2011

The Effects of Chronic Prophylactic Naproxen Sodium on Skeletal Muscle Adaptation to Resistance Exercise in Recreationally-Trained College-Aged Males

Christi Brewer

THE EFFECTS OF CHRONIC PROPHYLACTIC NAPROXEN SODIUM ON SKELETAL MUSCLE ADAPTATION TO RESISTANCE EXERCISE IN RECREATIONALLY-TRAINED COLLEGE-AGED MALES

A Dissertation
presented in partial fulfillment of requirements
for the degree of Doctor of Philosophy
in the Department of Health, Exercise Science, and Recreation Management
The University of Mississippi

by

CHRISTI BREWER

December 2011

ABSTRACT

Resistance exercise causes exercise-induced muscle injury (EIMI), triggering an acute local inflammatory response associated with delayed-onset muscle soreness (DOMS). DOMS is characterized by a predictable discomfort and decreased level of muscle performance and is believed to be the product of both mechanical and inflammatory processes. From the latter perspective, non-steroidal anti-inflammatory drugs (NSAIDs) have been investigated for their potential to alleviate DOMS-associated symptoms. Such analgesics exert their effects by blocking the formation of prostaglandins; however, prostaglandins, specifically prostaglandin $F_{2\alpha}$, also play an important role in skeletal muscle regeneration. While some have advocated the use of NSAIDs for the relief of EAP, research demonstrating the necessity of inflammation for adaptation and regeneration suggests their use may be counter-productive for those striving toward muscular fitness goals. In this double-blind design, twenty-three recreationally-trained college-aged males were randomly assigned to placebo or naproxen sodium treatment groups. Treatments were prophylactically administered twice per week, in conjunction with a supervised periodized upper body resistance training program. Main effects for time and exercise were seen for repeated acute systemic plasma $PGF_{2\alpha}$ metabolite concentrations, acute arm circumference response to exercise, strength, dominant arm lean and fat tissue, and non-dominant arm bone mineral content. No significant treatment effects were detected for any dependent variables, thus twice weekly naproxen sodium (440mg) does not appear to inhibit skeletal muscle adaptation to resistance exercise in recreationally-trained college-aged males over a 6-week time period.

LIST OF ABBREVIATIONS AND SYMBOLS

AA	Arachidonic acid
COX	Cyclooxygenase enzyme
DOMS	Delayed-onset muscle soreness
EIMI	Exercise-induced muscle injury
EAP	Exercise-associated pain
FSR	Fractional synthesis rate
MVC	Maximal voluntary contraction
NSAIDs	Non-steroidal anti-inflammatory drugs
PG	Prostaglandin
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
RM	Repetition maximum

ACKNOWLEDGEMENTS

This project would not have been possible without the support of my entire committee who took a leap of faith on the measurement technique employed in the study but believed in the importance of the study.

Great appreciation is expressed to Dr. Elaine Day, Assistant Professor of Biology, who generously offered her time and laboratory expertise to teach me to perform enzyme immunoassays. I had the desire to develop a skill set which I could take forward into my career, and she patiently facilitated this process.

I would also like to thank Lisa Huggins, RPh, who provided me with the placebo design and generously contributed pharmaceutical supplies.

I would like extend sincere thanks to my advisor and dissertation chair Dr. Dwight Waddell, who has been there since it all began and has provided financial support, encouragement, objectivity, patience, and whose love for research is inspiring.

Last but not least, I would like to acknowledge my parents (Gary and Linda Bouchillon) who made me believe I could accomplish anything. Also, to my husband (Paul) and son (Dempsey) who provided love, support, and patience as I completed this project.

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Chapter I

Introduction

Novel or intense exercise, particularly resistance training, causes exercise-induced muscle injury (EIMI). Such injury triggers an acute local inflammatory response and is associated with delayed-onset muscle soreness (DOMS). DOMS is characterized by a predictable discomfort and decreased level of muscle performance that peaks 24 hours and persists 48-72 hours post-exercise (Jones, Newham and Torgan, 1989; Donnelly, Maughan and Whiting, 1990; MacIntyre, Reid, Lyster, Szasz and McKenzie, 1996; Lecomte, Lacroix and Montgomery, 1998; Almekinders, 1999; Semark, Noakes, St Clair Gibson and Lambert, 1999; MacIntyre, Reid, Lyster and McKenzie, 2000; Baldwin, Stevenson and Dudley, 2001; Trappe, White, Lambert, Cesar, Hellerstein and Evans, 2002). DOMS is also characterized by diffuse muscle pain, swelling, and stiffness; and while several therapeutic options are available to ameliorate the discomfort associated with exercise-induced muscle injury, non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most popular. NSAIDs belong to a family of drugs that have achieved widespread use in sports medicine in both prescription and non-prescription forms (Hertel, 1997). Their use as a therapeutic treatment for EIMI has increased dramatically over the last 20 years (Lanier, 2003), and 40% of non-prescription analgesic users cite exercise-associated pain as their primary impetus to take NSAIDs (Wilcox, Cryer, and Triadafilopoulos, 2005).

Skeletal muscle adaptation to resistance exercise involves changes in nervous, musculoskeletal and endocrine systems, including enhanced motor unit recruitment, strengthened

muscle and connective tissue, and hormonal adaptations. The cumulative effects of these changes result in protection from damage incurred during subsequent bouts of similar exercise, a phenomenon referred to as the repeated bout effect (Ebbeling and Clarkson, 1989; McHugh, Connolly, Eston and Gleim, 1999). Specific patterns of nervous system stimulation during resistance training result in the simultaneous activation of hormonal changes related to recovery and adaptation of skeletal muscle tissue (Kraemer, Vingren and Spiering, 2008). The type of resistance training program performed dictates hormonal responses, which are intimately involved in protein synthesis and degradation, as well as the shifts between the two during remodeling (Kraemer, Vingren, Spiering, 2008). This is particularly true of prostaglandins (PG), hormones synthesized from skeletal muscle constituents upon membrane disruption and whose response has been shown to be affected by stimulus intensity (Boushel, Langberg, Gemmer, Olesen, Crameri, Scheede, Sander and Kjaer, 2002).

Animal research employing electrical stimulation and passive movement has demonstrated adaptation to muscular contraction relies on inflammation (Lapointe, Fremont and Cote, 2002; Pizza, Koh, McGregor and Brooks, 2002), making suppression of the acute inflammatory response controversial. While some have advocated the use of NSAIDs for the relief of exercise-associated pain (Donnelly et al., 1990; Gulick, 1996; Pizza, Cavender, Stockard, Baylies and Beighle, 1999; Baldwin et al., 2001; Lanier, 2003), aforementioned research demonstrating the necessity of inflammation for adaptation and regeneration suggests their use may be counter-productive for those striving toward muscular fitness goals (Lapointe et al., 2002; Pizza et al., 2002). Additional animal research has also demonstrated that suppression of the acute inflammatory response may provide immediate benefit, but this was followed by a decrement in muscle function at both early (3-7 days) and delayed (1 month) time points post-passive movement and laceration injury models, respectively (Mishra, Friden, Schmitz and Lieber, 1995; Shen, Li, Tang, Cummins and Huard, 2005).

It is important to acknowledge inherent pathophysiological differences between certain inflammatory responses and the necessity of NSAIDs in the associated injury models. In the clinical field, NSAIDs are prescribed to reduce inflammation resulting from muscle strains, contusions, or surgical lacerations. The substantial inflammation resulting from such types of injuries impedes rehabilitation and delays the return to activity, and NSAIDs are considered a requisite component of treatment. The inflammatory response associated with EIMI is less than that seen in the aforementioned injury models (Almekinders, 1999), and the use of NSAIDs is often optional. Due to these differences, the effects of NSAIDs on the inflammatory response associated with one type of injury cannot be assumed for other types of injuries. As an example, Almekinders, Banes, and Ballenger have cited a lack of fibroblast activation in exercise-induced injury models (1993). The current investigation will thus focus on the use of non-steroidal anti-inflammatory drugs in exercise-induced muscle injury, incorporating human and animal experimental research that investigated inflammation using muscular contraction models of injury.

Much of the existing literature concerning the role of NSAIDs in exercise-induced muscle injury has been investigated from the standpoint of DOMS treatment or prevention. While the majority has found no therapeutic benefit of NSAIDs on these symptoms (Donnelly et al., 1990; Gulick, 1996; Howell, Conatser, Chleboun, Karapondo and Chila, 1998; Pizza et al., 1999; Trappe et al., 2002; Peterson, Trappe, Mylona, White, Lambert, Evans and Pizza, 2003; Krentz, Quest, Farthing, Quest and Chilibeck, 2008; Mikkelsen, Langberg, Helmark, Skovgaard, Andersen, Kjaer and Mackey, 2009), some research has reported a positive effect of NSAIDs on exercise-induced muscle injury (Hasson, Daniels, Divine, Niebuhr, Richmond, Stein and Williams, 1993; Dudley, Czerkawski, Meinrod, Gillis, Baldwin and Scarpone, 1997; Baldwin et al., 2001). A less thoroughly investigated aspect of the role of NSAIDs in EIMI is the potential to unintentionally suppress

skeletal muscle regeneration or adaptation to resistance exercise in an attempt to alleviate symptoms associated with the acute inflammatory response.

Baldwin, Stevenson, and Dudley (2001) demonstrated an immediate preservation and maintenance of concentric and isometric strength in older males and females (n=15) who consumed naproxen sodium for 10 days (220 mg three times per day) after an acute bout of eccentric knee extensions. Dudley, Czerkawski, Gillis, Baldwin, and Scarpone (1997) reported reduced thigh soreness and improved recovery of concentric one-repetition maximum (1RM) in young males (n=8) who consumed naproxen sodium for 10 days after acute eccentric knee extensions. Using therapeutic administration of naproxen (500 mg twice per day for 6 days) after two 8-day bouts of eccentric single leg exercise separated by seven days for washout of the repeated bout effect, Lecomte, Lacroix, and Montgomery (1998) also reported decreased soreness and preserved peak quadriceps torque in naproxen-treated male participants (n=10) versus placebo-treated males (n=10). Hasson et al. (1993) also reported ibuprofen-treated participants perceived 40-50% less soreness and experienced significantly less decline in torque and EMG magnitude after acute exercise compared to placebo and control groups (N=20); however, animal research demonstrating long-term decrements accompanying initial short-term benefits should not be forgotten (Mishra et al., 1995; Shen et al., 2005). It is interesting to note that three of the four papers to report any beneficial effect of NSAIDs on DOMS employed naproxen sodium.

Mechanisms postulated to explain the phenomenon of DOMS are controversial. Some have argued DOMS is independent of inflammation (Almekinders, 1999; Semark et al., 1999), thus treatment with NSAIDs would be unlikely to yield dramatic results; however, the analgesic effect of these drugs confounds the assessment of muscle performance. Tidball, Berchenko, and Frenette (1999) pointed out there is little experimental evidence to demonstrate muscle injury and inflammation associated with muscle loading is exclusively attributable to mechanical factors.

Research that has reported a bimodal response in strength recovery after resistance exercise has postulated the initial decrease is attributable to mechanical events (Gulick, 1996), while the second decrease is due to phagocytic activity at the initial site of damage (MacIntyre et al., 1996; MacIntyre et al., 2000).

The inflammatory response is considered to begin with the influx of calcium into the cell through the abundant stretch-activated calcium channels located on the cell membrane. Neutrophils are the first of many inflammatory mediators to arrive at the site of an injury. Approximately 50% of neutrophils released from bone marrow are sequestered along blood vessel walls. Neutrophil infiltration is stimulated by chemotaxic factors, including prostaglandins, tumor necrosis factor-α, interleukin (IL)-1β, and IL-6 (Stupka, 2000). Neutrophils are activated as they circulate through a blood vessel in an injured area. One consequence of this activation is a "respiratory burst," during which neutrophils generate high concentrations of reactive oxygen species (ROS), or free radicals such as nitric oxide and hydrogen peroxide, which destroy engulfed pathogens (Martini, 2004; Butterfield, 2006). In addition to free radicals, neutrophils also release proteolytic enzymes such as elastase and lysozyme from their granules (Cannon, Orencole, Fielding, Meydani, Meydani, Fiatarone, Blumberg and Evans, 1990; Stupka, 2000). These molecules are chemically unstable compounds that attack and degrade cell membranes. This degradation is somewhat non-specific and may damage neighboring cells (Stupka, 2000). Very well-designed animal studies have clearly demonstrated secondary damage occurring as a result of these superoxide-dependent mechanisms (Tidball, Berchenko and Frenette, 1999; Toumi, F'guyer and Best, 2006).

Human research has also demonstrated a bimodal pattern of maximal voluntary contraction (MVC) recovery after isometric and concentric muscle action (Jones et al., 1989; Gulick, 1996) and eccentric torque after eccentric muscle action (MacIntyre et al., 1996; MacIntyre et al., 2000). While mechanical factors explain the initial and immediate decrease in muscle contractile ability, the second

decrease in muscle contractile ability is believed to be explained by the concomitant increase in white blood cells and associated free radicals in the hours after exercise (Cannon et al., 1990; Fielding, Manfredi, Ding, Fiatarone, Evans and Cannon, 1993; MacIntyre et al., 1996; Malm, 1999; MacIntyre et al., 2000; Malm, 2000). A positive correlation (rho=0.66, $p<0.001$) between neutrophil infiltration and intracellular z-band damage has been reported post-downhill running in males (n=9) (Fielding et al., 1993).

Further evidence for non-mechanical factors in exercise-induced muscle injury has been provided by research demonstrating neutrophil activation in ischemic and inactivity injury models. Using Western blot analysis and electron microscopy of myotubes co-cultured with neutrophils and/or macrophages muscle, non-exercise models of injury in animals have shown secondary damage resulting from neutrophils and macrophages (Nguyen and Tidball, 2003). Their action in non-contractile injury models strongly suggests the primary mechanism of tissue destruction is their release of cytotoxic agents (Butterfield, Best et al. 2006). Further evidence for this cytotoxic damage has been provided by Brickson et al. (2003) and Nguyen and Tidball (2003), who demonstrated significantly less myofibril damage in animals treated with a specific antibody to block neutrophil oxidation post-electrical stimulation and in myotubes cultured with specific free radical blockers, respectively. Human research has suggested eccentric exercise causes an up-regulation in the functional capacity of neutrophils to produce reactive oxygen species (Pizza et al., 1999).

Regardless of the exact mechanism of injury, inflammation is always followed by an attempt at regeneration (Martini, 2004; MacIntosh, 2006). The two processes are mechanistically linked, and suppression of initial inflammatory response could theoretically blunt the subsequent regenerative response. For instance, while macrophages, like neutrophils, have also been shown to elicit damage through free radical–mediated mechanisms, they also stimulate repair and release cell growth

regulatory cytokines such as fibroblast growth factor (FGF), insulin-like growth factor (IGF-1), and transforming growth factor-β1 (TGF- β1) (Butterfield, Best et al. 2006).

Prostaglandins (PGs) are a particularly interesting molecule in the inflammatory response. While it has been suggested virtually all mammalian cells may produce and respond to prostaglandins, they are not considered to exist pre-formed in any cellular reservoir (Pettipher, 1998). Rather, the precursor C_{20} unsaturated fatty acid is present in tissues and is hydrolyzed by phospholipase A_2 (Rifai, Bachorick and Albers, 1999). Prostaglandins' role in inflammation is vital, as they contribute to increased blood flow, vascular permeability, and pain sensation resulting from injury. It is for these reasons that prostaglandins are the primary target of non-steroidal anti-inflammatory drugs.

Prostaglandins, however, also stimulate satellite cell proliferation ($PGF_{2\alpha}$), differentiation and fusion (PGE_2), as well as the incorporation of new proteins into existing muscle (Zalin, 1987; Phillips, Tipton, Aarsland, Wolf and Wolfe, 1997; Trappe, Fluckey, White, Lambert and Evans, 2001; Trappe et al., 2002). Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) specifically, has been described as an endogenously-produced growth factor requisite for muscle growth (Zalin, 1987; Horsley and Pavlath, 2003).

Human research has demonstrated local increases in $PGF_{2\alpha}$ concentrations (Trappe et al., 2001; Trappe et al., 2002), with concomitant increases in skeletal muscle fractional synthesis rate (FSR) after resistance exercise (Phillips et al., 1997; Phillips, Tipton, Ferrando and Wolfe, 1999). Research by Phillips et al. (1999) demonstrated effects of training status on fractional synthesis rate, with trained participants exhibiting less protein synthesis post-resistance exercise compared to sedentary subjects after an acute bout of resistance exercise. These findings hold implications for interpretation of research by Trappe et al. (2001, 2002), who reported a significant increase in FSR

post-acute resistance exercise in a mixed participant pool of both sedentary and recreationally-trained males (n=24).

NSAIDs similar to ibuprofen have been shown to blunt protein metabolism in human and animal skeletal muscle via mechanisms which inhibit prostaglandin production (Rodemann and Goldberg, 1982; Palmer, 1990; Trappe et al., 2001; Trappe et al., 2002). Trappe et al. (2001, 2002) reported significant acute inhibitory effects of analgesics on fractional synthesis rate after one bout of resistance exercise, but the effects of continued consumption of NSAIDs in conjunction with resistance exercise are not known.

Like ibuprofen, naproxen sodium is a proprionic acid. While most human research examining post-exercise effects of anti-inflammatory drugs has employed ibuprofen (Donnelly et al., 1990; Hasson et al., 1993; Howell et al., 1998; Trappe et al., 2001; Trappe et al., 2002), less human research has been conducted using naproxen sodium (Dudley et al., 1997; Lecomte et al., 1998; Bourgeois, MacDougall, MacDonald and Tarnopolsky, 1999; Baldwin et al., 2001). Limited investigations of the effects of naproxen sodium on various indices of muscle damage, performance, and recovery have been conducted in animals, with Thorsson, Rantanen, Hurme, Kalimo (1998) employing naproxen in a study of the inflammatory response resulting from imposed contusion injury in rats. A considerable amount of research has also been conducted using formulations not commonly available in non-prescription form (ie, flurbiprofen, meclofename, ketoprofen).

Those human studies employing naproxen sodium have examined the efficacy of such drugs on exercise-associated pain. Of those to utilize naproxen, all looked at functional measures of muscle recovery, such as soreness, strength, torque, and/or EMG magnitude. Only one examined the response of an inflammatory molecule (creatine kinase) in response to exercise and naproxen sodium (Bourgeois et al., 1999). In light of the paucity of research examining the influence of naproxen sodium on inflammatory mediators, as well as previous research to demonstrate its

efficacy in DOMS (Dudley et al., 1997; Lecomte et al., 1998; Baldwin et al., 2001), research examining the influence of naproxen sodium on inflammatory molecules related to protein synthesis and satellite cell activity seemed warranted.

The hypertrophic process involves an increase in the net accretion of the contractile proteins actin and myosin within the myofibril, as new myofilaments are added to the periphery of the myofibril, resulting in an increase in its diameter (Kraemer et al., 2008). Muscular hypertrophy can be accomplished by more than one mechanism, including either an increase in protein synthesis or a decrease in protein degradation. However, hypertrophy is usually achieved by an increase in protein synthesis, often with a proportionately smaller increase in degradation (Palmer, 1990). Likewise, while atrophy is usually characterized by a decrease in protein synthesis or an increase in protein degradation, rates of protein synthesis may still be elevated during atrophy, as demonstrated by Goldspink (1977) in an immobilization model of injury in rats. It is the overall net balance between synthesis and degradation that determines the ultimate outcome, and muscle growth can only occur if there is net anabolism within the muscle (Phillips et al., 1997). The magnitude of protein synthesis is influenced by a host of variables including amount and timing of macronutrient intake, amino acid availability, intensity of the imposed mechanical stress, muscle cell hydration levels, and the anabolic hormonal response (Kraemer et al., 2008).

In terms of impacts on muscle function and inflammatory cells, it has been suggested a therapeutic dose of an NSAID is not as effective as a prophylactic dose, due to its administration after the injurious event (Hasson et al., 1993; Gulick, 1996; Pizza et al., 1999; Willoughby, 2000; Lanier, 2003; Lapointe, Fremont and Cote, 2003). Theoretically, prophylactic treatment should reduce the immediate inflammatory response. A prophylactic approach to NSAID administration has been shown to attenuate muscle soreness and dysfunction in humans (Hasson et al., 1993) and inflammatory cells in rats after induced pleurisy (Gilroy, Colville-Nash, Chivers, Paul-Clark and

Willoughby, 1999). Prophylactic indomethacin (non-selective acetic acid) given to rats prior to endotoxin injection inhibited the onset of fever, the increase in PGE_2, and the resulting increase in protein degradation (Goldberg, 1988). In contrast, when indomethacin was administered therapeutically two hours after endotoxin injection, PGE_2 production was inhibited, but the elevated rate of protein breakdown was unaffected (Goldberg, 1988).

Hasson et al. (1993) reported subjects treated prophylactically with ibuprofen perceived 40-50% less soreness and experienced significantly less decline in isometric, concentric, and eccentric torque 24 hours post-eccentric exercise compared to those treated therapeutically or with placebo. These results indicate therapeutic use of NSAIDs may be ineffective in inhibiting protein degradation and soreness if the contributing factors are elevated before drug administration, creating an argument for a prophylactic approach. At 48 hours, both drug groups (prophylactic and therapeutic) reported significantly less soreness and torque decline than placebo and control groups.

Since the inflammatory and regenerative phases are coupled, it is logical to suppose drug-induced inhibition of the initial phase could negatively affect the subsequent phase. This mechanistic link, along with research highlighting the necessity of inflammation for muscular adaptation (Lapointe et al., 2002; Pizza et al., 2002), seem to indicate inhibition of inflammation may be undesirable for those pursuing muscular fitness goals. The purpose of this study was to investigate the effects of a six-week period of resistance training coupled with prophylactic naproxen sodium (Aleve®) consumption on skeletal muscle response to exercise in recreationally-trained college-aged males. Like ibuprofen, naproxen sodium is a proprionic acid; however, unlike ibuprofen, naproxen sodium has a long half-life of 12-15 hours post-oral administration. In fact, naproxen sodium is the only non-prescription NSAID with a long half-life. While these pharmacokinetics are slower in comparison to ibuprofen, research has shown patients tend to be more compliant in taking medication when prescribed drugs with longer half-lives (Leadbetter,

1995). Indeed, recent marketing campaigns by Procter and Gamble-owned Bayer Healthcare, the company responsible for the manufacture and distribution of Aleve®, highlight this unique feature of naproxen sodium.

Skeletal muscle adaptation was examined through both hormonal, performance, and anthropometric changes in response to exercise over time. Hormonal adaptations were studied through examination of repeated systemic plasma prostaglandin $F_{2\alpha}$ metabolite concentrations (pg/ml) pre- and post-exercise at three times points over 6 weeks. Strength was studied by examining changes in one and ten repetition maximum (1- and 10-RM) testing at three time points over 6 weeks. Acute changes in dominant upper arm circumference in response to exercise were taken as an indirect marker of exercise-induced hyperemia. Basic anthropometrics were studied through comparison of pre- and post-study DXA measurements. The main outcome variables included the acute $PGF_{2\alpha}$ response to exercise, acute arm circumference response to exercise, strength development, and arm tissue composition.

Chapter II

Literature Review

Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Aspirin (acetylsalicylic acid) was the first non-steroidal anti-inflammatory drug (NSAID). Although it was developed in the 1800s, its use dates back thousands of years, when patients were advised to chew on salicin-containing white willow bark to reduce fever and inflammation. Although aspirin was used for centuries, its mechanism of action was not discovered until 1971, when Vane determined it inhibited production of all prostaglandins (Vane, 1971; Vane and Botting, 1987), work which coincidentally garnered him and his colleagues the Nobel Prize for Medicine in 1982. Vane (1971) also determined a linear relationship between percent inhibition of prostaglandin $F_{2\alpha}$ activity and the log concentration of indomethacin, aspirin, and sodium salicylate.

Although heralded as a wonder drug and used to treat a variety of ailments, aspirin is also associated with adverse side effects, particularly the risk of stomach bleeding. These side effects inspired the search for chemically similar drugs with similar analgesic and anti-inflammatory properties but without associated side effects. While aspirin is technically an NSAID, it is not included in the generic term generally reserved for the newer agents, as its effects on the cyclooxygenase (COX) enzyme differ from those of the newer generation NSAIDs. In contrast to newer generation NSAIDs whose COX inhibition is reversible, aspirin-induced COX inhibition is irreversible due to the fact platelets have no DNA and are thus unable to synthesize new COX enzymes.

Most NSAIDs inhibit COX by transacetylation of the active center of the enzyme, competing with the cell membrane substrate arachidonic acid for the enzyme's active site. Specifically, drugs such as aspirin and indomethacin, acetylate prostaglandin endoperoxide synthetase (PGG and PGH), resulting in loss of COX activity, which subsequently inhibits prostaglandin production.

COX exists in two distinct isoforms- COX-1 and COX-2. COX-3 is a third isoform recently identified as a variant of the COX-1 gene, and it appears to regulate fever within the central nervous system (Vane and Botting, 1987; Vane and Botting, 1996; Chandrasekharan, 2002). Despite a high sequence of homology between the two isoforms, differences in the active sites of the enzymes have been pharmaceutically exploited to develop inhibitors selective for COX-2 (Willoughby, 2000). As shown in Tables 1 and 2, NSAIDs may be classified according to their COX selectivity or chemical structure. COX-2-selective indicates selective and sole inhibition of COX-2, while non-selective indicates inhibition of both isoforms. NSAIDs are most commonly administered in oral form; however, transdermal patches have recently been developed and represent a novel mode of administration.

Table 1
Non-Steroidal Anti-Inflammatory Drug COX Classification System

Non-Selective[a]	COX-2 Selective[a]	COX-1 Selective[b]
Chemical Name (*Brand®*)	Chemical Name (*Brand®*)	Chemical Name (*Brand®*)
Diclofenac (*Voltaren*)	Celecoxib (*Celebrex*)	Aspirin
Diflunisal (*Dolobid*)	Valdecoxib (*Bextra*)	SC-560
Etodolac (*Lodine*)	Rofecoxib (*Vioxx*)	FR122047
Fenoprofen (*Nalfon*)	NS-398	Mofezolac
Flurbiprofen (*Ansaid*)		P6
Ibuprofen (*Motrin, Advil*)		TFAP
Indomethacin (*Indocin*)		
Ketoprofen (*Orudis*)		
Ketorolac (*Toradol*)		
Naproxen (*Aleve, Naprosyn, Anaprox, Naprelan*)		
Oxaprozin (*Daypro*)		
Piroxicam (*Feldene*)		

[a]Information from Food & Drug Administration
[b]Perrone, Scilimati, Simone, & Vitale (2010)

Table 2
Brief Non-Steroidal Anti-Inflammatory Drug Chemical Classification System

Acetic Acid	Proprionic Acid
Chemical Name (*Brand®*)	Chemical Name (*Brand®*)
Diclofenac sodium (*Voltaren*)	Fenoprofen calcium (*Nalfon*)
Etodolac (*Lodine*)	Flurbiprofen (*Ansaid*)
Indomethacin (*Indocin*)	Ibuprofen (*Motrin*)
Ketorolac (*Toradol*)	Ketoprofen (*Orudis*)
Sulindac (*Clinoril*)	Naproxen (*Naprosyn, Anaprox*)
Tolmetin sodium (*Tolectin*)	Oxaprozin (*Daypro*)

COX-1 is a constituent enzyme found in most tissues and is responsible for the regulation of normal cell activities in the gastrointestinal (GI) tract, kidneys, liver, and other organs (Vane and Botting, 1996). The concentration of COX-1 remains largely stable within the body. In the GI tract, prostaglandins decrease secretion of gastric acid while increasing secretion of bicarbonate and cytoprotective mucus (Miller, 1983; Vane and Botting, 1987). GI tract issues represent the most common side effects associated with NSAID use and stem from COX-1 inhibition and the resulting inhibition of protective prostaglandin activities.

COX-2 is found in the kidneys and central nervous system. Novak et al. (2009) also revealed its expression in the skeletal muscle fiber periphery, as well as in and near nuclei of myogenic precursor cells (satellite cells). This discovery was important in clarifying the isoform present in skeletal muscle, as questions concerning the specific isoform(s) present in skeletal muscle had been raised by Trappe et al. (2002). Unlike COX-1, whose concentration remains largely stable (Vane and Botting, 1996), COX-2 is not normally present in resting cells. Its expression can increase

dramatically after exposure of fibroblasts, vascular smooth muscle, or endothelial cells to growth factors, cytokines, and highly immunogenic lipopolysaccharides in monocytes/macrophages (Vane and Botting, 1996). COX-2 expression is tightly regulated and induced by inflammatory mediators such as growth factors, cytokines, and endotoxins. This specificity to the inflammatory process led to considerable interest in COX-2 inhibition and the subsequent introduction of Celecoxib (Celebrex®), Valdecoxib (Bextra®), and Rofecoxib (Vioxx®).

Generally, selective COX-2 inhibitors reduce pain and inflammation without the gastrointestinal side effects associated with non-specific NSAIDs. However, animal research has demonstrated the necessity of COX-2 activity for skeletal muscle successful regeneration in cryogenic, laceration, and synergist ablation models of injury, respectively (Bondesen, Mills, Kegley and Pavlath, 2004; Shen et al., 2005; Novak, Billich, Smith, Sukhija, McLoughlin, Hornberger and Koh, 2009). Research has demonstrated COX-2 inhibition after mouse laceration injury results in increased fibrosis, transforming growth factor-β1, and myostatin, a negative regulator of skeletal muscle growth (Shen et al., 2005). These findings suggest negative implications for COX-2 inhibition in terms of impacts on muscle growth, corroborating research demonstrating the necessity of COX-2 activity in regeneration and hypertrophy (Bondesen et al., 2004; Novak et al., 2009). In addition to selective pharmacological inhibitors, COX-2 is also regulated by mechanical deformation. While cyclical stretch of cultured myoblasts has been shown to have no effect on COX-1 mRNA, it was shown to significantly increase COX-2 mRNA expression 3.5 fold (Otis, Burkholder and Pavlath, 2005).

NSAIDs differ in their anti-inflammatory effectiveness as well as their ability to inhibit COX-1 and COX-2. Several commonly known NSAIDs such as piroxicam, indomethacin, and aspirin preferentially inhibit COX-1 and are associated with a greater risk of gastrointestinal and renal complication. Non-selective NSAIDs (Table 1) inhibit both COX isoforms, but it is their

preferential inhibition of COX-2 that is their primary analgesic mechanism. This selective COX-2 inhibition also tends to result in fewer GI complications (Vane and Botting, 1996).

Activity ratios represent inhibition of COX-1 versus COX-2. Compounds with lower activity ratios are more potent inhibitors of COX-2 and have fewer GI and renal side effects (Vane and Botting, 1996). Naproxen has the lowest activity ratio of 0.6 versus piroxicam, which has an activity ratio of 250. This value indicates the drug is 250 times more active on COX-1 than COX-2. Epidemiological data indicate that among non-selective NSAIDs, ibuprofen has one of the most favorable GI safety profiles, with an activity ratio of 15. Comparison of activity ratios for naproxen and ibuprofen suggest naproxen should result in fewer gastrointestinal complications and support its use in the current investigation.

The effects of COX–inhibiting drugs are tissue-specific. Ibuprofen, for instance, has a major effect on COX in the peripheral tissues, while acetaminophen performs its COX–inhibition in the central nervous system. The effects of COX-inhibiting drugs are also dose-dependent, and analgesic doses tend to be 50-75% of anti-inflammatory doses, both in terms of dosage and duration of treatment (Vane and Botting, 1987; Amadio, 1993; Koester, 1993). Over-the-counter, or non-prescription NSAIDs are available in doses that primarily yield analgesic and antipyretic, but not anti-inflammatory, effects.

Applications of Use and Pharmacokinetics of Naproxen Sodium

According to MICROMEDEX DRUGDEX® database, naproxen sodium is a member of the following dug classes: analgesic, antimigraine, antirheumatic, central nervous system agent, musculoskeletal agent, NSAID, and a proprionic acid (Table 2). Naproxen sodium provides its initial analgesic response in ~30 minutes, reaches peak blood concentrations ~1-2 hours, and endures ~12-15 hours after oral administration. Naproxen ranked twelfth on a list of the top forty

most commonly used prescription and non-prescription drugs (Kaufman, 2002). Between 2008 and 2009, use of ibuprofen increased 6.9%, while use of naproxen increased 0.4% (SDI/Verispan, 2009). Naproxen sodium is the only non-prescription NSAID with a long half-life, a characteristic which seems to improve compliance (Koester, 1993; Leadbetter, 1995). Its bioavailability is complete, and effects of food on its absorption are clinically insignificant. After absorption, 99% is protein-bound; however, some distributes to synovial fluid. Naproxen sodium undergoes extensive metabolism in the liver, where it is broken down into two inactive metabolites: 6-desmethyl-naproxen and glucuronide conjugate. After undergoing metabolism in the liver, nearly all (95%) of naproxen is excreted in the kidneys, with 28% of that being demethylated naproxen and the remainder being glucuronidated naproxen (MICROMEDEX, 2011)

Incidence of Use of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Non-steroidal anti-inflammatory drugs are used for their analgesic, antipyretic, anti-inflammatory, and anticoagulant effects (Hertel, 1997). The use of NSAIDs as a therapeutic modality for EIMI has increased dramatically over the last 20 years, in conjunction with the growing popularity of exercise and sport (Lanier, 2003). NHANES data revealed the number of Americans using NSAIDs on a frequent monthly basis doubled between 1994 and 2000 (Paulose-Ram, Hirsch, Dillon and Gu, 2005).

Exercise-associated pain has been described as a "lurking threat" to movement fluidity and maximum performance (Reider, 2009). While various treatment modalities exist for the relief of exercise-associated pain, NSAIDs remain the most popular choice. Numerous studies have revealed a pervasive use in a range of populations, much of this without regard for label directions and the potential for adverse reactions (Chambers, Reid, McGrath and Finley, 1997; Tricker, 2000; Kaufman, 2002; Warner, 2002; Corrigan and Kazlauskas, 2003; Paulose-Ram et al., 2005; Wilcox,

Cryer and Triadafilopoulos, 2005; Gorski, Cadore, Pinto, da Silva, Correa, Beltrami and Kruel, 2009; Tscholl, Feddermann, Junge and Dvorak, 2009; Wolf, 2011) (Table 3).

