
R. JAYARAMAN
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This is to certify that the

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THE USE OF FUNCTIONAL MAGNETIC RESONANCE IMAGING
IN THE STUDY OF DELAYED MUSCLE SORENESS

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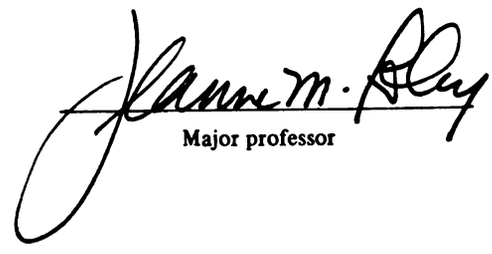

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THE USE OF FUNCTIONAL MAGNETIC RESONANCE IMAGING IN THE STUDY OF DELAYED MUSCLE SORENESS

by

Roop Jayaraman

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ABSTRACT

THE USE OF FUNCTIONAL MAGNETIC RESONANCE IMAGING IN THE STUDY OF DELAYED MUSCLE SORENESS

by

Roop Jayaraman

The purposes of this study were (1) to examine the acute T2 response to a bout of concentric exercise performed before and after eccentric exercise had been used to induce a delayed T2 increase and (2) to document the early time course of the delayed T2 development. Eight non-weight trained subjects were imaged before and after each exercise session, concentric #1, eccentric, and concentric #2 at 24 h post eccentric. In addition, subjects were imaged at 1, 2, 4, and 6 hours following eccentric exercise. The acute ΔT2 following the first concentric exercise was found not significantly different from the second bout. The delayed T2 increase was observed as early as 4 and 6 h post eccentric exercise. The findings of this study suggest that the underlying mechanism of the acute and delayed T2 increase is the same and that the acute T2 has an upper limit.

DEDICATION

To my grandparents, Govindammal and Govindarajan, for a lifetime of unconditional love, support and discipline.

ACKNOWLEDEMENTS

I wish to express my deep appreciation to Dr. Jeanne Foley, my advisor, for her guidance and advice. Her thoughtful discussions on teaching, research, and life in general have made the last three years a true learning experience. Her understanding, patience, and willingness to help me improve as a researcher and as an educator will not be forgotten. I would also like to thank the members of my committee, for their timely and thoughtful suggestions: Dr. James Pivarnik and Dr. Eugene Brown. I would also like to express my deepest gratitude to Dr. Ron Meyer for opening his laboratory to me and donating valuable time. My profound thanks to Dr. Roger Haut for financially supporting me early in my graduate studies and for your contribution to my learning experience as a graduate student.

Finally, I would also like to thank my family, mom, dad and Vijay (my best friend) for their support and kind words of encouragement, and my friends, especially Augustine Hernandez, for all those late night shoot arounds.

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CHAPTER I

THE PROBLEM

Delayed muscle soreness (DMS) is the sensation of discomfort and/or pain in skeletal muscles following unaccustomed muscular exercise. Nearly every adult has experienced DMS, most on numerous occasions. Unaccustomed physical activity of even moderate intensity or duration will result in the sensation of DMS, regardless of the individual's general fitness level. The most predictable method of inducing delayed muscle soreness is to subject muscles to repetitive, strenuous eccentric or lengthening contractions (5,6,7,20). Although, concentric or shortening contractions require more metabolic energy, they generally induce little or no soreness. The high mechanical forces produced during eccentric contractions have been shown to cause ultrastructural muscle damage which develops gradually and has been implicated with the sensation of DMS.

Muscles adapt to an initial bout of eccentric exercise, becoming resistant to damage from subsequent bouts. Muscle soreness is often localized in the distal portion of the muscle at the muscle/tendon junction and is accompanied by reduced mobility (6,7,38). The sore muscles become extremely sensitive to palpation and movement. Severity varies from slight stiffness to severe pain

which inhibits movement. Soreness is accompanied by swelling, decreased muscular performance, and a reduced range of motion at the joint. Since muscle soreness and swelling are only temporary, most DMS sufferers do not seek medical attention. Studies using animal models suggest that injured muscles are able to recuperate completely within 2 weeks (6,7).

A review of the literature to date will show that there is no firm consensus on the pathophysiological mechanisms responsible for DMS. At present, there is universal agreement that the muscle damage is initiated by the high mechanical forces produced during muscular exercise, particularly in eccentric exercise (6,20,36,60). Although the time course of the pathological changes associated with DMS is well established, it remains to be determined how the sequence and timing of DMS relate to a possible pathological mechanism. Thus far three mechanisms have been proposed: (a) "torn tissue" theory, (b) calcium-induced cell damage theory, and (c) acute inflammation theory (6).

Neither the "torn tissue" theory nor the calcium-induced cell damage theory is able to fully explain the relation between tissue injury initiated during the eccentric exercise bout and the soreness first experienced approximately 8 h after exercise that reaches a peak at 24-48 h post-exercise (20). In an attempt to better explain the unusual delay, several investigators have proposed that the mechanism underlying DMS is acute inflammation, based on the similar time course of DMS and inflammation (74). However, the time course data on the accumulation of inflammatory markers and DMS do not correlate well, ruling out acute inflammation as the sole mechanism of DMS.

The controversy over the mechanism(s) of DMS remains unresolved partly due to the fact that two parameters most often used to measure or document DMS, muscle performance and perceived muscle soreness, do not provide a measure of intracellular activity. A promising path to resolving this controversy is to accurately measure changes in intramuscular pressure, muscle compartment cross-sectional area, muscle recruitment, and muscle damage following eccentric exercise. Advances in the field of magnetic resonance imaging (MRI) have allowed investigators to measure muscle recruitment, muscle damage, and changes in muscle compartment cross-sectional area following eccentric exercise.

MRI has quickly become the method of choice for medical imaging in many orthopedic studies of soft tissue and skeletal muscle pathology. MRI exploits the changes in the tissue's hydrogen ion chemical environment (1H-NMR) that occur during normal activity. An NMR signal is the net magnetization of all the hydrogen nuclei in a given sample when it is placed in a strong static magnetic field. MRI is based on three different and independent fundamental NMR parameters: spin density, T1 relaxation time, and T2 relaxation time (12). A detailed explanation of each parameter can be found in Chapter II. Generally, tissues and fluids with more free water have longer T2 relaxation times and appear as brighter areas on T2-weighted images (3).

In 1988, Fleckenstein, Canby, Parkey, and Peshock published the first report documenting increased signal intensity in T2-weighted spin echo images of exercised muscles immediately following exercise (32). Soon thereafter,

Fisher, Meyer, Adams, Foley, and Potchen demonstrated that the extent of the acute contrast change in T2-weighted images of exercised muscles is dependent on the average force generated by the muscles during exercise (31). This acute increase in T2 signal intensity decays at a relatively rapid rate following exercise ($t_{1/2}$ < 20 min). More recent studies have shown that the acute increase in T2 signal intensity is a good qualitative index of muscle recruitment (2,49).

In addition, MRI is also able to document the changes associated with exercise-induced muscle trauma that develop more gradually with DMS (34,62,71,77,81). The change is seen as a delayed increase in the T2 signal intensity that begins to be visible approximately 12 h post-exercise and peaks 24-48 h post-exercise, paralleling the development of muscle soreness and damage associated with DMS. The strong correlation (r = 0.99) between T2 signal and ultrastructural damage was reported by Nurenberg et al. in 1992. Their results suggest that the extent of the delayed T2 increase can be utilized as an index of muscle damage.

Although there is universal agreement that the acute T2 change is an index of muscle recruitment and delayed T2 elevation is an index of muscle trauma, the mechanism of the contrast change remains unresolved. What is known is that exercise-induced changes in MR images are related to complex intracellular events and/or alterations in intracellular water concentration or chemistry. Therefore, at present, acute and delayed T2 increases can only be utilized as qualitative measures of muscle recruitment and trauma.

Need for the Study

The increase in T2 signal intensity following eccentric exercise has been reported to follow a bimodal pattern (81). Immediately following exercise there is a drastic increase in T2 signal intensity which returns to pre-exercise levels by approximately 30-35 min post-exercise (31,49). Approximately 12 h post-exercise, T2 signal intensity begins to increase again and reaches peak values 24-48 h following eccentric exercise.

Fleckenstein et al. proposed that the acute increase in T2 following exercise is due to increased water movement into the extracellular muscle compartment and that the acute increase in T2 reaches some upper limit with increasing exercise (32). Fisher and coworkers found that the acute increase in T2 signal following exercise increased as a function of exercise intensity (31). In an attempt to examine the changes in extracellular volume, these investigators used venous occlusion to increase extracellular volume. Fisher et al. reported similar increases in muscle volume following low intensity exercise and venous occlusion at rest (31). However, exercise during venous occlusion did not cause a large increase in T2 signal. This led to the conclusion that exercise-induced changes in MR images are probably related to alterations in intracellular water concentration and/or chemistry (31,32,49). A later study using a new rapid-scanning MR method called echo-plannar imaging confirmed this result (84).

On the other hand, the second increase or delayed increase in T2 was only observed in subjects experiencing DMS following eccentric exercise. Shellock, Fukunaga, Mink, and Edgerton suggested that the delayed T2 increase

is the result of damaged muscle fibers (70). Direct evidence of this relationship was reported by Nurenberg et al., who reported a strong correlation between the T2 signal intensity and ultrastructural damage (62). This study clearly showed that the delayed T2 increase can be utilized as an index of muscle damage.

Based on the combination of these two studies, Fleckenstein et al. proposed that the underlying mechanism of delayed T2 increase is edema (free water) which occurs in DMS. The increase in free water in skeletal muscle during DMS is likely due to the following factors: (a) damaged connective tissue, (b) increased permeability of the capillaries, and (c) increased permeability of the sarcolemma. As a result, the chemical environment of the water in damaged muscle fibers will be different than in undamaged fibers. However, Shellock and colleagues published another report in which delayed T2 signal intensity remained elevated up to 80 days following exercised-induced muscle damage, well after edema had subsided (71). Therefore, the delayed T2 increase following eccentric exercise is not solely the result of edema.

This led several investigators to speculate that the acute and delayed T2 changes might result from different mechanisms. A review of MRI literature showed that no experiments have been reported which specifically tested this hypothesis. In an attempt to address the mechanism of the T2 change, Sorichter et al. in 1994, examined the effects of concentric contractions prior to and during DMS in the quadriceps muscle (77). Subjects in the eccentric group performed a single bout of seven heavy eccentric contraction and subjects in the eccentric/concentric group performed additional concentric contractions before

and 2 h after eccentric exercise, and 1, 2, 3, 6, and 9 days after the initial eccentric exercise. One leg was randomly assigned as the control limb (unloaded leg) and the contralateral leg was used to perform the exercise (loaded leg). Several inflammatory markers (e.g. circulating leukocyte and neutrophil counts) and serum creatine kinase (CK) levels were measured before eccentric exercise, 2 h after exercise, and 1, 2, 3, 6, and 9 days after exercise. MR images were acquired from the loaded and unloaded legs of both groups at 3, 6, and 9 days after exercise.

Across all time points, CK levels were significantly higher in the eccentric/concentric group than in the eccentric group, suggesting more damage in the skeletal muscle. T2 relaxation times and inflammatory markers were not significantly different between the two groups. These results suggest that the elevated T2 signal might limit any additional increase in T2 signal during activity of the damaged muscle, consistent with early findings of Fleckenstein and coworkers.

Investigators analyzed the calculated mean T2 relaxation times at different time points using the student's t-test to compare the two groups. A more appropriate method would have been to look at the change in T2 relaxation times (ΔT2), using a t-test on the difference scores which would have limited the influence of subject heterogeneity. To measure the change in T2 relaxation times from muscles experiencing DMS more accurately, the difference should have been calculated by subtracting the T2 value measured before performing the additional concentric exercise from the T2 value measured after performing

the concentric exercise. However, these investigators did not acquire pre-exercise images from the leg that performed additional concentric contractions. Both legs, loaded and unloaded, in the eccentric/concentric group, were imaged after performing the additional concentric contractions.

Therefore, it is still unclear what influence, if any, an elevated T2 will have on the acute T2 response to additional concentric contractions. Furthermore, as in most other studies relaxation times were first measured 3 days post-exercise, there is little information documenting the early time course of the development of delayed T2 increase.

Purpose of the Study

The purpose of this investigation was to examine the acute T2 response to a bout of light concentric exercise performed before (control) and after eccentric exercise had been used to induce a delayed T2 increase. The secondary or descriptive purpose of this study is to document the early time course of the delayed T2 contrast development.

Research Hypotheses

The hypothesis tested in this study was that the mechanisms underlying both acute and delayed T2 changes are the same. If this hypothesis holds, and if acute T2 has an upper limit, as previously suggested, the combined T2 change from damage plus concentric exercise (Con2) will not be different from the response due to exercise alone in normal muscle (Con1).

The hypothesis leads to the prediction that concentric exercise performed when muscle T2 is already elevated will not result in a T2 response different from

that produced by the same concentric exercise performed in a baseline or resting condition. If the mechanism of the T2 response is identical for the acute exercise contrast change and for the delayed response to eccentric exercise, it is likely that the combined effects of the two conditions would not be additive. This is illustrated by column C in Figure 1, where the T2 change after concentric exercise in muscle already elevated in T2 from prior eccentric exercise is the same as that seen in the resting condition (column A). If, however, the two mechanisms are different, it is likely that the T2 changes from the different conditions will be additive. This would produce a net change equal to the sum of column A plus column B, as illustrated by column D.

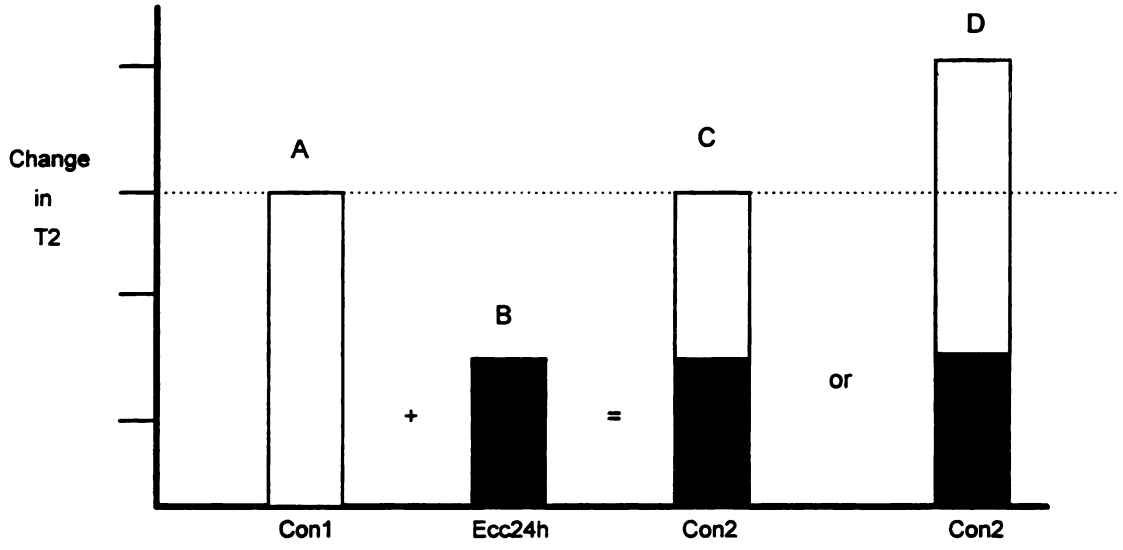

Figure 1. Hypothesized Changes in Calculated T2 following concentric exercise (Con1, A), elevated T2 at 24 h post-eccentric exercise (Ecc, B), and concentric exercise at 24 h post-eccentric exercise (Con2, C or D).

Research Plan

Eight healthy non-weight trained adults between the ages of 21 and 40 years were recruited to participate in this study. Each subject will serve as his/her own control. MR images of the biceps muscle will be acquired before (PreCon1) and after (PostCon1) an initial bout of light concentric biceps curl exercise to measure the acute T2 increase in "normal" muscle. Following a 2 h rest period, muscles will also be imaged before (PreEcc), immediately after (PostEcc), and every 2 h for the first 6 h following a single bout of eccentric biceps curl exercise to document the acute T2 increase following eccentric exercise and to document development of the delayed T2 increase. At 24 h post-exercise, subjects will again be imaged before (PreCon2) and immediately after (PostCon2) performing another bout of light concentric exercise.

Subjective muscle soreness scores will be collected using a questionnaire (20) starting at 24 h after eccentric exercise and for seven days following the eccentric exercise. Overall mean T2 value will be calculated for the biceps brachii in each subject. Cross-sectional area of the biceps brachii muscle will be calculated at 24 h post-eccentric exercise to measure muscle compartment swelling.

Significance of the Problem

The results of this study will provide insight into the unresolved question of whether a single mechanism is responsible for the increase in acute T2 and delayed T2 signal intensity. In recent years, several investigators have suggested the use of acute T2 increase as a qualitative measure of muscle

recruitment following exercise, and delayed T2 increase as qualitative measure of muscle damage following exercise. However, it may not be appropriate to employ the acute T2 increase as an index of muscle recruitment in muscles that already have an elevated T2 induced by exercise-induced damage and/or neuromuscular disease or trauma. If the research hypothesis is rejected, i.e. if the acute T2 response to concentric exercise is larger when performed after muscle T2 is already elevated due to eccentric damage, then the existence of distinct mechanism is strongly supported. In addition, documenting the early time course of the development of delayed T2 will fill gaps in the published data on T2 time course, as well as provide data on swelling during this intermediate period.

CHAPTER II

REVIEW OF RELATED LITERATURE

Delayed muscle soreness (DMS) is the sensation of discomfort and/or pain in the skeletal muscles following unaccustomed muscular exercise. The most predictable method of inducing delayed muscle soreness is to subject the muscles to repetitive, strenuous eccentric or lengthening contractions (5,6,7,20). Muscles adapt to an initial bout of exercise, becoming resistant to damage from subsequent bouts. The muscle soreness is often localized in the distal portion of the muscle at the muscle/tendon junction and is accompanied by a sense of reduced mobility (6,7,38). The sore muscles become extremely sensitive to palpation or movement. Severity varies from slight stiffness to severe pain inhibiting movement.

There is no evidence to suggest that the pathology associated with DMS is long term. Studies using animal models suggest that injured muscles are able to recuperate completely within 2 weeks (6,7). A review of the literature to date will show that there is no firm consensus on the pathophysiological mechanisms responsible for delayed muscle soreness. Although the time course of the pathological changes associated with DMS is well established, it remains to be

determined how the sequence and timing of DMS relate to a possible pathological mechanism.

Time Course of the Pathological Changes Associated with DMS

Nearly every adult has experienced delayed muscle soreness, most on numerous occasions. Unaccustomed physical activity of even moderate intensity or duration will result in the sensation of DMS, regardless of the individual's general fitness level. Since the muscle soreness or pain is only temporary, most people do not seek medical attention.

Muscle soreness usually increases in intensity in the first 24 h after unaccustomed and/or eccentric exercise and peaks at 24-72 h post-exercise. Soreness slowly dissipates and does not completely subside until 5-7 d post-exercise according to most reports (6). Recently, Clarkson, Nosaka, and Braun (1991) reported that following eccentric exercise of the forearm flexor muscles the soreness did not completely subside until 8-10 d post-exercise (20). This discrepancy might be due to the fact that the soreness data from 6-10 d post-exercise was based on a small sample (15 subjects), while the soreness from pre-exercise to 5 d post-exercise was based on 109 subjects.

Accompanying soreness, there was a dramatic decrease in muscular performance following eccentric exercise (20). The greatest reduction in isometric strength was reported to have occurred immediately post-exercise, a loss of over 50%, refer to Figure 2. In the following days, strength was gradually restored but a small deficit remained at 10 d post-exercise. On the other hand,

strength loss following concentric and isometric exercise has been shown to recover within a few hours post-exercise (20).

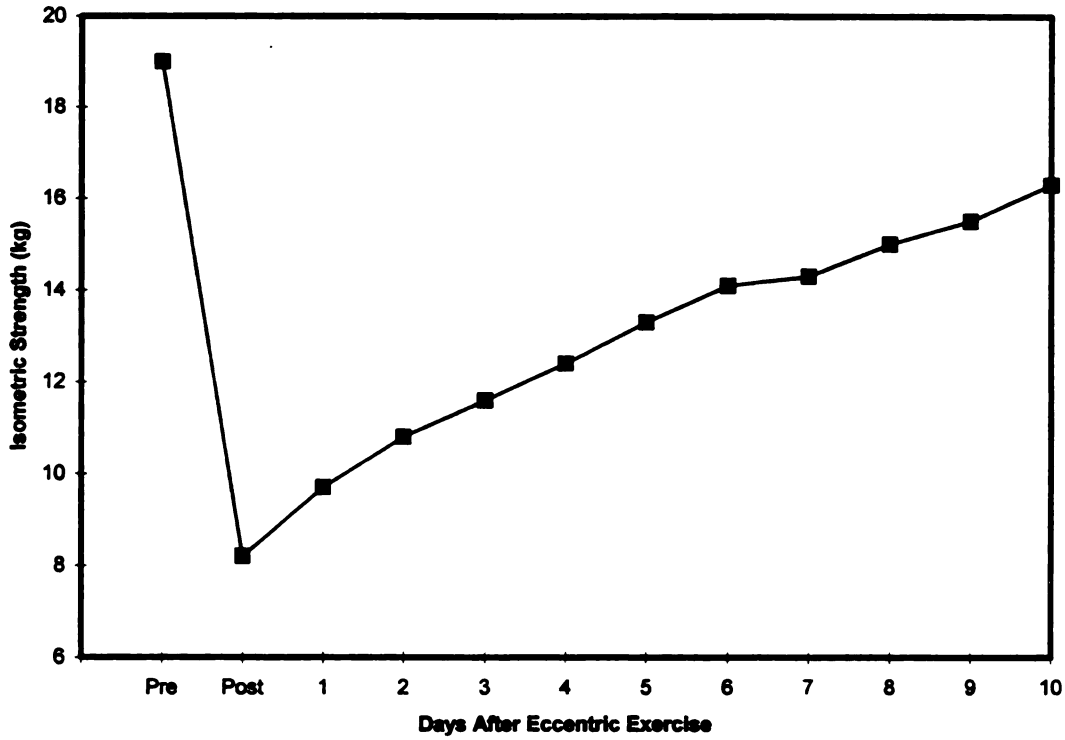

Figure 2. Isometric strength loss after performing eccentric exercise (20). Note the gradual recovery of strength over a period of days. At 10 d post-exercise, the isometric strength has not yet completely recovered.

