while you are filling out The Food Questionnaire. You may use <u>either the plates or the bowls</u> to help you choose your usual portion size.

Choose A, B, C or D: **A** = 1/4 Cup of Food **B** = 1/2 Cup of Food **C** = 1 Cup of Food **D** = 2 Cups of Food



APPENDIX H:
2015 BLOCK ADULT PHYSICAL ACTIVITY QUESTIONNAIRE



Work and Home Activities Survey

PLEASE DO NOT WRITE IN THIS AREA

SERIAL #

INSTRUCTIONS

- Please answer each question as best you can. Estimate if you aren't sure.
- **USE A NUMBER 2 PENCIL ONLY**
- Fill in the circles completely, and erase completely if you make any changes.

ABOUT YOU

RESPONDENT ID NUMBER

SEX
○ Male
○ Female

If female, are you pregnant or breast feeding?
○ No
○ Yes
○ Not female

AGE years

WEIGHT pounds

HEIGHT ft. in.

YOUR JOBS

The first questions are about what you do on the job, for pay. (If you are a student, count that as a job)

1 **Do you have a job outside the home, or a paid job you do from your house?**
○ Yes ○ No (IF NO, SKIP TO THE NEXT PAGE)

2 **How many days a week do you go to your job?**
○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7

3 **How many hours a day do you usually spend at your job?**
○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8 ○ 9 ○ 10+

4 **Please mark the job that is most like your job. If you do two different jobs, you may mark two job descriptions. If your exact job isn't listed below, please choose the one that is closest to your job.**

○ Working in an office
○ Work in a plant or factory
○ Work at a construction site
○ Driver, like bus driver, truck driver, taxi
○ Teacher
○ Student
○ Daycare, taking care of children, as a job
○ Cleaning staff, janitor, house cleaning
○ Feeding or working with large animals, livestock
○ Farm work, like baling hay, picking, planting
○ Moving boxes, packing
○ Mechanic
○ Painter
○ Jobs involving some very heavy work, like firefighter, oil well work
○ Any other jobs involving walking, like real estate, mail carrier, inspector
○ Any other jobs involving standing, like store clerk, hair stylist, bartender, cook

YOUR ACTIVITIES NOT COUNTING YOUR JOB: The following questions are about all the activities you did in the last 12 months, **NOT COUNTING YOUR JOB.** Think about the hours and days when you are not at your job.

EXAMPLE: This person plays golf 1-2 times a week, for about 2 hours each time:

Activities NOT counting the time on your job	HOW OFTEN in the past year?						How much time, ON THOSE DAYS?						
	Rarely or Never	A few times a MONTH	1-2 times a WEEK	3-4 times a WEEK	5-6 times a WEEK	EVERY DAY	Less Than 30 Minutes	30-59 Minutes	1 Hour	1½ Hours	2 Hours	3-4 Hours	More Than 4 Hours
Playing golf	○	○	●	○	○	○	○	○	○	○	●	○	○

For example, if you work from 9 to 5, think about mornings before work (including getting to work), evenings after work, and weekends.

Please mark one bubble for **HOW OFTEN** you did each activity. And, if you did it, mark one bubble for **HOW MUCH TIME** you usually spend doing it.

How often do you do these activities, NOT counting the time on your job. Things you do for exercise, fitness or fun	HOW OFTEN in the past year?						How much time, ON THOSE DAYS?						
	Rarely or Never	A few times a MONTH	1-2 times a WEEK	3-4 times a WEEK	5-6 times a WEEK	EVERY DAY	Less Than 30 Minutes	30-59 Minutes	1 Hour	1½ Hours	2 Hours	3-4 Hours	More Than 4 Hours
Walking fast, for exercise	○	○	○	○	○	○	○	○	○	○	○	○	○
Other walking, like walking the dog, doing errands, walking to school	○	○	○	○	○	○	○	○	○	○	○	○	○
Running, jogging, bike riding	○	○	○	○	○	○	○	○	○	○	○	○	○
Swimming for exercise	○	○	○	○	○	○	○	○	○	○	○	○	○
Exercise on treadmill, stair climber, exercise bike, rowing machine, other cardio machines	○	○	○	○	○	○	○	○	○	○	○	○	○
Other exercise, like weight machines, free weights, push-ups	○	○	○	○	○	○	○	○	○	○	○	○	○
Sports like basketball, softball, tennis, skiing	○	○	○	○	○	○	○	○	○	○	○	○	○
Fishing, hunting, camping, hiking	○	○	○	○	○	○	○	○	○	○	○	○	○
Playing golf	○	○	○	○	○	○	○	○	○	○	○	○	○
Any other fitness activity not mentioned **How often?**	○	○	○	○	○	○	○	○	○	○	○	○	○