Table 3
Prevalence of NSAID Use in Various Populations

Study	Population (sample size)	Prevalence (%)
Corrigan & Kaslauskas (2000)	Olympic athletes (n=2,758)	25.6
Chambers et al. (1997)	Junior high students (n=651)	58.7-95.9
Gorski et al. (2009)	Ironman athletes (n=327)	59.9
Kauffman et al. (2002)	US adults (≥18 yrs.) (n=2590)	17-23
Paulose-Ram et al. (2005)	US adults (≥20 yrs.) (n=4,880)	20-34
Tricker (2000)	Collegiate athletes (n=563)	55-62
Tscholl et al. (2009)	Elite soccer players (n=2,488)	17.3-30.7
Warner et al. (2002)	High school football players (n=651)	75
Wilcox et al. (2005)	US adults (≥18 yrs.) (n=9,062)	17-83
Wolf et al. (2010)	Collegiate football players (n=144)	73

Furthermore, much literature has revealed the majority of users independently decide to medicate. Aside from analgesia, a desire to accelerate healing, to permit an earlier return to competition, to mask injury with prophylactic use, and to improve performance are oft-cited reasons for using analgesics (Tricker, 2000; Reider, 2009). While NSAIDs are useful for treating pain and inflammation, the ease with which these drugs can be procured, along with a lack of knowledge concerning potential adverse effects, create a potential for misuse and abuse. The next few sections will describe use in a range of populations.

NSAID Use - U.S. Adult Population

Several important surveys examining the frequency of use of NSAIDs have been conducted over the past several years. Wilcox, Cryer, and Triadafilopoulos (2005) examined patterns of use and public perceptions of non-prescription NSAIDs using combined data from the 1997 Roper survey (n=4,799) and the 2002 National Consumers League (NCL) survey (n=4,263). The Roper survey was commissioned by the American Gastroenterological Association and conducted by Roper Starch Worldwide Proprietary Telephone Research Center in September and October 1997. The NCL survey was conducted December 16-29, 2002 by Harris Interactive, one of the largest market research and consulting firms in the world. The Roper survey included both prescription and non-prescription analgesics, while the NCL survey examined only non-prescription analgesics. Although not an NSAID, acetaminophen was included in both surveys; however, those who used acetaminophen exclusively were excluded from the Roper results.

With *use* defined as having used prescription or non-prescription pain relievers for any type of pain on at least 2 occasions in the past 12 months for at least 5 consecutive days at a time, the Roper survey reported non-prescription analgesic use in 17% of respondents. Of these, 30% used prescription only, 32% used non-prescription only, and 38% used a combination. Of those who used non-prescription NSAIDs exclusively, the median duration of use was 5 years. Seven percent reported use for $\leq$2 years, while 16% reported use for $\geq$15 years. Twenty-seven percent used non-prescription NSAIDs on a daily basis, while 73% used on an as-needed basis. Twenty-six percent consumed more than the recommended amount, while 57% used the exact dosage. Compared to the values presented for exclusive non-prescription users, prescription users' mean duration of use was 6.6 years, with 44% reporting daily use, and 8% reporting a tendency to consume a dose larger than the one prescribed.

The NCL survey found 83% of respondents reported use of a non-prescription analgesic in the past year. Fifteen percent reported daily use, 29% reported frequent weekly use, and 27% report frequent monthly use. Of daily users, the frequency of daily use ranged from once (44%) to five or more (5%) times per day, with a median of two. Alarmingly, it was daily users who were less likely to be concerned about potential side effects from non-prescription medications compared to those taking non-prescription medications on a frequent monthly basis. Of the 27% of respondents who reported frequent monthly usage, the median uses per month were two, with 24% using non-prescription NSAIDs five or more times per month. Forty-four percent consumed more than the recommended dosage, which could be achieved by taking the next dose sooner, taking more pills at a single time, or taking more doses per day than directed by the label. The most often reported reasons for non-prescription NSAID use included arthritis or joint pain (42%), back pain (41%), and muscular ache (40%).

The NCL survey reported 63% of non-prescription analgesic users selected NSAIDs (versus acetaminophen) when taking non-prescription analgesics, with ibuprofen-based medicines chosen by 38% of non-prescription users. The popularity of ibuprofen-based drugs was also seen in the Roper survey, with 57% of respondents selecting ibuprofen-based drugs most often. The NCL survey also highlighted gender differences in reasons for non-prescription NSAID use, with women being more likely to consume NSAIDs for headaches, migraines, and arthritis, and men being more likely to use them for sport or exercise-related pains. Opposite findings have been reported in athletic female populations, however, with female soccer players reporting greater NSAID use than their male counterparts (Tscholl et al., 2009). By demographically weighting results according to U.S. Census Bureau figures, Wilcox et al.'s (2005) extrapolation of their finding to the general population suggested 36 million Americans use non-prescription pain relievers daily, with approximately 23 million using NSAIDs. Twenty-five percent of these users exceed the recommended dosage.

Kauffman, Kelly, Rosenberg, Anderson, and Mitchell (2002) conducted the Slone Survey (n=2,590) between February 1998 and December 1999. With a sample reflecting the general population according to U.S. Census Bureau data comparison, participants were asked about use of prescription and/or non-prescription drugs, vitamin/mineral supplements, and any herbal supplement taken seven days preceding telephone contact. Eighty-one percent (81%) of participants had taken at least one medication during the preceding week, and 25% took at least 5 medications. According to the authors, these percentages reflect use in 169 million and 52 million individuals, respectively. Rates of use of these medications increased with age and were greater in women than in men in every age group. The finding of increased rates of use with age has been confirmed in other studies of differing populations (Chambers et al., 1997; Tscholl et al., 2009; Wolf, 2011).

Among FDA-regulated drugs, non-prescription analgesics were the most frequently used individual products, taken by 17-23% of the population. Of the three categories of medication inquired, acetaminophen, ibuprofen, and aspirin were the top three drugs used. Other research demonstrated these drugs are also the analgesics most commonly used on a frequent monthly basis, in addition to being the three products most commonly obtained without a prescription (Paulose-Ram et al., 2005). The Slone Survey revealed ibuprofen was more frequently used by younger (18-44 years) subjects. Estimates provided by the Slone Survey were only for aspirin, acetaminophen, and non-aspirin NSAIDs (ibuprofen, piroxicam, diclofenac, sulindac, indomethacin, naproxen, and tolmetin). Of the non-aspirin NSAIDs, ibuprofen was chosen by 80% of lifetime frequent monthly users, confirming findings by others (Kaufman, 2002; Wilcox et al., 2005). The data also revealed males had a greater tendency to use aspirin on a frequent monthly basis, while females had a greater tendency to use acetaminophen and non-aspirin NSAIDs on a frequent monthly basis.

In a report generated from 1999-2000 National Health and Nutritional Examination Survey (NHANES), Paulose-Ram, Hirsch, Dillon, and Gu (2005) presented data concerning frequent

monthly use of non-prescription and prescription non-narcotic analgesics among U.S. adults. The cross-sectional survey (n=4,863) was characterized by detailed in-person home interviews and a standardized health examination. 'Lifetime' frequent monthly users were defined as individuals who *ever used* a pain reliever nearly every day for as long as a month. 'Current' frequent monthly users were defined as lifetime frequent monthly analgesic users who *were currently using* a pain reliever daily or nearly every day (Paulose-Ram et al., p. 258).

According to 1999-2000 NHANES data, approximately 20% of U.S. adults met the criteria for lifetime frequent monthly use, doubling NHANES III (1988-1994) data, in which only 10% of U.S. adults met the criteria for frequent monthly use. Seventy-two percent of these lifetime frequent monthly analgesic users reported currently using an analgesic nearly every day for as long as a month. Additional comparisons to NHANES III data also revealed an increase in current frequent monthly use in 20-39 and 40-59 year old age groups. In addition to the 20% meeting criteria for lifetime frequent monthly use, another 14% (27 million) were current frequent monthly users. Seven percent (2 million) of these current frequent monthly analgesic users reported using two or more analgesics nearly every day during the month. Thirty-six to forty percent of all lifetime frequent monthly users reported a duration of use ranging 1-4 years.

NSAID Use - Pre-Adolescent and Adolescent Populations

To examine non-prescription medication use and self-administration among adolescents, Chambers, Reid, McGrath, and Finley administered a survey to seventh, eighth, and ninth graders (n=651). Their purpose was to determine the prevalence of non-prescription medication use for different types of pain, source of medications, the level of responsibility adolescents assume for self-administering analgesics, as well as potential relationships between both personal and pain characteristics and self-administration.

Fifty-eight to seventy-five percent of junior high students reported having taken a non-prescription medication for pain without first checking with an adult within the past three months. It was suggested this large percentage evolved both from the observation of adults using non-prescription medications as well as direct instruction. Surprisingly, the mean age at which children begin self-administering medication for muscle, joint, and back pain was reported to be 11.5 $\pm$ 2 years of age. The study also revealed the tendency for self-administration increased with grade. Ninety-seven percent (97.8%) of students surveyed reported experiencing muscle pain across a range of frequencies over the previous three months. Of these, 83.8% chose to self-medicate, with acetaminophen and ibuprofen being chosen by 85.3% and 26.7%, respectively. Of the different types of pain assessed, muscle, joint, and back pain were second only to menstrual pain in terms of highest pain intensity. While the muscle pain received the second highest average pain intensity score, this type of pain was experienced by the largest number of participants (97.8%). Interestingly, higher levels and frequencies of pain were related to higher scores for self-administration in all cases except muscle, joint, and back pain. The authors suggested this may be due to the fact alternative treatment strategies exist for such pain. Such information is meaningful given the potential for adverse complications associated with long-term use of such drugs.

NSAID Use - U.S. Adolescent Athletic Population

Additional data highlighting the early self-administration of analgesics during adolescence was provided by Warner, Schnepf, Barrett, Dian, and Swigonski (2002), who analyzed self-administered questionnaires given to high-school football players (n=681). Attitude was assessed through questions concerning potential beliefs that NSAIDs benefited performance by decreasing pain, lessening fatigue, and/or reducing cramping. Behavior was assessed through questions concerning independent or guided/directed use, prophylactic versus therapeutic use, and use before

versus after practice or games. Of those surveyed, 90% were white males (mean age 15.8 years). Seventy-five percent reported using an NSAID over the past 3 months, and 15% reported daily use. Daily use was greater in those with the attitude NSAIDs aided performance, those who decided independently, and those who used NSAIDs to block pain. The survey was distributed by the coach, which the authors cited as a limitation of the study, as the possibility for dissuasion by the coach could result in underreporting.

Similar rates of use were revealed by Wolf, Miller, Pescatello, and Barnes (2011), who examined use of non-prescription analgesics by NCAA Division I-A football players (N=144). The Over the Counter Drug Screen for Athletes, a 23-item survey, was utilized to assess use, dose, and consecutive days of use. Seventy-three percent of participants reported using non-prescription analgesics for football-related pain, with offensive players (85%) reporting greater use than both special teams' players (75%) and defensive players (59%). Those with more years of football experience were also more likely to use non-prescription analgesics than those with less experience, corroborating research showing use increasing with age (Chambers et al., 1997; Kaufman, 2002).

Ibuprofen was the most commonly chosen analgesic, selected by 80% of football players, confirming the analgesic's popularity as reported in other surveys of the general adult population (Kaufman, 2002; Paulose-Ram et al., 2005; Wilcox et al., 2005). Ibuprofen was followed by aspirin (71%), acetaminophen (29%), naproxen sodium (20%), and ketoprofen (3%). Six percent of those using non-prescription analgesics reported use for more than 10 consecutive days or daily. Of these, 78% believed they could take non-prescription analgesics as long as they wanted. Sixty-five percent used the recommended dose or less, while 35% used more. Twenty-five percent indicated they read labels every time they used non-prescription analgesics, while 22% read them only prior to the first use, and 15% never read label directions. Not surprisingly, those who did not read label directions were more likely to take a dose larger than that recommended.

Sixty-four percent self-decided to use non-prescription analgesics, with the remainder using such medicines at the recommendation of individuals ranging from the team physician to a teammate. Nearly half (47%) self-purchased these medication versus reception from an athletic trainer or physician. Those who self-purchased were also more likely to use more than the recommended dose (51%). Forty-one percent reported using non-prescription analgesics in anticipation of pain, and 31% used them to avoid missing a practice or game.

Tricker (2000) examined the frequency of use, as well as knowledge and attitudes concerning use of prescription and non-prescription analgesics in student athletes (N=563) from two NCAA Division I universities. The sample was comprised of males and females representing all sports in which the universities participated. Fifty-five percent reported use of painkilling drugs to alleviate soreness after workouts or competition. Fifty-eight percent regularly used painkillers throughout the duration of the season, and 62% used them after tough workouts. Fifty-eight to sixty percent obtained these medications from teammates or friends, while only 14% obtained from a physician.

Based upon responses to a set of attitude questions, nearly one-third (29%) of the total sample was defined as high risk for painkiller abuse (n=165). These student athletes perceived nothing wrong with the use of painkilling medications on the day of competition to prevent competing in pain, and this perception was significantly higher in males than females. Twenty-one percent reported they would take painkillers when injured to facilitate participation, while 32% were undecided on this matter. Thirty-three percent of this subset reported they felt painkilling drugs were necessary to facilitate recovery from participation in sporting events, while 33% were undecided. Analysis revealed many athletes felt that not only are painkilling drugs necessary to overcome soreness but also are necessary to achieve success in sport.

NSAID Use - International Athletic Population

To describe the use of analgesics in female and adolescent male soccer players, Tscholl, Feddermann, Junge, Dvorak (2009) performed a descriptive epidemiological study, gathering data obtained from team physicians at six FIFA (Federation Internationale de Football Association) World Cup tournaments. Information was collected in connection with doping controls carried out during the FIFA Women's World Cup 2003 and 2007, and the FIFA U-17 and U-20 World Cup 2005 and 2007. All team physicians were asked to give a certification of medication that s/he prescribed to all qualified players in the 72-hours preceding match play. Substances were classified as painkilling and NSAIDs, other analgesics, injected corticosteroids and local anesthetics, muscle relaxants, respiratory agents, intestinal medication, antimicrobial medication, contraceptive medication, and other medication, including psychotropics and topical corticosteroids.

NSAIDs were the most frequently prescribed type of medication in all tournaments. Compared to adolescent males, a larger percentage of females used NSAIDs per match, with 30.7% of females reporting use versus 17.3% of U-17 males and 21.4% U-20 males. Females in this study were found to take NSAIDs at a rate comparable to professional male soccer players, which had previously been reported at 30.8% before tournament play (Tscholl, Junge and Dvorak, 2008). Rates of use over the entire tournament duration (~1 month) were expectedly higher, with 41.9% of U-17 males, 45% of U-20 males, 51.1% of professional females, and 54.5% of professional males using NSAIDs. Of all players taking NSAIDs, 8.9% were using at least two preparations. The liberal use of NSAIDs highlighted by Tscholl et al. (2009) led to speculation whether these medications were being prescribed solely for therapeutic purposes. Indeed a discrepancy between the frequency of drug use and the rate of acute injuries, as reported by team physicians' medical diagnoses, was noted to exist. These discrepancies were believed to suggest a prophylactic approach

to analgesic prescriptions (Reider, 2009). Corrigan and Kazlauskas (2003) also reported an abusive and dangerous use of NSAIDs in Olympic athletes during the 2000 Sydney Olympics.

In summary, research has shown use of NSAIDs begins at an early age and continues throughout the lifespan. Unfortunately, many consumers do not adhere to dosing guidelines and many are unaware of the consequences of long duration and/or larger-than-recommended dosages. The next sections will describe the structure of skeletal muscle, with subsequent description of the effects of NSAIDs on various indices of muscle physiology and performance.

Skeletal Muscle Anatomy & Physiology

Individual skeletal muscle cells (muscle fibers) develop as a result of the fusion of embryonic stem cells called myoblasts, whose growth into large multi-nucleated cells requires multiple steps. Each nucleus reflects the contribution of a single myoblast. The multi-nucleated characteristic of muscle cells allows protein metabolism within a given fiber to be differentially regulated along its length by the respective nuclei in a given nuclear domain (Kraemer, Vingren and Spiering, 2008). Upon receipt of the appropriate stimulus, new proteins are incorporated as either non-contractile structural elements or as contractile proteins actin and myosin into existing or new sarcomeres (Kraemer et al., 2008).

Nucleic genes direct the production of enzymes and structural proteins required for normal muscle contraction. The presence of multiple copies of these genes accelerates this process and is an important feature due to rapid metabolic turnover in skeletal muscle fibers. Metabolic turnover is the continual removal and replacement of temporary organic molecules and reflects cellular adaptation to environmental stimuli. The average recycling time for muscle cell total protein is 30 days (Martini, 2004).

Mature skeletal muscle fibers are incapable of dividing, so new muscle fibers are produced through divisions of adult myoblasts or satellite cells. Satellite cells have aptly been described as a population of muscle-derived stem cells whose activity provides the potential for skeletal muscle to repair after injury (Crameri et al. 2004; Mendias et al. 2004). Satellite cells provide new myonuclei during hypertrophy and assist in repair of damaged muscle fiber segments (Mendias, Tatsumi and Allen, 2004; Mikkelsen et al., 2009). Satellite cells are generally located under the basal lamina but can also found outside the fiber within the endomysium (Mackey, Kjaer, Dandanell, Mikkelsen, Holm, Dossing, Kadi, Koskinen, Jensen, Schroder and Langberg, 2007; Novak et al., 2009).

Once activated, satellite cells give rise to myoblasts. While satellite cell activation is necessary for skeletal muscle development, it represents only the first stage of the cell cycle, which sequentially includes proliferation, differentiation, alignment, and fusion. Fusion allows increased protein synthesis and increases in cell size (Horsley and Pavlath, 2003). After satellite cell fusion with the existing myofiber, muscle cell size continues to increase through enhanced protein synthesis, which is regulated by specific prostaglandins and their effects on protein degradation and synthesis (Smith, Palmer and Reeds, 1983; Palmer, 1990; Trappe et al., 2002).

Each phase of the cell cycle is necessary for the incorporation of new proteins into a muscle in order to maintain a constant nuclear domain ratio (Crameri et al. 2004), and each phase of the cell cycle is dependent upon myogenic regulatory factors (MRFs), including myo-D, myf-5, pax-7, myogenin, and mRF-4 (myf 6). Myoblast proliferation is governed by a multitude of signaling cascades initiated by various cytokines and autocrine/paracrine growth factors, including prostaglandins (Otis et al., 2005). During the repair process, satellite cells proliferate and migrate into the necrotic area, where they differentiate into myoblasts. If the endomysium is still intact, satellite cells will fuse to damage fibers; however, if the endomysium is ruptured, satellite cells will escape into interstitial fluid where they will align and fuse to produce a new fiber (MacIntosh, 2006).

It is probable that the interaction of myogenic genes (genes that code for myogenic regulatory factors) and growth factors during regeneration is similar to that occurring during embryological muscle development (MacIntosh, 2006), although the role for $PGF_{2\alpha}$ has been shown to be dependent on the developmental stage of the myotube (Horsley and Pavlath, 2003).

It has been suggested primary activation of satellite cells most likely occurs roughly one week after resistance and aerobic exercise (Crameri, Langberg, Magnusson, Jensen, Schroder, Olesen, Suetta, Teisner and Kjaer, 2004; Mackey et al., 2007; Mikkelsen et al., 2009). It appears the extent of satellite cell proliferation is influenced by both the prior training status of the individual as well as the type of exercise, with a single bout of maximal eccentric muscle actions on an isokinetic dynamometer inducing a much stronger satellite cell response than a bout of regular resistance training (Crameri et al., 2004; Mackey et al., 2007; Mikkelsen et al., 2009). Aerobic exercise also stimulates satellite cell activation, as demonstrated by Mackey et al. (2007), who reported a significant increase in satellite cells in male endurance athletes (n=14) eight days post-36 km run.

Satellite cells are not only needed for the regeneration of damaged muscles fibers but also for the general adaptation to muscle loading (Mikkelsen et al., 2009). Force itself may directly activate satellite cells by force transduction via integrins (plasma membrane proteins that anchor actin and myosin myofilaments) on the satellite cell surface (MacIntosh, 2006; Mikkelsen et al., 2009). Crameri et al. (2004) reported a single bout of eccentric exercise performed by sedentary males (n=8) is sufficient for satellite cell activation, but terminal differentiation of these cells is dependent upon additional bouts of exercise. Taken together, the results of Crameri et al. (2004), Mackey et al. (2007), and Mikkelsen et al. (2009) indicate satellite cell activity is influenced by both the intensity and frequency of exercise.

Pathophysiology of Muscle Injuries – Inflammation & Regeneration

Skeletal muscle inflammation can result from a variety of stimuli, including biological (venoms, streptococcus), thermal, chemical (pathogens), and mechanical sources. Examples of the latter include strains, contusions, lacerations, and exercise-induced muscle injury (EIMI). The pathophysiology of mechanical modes of injury varies somewhat between strain, contusion, and laceration models and that produced by exercise, with the former injury models being characterized by an inflammatory response of a larger magnitude. Although muscle fiber damage occurs with aerobic forms of exercise such as downhill running (Donnelly et al., 1990), the eccentric phase of resistance training is particularly associated with the occurrence of exercise-induced muscle injury.

The initial inflammatory response is directed at the tissue level and is characterized by the cardinal signs of inflammation - swelling, redness, warmth, and pain (Martini, 2004). With EIMI specifically, diffuse muscle pain, swelling, and stiffness characterize the acute inflammatory response. Regardless of the exact mechanism of injury, each results in the unregulated influx of calcium into the muscle through abundant stretch-activated calcium channels located on the fiber membrane. Once inside the muscle cell, calcium ions activate a calcium-activated protease known as calpain and phospholipase A_2, which digest structural proteins and lipid membranes, respectively (MacIntosh, 2006). Along with calcium, the increased blood flow occurring during exercise also activates membrane-bound G-protein coupled receptors associated with various prostaglandins, further contributing to the activation of phospholipase A_2, which frees arachidonic acid from the phospholipid bilayer with subsequent metabolism within the COX pathway to produce prostaglandins (Boushel et al., 2002).

Damaged cells release prostaglandins, proteins, and postassium ions (Martini, 2004). These changing chemical conditions alter the composition of the interstitial fluid and potentiate the inflammatory response through the activation of mast cells, small mobile connective tissue cells

found near blood vessels. Like COX-2, mast cells play a pivotal role in the inflammatory response, being stimulated by mechanical stress or chemical changes in the local environment. Mast cells contain histamine, heparin, and prostaglandins, all of which further potentiate the local inflammatory response (Martini, 2004). PGE_2 and PGI_2 trigger vasodilation (Boushel, Langberg, Risum and Kjaer, 2004), while histamine and bradykinin increase capillary permeability and accelerate the flow of blood into the injured area (Martini, 2004). This increased blood flow elevates local temperature, increasing the rate of enzymatic reactions and accelerating phagocytic activity (Martini, 2004).

Strains and contusions are characterized by a disruption of fiber architecture, including both ruptured muscle fibers and connective tissue. Hematoma, an abnormal localized collection of clotted or partially clotted blood, and necrotic tissue are generally obvious at the site of injury. The initial acute inflammatory response is followed by a more intense inflammatory response within 2-3 days. Myoblasts provide evidence of regeneration within the first week, and fibroblasts mount a scar response (Almekinders, 1999). The combined regenerative and scar responses result in a healed muscle that has fewer and smaller muscle fibers in the injured area as well as an increased amount of collagenous tissue between the fibers (Almekinders, 1999).

Dynamic resistance training is characterized by repeated concentric and eccentric muscle actions. Although the metabolic demands of eccentric activity are less than that for concentric and isometric activity, eccentric activity causes the greatest change in muscle function (Jones et al., 1989). Although the number of strongly attached cross bridges during eccentric activity is only 10% greater than that during isometric activity, the force developed during eccentric activity is approximately twice that developed during isometric activity (MacIntyre et al., 1996). Furthermore, although EMG activity is significantly greater (~40%) during concentric action, as demonstrated by Gibala, MacDougall, Tarnopolsky, Stauber, and Elorriaga (1995), approximately 40% of skeletal muscle

damage occurs during the eccentric phase, as demonstrated by magnetic resonance imaging (Baldwin et al., 2001) and electron microscopy (Gibala et al., 1995). However, the rates fractional synthesis, fractional breakdown, or the net balance between the two, has not been found to be significantly different between concentric versus eccentric activity (Phillips et al., 1997).

In a comparison of the effects of concentric versus eccentric muscle actions in untrained men, Gibala et al. (1995) reported eccentric exercise resulted in significantly more myofiber disruption, as well as a persistent depression of voluntary and evoked strength measures compared to concentric activity. Jones, Newham, Torgan (1989) also reported eccentric activity caused greater fatigue and DOMS than either isometric or concentric activity. Contraction type is an important study characteristic to note, as static muscle activity has not been shown to increase protein synthesis as seen in dynamic muscle action (Smith et al., 1983).

In comparison to the pathophysiology of strains and contusions, DOMS is characterized by a limited inflammatory response (Almekinders, 1999). Morphological changes are barely evident within the first 24-hours and are then restricted to Z-disk streaming (MacIntosh, 2006); however, Mishra et al. (1995) reported myofibrillar damage in the A-band of the sarcomere as well. According to MacIntosh (2006), 10-15 days post-exercise-induced injury, a significant proportion of skeletal muscle fibers have undergone necrosis; however, Crameri et al. (2004) reported necrosis in only one of eight sedentary male subjects after one session of intense isokinetic eccentric exercise, demonstrating that necrosis is not necessary for satellite cell activation. Fibroblast activation does not seem to occur in DOMS as seen in strain and contusion injury models (Almekinders, 1999). Furthermore, the regenerative response associated with EIMI appears fully capable of restoring the muscle's architecture in contrast to strain and contusion models (MacIntosh, 2006).

Symptoms of EIMI are resolved at different rates. Acute soreness subsides before muscle function recovers. Human research (n=3, males) has reported recovery of maximal voluntary

contraction (MVC) strength in response to elbow flexion required 24-48 hours, while long-lasting fatigue required 3-4 days to fully dissipate (Jones et al., 1989). Donnelly, Maughan, and Whiting (1990) found isometric MVC required 72 hours to recover in untrained males (n=40; 18-30 years) post-downhill running. Other human research has demonstrated significant decreases in isometric MVC persisting 10 days post-resistance exercise (Pizza et al., 1999), while others have reported recovery of isometric MVC 8 days post-acute resistance training (Mikkelsen et al., 2009). Lanier et al. (2003) reported strength reductions of 30-60% 24-48 hours post-exercise followed by a continued depression for two weeks. Crameri et al. (2004) found necrosis resulting from a single bout of high intensity eccentric work in sedentary males required 8 days for full recovery. Howell, Conatser, Chleboun, Karapondo, and Chila (1998) found isometric strength had recovered only 50% 14 days post-acute elbow flexion exercise (70-90% maximum voluntary isometric force performed for an average of 18.6 repetitions) in a young untrained co-ed population (n=24). Animal studies employing in situ eccentric activity injury models have demonstrated resolution of exercise-induced inflammation by two weeks and complete regeneration by 3 weeks (Mishra et al., 1995; Pizza et al., 2002). It appears the length of the recovery phase is dependent not only on the parameter of muscle recovery examined but also on the model of injury.

In defense of a prophylactic approach to NSAID use for exercise-associated pain, some have cited an increased risk for injury if activities are undertaken while symptoms of DOMS are evident, while others have cited the potential for exercise-associated pain to discourage future participation in exercise (Donnelly et al., 1990; Baldwin et al., 2001). Furthermore, a prophylactic approach has been demonstrated to be more effective for exercise-associated pain relief in humans (Hasson et al., 1993) and fever resulting from endotoxin injection in rats (Goldberg, 1988).

While certain characteristics of the inflammatory response are dependent upon stimulus characteristics, the inflammatory response is also influenced by individual factors such as sex and

training status. One study found significantly higher radionuclide-labeled neutrophils in females (n=10) versus males (n=12), as well as a biphasic pattern of eccentric torque recovery in females that was not exhibited in males (MacIntyre et al., 2000). Neutrophils were significantly higher in females 2 hours post-eccentric exercise, but sex difference were no longer evident at 4 hours (MacIntyre et al., 2000). In this study, recreationally-trained was defined as less than but not greater than or equal to 6 hours of recreational exercise, not including jogging or weight training, and damage was produced by the performance of 300 fatiguing eccentric contractions on an isokinetic dynamometer.

Conversely, using a muscle biopsy technique, another study of males (n=8) and females (n=8) reported plasma granulocyte (neutrophils, basophils, eosinophils) counts decreased in females but increased in males 48 hours after eccentric exercise (Stupka et al., 2000). In this study, all participants were engaged in some form of moderate cardiovascular training but no resistance training. Damage was produced by performance of 12 sets of 12 eccentric muscle actions of lower body musculature at 120% concentric one repetition maximum. Stupka et al. (2000) concluded muscle damage resulting from eccentric exercise is similar between the sexes, but the inflammatory response is attenuated in women versus men. These findings are important to bear in mind when considering research involving exercise-induced inflammation, NSAIDs, and co-ed subjects.

Necessity of Inflammation

To test the hypothesis that suppression of the inflammatory reaction after an initial bout of eccentric muscle actions attenuates adaptation, Lapointe, Fremont, and Cote (2002, 2003) investigated the effects of twice–daily diclofenac (non-selective acetic acid NSAID) given therapeutically for either two or seven days beginning six hours after an initial bout electrical stimulation of rat skeletal muscle. Diclofenac almost completely abolished the increase in serum PGE_2 levels normally accompanying electrical stimulation and voluntary contraction.

After the initial bout of stimulation, the placebo group suffered a significant decrease in absolute and specific tension compared to its pre-bout value. The 2–day diclofenac group also suffered a significant decrease in tension compared to its pre-bout value; however, its deficit was significantly less than that experienced by the placebo group, seemingly demonstrating a short-term protective effect of diclofenac treatment.

A second bout of electrical stimulation was performed fourteen days later, at which time all groups had returned to baseline. Two days after this second bout, only the 7–day diclofenac group showed significant deficits in absolute tetanic tension compared to its corresponding pre-bout value. Both treatment durations resulted in significant decreases in specific tension compared to corresponding pre-bout values; however, the 7-day diclofenac group showed significantly less specific tension versus the 2-day treatment group.

Inflammatory molecules, including ED1+ and ED2+ macrophages, were measured to examine the acute inflammatory response. ED1+ macrophages circulate as monocytes until undergoing activation and appear 3-24 hours post injury, while ED2+ macrophages are the primary resident macrophage population in skeletal muscle and appear to be elevated 24-48 hours post-laceration injury (McLennan, 1996). Over the fourteen days between bouts, ED1+ macrophages remained significantly elevated in both NSAID-treated groups compared to placebo. These elevated macrophage concentrations were taken to indicate a persistent inflammatory response. This significant difference in macrophage concentration persisted even after the second bout. After the second bout, ED2+ macrophages significantly increased only in drug groups. Both 2- and 7-day drug groups exhibited significant increases in ED2+ concentrations compared not only to their corresponding pre-bout value but also compared to placebo at that time point. The authors suggested the lack of significant increase in the concentration of either subpopulation of macrophages in the placebo group after the second bout could indicate a lack of muscle damage,

indicating stimulus adaptation had occurred. Conversely, adaptation in the treated groups seemed to have been impeded by diclofenac. According to the authors, the repeated bout effect was most prominent in terms of force production in the control rats which experienced a "non–existent" inflammatory response to the second bout. The authors concluded by stating inflammation should be viewed as an integral component of skeletal muscle repair and adaptation.

Animal research has reported a more complete functional recovery three and seven days post-electrical activation in rabbits (Mishra et al., 1995), attenuated force decrements in rats two days post-electrical stimulation (Lapointe et al., 2002; Lapointe et al., 2003) and controlled muscle strain injury in NSAID-treated animals (Obremsky, Seaber, Ribbeck and Garrett, 1994). Although these acute results appear beneficial, measurements performed at later time points showed disparities between drug and control groups. Mishra et al. (1995) reported force and torque deficits in NSAID-treated animals versus controls twenty-eight days post-electrical stimulation. These results were corroborated by Lapointe, Fremonte, and Cote (2002, 2003) who reported NSAID-treated rats continued to exhibit significant force decrements after subsequent bouts of stimulation compared to controls. Shen, Li, Tang, Cummins, Huard (2005) also reported disadvantages to NSAID ingestion, manifested in delayed and blunted muscle regeneration and increased fibrosis seven and fourteen days post-laceration.

Similar to animal research, human research has also reported initial short–term advantages of NSAID ingestion after contraction-induced injury (Hasson et al., 1993; Dudley et al., 1997; Bourgeois et al., 1999; Baldwin et al., 2001). Concentric and isometric strength was reported to be preserved three days post-acute exercise accompanied by consumption of naproxen sodium in older males and females (Baldwin et al., 2001). What remains to be known is if these short term improvements are followed by performance decrements at later time points, as demonstrated in animal literature.

Prostaglandins

Hormones can be divided into four groups: peptide and protein hormones, steroid hormones, monoamines, and lipid-based hormones. Prostaglandins (PGs) are a type of eicosanoid, or lipid-based hormone, derived from the unsaturated fatty acid arachidonic acid, Figure 1.

