This is to certify that the

dissertation entitled

MAGNETIC RESONANCE IMAGING STUDIES OF
DELAYED MUSCLE SORENESS AND
THE REPEATED BOUT EFFECT

presented by

Roop Chand Jayaraman

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Kinesiology

Major professor

Date 9/30/2001

MAGNETIC RESONANCE IMAGING STUDIES OF DELAYED MUSCLE SORENESS AND THE REPEATED BOUT EFFECT

By

Roop Chand Jayaraman

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSPHOY

Department of Kinesiology

2001

ABSTRACT

MAGNETIC RESONANCE IMAGING STUDIES OF DELAYED MUSCLE SORENESS AND THE REPEATED BOUT EFFECT

By

Roop Chand Jayaraman

The purposes of this study were, first, to compare T2 relaxation computed by the non-negative least squares algorithm (NNLS) from muscles after two bouts of damaging eccentric exercise separated by 8 wk, and second, to examine the effect of eccentric exercise-induced muscle damage on the acute T2 response to bouts of milder concentric exercise. The first aim was accomplished by obtaining conventional T2-weighted axial spin-echo MRI images and echo-planar images of the upper arm before, and 1, 2, 4, 7, 12, 21, and 56 d after eccentric bout 1 (Ecc 1), and at 2,4,7, and 15 d after eccentric bout 2 (Ecc 2). Conventional spin-echo images were also obtained immediately before and after concentric exercise bouts performed before and at 2 and 21 d after Ecc 1. The prolonged T2 increase measured by conventional imaging after Ecc 1 (46.7 ± 3.8 ms at 2 d, mean ± SE, n=6) was correlated with the presence of slow-relaxing, long T2 components (60-300 ms) in the NNLS T2 spectra. However, T2 remained elevated by 2.5 ms even after 56 d, when distinct slow-relaxing components were no longer detectable. The magnitude of slow-relaxing components was less after Ecc 2 compared to Ecc 1, consistent with the previously reported protective effect of prior eccentric exercise. The acute T2 increase after Con 2 (1.5 ± 0.5 ms, mean ± SE, n=6) was significantly less ($p < 0.05$) compared to Con 1 (3.2 ± 0.5 ms) and Con 3 (5.0 ± 0.4 ms). The results confirm that the prolonged T2

increase observed by conventional imaging methods after eccentric exercise is primarily caused by edema, and show that this edema diminishes the acute T2 change measured during exercise using conventional spin-echo imaging methods.

The initial aim of the second set of experiments was to monitor the effects of topical heat and/or static stretch treatments on the recovery of muscle damage from intense eccentric exercise. For this purpose, 32 untrained male subjects performed intense eccentric knee extension exercise, followed by 2 wk of treatment (heat, stretch, heat plus stretch) or no treatment (control, n =8/group). Isometric strength testing, pain ratings, and multiecho MR images of the thigh were performed before and at 2, 3, 4, 8, and 15 d post-exercise. Increased T2 relaxation time, muscle swelling, pain ratings, and strength loss confirmed significant muscle damage during the post-exercise period. Pain ratings and muscle volume recovered to baseline by 15 d, although strength remained lower (19 ± 4%) and T2 values higher (12 ± 2%).

Treatment modality showed no effect on extent of recovery in any measured parameter, allowing pooling of data for a follow-up study in which a subset of nine subjects returned for testing 6 wk post exercise. Results showed a persistent elevation in T2 (6 ± 2%) and a small but significant muscle volume loss (12 ± 4%), confirming a previous report on arm muscle. These changes were accompanied by a persistence of muscle weakness (17 ± 4% below baseline). These long term changes are consistent with the hypothesis that intense eccentric exercise partially or totally destroys a small subpopulation of mechanically weak fibers.

To my parents, Drs. Gopal and Manimegalai Jayaraman

ACKNOWLEDGEMENTS

First and foremost I wish to express my deep appreciation to Dr. Jeanne Foley, my advisor, for her guidance and thoughtful critiques of my work through out my graduate career. Our many discussions on teaching, research, and life in general have contributed greatly to my development both as a scientist and person.