The prolonged decrease in performance following eccentric exercise is thought to be the result of both a reluctance to use the sore muscles and a reduction in the inherent capacity of the muscle to produce force (6,20). However, the soreness is not likely the cause of the decrements in strength since soreness peaks at 24-72 h after exercise, during which strength is gradually

recovering. To evaluate the reduction in the intrinsic ability of the muscles to produce force, investigators used direct electrical stimulation, by-passing voluntary control, which also resulted in a reduction in force generation (61). Therefore, the prolonged reduction in strength following eccentric exercise is more likely the result of a lowered inherent capacity of the muscle to produce force. Another symptom of DMS is a reduction in the range of motion at the joint or joints that are involved in the high-force eccentric exercise. Clarkson et al. assessed the reduction in the range of motion about the elbow joint in two ways: a) flexed elbow angle, and b) relaxed elbow angle (20).

Muscle shortening ability has been reported to decrease following eccentric exercise (20). Clarkson et al. assessed muscle shortening ability by measuring the subject's ability to their elbow, flexed elbow angle, which is the angle at the elbow after the subject has fully flexed the elbow, the arm being fixed at the side. The flexed elbow angle increased dramatically immediately after the eccentric exercise, indicating that the subjects were unable to fully flex the elbow. The flexed elbow angle decreased gradually in the days following but remained elevated above baseline at 10d post-exercise.

Along the same lines, Clarkson et al. observed that after eccentric exercise muscles spontaneously shortened (20). They assessed spontaneous muscle shortening by measuring the relaxed elbow angle. This angle was measured at the elbow after the subjects were instructed to let their exercised arm to hang freely. The relaxed elbow angle was dramatically decreased immediately following the eccentric exercise. The relaxed elbow angle continued

to decrease over the next few days, reaching the maximal decrease at 3 d post-exercise. At 10 d post-exercise the relaxed elbow angle had returned to baseline.

Lastly, several investigators have documented swelling following eccentric exercise (6,20). Most of the studies documented swelling by measuring the changes in circumference of the limb involved in the eccentric exercise. Swelling was reported to gradually increase in the days following eccentric exercise and does not peak until 5 d post-exercise (20). Table 1, summaries the time course changes in muscle soreness, strength, muscle shortening ability, spontaneous muscle shortening and swelling associated with DMS. Based on the time course changes in muscle function after performing eccentric exercise, several investigators began to speculate that these changes in muscle function were the result of damage, induced by eccentric contractions, at the cellular level.

Table 1. Summary of the changes in muscle performance following eccentric exercise.

Exercise induced changes in muscle performance	**Time to peak following eccentric exercise**
Muscle Soreness	2-3 d
Isometric Strength Loss	Immediately following exercise
Muscle Shortening Ability	Immediately following exercise
Muscle Spontaneous Shortening	3 d
Swelling	5 d

Morphological Changes Associated with DMS

In 1902, Theodore Hough published the first report on exercise induced muscle soreness and was the first to suggest that exercise induced muscle soreness is the result of morphological changes at the cellular level. Subjects in his study reported muscle soreness 8-10 h after performing rhythmical exercise using the flexor muscles of the middle finger (45). Hough hypothesized that DMS is the result of structural damage to muscle fibers or connective tissue within the muscle during exercise.

Evidence favoring Hough's "torn tissue " theory has come from recent studies that have looked at morphological changes associated with DMS. Friden, Sjostrom, and Ekblom in 1981 found disorganization of the myofibrillar Z-bands in human soleus muscles three days after downhill running (39). They found that the ultrastructural damage resulting from eccentric exercise involves streaming, broadening, and occasional disruption of Z-lines. In some cases A-bands were out of register within affected sarcomeres, and in several cases the thick filaments were completely absent. Armstrong, Ogilvie, and Schwane in 1983 also observed disruption of the striation pattern of rat slow-twitch extensor muscles after downhill running (7).

In 1985, Ogilvie, Armstrong, Baird, and Bottoms documented three types of lesions in rat soleus muscle following eccentric exercise: (a) Z-line dissolution, (b) focal disruptions of the A-band, and (c) clotted fibers (63). In addition, they found that 90% of the morphological changes were A-band disruptions. Newham, McPhail, Mills, and Edwards conducted a study in 1983 to examine the

changes associated with eccentric exercise (60). They noted extensive sarcomeric disruptions in biopsies taken from the human quadriceps muscles following eccentric exercise. This same type of Z-line streaming has been reported in control subjects by Fischman et al. and Meltzer and colleagues (36). However, Friden and Lieber, in a recent review, suggest that Z-disk streaming is primarily a myofibrillar response to high-tension physical exercise and altered metabolic situations (36).

Evidence accumulated, thus far, clearly shows that, following eccentric exercise, there are marked structural changes in the contractile and connective tissues in both animals and humans. Using sophisticated analytical techniques, several investigators have documented the different types of lesions that occur as a result of eccentric contractions in both humans and animals. The sensation of DMS appears to be related to this muscle and/or connective tissue disruption (6,74). Although there is no proof, it is assumed that the morphological damage observed in rodent muscle and DMS in humans are manifestations of the same exercise - induced cellular events (7,74).

Metabolic Changes

Hough's "torn tissue" theory was validated by Asmussen's 1956 study on concentric (positive) versus eccentric (negative) work (8). In this study, Asmussen had volunteers perform hard negative work (a step down exercise for the quadriceps and a flexion exercise for the triceps muscle) and positive work (step up exercise for the quadriceps and an extension exercise for the triceps)

until fatigue. In positive work, the muscles shorten during contraction and in negative work the muscles lengthened during contraction.

In order to place the same amount of tension on the muscles during positive and negative work the investigators instructed the volunteers to maintain a steady speed of movement throughout the different exercise regimens. As fatigue developed during positive work, some volunteers reported ischemic pain which disappeared shortly after the cessation of work and did not reappear. On the other hand, the volunteers performing negative work did not report any sensation of pain during or immediately following the bout of exercise, but 12h to 36h post-exercise the muscles were extremely sore. Asmussen found that fatigue always developed first in the muscles that performed positive work, suggesting that the metabolic cost of positive work is much greater. Therefore, this study effectively ruled out the possibility that DMS resulted from the accumulation of metabolic by-products.

Mechanical Aspects of DMS

More recently, Bigland-Ritchie and Woods in 1976 reported that eccentric contractions recruit fewer motor units than concentric contractions at a given muscle load (10). Therefore, the force generated during the eccentric contraction is distributed over a smaller cross-sectional area of muscle. Armstrong suggested that this relatively increased tension in the active contractile and elastic elements during eccentric contractions resulted in physical damage to the structural components of the muscle fibers (6).

Force -- Velocity Relationship in Eccentric Exercise

Armstrong's hypothesis was based on the work done by Katz in 1939 on the force-velocity relation in muscular contraction (51). Katz found that when muscles shorten, the dependence of load (P_o) or initial tension on the muscle contraction speed was approximately 4%P_o / % muscular velocity (V_{max}). The relationship between force and velocity in lengthening muscles is much more drastic. A muscle lengthening at 1%V_{max} could increase the tension placed on the fibers by 50%. The classic force-velocity relationship curve (Figure 3) demonstrates this 10-fold faster rise in tension for eccentric contractions than for an equivalent velocity of concentric contractions.

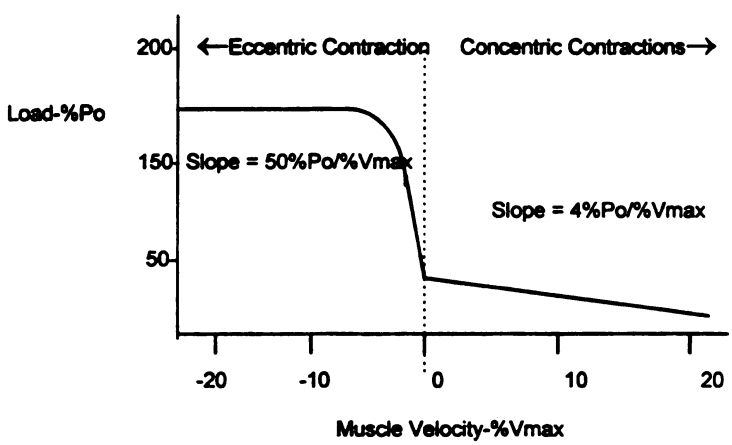

Figure 3. The classic force-velocity relationship curve (51). Note that for lengthening velocities (velocities less than zero) force increases much more rapidly than for shortening velocities (velocities greater than zero). Also note the discontinuity in the force velocity relationship around zero velocity.

As previously noted, the high tensions associated with eccentric contractions have been speculated to cause ultrastructural damage. Hough found that the degree of perceived pain was directly related to peak forces generated and to the rate of force development during the rhythmic contractions used to induce the delayed soreness. According to Friden and Lieber, discontinuity in the force-velocity relationship curve around zero velocity might cause mismatches between adjacent sarcomeres in terms of the forces that are placed on them, refer to Figure 3 (51).

Huxley and Peachey in 1961 showed that sarcomere length along the axis of the muscle might vary by 1% (47). In 1983 Lieber and Baskin used isolated frog muscle fibers to investigate sarcomere length as a function of time during "fixed-end" contraction; in which isolated semitendinosus muscle fibers were held at a constant length (54). They found that the sarcomeres near the ends of the muscle fiber are shorter in length and shorten at a higher velocity. Approaching the center of the muscle fiber, the starting sarcomere lengths become longer and the shortening velocity decreased. At the center of the muscle fiber, the sarcomere was fairly long and actually elongated (rather than shortening) at a slow rate. The force-velocity relationship near zero velocity was such that a small difference in muscle velocity during shortening caused minimal difference in the force sustained by the adjacent sarcomeres (36). However, a muscle lengthening at 1% V_{max} increased the tension by 50% P_o on adjacent sarcomeres.

Tension Imbalance Theories

If high tension is the principle cause of myofibrillar disruption, one would expect to observe similar muscle damage in passive stretching. Lieber, Woodburn, and Friden in 1991 found that cyclic passive stretching caused only a 10% increase in maximum tetanic tension (55). This prompted Friden and Lieber to conduct an experiment in which they varied the strain ($[L-L_o]/L_o$) placed on rabbit tibialis anterior muscle (36). One group was strained at 12.5% of fiber length and the second group was strained at 25% of fiber length. The 12.5% strain produced a 45% decrease in muscle load (P_o), while 25% strain produced a 65% decrease in muscle load. Although an earlier study by Lieber et al. had dismissed strain as a possible cause of muscle damage, this differential response to strain suggests that tension imbalance rather than absolute tension might be responsible for the eccentric exercise induced muscle damage.

Reviewing these results, Friden and Lieber proposed that fiber stress (load/cross-sectional area) rather than absolute tension might be determining the extent of muscle damage. Lieber and Friden conducted an experiment to test this above hypothesis by imposing cyclic length changes on actively contracting rabbit tibialis anterior muscle (TA) using a standard stretch magnitude of 20% of TA fiber length and a constant strain rate of 60%/second. Two protocols were used: (1) an early stretch (ES) group's TA was stretched immediately following muscle activation and (2) in the late stretch (LS) group, muscle stretch was delayed by 200 ms. The delay in the LS group allowed the muscle to develop tension and reach a peak force twofold higher than in the ES group. Despite the

fact that the LS group experienced over twice the total stress, maximum tetanic tension for the two groups was almost identical. Although it is well accepted that the muscle stress is high during eccentric contraction, these results indicate that muscle damage induced by eccentric exercise might not be directly related to the stress placed on the muscle.

Friden and Lieber propose that sarcomeres have a physical threshold for damage that is more likely to be reached during an eccentric contraction. The data presented thus far indicates that neither strain nor stress alone produces ultrastructural damage associated with eccentric exercise. Friden and Lieber suggested that active straining combined with increased tension per unit area increases the likelihood of ultrastructural damage. The physical sensation of DMS appears to be related to this ultrastructural muscle and/or connective tissue disruption.

Biochemical Aspects of DMS

Complementing these mechanical studies, evidence for ultrastructural changes associated with DMS has come from biochemical studies that have shown an increase in intramuscular enzymes and myoglobin in the blood following eccentric exercise (27). Of these, the most frequently studied muscle enzyme is creatine kinase (CK). There are several isoforms of CK in the body. This discussion will focus on the skeletal muscle isoform. It has been well documented that increased skeletal muscle CK activity in the blood indicates skeletal muscle injury, and increased cardiac muscle CK activity in the blood is

often observed after a myocardial infarction. The CK response varies considerably with different forms of exercise; downhill running, marathon running, eccentric and isometric exercise have been the modes most frequently studied (20).

The increase in CK activity in the blood is significantly lower after downhill running at a -10% grade for 30 minutes at a speed that elicited a heart rate of 170 beats per minute (approximately 300 units/liter) than with eccentric exercise of the forearm flexors (about 2,500 U/L) (14,20). These results are particularly interesting when one compares the amount of muscle mass involved in the two different exercise regimens. Although the forearm flexors are very small compared to the muscles involved in downhill running, the CK increase seen is much greater than for intense leg exercise. In the downhill running condition, the peak CK response occurred around 24 hours post-exercise. However, after eccentric forearm exercise there was no notable increase in the CK levels in the blood for two days after exercise, and peak CK values occurred around four days post-exercise.

On the other hand, changes in blood CK levels following forearm flexion isometric exercise were similar to the changes found following downhill running (18,19), but CK activity in the blood after marathon running was considerably higher. In addition to reaching higher levels, the CK level increased more rapidly in marathon runners than after downhill running or eccentric exercise (4). Blood levels of other muscle proteins, including lactate dehydrogenase,

asparate aminotransferase, myoglobin, and alanine aminotransferase, also follow the same time course as CK.

Muscle Damage Inferred from Serum Creatine Kinase Activity

It has been suggested that the extent of CK response represents the magnitude of the injury induced by different forms of exercise. This would explain the larger increases in CK levels in the blood following marathon running and eccentric exercise. However, there is no explanation as to why there was no relationship between CK response time course and other indicators of muscle damage (e.g., muscle soreness level, isometric strength, and relaxed arm angle).

Part of this problem was addressed in 1992 by Clarkson, Nosaka, and Braun (20). They suggested that the studies looking at CK response after exercise did not take into account the large inter--subject variability in CK response and used too small a sample. To account for this, they conducted a study in which they separated their subjects into three groups based upon the amount of increase in CK following eccentric exercise of the foreman flexors. If subjects had a peak CK response of over 2,000 U/L, they were classified as high responders; peak CK response between 500 and 2,000 U/L was grouped as medium responders; and peak CK response less than 500 U/L was the low responders.

Significant differences were found between the low CK group and high CK group on muscle soreness, isometric strength, and relaxed arm angle. The medium CK group fell in between the low and high CK group on all indirect

indicators of muscle damage. Statistically there was no difference on muscle soreness, isometric strength, or relaxed arm angle between the high and medium CK groups.

The authors believe that the low CK group might have been preadapted resulting in less muscle damage. As to why there was no difference between the medium and high CK groups, the authors quote Evans and Cannon (30): "the post-exercise rise in circulating CK activity is a manifestation of skeletal muscle damage but not a direct indicator of it."

Another factor to consider when evaluating the CK response after exercise is that the CK activity in the blood is a steady state; dependent on the amount of CK released by the muscle and the rate of CK clearance by the reticuloendothelial system. Therefore, high levels of CK in the blood does not necessarily indicate actual muscle damage.

Role of Calcium in DMS

Although the time course of DMS is well established, there is still controversy over the mechanism. Various hypotheses have been put forward to account for the unusual delay between the exercise, first soreness, and peak soreness, no single explanation shows more promise than disturbance in calcium (Ca^{2+}) homeostasis. In muscles Ca^{2+} plays a vital role in the regulation of the contractile cycle and more importantly maintains the integrity of the sarcolemma.

Muscle contraction is initiated by an increase in intracellular free calcium ion concentration. Ca^{2+} is rapidly bound to troponin C and other cytosolic calcium binding proteins and is removed by the ATPase pump of the sarcoplasmic reticulum during relaxation. A low intracellular free Ca^{2+} concentration (0.1 to 0.2 μM) is essential for normal cell function, but an excessively high concentration of free Ca^{2+} has been associated with cell dysfunction and cell necrosis (i.e., ischemia in the myocardium and specific types of degenerative muscular dystrophies) (5).

The high mechanical forces generated during eccentric exercise have been documented to cause structural damage to the sarcolemma and/or alterations in permeability of the cell membrane, which results in a net influx of Ca^{2+} from the interstitium into the muscle cell. In an attempt to buffer the high cytosolic concentration of Ca^{2+}, mitochondria take up large amounts of the excess Ca^{2+} ions. However, while reducing the potentially damaging effects of high cytosolic Ca^{2+}, the uptake of Ca^{2+} impairs mitochondrial respiration and ATP production. The attenuated levels of ATP could subsequently reduce Ca^{2+} extrusion from the cytoplasm via ATP-dependent Ca^{2+} pumps in the sarcolemma, mitochondria, and sarcoplasmic reticulum, as well as limiting force due to reduced ATP supply for the actin-myosin ATPase (5). However, this is unlikely to occur in exercise employing eccentric contractions since the energy (ATP) cost is relatively low.

The mechanism(s) by which Ca^{2+} is elevated in the muscle cells following

eccentric exercise is not known. Several possible mechanisms have been suggested. First, the source of the Ca^{2+} could either be intracellular or extracellular. The majority of the intramuscular Ca^{2+} is found in the sarcoplasmic reticulum. It is entirely possible that the reuptake of the Ca^{2+} by the sarcoplasmic reticulum was some how compromised following eccentric exercise, resulting in the accumulation of Ca^{2+} in the cytosol (27).

However, if the source of Ca^{2+} is extracellular, two mechanisms of entry have been proposed. First, the extracellular Ca^{2+} could gain entry into the cytosol through the stretch-activated Ca^{2+} channels in the sarcolemma (27). Ducan et al. , based on unpublished data, proposed that the increased active strain during eccentric exercise stimulates the stretch-activated Ca^{2+} channels, and Ca^{2+} moves through the sarcolemma down its concentration gradient. To date, stretch-activated Ca^{2+} channels have not been described for human skeletal muscles.

A second mechanism by which extracellular Ca^{2+} could gain entry into the cell was proposed by Armstrong, Ogilive, and Schwane. Armstrong et al. observed a marked elevation in creatine kinase and lactate dehydrogenase in the plasma of sedentary rats after walking downhill (eccentric exercise) (7). They interpreted these results as evidence that eccentric exercise caused disruption of the sarcolemma, allowing intracellular proteins to exit down their concentration gradient. Based on these results, Armstrong et al. proposed that the ruptures in the sarcolemma could allow the extracellular Ca^{2+} to enter the cytosol down its concentration gradient. In a more recent study, Ducan, Delp, Hayes, Delp, and

Armstrong noted that there was an accumulation of Ca^{2+} in injured rat muscles immediately following downhill walking (27).

However, the relationship between the initial influx of Ca^{2+} and the degradation of muscle fibers during the post-exercise period remains unclear. In a 1984 review of DMS, Armstrong proposed the following as a possible sequence of events in the production of DMS following the initial influx of Ca^{2+}. First, the elevated intracellular Ca^{2+} concentration increases the activation of phospholipase A2, resulting in the production of lysophospholipids, leukotrienes, and prostaglandins; all of which could lead to degradation of cellular structures (6). Also, high Ca^{2+} concentration activates proteases and stimulates production of free radicals, resulting in the preoxidation of membrane lipids. As a result, the sarcolemma becomes leaky (27). In addition, the calcium-dependent proteolytic enzymes that preferentially degrade Z-discs, troponin, and tropomyosin are activated by the abnormally high cytosolic Ca^{2+} concentration (6).

Second, the progressive deterioration of the sarcolemma in the post-exercise period is accompanied by a diffusion of intracellular components into the interstitium and plasma. Many of these substances, including prostaglandins, attract neutrophils and monocytes that convert to macrophages. In addition, these substances also activate mast cells and histocytes. As a result of the active phagocytosis and cellular necrosis; histamine, kinnis, and potassium could accumulate in the regions of group IV free nerve endings. This would then activate the nocieptors and cause the sensation of DMS (6).

The question that merits further investigation is whether the elevated Ca^{2+} in the muscle fibers is simply a symptom of DMS or whether Ca^{2+} is mechanistically involved in the etiology of the DMS. As a result, two separate experimental models have been developed to study the role of Ca^{2+} in DMS, non-exercised induced muscle injury and exercise induced muscle injury.

Chemically Induced Muscle Damage

Non-exercise experimental muscle injury studies have contributed to our understanding the function of Ca^{2+}. Jackson, Jones, and Edwards in 1984 conducted a series of in vitro experiments on mouse muscle using dinitrophenol (DNP)- an uncoupler of oxidation and phosphorylation in the mitochondria. In the first series of experiments, they stimulated mouse muscle for 30 min with both DNP and Ca^{2+} in the incubation medium (48). In order to significantly impair mitochondrial respiration and ATP production, both DNP and Ca^{2+} were included in the incubation medium. This resulted in the loss of lactate dehydrogenase (LDH) into the medium during the period of stimulation and with peak loss occurring within 30 min of the contractions. The loss of LDH into the medium is an indirect measure of sarcolemmal damage.