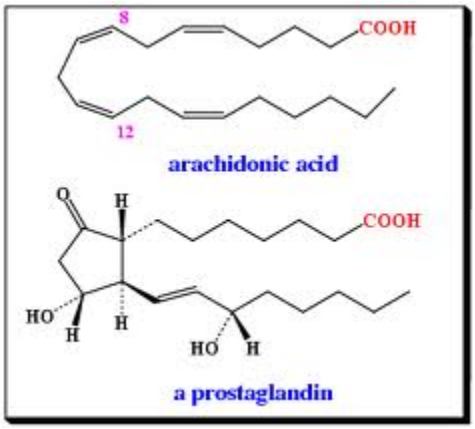

Figure 1 Chemical Structure of Arachidonic Acid and Prostaglandin

In contrast to traditional hormones, prostaglandins are not secreted from a specific gland. Prostaglandins may be produced by all cells except erythrocytes (Ciccone, 1990; Koester, 1993; Pettipher, 1998; Rifai et al., 1999), but the majority are produced from the 20-carbon (C_{20}) fatty acid known as arachidonic acid (C_{20}:4) located within the phospholipid bilayer by the COX enzyme (Rifai et al., 1999; Boushel et al., 2004). Within the circulation, the vascular endothelium is the most prominent source of prostaglandin formation (Boushel et al., 2004), with PGI_2 being the major COX product in endothelial cells (Pettipher, 1998).

The majority of prostaglandins operate in autocrine or paracrine fashion, indicating local production and exertion of local effects. According to Mikkelsen, Helmark, Kjaer, and Langberg (2008), concentration of prostaglandins increases locally in response to exercise. Almekinders, Banes, and Ballanger (1993) found PGE increased 1.3 – 1.8 fold as a result of cyclic deformation of cultured human tendon fibroblasts. Likewise, Otis, Burkholder, and Pavlath (2005) found levels of PGE_2 and $PGF_{2\alpha}$ significantly increased in stretched culture media 6 hours after cessation of stretch.

PGE_2 itself is considered a pro-inflammatory molecule. It is produced and released by ED1+ macrophages (Scott, Khan, Roberts, Cook and Duronio, 2004) and in response to in vitro cyclic deformation (Almekinders, Banes and Ballenger, 1993). It is the predominant eicosanoid detected in humans in inflammatory conditions ranging from acute edema to chronic arthritis (Vane and Botting, 1996). Prostaglandins are extremely potent and are capable of producing physiological actions at concentrations as low as 1μg/L (Rifai et al., 1999).

Skeletal muscle membrane disruption leads to an increase in intracellular calcium. This calcium then activates phospholipase A_2, which cleaves arachidonic acid (AA) from the membrane phospholipid bilayer. Once arachidonic acid is free, it can proceed either of two ways: it may be converted into leukotrienes through the lipoxygenase pathway or into prostaglandins or thromboxanes through the cyclooxygenase pathway. Exactly what controls entry into a specific pathway remains speculative (Rifai et al., 1999); however, it has been shown that production of leukotriene B4 (LTB4) is increased when prostaglandin production is inhibited (Almekinders et al., 1993). Free arachidonic acid is then acted upon by the PG synthetase complex, an enzyme complex that includes COX. COX is required to produce the key cyclic endoperoxides intermediates - PGG_2 and PGH_2. PGH_2 is then acted on by various isomerases to produce the various PG subclasses. Exactly which prostaglandin is produced depends on enzymes present in the biosynthetic pathways (Norman, 1997). Substitutions on the five-membered ring structure (Figure 1) determine the specific subclass and activity. Sixteen naturally-occurring prostaglandins have been described. While seven are commonly found throughout the body (Rifai et al., 1999), only three - PGF, PGE, and PGA - occur in abundance (Norman, 1997).

In denotation of prostaglandins, "F" stands for phosphate buffer-soluble structures, which are the most hydrophilic or polar of the PGs. The E (ether soluble) series is intermediate in solubility. Numerals and Greek symbols are two subscripts that may be used when designating PGs.

The number denotes the number of double bonds in the structure. For instance, $PGF_{2\alpha}$ has two double bonds. The Greek letter following the number refers to the steric position of the C-9 substituent, with α specifically indicating extension behind the plane of the ring (Figure 1) (Norman, 1997).

$PGF_{2\alpha}$ is one of five primary prostaglandins enzymatically derived from the cyclic endoperoxide PGH_2. $PGF_{2\alpha}$ is rapidly metabolized to 13, 14-dihydro-15-keto PGF_2 in vivo by the enzymes 15-hydroxy PG dehydrogenase and Δ^{13}-reductase (Trang, Granstrom and Lovgren, 1977). Due to this rapid metabolism, estimated primary prostaglandins, including $PGF_{2\alpha}$, in the peripheral circulation do not reflect the true endogenous levels of these compounds. However, the main metabolites in plasma (13,14-dihydro-15-keto $PGF_{2\alpha}$) and urine (5α,7α-dihydroxy-11-ketotetranorprosta-1,16-dioic acid) are much more stable, are not formed during sample collection, and are the recommended target of measurement for $PGF_{2\alpha}$ (Trang et al., 1977).

Prostaglandin synthesis can be regulated by controlling the activity of phospholipase A_2 or the COX enzyme. These two different mechanisms of control account for the differing actions of steroids versus non-steroidal anti-inflammatory drugs. Steroids act early to inhibit phospholipase A_2 through production of lipocortin (Norman, 1997), while NSAIDs act at a later stage to inhibit the COX enzyme, through which arachidonic acid is converted into prostaglandins. Steroids inhibit the formation of all prostaglandins, thromboxanes, and leukotrienes (Vane and Botting, 1996), while NSAIDs do not affect leukotrienes or lipoxins (Norman, 1997). The COX enzyme is regulated by analgesic drugs, as well as mechanical stimulation (Mendias et al., 2004; Otis et al., 2005).

After synthesis, prostaglandins are released into the cellular cytoplasm or cell exterior. In either case, PGs mediate signaling through G protein-coupled receptors that are distinct for each prostaglandin (Horsley and Pavlath, 2003). There are specific receptors for PGD_2, PGE_2, $PGF_{2\alpha}$, and PGI_2. These receptors can raise or lower cellular levels of cyclic AMP or stimulate

phosphatidylinositol (IP_3) metabolic pathway, both of which are major secondary signal transduction pathways for G-protein coupled receptors (Norman, 1997). $PGF_{2\alpha}$ facilitates increases in intracellular calcium by binding to its receptor, FP (Breyer, Bagdassarian, Myers and Breyer, 2001; Horsley and Pavlath, 2003). Horsely and Pavlath (2003) reported skeletal muscle cell growth is mediated through the $PGF_{2\alpha}$ receptor, which activates stretch activated calcium channels on the skeletal muscle membrane.

Prostaglandins have pleiotropic effects in a range of cell and tissue types (Horsley and Pavlath, 2003). Complicating the situation is the fact that a single prostaglandin may have opposing effects at different times in the inflammatory response (Willoughby, 2000). PGs play an integral role in the inflammatory response, sensitize nociceptors in a pain response, stimulate parturition, contribute to the control of blood pressure (Norman, 1997), contribute to vasodilation and the local control of blood flow during exercise (Wilson and Kapoor, 1993; Boushel et al., 2002; Mikkelsen, Helmark, Kjaer and Langberg, 2008) stimulate in vitro proliferation and differentiation of human and animal myoblasts (Zalin, 1987; Otis et al., 2005), stimulate in vivo human satellite cell proliferation after exercise (Mackey et al., 2007; Mikkelsen et al., 2009), stimulate nuclei accretion to existing myotubes (Horsley and Pavlath, 2003), and stimulate skeletal muscle protein metabolism (Trappe et al., 2001; Trappe et al., 2002). Along with bradykinine, histamine, and nitrous oxide, PGE_2 and PGI_2 are the two vasodilatory prostaglandins that facilitate shifts in fluid volume from the blood to the intracellular compartment and cells as a result of exercise (Koester, 1993; Boushel et al., 2002).

Prostaglandins and Pain

Prostaglandins, especially PGE_2, play a crucial role in inflammatory reactions, signaling a cascade of pro-inflammatory events including induction of vasodilation, increased vascular

permeability, increased local blood flow, and increased body temperature via action on the hypothalamus (Hertel, 1997). PGEs are released upon stimulation of sympathetic nerves and inhibit adrenergic transmission by depressing responses to norepinephrine at a post-junctional synapse and may also inhibit transmitter release from nerve terminals (Norman, 1997). Opposite effects are seen with PGFs, which often heighten the responses of effectors to norepinephrine. $PGF_{2\alpha}$ can facilitate the release of epinephrine from the adrenal medulla (Norman, 1997).

PGE_2 sensitizes type IV (C) nociceptors which transmit dull, aching pain to higher centers. Along with Aδ fibers, C fibers represent the two classes of nociceptive afferents. Aδ fibers are associated with sharp, focused pain, while C fibers are associated with dull, burning, diffuse pain, as exemplified in DOMS (Norman, 1997). Endogenous chemicals such as PGE and bradykinin facilitate spatial and temporal summation of nociceptor impulses, causing them to discharge at stimulation levels below that eliciting a "frank sensation of pain" (Norman, 1997). These pro-inflammatory and algesic characteristics of prostaglandins are the fundamental reasons they are the targets of non-steroidal anti-inflammatory drugs.

Prostaglandins and Skeletal Muscle Protein Metabolism

In addition to their roles in inflammation, prostaglandins (PGs) have been shown to modulate skeletal muscle protein metabolism (Rodemann and Goldberg, 1982; Smith et al., 1983; Trappe et al., 2001; Trappe et al., 2002). The following section will discuss human and animal research demonstrating prostaglandins' role in this activity vital to skeletal muscle adaptation to resistance exercise.

Smith, Palmer, and Reeds (1983) proposed the primary effect of mechanical activity on protein synthesis is the release of arachidonic acid from membrane-bound phospholipids. Human and animal research has demonstrated arachidonic acid metabolites PGE_2 and $PGF_{2\alpha}$ increase locally

as a result of resistance exercise (Smith et al., 1983; Langberg, Skovgaard, Karamouzis, Bulow and Kjaer, 1999; Trappe et al., 2001; Boushel et al., 2002; Trappe et al., 2002; Mikkelsen et al., 2008), and protein synthesis and degradation are then modulated through metabolites of this fatty acid (Rodemann and Goldberg, 1982). Langberg et al. (1999) reported a 100% increase in the interstitial concentration (ng/ml) of PGE_2 in response to intermittent static plantar flexion. Trappe et al. (2001) reported 70-80% and 60% increases (pg per mg wet weight) in $PGF_{2\alpha}$ and PGE_2 respectively in response to acute intense eccentric knee exercise. Boushel, Langberg, Gemmer, Olesen, Crameri, Scheede, Sander, and Kjaer (2002) reported an intensity-dependent release of PGs, with heavier loads of dynamic knee extension (15W versus 45 W) eliciting a two-fold increase in interstitial PGE_2 content (ng/ml). Using multiple microdialysis catheters inserted directly into exercising musculature, Mikkelsen et al. (2008) reported PGE_2 concentrations during exercise of 1,722 $\pm$ 259 in a control leg versus 879 $\pm$ 183 and 400 pg/ml in a leg subjected to two local prostaglandin blocks (indomethacin) via catheters at 4 and 1 cm sites. After exercise, PGE_2 concentrations in a control leg were 1,201 $\pm$ 204 versus 876 $\pm$ 168 and 318 pg/ml at 4 and 1 cm locations, respectively, in an NSAID-blocked leg.

Prostaglandins and Skeletal Muscle Protein Metabolism – Animal Research

Some of the initial research illuminating prostaglandins' role in protein turnover was performed by Rodemann and Golderg (1982), who provided evidence for a role of PGE_2 and $PGF_{2\alpha}$ in the acceleration of muscle protein metabolism in isolated rat skeletal (soleus and extensor digitorum longus), atrial, and diaphragm muscles. With no mechanical stimulus, exogenous arachidonic acid was supplied in vitro, bypassing phospholipase A_2 activity. Production of the known arachidonic acid metabolites - PGE_2 and $PGF_{2\alpha}$ - was measured by their release into incubation medium during a 2-hour period using radioimmunoassay. In vitro incubation with

arachidonic acid elicited a 20-35% increase in degradation in soleus, EDL, and diaphragm muscles. Addition of arachidonic acid caused no significant change in net protein synthesis in any of muscles except the soleus (slow twitch), which repeatedly responded not only with a significant increase in degradation but also a significant increase in synthesis (53%) upon addition of arachidonic acid. In the soleus, however, the net effect of arachidonic acid was to shift the balance to a more catabolic state. Differences in the response between the soleus and EDL may indicate potential differences in patterns of prostaglandin synthesis between fast and slow twitch muscle fibers, but there is no consensus linking fiber type and prostaglandin response (Palmer, 1990). However, type II fibers have been reported to be more vulnerable to exercise–induced muscle damage (Lapointe et al., 2002).

Rodemann and Goldberg (1982) additionally examined the effect of temperature on basal and AA-stimulated degradation. Lower incubation temperatures (30-33°C versus 37°C) were found to decrease basal protein degradation 20-30% without significantly affecting protein synthesis. Adding arachidonic acid to the medium at this lower incubation temperature resulted in a greater relative increase (40-50%) in net protein degradation compared to 37°C.

Three COX inhibitors (indomethacin, aspirin, meclofenamate) were then tested to elucidate whether arachidonic-acid stimulation of protein degradation was due to increased prostaglandin production. Theoretically, protein degradation should be blocked by COX inhibitors, which prevent the formation of prostaglandins via COX inhibition. Similar to Lapointe et al. (2003), Rodemann and Goldberg found indomethacin reduced arachidonic acid-induced stimulation of both protein synthesis and degradation. When these tissues were incubated with indomethacin in the absence of arachidonic acid, rates of protein synthesis and degradation were not altered. Therefore, indomethacin, aspirin, and meclofenamate exert their specific inhibitory effects on protein synthesis

and degradation through their actions on arachidonic acid metabolites generated in the COX pathway.

Evidence to support this was provided even earlier by Vane (1971) who examined guinea pig lung tissue supernatants incubated with arachidonic acid. Zero-point time samples contained 60-150 ng/ml of "$PGF_{2\alpha}$-like activity" and 120-750 ng/ml of "PGE_2-like activity" (p.233). $PGF_{2\alpha}$ and PGE_2 did not increase when incubated in the absence of arachidonic acid; however, increases of 220-520 ng/ml and 100-500 ng/ml in $PGF_{2\alpha}$ and PGE_2, respectively, were seen when incubated with arachidonic acid for 30 minutes. Vane further reported addition of indomethacin (5μg/ml) and aspirin (40μg/ml) to samples resulted in little or no increase in $PGF_{2\alpha}$ and PGE_2 over time-zero samples. Lower concentrations (1μg/ml indomethacin and 20μg/m aspirin) resulted in inhibition, but curiously the inhibitory effects of both indomethacin and aspirin on $PGF_{2\alpha}$ were greater than those seen on PGE_2. Peterson, Trappe, Mylone, White, Lambert, Evans, and Pizza (2003) similarly found ibuprofen and acetaminophen reduced prostaglandin $F_{2\alpha}$ after eccentric exercise but had no effect on muscle biopsy PGE_2 concentrations. Peterson et al.'s (2003) report of no NSAID-induced PGE_2 inhibition stands in contrast to Lapointe et al.'s (2002, 2003) animal research which reported diclofenac treatment completely abolished the typical serum increases in PGE_2 levels normally accompanying electrical stimulation and voluntary contractions. Possible explanations for the discrepancy between the two studies include the species examined and the NSAID employed.

To determine exactly which metabolites of arachidonic acid affect protein metabolism, Rodemann and Goldberg (1982) tested six metabolites separately. Of these, only PGE_2 and $PGF_{2\alpha}$ were found to have significant effects on protein metabolism. PGE_2 consistently stimulated protein degradation by ~22%. In contrast $PGF_{2\alpha}$ had no effect on degradation but consistently increased protein synthesis by ~35%. Additional in vivo studies performed by Rodemann and Goldberg (1982) demonstrated arachidonic acid stimulated PGE_2 synthesis 5-6 fold and $PGF_{2\alpha}$ synthesis

approximately 3-fold. These levels were dramatically reduced when indomethacin was added to the medium, corroborating findings by Lapointe et al. (2003). Rodemann and Goldberg (1982) concluded the effects of arachidonic acid and PGE_2 on protein degradation seen in their study may well underestimate changes occurring in vivo in well-nourished active organisms.

One year later, Smith, Palmer and Reed (1983) conducted a similar study to further understand the prostaglandins' role in protein synthesis specifically. Their study provided additional evidence for the involvement of arachidonic acid metabolites in the stretch-induced stimulation of muscle protein synthesis. In contrast to Rodemann and Goldberg (1982), Smith et al. (1983) utilized isolated rabbit forelimb muscles and a tension stimulus. Muscles were incubated either under intermittent mechanical stretching or constant tension for 90 minutes. Exogenous $PGF_{2\alpha}$, PGE_2, and arachidonic acid were added to the medium at the onset of incubation, and protein synthesis was measured during 10-30 minutes period using radioimmunoassay.

Intermittent mechanical stretching significantly increased rates of protein synthesis (%/day) with a simultaneous increase in $PGF_{2\alpha}$, compared to muscles held under constant tension (2.47 ± 0.33 versus 5.21 ± 0.77). The addition of indomethacin and meclofenamic acid to the incubation medium significantly reduced rate of protein synthesis in the intermittently stretched muscle but did not affect the basal rates of protein synthesis in the unstimulated muscle. In the intermittently stretched muscle, rates of protein synthesis were significantly reduced 42.2% ($p<0.05$) upon addition of indomethacin. Likewise, meclofenamate significantly reduced rates of protein synthesis 35.4%.

To confirm the potential of arachidonic acid metabolites to induce protein synthesis, muscles were incubated, without stretching, with arachidonic acid in the presence and absence of indomethacin. Arachidonic acid was found to mimic the stretch-induced stimulation of protein synthesis, eliciting a significant increase in anabolic processes. The addition of indomethacin (50μM) to muscles incubated with arachidonic acid caused a significant decrease in protein synthesis

compared to the effect of arachidonic acid alone, and this decrease was not dependent on the concentration of arachidonic acid. Even at lower concentrations (20μM), indomethacin decreased protein synthesis rates by 30%.

Smith et al. (1983) determined activation of muscle protein synthesis persists 10-20 minutes after removal of a 30 minute stretch stimulus, in what the authors referred to as residual activation. Human evidence supporting these findings has been provided by Phillips, Tipton, Aarsland, Wolf and Wolfe (1997), who demonstrated elevated levels of muscle protein synthesis 48 hours after exercise in relatively untrained subjects (n=8). Smith et al. (1983) felt this was explained by assuming arachidonic acid released by lengthening activity is retained intracellularly and continues to be metabolized into prostaglandins. So while mechanical activity results in an initial increase in prostaglandins and thus in vitro protein synthesis, additional protein synthesis continues as a result metabolic activities occurring after removal of the stimulus. This effect has been reported to fall progressively after the stimulus is withdrawn, and Irvine (1982) postulated this was due to the re-esterification of free arachidonic acid. Recall, levels of free arachidonic acid in cells are low, but it is stored in high concentrations in an esterified form in the membrane phospholipids (Pettipher, 1998). The temporal sequence of residual activation may differ between models of mechanical stimulation (Palmer, 1990). Smith et al. (1983) concluded their findings, along with those of Rodemann and Goldberg (1982), support the hypothesis that stretch-induced stimulation of protein synthesis is mediated by changes in intracellular concentrations of arachidonic acid metabolites. This study provides additional evidence protein synthesis increases when skeletal muscles are subjected to mechanical deformation, and the addition of a COX-inhibitor significantly decreases this increase in protein synthesis. The negative implications of this are obvious if one is pursuing increases in muscle size or strength.

Novak, Billich, Smith, Sukhija, McLoughlin, Hornberger, and Koh (2009) found NS-398, a selective COX-2 inhibitor, reduced compensatory hypertrophy in mice. A synergist ablation model of mechanical overload was achieved by removing the soleus and gastrocnemius. Treatment groups included non-ablated vehicle-treated controls, non-ablated NS-398-treated, ablated vehicle-treated controls, and ablated, NS-398-treated mice. NS-398 was delivered by intraperitoneal injection 2 hours prior to ablation and at the same time daily until plantaris muscles were harvested. Controls were treated with a vehicle solution of dimethyl sulfoxide in saline. In vehicle-treated mice, synergist ablation resulted in an ~80% increase in the mass of the plantaris muscle and a ~50% increase in total protein content 2-weeks post-ablation compared to non-ablated controls. In NS-398-treated mice, the hypertrophic response was reduced 75% compared with vehicle-treated ablated controls. Total protein content was also reduced in NS-398 treated mice relative to vehicle-treated and did not differ significantly from non-ablated controls. In mice not subjected to synergist ablation, treatment with NS-398 for 14 days did not alter plantaris mass or protein content, corroborating the lack of effect of indomethacin and meclofenamic acid on basal protein synthesis in unstimulated musle (Smith et al., 1983).

Novak et al. (2009) suggested the increase in muscle protein content during compensatory hypertrophy was likely due to a combination of increased protein synthesis and decreased degradation, as suggested by Sandri (2008), and NS-398 caused either a decrease in synthesis or increase in degradation. Previous research would support an increase mediated by proportionately greater synthesis, as hypertrophy is usually achieved by an increase in protein synthesis with a relatively smaller increase in degradation (Palmer, 1990). However, human research has demonstrated both synthesis and degradation are increased after mechanical loading, and ibuprofen and acetaminophen blunt the increase in both associated prostaglandins (Trappe et al., 2001; Trappe et al., 2002).

Immunofluorescence analysis was employed to determine local expression of COX-2 during compensatory hypertrophy. Novak et al. (2009) reported a sparse distribution at the muscle fiber periphery in non-ablated control mice, while synergist ablation resulted in COX-2 expression in or near nuclei, within or closely associated with muscle fibers or myogenic precursor cells, suggesting that muscle fibers and/or myogenic precursor cells (satellite cells) are the primary source of COX-2 during hypertrophy. COX-2 inhibition also resulted in a 20% reduction in proliferating cells relative to vehicle-treated mice, leading the authors to conclude COX-2 activity is required for compensatory muscle hypertrophy.

Prostaglandins and Skeletal Muscle Protein Metabolism – Human Research

Trappe et al. (2001, 2002) extended Rodemann and Goldberg's (1982) and Smith et al.'s (1983) findings of prostaglandins' role in protein metabolism to humans in two studies examining fractional synthesis rate (2002) and $PGF_{2\alpha}$ concentrations (2001) in subjects of a mixed training status after an acute intense resistance training intervention. As a result of the exercise intervention, both PGE_2 and $PGF_{2\alpha}$ increased significantly. Since these studies involved two analgesic treatment groups in addition to a control group, they will be discussed in greater detail in the subsequent section entitled "Effects of NSAIDs on Skeletal Muscle Fractional Synthesis Rate."

Skeletal Muscle Fractional Synthesis Rate and Fractional Breakdown Rate

Fractional synthesis rate (FSR) represents the rate at which new proteins are incorporated into a muscle. The essential amino acid phenylalanine is often utilized as the tracer to measure FSR and FBR (fractional breakdown rate) because it is not oxidized in muscle or synthesized in the body; thus, its appearance results entirely from protein breakdown (Phillips et al., 1997). Primed constant infusions of [$^2H_5$] phenylalanine and ^{15}N-phenylalanine were used by Phillips, Tipton, Aarsland,

Wolf, and Wolfe (1997) to determine fractional synthesis rate (FSR) and fractional breakdown rate (FBR), respectively. Phillips et al. (1997) sought to examine the time course of muscle protein synthesis and breakdown after an isolated bout of resistance exercise in a co-ed population of aerobically-trained subjects, along with a secondary purpose of investigating potential differences in FSR and/or FBR resulting from contraction type (concentric versus eccentric). Participants were studied in a fasted stated at rest, 3, 24, and 48 hours after eight sets of eight concentric or eccentric repetitions at 80% concentric 1RM with 2 minutes rest between sets.

FSR was significantly elevated in response to exercise at all time points: 3 hours, 122%; 24 hours, 65%; 48 hours, 34% ($p<0.01$). No significant differences were seen between the 24- and 48-hour assessments of FSR. FBR was significantly elevated in response to exercise at 3 and 24 hours but had returned to baseline levels by 48 hours. Exercise resulted in not only an initial increase in FSR, but this heightened state of activity persisted 48 hours after exercise, resulting in a positive change in net protein balance, findings in support of the residual activation reported previously (Smith et al., 1983). It was not clear whether this time course of activation would be the same across all fitness levels, so Phillips et al. sought to clarify this in a 1999 study, which will be discussed next.

Phillips et al.'s (1997) comparison of concentric and eccentric muscle activity revealed no significant differences in FSR, FBR, or muscle net balance between the two types of muscle actions, a somewhat surprising finding in light of the wealth of information demonstrating greater myofibrillar damage and enzyme release resulting from eccentric activity. Phillips et al. (1997) also reported a highly significant correlation ($r=0.88$, $p<0.001$) between FSR and FBR.

To clarify the time course of protein turnover in trained and untrained subjects, Phillips, Tipton, Ferrando, and Wolfe (1999) equally divided co-ed subjects ($N=12$) into untrained ($n=6$) and trained ($n=6$) groups. Untrained subjects were recreationally active, engaging in no more than 1.5 hours per week of exercise and participating in no resistance exercise. Trained subjects were

performing a minimum of three resistance training sessions per week and had been doing so for $\geq$5 years.

After one bout of resistance exercise (8 sets of 10 unilateral eccentric leg extensions at 120% 1RM; 3 minutes rest between sets), FSR and FBR were determined in muscle biopsy samples after amino acid infusions of [$^{2}H_{5}$] and [^{15}N] phenylalanine. Both isotopic infusions were delivered at a rate of 0.05 μmol • kg • min and were designed to achieve steady state in muscle and plasma pools. Biopsies were taken from both the resting and exercise leg to compare the effects of resistance exercise between trained and untrained subjects. Biopsy samples were analyzed for protein-bound and free intracellular enrichment and concentration. Free and protein-bound enrichments were determined by taking heptafluorobutyric (HFB) derivatives of the pooled intracellular fractions and hydrolyzed muscle protein pellets. According to the authors, the HFB derivative of phenylalanine facilitates the determination of phenylalanine enrichment using chemical ionization in gas chromatography-mass spectrometry. Mixed muscle protein FSR was determined using the free intracellular phenylalanine enrichment. Actual mixed muscle protein FSR was calculated as the mean of the incorporation of [$^{2}H_{5}$] phenylalanine into mixed muscle proteins over the 4- hour duration after exercise divided by the free intracellular enrichment.

There were no significant differences between trained and untrained subjects in FSR at rest (0.045% per hour trained; 0.036% per hour untrained) or after exercise (0.067% per hour trained; 0.080% per hour untrained). However, both groups responded to exercise with significant within-group increases in FSR. Untrained subjects' FSR increased 118 $\pm$ 18% (0.036 to 0.080 % per hour) ($p<0.01$), while trained subjects' FSR increased 48 $\pm$ 6% (0.045 to 0.067% per hour) ($p<0.01$) as a result of exercise. This compares similarly to Trappe et al. (2001), who reported a 77% increase in FSR in muscle biopsy taken 24-hours after an acute bout of resistance training in a mixed sample of sedentary and recreationally-trained males.

There was also no significant difference in resting FBR between trained and untrained subjects; however, untrained subject exhibited a significant 37 $\pm$ 5% increase in FBR as a result of exercise. Conversely, trained subjects responded to exercise with a non-significant increase of 15 $\pm$ 3% increase in FBR. Phillips et al. (1999) again reported a significant correlation ($r=0.84$, $p<0.001$) between mixed muscle FSR and FBR, as well as a significant correlation between the change in the two variables as a result of exercise ($r=0.91$, $p<0.001$). Such correlations, the authors concluded, suggest a coupling between the two processes much like the coupling between inflammation and regeneration (Martini, 2004).

Compared to rest, exercise resulted in a significant improvement in muscle protein net balance for both groups, with reductions in the negative balance of 37 $\pm$ 4% and 34 $\pm$ 11% for untrained and trained subjects, respectively. Although FSR increased in both groups as a result of exercise, the overall net balance (FSR-FBR) remained negative compared to time zero, which marked the onset of [$^2H_5$] phenylalanine infusion. According to the authors, this was the expected outcome due to the fact participants were studied in a fasted state. The authors did not report an effect of training status on muscle net balance at rest, after exercise, or on the magnitude of change in net balance.

Smaller increases in FSR and FBR in trained subjects in response to exercise led the authors to conclude repeated resistance training seems to decrease muscle protein turnover after exercise. This conclusion adds to the understanding of the repeated bout effect, suggesting a "training-induced suppression of muscle protein turnover is related to a training-induced reduction in exercise-induced muscle injury" (pE122). The number of bouts of resistance training required to elicit this reduction in post-exercise muscle protein synthesis is not known, but even one bout appears to afford protection, as demonstrated in animal research by Lapointe et al. (2002). These

findings are important to bear in mind when considering Trappe et al.'s research (2001, 2002), which employed both sedentary and recreationally-active males.

Effects of NSAIDs on Skeletal Muscle Fractional Synthesis Rate

Trappe, White, Lambert, Cesar, Hellerstein, & Evans (2002) examined the effects of maximal over-the-counter doses of ibuprofen (1,200 mg/day), acetaminophen (4,000 mg/day), and placebo on muscle protein synthesis and soreness in sedentary or recreationally-active males (n=24, 25 $\pm$ 3 years) after an acute bout of 10–14 sets of 10 eccentric knee extensions at 120% concentric 1RM. Sixty seconds rest was allowed between sets, and the set was concluded when the weight was lowered in less than 0.5 seconds. Drugs were administered in three equivalent doses each day (8 AM, 2 PM, 8 PM), corresponding to the maximal non-prescription dose. The first dose was given at the start of the eccentric exercise protocol, and a final fourth dose was given the morning following the exercise session, ~5 hours before the second muscle biopsy. Biopsies were taken from the vastus lateralis of the dominant leg two days before the exercise and from the non–dominant leg 24 hours after exercise.

Single doses of ibuprofen and acetaminophen at or near those provided in the study are characterized by similar pharmacokinetic parameters. Such doses of ibuprofen are purported to elicit only analgesic activity, with anti-inflammatory effects only seen with larger doses. Acetaminophen (paracetamol) is considered a pure analgesic and antipyretic and is not believed to elicit any anti-inflammatory activity (Vane and Botting, 1996). The two drugs are purported to relieve muscle soreness and pain through separate mechanisms. Ibuprofen blocks cyclooxygenase, reducing prostaglandins, which are responsible for inflammation and algesia. Acetaminophen is believed to exert its action within the central nervous system, thus it would not be expected to interfere with muscle protein metabolism in peripheral tissues after exercise.

A catheter was placed in the antecubital vein for [$^2H_5$] phenylalanine infusion for measurement of skeletal muscle fractional synthesis rate. Blood samples were analyzed for [$^2H_5$] phenylalanine enrichment, and muscle samples were analyzed for free intracellular and protein-bound [$^2H_5$] enrichment by mass spectrometry. Creatine kinase was an additional measure found to be significantly elevated in all three groups, with no significant differences between the treatment groups due to the large and highly variable response seen in this measure.

Post–exercise skeletal muscle fractional synthesis rate (FSR) was significantly increased after exercise in the placebo group but remain unchanged in both the ibuprofen and acetaminophen groups. Variables than can potentially affect FSR, including training status, dietary state, and exercise amount, were controlled in this study. Their results suggested non-prescription doses of ibuprofen and acetaminophen suppressed the normal protein synthesis response in skeletal muscle after eccentric resistance exercise, extending Rodemann and Goldberg's (1982) findings to humans.

Acetaminophen's inhibitory effect on post-exercise FSR was surprising due to the belief its effects are exerted in the central nervous system. To account for this finding, the same muscles used for FSR measurement were measured for $PGF_{2\alpha}$, since animal research has demonstrated its role in skeletal muscle protein synthesis (Rodemann and Goldberg, 1982; Palmer, 1990). $PGF_{2\alpha}$ levels corroborated FSR levels, being significantly increased in the placebo group but unchanged in the acetaminophen and ibuprofen groups. This led Trappe et al (2002) to conclude acetaminophen and ibuprofen attenuate the increase in FSR by blocking production of $PGF_{2\alpha}$ via the COX enzyme. Furthermore, Trappe et al. (2002) concluded by stating continued inhibition of the normal increase in protein synthesis after each session of resistance training could blunt the hypertrophic response to exercise. The proposed study intended to address this possibility.

Krentz, Quest, Farthing, Quest, and Chilibeck (2008) proclaimed their study to be the only study to investigate the chronic NSAID consumption on size and strength development in humans.

In a seemingly convoluted design, co-ed subjects with roughly six years of resistance training experience trained their right and left biceps on alternate days, 5 days per week for six weeks. The exercise consisted of 3 sets of 8-10 concentric repetitions at 70% 1RM and 3 sets of 4-6 eccentric contractions (3 seconds) at 100% 1RM, with 1 minute rest between sets. Of note, the exercise intervention was not performed in a laboratory setting or under the supervision of the research team. Rather, subjects were given guidelines to follow in terms of weight increases to personally implement as strength improved over the duration of the study. Furthermore, 1RM testing was performed with a unilateral bicep curl, an exercise which does not fulfill requirements for 1RM testing. It appears subjects reported only for drug or placebo treatment and pre- and post-measurements of size and strength.

Using one third of the dose administered by Trappe et al. (2001, 2002), ibuprofen (400mg) was given immediately after training the biceps of one arm and placebo was given after training the other arm the following day. Subjects receiving ibuprofen after training their dominant arm was equal to the number receiving ibuprofen after training their non-dominant arm in what the authors described as a counter-balancing measure. According to the authors, ibuprofen is virtually entirely excreted within 24 hours of administration; thus, it was purported to have a negligible effect on the arm receiving placebo the subsequent day. Krentz et al. (2008) cited this as a potential weakness of their study.

Krentz et al. (2008) reported no effect of ibuprofen consumption on muscle thickness as determined by ultrasound or strength development as determined by 1RM. Although this is in discord with Trappe et al.'s conclusion that medications such as ibuprofen and acetaminophen could blunt the anabolic response to exercise, numerous methodological differences exist between the two studies (Trappe et al., 2001; Trappe et al., 2002). While Krentz et al.'s (2008) findings do not demonstrate a negative long term effect as suggested by Trappe et al. (2001), there are substantial

methodological differences between the two studies that undoubtedly account for some of this discord. Krentz et al. (2008) called for the need for research measuring blood markers of inflammation and protein synthesis over the course of a strength training program was called for at the conclusion of their study.