I would also like to thank Dr. Ronald Meyer, his many hours of technical advice and guidance made my dissertation possible. He not only taught me the basic principles of magnetic resonance imaging, but also gave me down to earth advice and helped me navigate some of the rough waters during my graduate career.

I owe a special thank you to Dr. James Pivarnik, who had a hand in guiding my graduate career long before joining my committee. My interactions with Dr. Anthony Paganini, guidance committee member, contributed greatly to my development both as a scientist and an educator.

I would also like to express my deep appreciation to Dr. Laura McCabe for giving me the opportunity to learn molecular biology techniques in her lab and for the timely words of encouragement.

Thanks also to Mr. Rob Reid, and Dr. Barry Prior, fellow MRI laboratory colleagues, whose help and friendship was invaluable. I would also like to thank Jo Ann Janes, the graduate secretary, for all her help and encouragement during my graduate career. I owe a special thank you to the UCIRHS staff and co-workers.

Finally, I wish to express by deepest gratitude to my entire family, especially, mom and dad, my brother - Vijay, and my fiancée - Kathleen for their love,

encouragement, support and understanding as I pursed this goal. Special thanks to Kathleen, for being understanding of my absence at numerous wedding and family related activities.

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCION

Delayed muscle soreness (DMS) is the sensation of discomfort and/or pain experienced 24-48h following unaccustomed physical activity (6-8, 21, 45, 58, 77). DMS occurs most often in individuals who engage in sporadic, vigorous physical activity. Research has shown that eccentric or lengthening contractions cause structural damage to the muscles. It is this muscle damage that results in the sensation of pain or soreness that gradually develops in the ensuing days (6, 8, 9, 12, 46, 77).

Several studies have documented exercise-induced muscle damage after lengthening contractions by tracking different indices, including serum levels of muscle enzymes, inflammation markers, limb swelling, and decrements in muscle performance (7, 12, 17, 21, 22, 44, 53, 64, 76, 78, 80, 85, 99, 101). In addition, it is well established that performance of the same eccentric exercise 1-6 wk later produces more modest changes in the indicators of damage and muscular performance (10, 17, 21, 22, 35, 76, 79, 80). In the literature, this protective effect of a single bout of eccentric exercise is commonly referred to as the "repeated bout effect."

A review of the literature will show that several distinct hypotheses have been proposed to explain the mechanism underlying DMS and the "repeated bout effect". Smith (99) has presented a plausible argument for acute inflammation as the sole underlying mechanism of DMS. However, several key studies have failed to support Smith's hypothesis (8, 47, 95). On the other hand, Armstrong's model (8) divides the muscle injury process in DMS into four distinct stages: 1) initial, 2) autogenetic, 3)

phagocytic, and 4) regenerative. Armstrong's group has primarily focused their research on the first two stages of the model. Although the experimental data failed to support acute inflammation as the exclusive mechanism of DMS, the acute inflammatory response fits well with the phagocytic and regenerative stages proposed in Armstrong's model of DMS. Furthermore, no single explanation shows more promise than Armstrong's theory combined with Smith's acute inflammation model of DMS.

In an attempt to explain the "repeated bout effect", Armstrong proposed the "stress-susceptible" fiber theory. The "stress-susceptible" fiber model states that a sub-population of fibers in healthy muscles (<5%) nearing the end of their functional life are more susceptible to irreversible ultrastructural damage during a bout of unaccustomed eccentric exercise, which leads to degeneration of these cells in the days following the exercise. In addition, microinjury occurs in the "normal" or non-susceptible muscle cells that were recruited during the lengthening contractions but does not result in necrosis of these entire fibers. Accordingly, there is a prolonged decrease in muscle strength and range of motion, muscle soreness, and a significant increase in the serum levels of muscle proteins following the initial bout of eccentric exercise. During the degeneration-regeneration process, necrotic fibers are replaced with newly assembled fibers and the damaged ones are repaired. Therefore, the process of degeneration-regeneration should result in fewer stress-susceptible fibers to be destroyed by subsequent eccentric exercise bouts.