However, when the incubation medium lacked Ca^{2+}, LDH release was significantly reduced both during the exercise and in the post-exercise period. The role of DNP in the above scenario is not known, but has been speculated to cause the release of mitochondrial Ca^{2+}. This is plausible, as it has also been

suggested that DNP's mode of action is to make mitochondrial membranes "leaky" to proton flux and possibly other ions.

In a second series of experiments, Jackson et al. examined the influence of phospholipase A2 inhibitors on the release of LDH with DNP and Ca^{2+} in the incubation medium. Phospholipase A2 has several deleterious effects on the cell, in that it produces detergent fatty acids and lysophospholipids which are involved in cell-membrane degradation. Phospholipase A2 inhibitors significantly reduced the loss of LDH, although various other protease inhibitors were not able to produce the same result (48). These results suggest that sarcolemmal damage following contractions in the presence of DNP involves a Ca^{2+} mediated pathway. More importantly, they intimate that the underlying mechanism of injury to membrane involves a Ca^{2+} activated phospholipase A2.

In 1987, Chang, Musser, and McGregor determined that phospholipase A2 has a Ca^{2+} binding site and that the activity level increases as a function of the Ca^{2+} concentration (17). In the same year Duncan and Jackson established that inhibiting phospholipase A2 provides a protective effect to the cell membrane but does not protect against myofibrillar damage (28). They noted that the chemically induced muscle injury is much more rapid (5-30 min) than muscle injury associated with DMS, and involves two distinctive Ca^{2+} mediated pathways: (1) Ca^{2+} activated phospholipase A2 mediated injury to the sarcolemma , and (2) ultrastructural damage to the myofilaments by an unknown mechanism.

The Role of Calcium in Exercise Induced Muscle Damage

Although studies of chemically induced muscle damage were not able to explain the role of Ca^{2+} in DMS, these studies have set the stage for others to look more critically at the exercise-induced sarcoplasmic reticulum dysfunction and mitochondrial Ca^{2+} concentration ($[Ca^{2+}]_{MITO}$) as possible factors involved in DMS etiology. $[Ca^{2+}]_{MITO}$ is relatively low compared to the calcium levels in the sarcoplasmic reticulum in the resting muscle cell (5). As early as 1952, it was established that $[Ca^{2+}]_{MITO}$ is an indirect indicator of cytosolic $[Ca^{2+}]$ in the muscle cell (78). Sembrowich, Quintinskie, and Li reported in 1982 that Ca^{2+} uptake kinetics of the mitochondria in slow twitch muscles are adequate to play a vital role in regulation of Ca^{2+} concentration in the muscle fibers (69).

In fast twitch muscles, the cystolic Ca^{2+} concentration increased considerably after maximal contraction, while the $[Ca^{2+}]_{MITO}$ remained fairly low (76). In contrast, Tate, Bonner, and Leslie in 1978 exercised trained rats to exhaustion and found that $[Ca^{2+}]_{MITO}$ increased by 132% (83). The authors suggested that the abnormally high cystolic Ca^{2+} levels resulting from exhaustive exercise were buffered by the mitochondria.

These findings lead Duan et al. in 1990 to conduct a series of experiments in order to evaluate the relationship between the $[Ca^{2+}]_{MITO}$ and muscle injury in rat soleus and vastus intermedius from eccentric exercise (27). Duan et al. hypothesized there would be a direct relationship between $[Ca^{2+}]_{MITO}$ and muscle injury. Several studies have reported that Ca^{2+} antagonists attenuate the incidence of damage during the post-exercise period, suggesting

that Ca^{2+} might have a direct causal role in DMS etiology.

Duan and co-workers addressed this issue by using three different Ca^{2+} antagonists -- verapamil, EDTA (ethylenediaminetetraacetic acid), and EGTA (ethylene glycol-bis-*N*,*N*,*N'*,*N'*-tetracetate) in their study. Verapamil blocks slow Ca^{2+} channels, whereas EDTA and EGTA chelate Ca^{2+} and are excreted, therefore lowering total body Ca^{2+} (27). Muscle injury was evaluated by determining the number of undamaged fibers per unit area of muscle cross section.

Results showed that $[Ca^{2+}]_{MITO}$ was inversely related to the number of intact fibers per square millimeter of muscle cross-section. They reported that verapamil reduced the increase in $[Ca^{2+}]_{MITO}$ but did not reduce the occurrence of injury. Verapamil results must be interpreted with care, because relatively high concentrations can have harmful direct effects on the muscle fibers and the dose-response relationships have not been completely established for this drug. The investigators suggested that these side-effects of verapamil may have contributed to the muscle injury, and thus might explain why verapamil reduced the $[Ca^{2+}]_{MITO}$ but did not lower the incidence of muscle injury (27).

The Ca^{2+} chelators, EDTA and EGTA, significantly reduced the elevation of $[Ca^{2+}]_{MITO}$ and reduced the occurrence of injury but did not affect serum Ca^{2+} concentration. The mechanism by which chelators blocked Ca^{2+} from entering the mitochondria is not completely clear. At two days after the first introduction of chelator, serum Ca^{2+} concentration did not change. Therefore, the extracellular-to- intracellular Ca^{2+} concentration gradient did not limit the influx of

Ca^{2+} into the cell, if it is assumed that serum Ca^{2+} concentration is equivalent to interstitial Ca^{2+} concentration (27). These authors proposed that the chelators may have accumulated within the affected muscle cells and restrictively bound Ca^{2+} in some compartment within the muscle cell, thereby preventing it from entering the mitochondria.

As an alternative explanation for the effects of Ca^{2+} chelators, it was proposed that chelation might influence the recruitment of motor units in the muscles. This is based on the fact that alpha-motor neuron's ability to release neurotransmitters is dependent on Ca^{2+} influx from the interstitium. If the release of acteylcholine is limited from the initially recruited pool of motoneurons, then additional motor units are recruited to produce the required force. In effect, this will significantly reduce the active strain generated within the motor unit and decrease the potential for mechanical damage to the constituent fibers. Since the authors reported no change in the interstitial Ca^{2+} concentration following EDTA treatment, it is unlikely that the recruitment pattern in muscles changed as a result of chelator treatment (27).

A very recent study focused on the natural ameliorative process that attenuates the development of muscle injury and soreness (56). This work demonstrated that following eccentric exercise mouse soleus muscles were able to buffer the increased influx of extracellular Ca^{2+} and avoided the activation of Ca^{2+} sensitive degradative pathways (56).

Lowe et al. conducted a series of experiments to examine the relationship between extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) and the extent of muscle

injury. The investigators hypothesized that by increasing $[Ca^{2+}]_o$, they could increase the electrochemical gradient for Ca^{2+} across the cell membrane and cause a greater influx of Ca^{2+} through damaged sarcolemma. In a normal resting muscle, cell the Ca^{2+} concentration lies in the range of 0.1 to 0.2 μM and in the extracellular fluids the concentration is about 1 m*M* (79). Lowel et al. found that the extent of muscle injury following eccentric contractions was not affected by $[Ca^{2+}]_o$ in the range of 0.05-5.0 m*M* (56). The investigators chose this range so as to avoid adverse effects on contractility/force production when $[Ca^{2+}]_o$ is either too low or too high.

Lowel et al. observed an initial decrement in force production following their eccentric exercise protocol. The authors suggested that this decrease in force production was due to the damaged force-producing and transmitting structures and to a loss of excitation-contraction coupling. Interestingly, after eccentric exercise, the total muscle Ca^{2+} concentration increased, although no increase was seen in the protein degradation process. The muscles that performed eccentric exercise showed neither a decrease in the contractile properties nor was there an accumulation of LDH, leukotriene B4, prostaglandin E2, or tyrosine in the incubation medium. Leukotriene B4, LDH and prostaglandin E2 were measured because they are the end products of the proposed Ca^{2+}-mediated degradative processes (56).

More importantly, the muscles incubated in the higher $[Ca^{2+}]$ solution did not show a higher incidence of muscle injury than the muscles in the normal $[Ca^{2+}]$ solution. The muscles that performed eccentric contractions had a higher

total muscle [Ca^{2+}] than either muscles that performed isometric contractions or control (unexercised muscles). This is consistent with other studies that have compared total muscle [Ca^{2+}] of normal muscles and damaged muscles (56).

It has been assumed that the critical variable that activates the proposed Ca^{2+} mediated degradative pathways is intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Lowel et al. have clearly demonstrated that the $[Ca^{2+}]_i$ in the injured muscle did not increase into the pathological range of 10 - 50 μM $[Ca^{2+}]_i$ (15). The resting contraction threshold for skeletal muscle was around 1 μM $[Ca^{2+}]_i$. If the $[Ca^{2+}]_i$ surpasses this value, the resting tension would automatically increase.

Since the resting tension did not change during the incubation period, it's unlikely that the injured muscle's $[Ca^{2+}]_i$ approached the pathological range. The authors argued that the injured muscles were able to buffer the increased total muscle [Ca^{2+}], since the $[Ca^{2+}]_i$ showed only a minimal increase (56). It has been suggested by others that the mitochondria, sarcoplasmic reticulum, and endoplasmic reticulum are primarily responsible for buffering the Ca^{2+} ions (10). Unfortunately Lowel et al. did not measure any of the above organelle's Ca^{2+} content.

The results of this study need to be interpreted with care since the investigators incubated the muscle for only 120 minutes after the contraction protocol prior to collecting the data. This time period may not be long enough for the influx of Ca^{2+} to overcome the buffering capacity of the mitochondria and activate the endogenous proteolytic pathways. Previous studies have clearly demonstrated that the exercise induced muscle injury is more apparent two days

after exercise than immediately post-exercise (7). Armstrong et al. have shown that local disruptions of the banding pattern in the injured fibers are visible immediately post-exercise. However, two days after exercise, the regions of degeneration are more apparent because they are marked by the accumulation of macrophages.

Lowel et al. acknowledged the limitations of a short incubation period but provided no explanation as to why they chose that particular incubation time period, nor did they propose which of the injured muscle cell organelle(s) was responsible for buffering the Ca^{2+} ions. Furthermore, there was no histochemical analysis performed on the muscle samples following the incubation period to document damage.

A Comparative Analysis of the "Torn Tissue" Theory and Calcium Induced Cell Damage Theory

Thus far, two hypotheses have been presented: (1) the "torn tissue" theory, and (2) calcium induced cell damage theory. Neither of the two fully explains the relation between tissue injury initiated during the exercise bout, and the soreness first experienced 8 h and reaching a peak at 24 h post-exercise. At present, most investigators agree that the muscle damage is initiated as the result of high mechanical forces produced during muscular exercise, particularly in eccentric exercise.

Experimental research conducted over the past 90 years, since Hough's "torn tissue" theory was first published, generally have supported his contentions.

However, the studies focusing on the ultrastructural damage following eccentric exercise are not able to elucidate why the tissue damage is not immediately followed by the perception of pain, like other tissue lesions. Although with limited success, the calcium induced cell damage theory predicts that the delay in the sensation of pain is partly due to the slow influx of Ca^{2+} into the muscle cell. This critical influx of Ca^{2+} into the cell is the result of the ultrastructural damage to the sarcolemma and the contractile tissues following eccentric exercise. A review of the Ca^{2+} literature suggests that increase in the intracellular calcium concentration within the physiologically relevant range does not elicit the indigenous calcium mediated destructive process. Yet to be resolved is the mechanism and organelle(s) that are primarily responsible for buffering the increased total muscle Ca^{2+} concentration following eccentric exercise.

In an attempt to better explain the unusual delay, several investigators have proposed that the underlying mechanism of DMS is acute inflammation, based on the similar time courses of the two conditions. The following sections describe the mechanism of the inflammatory response and the evidence relating this process to DMS.

Acute Inflammation as the Underlying Mechanism of DMS

Acute inflammation is a generalized response of the body to tissue injury which confines the injury and promotes the healing process. The entire process will generally last for approximately a week (74). The superficial characteristics of inflammation include redness, swelling, heat, pain, and loss of function. The

inflammatory response in the classic sense is composed of two separate but related processes: (1) the vascular response and (2) the cellular response.

First, the cellular component of inflammation involves predominately two types of leukocytes: neutrophils and monocytes (74). Within a few hours of injury, the number of circulating neutrophils increases dramatically. Neutrophil concentration at the site of injury peaks between 1 and 4h post - injury, depending on the intensity of the damage, and then declines rapidly. Neutrophils are the first phagocytic leukocytes to arrive at the site of injury and they participate in the digestion of necrotic cells and cellular debris. Several hours following the neutrophil migration, the monocytes begin to accumulate at the site of injury. Once the monocytes leave the blood and enter the injured tissue compartment, they mature into macrophages. Unlike the neutrophil concentration, the monocyte concentration at the site of injury increases continually for 48 h. The monocytes, like the neutrophils, also digest dead cells and cellular debris.

Second, the vascular component of inflammation involves the arterioles near the site of injury constricting for approximately 5 - 10 minutes. Vasoconstriction is followed several hours later by vasodilatation, which increases blood flow to the injured tissue and increases the vascular permeability in the surrounding blood vessels. This increased vascular permeability continues throughout acute inflammation resulting in a continuous outward leakage of blood cells and protein - rich plasma or exudate into injured tissues. The end result of the increased vascular permeability is swelling and the

sensation of pain in the affected area. The following sections will evaluate the evidence that suggests that similar changes also occur in association with DMS.

Swelling

Swelling is one cardinal symptom that is always present to some extent in acute inflammation (74). The swelling or edema is a result of increased permeability of the small blood vessels which allow the exudate to escape into the damaged tissues. Evidence linking inflammation to DMS includes reports of increased permeability in rat skeletal muscle following eccentric exercise (80). Other studies reported increases in limb volume at 24 , 48, and 72 h after eccentric exercise (45,79). More importantly, these investigators observed that the increase in limb volume was specific to eccentric muscle action. The limb volume did not change following isometric or concentric contractions.

This lead some investigators to speculate that swelling might play a causal role in the sensation of DMS by increasing the intramuscular pressure. Newham and Jones in 1985 reported no significant increases in intramuscular pressure in the human biceps muscle following eccentric exercise (59). However, a study conducted by Friden, Sfakianos, and Hargens in 1986 found significant increases in intramuscular pressure (37).

It should be noted that Newham and Jones monitored intramuscular pressures in less restricted compartments and during rest. Smith suggested that the largest increases in intramuscular pressure will only occur during a contraction (74). In addition, it has been shown repeatedly by several

investigators that the sensation of pain in DMS is only experienced during contraction or palpation and not during rest. Based on this, Smith proposed that increases in intramuscular pressure during a contraction or palpation will provide a mechanical stimulus for the PGE_2 - sensitive receptors (pain receptors) (74). This in turn results in the sensation of DMS.

The development of swelling / edema in the damaged tissue fits well with the time course for the development of DMS, both showing marked increases at 24, 48 and 72 h after exercise. In contrast, a study in 1972 by Talag found the greatest increases in limb volume occurred at 72 h after exercise, while peak soreness occurred at 48 h (82). Smith makes use of the acute inflammation model proposed by Ryan and Majno to explain the results reported by Talag (82).

Ryan and Majno suggesed that during the early stages of inflammation swelling is brought about by the accumulation of fluid in the tissue (67). During later stages of inflammation, the increase in limb volume is primarily due to the production of new connective tissue. Based on this model Smith suggested that the increases in limb volume at 24 and 48 h after exercise is the result of fluid accumulation, and the increases in limb volume at 72 h post - exercise (reported by Talag) was primarily due to the synthesis of new connective tissue (82). In addition, several investigators, including Smith, have suggested that the adaptation observed in response to a single bout of eccentric exercise and subsequent DMS is due to changes in the properties of connective tissue (74).

In summary, swelling is present in both acute inflammation and DMS.

The time course data on the development of swelling and sensation of pain in DMS correlated very well, lending support to the notion that the underlying mechanism of DMS might be acute inflammation. Unfortunately, the studies that have examined the causal role of swelling in initiating the sensation of DMS by increasing the intramuscular pressure are not conclusive.

Pain

Another cardinal symptom of DMS as well as acute inflammation and tissue injury is pain (74). The sensation of pain involves the activation of pain afferents, most likely Type III and IV nerve fibers. In addition, certain chemical compounds are associated with the sensation of pain. Histamine, acetylcholine, bradykinin, potassium, serotonin and prostaglandins of the E series (PGE) have been proposed as possible candidates.

The non - exercise literature suggests that the most likely candidates are the prostaglandins of the E series. PGE does not directly stimulate the nociceptors but rather it produces a state of hyperalgesia (increased sensitivity) in the nociceptors. Therefore, a previously benign chemical, mechanical, or thermal stimuli might be able to activate the afferent pain fibers. In particular, the exercise induced muscle damage models have documented elevated levels of PGE_2 following a bout of eccentric exercise.

More importantly , Smith and colleagues have documented a similar time course for the increase in PGE_2 and DMS 24 h after a bout of eccentric exercise. Suggesting that the elevated levels of PGE_2 are responsible for the sensation of

DMS (74). The mechanism responsible for increasing the biosynthesis of PGE_2 following eccentric exercise remains unresolved.

Interleukin - I , Ca^{2+}, and macrophages have been shown to stimulate local PGE_2 synthesis and have also been shown to increase after eccentric exercise. However, a study conducted by Cannon and colleagues using human subjects in 1989 reported that after unaccustomed eccentric exercise, the increases in interleukin - I levels do not correspond with the time frame for PGE_2 increases (16). As mentioned earlier, mitochondrial Ca^{2+} concentration (Ca^{+2}_{MITO}) was used as an indicator of muscle Ca^{2+} levels. [Ca^{+2}_{MITO}] has been shown to reach peak levels 48 h after eccentric exercise in rodents (27), a time when PGE_2 and muscle soreness are also significantly elevated in humans (74).

On the other hand, elevation in [Ca^{+2}_{MITO}] was also seen immediately after downhill running (27). Since PGE_2 levels were not measured, it is not known whether PGE_2 levels were elevated immediately following downhill running, but clearly the onset of DMS has not yet occurred. Therefore, it appears that, during DMS, elevated levels of Ca^{2+} may not play a critical role in stimulating local PGE_2 synthesis.

Cellular Infiltrates in DMS as well as Acute Inflammation

Of the three proposed stimulators of local PGE_2 synthesis, macrophages show the most promise. Under inflammatory conditions macrophages begin synthesizing and releasing large quantities of PGE_2 within 30 min and continue for at least 24h (22). It has been proposed by several investigators including

Smith , Tullson, and Armstrong that the presence of the macrophages at the site of injury, during acute inflammation and after eccentric exercise in rodents, is most likely responsible for the biosynthesis of PGE_2 (74). In both conditions, the macrophages are present in large numbers after 24 h and 48 h of tissue damage. Conveniently, this is also the period when peak levels of soreness are experienced. However, a study conducted in 1983 by Armstrong, Ogilvie, and Schwane rarely found any neutrophils at the site of injury (7). The inflammation literature suggests that the most important factor in acute inflammation in the classic sense is the accumulation of neutrophils at the site of injury (74). Accordingly, Armstrong et al. concluded that the absence of neutrophils indicated that the pathology of DMS did not involve inflammation in the classic sense.

Kuipers, Drukker, Frederik, Geurten, and Kranenburg in 1983 reported margination and infiltration of neutrophils in the soleus muscle of the rat between 0 and 2 h after eccentric exercise (52). This time course for the neutrophil infiltration fits well with the acute inflammation scenario. In this study, the eccentric exercise involved running at a 10^0 upward incline, which maintains the soleus muscle in a lengthened position during the run. In addition, the muscle damage observed in the soleus during incline running is similar to the damage seen in downhill running.

In contrast, a study conducted in 1983 by Schwane, Johnson, Vandenakker and Armstrong performed total and differential white blood cell counts on blood samples from seven male volunteers taken before the exercise and at 5 min and 24, 48, and 72 h following downhill running. The volunteers ran

on a -10% grade at 57% of their VO_2 max for 45 minutes. Schwane et al. reported no significant increase in peripheral white blood cell count, a marker of acute inflammation, following downhill running (68).

More recently Smith, McCammon, Smith, Chamness, and O'Brien reported significant increases in the peripheral neutrophil count at 1 and 2 h after 40 minutes of downhill jogging (75). Smith et al. suggest that the elevated levels of peripheral white blood cells are the result of the increased levels of circulating polymorphs (a type of neutrophil), which is also seen 1 to 2 h after exercise. The acute inflammation literature has also reported elevated levels of polymorphs during the same time period. The authors interpreted these results as evidence to support the theory that acute inflammation is the underlying cause of DMS.

However, a key study conducted by Friden, Sjostrom, and Ekblow in 1983 did not support the hypothesis that acute inflammation is the underlying cause of DMS (40). Friden et al. obtained muscle biopsies from the vastus lateralis muscles of human subjects following eccentric exercise. Although the muscle biopsies exhibited the classic ultrastructural damage that is associated with DMS, no invasive macrophages were present. Friden et al. concluded that the absence of macrophages suggests that acute inflammation is not the underlying mechanism for the sensation of DMS.

In contrast, two fairly recent studies have reported the presence of invasive macrophages in the muscle of human subjects following eccentric exercise (50,66). However, the time frame for the appearance of the

macrophages was much later than what is usually seen during a classic inflammatory response and was also much later than what is reported in the exercise induced muscle damage literature using rodents. The discrepancy might be explained by the fact that muscle biopsies require removal of tissue from the belly of the muscle; whereas it is possible that the damage is initiated in the myotendinous joint, and only later spreads to the belly of the muscle (7). Therefore it is more than likely that the human muscle biopsy samples missed the sites of injury especially during the early stages of DMS.