Prostaglandins and Satellite Cells

According to Petrella et al. (2007), the general consensus is that successful skeletal muscle regeneration following injury or intense exercise is dependent upon protein synthesis mechanisms and satellite cell recruitment. Research has shown prostaglandins stimulate satellite cell activity, and evidence is mounting that NSAIDs interfere with satellite cell proliferation, differentiation, and fusion (Zalin, 1987; Shen et al., 2005; Mackey et al., 2007; Mikkelsen et al., 2008; Mikkelsen et al., 2009; Novak et al., 2009).

Some of the seminal research highlighting the role of prostaglandins in human myoblast proliferation and differentiation was performed by Zalin (1987). Aborted human fetuses were cultured and studied in vitro. Addition of $PGF_{2\alpha}$ to the culture positively affected myoblast proliferation, suggesting that $PGF_{2\alpha}$ is an endogenously-produced growth factor. Stimulation of cell increase was most marked 24-48 hours after hormone addition. Addition of PGE_2 was found to stimulate myoblast fusion and differentiation, with fusion seen 2 hours earlier versus control cells not exposed to PGE_2. The relationship between prostaglandin production and satellite cell proliferation was evidenced by the addition of two prostaglandin inhibitors, indomethacin and aspirin. These resulted in some inhibition as early as 24 hours and complete inhibition 48-72 hours after exposure. The reason for the delay between the addition of prostaglandin antagonists and inhibition was concluded to be unknown, although it was suggested effective levels of prostaglandin inhibition may not be reached until successive (third) administrations. If true, chronic use would

theoretically blunt the regenerative response, and this was indeed demonstrated in comparisons of 2- versus 7-day NSAID treatments (Lapointe et al., 2003)

To test this, Zalin (1987) exposed some cells to consecutive 24 hour doses of indomethacin, while others were exposed to only one dose at 48 hours. Indomethacin added to cell cultures in a single dose (10^{-4} M) at 48 hours was not found to inhibit the increase in cell number normally occurring at that time (48-72 hours). In contrast, less than that same amount added daily resulted in a marked inhibition when administered from time zero. Since a smaller amount resulted in marked inhibition of proliferation, the authors concluded the time delay was not due to insufficient prostaglandin antagonists. These findings suggest chronic low dose administration of NSAIDs may have negative impacts on myoblast proliferation and hold serious implications for those persons chronically consuming NSAIDs while anticipating hypertrophic gains.

Both aspirin and indomethacin were found to markedly and rapidly reduce rate of fusion. These drugs were found to inhibit cell fusion even when added 1 day after the initial detection of fusion in cell cultures. Zalin (1987) reported greater inhibitions of fusion could be obtained with earlier exposure, with maximum inhibitions achieved when cells are exposed to the PG antagonists 24 hours or more before the expected time of fusion onset. During fusion, cell content of PGE decreased, while the content of the culture medium increased two-fold. This finding holds implications for the metabolism of PGE and suggests the rate of secretion exceeds the rate of metabolism in the culture medium and lends credence to research by Pradelles et al. (1985) and Trang et al. (1977), which validated measurement of prostaglandin metabolites in blood plasma and joint fluid.

Horsley and Pavlath (2003) examined the role of $PGF_{2\alpha}$ on satellite cell activity in an in vitro study of primary (adult) myoblast cultures of mice tibialis anterior muscles. To investigate $PGF_{2\alpha}$'s role in muscle growth, differentiating primary muscle cultures of mice tibialis anterior muscles were

treated with vehicle or $PGF_{2\alpha}$. Twenty-four hour assessments of myoblast size revealed no apparent differences between vehicle- and prostaglandin-treated cells; however, by 48 hours, prostaglandin-treated myoblasts were larger, suggesting a role for $PGF_{2\alpha}$ in skeletal muscle growth. Unlike Zalin (1987) who found a role for $PGF_{2\alpha}$ in myoblast proliferation, Horsely and Pavlath (2003) reported no effect of $PGF_{2\alpha}$ on cell proliferation (determined by quantification of DNA content), cell differentiation (determined by embryonic myosin heavy chain staining), or cell fusion index (determined by dividing the total number nuclei within myotubes (2 or more nuclei) by the total number analyzed (100-250). While these results are different from Zalin's (1987), it is important to note Zalin (1987) examined fetal human myoblasts, while Horsely and Pavlath (2003) examined primary (adult) mice myoblasts.

Horsely and Pavlath (2003) determined the role of $PGF_{2\alpha}$ on skeletal muscle growth is dependent on the developmental stage of the myotube. During the embryonic developmental period, $PGF_{2\alpha}$ enhances cell fusion processes that initially form myotubes. During regeneration periods, $PGF_{2\alpha}$ enhances the accretion of myonuclear cells. Specifically, compared to vehicle-treated controls, $PGF_{2\alpha}$-treated cells demonstrated a significant increase in the percentage of myotubes expressing five or more nuclei per fiber at a 48 hour assessment. Another remarkable finding of Horsley and Pavlath's (2003) study was the effect of time on $PGF_{2\alpha}$'s role in fusion. These effects were examined by treating differentiating muscle cells with exogenous $PGF_{2\alpha}$ at different stages of fusion, which itself involves the processes of cell motility, alignment, recognition, adhesion, and membrane union (Horsley and Pavlath, 2003). Cells were treated either at the onset of differentiation (0 h) or at 24 hours, a time when fusion processes begin and a few multinucleated muscle cells are present. In both cases, myotubes were analyzed 48 hours later. When given at 0 h, no significant differences in the percentage of myotubes expressing 5 or more nuclei were seen between vehicle- or $PGF_{2\alpha}$-treated myofibers. Conversely, at 24 hours, $PGF_{2\alpha}$-treated cells displayed

significantly more nuclei than vehicle-treated, indicating $PGF_{2\alpha}$ acts at a later stage of fusion to facilitate an increase in cell size. This holds important implications for use NSAIDs therapeutically. According to Horsley and Pavlath's findings (2003), inhibition of $PGF_{2\alpha}$ 24 hours post-injury would be detrimental to muscle cell growth. Coincidentally, this 24 hour time point coincides with the initial onset of symptoms of DOMS.

Both Mackey et al. (2007) and Crameri et al. (2004) found a single bout of high intensity exercise sufficient for satellite cell activation. In Crameri et al.'s (2004) study, the experimental design included eccentric exercise (50 single leg drop jumps, 8 sets of 10 maximal eccentric contractions performed at 30 degrees • sec and 8 sets of 10 maximal isokinetic eccentric knee extensions performed 180 degrees • sec.) performed by sedentary young males (n=8). Conversely, Mackey et al. (2007) used trained males and a long-distance (36-km) aerobic intervention. While both found a single bout of exercise to be sufficient for satellite cell activation, Crameri et al. (2004) stated a single bout is not sufficient for terminal differentiation. Crameri et al. (2004) suggested that the number of cells expressing satellite cell protein markers, including neural cell adhesion molecule (N-CAM) and fetal antigen 1 (FA1,) can be up-regulated in readiness for a second bout of exercise. However, unless subsequent bouts of activation occur, the satellite cells will again become quiescent. Similar to Mackey et al. (2007), Crameri et al. (2004) identified satellite cells through N-CAM and FA1 staining, while necrosis was identified by the presence of desmin-negative cell, dystrophin-negative cells, fibronectin-positive cells, and centralized nuclei. There is still debate concerning the ideal satellite cell marker (Bamman, 2007).

Crameri et al. (2004) reported a significant increase in cells positively staining for N-CAM and FA1 on days 4 and 8 post-eccentric dominant resistance exercise. Mackey et al. (2007) also reported a significant increase (27%) in the number of N-CAM+ cells eight days post-36 km run but no significant changes over time in FA1+ cells. The training intervention (36-km aerobic versus

210 voluntary contractions) and the subject population (trained versus sedentary males) are two key differences between Crameri et al.'s (2004) and Mackey et al.'s (2007) research. Particularly in regards to the training status, Crameri et al. (2004) reported 200% increases in N-CAM+ cells on days 4 and 8 post-exercise in sedentary participants, while Mackey et al. (2007) reported increases of 27% in N-CAM+ cells by day eight in trained participants. The difference in N-CAM+ cells between trained and untrained populations is reminiscent of Philips et al. (1999) findings of significantly different exercise-induced fractional synthesis and breakdown rates between trained and untrained populations. Thus, not only does the magnitude of the response appear to be substantially different between trained and untrained populations, but the time course of peak activation seems different between the two exercise modalities as well.

In Mackey et al.'s (2007) study of satellite cell activation post-36 km run, indomethacin (100 mg/day for 4 days pre-run to 8 days post run) was found to blunt the increase in N-CAM positive cells after exercise, but differences between indomethacin and placebo groups were not statistically significant. Even in the absence of significant differences, the authors argued their findings provided in vivo support for the COX pathway in satellite cell activity.

Novak et al. (2009) reported satellite cells are the primary source of COX-2. Curiously, Mackey et al. (2007) reported FA1 stained 25% more satellite cells in healthy untrained individuals versus trained individuals. Of these additional FA1+ satellite cells, some were located in the classic position under the basal lamina, while others were located outside the muscle fiber, finding similar to Novak et al. (2009), who found COX-2 expressed in the muscle fiber periphery in control mice; while in those subjected to mechanical overload, COX-2 was found in or near nuclei associated with satellite cells. According to Mackey et al. (2007), this may support the theory that skeletal muscle is comprised of different subpopulations of myogenic precursor cells.

Similar to Philips et al. (1999), Mackey et al. (2007) suggested the findings of lower numbers of FA1+ cells in trained versus untrained could represent a loss of regenerative capacity for cell division; however, Mackey et al. (2007) reported no necrotic fibers in the muscles post-run, similar to Crameri et al. (2004) who reported necrosis in only one of eight males. In the one case of necrosis, expression of myogenic regulatory factors (myogenin) was coincident. Taken together with Crameri et al.'s findings (2004), it appears satellite cell activation is not dependent on muscle damage. Bamman (2007) stated "the fact that N-CAM+ satellite cells number was inhibited by NSAID treatment, even in the absence of visible myofiber necrosis or detectable increases in regeneration, suggests that COX-mediated prostaglandin synthesis may be initiated simply by exercise" (p 415), a conclusion which, of course, been demonstrated by previous research (Rodemann and Goldberg, 1982; Smith et al., 1983).

Mikkelsen et al. (2009) also examined satellite cell activation post-resistance exercise in males (n=8) who were described as well-trained yet unaccustomed to intense eccentric exercise. Immunohistochemistry was utilized to investigate satellite cells, which were identified by N-CAM and Pax7 staining of muscle biopsies before and eight days after an acute exercise intervention consisting of 200 eccentric knee extensions on an isokinetic dynamometer. A microdialysis catheter was used to locally infuse indomethacin into one leg, while the other leg served as the control. Indomethacin infusion began during exercise and continued for 4.5 hours after completion of knee extensions. Compared to baseline, exercise resulted in an impressive 96% increase in the number of Pax7+ cells (expressed per myofiber) eight days post-exercise in the control leg, while the number remained unchanged in the leg infused with indomethacin. Mikkelsen et al. (2009) reported a 22% increase in N-CAM+ cells in the control leg, results similar to the Mackey et al. (2007) who reported a 27% increase in N-CAM+ cells in a trained population. Mikkelsen et al. (2009) concluded their

results provided evidence that NSAIDs negatively affected satellite cell proliferation during an 8 day period post-exercise when administered before, during, and 4.5 hours after exercise.

Mendias, Tatsumi, and Allen (2004) examined the role of COX-1 and COX-2 on satellite cell proliferation in an in vitro study of isolated rat skeletal (hindlimb) muscle. The authors hypothesized if COX-2 is involved in satellite cell regulation during muscle repair, it should be expressed in activated satellite cells. Of course, this was confirmed later by Novak et al. (2009). Satellite cells were isolated and cultured, and were examined for proliferation, differentiation, and fusion. To examine the effects of COX-inhibitors on satellite cell activity, cultures were grown in the presence of naproxen sodium (non-selective COX-inhibitor), NS-398 (selective COX-2 inhibitor), or SC-560 (selective COX-1 inhibitor) from 24 h to 120 hours. Satellite cell lysates from cultures at 30 h, 72 h, 120 h, and 168 h were all positive for COX-2. Results revealed a significant increase in proliferation from 72-120 hours in control cell cultures. Cultures grown in the presence of naproxen sodium and NS-398 revealed a significant decrease in proliferation. Cultures grown in the presence of naproxen sodium, NS-398, and SC-560 revealed a significant decrease in differentiation as well as fusion. In summary, Mendias et al. (2004) found inhibition of COX-2 alone resulted in decreased proliferation, and inhibition of both isoforms of COX resulted in decreased differentiation and fusion. These findings are in accord with McLennan (1987), who found chicken egg embryos treated with aspirin or indomethacin exhibited less proliferation than vehicle-treated embryos; but not in accordance with Thorsson et al. (1998), who reported no effect of naproxen on satellite cell proliferation in female rat skeletal muscle subjected to contusion injury.

To investigate the effects of mechanical stretch on cultured primary mice myoblasts, Otis, Burkholder, and Pavlath (2005) subjected them to 5 hours of cyclical stretch at 5 Hz. The stretch stimulation resulted in a 32% and 41% increase in proliferation (BrdU+ cells) 24 hours and 48 hours after stretch, respectively. However, stretch was found to decrease mRNA levels of myogenin, one

of primary myogenic regulatory factors. According to the authors, these results suggested mechanical deformation delays differentiation to facilitate increased cell proliferation. The authors postulated this "stretch-induced delay of differentiation allows for increases in the myoblast pool and ultimately may lead to enhanced myotube formation" (p423). Otis, Burkholder, and Pavlath (2005) determined stretched myoblasts secrete a soluble factor that facilitates cell proliferation and sought to determine if this factors was a product of the COX-2 pathway. To test this, COX2 activity was disrupted through treatment with SC-236, a selective COX-2 inhibitor, or through removal of the COX2 gene. Both myoblasts treated with SC-236 and those deficient for the COX2 gene ($COX2^{-/-}$) failed to proliferate upon stretch stimulation. In fact, in those cells treated with SC-236, prolilferation was actually decreased compared to non-stretch controls.

Compared to vehicle-treated (95% ethanol), there was a significant decrease in proliferative cells. To determine if products of the COX-2 pathway are required for stretch-induced proliferation, PGE_2 and $PGF_{2\alpha}$ were added to COX-2 deficient myoblasts to determine if these growth factors could correct the proliferative deficiency in these mice. While PGE_2 only partially compensated for this lack, $PGF_{2\alpha}$ corrected a significant portion of this deficit. According to the authors, these pharmacologic and genetic approaches to COX-2 inhibition confirm the idea that COX-2 pathway products, PGE_2 and $PGF_{2\alpha}$, are necessary for myoblast proliferation.

With human and animal research demonstrating a role for $PGF_{2\alpha}$ in skeletal muscle protein synthesis (Rodemann and Goldberg, 1982; Smith et al., 1983; Trappe et al., 2001; Trappe et al., 2002) and satellite cell activity (Zalin, 1987; Mackey et al., 2007; Mikkelsen et al., 2009) and the current idea that $PGF_{2\alpha}$ is an endogenously produced growth factor (Trappe et al., 2001; Horsley and Pavlath, 2003), a strong line of previous research has led to the current investigation. While negative effects of prostaglandin-inhibiting drugs have been seen after acute resistance training, whether these acute effects continue to suppress parameters of adaptation is not known. In consideration of

the ability to self-medicate with a readily-accessible drug that may potentially inhibit reparative processes in the muscle, the purpose of this study was to investigate the effects of a chronic prophylactic dose of naproxen sodium on skeletal muscle adaptation to resistance exercise in recreationally-trained college-aged males.

Chapter III

Methodology

The purpose of this study was to investigate the effects of chronic prophylactic naproxen sodium on skeletal muscle adaptation to resistance exercise in recreationally-trained college-aged males. Recreationally-trained was defined as individuals who were currently performing resistance exercise 3-5 times per week and had been doing so a minimum of 6 weeks. Performance of a total body workout three times per week, upper/lower split routine totaling four sessions per week, or push/pull/lower split routines totaling five sessions per week qualified a participant for assignment as recreationally-trained. Participants (n=23) were recruited from the University of Mississippi to engage in a 6-week resistance training protocol designed to develop size and strength of upper body musculature.

Due to the potential confounding effects of sex hormones, as well as sex differences in the inflammatory response associated with exercise-induced muscle injury (MacIntyre et al., 1996; MacIntyre et al., 2000; Stupka, 2000), only males were recruited for this investigation. Further rational for this decision came from a study that reported males are more likely to take over-the-counter analgesics for muscle ache (Wilcox et al., 2005). Primary outcome variables included acute and chronic changes in systemic plasma $PGF_{2\alpha}$ metabolite concentrations, acute and chronic changes in dominant arm circumference, strength, and arm tissue (bone, adipose, and lean skeletal) composition as a result of exercise (placebo) or exercise and NSAID (naproxen sodium).

Pilot Study

Due to a lack of literature describing normal resting or exercise-induced $PGF_{2\alpha}$ metabolite concentrations or using a systemic approach to its measurement, the study was piloted for a 2-week period. During the pilot study, male participants (n=4) were randomly assigned to placebo (n=2) or naproxen sodium (n=2) treatment groups, subjected to baseline strength testing, and supervised in the performance of upper body resistance exercise similar to that used in the formal 6-week study. During the pilot study, each participant provided a pre- and post-exercise blood sample (3.0 ml) with every supervised workout, resulting in a total of 8 samples for each subject. Pilot study drug assignment was double blind, and the blind was not broken until after enzymeimmunoassay (EIA) and statistical analyses were completed. Blood samples obtained during the pilot study were analyzed for 13,14-dihydro-15-keto $PGF_{2\alpha}$ concentrations using the EIA kit intended for analysis of formal study samples (Cayman Chemical, Catalog No. 516671, Ann Arbor, MI). The results of the pilot study supported the systemic measurement approach employed during the formal study, for which the following methodological sections detail.

Screening

Participants were screened using IRB-approved documentation, consisting of a general physical activity readiness questionnaire and an expanded health status questionnaire, which included a series of questions to determine characteristics of their exercise program, current supplement use, and contraindications to the use of naproxen sodium. After explanation of all procedures, risks, and benefits, IRB-approved informed consent was provided by all participants. Nine participants did not pass pre-screening qualification for reasons including frequent indigestion, failure to meet recreationally-trained status, and/or use of numerous and varied ergogenic supplements.

Recent (within 1 month) regular use of non-prescription or prescription NSAIDs precluded participation, as did any history of sensitivity to NSAIDs, gastric ulcers, and heart, kidney, or liver disease. Potential participants who were using any type of performance enhancing supplements, including creatine, ephedrine, amphetamines, or anabolic-type products were also excluded from participation. Individuals who regularly consumed alcohol in excess of moderate amounts (>3 drinks per day for a male) were also precluded due to liver concerns. Subjects were randomly assigned to placebo or naproxen sodium treatment groups, and drugs were administered in double-blind fashion prior to each biweekly workout.

Familiarization Session

An initial familiarization session was first performed to acquaint the participants with specific characteristics of each exercise and to conduct baseline testing. This familiarization session occurred 1-8 days prior to the onset of the 6-week intervention. Within this session, dual energy x-ray absorptiometry (DXA) was used to determine baseline body composition, with specific emphasis on bone mineral content, lean tissue (g), fat tissue (g), and fat percentage in right and left arms. DXA measures tissue absorption of high- and low-energy x-ray beams. Mazess, Barden, Bisek, and Hanson (1990) reported precision errors (1 SD) for total body BMD, percent fat in soft tissue, fat mass, and lean tissue mass were <0.01 g/cm^2, 1.4%, 1.0 kg, and 0.8 kg, respectively. These precision error rates corresponded to a relative error of 0.8% for total body BMD and 1.5% for lean body mass. In addition to its initial use during the familiarization session, DXA was also performed at the conclusion of the 6-week study to determine changes in arm tissue composition.

Dominant upper arm circumference (cm) and body weight (kg) were recorded during the familiarization session. A minimum of two circumference measurements were performed pre- and post-exercise to examine the acute change in arm circumference in response to exercise. If the two

circumference measures differed by more than 0.3 cm, a third measurement was taken, and the average of the three was recorded. Upper arm circumference was measured to the nearest tenth of a centimeter. To measure circumference, participants were directed to first extend the arm shoulder level in the frontal plane and then contract the bicep. A Gulick tape measure was placed around the visually-determined widest circumference of the dominant upper arm, which was reported as the right arm by all participants. Resting blood pressure was assessed during the familiarization session. In addition to its initial measurement during the familiarization session, body weight (kg) was also measured prior to each exercise session. An initial 6-ml blood sample was also collected during the familiarization session.

During the familiarization session, participants' 1-repetition maximum (1RM) was determined for each exercise performed in the study (Figures A1-4). Participants were also initially 1RM tested in the bench press and plate-loaded leg press, and these results were used to formulate an individualized, periodized, and unsupervised chest and leg exercise prescription for the participants to perform during the six weeks. The chest, shoulder, tricep workout included (1) flat barbell bench press, (2) dumbbell military press, (3) machine chest fly, and (4) cable tricep extension. The lower body workout included the (1) plate-loaded leg press, (2) Romanian deadlift, (3) leg extension, and (4) bent-knee calf raise. Participants were verbally provided with specific performance guidelines for all unsupervised exercise sessions to ensure safety. While this had been offered as an incentive for participation, most participants performed their own personally-developed chest and leg programs during the 6-week training study.

One repetition maximum testing followed NSCA guidelines. One repetition maximum was directly determined for the Nautilus® lat pulldown, t-bar row, bench press, and plate-loaded leg press. Procedures for one repetition maximum testing entail the following:

1. Warm-up set allowing 10 repetitions.
2. 1 minute rest.
3. Addition of absolute or relative increase of 10-20 lbs. or 5-10%, respectively, for performance of first set, for which the intention is to complete 3-5 repetitions.
4. 2 minute rest.
5. Addition of absolute or relative increase of 10-20 lbs. or 5-10%, respectively, for performance of second set, for which the intention is to complete 2-3 repetitions.
6. 2-4 minute rest.
7. Addition of absolute or relative increase of 10-20 lbs. or 5-10%, respectively, for performance of third set, for which the intention is to complete 1 repetition.

Care was taken to ensure 1RM was determined within three sets, not including the warm-up set. A 10-repetition warm-up set was performed only for the first exercise tested (lat pulldown). For those unable to complete the goal repetitions for each respective set in the 1RM process, absolute or relative weight was decreased 5-10 lbs. or 2.5-5.0%, respectively, until only one repetition could be completed with good technique.

Due to their classification as assistance exercises, the barbell bicep curl and barbell upright row 1RM were determined indirectly via a 10RM test. Guidelines for 10RM are similar to those for 1RM, except load increases over sets are 50% of the amounts used during 1RM testing (Brzycki, 1993) and goal repetitions are always ten. Assessment of barbell bicep curl and upright row 10RM

were not preceded by warm-up sets, and 10RM was determined in 1-2 testing sets. Exercises were always tested in the same order.

Prior to determination of 1RM, subjects were specifically instructed on technicalities of each exercise, including body posture, joint position, range of motion, and tempo of movement, all of which were standardized. Standardization information has been provided as captions along with each respective figure (Figures A1-4).

Exercise Sessions

The resistance training program consisted of four resistance training exercises targeting the upper back and bicep musculature. These included the lat pulldown (Figure A1), t-bar row (Figure A2), barbell upright row (Figure A3), and barbell bicep curl (Figure A4). Specific details for the performance of each exercise accompany each picture in Appendix A. Prostaglandins are considered to act locally, and these specific exercises load musculature near the venipuncture site. Thus, it was believed a relatively large prostaglandin response would be seen in an antecubital vein after performance of these exercises, as exercised-induced PG increases in local tissues have been reported (Mikkelsen et al., 2008) and diffusion from in vitro cells into incubation medium has been shown (Zalin, 1987). Furthermore, muscle contractions >45% 1RM results in venous pooling in the peripheral circulation, which effectively increase hormonal concentrations in the venous blood (Kraemer et al., 2008).

Participants performed two workouts per week, with workouts separated by 48 hours. Participants performed 3 sets of 6-10 repetitions of each exercise at 66-86% 1RM with 90 seconds rest between each set and each exercise. Following a "heavy-light" approach to the resistance workout, the first workout of the week utilized a greater percentage of 1RM (76-86% 1RM), while

the second workout utilized a lighter percentage of the 1RM (66-76% 1RM). The periodization protocol is provided in Table 4.

Table 4

Periodization Program

	Workout 1		Workout 2	
	Intensity	Sets x Reps	Intensity	Sets x Reps
Week 1	76%	3 x 8	66%	3 x 10
Week 2	78%	3 x 8	68%	3 x 10
Week 3	80%	3 x 6	70%	3 x 10*
Week 4	82%	3 x 6	72%	3 x 10
Week 5	84%	3 x 6	74%	3 x 10
Week 6	86%	3 x 6	76%	3 x 10

*Second strength assessment conducted prior to workout. Subsequent intensities based on results of this assessment.

Participants were instructed to complete the concentric phase of the lift in ~1 second and the eccentric phase slightly slower. With few exceptions, participants performed each supervised workout at the same daily time throughout the 6-week study in order to standardize measurement.

In anticipation of strength increases, participants' 1RM was re-evaluated for each exercise at week 3 to determine appropriate load adjustments for the final three weeks of the program. This second 1RM test occurred prior to the second, or light, workout of the week. On this re-assessment day, 1RM was first assessed according to aforementioned guidelines, treatments (placebo or naproxen sodium) were orally administered with nutritional supplement, thirty minutes rest was provided, and the scheduled workout was completed. Subjects were prohibited from performing

any additional back or bicep resistance training exercise over the 6-week period. Subjects were also prohibited from performing any type of exercise (aerobic or resistance) prior to lab workouts.

Blood Sampling

Blood was sampled at three time points: workout 1 of week 1, workout 7 of week 4, and workout 11 of week 6. A pre- and post-exercise blood draw was performed at each of these time points, resulting in a total of three pre-exercise and three post-exercise blood samples per subject, in addition to the baseline sample obtained during the familiarization session. Arms used for pre- and post-exercise blood draws were alternated over the seven venipunctures. Venipuncture was performed using a 25 to 23 gauge BD Vacutainer® Push Button Blood Collection Set (BD, Franklin Lakes, NJ) to draw 6 milliliters (mls) of blood from an antecubital vein of participant's arm. Pre-exercise and post-exercise blood samples were collected in BD Vacutainer® K2 ethylenediaminetetraacetic acid (EDTA)-treated tubes. Samples were placed on a brief ice bath and promptly centrifuged (15 minutes, 2600 rpm, 18°C), aliquoted, and frozen at -80°C.

After the pre-exercise blood draw during workouts 1, 7, and 11, and at the beginning of all other non-blood collection sessions, each subject was provided an 8-ounce serving of a commercially-available meal replacement (Boost®) to standardize nutritional intake and buffer potential gastrointestinal effects of naproxen sodium. Both Aleve® and placebo were placed inside identical quick-dissolving gelatin capsules (Health Care Logistics, Inc. Item #14012W), and drugs were orally administered in double-blind fashion immediately after the pre-exercise blood draw, if draws were scheduled, or immediately upon reporting to the lab if draws were not scheduled. A 440mg dose of Aleve® was administered, in accordance with the manufacturer's over-the-counter dosing regimen. This dose is delivered in two Aleve® caplets, which contain 400mg of the active ingredient naproxen plus 40mg of sodium to accelerate absorption. The non-prescription dosing

regimen for pain is an initial oral dose of 200-400 mg followed by 200 mg every 8-12 hours as needed (MICROMEDEX, 2011). The double-blind was not broken until after all blood assays and statistical analyses had been completed.

Although blood was only drawn in conjunction with workouts 1, 7, and 11, treatments were administered at both supervised exercise sessions each week. After allowing 30 minutes for the initial analgesic action of naproxen sodium, participants were supervised in the performance of the four resistance exercises. Seventeen participants were trained solely by the primary investigator. Six participants were trained by a research assistant, who was first trained by the primary investigator to ensure consistency in the implementation of the supervised workouts.

Enzyme Immunoassay (EIA)

The primary metabolite of prostaglandin $F_{2\alpha}$ metabolite in the peripheral circulation is 13, 14-dihydro-15-keto $PGF_{2\alpha}$ (Trang et al., 1977; Guthrie and Rexroad, 1981), and measurement of 13,14-dihydro-15keto $PGF_{2\alpha}$ in plasma can be used as a marker of the in vivo production of $PGF_{2\alpha}$ (Del Vecchio, Maxey and Lewis, 1992). Enzyme immunoassay (EIA) has been shown to be a viable alternative to radioimmunoassay (Pradelles, Grassi and Maclouf, 1985; Del Vecchio et al., 1992) for the quantification of this metabolite. Plasma samples analyzed with both EIA and RIA have shown a correlation of 0.95 (Del Vecchio et al., 1992).

Plasma concentration of prostaglandin $F_{2\alpha}$ metabolites were analyzed with a commercially available EIA kit (13,14-dihydro-15-keto $PGF_{2\alpha}$ EIA Kit, Catalog No. 516671, Cayman Chemical, Ann Arbor, MI). This is a competitive assay validated for determination of this downstream metabolite in plasma and other sample matrices. Manufacturers state the EIA displays an IC_{50} value (50% B/B_0) of approximately 120 pg/ml and a detection limit (80% B/B_0) of approximately 15 pg/ml. The assay is based on competition between free 13,14-dihydro-15-keto $PGF_{2\alpha}$ and a 13,14-

dihydro-15-keto $PGF_{2\alpha}$ tracer linked to acetylcholinesterase (AChE) for a limited number of 13, 14-dihydro-15-keto $PGF_{2\alpha}$-specific anti-serum binding sites. Concentration of 13,14-dihydro-15-keto $PGF_{2\alpha}$ tracer remains constant, while the concentration in the sample varies. The amount of 13,14-dihydro-15-keto $PGF_{2\alpha}$ tracer that is able to bind to the available binding sites is inversely proportional to the concentration of free 13, 14-dihydro-15-keto $PGF_{2\alpha}$ in the well.

Using one pre- and post-exercise sample obtained from one participant who chose to discontinue participation after completion of baseline testing and the first two supervised workouts, a second preliminary EIA was performed to examine potential interference in plasma samples and appropriate dilutions for the assay. Along with the pilot study experiment, this provided a second opportunity for refinement of the procedures and techniques associated with the specific EIA kit. Based on the results of this experiment, purification protocols were not deemed necessary and a single dilution factor of one (undiluted) was deemed acceptable due to consistency in absorbance readings.

EIA kits were stored at -20°C. Plasma samples (1.5 ml) were removed from -80°C freezer and allowed to thaw on an ice bath (approximately 30 minutes). Samples were then centrifuged at 1500 rpm for 6 minutes at 20°C. UltraPure water (Cayman Chemical, Item No. 400000) was used not only to prepare all buffers and reagents but also to wash all glassware used during the EIA procedure. Diluted buffers were stored at 4°C.

Not including 24 baseline samples, a total of 160 blood samples were analyzed. Processing was divided into 3 batches, with 2 plates being run simultaneously in each batch. An 8-point standard curve was generated for each 96-well plate using the 13,14-dihydro-15-keto $PGF_{2\alpha}$ kit standard. Each standard was run in duplicate. Plasma samples (50µl) were then added to the wells. Sample numbers 25-68 were run in duplicate, and samples 69-168 were run in triplicate. Standard and sample addition to the plate was followed by addition of AchE tracer, which consists of one

molecule of 13, 14-dihydro-15-keto $PGF_{2\alpha}$covalently attached to one molecule of AChE. $PGF_{2\alpha}$ antiserum was then added to each well. Plates were covered and incubated 18 hours at 4°C. Immediately after incubation, each 96-well plate was washed five times with wash buffer containing potassium phosphate, UltraPure water, and Tween 20 using an automated washer (BioRad, Model 1575 ImmunoWash).

Ellman's reagent (acetylthiocholine and 5,5'-dithio-bis-(2-nitrobenzoic acid)), which is used to measure AChE activity and thus quantify the amount of tracer bound to the well, was reconstituted with UltraPure water immediately before use on day two of the EIA procedure. After addition to each well, plates were covered and incubated an additional 2 hours in the dark at room temperature on an orbital shaker. When acetylthiocholine is hydrolyzed by AChE, thiocholine is produced. The reaction of thiocholine with 5,5'-dithio-bis-(2-nitrobenzoic acid) produces 5-thio-2-nitrobenzoic acid, which produces a distinct yellow color that absorbs strongly at 412 nm.

Spectrophotometry was then employed to measure the intensity of the color, which is proportional to the amount of 13, 14-dihydro-15-keto $PGF_{2\alpha}$ tracer bound to the well and indirectly proportional to the amount of free 13, 14-dihydro-15-keto $PGF_{2\alpha}$ in the well. An endpoint assay was conducted, and optical density of $PGF_{2\alpha}$ was read at 415 nm using an automated microplate spectrophotometer (BIO-RAD, Model 680, Hercules, CA) operating Microplate Manager Software (BIO-RAD, Version 5.2.1, Hercules, CA).

Using MyAssays Analysis Software Solutions, a four parameter logistic (4PL) curve was generated for each 8-point standard curve, and the resulting equation was used to determine concentration of the unknowns. A standard 4PL regression equation is given by

$y = [(A-D)/(1 + (x/C)^{\wedge B})] + D$, where

y = concentration

x = absorbance at 415 nm

A = minimum asymptote or lower boundary

B = slope

C = inflection point

D = maximum asymptote

Concurrent Medication Use

During the 30 minute wait after treatment administration, participants completed a brief recall of any recent use of additional NSAIDs and/or any other medications. If a participant reported taking anti-inflammatory drugs or other medicines believed to affect plasma hormonal measurements prior to blood collection, the lab session was rescheduled for the following day. While this necessitated a rescheduled lab session only twice, there were two participants who received steroid shots from the student health center 4 and 6 days before blood collection. One participant used Flonase®, but this did not overlap blood collection. There were nine reports of non-prescription analgesic (ibuprofen or acetaminophen) for personal reasons not related to the exercise intervention. Of these 9 instances of analgesic self-medication, only one occurred at a time such that the blood collection overlapped the presence of the medication in the system. Three subjects took one 7- or 10-day cycle of antibiotics. There were also two instances of Sudafed® use, one of which coincided with a blood draw for one subject; four instances of antihistamine use

(Benadryl®, Claritin D®, Zyrtec®), none of which overlapped blood collection; four instances of multi-ingredient cold/flu medications, none of which interfered with sample collection; and two instances of prescription narcotic/prescription analgesic use (codeine, ultram), with neither interfering with blood collection.