What was it? _____

Was it ○ Not vigorous ○ Somewhat vigorous ○ Very vigorous

Activities, NOT counting the time on your job. Other things you do when you're not on the job	HOW OFTEN in the past year?						How much time, ON THOSE DAYS?						
	Rarely or Never	A few times a MONTH	1-2 times a WEEK	3-4 times a WEEK	5-6 times a WEEK	EVERY DAY	Less Than 30 Minutes	30-59 Minutes	1 Hour	1½ Hours	2 Hours	3-4 Hours	More Than 4 Hours
Going to and from your job in a car or bus, (not counting during your job)	○	○	○	○	○	○	○	○	○	○	○	○	○
Other driving or riding in a car or bus for other reasons, like going shopping, going out	○	○	○	○	○	○	○	○	○	○	○	○	○
Watching TV or movie	○	○	○	○	○	○	○	○	○	○	○	○	○
On the Internet, email, video games or computer work (Not counting your job)	○	○	○	○	○	○	○	○	○	○	○	○	○
Just sitting: for example, reading the paper or a book, or writing	○	○	○	○	○	○	○	○	○	○	○	○	○
Taking care of a child, such as feeding, bathing, dressing	○	○	○	○	○	○	○	○	○	○	○	○	○
Food preparation: for example, cooking, baking, setting table	○	○	○	○	○	○	○	○	○	○	○	○	○
Shopping for food, putting groceries away	○	○	○	○	○	○	○	○	○	○	○	○	○
Shopping for other things, not food, like clothes	○	○	○	○	○	○	○	○	○	○	○	○	○
Washing dishes, cleaning the kitchen	○	○	○	○	○	○	○	○	○	○	○	○	○
Any other house cleaning: for example, sweeping, making beds, cleaning bathroom	○	○	○	○	○	○	○	○	○	○	○	○	○

SERIAL #

PLEASE DO NOT WRITE IN THIS AREA

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Activities, NOT counting the time on your job. Other things you do when you're not on the job	HOW OFTEN in the past year?						How much time, ON THOSE DAYS?						
	Rarely or Never	A few times a MONTH	1-2 times a WEEK	3-4 times a WEEK	5-6 times a WEEK	EVERY DAY	Less Than 30 Minutes	30-59 Minutes	1 Hour	1½ Hours	2 Hours	3-4 Hours	More Than 4 Hours
Working outside (not on the job): for example, yard work, mowing, weeding	○	○	○	○	○	○	○	○	○	○	○	○	○
House repairs, maintenance, painting, workshop	○	○	○	○	○	○	○	○	○	○	○	○	○
Talking with friends, socializing, visiting, in person or on the phone	○	○	○	○	○	○	○	○	○	○	○	○	○
Attending meetings, social events, church, temple, PTA and so forth	○	○	○	○	○	○	○	○	○	○	○	○	○
Any other home or leisure activity not mentioned													
How often? →	○	○	○	○	○	○	○	○	○	○	○	○	○

What was it? _____

Was it ○ Not vigorous ○ Somewhat vigorous ○ Very vigorous

YOUR SLEEP

On average, how many hours a night do you sleep (from lights out to when you get up)?

○ 4 ○ 4½ ○ 5 ○ 5½ ○ 6 ○ 6½ ○ 7 ○ 7½ ○ 8 ○ 8½ ○ 9 ○ 9½ ○ 10+

THANK YOU for filling out this survey.

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