The Effect of Anti-inflammatory Drugs on DMS

In a continued effort to determine the role of acute inflammation in the etiology of DMS, several investigators have used anti-inflammatory drugs to study the mechanism of DMS. The following section provides a critical review of five studies that have employed non-steroidal anti-inflammatory agents to evaluate their role on muscle soreness, muscle weakness, and muscle damage inferred from serum enzyme activity changes that accompany DMS. Although four out of the five studies employed different exercise protocols and used different anti-inflammatory agents, their main hypothesis remained the same: If the mechanism of DMS is acute inflammation, then administering anti-inflammatory drugs will reduce DMS.

Effects of Aspirin on DMS after Performing Elbow Extensions

Francis and Hoobler in 1987 investigated whether the intensity of DMS induced by eccentric exercise is attenuated by aspirin (35). Aspirin via a dose-dependent mechanism inhibits prostaglandin synthesis which in turn results in a decrease in white blood cell activation and inhibits release of lysosomal enzymes. Twenty healthy female subjects were randomly assigned into either the control group (n=10) or the aspirin group (n=10) which took 10 grains (650 mg) of aspirin 4 times for 48 h starting 4 h prior to the exercise (n=10). Pain scores were measured at 24 h and 48 h after exercise using the Likert scale, 0 representing no soreness and 6 representing unbearable soreness. The subjects were asked to verbally report the sensation of pain in the elbow flexor muscle group. Elbow range of motion was measured at 24 h and 48 h using a goniometer. Maximal elbow flexion troque was measured using the Cybex II isokentic dynamometer at 60°/s and 180°/s at both times.

The eccentric exercise was performed with the Nautilis upper body biceps machine. The peak torque developed during the maximal elbow flexion at 60°/s was used as the starting weight for the eccentric exercise. Each subject performed as many repetitions as possible at this predetermined weight until the subject was unable to lower the weight slowly in a controlled fashion. The weight was then lowered by 5 pounds and the procedure was repeated at the new weight. The weight was continually decreased until the final weight with which the subject exercised was 5 pounds.

Francis and Hoobler found that muscle soreness was not significantly

different between the two groups at 24 h; whereas, the muscle soreness of the aspirin group was approximately 25% ($p<0.05$) less than the control group at 48 h. Both groups exhibited a significant decrease in the range of motion at the elbow at 24 h and 48 h compared to the pre-exercise values. The aspirin group exhibited approximately 50% less change in the range of motion at the elbow than the control group at 24 h and 48 h. Both groups showed a dramatic decrease in maximal elbow flexor force at both time periods, but the change was not significantly different when compared between groups. Based on these findings, the investigators concluded that the reduced soreness and improved elbow range of motion in the aspirin group was the result of aspirin inhibiting prostaglandin synthesis and release.

There are several flaws with the Francis and Hoobler study that need to be addressed before one can conclude that administering aspirin reduces the sensation of DMS. First, the investigators did not screen the subjects for resistance training prior to the study. It has been well established that exposure to even a single bout of eccentric exercise causes a protective effect in the muscles involved for up to 6-10 wk (14). Second, Francis and Hoobler collected general soreness data which relates to the overall soreness of the muscle at rest. It has been shown repeatedly by several investigators that the sensation of pain in DMS is more pronounced during contraction or palpation and not during rest (5,6,20,74). This effect is the result of increased production of prostaglandins and swelling that accompanies DMS which in turn produces a state of hyperalgesia (increased sensitivity) in the nociceptors. As a result, a

previously benign increase in intramuscular pressure might now be able to activate the afferent pain fibers. Therefore, a more appropriate measure of soreness would be one that documents the perception of pain during a contraction or palpation.

In addition, the eccentric contraction rate was controlled using a verbal 3 count. This method allows for a great degree of variability between the subjects in terms of total work performed. The investigators did not provide any data in terms of the subjects' height, weight, and pre-exercise maximal elbow flexor force; and they did not report if there was any significant difference between the two groups. Lastly, Francis and Hoobler in the methods section incorrectly referred to the control group as the placebo. Therefore, the conclusions drawn by Francis and Hoobler on the effects of aspirin are limited.

Effects of Diclofenac on DMS after Downhill Running

In an attempt to conduct a more controlled study, Donnelly, McCormick, Maughan, Whitting and Clarkson used a double blind crossover design to evaluate the effects of a non-steroidal anti-inflammatory drug on DMS (26). They used 20 healthy, untrained subjects who were required to run downhill (12°) for 45 min on two occasions separated by 10 wk. The running speed, which was the same for the two running sessions, was set to elicit a heart rate equivalent to 75% of the age-adjusted maximum heart rate (220-age). General perceived soreness, serum activities of several enzymes, and soreness elicited by the application of minor pressure over specific areas, were measured before

and at 6, 24, 48, and 72 h after the exercise.

Each subject took either 50mg of diclofenac or placebo before and for 72 h at 8-hour intervals after each run according to a randomized double blind crossover design. Therefore, each subject took a total of 500mg of diclofenac either during the first or second exercise bout. Diclofenac, like aspirin, is a non-steroidal agent that possesses analgesic, antipyretic and anti-inflammatory properties (41). Diclofenac inhibits cycoloxygenase and also appears to reduce the intracellular concentrations of free arachidonate in leukocytes. Donnelly et al. chose diclofenac because its potency is considerably greater than that of other anti-inflammatory agents.

Donnelly et al. reported that administration of diclofenac did not significantly reduce the magnitude of the enzyme response to exercise. However, the serum enzyme response to the second run was reduced indicating that a single bout of downhill running for 45 min. was able to produce a protective effect lasting at least 10 wk. Diclofenac did not significantly reduce total soreness at either period of the study nor did it consistently reduce probe-induced soreness at any of the specific sites. It did, however, reduce the general soreness in the front lower leg and back thigh and also reduced the probe-induced soreness in the middle of the back thigh after the first exercise bout.

The researchers tried to explain the inconsistency in the soreness data by suggesting that there might be some form of interaction between the action of diclofenac and the protective effect of eccentric exercise. The possibility of such an interaction was addressed by Evans (29). He suggested that since

prostaglandins increased muscle protein synthesis, inhibiting their production would hinder muscle repair, making the muscle susceptible to further damage. The results from the Donnelly et al. study seemed to indicate the opposite- inhibiting prostaglandin synthesis provided a protective effect against further soreness and muscle damage. Another explanation for the difference in soreness is that downhill running produces more muscle damage in certain muscles of the lower leg. Its more than likely that the muscles of the lower leg that did not change in soreness were contained in expandable compartments where the exercise induced swelling did not produce a state of hyperalgesia.

Along the same line, it is entirely possible that the reason why diclofenac did not reduce soreness is due to the fact that the 8 h interval between doses might have been too long. Diclofenac extensively binds to plasma proteins and has a half-life in plasma of about 1 to 2h. On the other hand, diclofenac has been reported to accumulate in the synovial fluid, which might prolong the therapeutic effects beyond the plasma half-life. Regardless of the mechanism, therapeutic doses of diclofenac in a double blind crossover design did not reduce serum enzyme changes but might reduce soreness at specific locations in the lower leg.

Effects of Ibuprofen on DMS after Downhill Running

In a continued effort to determine the role of anti-inflammatory drugs on muscle soreness, muscle weakness, and muscle damage inferred from serum enzyme activity changes, Donnelly, Maughan, and Whitting in 1990 administered

ibuprofen or placebo to forty healthy untrained subjects (25). They had two treatment groups: group 1 received ibuprofen at the first exercise bout and placebo at the second exercise bout, and group 2 received placebo at the first exercise session and received ibuprofen at the second exercise session. The experimental design and exercise protocol were very similar to their pervious study from 1987 with minor changes. Most obvious is that they used ibuprofen rather than diclofenac in the current study. Second, the dose administered prior to each exercise bout was increased to 1200mg of ibuprofen, which was then followed by 600mg every 6 h up to 72 h post-exercise. Therefore, each subject took a total of 8400mg of ibuprofen. Third, Donnelly et al. measured isometric endurance time at 50% of maximum voluntary contraction, which is an indicator of the subject's ability to sustain a muscle contraction. Fourth, the running was decreased to elicit a heart rate equivalent to 70% of the age adjusted maximum.

Donnelly et al. found that the total muscle soreness, the sum of all 20 soreness sites, increased significantly after both downhill runs. However, there was no difference in the soreness between the two treatment groups. The soreness data was collected on a scale of 20 (no soreness) to 200 (very, very, sore at all points). The highest reported mean soreness was 50 ± 6 (mean ± SEM) for both treatment groups, suggesting that the damage induced by downhill running was not severe. The changes reported in isometric strength and 50% endurance time also support this notion that downhill running at the prescribed speed did not cause extensive damage.

First, isometric strength decreased significantly after each exercise

session. However, ibuprofen did not significantly affect the loss in isometric strength. The decline in strength was no more than 10% percent of the pre-exercise isometric strength and was maximal at 24 h post-exercise for both treatment groups. The decline in strength was not significantly different between the first and second exercise sessions. More importantly, Donnelly et al. reported that strength returned to normal level by 72 h post-exercise for both treatment groups. In contrast, Clarkson et al. reported that following eccentric arm exercise there was a 50% loss in isometric strength and strength was gradually restored by 10 d post-exercise (20). Based on the assumption that strength loss following eccentric exercise is related to muscle fiber damage, it appears that the downhill running protocol employed by Donnelly et al. did not cause nearly as much muscle damage as the eccentric arm exercise employed by Clarkson et al.

Second, the 50% endurance time decreased significantly following both exercise sessions. The most drastic decrease occurred at 6 h post-exercise but returned to pre-exercise levels by 72 h post-exercise. The authors suggested that the reduced ability of the muscle to sustain a contraction following downhill running was the result of myofibril damage. Although the decrease in 50% endurance was significant, it was a relatively small decrease. Therefore, it is not certain if the decrease in 50% endurance time reflects muscle fiber damage.

In contrast, the measured serum activities of creatine kinase, lactate dehydrogenase, creatinine, urea, and aspartate transaminase before and after each exercise session at 6, 24, 48, and 72 h suggest that muscle damage did

indeed occur after downhill running. Serum levels of creatine kinase and aspartate transaminase increased to a maximum at 24 h post-exercise after both exercise sessions. The lactate dehydrogenase, creatinine, and urea reached the maximum at 6 h post-exercise after both exercise bouts. However, only creatine kinase, lactate dehydrogenase, and aspartate transaminase were significantly lower after the second exercise session. The investigators did not provide any explanation as to why creatinine and urea did not show this decrease in serum activity after the second downhill run. It is more than likely due to the fact that creatinine and urea are less sensitive indicators of muscle damage.

More importantly, it was reported that the serum activity of creatine kinase and serum urea level were higher in subjects receiving ibuprofen than in subjects receiving placebo. The authors suggested that this cannot be interpreted to indicate increased muscle damage in the subjects receiving ibuprofen, since the other indicators of damage did not increase. In addition, Clarkson et al. reported that serum creatine kinase activity after downhill running varies a great deal between subjects and did not necessarily represent the extent of muscle injury (20).

Donnelly et al. suggested that the increase in serum creatine kinase and urea concentration were the result of ibuprofen's action on plasma protein and plasma volume, since it has been reported that ibuprofen administered immediately after exercise reduces plasma protein content. This in turn would facilitate the movement of intracellular proteins down the concentration gradient into the blood stream. Again, they were unable to explain why the other serum

proteins did not change in a similar fashion. Regardless, Donnelly et al. concluded ibuprofen did not reduce DMS, since neither muscle soreness nor strength loss were reduced. This is consistent with Donnelly et al.'s 1987 study in which diclofenac was used to treat DMS following downhill running.

Effects of Flurbiprofen on DMS after Performing Eccentric Exercise on a Motor-Driven Bicycle

Kuipers, Keizer, Verstappen, and Costill investigated whether the intensity of DMS induced by eccentric exercise was significantly reduced by the anti-inflammatory drug flurbiprofen (53). They used six trained cyclists to perform two bouts of negative exercise on a motor-driven bicycle 24 h after ingesting a placebo or a drug in a double blind, randomized crossover design, with 3 to 6 wk intervening between trials. Kuipers et al. reported that the flurbiprofen did not significantly reduce the intensity of the DMS and concluded that that sensation of DMS may not be the result of an inflammatory response.

However, a more critical review of the results revealed that all subjects experienced significantly less soreness after the second trial, suggesting an order effect. This effect can be explained by the fact that a single bout of negatively biased exercise can provide a protective effect for up to 10 wk following the initial bout of eccentric exercise. In addition, the subjects were trained cyclists, therefore, they were very likely to be preadapted to the eccentric component of the cycling exercise.

Effects of Therapeutic and Prophylactic Doses of Ibuprofen on DMS after Performing a Negative Bench Stepping Exercise

More recently, Hasson, Daniels, Divine, Niebuhr, Richmond, Stein, and Williams in 1993 reported that prophylactic and therapeutic doses of ibuprofen significantly reduced muscle soreness perception, and significantly increased isometric, concentric, and eccentric torque at 48 h when compared with placebo and control (42). Ibuprofen produces anti-inflammatory, analgesic, and antipyretic effects. At doses greater than 400 mg q.i.d. (four times a day), ibuprofen is a powerful anti-inflammatory drug that interferes with the metabolism of arachidonic acid by inhibiting the enzyme cycloxygenase. This in turn inhibits the production of endoperoxides and the inflammation mediating prostaglandins PGE2 and PGF2A.

Hasson et al. employed four different treatment groups, each with five randomly assigned healthy subjects (23.8 ± 4.3 yr; 71.8 ± 10.6 kg; 172.3 ± 8.6 cm), to evaluate the effects of ibuprofen at 24 h and 48 h post-exercise. Subjects in group one (prophylactic) were given three 400 mg doses of ibuprofen in 24 h for a total of 1200 mg (400 mg-TID). The initial dose of ibuprofen was given 4 h prior to the exercise bout and was followed by placebo for the reminder of the study. The subjects in group two were also given 400 mg-TID with the initial ibuprofen dose given at 24 h after the exercise bout (therapeutic), but were given placebo prior to the 24 h dose. In the third treatment group the subjects received placebo throughout the entire experiment (placebo). The subjects in the control group received no treatment during the experiment.

The investigators used powered sugar as the placebo. The drug and placebo were packaged in gel capsules for two reasons: (a) so that the subjects would not be able to taste the difference between the drug and placebo, and (b) it made the drug and placebo almost identical in appearance. A double-blind procedure was employed throughout the experiment, in which the investigators providing the treatment and the subjects were unaware of the treatment protocol.

The muscle soreness perception was measured every day using the following protocol. The entire quadriceps muscle was divided into 2cm x 2cm sites using a transparent grid sheet. At each site, a gradually increasing force was applied by a 2cm metal probe up to a maximum of 50 N. The subjects were asked to verbally respond when the pressure changed to one of discomfort and the force was recorded. If the subject did not report any discomfort up to 50 N, soreness was not considered to be present. The intensity of the pain was determined as the inverse of the amount of force applied to each site that elicited discomfort. The mean intensity for each subject was determined by the summation of all the forces that elicited discomfort divided by the number of individual 2cm sites that were reported to be sore. The area of muscle soreness was calculated by dividing the number of sites that were reported to be sore by the total number of sites covering the entire quadriceps muscle. Muscle soreness for each subject was reported as a product of intensity and area.

Hasson et al. measured the following variables prior to the ibuprofen/ placebo treatment and at 24 h and 48 h after exercise: (a) maximal isometric force, peak eccentric and peak concentric torque production using the Lido

isokinetic dynamometer; (b) electromyography activity, documented during peak torque production; (c) skeletal muscle creatine kinase levels in the blood; and (d) muscle soreness. The exercise protocol employed by Hasson et al. was a 10 min. step exercise--stepping up on a bench with the right leg and lowering the body weight down with the contralateral leg. The step frequency was set at 15 cycles per min. Bench height was set at 110% of the lower leg length (floor to knee joint line) and the subjects carried an additional load of 10% body weight.

All four treatment groups showed a significant decrease in maximal isometric force, peak concentric and eccentric torque at 24 h compared to the baseline data ($p<.005$). At 48 h, maximal isometric force of the prophylactic and therapeutic ibuprofen groups were not significantly different from the baseline or between each other. However, the maximal isometric force at 48 h of the placebo and control groups were significantly less compared to the prophylactic and therapeutic ibuprofen groups.

At 24 h, the decrease in the prophylactic ibuprofen group's peak eccentric and concentric torque from baseline was significantly less compared to the decrease from baseline in the other treatments, and at 48 h was significantly less compared to the placebo and control. At 24 h, the decrease in the therapeutic group's peak eccentric and concentric torque from baseline was not significantly different compared to the decrease from baseline in the other treatment groups and at 48 h was significantly less compared to the placebo and control. These results suggest that the prophylactic treatment improved muscular performance at 24 h and up to 48 h.

The results from the creatine kinase analysis showed that there was a significant increase in all groups at 24 h and 48 h compared to baseline. There were no significant differences between groups at any time. Hasson et al. interpreted these results as evidence that ibuprofen did not limit the extent of muscle damage and that quantification of muscle damage could not be detected through creatine kinase analysis.

All subjects consistently reported muscle soreness in the distal head of the vastus medialis and proximal head of the vastus lateralis. The rectus femoris was not affected by the stepping exercise. The muscle soreness perception was significantly less for the prophylactic ibuprofen group than the therapeutic, placebo or control groups at 24 h post exercise ($p < 0.05$). At 48 h post-exercise the muscle soreness perception was significantly less for the prophylactic and therapeutic ibuprofen groups than the placebo or control groups ($p < 0.05$). However, muscle soreness perception at 24 h and 48 h post exercise was not significantly different between the therapeutic and prophylactic ibuprofen groups.

Hasson et al. suggested that the mechanism behind the improved muscular performance and decreased DMS at 48 h in the therapeutic group was due to the analgesic effect of ibuprofen. On the other hand, Hasson et al. suggested that the mechanism behind the decrease in DMS and improved muscular performance at 48 h in the prophylactic group was less likely the effect of ibuprofen, based on the assumption that the drug should have been completely eliminated from the system by 48 h. Unfortunately, they did not measure plasma levels of ibuprofen. As an alternative explanation the

investigators suggested that the improvement in muscular performance at 24 h for the prophylactic and at 48 h for both groups, based on EMG data, was the result of increased motor unit activation compared to the control and placebo groups. However, it must be noted that the exact relationship between motor unit activation and pain perception remains unresolved.

Clearly, therapeutic and prophylactic administration of ibuprofen was effective in reducing DMS and improving muscular performance at 48h post-exercise. As noted by the authors, this effect was partially due to the analgesic response of ibuprofen. Since the analgesic effects of ibuprofen is closely related to the anti-inflammatory effects, it was not possible to partition the effects of each using the measurements made by Hasson et al.

Summary of the Effects of Anti-inflammatory Drugs on DMS

Thus far, based on the two studies by Donnelly et al. and Kuipers et al., it appears that treatment of DMS with anti-inflammatory drugs is not beneficial. In contrast, Francis and Hoobler and Hasson et al. reported that administering anti-inflammatory drugs reduced DMS. The conflicting findings might be the result of the different exercises employed by the investigators to induce DMS. Francis and Hoobler employed elbow extension exercise to induce muscle damage, while Hasson et al. used an eccentric or negative bench stepping exercise. The two studies by Donnelly and co-workers used downhill running, and Kuipers et al. used a motor-driven cycle to induce muscle damage, both of which have limited eccentric loading, therefore limit damage induced by eccentric contractions. This

is supported by the fact that the increase in serum creatine kinase activity was considerably greater after eccentric arm exercise compared to downhill running (20). In addition, isometric strength recovered much faster, 72 h post-exercise, after downhill running compared to 10d post-exercise after eccentric arm exercise.

In a recent review, Clarkson et al. suggested that the metabolic stress of downhill running is considerably higher and the immune response to downhill running is much different compared to an eccentric exercise that involves a smaller muscle group, for example elbow flexors or quadriceps muscle (20). In addition, people are generally more likely to be preadapted to eccentric exercises like downhill running and cycling. Thus, it is not appropriate to conclude that nonsteriodal anti-inflammatory drugs do not reduce DMS based on the data from downhill running or cycling studies. On the other hand, it appears that anti-inflammatory drugs reduce DMS when exercise involves eccentric contractions of a small muscle group for a short duration so as not to drastically affect metabolism or the immune system.

Analgesic Effect Versus Anti-inflammatory Effect

Studies discussed thus far were unable to resolve the controversy of how the sequence and timing of DMS relate to acute inflammation as a possible pathological mechanism. This is primarily due to the fact that the parameters most often used to measure or document DMS were muscle performance and perceived muscle soreness, which cannot be used to separate the analgesic

effects of the drug from the anti-inflammatory effects. The best path to resolving this controversy is to measure intramuscular pressure, muscle compartment cross-sectional area, muscle recruitment, and muscle damage following eccentric exercise.

Muscle damage and intramuscular pressure are seldom measured since they require invasive procedures, for example muscle biopsies. There are several disadvantages with using muscle biopsies. First, muscle biopsy itself can cause muscle damage and is a painful procedure. Second, muscle biopsy samples a small area of the muscle which might not be damaged by eccentric exercise. Therefore, a noninvasive procedure that samples a larger area of the muscle may provide more complete information.

Muscle recruitment has been measured using surface and intramuscular electromyography (EMG) following eccentric exercise. Validity of EMG to measure small changes in muscle recruitment has been questioned by several investigators (2). However, the controversy regarding use of EMG to detect changes in muscle recruitment is beyond the scope of this review of literature. Muscle recruitment is an important variable because several investigators have suggested that the improvement in muscle performance with anti-inflammatory drugs is the result of increased motor recruitment.

Lastly, measuring muscle compartment cross-sectional area will allow investigators to evaluate the role of swelling development at the cellular level and whether or not anti-inflammatory drugs reduce swelling associated with DMS.

Thus far, swelling has been documented by measuring changes in limb circumference, which gives no information on the distribution of swelling amongst muscle compartments or between intra- and extra-cellular spaces. Therefore, a noninvasive procedure to measure changes in muscle compartments will provide addition information regarding the effects of anti-inflammatory drugs on swelling.