Physical Activity and Dietary Measurement

Participants were provided with instructions for participation (Appendix B), which included exercise restrictions, alcohol consumption guidelines, and a comprehensive list of all potential serious and less serious reactions to naproxen sodium. During the 30-minute wait after pill administration, information concerning the most recently performed aerobic and resistance exercise was documented. Physical activity was also measured for two 3-day time periods using the BodyMedia SenseWear PRO^2 armband (Figure A5). This device is worn on the posterior surface of the upper dominant arm. It has been validated as a tool to assess physical activity in free-living conditions, and significant correlations between indirect calorimetry were determined for supine resting and walking conditions have been reported (Hallam, Lacey, Hatchett and Wadsworth, 2007).

The first and second 3-day physical activity collection periods were scheduled to overlap the second (week 4), and third (week 6) blood draws. Participants were provided the armband and instructed to wear it continuously for those three days. Each participant thus provided six days of physical activity data. The BodyMedia SenseWear PRO^2 armband uses a proprietary formula to estimate energy expenditure. Biaxial accelerometers, thermistors to record not only the exchange of heat with the environment but also the galvanic skin response, and a pedometer are used to obtain the raw data for the estimation of energy expenditure. Galvanic skin response is a measure of the electrical conductivity between two points on the skin and is used to quantify maximal sweat rates. Daily and average total energy expenditure, active energy expenditure, physical activity duration,

number of steps taken, and duration of sleep were all measured. Using skin thermistors, the armband is able to detect when it has been removed from the body. Participants were compliant in wearing the armband for the majority of the 3-day period. Participants in placebo treatment group kept the device on their body 88 $\pm$ 13.8% and 86.2 $\pm$ 19.2% of the first and second 3-day collection period, while those in the placebo treatment group two kept the device on their body 90.5 $\pm$ 12.4% and 91.5 $\pm$ 9.1% of the first and second 3-day periods.

Two 3-day dietary recall collection periods were attempted, with an initial plan to measure intake over a weekend (Friday-Sunday) and weekday (Monday-Thursday) period. Participants were provided with Nutrient Data System for Research diet recall documents and instructed to record the type and amount of food and beverages consumed for three days, which overlapped 3-day physical activity measurement (3-day period during weeks 4 and 6) and the blood draws accompanying workouts 7 (week 4) and 11 (week 6). The intent of overlapping collection of dietary and physical activity data with blood collection was to collect ancillary data which could potentially affect plasma concentrations of $PGF_{2\alpha}$ metabolites.

After receiving instructions on how to complete the recall documents, participants personally recorded their dietary intake for 3-days. Immediately after the collection period, participants were interviewed by a dietetic professional to complete the information collection process. The Nutrient Data System for Research (NDSR) was utilized to analyze each participant's diet for total energy (kilocalories), total protein (g), percent calories from protein, arachidonic acid (g), total polyunsaturated fatty acids (g), calcium (mg), vitamin D (μg), and antioxidant content.

Mixed-design repeated measures ANOVAs were utilized to determine potential interactions between treatment and time, which was measured both acutely and chronically. Acute responses to exercise are represented in the pre-to-post exercise change. Chronic responses are represented by the changes exhibited over the 6-week training period.

The following null hypotheses were tested:

Ho_1: There will be no significant difference in the acute $PGF_{2\alpha}$ response to exercise between groups over time. This will be tested using a 2 x 2 x 3 mixed-design repeated measures ANOVA. The potential three-way interaction (treatment x pre-post x time) was interpreted first.

Three potential two-way interactions were examined after the omnibus test.

Ho_{1a}: There were will be no significant difference in the acute $PGF_{2\alpha}$ response to exercise between treatment groups, when all three pre-exercise concentrations and post-exercise concentrations are averaged within treatment group. This will be tested using a 2 x 2 (treatment x pre-post) ANOVA.

Ho_{1b}: There will be no significant difference in the acute $PGF_{2\alpha}$ response to exercise over time, when pre-exercise concentrations and post-exercise concentrations are averaged across treatment groups at each blood sampling time. This will be analyzed using a 3 x 2 (time x pre-post).

Ho_{1c}: There will be no significant difference in the $PGF_{2\alpha}$ response to exercise over time, when pre- and post-exercise data are averaged within each treatment group at each blood sampling time point. This will be analyzed using a 2 x 3 (treatment x time) repeated measures ANOVA.

After two-way interactions, main effects of time, treatment, and exercise were examined.

Ho_{1d}: There will be no effect of time on acute $PGF_{2\alpha}$ response to exercise.

Ho_{1e}: There will be no effect of treatment on acute $PGF_{2\alpha}$ response to exercise.

Ho_{1f}: There will be no effect of exercise on the acute $PGF_{2\alpha}$ response to exercise.

Ho_2: There will be no difference in acute arm circumference response to exercise between groups over time. This was tested using a 2 x 2 x 6 mixed-design repeated measures ANOVA.

The potential three-way interaction was interpreted first (treatment x pre-post x time), followed by examination of potential two-way interactions.

Ho_{2a}: There will be no significant difference in the acute arm circumference response to exercise between treatment groups, when all pre-exercise measurements and post-exercise measurements are averaged within treatment group. This will be tested using a 2 x 2 (treatment x pre-post) repeated measures ANOVA.

Ho_{2b}: There will be no significant difference in the acute arm circumference response to exercise over time, when pre-exercise measurements and post-exercise measurements are averaged across treatment groups at each measurement time point. This will be analyzed using a 2 x 6 (pre-post x time) repeated measures ANOVA.

Ho_{2c}: There will be no significant difference in the arm circumference response to exercise over time, when pre- and post-exercise data are averaged within each treatment group at each measurement time point. This will be tested using a 2 x 6 (treatment by time) repeated measures ANOVA.

Main effects of time, treatment, and exercise were also examined.

Ho_{1d}: There will be no effect of time on acute arm circumference response to exercise.

Ho_{1e}: There will be no effect of treatment on acute arm circumference response to exercise.

Ho_{1f}: There will be no effect of exercise on the acute arm circumference response to exercise.

Ho_{3}: There will be no difference in strength development between treatment groups over time. This will be tested using a 2 x 3 repeated measures ANOVA.

Main effects of treatment and time were also examined.

Ho_{3a}: There will be no main effect of treatment on strength development.

Ho_{3b}: There will be no main effect of time on strength development.

Ho_4: There will be no significant difference in arm tissue composition changes between groups over time. Arm tissue includes bone mineral content (BMC), adipose, and lean tissue, and each tissue component was analyzed separately with a 2 x 2 repeated measures ANOVA. After the interaction term, main effects of treatment and time were examined.

Ho_{4a}: There will be no main effect of treatment on arm tissue composition changes.

Ho_{4b}: There will be no main effect of time on arm tissue composition changes.

All assumptions inherent to analysis of variance were tested to ensure appropriate use of the statistical tool. To account for correlation and consequent lack of independence between repeated measures over time for each subject, Mauchly's test of sphericity was utilized to assess the homogeneity of the variances across time points. If sphericity was violated, the F statistic was adjusted using either the Greenhouse-Geisser or Huynn-Feldt estimates. If any null hypotheses were rejected, significant omnibus F tests were followed with multiple dependent t tests, in which the alpha level was adjusted using the Bonferonni correction. Trend analysis was also employed to see any potential trends that emerged over the 6-week period. Statistical tests were chosen to elicit a medium effect size of 0.25 (Cohen's F). Power was set a priori at 0.90, and significance was accepted below 0.05. With the aforementioned effect size and power, analyses revealed twenty-four subjects were necessary to detect statistical significance. SPSS 17.0 (Chicago, IL) was used to perform all statistical analyses, and MyAssays Analysis Software Solutions was used to calculate plasma $PGF_{2\alpha}$ metabolite concentrations fit to a 4-parameter logistic regression fit.

Chapter IV

Results

Twenty-four participants were successfully recruited; however, one subject discontinued participation due to scheduling conflicts after completion of baseline testing and the first two supervised exercise session. Data from these three sessions was not included in any statistical analyses. Participants were randomly assigned to placebo (n=11) or naproxen sodium (n=12) treatment group. Two hundred ninety-nine supervised exercise sessions were scheduled over the 6-week period, and 295 (98.7%) were completed.

Participant Anthropometrics

Participant anthropometrics are provided in Table 5. With the exception of one participant in the placebo treatment group who failed to undergo initial measurement, all participants completed pre- and post-study DXA analysis. One-way ANOVA revealed no significant differences between treatment groups in initial total body, left arm, or right arm skeletal muscle tissue (g), which is important to note in preparation for interpretation of data concerning gains in strength. Likewise, one-way ANOVA revealed no significant differences in initial total, left arm, or right arm adipose tissue (g).

Table 5
Participant Anthropometrics

	Placebo (n=11)	Naproxen Sodium (n=12)
Age (years)	20.6 ± 1.3	20.9 ± 1.4
Height (cm)	174.2 ± 9.9	178.8 ± 5.6
Initial Body Mass (kg)	77.9 ± 11.5	80.7 ± 9.5
Final Body Mass (kg)	77.6 ± 10.4	80.8 ± 8.8

Table 6
DXA Tissue Composition

	Initial	Final
Placebo		
Total BMC (g)	2937.845 ± 490.164	2936.496 ± 437.156
Left Arm BMC	204.098 ± 33.750	202.437 ± 33.794
Right Arm BMC	228.303 ± 31.300	230.930 ± 35.669
Total Fat (g)	12244.570 ± 5581.916	12719.882 ± 5012.037
Left Arm Fat	576.370 ± 257.125	567.236 ± 235.917
Right Arm Fat	596.930 ± 269.441	618.300 ± 293.795
Total Lean (g)	61301.400 ± 7789.383	60799.036 ± 7221.434
Left Arm Lean	3852.540 ± 488.235	3752.655 ± 490.735
Right Arm Lean	4065.210 ± 518.816	4141.600 ± 526.297
Total Body % Fat	15.570 ± 5.325	16.327 ± 5.286
Left Arm % Fat	12.240 ± 4.549	12.391 ± 4.485
Right Arm % Fat	12.000 ± 4.516	12.127 ± 4.841
Naproxen Sodium		
Total BMC (g)	2917.416 ± 354.459	2920.628 ± 363.646
Left Arm BMC	207.888 ± 18.208	204.149 ± 24.868
Right Arm BMC	226.761 ± 34.737	232.518 ± 30.660
Total Fat (g)	13801.892 ± 4588.963	13646.317 ± 4557.519
Left Arm Fat	616.350 ± 219.334	610.092 ± 203.905
Right Arm Fat	662.175 ± 217.552	647.100 ± 221.934
Total Lean (g)	61996.433 ± 654.015	62458.392 ± 6719.558
Left Arm Lean	3982.933 ± 643.414	3960.917 ± 513.099
Right Arm Lean	3997.625 ± 471.430	4235.167 ± 528.532
Total Body % Fat	17.283 ± 4.662	17.058 ± 4.713
Left Arm % Fat	13.000 ± 4.098	12.792 ± 4.070
Right Arm % Fat	13.192 ± 3.994	12.617 ± 4.027

Values provided as means ± standard deviations.

Plasma 13, 14-dihydro-15-keto Prostaglandin $F_{2\alpha}$ Concentrations

Participants (n=23) provided one baseline blood sample (6ml) during the familiarization session, prior to any exercise testing. In addition, participants provided a pre- and post-exercise blood sample in conjunction with workouts 1, 7, and 11, resulting in a total intended collection of 161 blood samples (23 baseline samples, 138 pre- and post-exercise samples).

One participant did not complete a scheduled draw due to a potential contraindication reported after the final pre-exercise sample was obtained. This potential adverse event was reported to the medical advisor of the Institutional Review Board, and the participant was not allowed to perform the scheduled workout or post-exercise blood draw. In an attempt to retain this data, an intent-to-treat analysis was employed by using the average of his first two post-exercise concentrations for his third and final concentration.

Concentrations were determined using the 4PL regression equations determined by MyAssays Data Analysis Software. An 8-point standard curve was created, with the resulting 4PL regression equations from which concentrations were calculated:

Samples 25-68: $y = \{(-11.467 - 562.821) / 1 + (x/0.193)^{\wedge -0.319}\} + 562.821$

$R^2 = .9908$

Samples 69-118: $y = \{(5.790 - 3682.54) / 1 + (x/0.004)^{\wedge -0.420}\} + 3682.54$

$R^2 = .9399$

Samples 119-142: $y = \{(-3.500 - 125.491) / 1 + (x/52.979)^{\wedge -0.483}\} + 125.491$

$R^2 = .9857$

Samples 143-168: $y = \{(0.663 - 204.382) / 1 + (x/37.714)^{\wedge -0.485}\} + 204.382$

$R^2 = .9955$

Pre-exercise plasma concentrations represent resting conditions. Post-exercise concentrations represent the response to exercise (placebo) or exercise and medication (naproxen sodium). The acute change in $PGF_{2\alpha}$ metabolite concentrations (pg/ml) were examined by application of a 2 x 2 x 3 repeated measures ANOVA. Pre- and post-exercise systemic plasma concentrations (pg/ml) for each treatment group and for workouts 1, 7, and 11 are provided in Table 7, with the log concentration of each respective draw presented graphically in Figure 2.

Table 7
$PGF_{2\alpha}$ Metabolite Concentrations (pg/ml)

	Placebo	Naproxen Sodium
Pre-Workout 1	71.75 $\pm$ 40.30	119.08 $\pm$ 65.38
Post-Workout 1	92.72 $\pm$ 44.86	125.89 $\pm$ 110.48
Pre-Workout 7	50.06 $\pm$ 19.12	69.14 $\pm$ 51.56
Post-Workout 7	57.58 $\pm$ 18.82	64.08 $\pm$ 54.57
Pre-Workout 11	163.14 $\pm$ 100.13	160.51 $\pm$ 158.83
Post-Workout 11	175.24 $\pm$ 112.32	166.96 $\pm$ 137.60

Values provided as mean $\pm$ standard deviation.

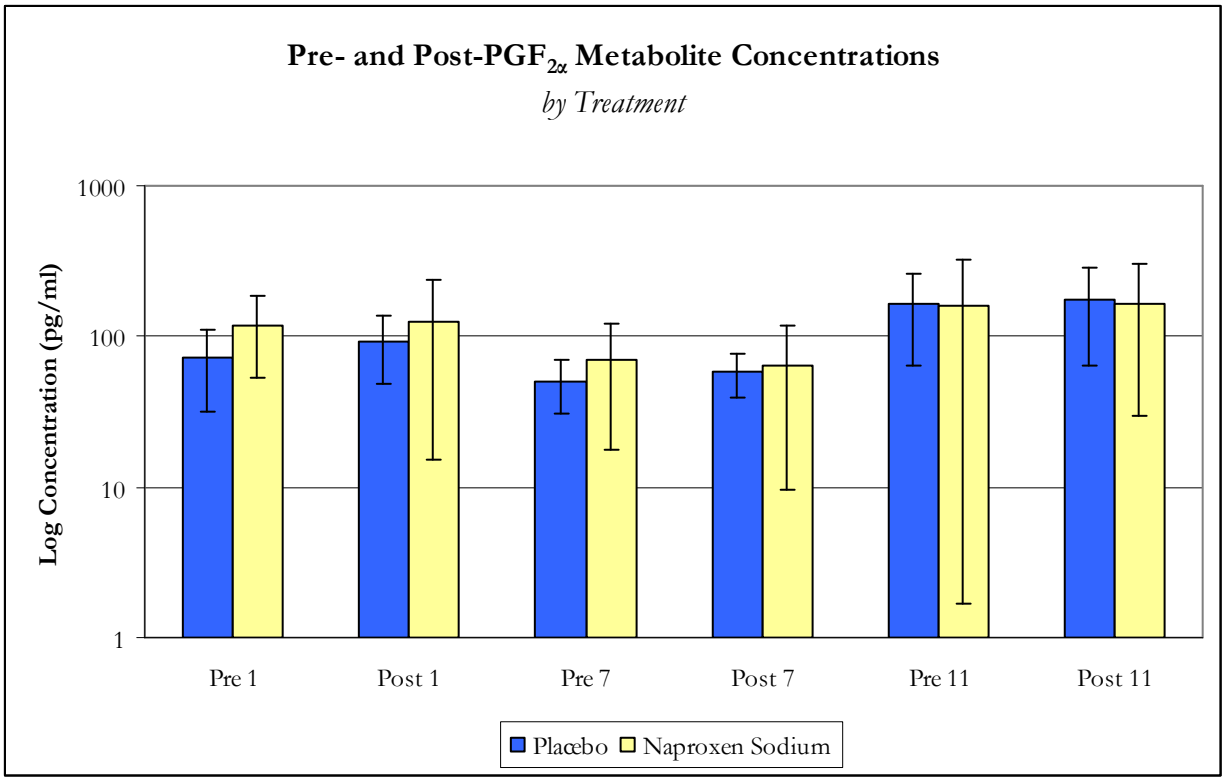

Figure 2
Error bars represent standard deviation.

Interpretation of the three-way interaction term (treatment x acute change x time) was non-significant (p=0.937), indicating no significant difference in the acute $PGF_{2\alpha}$ metabolite response between groups over time. All two-way interaction terms (time x treatment (p=0.381), acute change x treatment (p=0.093) and acute change x time (p=0.596)) were also non-significant. These results led to the decision to fail to reject Ho_1 and $Ho_{1a\text{-}c.}$

Main effects for treatment, time, and exercise were then examined, with significant effects for time and exercise revealed. With Mauchly's test of sphericity violated by time, the significance value was appropriately adjusted. Both Greenhouse-Geisser and Huynh-Feldt corrections yielded significant p values ($p<0.05$), indicating the acute response (average of pre- and post-exercise concentrations) changed over time, regardless of treatment group (Figure 3). This led to the conclusion to reject Ho_{1d}. Post hoc analysis using the Bonferroni correction showed all time points

were statistically different from one another (workout 1 and 7, $p<0.005$; workout 1 and 11, $p<0.008$; workout 7 and 11, $p<0.005$). Trend analysis revealed both significant linear ($p=0.003$) and quadratic ($p<0.005$) trends for time. The quadratic trend indicated a significant reduction in the average of pre- and post-exercise concentrations at the second measurement time (workout 7, week 4), which was followed by an increase in the pre- and post-exercise average at the final measurement time (workout 11, week 6).

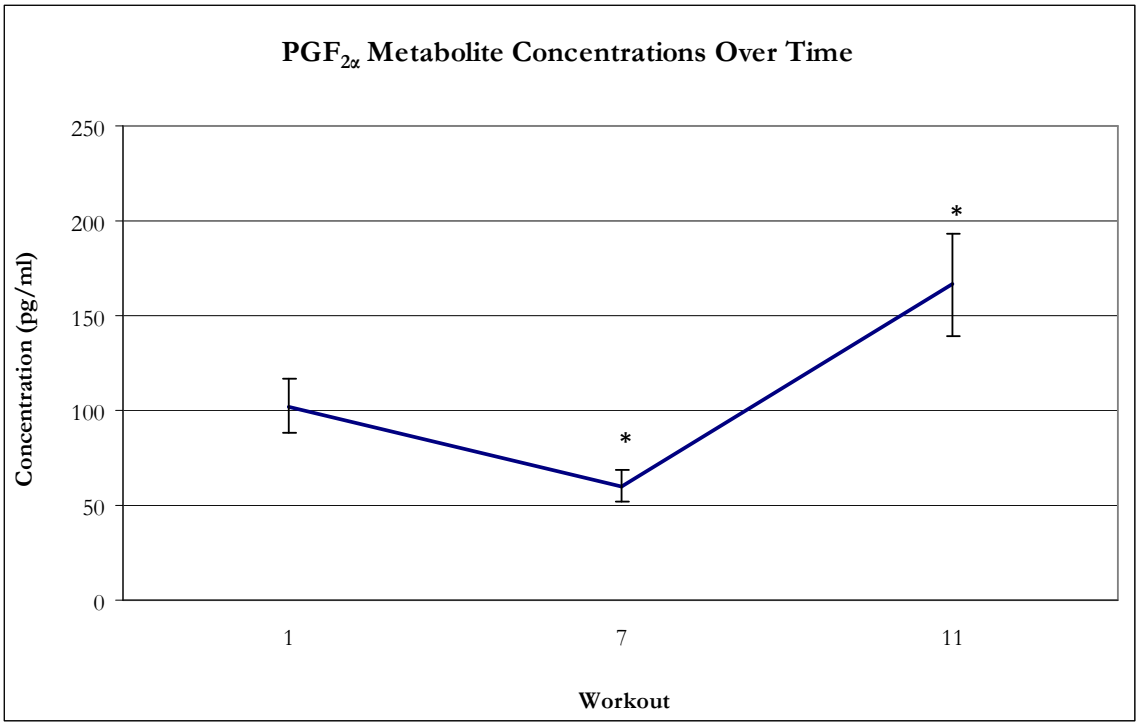

Figure 3 – Average pre-exercise and post-exercise concentrations collapsed across treatments. Error bars represent standard error.
*All time points significantly ($p<0.05$) different from one another.

Statistics also revealed a significant main effect of exercise on plasma $PGF_{2\alpha}$ metabolite concentrations, with trend analysis revealing a linear trend ($p=0.015$). Thus, regardless of treatment group, average post-exercise plasma concentrations were higher than average pre-exercise concentrations (pre-exercise mean $\pm$ standard error 105.61 $\pm$ 14.90; post-exercise 113.74 $\pm$ 16.25 pg/ml). Placebo-treated participants demonstrated an average acute increase of 13.5 $\pm$ 11.3 pg/ml, versus 2.7 $\pm$ 24.2 pg/ml in naproxen-treated. Without a significant treatment effect, the overall average increase was 8.13 $\pm$ 15.58 pg/ml or approximately 7.15% as a result of exercise. This is presented graphically in Figure 4.

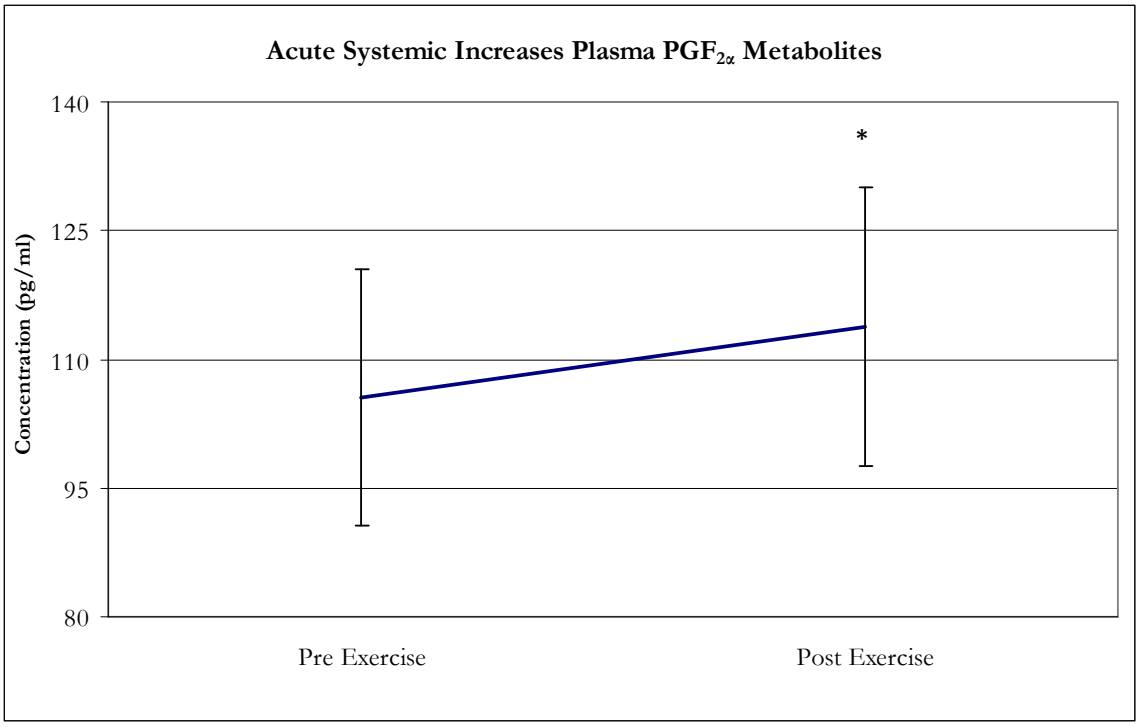

Figure 4
Error bars represent standard error.
*Significantly different from pre-exercise.

With substantial variability in systemic $PGF_{2\alpha}$ metabolites, concentrations were normalized to pre-exercise values and analyzed as a percent change in response to the intervention. The percent change in plasma $PGF_{2\alpha}$ metabolites for each treatment group resulting from exercise (placebo) or exercise and drug (naproxen sodium) are presented in Table 8 and Figure 5. Statistical analyses revealed a significant ($p=0.04$) treatment effect with data expressed as a percent change from pre- to post-exercise.

Table 8
Plasma $PGF_{2\alpha}$ Metabolite Concentration Response to Treatment – Expressed as % Change

	Workout 1	Workout 7	Workout 11
Placebo	23.62 ↑	13.06% ↑	6.91% ↑
Naproxen Sodium	5.41% ↑	-7.91% ↓	3.86% ↑

*Significantly ($p<.05$) different from placebo.

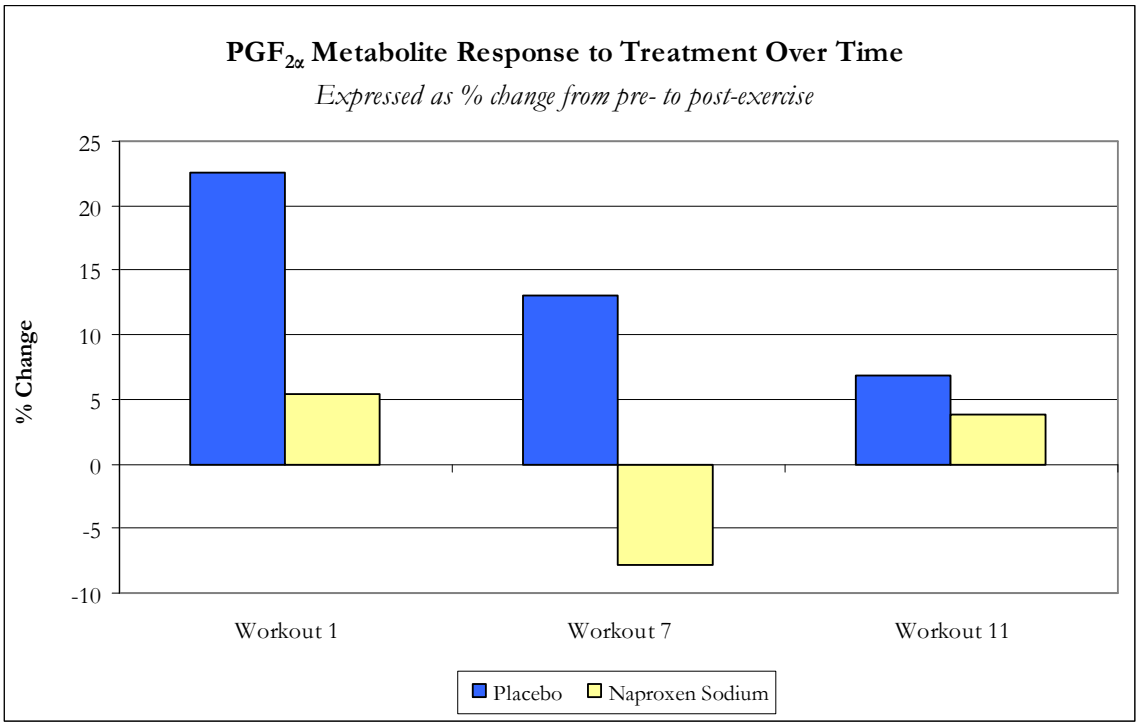

Figure 5

The effects of naproxen sodium, expressed as a percent change from pre- to post-exercise and in comparison to placebo, revealed reductions in exercise-induced increase in $PGF_{2\alpha}$ metabolites of 76.08, 160.60, and 44.14%, at workouts 1, 7, and 11, respectively.

Arm Circumference

For each participant, upper arm circumference was measured before and after each of the three strength assessments and before and after all exercise sessions, resulting in a total of 28 circumference measures for each participant. Seven participants were initially excluded from the 2 x 2 x 6 repeated measures ANOVA due to one or two missing data points. To prevent the loss of this data, an intent-to-treat analysis was employed. Pre- and post-exercise arm circumference averages were calculated for each participant for each week. The average calculated from the closest time points was then inserted at the appropriate time point for the participants for whom data was missing. Statistical analyses were thus conducted on weekly average pre- and post-exercise measurements rather than individual workout measurements.

The three-way interaction term (treatment x pre-post x time, 2 x 2 x 6) approached significance ($p=0.060$); however, the decision was made to fail to reject H_{o2}. This conclusion indicated that the acute response to exercise (pre-to-post-exercise change) did not differ significantly between groups over the 6-week training period. Interestingly, trend analysis revealed a significant fourth order polynomial trend for the three-way interaction ($p=0.035$). The data for the 3-way interaction is presented graphically in Figure 6, with the average weekly acute change (pre-to-post-exercise difference score) plotted for each treatment group.

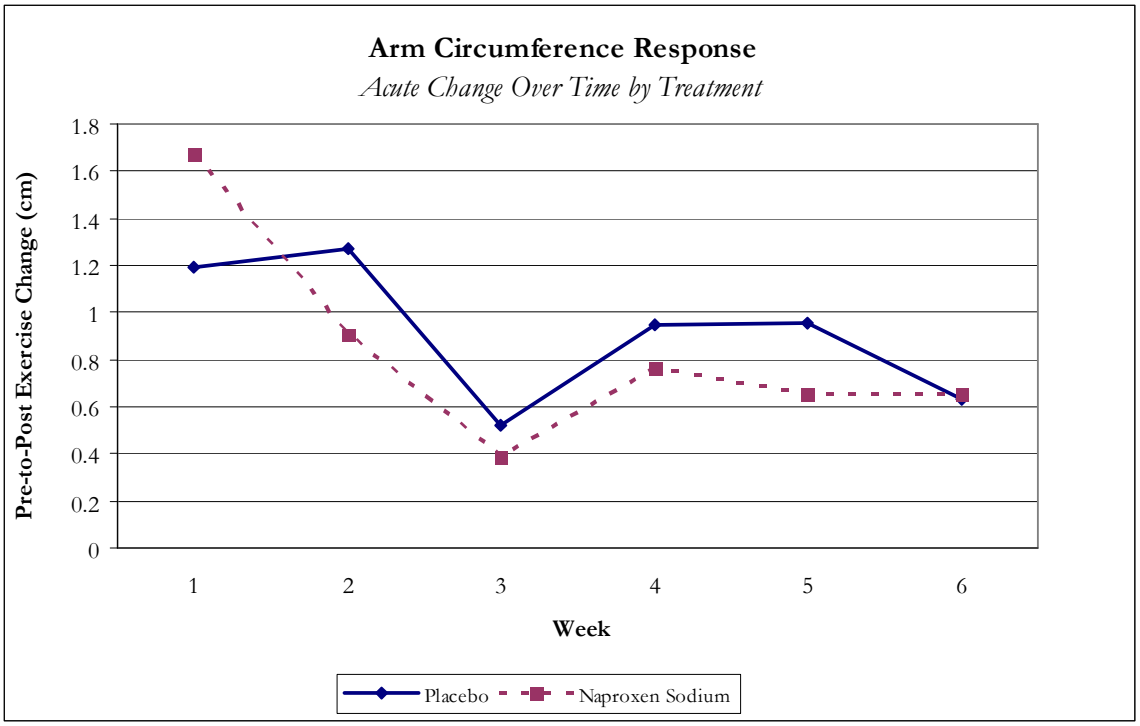

Figure 6

Two-way interactions were then interpreted. The treatment x acute response (2 x 2) interaction was non-significant ($p=0.567$), leading to the decision to fail to reject Ho_{2a}. This conclusion indicated there were no significant differences between treatment groups in the 6-week average of all pre-exercise and post-exercise arm circumference measures.

With Mauchly's sphericity test violated ($p<0.005$) in the acute response x time (2 x 6) interaction term, the significance value was appropriately adjusted. Both Greenhouse-Geisser and Huynh-Feldt corrections revealed a significant interaction ($p<0.005$), leading to the decision to reject Ho_{2b}. This conclusion indicated, regardless of treatment, there was a significant effect of time on the acute arm circumference response to exercise and/or naproxen sodium (Figure 7).

Trend analysis of the 6-week acute response x time interaction yielded significant p values for all possible polynomial contrasts, up to the fifth order (linear $p<0.05$, quadratic $p=0.011$, cubic $p=0.013$, fourth order $p=0.027$, fifth order $p=0.005$), indicating an undulating circumference response to the stimulus.