Recent advances in the field of magnetic resonance imaging (MRI) have allowed in vivo measurements of gross muscle recruitment, muscle damage, and changes in muscle compartment cross-sectional area during DMS (42, 43, 61, 81, 97, 103). Muscle

"functional" magnetic resonance imaging (fMRI) exploits the changes in tissue ^{1}H-NMR properties that occur with exercise-induced muscle trauma and develop more gradually with DMS (42, 43, 61, 81, 96, 103). Generally, tissues and fluids with more free water have longer T2 relaxation times and appear as brighter areas on T2-weighted images. The damaged muscles appear bright in MR images compared to undamaged or normal muscles. This contrast change in MR images is the result of increased T2 relaxation time of damaged muscle, which occurs as early as 12 h and peaks 48-72 h, post-eccentric exercise (61, 103). Several MRI studies have exploited this increase in eccentrically damaged muscle T2 relaxation time to document the time course of muscle damage after eccentric exercise, and the recovery of muscle damage in days following eccentric exercise (42, 43, 61, 97, 103). In addition, application of MRI software has allowed investigators to measure muscle volume changes during DMS (43, 61, 90). Although there is universal agreement that the increase in muscle T2 following eccentric exercise is an index of exercise-induced muscle damage, the exact physiological mechanism of the contrast change remains unresolved.

Recently, Foley et al. (43) reported a persistent elevation in muscle T2 at 56 d post-eccentric exercise. These investigators also found the increase in muscle T2 is significantly reduced in the days following the second bout of eccentric exercise, consistent with the repeated bout effect. Since edema has been reported to occur during DMS, it is plausible that the smaller increase in muscle T2 after the second bout of eccentric exercise is the result of more modest changes in the extracellular and/or vascular fluid compartments in the muscle. However, no studies to date have directly tested this hypothesis.

Furthermore, these investigators reported a significant decrease in muscle volume after recovery from eccentric exercise. If this reported volume contraction is truly the result of muscle loss, then a decrease in maximal isometric strength should also occur. However, Foley et al. did not measure isometric strength changes in the eccentrically damaged muscle.

In summary, three major questions remain unanswered related to the recent MRI studies on DMS. First, how does muscle volume loss, detected using MRI, relate to changes in functional capacity of the muscle? In our current study, we propose experiments to answer this question by determining how well muscle strength loss correlates with muscle volume loss following eccentric exercise. Secondly, no studies have tested directly either the hypothesis that the increase in muscle T2 during DMS is the result of exercise-induced edema or the hypothesis that the persistent elevation in muscle T2 long after apparent recovery from eccentric-induced DMS is due to changes in the intracellular compartment of the repaired and/or newly-synthesized muscle cells. Finally, this work will also allow the testing of the hypothesis that the smaller increase in muscle T2 after a repeated bout of eccentric exercise is due to reduced edema development after the second bout. We propose using echo-planar imaging and non-negative least squares algorithm to separate components of the multiexponential T2 decay in eccentrically damaged muscle. This analysis will allow us to localize the exercise-induced edema and measure the contribution of the exercise-induced edema to the well-documented increase in muscle T2 during DMS.

CHAPTER II

REVIEW OF RELATED LITERATURE

Nearly every adult has experienced delayed muscle soreness, especially those of us who engage in sporadic physical activity. Delayed muscle soreness (DMS) is the sensation of discomfort and/or pain following unaccustomed exercise. Regardless of the level of fitness, DMS in healthy adults occurs most commonly following repetitive, strenuous eccentric or lengthening contractions (6-8, 21, 45, 58, 77). In contrast, during an isomeric contraction the muscle length is relatively unchanged and during a concentric contraction the muscle shortens. DMS is accompanied by variable muscle damage, and the severity varies from slight stiffness to severe pain that inhibits movement. Additional studies indicate that performance of the same exercise 1-6 wk later produces more modest changes in the indicators of damage and muscular performance (10, 17, 21, 22, 35, 76, 79, 80). In the literature this effect is commonly referred to as the "repeated bout effect."

Numerous studies have documented the exercise-induced muscle damage following lengthening contractions by tracking different indices, including serum levels of muscle enzymes, markers of inflammation, swelling of limbs, and decrements in muscle performance (7, 12, 17, 21, 22, 44, 53, 64, 76, 78, 80, 85, 99, 101). More recently, several investigators have employed magnetic resonance imaging (MRI) to quantify eccentrically induced muscle damage from the elevated T2 (transverse or spin-spin) relaxation time in proton MR images (43, 81, 97, 103).