In the last 10 years, advances in the field of MRI have allowed investigators to measure muscle recruitment, muscle damage, and changes in muscle compartment cross-sectional area following eccentric exercise. The following sections will provide a detailed description of the accuracy of MRI to detect small changes in skeletal muscle function following exercise(i.e., muscle recruitment, damage, and swelling).

Use of Functional MRI to Study Muscle Use and Muscle Trauma with Resistance Exercise.

Despite its relatively recent development, MRI has quickly become the method of choice for medical imaging in many orthopedic studies of soft tissue and skeletal muscle pathology. Functional MRI exploits the changes in tissue 1H-NMR properties that occur during normal activity; as a result tissues and fluids with excess amounts of free water have long T2 relaxation times and appear as bright areas on T2-weighted images (3).

In 1988, Fleckenstein, Canby, Parkey, and Peshock published the first report documenting increased signal intensity in T2 -weighted spin-echo images of exercised muscle immediately following exercise (32). Soon thereafter,

several studies demonstrated that the increase in T2 signal, which occurs in exercised muscle, can be used to determine the extent of muscle recruitment for a given exercise by assessing the magnitude of change in image brightness (2,31,49). This change in image brightness or contrast is an acute change that occurs immediately following exercise and even during exercise but begins to wane within minutes, returning to resting values by approximately 40 to 45 min post-exercise.

In addition, MRI is also able to document the changes associated with exercise-induced muscle trauma which develops more gradually with DMS (71). These change are seen as a delayed increase in T2 signal intensity that begins to be visible approximately 12 hr post-exercise and peaks at 24-48 hr post-exercise, paralleling the development of soreness and swelling. The precise mechanism of acute and delayed contrast change following exercise is not yet clear, but is known to be related to changes in muscle water chemistry (43).

Fundamental Principles of Magnetic Resonance Imaging

MR imaging is based on three different and independent fundamental nuclear magnetic resonance parameters: spin density, T1 relaxation time, and T2 relaxation time (12). The following section is a brief summary of the fundamental principles of MRI, adapated from Bushong's textbook entitled Magnetic Resonance Imaging--Physical and Biological Principles (12).

A sample containing hydrogen compounds placed in a strong static magnetic field results in a net magnetization of all the nuclei. An NMR signal

generated after placing the sample within a strong static magnetic field depends on two variables: (a) concentration of hydrogen nuclei, and (b) environment of the hydrogen nuclei. Spin density is a measure of the concentration of free or loosely bound hydrogen nuclei available to produce the NMR signal. The relationship between MR image and spin density is as follows: a tissue with a high spin density will result in a stronger NMR signal which, in turn, will result in better resolution of the MR image.

T1 and T2 relaxation times are measured by placing samples within a strong static magnetic field and then applying radio-frequency energy in a pulsating fashion (2,3,43,). As a result, mobile hydrogen nuclei absorb the energy and the net magnetization vector changes in both magnitude and direction. This new magnetization state is a higher energy state, which produces a detectable signal that declines over time by way of two coexisting processes described as T1 and T2 relaxations, both of which are measured in milliseconds, typically in the range of 10-500ms in most tissues. T1 relaxation is the result of excited hydrogen nuclei's returning to the lower energy state or equilibrium, which is accomplished by releasing energy to neighboring hydrogen nuclei within the molecule or to neighboring molecules with similar resonant frequencies. The relationship between the MR image pixel intensity and T1 relaxation time involves a complex series of calculations, which is beyond the scope of this literature review. In simple terms, on T1-weighted images, the tissues with long T1 relaxation times will appear dark and tissues with short T1 relaxation times will appear bright.

T2 relaxation involves the decay of the phase-coherent oscillation of individual magnetic dipoles of excited hydrogen nuclei in the presence of a strong static magnetic field. Immediately after the initial excitation pulse, all of the individual nuclear magnetization vectors are rotating in synchrony, or "in pluse." This coherence of phase then begins to break down as some individual vectors begin to rotate slightly slower and some faster, producing vector components that partially cancel one another. The two main processes that contribute to the loss of phase-coherence are: (a) heterogeneity of the static magnetic field and (b) interactions between neighboring magnetic dipoles, which create local distortions of the field around each nucleus. Heterogeneity of the static magnetic field can be minimized by the use of a process called "spin-echoes." Therefore, the main contributor to the loss of phase-coherence is the interactions between neighboring magnetic dipoles.

In a pure water solution, rotational movements of the individual dipoles occur so fast that they average each other out. However, if the free mobile hydrogen nuclei are bound to proteins and/or other molecules, the rotational movements of individual dipoles are slowed down for brief periods of time (microseconds). As a result, these random interactions cause loss of phase-coherence, and T2 relaxation time characterizes the rate of this decay of the NMR signal following initial excitation. Therefore, a heterogeneous sample will result in a relatively short T2 relaxation time.

Exercise-induced Acute T2 Increase: Qualitative Index of Muscle Recruitment.

Using a low magnetic field (0.35T), Fleckenstein et al. reported increased signal intensity in both the T1 and T2 weighted MR images of exercised muscle, and that the changes in signal intensity correlated weakly with the level of exertion (32). The weak correlation between signal intensity and level of exertion was partly due to the low magnetic field utilized in the study. More recent studies have shown that, at 1.5T, the change in T1 signal is minimal compared to T2 signal changes following exercise (2,21). Regardless, results of the Fleckenstein et al. study clearly showed that, following exercise, changes in signal-intensity can be utilized to determine which muscle groups were recruited.

It was not until 1990 that the relationship between T2 signal intensity and exercise intensity was clearly established by Fisher, Meyer, Adams, Foley and Potchen (31). Fisher et al. had 8 untrained subjects lying supine in the GE 1.5T superconducting magnet perform three consecutive bouts of resisted ankle dorsiflexion against graded loads (load 1 < load 2 < load 3). Each subject dorsiflexed against each of the three loads for a total of 1.5 min at a constant rate of 1Hz. The loads were presented to the subjects in a random order in an attempt to minimize any load order effects that might affect T2 signal intensity. The investigators allowed subjects at least 30 min between sequential exercise bouts for recovery. Scans were collected before and immediately after each exercise bout and during the recovery period (5,10,15,20, and 30 min).

Data from a subset of subjects was used to evaluate the effect of venous

occlusion on T2 signal intensity in resting and exercised muscles; venous occlusion generally causes an increase in extracellular fluid volume. Three subjects underwent lower extremity venous occlusion for 5 min before, during, and after one bout of ankle dorsiflexion against load 2 for 1.5 min at a rate of 1 Hz. Another subset of subjects (n=3) underwent lower extremity venous occlusion for 20 min at rest.

These investigators reported that the mean forces generated against loads 1, 2, and 3 were 3.9 ± 0.4, 6.5 ± 0.5, and 9.2 ± 0.5 kg. T2 values of exercised muscles increased significantly compared to resting muscles. The extent of increase in T2 values of exercised muscles was dependent upon the mean force generated during exercise. Fisher et al. were able to adequately describe the relationship between force generated during exercise and T2 values by the following first order equation: T2 = 29.6 + 0.9 X Force. T2 values returned to near resting values by approximately 25 to 35 min, independent of mean force development.

Venous occlusion during exercise resulted in larger T2 values compared to T2 values without venous occlusion, although mean force generated was slightly lower with venous occlusion. Additionally, venous occlusion during exercise augmented the typical exercise-induced increase in muscle cross-sectional area. Other studies have demonstrated that the increase in muscle cross-sectional area following low-intensity exercise is primarily the result of increased extracellular volume (73). Venous occlusion alone, however, resulted in minimal increase in muscle T2 values. Therefore, a direct relationship could

not be established between the increases in T2 and muscle cross-sectional areas (extracellular volume), resulting from venous occlusion, exercise, and venous occlusion during exercise. Finally, venous occlusion did not affect the postexercise T2 recovery time or rate.

Because the extent of the contrast change in T2-weighted images of exercised muscles is dependent on average force generated by the muscles during exercise, Fisher et al. suggested that this phenomenon can be utilized to study muscle recruitment for a given exercise.

Exercise-induced Acute T2 Increase: Concentric vs Eccentric Exercise

In 1991 Shellock, Fukunaga, Mink, and Edgerton compared exercise-induced acute T2 increase following concentric versus eccentric exercise (70). Five subjects performed standing biceps curl exercise using a single dumbbell weighted to a normalized percentage of each subject's body weight (15% for women and 20% for men). One arm was randomly selected to perform concentric contraction (biceps curl) starting with the elbow in a fully extended position and bending the elbow to full flexion. The weight was then passed to the opposite arm by an assistant to perform the eccentric contraction, which was accomplished by lowering the weight with the arm starting at full flexion and lowering it to full extension. Throughout the entire range of movement the palm was supinated in both eccentric and concentric movements. Subjects were encouraged to maintain strict form and maintain a constant rate of eccentric and concentric contractions.

Subjects performed isolated alternating eccentric and concentric contractions until perceived exhaustion and "failure." As a result, both eccentric extremity and concentric extremity performed equal number of repetitions at approximately the same rate; therefore, both eccentric and concentric actions were performed at the same relative work level. MR images were acquired before and immediately following exercise of both extremities.

Shellock et al. reported a larger increase in signal intensity of MR images of the biceps and brachialis muscles immediately after performing concentric contractions, compared to a minimal change in signal intensity of the biceps and brachiallis immediately following eccentric contractions. Signal intensity of the triceps muscle (nonactive muscle) appeared unchanged compared to pre-exercise images in both extremities.

Shellock and coworkers noted that T2 relaxation times of the unexercised triceps muscle were not significantly different between the concentric and eccentric extremity. Significant increases in T2 relaxation times were reported for the biceps muscles following concentric and eccentric contractions. More importantly, the increase in T2 relaxation times for the biceps performing concentric contractions were significantly higher than those performing eccentric contractions. Since T2 values have been reported by Fisher et al. to be related to exercise intensity, and because eccentric contractions require less of an energy expenditure than concentric contractions at the same load, it is not surprising that Shellock et al. found T2 values following eccentric exercise significantly lower than T2 values following concentric exercise.

In a continued effort to determine the relationship between T2 signal and exercise intensity, in 1992 Adams, Duvoisin, and Dudley conducted a study to compare contrast changes in MR images with EMG following graded concentric and eccentric exercises(2). Prior to advances in the field of medical MR imaging, EMG was the noninvasive method of choice for analyzing muscle activation during exercise. Research has shown that there is a good correlation between the integrated root mean squared EMG (IEMG) and force development during exercise. Therefore, as pointed out by Adams et al., increases in IEMG reflect increased motor unit recruitment and not increased firing frequency of already recruited motor units.

Adams et al. collected both MRI and EMG data on seven subjects who performed eccentric and concentric exercises of increasing intensity. Each subject started the exercise session by performing five sets of 10 eccentric arm curls at 40% of their 10 repetition maximum for concentric curls. There was a 1.5 min rest period between sets and a 30 min recovery between bouts. Surface EMG signals were collected from both heads of the biceps brachii and the long head of the triceps brachii muscles during exercise. Subjects were imaged before and immediately after performing 5 sets of 10 repetitions. After the 30 min recovery period, the subject performed 5 sets of 10 concentric arm curls at the same relative resistance on the opposite arm. Again, there was a 1.5 min of rest between sets and 30 min of recovery between bouts, during which subjects were again imaged. This exercise protocol was followed at three different resistances of increasing magnitude: 60, 80, and 100% of the 10 repetition

maximum for concentric curls.

Adams et al. reported that the IEMG of the biceps brachii muscle was significantly less ($p < 0.05$) for eccentric than for concentric contractions at any given exercise intensity. IEMG increased as a function of relative resistance in both types of contractions. Rate of increase and absolute IEMG was significantly higher ($p < 0.05$) for concentric contractions, suggesting increased motor unit recruitment.

T2 changes were uniform throughout the 10 regions that were sampled in each image slice of the biceps brachii; as a result, values for the 10 regions within each image plus the five image slices were averaged prior to analyzing the T2 response to exercise. Shifts in T2 weighted MR images of the biceps brachii after concentric and eccentric exercise increased as a function of the relative resistance. However, rate of T2 increase and absolute T2 values were greater following concentric exercise. These results reinforce the fact that muscles have an inherent ability to generate greater force during eccentric actions with a given number of active motor units. Finally, there was a strong significant correlation ($r = .99$, $p < 0.05$) between IEMG and the shifts in T2 weighted MR images following both concentric and eccentric contractions.

These results were interpreted as evidence that the shifts in T2 weighted MR images increase as a function of exercise intensity. However, use of exercise-induced acute T2 contrast change as a quantitative index of muscle recruitment is limited because the exact mechanism of the T2 increase is unclear. It has been suggested that the exercise-induced T2 increase is related

to changes in muscle fiber water content.

Proposed Mechanism of Exercise-induced Acute T2 Increase

The contrast in MR images depends primarily on spin density of the tissue imaged. For example, bone, fat, and muscle have different spin densities which result in markedly different contrasts. In addition, T1 and T2 relaxation processes, independent of each other, have significant effects on the contrast of the final MR image. This effect has been illustrated in the studies discussed thus far. For instance, exercised muscles have longer T2 relaxation times than non-exercised muscles, and subsequently appear brighter on T2-weighted images. As Adams et al. noted, these changes in muscle T2 signals depend on the molecular environment of protons of either water or lipid, since these contain the largest spin density (2). Adams et al. reported that T2 of bone marrow did not change following exercise. According to the researchers this was evidence that there were no gross changes in lipid signal. Additionally, as suggested by the researchers, it is very unlikely that these acute bouts of exercise caused any changes in intramuscular fat content, in which case T2 signal intensity changes must be the result of one or more of the water compartments in the muscle.

Recent research has shown that exercise causes rapid alterations in skeletal muscle water content (72,73). Increase in water content of exercising muscle is the result of increased water movement across the capillary bed. Hydrostatic pressure in capillaries and osmotic forces in both capillaries and interstitial fluid contribute to the increased water content of skeletal muscle.

Furthermore, functional capillary surface area increases during exercise in active muscles, resulting in increased capillary pressure. Elevated capillary pressure then increases the rate of water filtration from the capillary bed into the interstitial space.

Low intensity exercise has been shown to cause a drastic increase in extracellular water volume (e.g., up to 100%) and only a minor increase in intracellular water volume (e.g., close to 10%) (73). On the other hand, with high intensity exercise majority of the increase in muscle water content is due to an increases in intracellular water content (72). Adams et al. and Shellock et al. reported a significant increase in T2 signal following high intensity concentric exercise (2,70). Both groups of researchers suggested that the increase in T2 signal following high intensity exercise was the result of increased intracellular water content.

Fisher et al. reported similar increases in muscle volume following low intensity exercise and venous occlusion. Venous occlusion generally causes an increase in extracellular volume. However, venous occlusion alone and venous occlusion during exercise had little additional effect on T2 signal intensity, prompting Fisher et al. to conclude that increases in T2 signal intensity following even low intensity exercise were related to intracellular water content. Therefore, it is naive to assume that exercise-induced contrast enhancement is solely the result of increased perfusion and extravascular movement of plasma water. The Fisher et al. findings suggest that exercise-induced changes in MR images is probably related to more complex intracellular events and/or intracellular water.

Delayed T2 Increase: An Index of Muscle Damage

Changes in T2 signal intensity discussed thus far have been acute T2 increases following exercise. Another observed phenomenon is a delayed increase in T2 signal intensity following eccentric exercise but absent after concentric exercise. Fleckenstein, Weatherall, Parkey, Payne, and Peshock were the first to report on the delayed T2 increase following eccentric exercise (34).

Fleckenstein et al. noted that all subjects (n = 6) reported muscle soreness (DMS) after unaccustomed calf plantar-flexion exercise, which involves concentric and eccentric loading. Two subjects were imaged before, immediately after, and 24, 36, 48, and 72 hours post-exercise and at 13, 23, and 48 days post-exercise. These investigators also measured plasma creatine kinase levels (CK), and collected subjective muscle soreness scores (on a scale of 1 to 10), both of which have been documented to increase with DMS.

Results from the linear regression analysis showed a positive correlation between the following pairs of variables: pain vs CK levels (r = .59, p = 0.2), pain vs T1 (r = .87, p = .001), pain vs T2 (r = .67, p = .005), CK levels vs T1 (r = .50, p = .05), CK levels vs T2 (r = .76, p = .001), and T1 vs T2 (r = .73, p = .002). In the first 72 hr post-exercise increase in CK levels lagged behind the increase in pain and MR signal intensity. Although there is universal agreement that DMS results in increased serum CK levels, several researchers have suggested that it is inappropriate to interpret the increase in serum CK levels as a direct measure of muscle damage. Signal intensity and pain peaked at 24-72 hr post-exercise.

Pain and serum CK levels returned to pre-exercise levels by approximately 2 weeks. However, signal intensity remained elevated for 48 days post-exercise.

Based on the positive correlations between pain, CK levels, and signal intensity during the first 72 hr post-exercise, Fleckenstein et al. concluded that the underlying mechanism of delayed increase in signal intensity is probably related to the muscle damage that gradually develops with DMS. Fleckenstein et al. were unable to provide an explanation as to why the signal intensity remained elevated at 48 days post-exercise.

Exercise-induced Delayed T2 Increase: Concentric vs Eccentric Exercise

Although, Fleckenstein et al. showed that the delayed increase in signal intensity only occurred in subjects that reported DMS, the experimental design was limited in terms of distinguishing damage resulting from concentric contractions versus eccentric contractions. Since eccentric contractions are most likely to cause DMS, Shellock, Fukunaga, Mink, and Edgerton conducted a study to evaluate the delayed T2 increase following concentric versus eccentric exercise (70).

Subjects (n = 5) performed isolated alternating eccentric and concentric biceps curls using a single dumbbell weighted to a normalized percentage of each subject's body weight (10% for women and 20% for men). One arm was randomly selected to perform the isolated eccentric actions and the opposite arm performed concentric actions. Subjects performed alternating eccentric and concentric contractions at a rate of 1 repetition every 2 seconds until perceived

exhaustion. This same exercise protocol was employed by Shellock et al. in a 1991 study which documented acute T2 changes following eccentric and concentric exercise (71). MR images were collected before, and 1 day, 3, 5, 10, 25, 40, 50, 60, and 80 days after exercise using a 1.5T 64-MHz unit and a quadranture-driven, transmit/receive body coil. Subjective muscle soreness scores and joint stiffness data were also measured before and on each day of imaging.

Muscles that performed concentric exercise did not show a significant increase in T2 signal intensity, pain, or joint stiffness on days 1-80 post-exercise compared to pre-exercise values. The biceps muscle that performed eccentric exercise had a significant increase in T2 signal intensity on days 1-80 post-exercise compared to baseline measurements. Highest mean T2 signal intensities were reported to occur on days 3 and 5 post-exercise and slowly decreased towards baseline values by day 80. Muscle soreness and joint stiffness following eccentric exercise returned to baseline values by day 10 post-exercise.

These investigators suggested that the time course of the increase in T2 signal intensity are consistent with other studies that have documented a similar time course for other indirect measures of muscle damage (e.g. pain, CK levels, and swelling) following eccentric exercise. Therefore, Shellock et al. suggested that the delayed increase in T2 signal intensity reported was most likely the result of muscle damage induced by eccentric contractions. Increase in T2 signal intensity in days 1-5 was interpreted as evidence that muscle damage

progressively increased in days following eccentric exercise, consistent with the acute inflammation theory of DMS.

Studies presented thus far have only suggested that the delayed increase in T2 signal intensity is related to the muscle damage associated with DMS. In 1992, Nurenberg, Giddings, Stray-Gundersen, Fleckenstein, Gonyea, and Peshock conducted a study to examine if there was a correlation between the degree of delayed increase in signal intensity following eccentric exercise and amount of ultrastructural damage and DMS (62). Nine untrained subjects ran on a treadmill set at a negative 8% grade for 30 min at a speed of 8km/h. MR images were collected using a 0.35T imager at 48 and 96 hr post-exercise. Muscle biopsy samples were taken from muscle regions that showed an elevated T2 at 48 hr post-exercise. Structural features of muscle fibers were assessed using electron microscopy.

Peak soreness was generally reported between 12-36 hr after exercise. Linear regression analysis showed a strong correlation between the degree of increase in T2 signal intensity at 48 hr post-exercise and the degree of ultrastructural injury. Highest correlations were reported between the signal intensity increases with the T1-weighted and spin-density pulse sequences and the degree of ultrastructural injury. As pointed out by Nurenberg et al., these results are not consistent with the findings of Fleckenstein et al. and Shellock et al., who showed a significantly larger increase in T2-weighted images than T1-weighted images.

Nurenberg et al. observed that the peak CK levels was considerably

greater in the study conducted by Fleckenstein et al.. Lower peak CK levels was interpreted as evidence that the damage induced by downhill running was less compared to the more concentrated eccentric exercises employed by Fleckenstein et al. and Shellock et al. Since, more severe muscle damage generally results in more edema (free water), and free water plays a significant role in the increase in T2-weighted signal intensity; Nurenberg et al. concluded that this was the cause of the large increases in T2-weighted images that were reported by Feckenstein et al. and Shellock et al.. Two flaws were identified with this reasoning: (a) increase in serum CK level is a poor indicator of muscle damage, and (b) more recent studies have shown that using a low magnetic field generally results in a more pronounced T1 signal than T2 signal (2,20,21).

In addition, there was poor correlation between perceived peak soreness and peak CK level, and between perceived peak soreness in the region of biopsy and degree of ultrastructural damage. The most significant finding of this study was that it showed a high correlation between areas with increased signal intensity and ultrastructural injury. These results suggest that the magnitude of delayed increase in T2 signal intensity is an index of the extent of muscle damage.