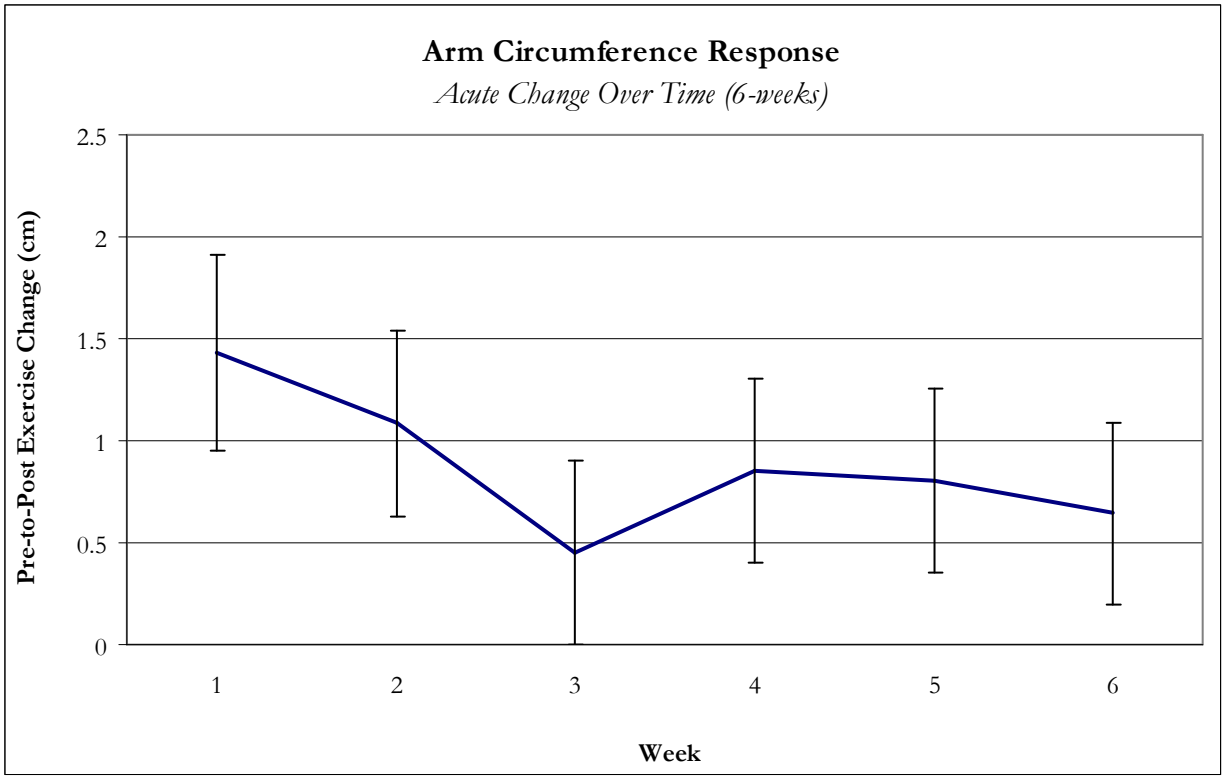

Figure 7
Error bars represent standard error.

Programs characteristics associated with week three were then subjected to closer examination in an attempt to understand the acute response which reached a 6-week low at the week 3. While week three consisted of the typical heavy and light intensity resistance workouts, the second strength assessment occurred prior to the second workout of week 3. While placebo-treated participants exhibited average acute increases in arm circumference of 1.18 cm versus 0.89 cm in naproxen-treated, the response was 0.29 and 0.04 cm in the respective treatment group after the second workout of week 3. It appears the re-assessment of strength prior to the second

workout attenuated the typical circumference response. With week three removed, the acute response decreased linearly over time (Figure 8).

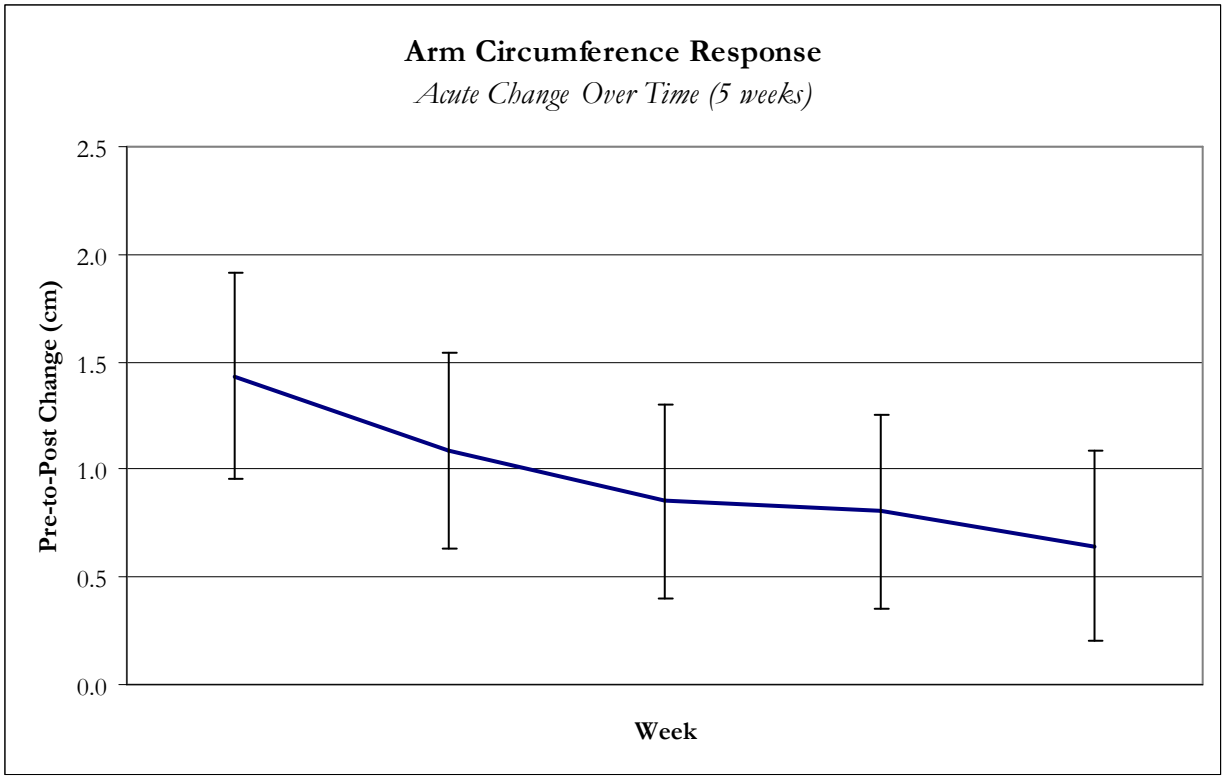

Figure 8
Error bars represent standard error.

The treatment by time interaction term was non-significant (p=0.614), leading to the decision to fail to reject Ho_{2c}. This indicated the weekly averages of pre *and* post-exercise circumferences were not statistically different between treatment groups over the 6-week training period.

Examination of main effects revealed a significant main effect of time on arm circumference response to exercise. With Mauchly's test violated for this within-subjects, both Greenhouse-Geisser and Huynh-Feldt corrections yielded significant p values (p=0.012 and 0.008) for time. Multiple comparison procedures using the Bonferonni correction revealed the response in week 2

was statistically different from the response in week 4 ($p<0.005$), week 5 ($p=0.002$), and week 6 ($p=0.003$). Trend analyses for time revealed three significant polynomial trends, including linear ($p=0.03$), cubic ($p=0.004$), and fourth order ($p=0.003$). Main effects for time are presented graphically in Figure 9.

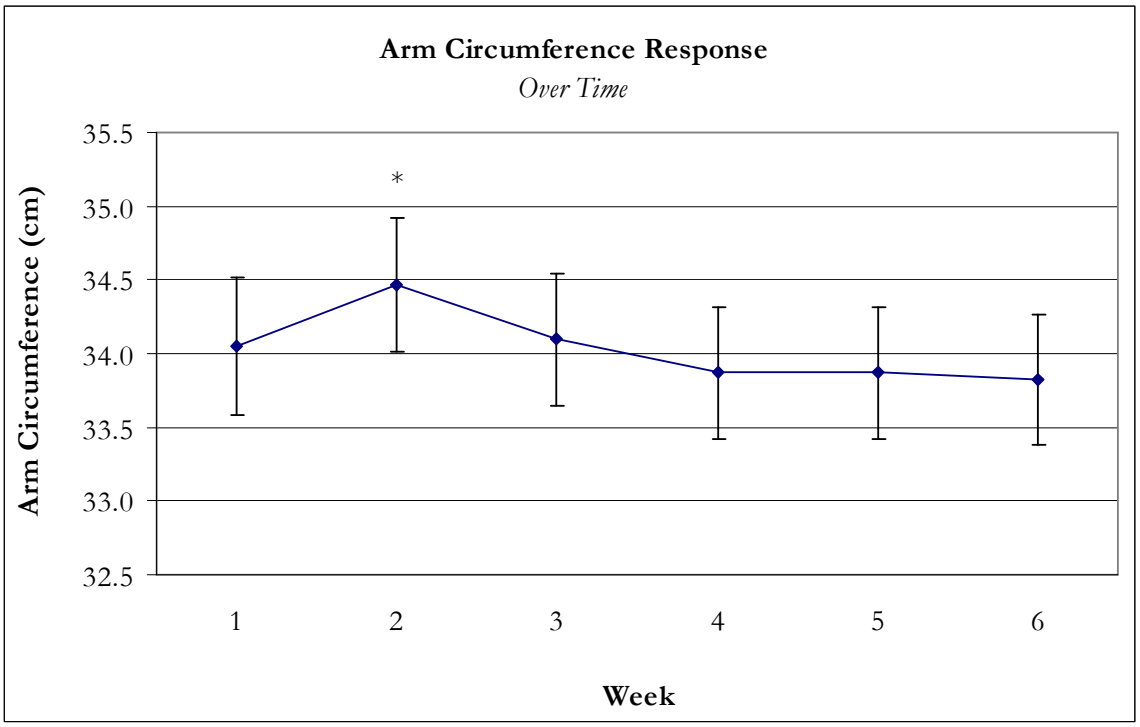

Figure 9
* Significantly different from weeks 4, 5, and 6.

A main effect for exercise was also demonstrated in the acute change pre- to-post-exercise ($p<0.005$). This indicated post-exercise circumference measures were greater than pre-exercise measures (Figure 10). Regardless of treatment group, the average change in arm circumference from pre-to-post exercise was 0.879 ± 0.641 cm (mean $\pm$ standard error).

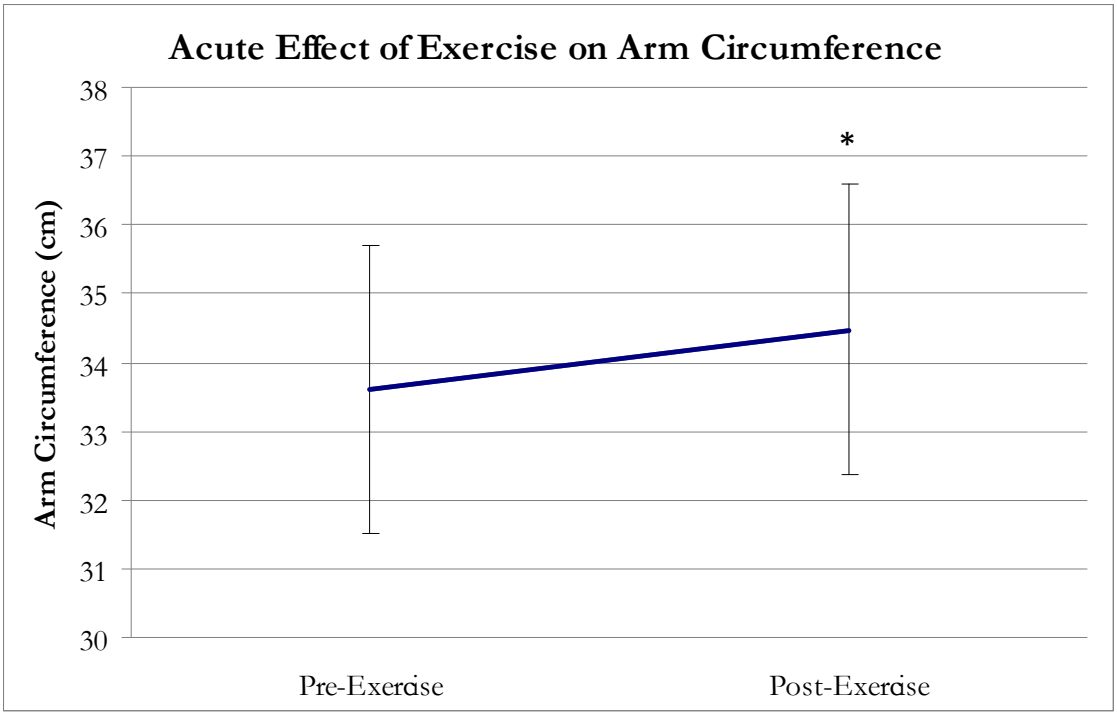

Figure 10
*Significantly greater than pre-exercise.

Over time, pre-exercise arm circumferences reflect hypertrophy resulting from the training intervention. Comparison of the first and last pre-exercise arm circumference revealed the naproxen sodium treatment group demonstrated a 0.58 $\pm$ 1.59 cm increase in pre-exercise arm circumference over the 6-week training period versus a 0.31 $\pm$ 1.15 cm increase in the placebo treatment group (Figure 11) The difference between groups did not reach statistical significance.

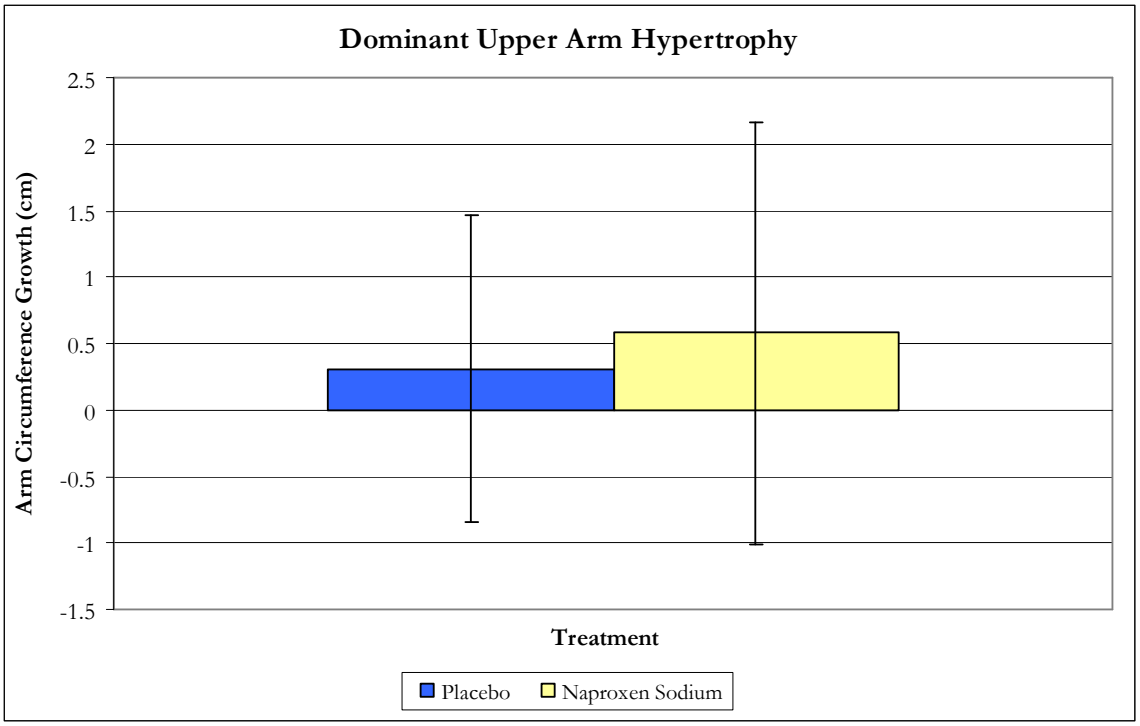

Figure 11
Error bars represent standard deviation.

Post-exercise arm circumferences are related to the hyperemic response to exercise. Post-exercise arm circumferences declined over time, with all groups exhibiting a final post-exercise arm circumference less than that exhibited after the first workout (Figure 12). In response to the first workout, participants in the placebo and naproxen sodium treatment groups exhibited a 1.47 $\pm$ 1.21 cm and 1.69 $\pm$ 1.22 cm increase, respectively. By the final workout, participants responded with

only a 0.58 ± 0.59 cm and 0.48 ± 0.36 cm increase in placebo and naproxen sodium treatment groups, respectively. This data can be seen in Figures 6, 7, 8, and 12.

Within Figure 12, several responses can be seen. First, the acute effect of exercise is seen, with post-exercise values being greater than pre-exercise values. Also, hypertrophy across groups can be seen by the slight but non-significant increase in pre-exercise measurements. The generally decreasing hyperemic response to exercise can be seen by the downward trend in post-exercise measurements.

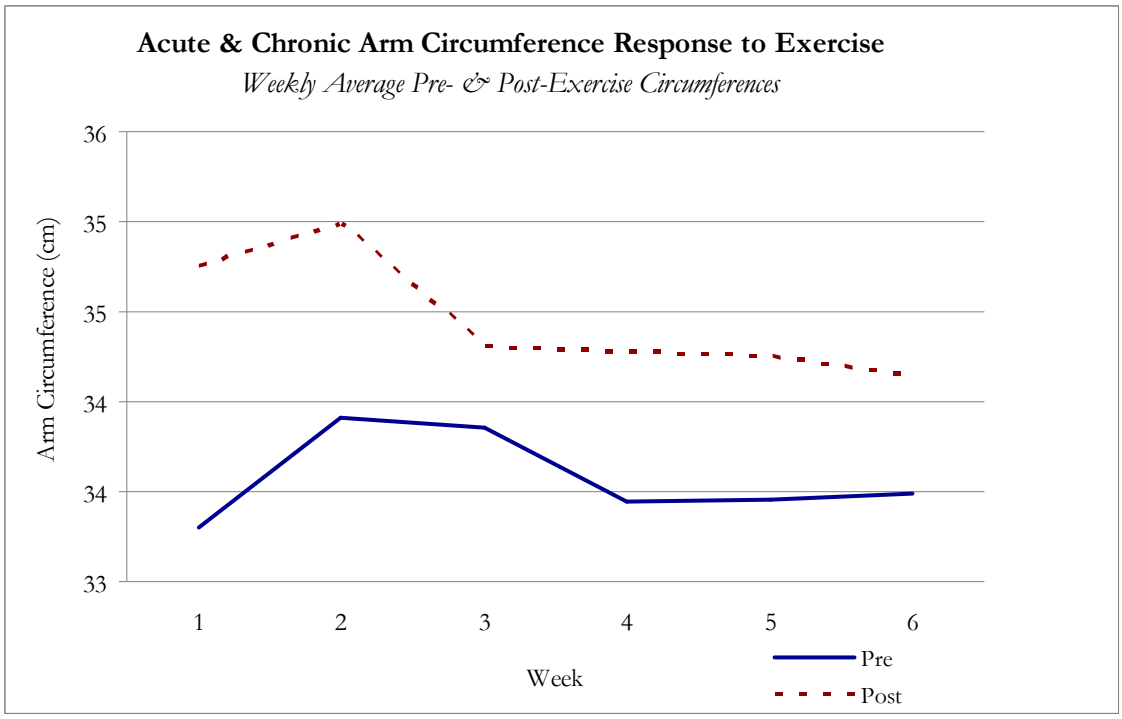

Figure 12

Strength

All subjects completed three strength assessments, which were conducted at (1) baseline, (2) week 3, pre-workout 6, and (3) week 6, workout 12. The final workout (12) consisted of the final strength assessment only, in contrast to workout 6, which entailed the second strength assessment in addition to the regularly scheduled workout for that day. Workout 6 began with the mid-point strength assessment. Subjects were then provided with their respective treatment and nutritional supplement and allowed 30 minutes rest for both metabolism of naproxen and recovery from strength testing.

The overall omnibus test (treatment x time interaction) for strength was not statistically significance, indicating no significant difference in the rate of strength development between treatment groups. Since the main effect for treatment was also determined to be non-significant, both Ho_3 and Ho_{3a} failed to be rejected.

For all four resistance training exercises, 2 x 3 repeated measures ANOVA revealed a significant main effect for time ($p<0.05$), a significant linear time trend, and statistically significant differences between all time points within each treatment group (Figures 13-20). Ho_{3b} was thus rejected. Overall absolute increases in strength, for each exercise and both treatment groups are provided in Table 9. Values are provided in both units of weight (mean $\pm$ standard deviation) and overall percent change. Table 10 details the rate of change in strength across 1RM testing intervals.

Table 9
Strength Increases - Overall

	Placebo		Naproxen Sodium	
	Absolute[a] (kg)	Absolute(%)	Absolute (kg)	Absolute(%)
LP	36.36 ± 11.94*	27.89	34.56 ± 10.63*	29.58
TB	19.21 ± 11.91*	23.08	22.54 ± 10.59*	29.38
UR	23.14 ± 4.68*	34.78	16.48 ± 3.89*	29.69
BC	25.0 ± 16.1*	22.56	11.78 ± 2.96*	25.36

* Significant at $p<0.05$ level.
[a] Values given as means ± standard deviations
LP – lat pulldown, TB – t-bar row, UR – upright row, BC – bicep curl

Table 10
Strength Increases (kg)[a] - Across Testing Intervals

	Placebo		Naproxen Sodium	
	Interval 1	Interval 2	Interval 1	Interval 2
LP	18.39 ± 9.98	17.98 ± 11.70	17.33 ± 8.14	17.24 ± 7.94
TB	10.33 ± 10.18	8.89 ± 5.88	10.42 ± 6.16	12.12 ± 6.81
UR	9.09 ± 3.11	10.95 ± 4.97	7.77 ± 4.59	8.71 ± 5.72
BC	3.35 ± 3.66	8.02 ± 6.02	5.79 ± 3.85	5.37 ± 3.26

[a]Values (kg) presented as means ± standard deviation.
LP – lat pulldown, TB – t-bar row, UR – upright row, BC – bicep curl
Interval 1 = 1st to 2nd assessment (3 weeks); Interval 2 = 2nd – 3rd assessment (3 weeks)

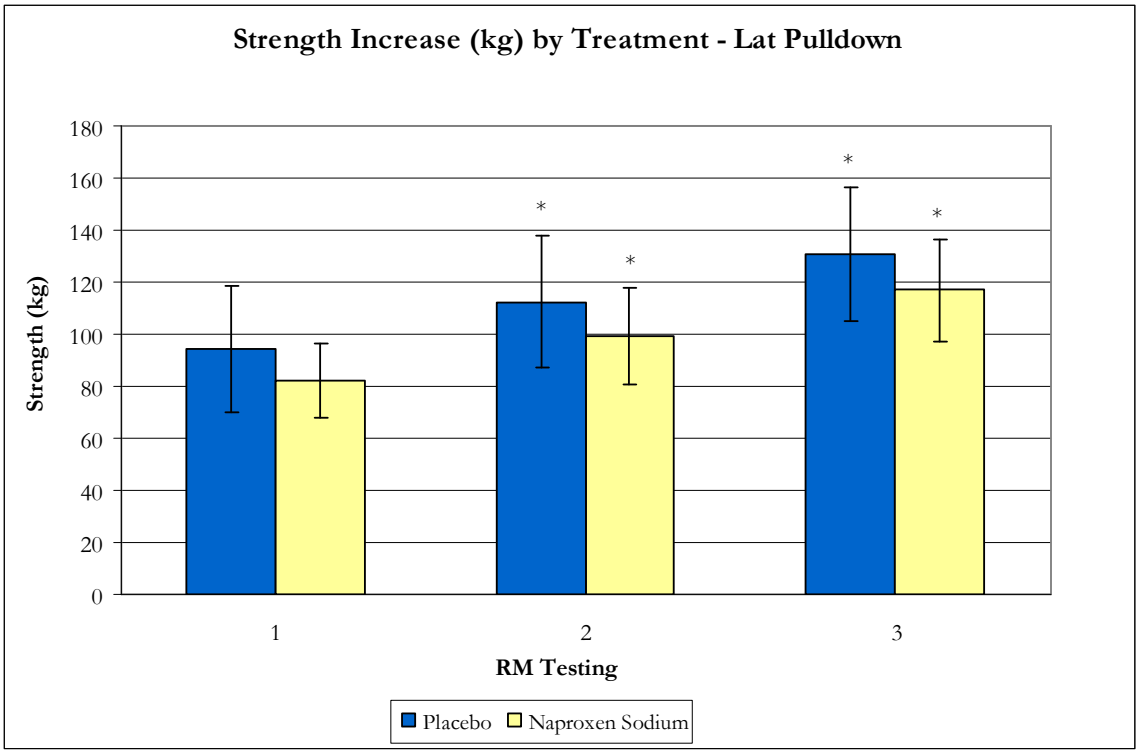

Figure 13
Error bars represent standard deviations.
*Significantly greater than preceding strength assessment(s) within group.

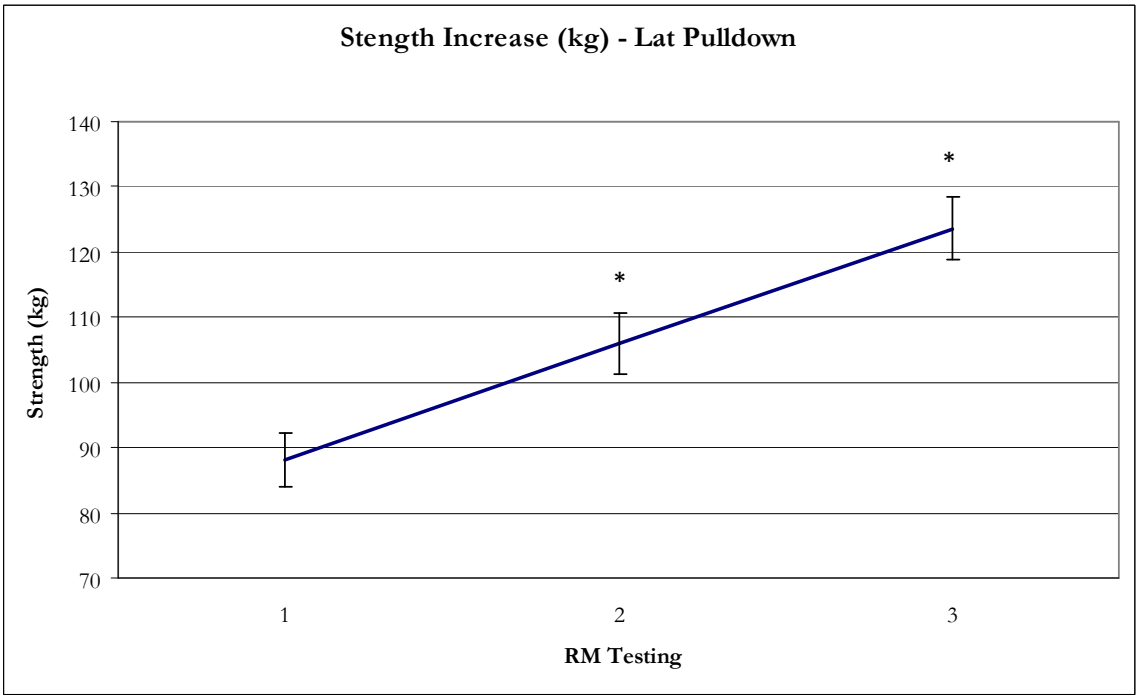

Figure 14
Error bars represent standard error.
* Significantly greater than preceding strength assessment(s).

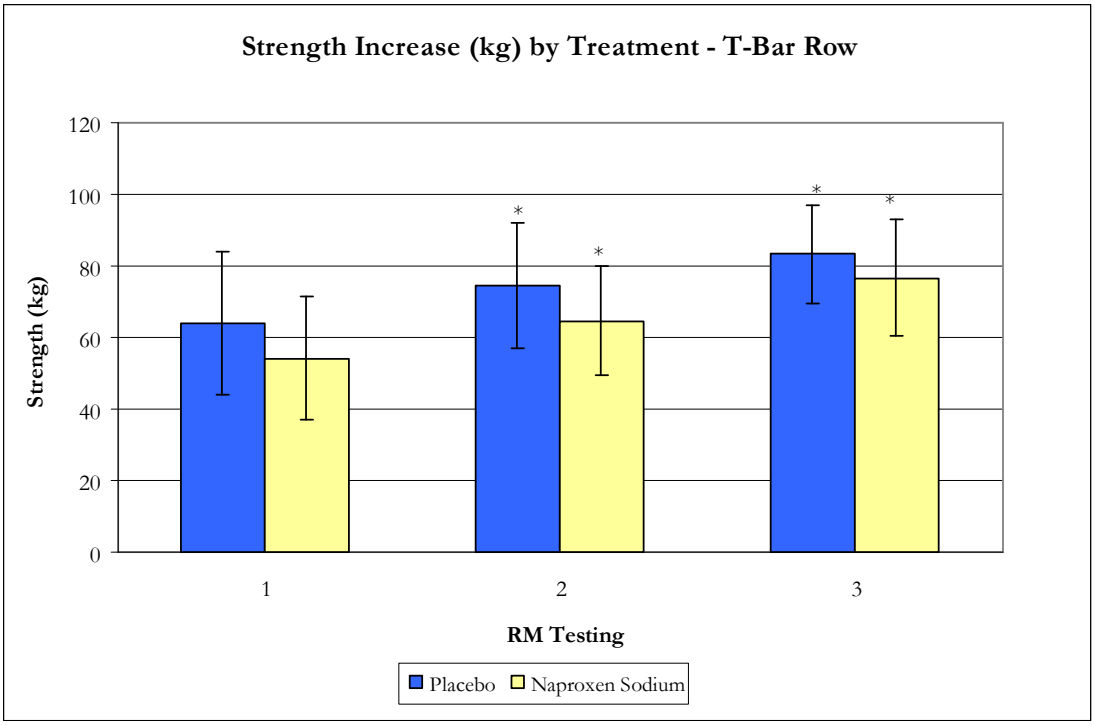

Figure 15
Error bars represent standard deviation.
*Significantly greater than preceding strength assessment(s) within group.

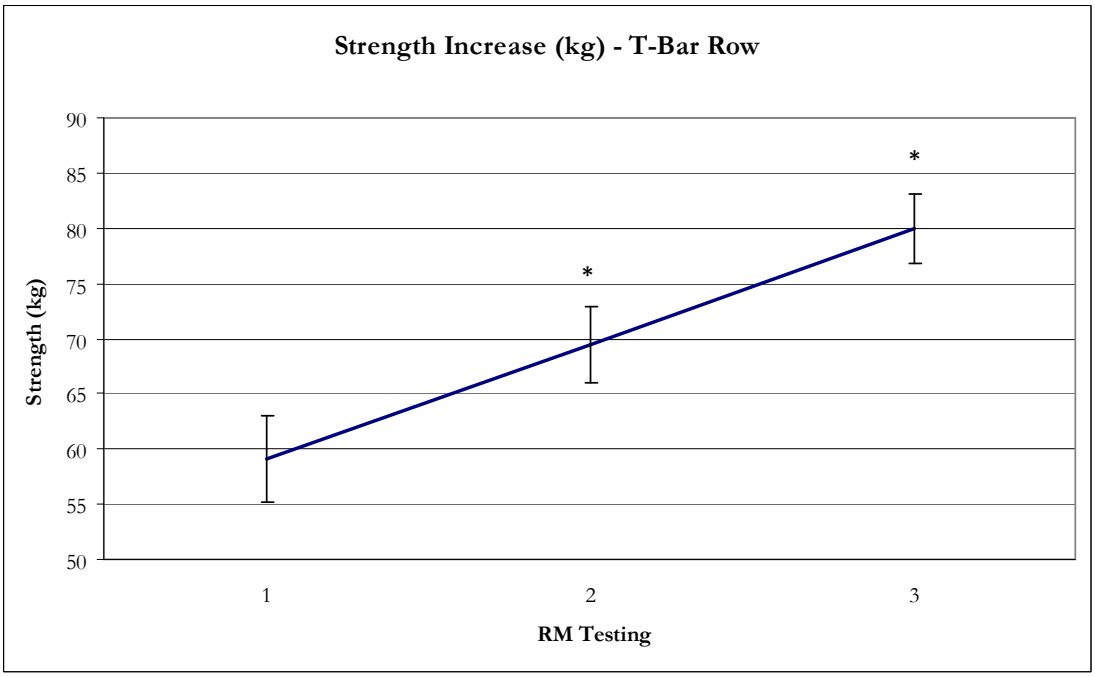

Figure 16
Error bars represent standard error.
* Significantly greater than preceding strength assessment(s).

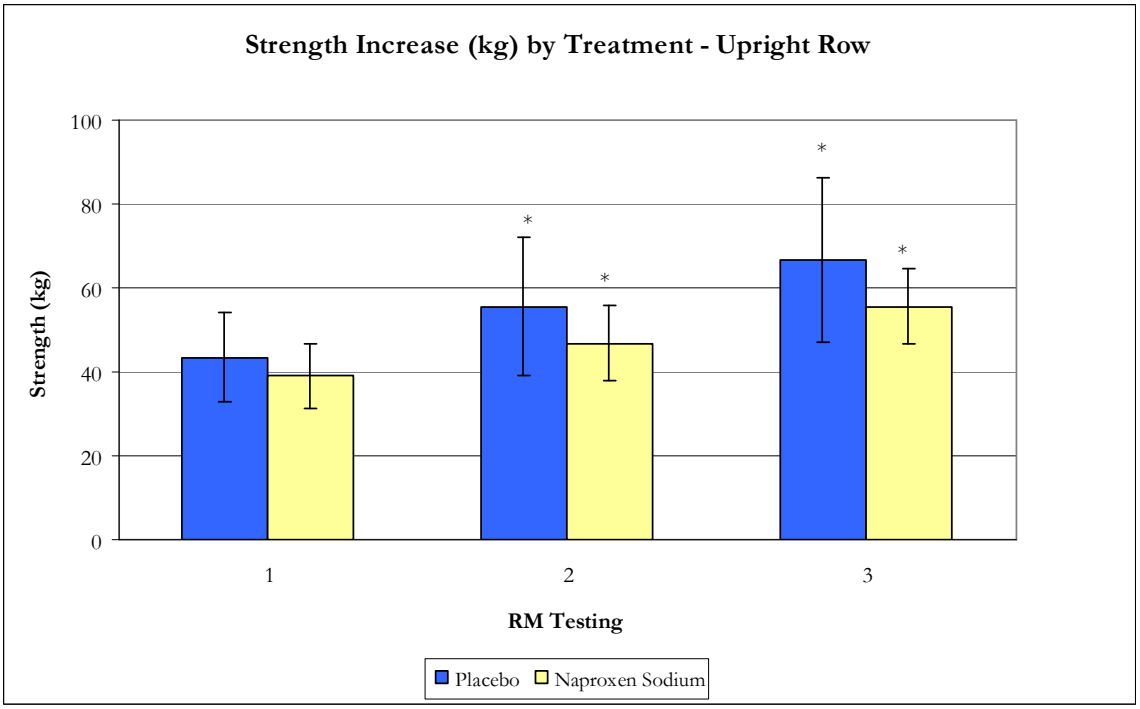

Figure 17
Error bars represent standard deviation.
* Significantly greater than preceding strength assessment(s) within group.