Time Course of the Changes in T2 Relaxation Time and Muscle Cross-sectional Area Following Eccentric Exercise

Majority of studies that have evaluated the delayed increase in T2 signal intensity associated with DMS have not documented the early time course

changes in the T2 signal intensity following the transient acute increase in the T2 signal. Takahashi, Kuno, Miyamoto, Yoshioka, Inaki, Akima, Katsuta, Anno, and Itai conducted a study in 1994 to examine the early time course data on the changes in T2 relaxation time and muscle cross-sectional area following eccentric exercise (81).

Six healthy male subjects were imaged before, immediately after, and at 7, 15, 20, 30, and 60 min and 12, 24, 36, 48, 72, and 168 hr post-exercise. Subjective muscle soreness scores were collected every day for 6 days after exercise. Plasma CK levels were measured before and at 5 and 60 min then 24 and 48 hr post-exercise. Subjects began the exercise in a seated position on a chair 42cm high, then stood up on both legs. From the standing position, left leg was slightly raised of the ground so that all the body weight was placed on the right leg. While the left leg was slightly raised, the subjects slowly crouched back down to the seated position without lowering the left leg. This movement was completed in a total of 4 seconds and was repeated 300 times by all the subjects. Takahashi et al. pointed out that during this type of movement the right quadriceps muscle undergoes eccentric contraction as the subject advances from the standing position to the sitting position. However, the investigators failed to recognize that the right quadriceps muscle also undergoes concentric loading as the subject goes from sitting position to standing position using both legs.

In most subjects peak soreness occurred 1-2 days after eccentric exercise. CK levels remained unchanged for the first 24 hr and then increased

significantly at 48 hr post-exercise. Immediately following the eccentric exercise there was a significant increase in the T2 signal intensity and muscle cross-sectional area of the quadriceps muscle as a whole, both muscle cross-sectional area and signal intensity returned to baseline values by 60 min post-exercise. At 12 hr post-exercise signal intensity increased significantly, reaching peak values at 24-36 hr after exercise. Cross-sectional area of the quadriceps muscle as a whole increased significantly at 12 hr post-exercise and reached peak values at 12-24 hr after exercise.

Muscle cross-sectional area and T2 signal following eccentric exercise appears to follow a bimodal response with the first peak occurring immediately after the exercise, and the second peak sometime after 12 hr post-exercise. These investigators also found that subjects with the lowest increase in T2 and muscle cross-sectional area at 12 hr post-exercise exhibited a faster decrease in muscle soreness. The bimodal response of muscle cross-sectional area and T2 signal was interpreted as evidence that two different mechanisms are responsible for the acute and delayed increase in T2 signal intensity, and muscle cross-sectional area following eccentric exercise.

Proposed Mechanism of the Delayed Increase in T2 Signal Intensity

Several investigators have suggested that the delayed increase in T2 signal intensity is the result of increased edema associated with DMS (34,62,81). The increase in edema in skeletal muscle during DMS is mainly due to the following factors: (a) damaged connective tissue, (b) increased permeability of

the capillaries, and (c) increased permeability of the sarcolema. As discussed by Takahashi et al., ultrastructural damage in muscle fibers results in accumulation of degraded proteins and release of protein bound ions. Therefore, the chemical environment of the water in damaged muscle fibers will be different compared to undamaged fibers. However, it is not clear as to which of the above mentioned changes is most responsible for the delayed T2 increase.

Takahashi and co-workers have suggested that the combination of changes in proton concentration and chemical environment of muscle water contribute to the increase in T2 signal intensity after 12 hr post-exercise. These investigators also found that subjects with the lowest increase in T2 and muscle cross-sectional area at 12 hr post-exercise exhibited a faster decrease in muscle soreness. In addition, Nurenberg et al. showed a good correlation between increases in signal intensity and extent of ultrastructural damage following eccentric exercise. Based on these findings, Takahashi and co-workers suggested that the extent of muscle damage and rate of soreness disappearance can be assessed by the magnitude of the delayed increase in T2 signal intensity.

According to this proposed mechanism of delayed T2, any additional damage during the recovery period should result in an additional increase in the delayed T2 signal intensity. Sorichter, Koller, Haid, Wicke, Judmaier, Werner, and Raas examined the consequences of a single bout of eccentric exercise with and without additional concentric exercises during recovery (77). One leg was randomly assigned as the control limb (unloaded leg) and the contralateral leg

was used to perform the exercise (loaded leg). Eccentric exercise was performed using a specially designed exercise equipment that forced the quadriceps femoris into a lengthening contraction. The eccentric load was set at 110% of the maximum voluntarily generated force. Subjects in the eccentric group performed a single bout of seven eccentric contractions, at a rate of 1 repetition every 1-2 seconds with 15 seconds of rest between each repetition.

Subjects in the eccentric/concentric group performed addition concentric contractions before and 2 h after exercise, and 1, 2, 3, 6, and 9 days after eccentric exercise. Subjects performed two voluntary maximum knee extensions at angular velocities of 30^0/s and 90^0/s and three voluntary maximum knee extensions at 180^0/s. Therefore, it is misleading to use the term light concentric in describing the addition concentric contractions performed by the eccentric/concentric group.

Several inflammatory markers and serum CK levels were measured before eccentric exercise, 2h after exercise, and 1, 2, 3, 6, and 9 days after exercise. MR images were acquired from the loaded and unloaded legs of both groups starting at 3, 6, and 9 days after exercise. The timing was chosen based on a pervious study in which the investigators on day 3 post-exercise found the first significant increase in signal intensity.

Over time, inflammatory markers did not increase above the reference range. Additional concentric contractions caused a five fold increase in the serum CK levels. T2 weighted relaxation times for the eccentric and eccentric/concentric group were not significantly different, over time. Sorichter et

al. noted that the maximum mean T2 values of the affected regions of muscle from the eccentric group and concentric group were 19.65 ms and 36.44 ms. It is possible that the variance in the data, indicated by the large standard deviation, contributed to the non-significant results.

Investigators analyzed the calculated mean T2 relaxation times at different time points using the student's t-test to compare the two groups. A more appropriate method would have been to look at the change in T2 relaxation times (ΔT2) using a t-test which would have limited the influence of variance in the data set. To measure the change in T2 relaxation times from quadriceps muscles experiencing DMS more accurately, the difference should have been calculated by subtracting the T2 value measured before performing the additional concentric exercise from the T2 value measured after performing the concentric exercise. However, these investigators did not acquire pre-exercise images from the leg that performed additional concentric contractions. Both legs, loaded and unloaded, in the eccentric/concentric group were imaged after performing the additional concentric contractions.

In any event, it is unclear what influence, if any, an elevated T2 will have on the acute T2 response to additional concentric exercise. Furthermore, a review of the MRI literature to date reveled that most of the studies acquired the first images starting at 3 days post-exercise, there is little information documenting the early time course of the development of delayed T2 increase.

CHAPTER III

RESEARCH METHODS

As discussed in the two preceding chapters, the precise mechanism of acute and delayed T2 contrast changes in MR images following exercise is not yet clear; but, it is known to be related to changes in the intracellular water chemistry (64). A review of the MRI literature to date has shown universal agreement that the extent of the acute T2 elevation following exercise is a good qualitative index of motor recruitment (2,31,49,21,70). Furthermore, the acute increase in T2 reaches some upper limit with increasing exercise (33). Several studies, focusing on the delayed T2 elevation following exercise-induced muscle trauma, have reported a strong correlation between delayed increase in T2 signal intensity, ultastructural damage, and sensation of DMS (2,31,62). Although acute and delayed T2 changes might result from different mechanisms, no experiments have been reported which specially test this claim.

The primary objective of the current study was to compare the changes in acute T2 following a bout of concentric exercise performed before vs. 24 h after eccentric exercise. Eccentric exercise has been shown to induce: (1) both an acute T2 change, which fades within an hour similar to the acute T2 elevation due to concentric exercise, and (2) delayed T2 change which peaks at 24-48 h

and then is sustained at an elevated level for a period of days to weeks (2,31,62).

Specifically, the present design tested whether or not the acute T2 response to concentric exercise is larger with an elevated resting T2 resulting from the exercise-induced muscle trauma. Based on the hypothesis that the underlying mechanism of the acute T2 and delayed T2 is the same and acute T2 has an upper limit at a given exercise intensity, one would expect that a standard bout of concentric exercise would cause the same total T2 response whether starting from a normal or an elevated baseline T2. In this scenario, acute concentric and delayed eccentric T2 changes occur via the same mechanism, so concentric exercise on top of an eccentrically-elevated baseline T2 will reach the same absolute T2 "saturation" or "plateau" level as a concentric bout performed from a normal baseline. This is illustrated by column C in Figure 4, where the T2 effects of concentric exercise after eccentric exercise is the same as for concentric exercise alone.

Alternatively, if the acute concentric and delayed eccentric T2 changes occur via different mechanisms, it is very likely that the effects would be additive. In this case, a concentric bout after T2 elevation due to eccentric exercise would be expected to produce an acute T2 change greater than the initial concentric exercise, and the difference should be approximately equal to the eccentrically-induced T2 elevation. This situation is depicted by column D of Figure 4.

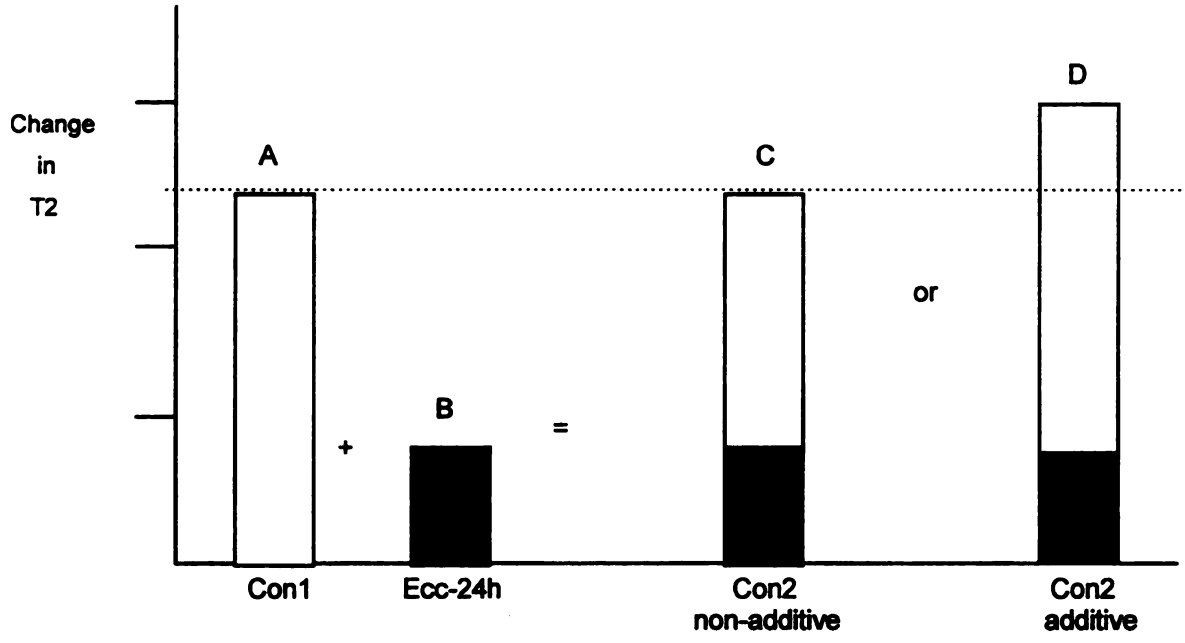

Figure 4. Additive vs. non-additive T2 changes. Column A represents acute T2 elevation following a single concentric exercise bout (Con1). Column B depicts the delayed T2 increase at 24 h after eccentric exercise (Ecc-24h). The predicted effect of a second concentric bout after eccentrically-induced T2 elevation is represented by column C if the mechanisms are identical (Con2, non-additive) or by column D if the mechanisms are different (Con2, additive).

A secondary purpose of this study is to examine the early time course of development of the T2 change following eccentric exercise. For this purpose, T2 data will be collected at 1, 2, 4, and 6 h following the bout of eccentric exercise to document the early time course of the acute T2 following eccentric exercise and the development of the delayed T2 increase. Although changes in this time span are unlikely to be large and perhaps not even discernible from resting values, documentation of these time points will fill gaps in the published data on T2 time course following eccentric exercise.

Subjects

Four males and four females between the ages 21-40 were recruited from the student population at Michigan State University. During the initial recruiting process, subjects were interviewed to ensure that they had not participated in any type of resistance training for a minimum of six months prior to the study. Furthermore, subjects who engaged in heavy lifting as part of their daily activity were also excluded from the study. Non-resistance trained subjects were used in an attempt to minimize any prior adaptation that might reduce the development of DMS and attenuate the T2 signal response. Subjects were also screened for any acute or chronic diseases or injuries that might interfere with their exercise performance (see Appendix A). Subjects with orthopedic implants and/or pacemakers were also excluded from the study. The strong magnetic field generated by the MRI magnet has been shown to interfere with pacemakers and orthopedic implants. A week before the scheduled experiment, the subjects met with the investigator on an individual basis to review the experimental procedures, including possible risks. All subjects signed an informed consent approved by the Michigan State University Committee on Research Involving Human Subjects (see Appendix B).

Exercise Protocol and Timing of MRI Scans

Each subject was imaged at rest to obtain baseline data on "normal" muscle and again before and after each exercise session. Therefore, the study was conducted as a repeated measures design. This design allowed each

subject to serve as his/her own control. After completion of the baseline image sets, subjects performed an isolated concentric standing biceps curl exercise with the left arm using a single dumbbell weighted to a normalized percentage of body weight (15% for women and 20% for men) (70).

Light concentric exercise began with the subject's elbow in a fully extended position. An assistant placed the dumbbell in the subject's grip; the subject then slowly flexed the elbow to full flexion. To control the rate of elbow flexion, subjects followed the investigator's verbal count of "one-one thousand, two-one thousand, three-one thousand." The weight was then removed from subject's grip so s/he could fully re-extend the elbow without external load. Subjects repeated this movement for 3 sets of 10 repetitions at approximately 1 repetition every 5 seconds. There was a 1.5 min. rest period between sets. Subjects were verbally encouraged to follow strict form (standing erect with feet shoulder-width apart) (9). Subjects were also encouraged to maintain a constant rate and keep the arms stationary throughout the entire range of motion. Palms remained supinated throughout the full range of motion to ensure full activation of the biceps brachii muscle (9). MR imaging was performed immediately (i.e., within a minute) following the completion of the concentric exercise bout, followed by a two hour rest period. The two hour delay allows sufficient time for the acute T2 signal to return to baseline (31). During the rest period, subjects were instructed not to perform any lifting movements with the exercised arm (e.g., lifting of book bags).

At the end of the two hour rest period, subjects were re-imaged to confirm that the acute T2 signal had returned to baseline values. Immediately following this scan, subjects performed the eccentric exercise. Since the muscle can tolerate a larger load during the eccentric contraction, the eccentric exercise was performed using a single dumbbell weighted to a higher normalized percentage of each subject's body weight (25% for women and 30% for men) (20,36).

Eccentric exercise began with the elbow in the fully flexed position. The assistant placed the dumbbell in subject's grip; the subject then fully extended the elbow. Again, in an attempt to control the rate of elbow extension, subjects followed the investigator's verbal count of "one-one thousand, two-one thousand, three-one thousand." The weight was then removed from subject's grip and the subject returned the unloaded arm to the starting position (i.e., elbow fully flexed). Subjects repeated this movement for 3 sets of 10 repetitions at approximately 1 repetition every 5 seconds, with a 1.5 min. rest period between sets. Subjects were again encouraged to follow strict form and the palms were supinated throughout the entire range of motion (9). Subjects were imaged immediately following the eccentric exercise (PostEcc) and at 1, 2, 4, and 6 h post-exercise.

At 24 h post-exercise, subjects were imaged before and immediately after performing the same light concentric exercise that was used at the start of the experiment.

General muscle soreness was evaluated using a questionnaire (20). Subjects were asked to rate their perception of overall soreness in the biceps

brachii muscle with the arm fully extended and relaxed on a scale of 1 (no soreness) to 10 (extremely sore) before each exercise session, and at 12 h post eccentric exercise and at 24 h intervals for 7 consecutive days (see Appendix C).

MR Imaging Parameters

Previous studies have shown that a standard two dimensional multiple spin-echo pulse sequence with flow compensation maximizes the visibility of exercise-induced contrast change (2,31,71,77,81). Pilot studies showed optimal results with two echo times of 30 and 60 ms, with a repetition time of 2000 ms and 0.75 NEX - number of excitations.

Images were acquired using a linear extremity coil and a 1.5T GE Signa whole body imager. Subjects were positioned head first and prone, with the left arm extended beyond the head. The coil housing was centered on a mark inked on the brachial region 7.5 cm from the elbow joint along a line between the olecranon and acromion processes.

An initial coronal scan was used to locate the distal end of the biceps muscle. From this point, ten axial slices 10 mm thick were acquired at 15 mm intervals (5 mm gap) along the length of the brachium. With the TR/TE 2000/30 and 2000/60 and other parameters as previously noted, the total scan time for a full series of 10 slices was 3 minutes and 32 seconds.

Images Analysis

Image sets were transferred to a Sun computer workstation to calculated the muscle cross-sectional area and T2. Specifically, muscle cross-sectional area and T2 were calculated using the analysis software package "x-vessel" developed by Dr. Ron Meyer at Michigan State University (61). Images were acquired over a 16 cm x 16 cm field of view using a 256x128 pixel matrix, giving a resolution of 1.28 mm^2 per pixel.

Cross-sectional area of the biceps brachii was calculated by the following method: first the investigator selected an image that was near the belly or gaster of the muscle, typically slice #6 out of the 10 slices acquired from the brachial region. Then a region of interest was established by manually tracing around the biceps brachii compartment, carefully excluding the subcutaneous fat and skeletal tissue. Once the region of interest was established, the software automatically calculated the number of pixels contained within this region. Lastly, the biceps brachii cross-sectional area was calculated by multiplying the total number of pixels within the region of interest by the per pixel area.

Past studies have used different methods of image analysis to calculate the muscle T2, but none have directly compared the results of the different methods within a single study (2,31,70,71,77,81). In the present study, three different methods were used to calculate muscle T2, with the operator blinded to subject identity in all cases.

In the first method, the investigator used a single image slice to outline a small rectangular region of interest ("box") within the biceps brachii muscle

compartment. The location of this region of interest or "box" was such that it did not include any adipose tissue, blood vessels, subcutaneous fat, or bone. Image slice #6 was selected out the 10 slices from the brachial region because pilot data showed that this slice to have the largest change in the T2 immediately following exercise and at 24 h post-eccentric exercise. Using x-vessel software, a T2 value was calculated for each pixel in the region of interest and an overall mean T2 value was reported.

Within each subject, the same slice and rectangular region of interest was used to calculate the mean T2 from the image sets acquired at the different time points. Although the same slice was utilized in all subjects, the size and location of the "box" varied slightly between subjects due to anatomical differences.

In the second method, the region of interest was defined by tracing around the entire biceps brachii muscle compartment excluding subcutaneous fat, bone, and residual blood flow artifacts within a single image slice. Therefore, the mean T2 that was reported for each subject by this method provided an overall average of the T2 values of the entire biceps muscle compartment within a single image. Slice #6 was also used in this method to calculate the mean T2 of the biceps brachii muscle compartment from the image sets acquired at the different time points.

The third method used the entire biceps brachii muscle compartment excluding only the subcutaneous fat and bone from all ten slices to calculate an overall average T2 at the different time points. Therefore, the mean T2 that was reported for each subject using the third method provides an overall average of

the entire imaged region of muscle using all ten image slices at the different time points.

Statistical Analysis

Initially, data were analyzed by using a repeated measures one way analysis of variance (ANOVA) to assess difference with time in muscle soreness, T2 relaxation times of the biceps muscle , change in T2 relaxation times from baseline value, and muscle cross-sectional areas. If a significant difference was noted ($p<0.05$) with the ANOVA, then post hoc tests were performed. These tests make pairwise comparisons of the means. The Bonferroni's adjusted paired t-test ($p<0.05$) was chosen for the post hoc analysis. Specifically, the Bonferroni's adjusted paired t-test was chosen over others because it allowed the investigator to adjust the alpha values based on the number of comparisons. Correlation analysis was conducted using the Spearman's correlation test. The results are presented as means ± standard error (S.E.) of the means.

CHAPTER IV

RESULTS AND DISCUSSION

The content of this chapter is divided into five major sections. Subject characteristics are presented first. Next, the acute T2 responses to a bout of light concentric exercise performed before and after eccentric exercise are described. Also included is a report on the time course of the delayed T2 increase following eccentric exercise. The third section presents the descriptive analysis of the changes in muscle cross-sectional area 24 h following eccentric exercise. This section is followed by a description of the self-reported muscle soreness ratings following eccentric exercise. A discussion of the results as well as comparisons with related literature are detailed in the final section. All results are shown as mean ± SE, with n = 8 for overall means or n = 4 for gender subgroup means.

Subject Characteristics

Eight healthy non-weight-trained adults, four males and four females, were recruited from the student population at Michigan State University. A summary of subject characteristics is presented in Table 2.

Table 2. Subject characteristics.

Subject ID	Gender	Age (yr.)	Height (cm)	Weight (kg)
Subject 2	F	21	174	72.7
Subject 4	F	24	151.6	54.8
Subject 6	F	27	158	57.2
Subject 8	F	21	158.5	54.1
Mean ± SE (females)		23.3 ± 1.4	161 ± 5	60 ± 4
Subject 1	M	30	171.6	75.3
Subject 3	M	23	165.8	67.8
Subject 5	M	27	178.4	91.1
Subject 7	M	26	182.9	76.6
Mean ± SE (males)		26.5 ± 1.4	175 ± 4	78 ± 5
Overall Mean ± SE		**24.7 ± 0.8**	**167 ± 3**	**68 ± 3**

Acute and Delayed T2 Responses

In the present study, three different methods were used to calculate muscle T2 values from the MR images acquired as described in Chapter 3. SE as a percent of the mean T2 was calculated using all three methods for the resting data set to determine which method minimized variance. SE as a percent of the mean T2 calculated using the entire muscle compartment from slice #6 and using all ten slices was 1%. On the other hand, SE as a percent of the mean T2 calculated using the "box" method was 0.4%. Since the T2

calculated using the "box" method reduced the variability considerably, all the T2 data presented in Table 3 and Figures 5, 6, and 7 were calculated using the "box" method.