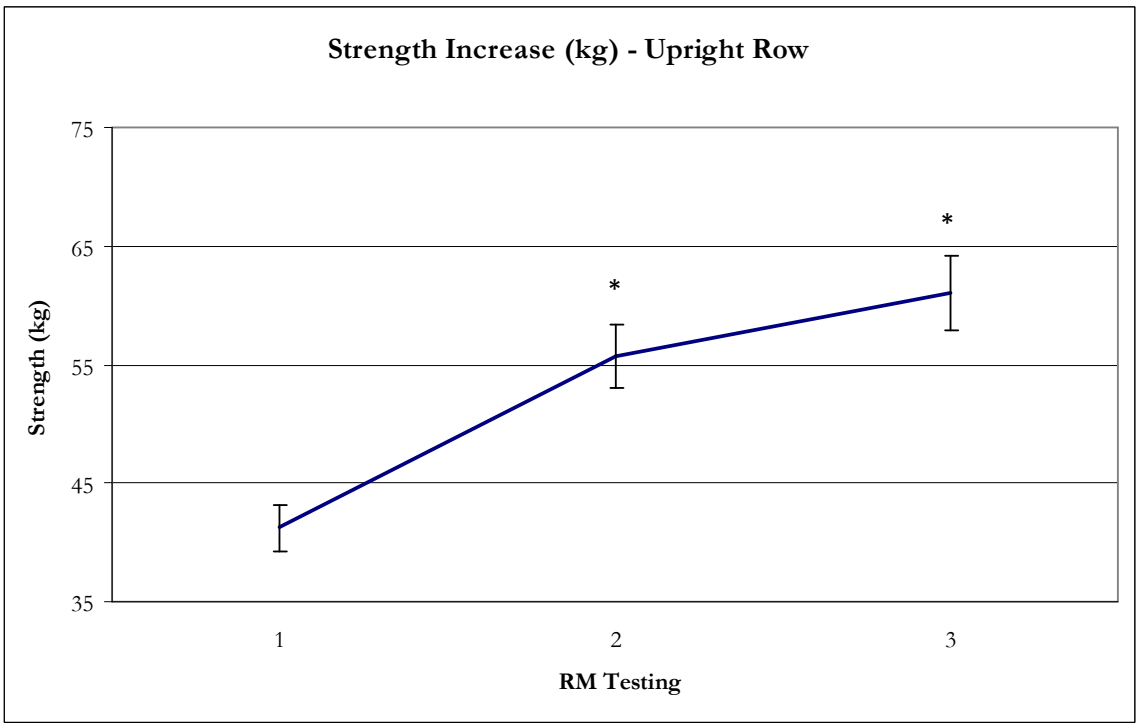

Figure 18
Error bars represent standard error.
* Significantly greater than preceding strength assessment(s).

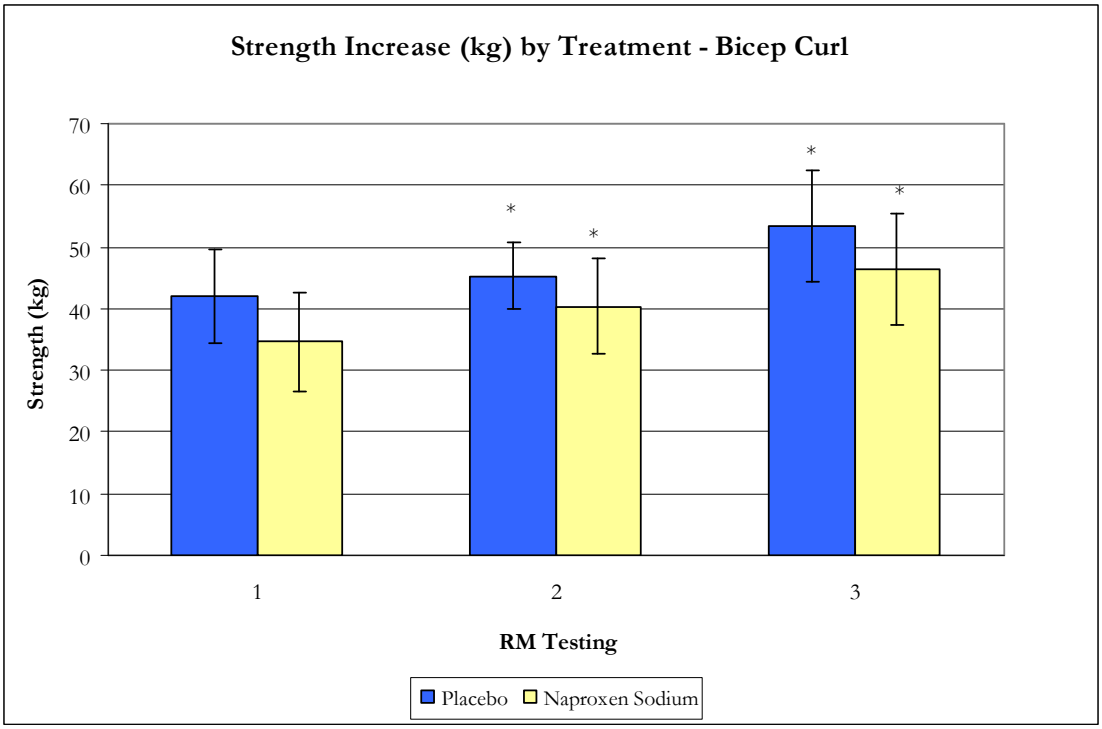

Figure 19
Error bars represent standard deviation.
* Significantly greater than preceding strength assessment(s) within group.

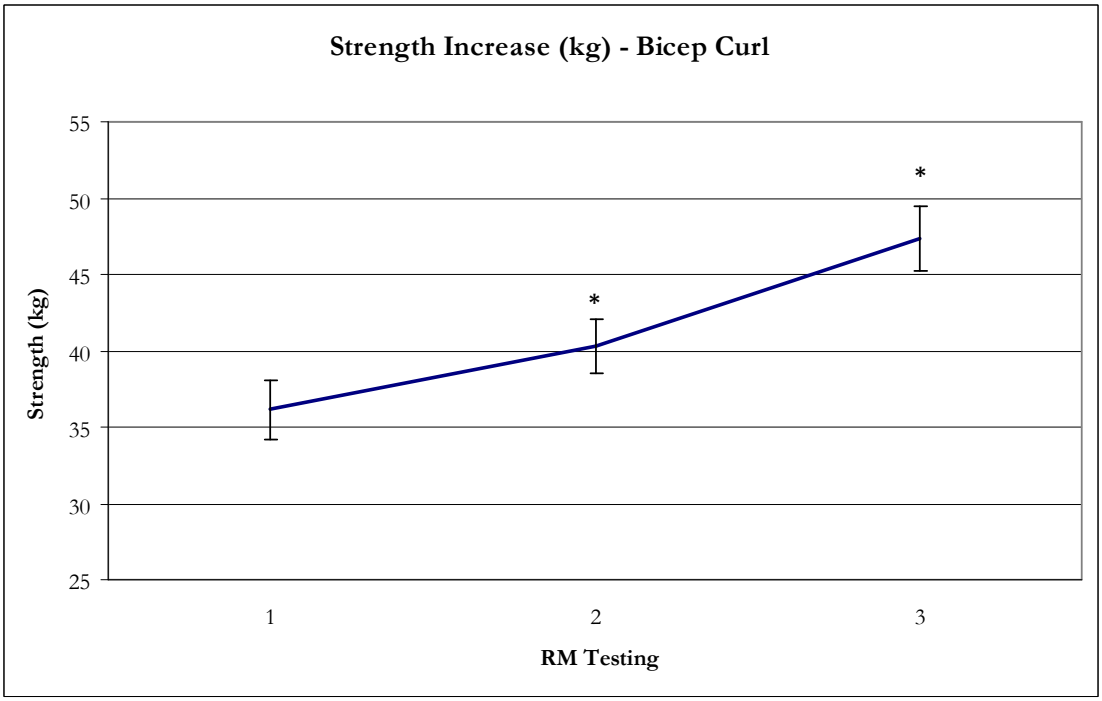

Figure 20
Error bars represent standard error.
* Significantly greater than preceding strength assessment(s).

Body Composition

DXA was performed both at the beginning and end of the 6-week intervention. One subject in treatment one did not complete the initial DXA scan. Statistical analysis of body composition was carried out on a per-case basis (placebo n=10; naproxen sodium n=12). All subjects reported the right arm as the dominant arm for the performance of all gross motor skills, a fact which is important in the interpretation of tissue changes in the right and left arms. Statistical analyses of each tissue type (skeletal muscle tissue, adipose tissue, and bone mineral content) were conducted separately. Examination of tissue changes was restricted to total body and right and left arms.

Total Body Tissue – Bone Mineral Content, Lean Tissue, Total Fat (g), Total Fat (%).

Repeated measures ANOVA (2 x 2) revealed no main effects for treatment, time or any treatment by time interaction for any total body tissue measures.

Left (Non-Dominant) Arm Bone Mineral Content

A 2 x 2 repeated measures ANOVA revealed a significant main effect for time ($p<0.05$), no significant main effect for treatment, and no significant difference in the rate of change in left arm bone mineral content between treatment groups over time. A significant linear time trend was revealed ($p<0.05$); and surprisingly, this was a significant decrease in left arm bone mineral content in both treatment groups over time (Figures 21-22). Based on individual participant results, bone mineral content declined 4.25 $\pm$ 6.38 g (1.64 $\pm$ 4.17%) in placebo-treated participants, and 3.74 $\pm$ 8.54 g (2.21 $\pm$ 4.40%) in naproxen-treated participants (Figure 21). Without significant treatment effects, this averaged to a 3.995 $\pm$ 7.46 g (1.93 $\pm$ 4.29%) decrease (Figure 22).

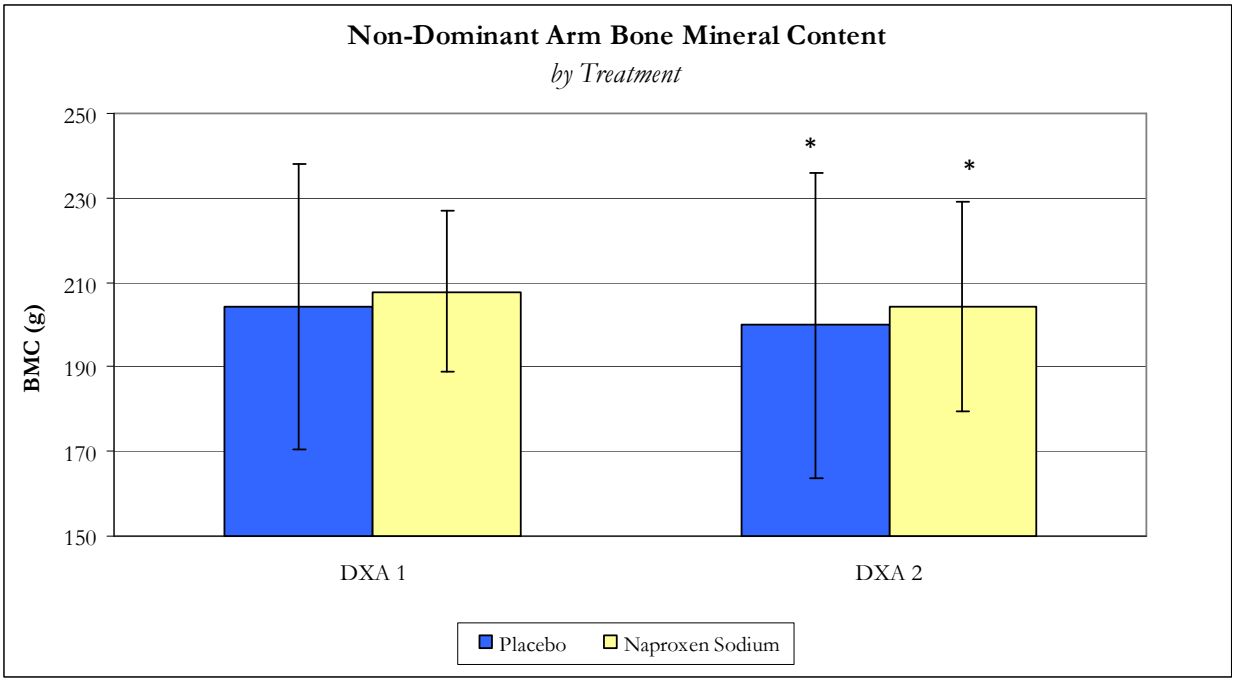

Figure 21
*Significantly less than within-group DXA 1.

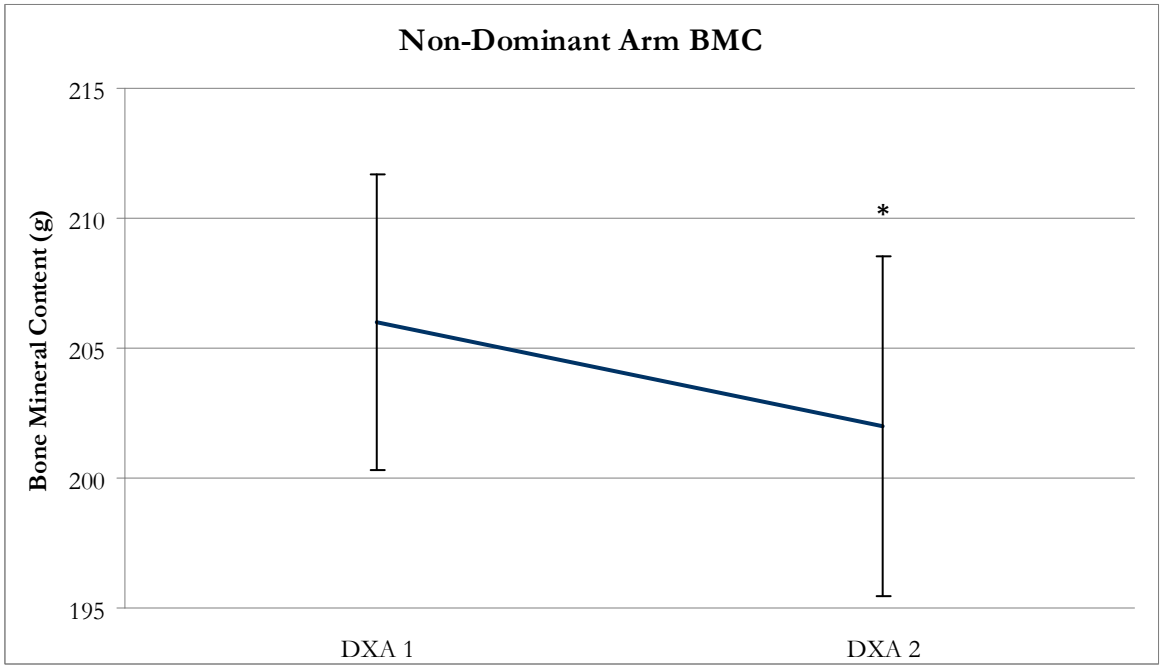

Figure 22
Error bars represent standard error.
*Significantly less than DXA 1.

Right Arm Bone Mineral Content

With all ANOVA assumptions being met, 2 x 2 repeated measures ANOVA revealed no main effects of treatment or time and no treatment x time interaction.

Left Arm Lean Tissue (g)

With all ANOVA assumptions being met, 2 x 2 repeated measures ANOVA revealed no main effects of treatment or time and no treatment x time interaction.

Right Arm Lean Tissue (g)

With all ANOVA assumptions being met, 2 x 2 repeated measures ANOVA revealed a significant main effect and linear trend for time ($p=0.037$) but no significant main effect for treatment or treatment by time interaction. Both treatment groups significantly increased dominant arm lean tissue. Participants in the placebo group gained an average of 117.88 ± 233.51 grams (2.82%) of lean tissue in their dominant arms, while those in the naproxen sodium group gained an average of 237.54 ± 452.66 grams (5.61%) of lean tissue (Figure 23).

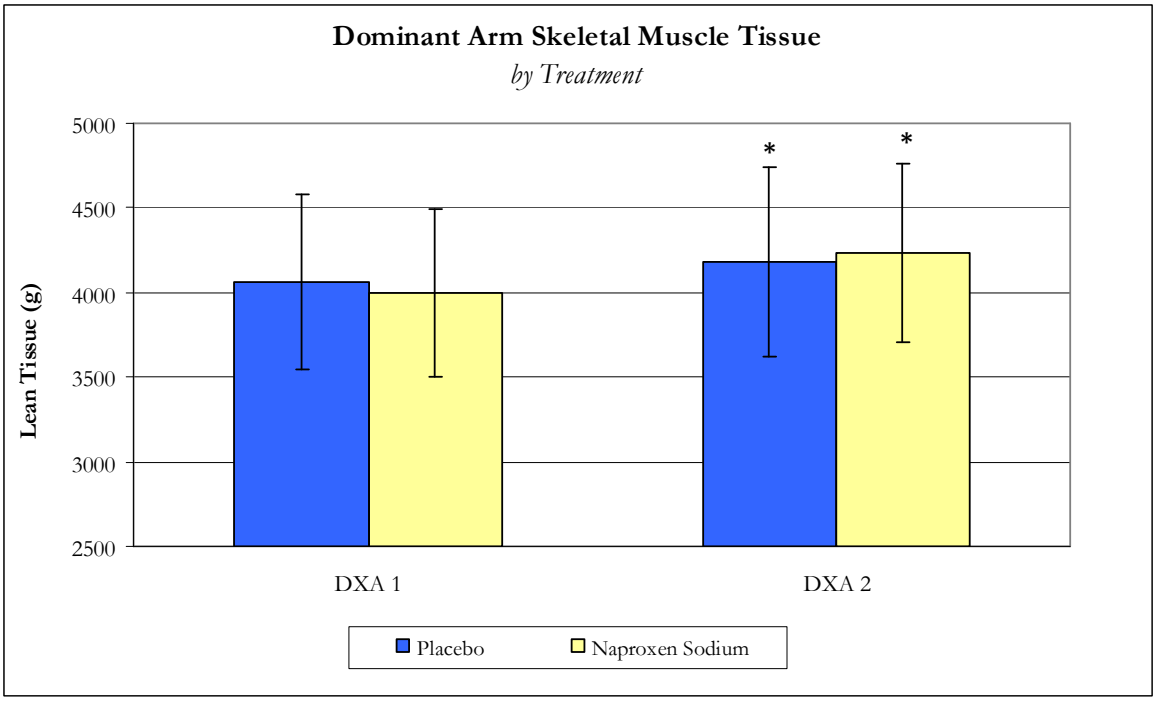

Figure 23
Error bars represent standard deviation.
*Significantly greater than within-group DXA 1.

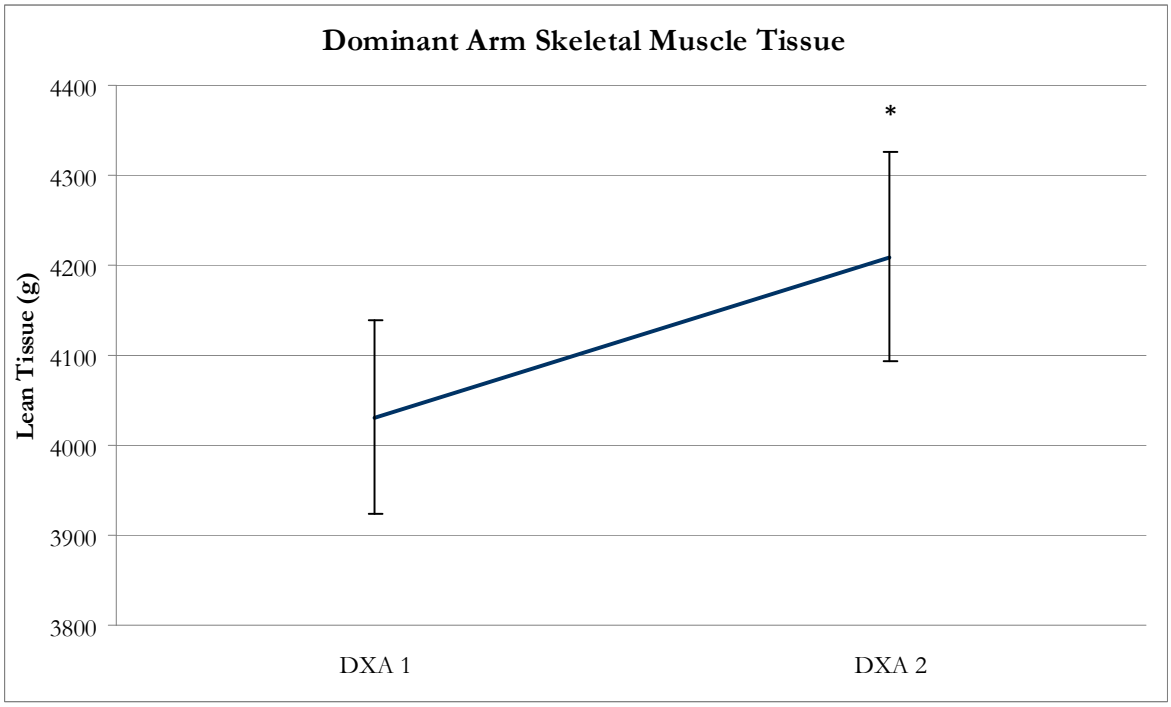

Figure 24
Error bars represent standard error.
*Significantly greater than DXA 1.

Left Arm Fat (g)

With all ANOVA assumptions being met, 2 x 2 repeated measures ANOVA revealed no main effects of treatment or time and no treatment x time interaction.

Right Arm Fat (g)

With all ANOVA assumptions being met, 2 x 2 repeated measures ANOVA revealed no main effects of treatment or time and no treatment x time interaction.

Left Arm Fat Percent

With all ANOVA assumptions being met, 2 x 2 repeated measures ANOVA revealed no main effects of treatment or time and no treatment x time interaction.

Right Arm Fat Percent

With all ANOVA assumptions being met, 2 x 2 repeated measures ANOVA revealed a significant main effect and linear trend for time ($p=0.035$), no significant main effect treatment, and no significant interaction. Both treatment groups significantly decreased dominant arm fat composition. Placebo treated participants decreased from $12.0 \pm 4.52\%$ to $11.29 \pm 4.48\%$, and naproxen sodium-treated participants decreased from $13.19 \pm 4.17\%$ to $12.62 \pm 4.03\%$ (Figure 25). With no significant treatment effect, the average decrease in dominant arm percent fat composition was $0.65\% \pm 0.92\%$ (Figure 26).

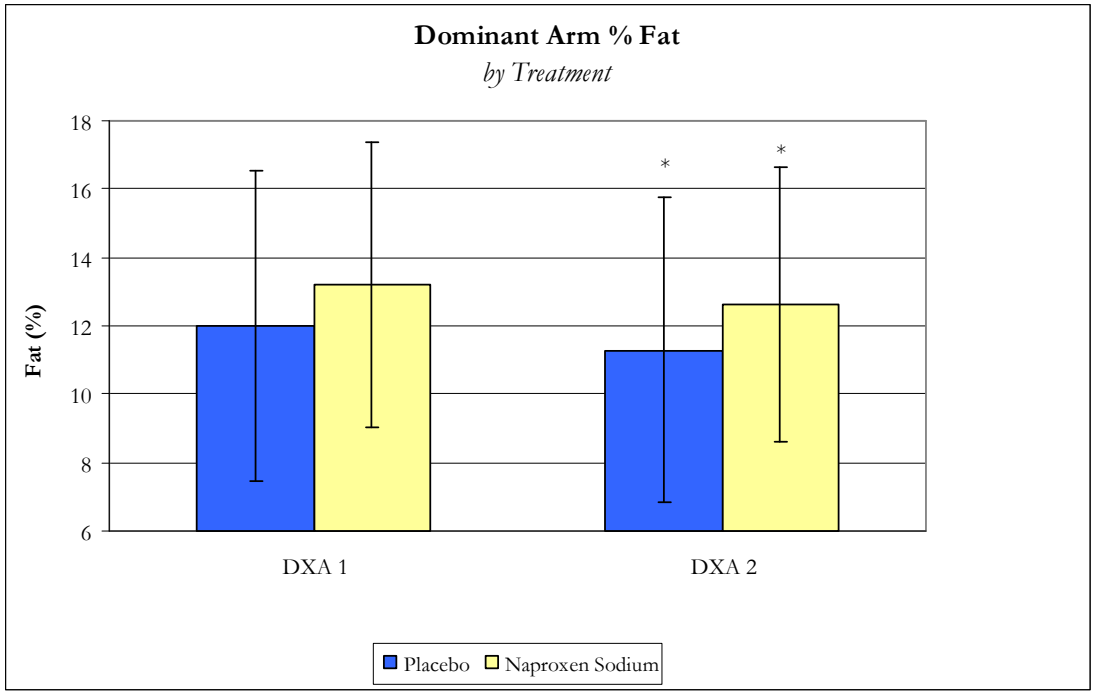

Figure 25
Error bars represent standard deviation.
*Significantly less than preceding within-group DXA.

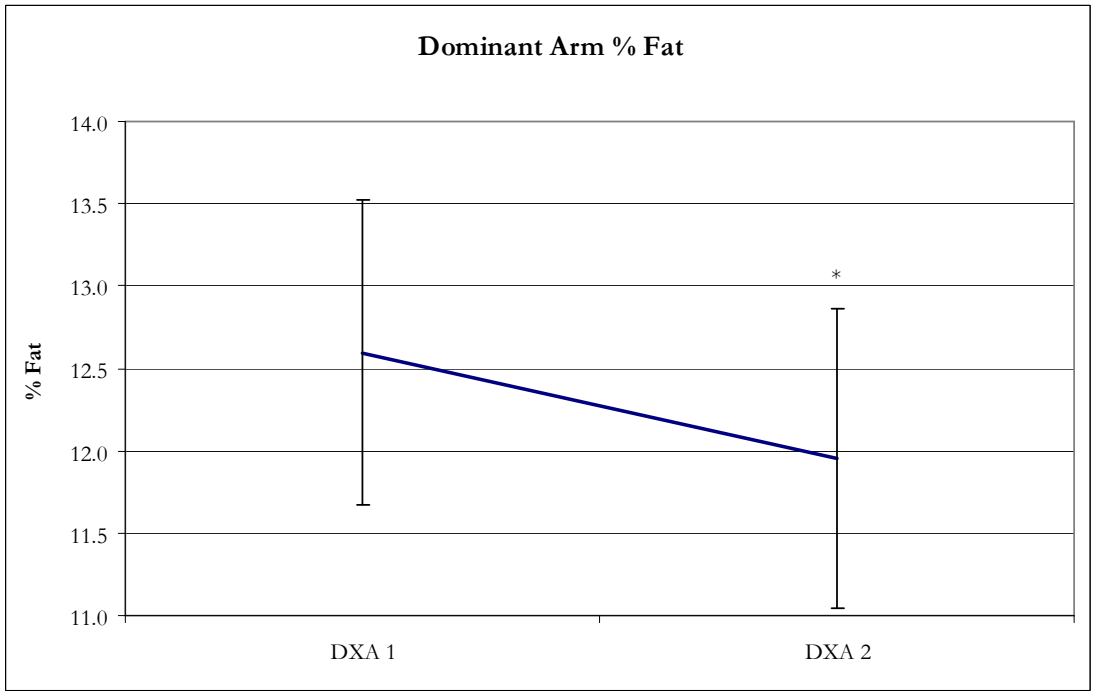

Figure 26
Error bars represent standard error.
*Significantly less than DXA 1.

Table 11
Summary of Tissue Changes in Dominant and Non-Dominant Arms

	Non-Dominant Arm	Dominant Arm
Bone Mineral Content (g)	↓*	↔
Skeletal Muscle Tissue (g)	↔	↑*
Adipose Tissue (g)	↔	↔
Adipose Tissue (%)	↔	↓*

↔ - no change; ↓ - decrease; ↑ - increase
* Significant at $p<0.05$ level.

Table 12
Average Daily Dietary Intakes

	Placebo	Naproxen Sodium
Total energy (calories)	2594.6 ± 559.6	2383.6 ± 754.4
Calcium (mg)	1175.8 ± 319.0	939.1 ± 369.7
Vitamin D (mcg)	5.8 ± 3.3	8.5 ± 9.5
Protein (g)	123.1 ± 44.0	134.2 ± 56.9
Kilocalories derived from protein (%)	19.7 ± 5.0	23.0 ± 6.4

Values presented as means ± standard deviation.

With four primary outcome variables, a condensed summary of the statistical results is presented in Table 13.

Table 13

Summary of Statistically Significant Findings

Outcome Variable	Statistical Results
Plasma $PGF_{2\alpha}$ Metabolite Concentrations	
Time *with Huynh-Feldt df correction*	$F_{(1.34,28.12)}=21.37$; $p<.005$
Exercise	$F_{(1,21)}=7.030$; $p=.015$
Upper Arm Circumference	
Time *with Huynh-Feldt df correction*	$F_{(2.56, 53.70)}=4.69$; $p=.008$
Exercise	$F_{(1,21)}=164.66$; $p<.005$
Time x Pre-Post *with Huynh-Feldt df correction*	$F_{(3.06, 64.27)}=11.09$; $p<.005$
Strength	
Time	
Lat Pull	$F_{(2,42)}=140.86$; $p<.005$
T-Bar Row *with Huynh-Feldt df correction*	$F_{(1.57,32.89)}=63.50$; $p<.005$
Upright Row *with Huynh-Feldt df correction*	$F_{(1.64,34.45)}=71.47$; $p<.005$
Bicep Curl	$F_{(2,42)}=84.47$; $p<.005$
Left Arm BMC	
Time	$F_{(1,20)}=5.96$; $p=.024$
Right Arm Skeletal Muscle Tissue	
Time	$F_{(1,20)}=5.02$; $p=.037$
Right Arm Percent Fat Composition	
Time	$F_{(1,20)}=5.12$; $p=.035$

Chapter V

Discussion

To the author's knowledge, this is the first study to examine the stable downstream metabolite of $PGF_{2\alpha}$ in the systemic circulation during the course of a training study in a strictly defined participant pool. All previous research involving this hormone is a mixture of acute training studies, animal models of imposed injury, and/or in vitro investigations. Both the chronic and systemic hormonal measurement characteristics of the study are unique factors that contribute to the existing body of literature concerning the $PGF_{2\alpha}$ hormonal response to chronic resistance exercise. This study sought to examine the anabolic growth factor at three time points over a 6-week periodized resistance training program and to attempt to clarify how its response to exercise and/or prostaglandin-inhibiting drugs may affect functional outcomes such as strength and hypertrophy. The combined measurement of systemic prostaglandin $F_{2\alpha}$ metabolites with functional parameters of muscle performance is also a unique characteristic of the study.

Systemic $PGF_{2\alpha}$ Metabolite Concentrations

While there are many hormones that may play a role in skeletal muscle growth, this paper sought to further investigate metabolites of an endogenously-produced growth factor shown to be intimately involved in satellite cell activity and the incorporation of new proteins into skeletal muscle. Along with Wilson and Kapoor (1993), it is one of only two studies to demonstrate a systemic increase in prostaglandin F levels in response to resistance exercise, validating a less

invasive approach to its measurement. This is the first investigation to demonstrate an acute exercise-induced increase in $PGF_{2\alpha}$ metabolites, using plasma samples derived from the general systemic circulation. Furthermore, the current study is the first to show a change in the acute $PGF_{2\alpha}$ metabolite response to exercise over a chronic (6-week) period of time.

There is no research to which these findings can be compared since no previous studies have sampled $PGF_{2\alpha}$ metabolites from the systemic circulation over time. The most similar study was performed by Wilson and Kapoor (1993), who reported significant five-fold increases in $PGF_{1\alpha}$ and PGE_2 ($pg.min^{-1} \bullet 100\ ml^{-1}$) in response to two levels of acute wrist flexion (0.2 and 0.4 W) in 12 normal subjects using arterial and venous catheter techniques. Their assumption was that the difference between arterial and venous circulations in the forearm reflected increases in synthesis, which are then reflected as "spillover" from the local muscular tissue.

The average increases of 13.5 $\pm$ 11.3 pg/ml and 2.7 $\pm$ 24.2 pg/ml in placebo- and naproxen-sodium-treated participants in this study are difficult to compare to previous studies, due to the different sources of measurement. It is not surprising, however, that concentrations were lower when derived from the general venous circulation. It is believed the measurement approach employed in the current investigation contributes to our understanding of the relationship between local and systemic values of this growth factor and may hopefully encourage more training studies that examine $PGF_{2\alpha}$ metabolites using less invasive techniques which facilitate repeated measurement in the same participant(s).

FSR has been shown to correlate $PGF_{2\alpha}$ concentrations (Smith et al., 1983; Trappe et al., 2002). With that correlation, increases of 48 $\pm$ 6% and 76 $\pm$ 19% in FSR ($PGF_{2\alpha}$) have been reported after acute exercise (Phillips et al., 1999; Trappe et al., 2002). There were strong similarities between Phillips et al.'s and Trappe et al.'s research, with both using similar participants who performed acute resistance exercise with FSR measured by phenyalanine incorporation into muscle.

While placebo-treated participants demonstrated ~77% increases in FSR and $PGF_{2\alpha}$ after acute resistance exercise, maximal non-prescription doses of ibuprofen (1,200 mg/day) and acetaminophen (4,000 mg/day) were found to blunt the typical increase, leading to the conclusion these medications attenuate the increase in FSR by inhibition of $PGF_{2\alpha}$ and that continued inhibition of the normal increase in synthesis after each session of resistance training would blunt the hypertrophic response to exercise (Trappe et al., 2002).