T2 relaxation times in milliseconds are given in Table 3. Baseline (resting T2 of "normal" muscle) averaged 27.1 ms, typical of values reported in human skeletal muscle (2,71). Concentric exercise resulted in a 30% rise in T2. Eccentric exercise produced a smaller acute increase (16%), but resulted in a delayed T2 elevation of about 1.5 ms at 24 hours post eccentric exercise.

Table 3. T2 data.

Time of Scan	T2 (ms)
PreCon1 (baseline)	27.1 ± 0.1
PostCon1	35.2 ± 1.0#
PreEcc	28.2 ± 0.4
PostEcc	32.7 ± 0.9#
PreCon2 (24h)	28.7 ± 0.3*
PostCon2	34.8 ± 0.7#

Values are mean ± SE, n=8.
significantly different from the pre-exercise T2 value ($p<0.05$).
* significantly different from baseline T2 value ($P<0.05$).

Acute and delayed T2 changes (Δ T2) were calculated by subtracting the baseline (PreCon1) T2 value from the T2s measured at the various post-exercise time points (Figure 5). The acute ΔT2 following the first bout of concentric exercise (Con1, Figure 5) was not significantly different from that following the second bout of concentric exercise (Con2, Figure 5). It should be noted that the results displayed in Figure 5 are consistent with the "non-additive effects" scenario presented in the previous chapter (Figure 4, page 87). Therefore these findings support the hypothesis that the mechanism underlying both acute and delayed T2 is the same and that acute T2 has an upper limit.

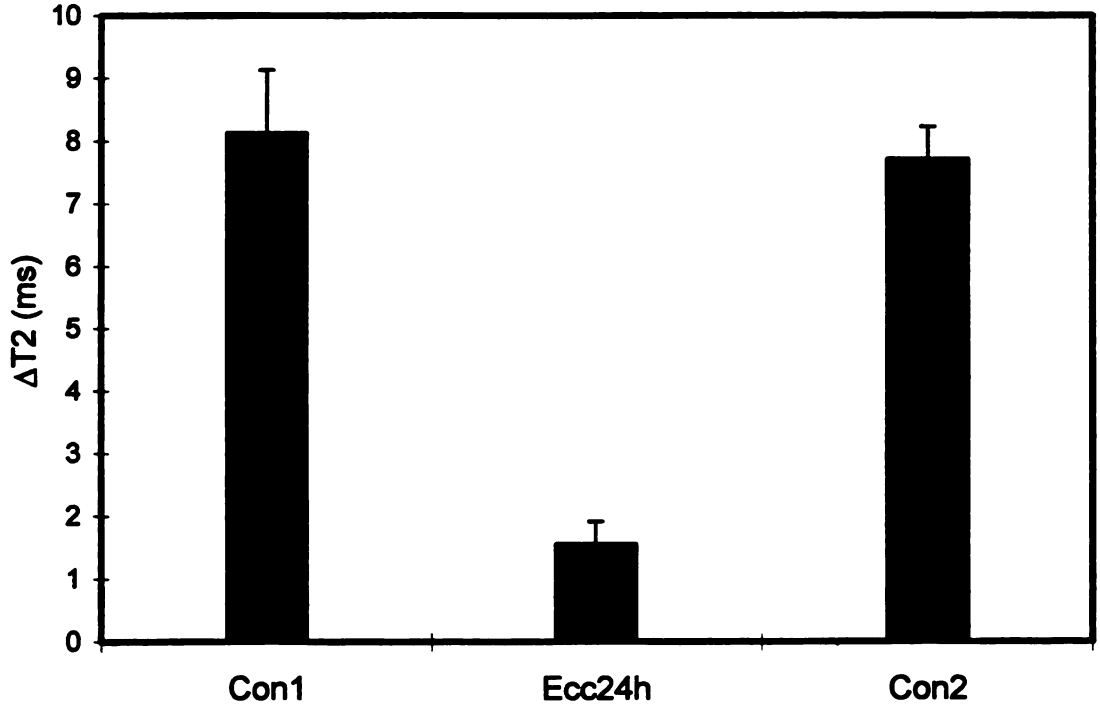

Figure 5. The change in the acute and delayed T2 following exercise. Values are means ± SE, n=8.

Figure 6 presents the ΔT2 values in relative terms (percent increase above baseline). This figure illustrates two additional points: first, the failure of T2 to return completely to baseline before the eccentric exercise (PreEcc), and secondly, the minimal magnitude of the delayed T2 increase (Ecc24h).

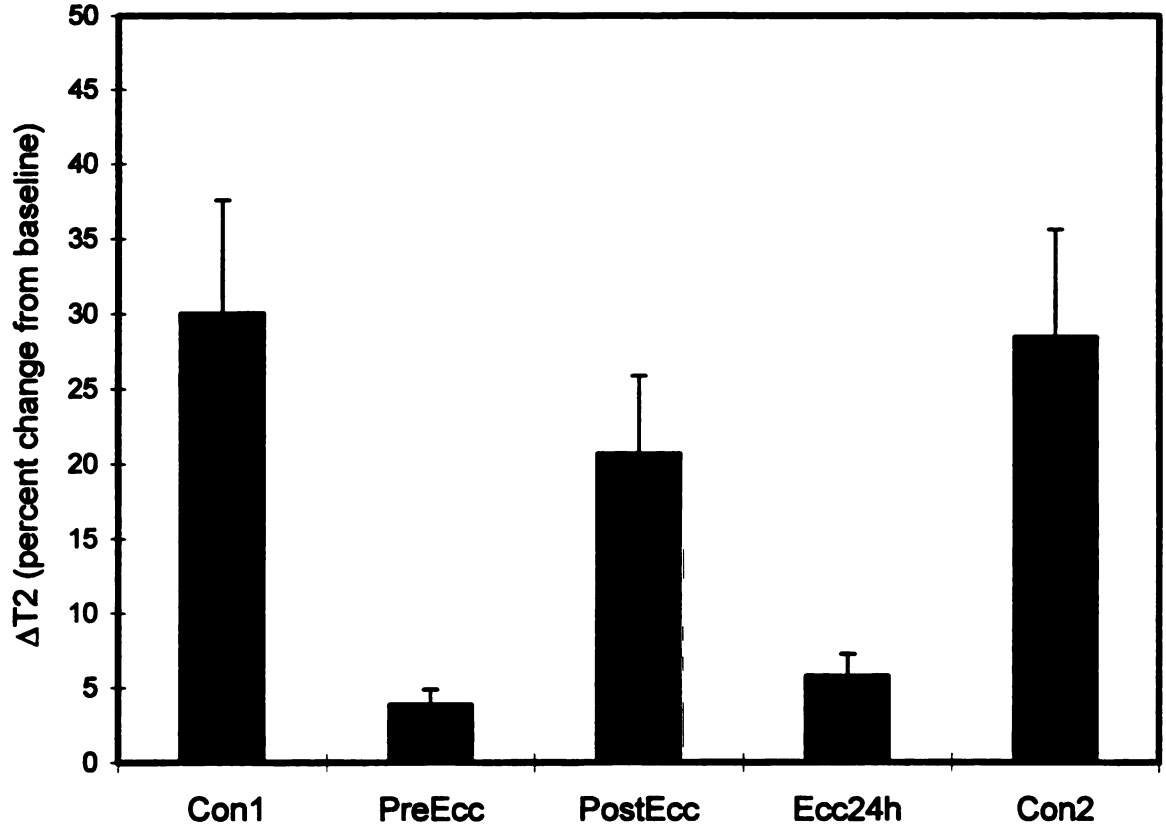

Figure 6. Percent change in T2 from baseline (PreCon1). Values are means ± SE, n=8.

To document the early time course of the delayed ΔT2 following eccentric exercise, subjects were imaged immediately following eccentric exercise and at 1, 2, 4, 6, and 24 h post exercise. Figure 7 illustrates the finding that the delayed

T2, which peaks 24-48 h post eccentric exercise, begins to increase as early as 4 h post exercise. It should be noted that the elevation evident at 1 h is likely due to the incomplete return of the acute T2 response to baseline, since the T2 value continues to fall between 1 h and 2 h.

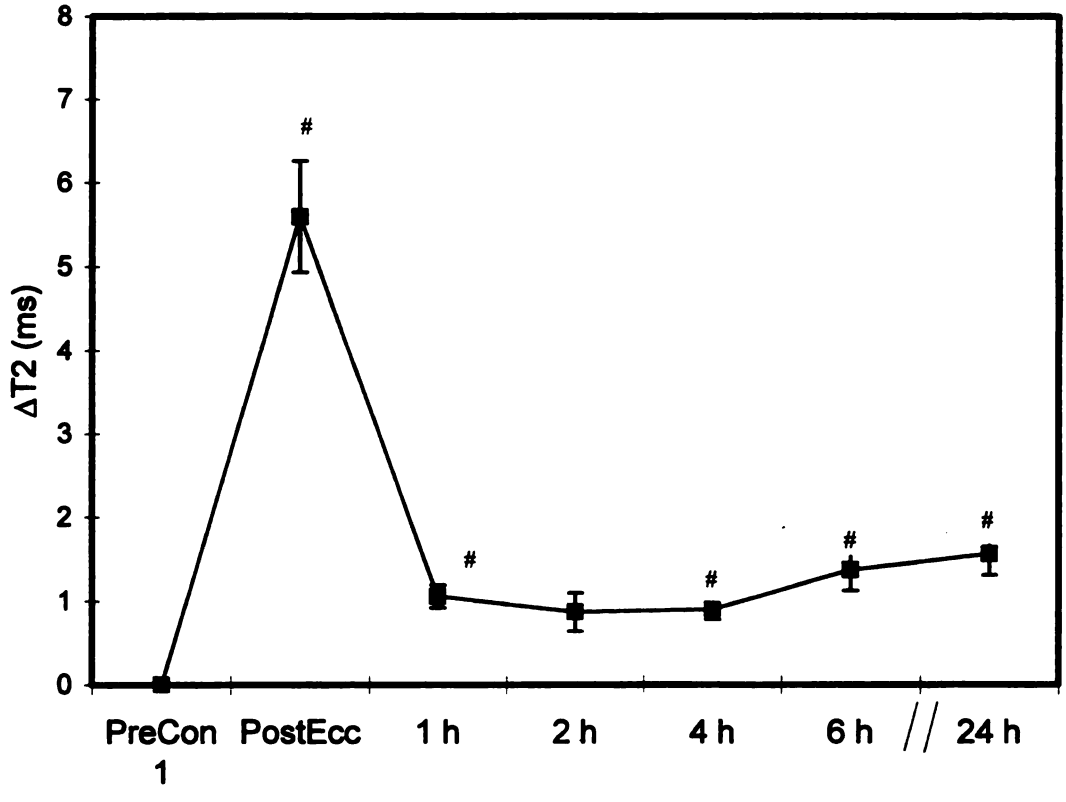

Figure 7. Change from baseline (PreCon1) in T2 following eccentric exercise. # significantly different from PreCon1 ($p<0.05$). Values are means ± SE, n=8.

Figure 8 displays regional T2 changes along the imaged length of the brachium. Relative T2 changes are given for acute responses to both concentric and eccentric exercise as well as the delayed response to eccentric exercise. These T2 numbers were derived using the entire biceps muscle compartment

rather than a small box as in the previous figures. Several investigators have suggested that most of the damage caused by eccentric contractions occurs at the belly of the muscle, whereas others report greater damage at the myotendious junctions at the ends of the muscle. Using delayed $\Delta T2$ as an indirect index of muscle damage, our findings show that the damage induced by eccentric contractions (Ecc24h, open bars in Figure 8) occurred throughout the length of the biceps muscle compartment. Using acute $\Delta T2$ as a measure of muscle recruitment, our results suggest that concentric contractions recruited more motor units compared to eccentric contractions (Con1 vs. PostEcc, Figure 8). In addition, there was a trend towards increased motor activity towards the belly of the muscle (slices 3 through 8) in both concentric and eccentric contractions.

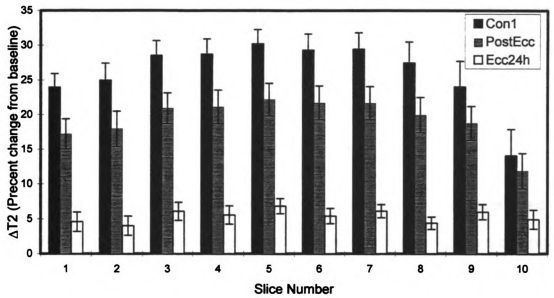

Figure 8. Percent change in acute and delayed T2 in each slice along the arm. Slices are numbered from distal (1, towards elbow) to proximal (10, towards shoulder). Values are mean ± SE, n=8.

Muscle Cross-sectional Area

MR imaging allowed us to directly assess specific muscle compartment swelling, vs. the total arm girth measurements utilized in previous studies. Since girth data suggests maximal swelling occurs near the belly of the muscle (20,50,74), we performed area analysis in image slice #6 at the middle of the longitudinal axis of the biceps muscle. The percent change in the muscle compartment cross-sectional area was calculated by subtracting the resting muscle compartment cross-sectional area from the 24 h post eccentric muscle compartment cross-sectional area and dividing it by the resting compartment cross-sectional area. The overall mean percent increase in muscle compartment cross-sectional area at 24 h post eccentric exercise was

11 ± 2% (n=8). Males showed a larger increase in muscle compartment cross-sectional area at 24 h post eccentric exercise compared to females (13% vs. 9%). However, this difference was not statistically significant.

Muscle Soreness

Muscle soreness was evaluated using a questionnaire. Subjects rated their perception of soreness in the biceps muscle on a scale of 1 (no soreness) to 10 (extremely sore) with the arm fully extended before and after each exercise session and at 24 h intervals for 7 days following the eccentric exercise (reference 20; also see Appendix B). All subjects reported no soreness prior to the start of the experiment and immediately after performing the first bout of concentric exercise. At 12 h post eccentric exercise all subjects reported mild soreness (2 ± 0). Males reported an increase in their mean soreness immediately following the second bout of concentric exercise (5 ± 0.3) compared to before performing the second bout of concentric exercise (3 ± 0.5). However, this difference was not statistically different. On the other hand, females reported similar mean soreness ratings before (3 ± 0.9) and after (3 ± 0.7) the second bout of concentric exercise. Due to the large variance in the soreness scores, these gender differences were not statistically significantly.

Figure 9 shows the mean muscle soreness ratings by gender at 24 h intervals following eccentric exercise. Males reported peak soreness earlier (48 h post eccentric exercise) compared to females (72 h), although the peak value and variation were identical in both groups (5 ± 2.0).

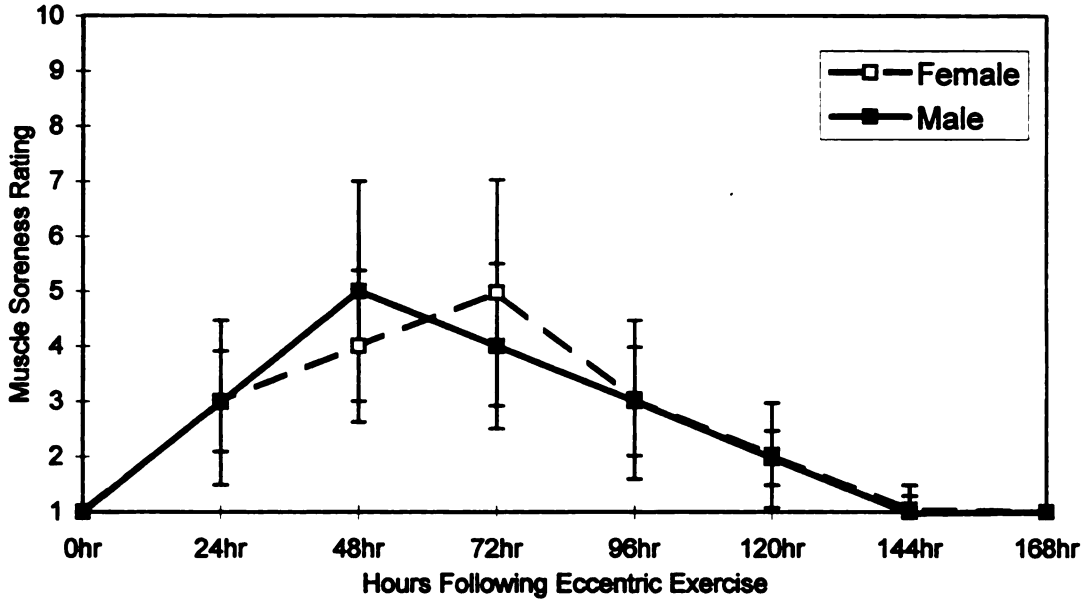

Figure 9. Mean muscle soreness ratings (1-no soreness to 10-extremely sore) at 24 h intervals following eccentric exercise. Note that the males reported peak soreness at 48 h and females reported peak soreness at 72 h post eccentric. Values are means ± SE, n=4.

According to the acute inflammatory theory, the general muscle soreness experienced during DMS is mostly due to the swelling. Figure 10 shows the plot of mean muscle soreness ratings against the percent change in muscle cross-sectional area (swelling) at 24 h post eccentric exercise. Spearman's correlation test revealed poor correlation between mean soreness ratings and the increase in muscle cross-sectional area ($r = .356$). Mean muscle soreness ratings at 24 h after eccentric exercise plotted against the 24 h T2 value are shown in Figure 11. Spearman's correlation test did not show a significant correlation between muscle soreness ratings and delayed T2 ($r = -.074$), as can be seen by the nearly flat slope of the regression line in Figure 11.

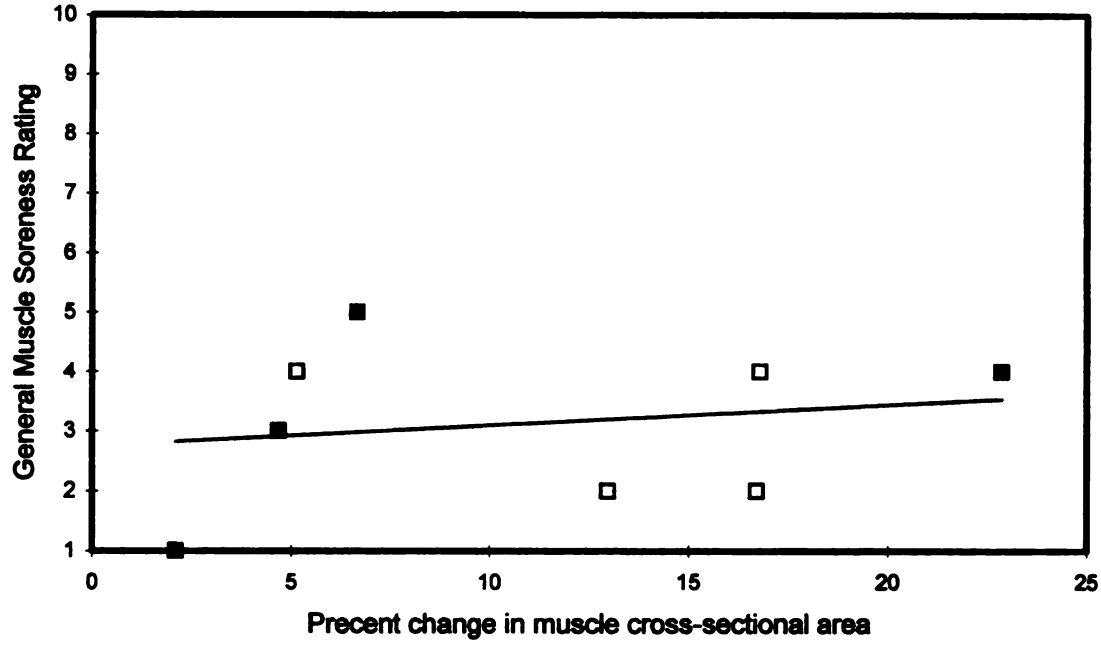

Figure 10. General muscle soreness rating (1-no soreness to 10-extermely sore) vs. percent change in muscle cross-sectional area at 24 h post eccentric exercise (■ females □males). Values are means, n=4.

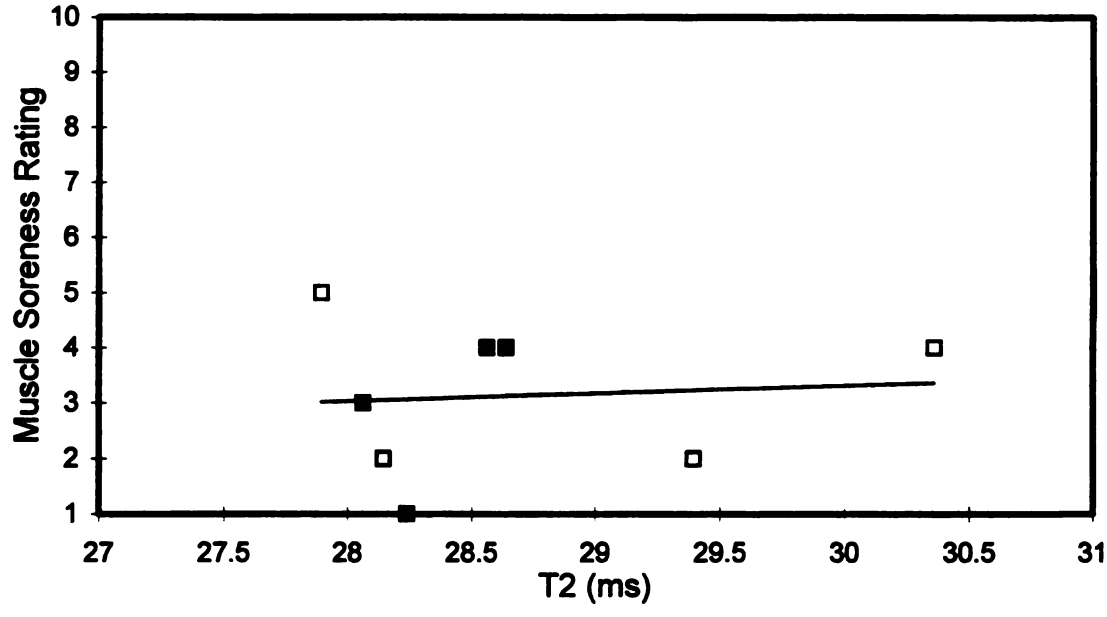

Figure 11. Mean muscle soreness ratings (1-no soreness to 10-extermely sore) vs. T2 at 24 h post eccentric exercise (■ females □males). Values are means, n = 4.