Using one-third of the dose administered by Trappe et al. (2001, 2002), Krentz et al. (2008) found no significant effect of ibuprofen (400 mg) on strength or hypertrophy but measured no biochemical markers of inflammation or protein synthesis. With findings that were in opposition to Trappe et al. (2001, 2002), Krentz et al. (2008) stated the importance of measuring biochemical markers of inflammation and protein synthesis over the course of a strength training program. Thus, the primary purpose of this investigation was to address the issues presented by these researchers.

While acute response revealed an increase post-resistance exercise, the chronic response, when expressed in picograms per ml of blood, demonstrated an unexpected quadratic trend over time (Figure 3). There are several factors which may explain this trend. First, it may be a function of stimulus intensity, as demonstrated by previous acute studies (Wilson and Kapoor, 1993; Boushel et al., 2002). The intensities of the workouts when blood was sampled were 76%, 82%, and 86% 1RM, respectively. Thus, the smallest acute response, and coincident lowest concentration (pg/ml), occurred when a 6% increase in intensity had been progressively imposed over 3.5 weeks. This was followed by a dramatic increase in the response after an additional 4% increase was imposed over the last 2.5 weeks of training. Strength had been re-assessed at the end of week three, and training load intensities for weeks 4-6 were set on the basis of this re-assessment; thus the decrease in concentration was unexpected and difficult to explain.

Dietary factors may also partially explain $PGF_{2\alpha}$ metabolite concentrations. The prostaglandin precursor, arachidonic acid, is found in all animal tissue; thus a diet high in meat should also be high in arachidonic acid. While it was planned for the first and second 3-day dietary collection periods to overlap the first and final blood draw, only the second dietary collection period actually overlapped the final blood draw. A bivariate correlation was performed to examine a potential relationship between dietary arachidonic acid content (g) and $PGF_{2\alpha}$ metabolite concentrations (pg/ml); however, analysis revealed no significant correlation was revealed ($p=0.313$). This analysis was conducted only for the final blood draw, as it was the only draw for which coincident dietary data was available.

It is important to remember the hormone was only measured before and after three of the twelve supervised resistance training sessions. It is not possible to know the activity of the hormone at intervals between. However, a strength of the study's design that adds to the validity of the three blood collections is the consistent conditions under which blood was sampled. To account for the circadian rhythm of many hormones (ie., cortisol), blood was always sampled at the same time. Also, in light of the fact stimulus intensity is known to influence the hormonal response (Boushel et al., 2002), blood was always sample before and after the most intense weekly workout.

The large response in $PGF_{2\alpha}$ metabolites exhibited from the second to third blood draws may also be explained by other phenomenon. Histological markers in Mishra's (1995) study of isolated intact rat skeletal muscle followed a somewhat curious time course. While significantly less embryonic myosin was seen in NSAID-treated rabbits 3-day post-stimulation, 7-day observations showed expression of this regenerative marker to be greater in treated versus untreated animals. While the authors purported this finding suggested a "delayed or ineffectual regenerative response" treated animals, it may also be considered super-compensatory type of response. It is possible $PGF_{2\alpha}$ metabolites also exhibited a super-compensatory type of response, reaching a low at the

seventh workout but dramatically rebounding by the final workout and blood sample (Figure 3). One explanation for the significant increase in $PGF_{2\alpha}$ metabolites measured at workout 11 of week 6 may reside in the significant increase in lean muscle tissue in the dominant arm. Comparatively larger muscle mass presents a greater source of the PG precursor arachidonic acid.

The lock-and-key hypothesis is a basic tenet of endocrinology. According to this theory, all hormones must interact with their receptors to produce their effects. $PGF_{2\alpha}$ exerts its effects by binding to its receptor, FP. With no previous research concerning the FP response to exercise, it is not possible to say how it might be affected by exercise and/or naproxen sodium; however, skeletal muscle growth is mediated through FP, which activates stretch-activated calcium channels on skeletal muscle membrane (Horsley and Pavlath, 2003). Eccentric exercise has been postulated to up-regulate neutrophil's ability to produce reactive oxygen species (Pizza et al., 1999). While there is no literature describing the effect of exercise or analgesics on the FP receptor, the potential for up- or down-regulation of FP receptor sensitivity as a result of these stimuli cannot be ignored. It may be possible a similar phenomenon to that seen in neutrophils could be seen in $PGF_{2\alpha}$-producing cells. The hormonal response exhibited could be attributed to up- and/or down-regulation of FP receptor sensitivity due to exercise intensity, naproxen, or other adaptive mechanisms.

In both an attempt to relate our data to that presented by previous studies and to account for substantial intra-individual variability in $PGF_{2\alpha}$ metabolites, post-exercise concentrations were normalized to pre-exercise concentrations. With data expressed as a percent change from pre- to post-exercise, significant treatment effects were revealed ($p=0.04$) (Table 8 and Figure 5).

Exercise resulted in $PGF_{2\alpha}$ metabolite increases in placebo-treated participants at each measured time point, but this response decreased linearly over time. Within the placebo group, each workout for which blood was sampled resulted in a progressively smaller increase in $PGF_{2\alpha}$ over time, with increases of 22.6, 13.1, and 6.9% after workouts 1, 7, and 11, respectively (Figure 5).

These findings support those of Phillips et al. (1999), who demonstrated trained participants respond to exercise with a smaller increase in FSR and may suggest hormonal adaptation to the exercise intervention. It would be reasonable to assume continued training in the current participant sample would result in progressively smaller increases in $PGF_{2\alpha}$, which correlate FSR (Trappe et al., 2002).

Conversely, the naproxen sodium treatment group demonstrated a non-linear chronic response in $PGF_{2\alpha}$ activity, with an initial 5.4% increase in $PGF_{2\alpha}$ metabolites. This was followed by complete inhibition of the typical increase in $PGF_{2\alpha,}$ with levels completely abolished, ~8% below pre-exercise levels. The final naproxen sodium blood sample showed only a slight 3.9% increase in $PGF_{2\alpha}$ metabolite concentrations. It is not clear, however, why levels were so drastically reduced at only the second measured time point (workout 7); however, it may lie in individual responses to medications.

Arm Circumference

The acute change in dominant arm circumference was used as an indirect measure of exercise-induced hyperemia. The significant time x pre-post response interaction indicated this response decreased over time, even in a periodized resistance training program in which intensity was increased 2% each week. While both groups exhibited a significant increase in the acute response (cm) from week 1 to week 2 (Figure 9), all subsequent pre- and post-exercise measurements decreased toward their respective initial baseline value after week two. Participants in the placebo treatment group demonstrated an average increase in upper arm circumference of 1.47 $\pm$ 1.21 cm (4.05%) in response to the first workout, versus those in the naproxen sodium treatment group, who demonstrated an average increase of 1.69 $\pm$ 1.22 cm (4.69%). By the eleventh and final complete workout, the average increase was 0.58 cm $\pm$ 0.59 cm (2.5%) in the placebo treatment

group versus 0.48 $\pm$ 0.36 cm (1.45%) in the naproxen sodium treatment group. Whether or not this lends support to the use of pre-workout vasodilators is not clear; however, use of such ergogenic supplements is relatively common in certain subsets of recreationally-trained individuals.

Howell et al. (1998) reported modest (1%) swelling of the upper arm in response to acute elbow flexion consisting of 3 consecutive workloads of 90%, 80%, and 70% maximum voluntary isometric force. Repetitions at each workload were performed until the subject could no longer maintain a 2 second eccentric contraction. Progressively fewer repetitions were performed in each successive set, with an average total number of 18.6 repetitions. Howell et al. (1998) reported no differences between control, placebo, high dose ibuprofen (800 mg 4 x day for 6 days), or low-dose ibuprofen (400 mg 4 x day for 6 days) groups in the acute swelling of the upper arm in response to this exercise intervention. Our results were substantially greater than those reported by Howell et al. (1998), with those in the placebo treatment group responding with a 4.05% increase in arm circumference in response to workout one, and participants in the naproxen sodium treatment group responding with a 4.69% increase. In comparison to the average 18.6 repetition total performed by participants in Howell et al.'s study, the repetitions which elicited an approximate 4% increase in acute arm circumference totaled 96 in the current investigation.

An interesting finding related to the arm circumference response to exercise was the attenuation of the typical response by a preceding strength assessment, as seen in week 3. Although participants were provided 30 minutes recovery after the second strength assessment, the typical circumference response was attenuated by the prior muscular work. These results may suggest a refractory period for the hyperemic response to exercise, in that 30 minutes is not sufficient time for recovery of the hyperemic-related processes.

Strength

When strength gains were averaged for all four exercises, participants in the placebo treatment group demonstrated an average increase in strength of 27.1% compared to a 28.5% increase in strength for those in the naproxen sodium treatment group. This rate of strength development compares somewhat to Krentz et al. (2008), who reported a 21% increase in the strength of the elbow flexors after 6 weeks of unsupervised unilateral elbow flexion exercise performed by trained co-ed subjects 5 days per week. Krentz et al. (2008) only performed RM tested at the beginning and end of the study, a fact which may explain the larger gains in strength elicited by this study's periodized design and mid-point strength testing.

Recreationally-trained males in the current investigation consistently increased strength in each exercise throughout the duration of the study. As shown in Figures 14, 16, 18, and 20, the rate of strength development was maintained across 1RM testing intervals, with no diminution seen during the final 3-week 1RM testing interval. Although this consistent increase was likely due to the 3-week re-assessment in strength which allowed for appropriate adjustment of training loads, the continued increase in the absence of significant growth is somewhat surprising considering the relatively well-trained status of the participants.

These significant increases in strength were not accompanied by significant hypertrophy of the dominant upper arm (0.31 ± 1.15 cm increase in placebo versus 0.58 ± 1.59 cm increase in naproxen sodium). By selecting trained males, it was assumed participants were outside the adaptation window predominated by neurological adaptations. In the absence of significant anthropometric (cm) adaptations, neurological phenomenon, including increased motor unit recruitment and/or their discharge rate, may partially explain the increases in strength in the absence of hypertrophy.

It is important to distinguish between morphology, relating to structure or form, and hypertrophy, as the latter term certainly falls under the umbrella term of morphology. Although significant hypertrophy did not occur, there were significant morphological changes, evidenced in a significant increase in dominant arm lean tissue and a significant decrease in dominant arm fat composition (percent). It is believed the significant decrease in fat tissue composition balanced the significant increase in lean tissue, thus resulting in no significant gross changes in arm circumference.

Various responses to both aerobic and resistance exercise are known to be strongly dictated by the initial fitness status of the individual, with relatively larger gains in strength possible in sedentary and/or less-trained populations. Resistance-training status was one of the main pre-requisites for participation, and all participants were classified as recreationally-trained. While authors provide different definitions of recreationally-trained, in this study, recreationally-trained was operationally-defined as individuals who were currently performing resistance exercise 3-5 times per week and had been doing so a minimum of 6 weeks. Performance of a total body workout three times per week, upper/lower split routine totaling four sessions per week, or push/pull/lower split routines totaling five sessions per week qualified a participant for assignment as recreationally-trained.

In a meta-analysis of 37 studies of collegiate or professional athletes employing a strength training intervention, Peterson, Rhea, and Alvar (2004) reported that maximal strength gains in athletes are achieved with a mean intensity of 85% 1RM, frequency of twice per week, and mean training volume of 8 sets per muscle group. Eight set per muscle group training was reported to elicit strength increases of approximately one standard deviation above that of 1-set interventions in regards to magnitude of effect (Peterson, Rhea and Alvar, 2004).

The current investigation was characterized by an undulating (heavy-light) microcycle nested within a progressively periodized mesocycle. The heavy day was performed at 76-86% 1RM

intensity for 3 sets of 6-8 repetitions, and the light day was performed at 66-78% 1RM for 3 sets of 10 repetitions. Thus, the mean intensity was 76% 1RM over the 6-week mesocycle, and the number of sets per muscle group was always 12. The continued development of strength to an equal extent across both 3-week 1RM testing intervals supports the heavy-light approach to resistance exercise, which is useful when considering the potential for overuse injuries resulting from exercise. Also, continued increases in strength with 12 sets in the current study stands in contrast to the meta-analysis research indicating that the magnitude of strength gains are decreased with training above 8 sets per muscle group in a trained population (Peterson et al., 2004). There was no decrease in strength observed in this study's recreationally-trained participants. This discrepancy in dose-response may be explained by differences between participant pools (recreationally-trained athlete versus collegiate/professional athlete).

Segmental Tissue Composition

Changes in bone mineral content and skeletal tissue were inconsistent between dominant and non-dominant arms (Table 11). While the non-dominant arm in both treatment groups lost bone mineral content without significant increases in skeletal muscle tissue or decreases in adipose tissue (grams or percent), the dominant arm exhibited no significant changes in bone mineral content but a significant increase in lean tissue and significant decrease in adipose tissue, expressed as a percentage (Table 11).

The statistically significant decrease in non-dominant arm BMC fell within the standard error of the DXA machine ($\pm$2.0%). While it is possible to argue the decrease may not be statistically significant due to this fact, any decrease in BMC seen in recreationally-trained college-aged males performing resistance training seems clinically significant and warrants further inspection.

According to the 2004 Surgeon General's Report on Bone Health and Osteoporosis, bone mineral content is progressively developed up until age 30 in both sexes, provided daily intakes of calcium and vitamin D are sufficient and weight-bearing physical activity is performed (Carmona and Moritsugu, 2004). Other factors which may adversely affect bone mineral content include excessive alcohol consumption, smoking, underweight status, and thyroid medications (Carmona and Moritsugu, 2004).

The average age of participants in the current study was 20.8 years. Participants were pre-screened for alcohol consumption behaviors and cigarette smoking, thus these potential contributing factors do not seem to explain the decrease in bone mineral content in the non-dominant arm. Components of dietary intake which have the potential to affect bone mineral content were examined (Table 12). Due to scheduling conflicts, two participants in the placebo treatment group were unable to complete the first 3-day dietary collection period, and two additional participants in the placebo group were unable to complete the second 3-day collection period for follow-up interview with study nutritional personnel. Two participants in the naproxen sodium treatment group completed one 2-day collection (n=1) period and one 3-day collection period (n=1), but were able to complete the other respective 3-day collection period. For those with incomplete 3-day dietary assessments, an intent-to-treat analysis was employed, using the average intakes for the completed collection periods.

Averages for each collection period were calculated from the actual number of days of record. Average calcium, vitamin D, protein (g), and percent kilocalories from protein are presented in Table 12. Two-tailed independent samples t-tests revealed no significant between group differences in the intake for the presented macro- and micronutrients.

The majority (63.6%) of participants in the placebo treatment group met their daily recommended dietary allowance for calcium (1,000 mg/day), while exactly 50% of participants in the

naproxen sodium treatment group met their daily allowance. Thus, between the two treatment groups, more than half (56.5%) met the recommended dietary reference intake (DRI) for calcium. In contrast, only one participant in the placebo group and two participants in the naproxen sodium treatment group met the RDA for Vitamin D. Thus, only 13.4% (3) of participants met the 2011 Institute of Medicine RDA for vitamin D for persons 1-70 years. Group averages were well below the 2011 Institute of Medicine RDA of 15µg. The placebo group averaged 5.8µg, and the naproxen sodium treatment group averaged 8.54µg. Based on their training status and initial body mass, protein recommendations for study participants fall between 1.5 – 2.0 g/kg of body mass (Reimers, 2004). Using this value, recommended ranges were determined using the average initial body mass for each treatment group. Both treatment groups average daily protein intake fell within their respective recommended range (placebo treatment group recommendation 116.9–155.8 g/kg; naproxen sodium treatment group recommendation 121.05–161.4 g/kg).

Dietary deficiencies may partially explain the decrease; however, these deficiencies would be expected to manifest systemically, with decreases in bone mineral content seen throughout the body. This was not the case, however. While the change in dominant arm bone mineral content was not significant, values did increase, however slightly, in both treatment groups over the 6-week training intervention. It is believed the decrease in BMC exhibited by the non-dominant arm is best explained by the bilateral nature of the exercise intervention.

Strength was assessed bilaterally, but tissue composition was examined unilaterally with DXA. To make a connection between unilateral tissue changes in response to a bilateral resistance training intervention is difficult with the study's design. Had both bilateral and unilateral strength been assessed at the onset of the study, it may be more possible to speak to potential corresponding changes in strength and tissue.

One likely explanation for the decrease would be regular performance of unilateral exercises prior beginning the 6-week bilateral exercise intervention. While subjects had been pre-screened for resistance training, the specific type of exercise (barbell versus dumbbell) was a program characteristic participants were not asked to specify. Thus, subjects were contacted roughly 6 months after the study's conclusion to determine if a substantial number had indeed been performing regular unilateral upper body exercise (ie., dumbbell curl) prior to enrolling in the study. Nineteen of twenty-three participants responded. Eleven reported having been regularly performing unilateral upper body resistance exercises prior to beginning the study, while eight reported they were not. It is thus possible that non-dominant arm bone mineral content decreased as a result of the substitution of a unilateral exercise with a bilateral exercise. To support this theory, not only did lean skeletal muscle tissue not significantly increase in the non-dominant arm, but the trend was for a decrease in lean tissue. The average decrease in non-dominant arm lean skeletal muscle tissue was 60.95 grams. It is highly likely resistance trainers subconsciously exert greater force with the dominant arm when performing bilateral exercises.

Limitations

The Nautilus lat pulldown selectorized resistance training machine, or the exercise itself, revealed itself as a potential issue as the study progressed. During the initial 1RM assessment, the maximum weight allowed by the machine was beyond the strength capabilities of all participants; however, as strength increased, the maximum weight limit of the machine was exceeded by several of the participants. As a result, the planned 1RM test became a multiple RM assessment, with 1RM values estimated using the Brzycki equation (Brzycki, 1993). While meeting 1RM test prerequisites for being a core exercise, its gravity-assisted nature may artificially inflate strength measures. Also, due to pain, one subject substitute the seated cable-row for the t-bar row, and one subject

substituted an upright row performed with a rope for the barbell upright row. A few subjects reported discomfort using the straight barbell for bicep curls, and these were allowed to substitute the easy-curl bar. If the substitution was made, strength testing was carried out using the easy-curl bar.

A total of three researchers assisted in measuring arm circumference, and although the average of 2-3 measurements pre- and post-exercise were taken to determine a official pre- and post-exercise value, arm circumference measures were associated with large standard deviations. While the average of the measurements attends to intra-rater reliability concerns, inter-rater reliability was not established. Thus, the lack of this may explain the large measures of variance and subsequent lack of statistically significant findings. Finally, there were several instances of random medication use during the 6-week study. Ideally, all other medications other than that employed in the study would be prohibited; but, ethical concerns arise when prohibiting participants from pharmacological aide for ailments such as headaches or colds.

Conclusion

General consensus holds that skeletal muscle regeneration is contingent upon protein synthesis and satellite cell activity. Satellite cells are the foundation of myogenesis, and many well-controlled studies have presented convincing evidence for the role of prostaglandins in the cell cycle. Research has shown detrimental effects of prostaglandin-inhibiting drugs on satellite cell activity after various mechanical forms of acute muscle injury. While the author acknowledges the precision, sensitivity, and quality of this measure, this study sought to examine the more ultimate and functional outcomes of satellite cell activity, including muscular strength and hypertrophy. In addition, this study sought to investigate how a repeated consumption of naproxen sodium might affect the $PGF_{2\alpha}$ response to exercise and potentially corroborate strength and hypertrophy

measures. While muscle biopsy and catheter techniques to measurement more accurately reflect tissue-specific changes in this hormone, these approaches are also invasive, and as a result, are generally limited to measurement after acute interventions. As noted in the introduction, three of the four studies to examine the efficacy of NSAIDs in DOMS prevention/treatment and to report beneficial effects (preserved strength, torque, decreased soreness) used naproxen sodium after acute exercise (Dudley et al., 1997; Lecomte et al., 1998; Baldwin et al., 2001). In light of these findings, further investigation of treatment effects on chronic muscle adaptation seem warranted.

The results of the study indicate this dose does not interfere with the development of strength, size, or lean skeletal muscle tissue in recreationally-trained college-aged males. It is not possible to extrapolate these finding to other populations, other models of injury, or other time courses of analgesic use. Regenerative potential could logically be contingent upon whether NSAID administration is limited to the immediate post-injury period or continued during the regenerative phase, as longer duration treatments have been shown to impose even greater decrements in muscle performance (Lapointe et al., 2003). Our findings are now added to those reporting no negative effects of a non-selective NSAID on skeletal muscle adaptation over a 6-week period (Krentz et al., 2008).

The inflammatory response can be mediated through multiple cellular pathways. Arachidonic acid, once cleaved from the membrane, can be converted into prostaglandins and thromboxanes through the COX pathway, or into leukotrienes through the lipoxygenase pathway. In vitro studies of human tendon fibroblasts subjected to cyclic mechanical deformation with or without indomethacin have shown a concomittant increase in leukotriene B4 (LTB4) when PGE_2 is inhibited (Almekinders et al., 1993). It appears the marvelous human body finds a way to overcome exercise-induced inflammation.

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Appendix: A

Appendix A

Figure A1 Lat Pulldown

1. Sit with feet flat on floor hip-width apart with thighs supported by machine pads.
2. Assume a wide (slightly greater than shoulder-width) overhand closed grip on the bar.
3. Slightly recline the trunk so bar will pass just in front of the nose during the concentric phase of the lift.
3. With head in a neutral position and eyes forward, contract upper back musculature, pulling bar to breastbone (1 second).
4. Slowly (2 seconds) return the bar to starting position, allowing shoulder blades to protract slightly.
5. Ensure trunk remains stationary throughout exercise.
6. Exhale during the concentric phase; inhale during the eccentric phase.

Figure A2 T-Bar Row

1. Assume a prone position on machine, allowing pad to support chest (not shown).
2. Assume a hip-width stance with feet.
3. Assume a neutral closed grip on the diagonal grips.
4. With head in a neutral position and eyes down, contract upper back musculature, pulling bar to chest (1 second).
5. Slowly (2seconds) return the bar to starting position, allowing shoulder blade protraction.
6. Exhale during the concentric phase; inhale during eccentric phase.

Figure A3 Upright Barbell Row

1. Stand with feet in a hip-width stance with arms extended.
2. Assume a shoulder-width or slightly more narrow overhand closed grip on barbell.
3. With head in a neutral position and eyes forward, contract the shoulders (1 second), pulling bar to neck, allowing elbows the lead the movement.
4. Allow wrists to flex during this concentric phase.
5. Slowly lower (2 seconds) the bar to starting position.
6. Exhale during the concentric phase; inhale during eccentric phase.

Figure A4 Bilateral Barbell Bicep Curl

1. Stand with feet in a hip-width stance with arms extended.
2. Assume a shoulder-width underhand closed grip on the barbell.
3. With head neutral and eyes forward, flex the elbows (1 second), bringing bar to shoulders.
4. Slowly (2 seconds) lower arms to extended staring position.
5. Ensure upper arms, elbows, and trunk remain stationary throughout the exercise.
6. Exhale during the concentric phase; inhale during eccentric phase.

Figure A5 BodyMedia SenseWear PRO^2 Armband

Appendix: B

Appendix B

Participant Instructions

Exercise

(1) The day **before** you are scheduled to report to the lab, please do not perform resistance training exercises involving the upper back or biceps. This includes the following:

- Pull ups
- DB pullovers
- Lat pull downs
- Rows
- Bicep Curls
- Rear deltoid pulls
- Rear deltoid flys (reverse flys)

(2) The day you report, please do not perform any type of exercise (aerobic or resistance) 2 hours before you come in to exercise with me. Please wait until after our lab session to do your personal workout.

Alcohol/Medicines/Supplements

(3) Please do not drink more than 3 drinks the evening after your workout with me. Both Aleve and alcohol are metabolized by your liver. It is wise not to drink when taking any medicine. One drink is equal to 12 ounces beer, 5 ounces wine, or 1.5 ounces (~1 shot) of liquor.

(4) If your doctor prescribes any new medication to you while you are enrolled in this study, please tell me immediately. You should also tell your physician about being involved in this research study. S/he will need to know that you have been taking 440mg of Aleve two days per week.

(5) If you begin taking any new over-the-counter medicines while you are enrolled in this study, please tell me immediately.

(6) Please do not begin taking any new supplements (creatine, amino acid pills or powder) while you are enrolled in this study.

(7) Please do not ingest over-the-counter stimulants (energy pills) prior to exercising with me. Please do not drink any caffeine (coffee, coke, Red Bull, Rockstar, etc) in the 2 hours before working out with me.

(8) If you have a headache and need to take a pain reliever, take only the recommended dose of your preferred pain reliever. Report any additional use of pain relievers to me during the course of the study. You can email me at cbrewer2@olemiss.edu, text me at 801-9691, or tell me when you come in to exercise.

(9) The manufacturer of Aleve directs that no more than 3 Aleve caplets are to be taken in a 24 hour period, unless directed by a physician. I will be giving you 2 caplets. If you find yourself in need of a pain reliever later on during the days we work out, do not take more than 1 additional caplet within that 24 hour period. For sore muscles, often low-moderate

intensity exercise, such as walking or swimming, can alleviate pain. Other options include a warm shower and/or massage. It is wise to avoid taking additional pain relievers for muscle ache if at all possible while participating in this study.

Specific Patient Instructions for Oral Naproxen Sodium

(1) You should not use this medicine if you have had an allergic reaction (including asthma) to naproxen, aspirin, or other NSAID medicines, such as diclofenac, ibuprofen, Advil®, Celebrex®, Ecotrin®, Motrin®, or Voltaren®.
(2) It is best to take this medicine with food or milk, so it does not upset your stomach. Drink a full glass of water after a dose.
(3) If you see a doctor or dentist while participating in this study, make sure you inform them of your participation in this study. Tell them you are taking 2 Aleve caplets before exercise twice per week.
(4) This medicine may make you drowsy or dizzy. Avoid drinking, using machines, or doing anything else that could be dangerous if you are not alert.

Possible Side Effects While Using This Medicine

Call your doctor immediately if you notice any of the following signals of a serious reaction:
(1) Allergic reaction – itching, hives, swelling in your face or hands, swelling or tingling in your mouth or throat, chest tightness, trouble breathing
(2) Blistering, peeling, or red skin rash
(3) Blood in your urin
(4) Bloody or black, tarry stools
(5) Change in how much or how often you urinate
(6) Chest pain, shortness of breath, or coughing up blood
(7) Flu-like symptoms
(8) Numbness or weakness in your arm or leg or on one side of your body
(9) Pain in your lower leg (calf)
(10) Problems with vision, speech, or walking
(11) Redness or swelling of the body area where you have pain
(12) Severe stomach ache
(13) Shortness of breath, cold-sweat and bluish-colored skin
(14) Skin rash or blisters with fever
(15) Sudden severe headache
(16) Swelling in hands, ankles, or feet
(17) Trouble swallowing
(18) Unusual bleeding, bruising, or weakness
(19) Vision changes
(20) Vomitting blood or something that looks like coffee grounds
(21) Yellowing of your skin or whites of your eyes

Tell me immediately if you notice any of the following less serious side effects:
(1) Headache
(2) Mild nausea, stomach pain, heartburn, gas, diarrhea, constipation
(3) Mild skin rash or itching
(4) Ringing in your ears

VITA

Christi Brewer, Ph.D., CSCS

The University of Mississippi
School of Applied Sciences
Dept. of Health, Exercise Science, Recreation Management
PO Box 1848
University, MS 38677
Phone: 662.915.5595
Fax: 662.915.5525
Email: cbrewer2@olemiss.edu

ACADEMIC RECORD

The University of Mississippi — December 2011
Ph.D., Health & Kinesiology
Major Area: Exercise Physiology

Dissertation: The effects of chronic prophylactic naproxen sodium on skeletal muscle adaptation to resistance training in recreationally-trained college-aged males.

Committee: Dwight Waddell (Chair), Mark Loftin, John Garner, John Bentley, Elaine Day

The University of Mississippi — May 2008
Master of Science, Exercise Science
Major Area: Exercise Science

The University of Mississippi — May 2001
Bachelor of Science, Exercise Science
Minor: English

EMPLOYMENT RECORD

January 2007 - Present
Graduate Instructor
The University of Mississippi
Health, Exercise Science, and Recreation Management

- Primary instruction of 100-400 level exercise science and health promotion courses, including personal and community health, first aid and cardiopulmonary resuscitation, motor learning & control, and exercise leadership

August 2011 – Present
Adjunct Instructor
Itawamba Community College
Health, Physical Education, and Recreation
- Primary instruction of departmental health courses

August 2006 - January 2007

Graduate Assistant
The University of Mississippi
Baptist HealthPlex
- Perform fitness assessments & equipment orientations
- Develop and teach personalized exercise programs and group exercise classes

May 2005 - May 2006
Strength & Conditioning Coach
The University of Mississippi
- Assistant in administration of off-season workouts for university football team
- Independently develop, administer, teach, and evaluate strength and conditioning programs for three university squads

October 2001 - May 2005
Director of Fitness
Foxy's Health & Racquet Club; Baton Rouge, LA
- Administer fitness assessments and develop safe and effective personalized exercise programs for healthy and special populations
- Design and implement new youth exercise programs, including Gym 101, Gym 102, Family Fitness group exercise classes, and summer Kids' Camp exercise & nutrition classes
- Hire, train, evaluate, and manage large fitness staff
- Organize and promote fitness & nutritional seminar throughout community, drawing 80 attendants
- Public speaking engagements/interviews for local organizations to promote exercise, health, and wellness (American Legion, AM Radio, Baton Rouge's *The Advocate*, Baton Rouge's Annual Women's Expo)

August 2001 - October 2001
Health & Fitness Staff
YMCA, Baton Rouge, LA
- Fitness instructor, group aerobics instructor, home-school PE coach

January 2001 – May 2001
Cardiac Rehabilitation Intern
Baptist Memorial Hospital – North Mississippi
The University of Mississippi
- Assist with patient ambulations, pre- and post-operational teachings, and home exercise prescription
- EKG set-up and blood pressure measurement
- Conduct intake and retrieval of patient information and files

ABSTRACTS & PRESENTATIONS

Peer-Reviewed

Brewer, C., Waddell, D. (2009) The effects of cold temperature on gait initiation and dynamic balance in young and older females. *Medicine and Science in Sports and Exercise*, 41(5), Supplement.

Non-Peer Reviewed (Invited Presentations)

Brewer, C. University of MS – Lunch & Learn - New Beginnings Weight Management Presentation (November 2011)

Brewer, C. Law Enforcement Wellness - MS Law Enforcement Command College, University of Mississippi (July 2008)

Brewer, C. Exercise and Multiple Sclerosis - Multiple Sclerosis Local Support Group, Baptist Memorial Hospital – North Mississippi (August 2007)

Brewer, C. Health and Wellness Presentation – U.S. Army Corps of Engineers, Oxford Conference Center (March 2011)

HONORS

Graduate Student Academic Achievement Award in Health, Exercise Science and Recreation Management (2008)

Graduate School Dissertation Fellowship (Fall 2011)

FUNDING

Internal - Funded

2007 Graduate School Summer Research Grant, University of MS, $2,000

Internal – Non-Funded

2009 Graduate Student Council Research Grant, University of MS, $500
2010 Graduate Student Council Research Grant, University of MS, $500

RESEARCH PROJECTS

Use of the Profile of Mood States (POMS) Questionnaire to Assess Overtraining in Collegiate Females
Fall 2011
Role: Primary Investigator

Effects of prophylactic naproxen sodium on skeletal muscle adaptation to resistance exercise in recreationally-trained college-aged males
Dissertation study
Spring 2011
Role: Primary Investigator

Effects of prophylactic naproxen sodium on exercise-induced prostaglandin $F_{2\alpha}$ blood plasma concentrations
Dissertation pilot study
Fall 2010
Role: Primary Investigator
Frequency of use of over-the-counter non-steroidal anti-inflammatory drugs in recreationally-trained college students and collegiate student athletes
Spring 2010 - present
Role: Primary Investigator

Shock attenuation analysis to evaluate various gender specific insoles
Schering Plough Grant (Dwight Waddell, Ph.D., Primary Investigator)
Fall 2008
Role: Co-Investigator

The effects of cold temperature on gait initiation and dynamic balance in young and older females
Thesis
Fall 2007-Spring 2008
Role: Primary Investigator

SUPERVISION OF STUDENTS

- Aaron Proctor – Research Assistant, Exercise Biochemistry Laboratory (Fall 2010)
- Sarah Annand, Jonathan Collins, Christopher Hawkins – Research Assistants, Exercise Biochemistry Laboratory (Spring 2011)

PROFESSIONAL SERVICE

Delmar Cengage Learning - Book proposal review (April 2011)
UM Womens' Soccer - Consultant (May 2011-present)

PROFESSIONAL AFFILIATIONS

- National Strength and Conditioning Association – Certified member (2004-Present)

CERTIFICATIONS

- American Red Cross, CPR/AED, First Aid Instructor (May 2008-present)
- National Academy of Sports Medicine (NASM), Certified Personal Trainer (2003-2008)
- National Strength and Conditioning Association (NSCA), Certified Strength and Conditioning Specialist (CSCS) (2004-present)

COMMUNITY SERVICE

- DII U10 Boys Soccer Team Manager (2010-2011)
- Oxford Park Commission Youth Soccer Coach (Fall 2005, Spring 2006, Fall 2007, Spring 2008)
- FITNESSGRAM administration, Pope Elementary School (December 2007)
- Relay for Life - Participant & Fundraiser, Baton Rouge, LA (2005)
- BP Multiple Sclerosis Tour - Participant & Fundraiser (April 2005)
- Multiple Sclerosis Tour for Cure - Participant and Top Tour Fundraiser, Hammond, LA (October 2004)
-

UNIVERSITY SERVICE

- Facilitation of adult and child CPR/AED expedited courses for University groups, including Mississippi Teacher Corps (2009, 2010), Literacy for Life (2010), RebelQuest Summer Camp (2010), Human Resources (2010), and Physical Plant (2010)
- Jean Jones Run – Volunteer, University of Mississippi (April 2008)
- Rebel Man Triathlon - Volunteer, University of Mississippi (2007-2011)

CONTINUING EDUCATION AND CONFERENCES

- American College of Sports Medicine National Conference (May 2009)
- University of Mississippi Strength Clinic (February 2006)
- National Strength and Conditioning Association Regional Conference (August 2011)