Discussion

The main purpose of this study was to examine the acute T2 response to a bout of light concentric exercise performed before (control) and after eccentric exercise had been used to induce a delayed T2 increase. In addition, the early time course of the delayed T2 contrast development was documented. As reported in Table 3, the mean resting T2 of the biceps brachii muscle was 27.1 ± 0.1 msec, in agreement with previously reported values for the biceps brachii muscle (2,71). The concentric contractions induced a significant ($p<0.05$) increase in the acute T2 ($\Delta T2 = 8.1 \pm 1.0$ ms, 30% increase; see Figures 5 and 20). This elevation also falls within the range typically reported for acutely exercised human muscle (2,71).

The acute T2 increase that occurs in response to concentric exercise has been reported by Fisher et al. to return to resting levels by approximately 25 to 35 min post-exercise (31). However, in the present study, the T2 at the end of the 2 h rest period following the first bout of concentric exercise remained slightly elevated by about 4% compared to the resting or baseline value (Figure 6). Although this increase was not statistically significant, it suggests that the muscle had not completely recovered from the first bout of concentric exercise. Since the T2 response is sensitive to small increases in motor activity, it is possible that the elevation could be due to the casual use of the exercised arm during the rest period. Thus, the fact that the subjects in the Fisher et al. study spent most of the rest period laying supine with the exercised extremity fixed in position inside the magnet may account for the difference in the acute T2 recovery.

Alternatively, the small but statistically nonsignificant difference could be due to random statistical variation in this small sample size.

As shown in Table 3 and Figures 5 and 6, the acute T2 increase following eccentric contractions was significantly less than that induced by the concentric exercise. Several investigators have also reported similar results following eccentric contractions (1,2,10,57,70). This result is not surprising given that T2 increases have been shown to scale with exercise intensity (31) and because of the well-known fact that eccentric contractions consume less energy than concentric contractions at the same load (1,10,57). Although the concentric load was actually 10% less than the eccentric load, concentric contractions likely recruited more motor units compared to eccentric contractions in each slice along the upper arm. These results are due to the fact that muscles have an inherent ability to generate greater force during eccentric contractions with a given number of active motor units (10,51).

MR images were also acquired at 24 h post eccentric exercise to confirm that the new resting T2 was elevated, a condition crucial for testing the research hypothesis. There was in fact a significant increase in the biceps brachii muscle T2 at 24 h post eccentric exercise (PreCon2) compared to baseline (PreCon, Table 2). Shellock et al. reported similar results at 24 h post eccentric exercise (70). However, the very small magnitude of the delayed T2 elevation ($\Delta T2 = 1.6 \pm 0.4$ ms, 6% increase) reduced the capacity to discriminate between the additive and nonadditive conditions.

There are two possible explanations as to why the eccentric exercise protocol employed in the present study did not cause more notable muscle trauma at 24 h post eccentric exercise. One is that the subjects did not actively resist lengthening during eccentric contractions. In another words, they simply allowed the weight to drop. As a result, the individual biceps brachii muscle fibers would not be exposed to high enough mechanical strain to cause structural damage. The second possibility is that the eccentric exercise intensity in the present study was simply not high enough to induce structural damage even when performed correctly.

After the 24 h post eccentric images were acquired, each subject performed another bout of the original concentric exercise and was then re-imaged (PostCon2, Table 2). Acute ΔT2 following the first bout of concentric exercise (8.1 ± 1.0 ms, 30% increase) was not significantly different from the acute ΔT2 after the second bout of concentric exercise (7.7 ± 0.5 ms, 28% increase). Therefore, these findings support the hypothesis that the mechanism underlying both acute and delayed T2 is the same and that acute T2 has an upper limit.

The secondary or descriptive purpose of this study was to document the early time course of the delayed T2 contrast development. Takahashi et al. observed that the T2 signal following eccentric exercise has a bimodal response with the first peak occurring immediately after the exercise (acute T2), and the second peak sometime after 12 h post-exercise (delayed T2) (81). These investigators acquired MR images before, immediately after, and at 7,15,20,30,

and 60 min and 12, 24, 36, 48, 72, and 168 h post-exercise. In the present study, MR images were acquired before, immediately after, and at 1, 2, 4, 6 h following eccentric exercise (Figure 7).

In our results the characteristic first peak immediately following eccentric exercise was evident (Figure 7). At 1 h post eccentric exercise, T2 remained elevated compared to baseline ($p<0.05$). Takahashi et al. reported similar findings at 1 h post eccentric exercise. At 2 h post eccentric exercise, T2 remained slightly elevated compared to baseline, although the T2 values had decreased from the 1 h time point. It is our belief that the increase in T2 values measured at 4 and 6 h post eccentric exercise is the early development of the second peak in the bimodal response of T2 following eccentric exercise found by Takahashi et al. at 12 h post-exercise. No previous report has documented T2 values in the interval between 1 and 12 h after eccentric exercise. However, other investigators have reported evidence of damage as early as 1.5 h post eccentric exercise based on elevated levels of serum CK activity, circulating leukocytes and neutrophils (64).

In the present study, the time course of the development of soreness was consistent with past reports (6,11,20,61). Subjects reported mild soreness at 12 h post eccentric exercise, reached peak levels 2-3 d post-exercise, and by day seven the soreness had completely dissipated. Females in our study tended to report peak soreness a day later than the males (2-3 d vs. 1-2 d). However, this is still within the time frame reported in the literature of 2-3 d for peak soreness

development. In addition, there is no evidence in the literature to suggest that the time to reach peak soreness is longer in females.

In the present study, at 24 h post eccentric exercise, males reported a mean soreness rating of 3 ± 0.9 (Figure 9). Immediately following the second bout of concentric exercise they reported an increase in soreness, 5 ± 0.3. On the other hand, females reported similar soreness ratings before (3 ± 0.5) and after (3 ± 0.7) the second bout of concentric exercise. Due to the large variance in the soreness scores neither the increase in soreness reported by males following the second bout of concentric exercise nor the gender difference was statistically different. In Hough's 1902 report on delayed muscle soreness, he noted that a second bout of exercise performed while the muscles were already sore actually decreased the sensation of soreness (45). In addition, two studies conducted by De Vries found stretching exercises performed after eccentric exercise reduced muscle soreness (23,24). However, in a more recent study, High and Howely reported that static stretching and warm-up exercises did not reduce the development of DMS (44). The discrepancy in our results, as well as between studies, on the ameliorating effect of exercise on DMS can likely be attributed to the subjective nature of the muscle soreness scores as well as to the relatively low level of soreness induced in our study.

In an attempt to better explain the delay between the immediate tissue injury and later soreness development, several investigators have proposed that the mechanism underlying soreness development is acute inflammation, based on the similar time courses of the two conditions. The time course data on the

development of swelling and sensation of pain in DMS correlated very well, lending support to the notion that the pain reported during DMS might be the result of inflammatory swelling. Unfortunately, the studies that have examined the causal role of swelling in initiating the sensation of DMS by increasing the intramuscular pressure are not conclusive (74).

In the present study, muscle cross sectional area was calculated at 24 h post eccentric to evaluate the relationship between swelling and soreness in DMS. Overall there was a significant increase in muscle cross sectional area or swelling (11 ± 2%, n = 8). Although males showed a larger increase in muscle cross sectional area at 24 h post eccentric exercise compared to females, this difference was not statistically different. If this trend towards greater swelling in males is indeed real, it could possibly stem from the larger loads lifted rather than from a gender difference per se.

Spearman's correlation test revealed only a modest realtionship between mean soreness ratings and swelling (r = 0.356) at 24 h post eccentric exercise (Figure 10). Bar et al. also observed that the peak soreness was reported at 48 h post eccentric exercise, when signal intensity and the muscle surface area had only started to increase (9). Therefore, at least at 24 h post eccentric exercise, swelling is not likely to be the primary cause of soreness.

The relationship between soreness ratings and T2 signal intensity was also evaluated at 24 h post eccentric exercise in the current study (Figure 11). The nearly flat slope of the regression line in Figure 10 suggests that there is no correlation between T2 signal intensity and soreness ratings at 24 h post

eccentric exercise. Based on our results and Nurenberg et al.'s findings that there is a high correlation between areas with increased signal intensity and ultrastructural injury, it can be concluded that the soreness experienced at 24 h post eccentric exercise is not solely dependent on the extent of the initial structural damage.

In summary, the primary findings of our study were that (1) acute T2 elevation is not additive to chronic T2 elevation, and (2) the delayed T2 response begins as early as 4 h after eccentric exercise. Secondary results included (1) the observed trend towards a later soreness peak in females vs. males, (2) a confirmation of increased T2 activation in the belly vs. ends of the muscle and (3) lack of correlation of soreness with either swelling or T2 response. The final chapter of this thesis will discuss the significance of these findings as well as present recommendations for future experiments addressing the limitations of the current study.

CHAPTER V

CONCLUSIONS, SIGNIFICANCE, AND RECOMMENDATIONS

The purpose of this study was to examine the acute T2 response to a bout of light concentric exercise performed before (control) and after eccentric exercise had been used to induce a delayed T2 increase. The secondary purpose of this study was to document the early time course of the delayed T2 contrast development. As described in Chapter 4 and summarized briefly in the following paragraphs, the results of this study demonstrated partial success in achieving both objectives. In addition to the direct outcomes of the current study, this work has led to numerous suggestions for future refinements and additional studies.

Regarding the main study objective, the results indicated that the acute T2 change was not significantly different whether concentric exercise was performed using "normal" muscle or with muscle having an elevated resting T2. The immediate interpretation of these results would be that the mechanisms of both the acute and delayed T2 response are identical, hence non-additive. However, the very small magnitude of the eccentric-induced delayed T2 change resulted in a reduction in the power of the statistical test of the mechanism

hypothesis. Suggestions for remedying this loss of conclusiveness are presented near the end of this chapter.

Documentation of the early time course of the delayed T2 contrast development was somewhat more successful, although this objective would also have benefited from an increase in the eccentric stimulus. A statistically significant elevation in T2 values were observed as early as 4-6 h post eccentric exercise, vs. the 12-24 h initial rise reported by other researchers.

Additional secondary findings included (1) the observed trend towards a later soreness peak in females versus males, (2) a larger non-significant increase in muscle compartment cross-sectional area in males, and (3) no significant correlation between soreness and either swelling or T2 response at 24 h post eccentric exercise.

In addition to these findings, a pair of unexpected observations arose from the current study. The first of these was the failure of acute concentric T2 to return to baseline values at the end of the 2 h rest period. It is conjectured that the elevated T2 at the end of the rest period was due to casual use of the exercised arm during the rest period. The other unexpected finding was that the trend towards increased T2 activation in the belly of the muscle versus the ends of the muscle following both concentric and eccentric exercise. The increased T2 activation in the belly of the muscle suggests that the metabolic demand was greater in the belly of the muscle compared to the distal and proximal ends. Further studies directly measuring the regional energy utilization within a muscle

will be useful in confirming the trend towards increased metabolic activity in the belly of the muscle as indicated by T2 values.

Significance

In "normal" muscle the increase in acute T2 has been reported to be roughly linear at submaximal intensities. The findings of this study suggest that the acute T2 elevation following exercise in damaged muscle does not exhibit a similar dependence on exercise intensity as in normal muscle, as evidence by the smaller acute T2 response to the same bout of concentric exercise (PreCon2 (at 24h) minus PostCon2).

Clinically, the findings of the current study suggest that acute T2 elevation is not a good index of motor recruitment in muscles that exhibit an elevated T2 as a result of exercise-induced damage and/or neuromuscular disease. Therefore, the acute contrast change can only be used as a measure of the extent of muscle recruitment in "normal" muscles following various activities.

The findings of relatively early initiation of the second or delayed T2 response to eccentric exercise may shed some light on the mechanism of the damage induced by lengthening contractions. This early time course supports the inflammatory theory, since initial events of the inflammatory response also begin within 4-6 hours as documented in Chapter II. The fact that this T2 response becomes visible before CK normally begins to appear in the bloodstream is not surprising, since the relatively large kinase protein must transverse the slow-moving lymph system before finding its way into the

systemic circulation. Studies measuring myoglobin, a much smaller muscle protein, have in fact documented its appearance in the bloodstream within the 4-6 hour time frame of the initial T2 response.

In addition to these two potential applications of the results of this study, the other main contribution of the current results is to the development of more refined and powerful methods of utilizing the basic method of the current study to more effectively examine the question of mechanisms of the acute versus chronic T2 responses. These suggested refinements are described in the following section.

Recommendations

The results of this investigation support the research hypothesis of identical mechanisms for the acute and delayed T2 responses to exercise as described in Chapter I. However, the small magnitude of the delayed T2 elevation ($\Delta T2 = 1.6 \pm 0.4$ ms, 6% increase) reduced the power of the statistical test of the research hypothesis. The following recommendations are offered as points to be considered in future studies, both to remedy the low statistical power problem and to address other shortcomings of the present study.

1. Since a larger increase in the delayed T2 elevation has been reported at 48 h post eccentric exercise compared to 24 h, performing the second bout of concentric exercise at 48 h post exercise will increase the capacity to discriminate between the additive and nonadditive conditions.

2. The relatively small elevation in the delayed T2 at 24 h post eccentric exercise is partly due to the fact that the eccentric exercise intensity was not high enough. Instructing the subjects to actively resist the lengthening during eccentric contractions will increase the exercise intensity. In addition, the eccentric exercise intensity can be increased by raising the resistance and the number of repetitions, for example, five sets of 10 repetitions at 110% one repetition maximum (1RM), the maximum amount of weight lifted one time with proper form. Furthermore, setting the load relative to the 1RM value rather than body weight normalizes the exercise intensity between subjects. On the other hand, the concentric exercise intensity can be relatively low, for example, three sets of 10 repetitions at 15% of the 1RM, since the acute T2 is sensitive to small increases in concentric motor activity.

3. No experimental studies have documented the acute T2 recovery following the second bout of concentric exercise performed with an elevated resting or baseline T2. Likewise, a high time resolution study of the T2 recovery in the hour after eccentric exercise has never been reported. Such a study would help clarify the magnitude and time course of the chronic or delayed T2 response, as discriminated from the acute response.

4. It has been reported that the T2 signal following eccentric exercise follows a bimodal response with the first peak occurring immediately after the exercise (acute T2), and the second peak sometime after 12 h post-exercise (delayed T2). In the current study, as expected, the first peak was observed immediately following the eccentric exercise and by 2 h post exercise the T2 had nearly

returned to baseline. It is our belief that the elevations in the T2 at 4 and 6 h post eccentric exercise is the early development of the delayed T2, which peaks 24-48 h post exercise and is an index of damage. In order to confirm that the elevation in T2 at 4 and 6 h post eccentric exercise is due to muscle damage, it might be useful to document the development of the damage using blood borne inflammatory markers within the same time frame. Several investigators have used a combination of inflammatory markers to document damage, for example, serum CK activity, serum myoglobin, and circulating leukocyte and neutrophil counts.

5. According to the acute inflammatory theory, the soreness experienced during DMS maybe the result of swelling. In the current study, the correlation between soreness scores and swelling was not significant. Although it has been claimed by some investigators that swelling is not likely to be the primary cause of soreness at 24 h post eccentric exercise, this could be due to lack of precision in the methods applied to measure swelling. In the current study, swelling was calculated by measuring the change in the biceps brachii muscle compartment cross-sectional area at 24 h post eccentric exercise, excluding the subcutaneous fat compartment around the muscle. However, most of the swelling in DMS occurs in the interstitial space. Therefore, in future swelling should be calculated by measuring the cross-sectional area of the muscle compartment plus the subcutaneous fat compartment around the muscle. It would also be useful to compare swelling of the non-exercised (triceps) muscle compartment to that of the exercised compartment. It seems likely that the increased eccentric exercise

intensity recommended in item 2 above might in fact result in detectable swelling in the biceps compartment alone, which could then de discriminated from changes in the triceps or subcutaneous fat compartment.

6. In the present study, females reported peak soreness a day later than the males (72 h vs. 48 h). However, this difference was not statistically significant due to the large variance in the soreness scores. In general, soreness scores collected using a questionnaire are subjective in nature. Nonetheless, these soreness scores may be useful in documenting soreness development across different time points of a single subject. On the other hand, comparing soreness scores of different subjects is problematic since different individuals perceive pain differently. In an attempt to develop a more objective measure of soreness, several investigators have elected to measure soreness in the following fashion. First, the investigator applies a small amount of pressure directly on the muscle experiencing DMS. Next, the subject is asked to verbally rate the perception of pain. Since the soreness experienced in DMS has been reported to worsen during movement or palpation, researchers suggest that the pressure induced soreness rating is more valid because it measures soreness in a manner similar to that experienced in DMS. Therefore, in future studies, investigators should consider collecting pressure induced soreness scores in addition to general soreness ratings. Finally, an increase in the size of the gender subgroups would also provide an obvious boost to the power to discriminate gender differences.

Summary

The current study has provided preliminary evidence supporting the hypothesis of identical mechanisms underlying the acute and delayed T2 responses to exercise, as well as novel information on the early time course of the delayed T2 response. The major value of this work, however, has been to provide a platform from which to launch future studies of the T2 response to exercise. This project has this investigator about the theory and methods of magnetic resonance imaging. It has also brought into focus for the numerous potential pitfalls in empirical research. The research design and execution recommendations resulting from the present study are already being incorporated into an expanded project which will constitute the first phase of my doctoral dissertation research. It is this investigator's expectation that the skills and insights gained from the current study will pay off in more conclusive results and more extensive potential significance of these ongoing and planned future studies.

APPENDICES

APPENDIX A

APPENDIX A

APPLICATION FOR PARTICIPATION IN EXERCISE-INDUCED DELAYED MUSCLE SORENESS RESEARCH PROJECT

Name:_____
first middle last

Address:_____
street city state zip

Local Phone: ( ) -_____ - _____ Age:_____ Date of birth:___/___/___

Height :_____ Weight :_____

Have you lifted weights or done any strength or resistance training in the past two years? Yes_____ No_____
If yes, please describe (how long ago, duration of sessions, sessions per week, over how many months/years):

Please describe your typical weekly exercise/sports/physical activity:
(If none, please indicate when you last participated in such activity, if ever.)

Do you have any surgically implanted devices (e.g. metal plates/pins/screws /rods. Pacemaker, etc.) or other metal objects such as shrapnel or braces?
Yes_____ No_____

Do you suffer from any type of acute or chronic disease?
Yes_____ No_____
If yes, please describe in detail:

APPENDIX B

APPENDIX B

GENERAL MUSCLE SORENESS QUESTIONNAIRE.

Instructions: The following is a muscle soreness scale which will allow us to measure each individual's perception of pain/soreness in the exercised arm as a result of the eccentric exercise. Rate the level of soreness in the exercise arm (left) in the following position: standing with the arm hanging completely relaxed to the side. Write the number that corresponds to the perception of soreness in the space provided next to the appropriate date and time.

1 -- No soreness

2

3 – Mild soreness

4

5 – Moderately sore

6

7 - Very sore

8

9

10 - Extremely sore

APPENDIX C

APPENDIX C

INFORMED CONSENT

MRI analysis of muscle function. Departments of Physiology and Radiology, Michigan State University.

1. I have freely consented to participate in a study using Magnetic Resonance Imaging. I understand that there are no known hazards or risks associated with Magnetic Resonance Imaging in patients who do not have a pacemaker.

2. I understand that the study involves the following procedures: I will perform repeated bouts of maximal arm curls (biceps curls) on free weights. In some cases this exercise may result in the development of muscle soreness one to three days later. MR images of my muscles will be acquired immediately before and after the exercise bouts, with a follow-up MR imaging session 2 days later. Each MR exam will require about 20 minutes, and the exercise session will last about 15 minutes. My participation may also involve taking a standard therapeutic dose of the over-the-counter anti-inflammatory drug ibuprofen (three doses of 400mg each over 24 hours, with initial dose given four hours prior to the exercise bout; each dose to be taken with milk or food). I understand that if I am not asthmatic or allergic to aspirin, and if I take the drug with milk or food, the risk of side effects is very small.

3. I understand that any information I provide, and all data collected will be treated in strict confidence. No information or data will be traceable to my name.

4. I understand that I am free to discontinue my participation in this study at any time without penalty.

5. I understand that the results of this study will be made available to me at my request. Furthermore, on my request I can receive an additional explanation of this study after my participation is completed.

6. I understand that my participation in this study does not guarantee any beneficial results to me.

7. I understand that if I am injured as a result of my participation in this research project, Michigan State University will provide emergency medical care if necessary. I further understand that if the injury is not caused by the negligence of MSU I am personally responsible for the expense of this emergency care and any other medical expenses incurred as a result of this injury.

_____ _____

Participant's Signature Date

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