Although DMS has been studied extensively for over five decades, there is still considerable controversy regarding the pathophysiological mechanism(s) responsible for DMS. Many reports have been published regarding the time course of changes in various pathological indices associated with DMS, and it is generally accepted that the pathology associated with DMS is completely reversible. However, the relationship of the sequence and timing of the symptoms of DMS to a possible pathological mechanism remains unclear. In addition, the mechanism responsible for the repeated bout effect remains in question. The first section of this literature review discusses the symptoms of DMS. The second section examines how the repeated bout effect alters the symptoms of DMS, and the third addresses current concepts on the mechanism of DMS and the repeated bout effect. The last section will discuss the use of MR parameters to study the underlying mechanism(s) of DMS and the repeated bout effect.

Symptoms of DMS

The most commonly reported symptom of DMS is soreness in the exercised muscle; however, soreness or pain is only temporary. Muscle soreness generally increases in intensity in the first 24 h after a bout of unaccustomed and/or eccentric exercise (6, 14, 21, 22, 43, 45, 76, 79, 80). The soreness peaks at 24-72 h post-exercise and does not completely subside until 8-10 d post-exercise. Lieber and Friden (68) suggest that the muscle strain (Δ muscle length/initial muscle length) or lengthening distance is the critical factor in determining the magnitude of muscle injury, which in turn determines the intensity and time of peak soreness. On the other hand, McCully et al. (73) suggest that it is the number of lengthening contractions performed determines

the extent of muscle injury. More than likely, it is a combination of intensity and duration parameters that determines the magnitude of the muscle damage, and it is this damage to muscle that ultimately results in the sensation of soreness in the days following eccentric exercise. Time of peak soreness and the intensity of the soreness may vary between studies both because of the different exercise protocols employed on different muscles (i.e. elbow flexors versus knee flexors) and because of the large variability in the perception of pain between subjects.

A significant and persistent decrease in isometric strength accompanies the soreness following eccentric exercise (21, 22, 76, 80). The strength loss or fatigue following concentric or isometric exercise recovers within minutes to tens of minutes after the exercise. In contrast, a fraction of the strength loss following eccentric exercise can persist for many hours to days after the fatiguing bout. This persistent loss of strength may be the result of myofibrillar damage caused by the lengthening contractions. However, only a few studies have compared the long term time course of the recovery of strength with other indices of muscle damage in human subjects.

The prolonged decrease in isometric strength after eccentric exercise was once thought to be simply the result of reluctance to use the sore muscles. However, this appears unlikely, since strength actually recovers somewhat even before peak soreness develops, typically at 24-72 h post-exercise. To determine if the loss in isometric strength was due to sub-maximal activation of the sore muscles following eccentric exercise; Newham et al (76) employed the twitch interpolation technique which superimposes electrical stimulation on maximal voluntary contraction (MVC). Under these conditions, additional force will only be generated if the MVC was sub-maximal.

Because force did not increase with twitch interpolation during MVC, Newham and colleagues concluded that the subjects were able to fully activate their eccentrically damaged muscle during MVC despite any soreness. More importantly, Newham and co-workers suggest that the prolonged decrease in MVC after eccentric exercise is the result of changes in the contractile elements.

Another symptom of DMS is a reduction in the range of motion at the joint or joints that were involved in the eccentric exercise. Clarkson and others (21, 22, 80) have used the following two criteria to measure the reduction in the range of motion at the exercised joint. First, the muscle's ability to fully shorten was assessed by measuring, flexed joint angle, the angle at the joint following full voluntary flexion. The largest change in the flexed joint angle was reported immediately following eccentric exercise, suggesting that the eccentrically exercised muscles were unable to fully flex. The flexed joint angle gradually returns towards pre-exercise levels in the days following eccentric exercise. However, the flexed joint angle remained different from baseline at 10d post-eccentric exercise. The decrease in muscular strength and the inability to fully shorten muscles could be the result of over stretching of sarcomeres by the lengthening contractions. Consequently, when the sarcomeres are stretched beyond their optimum length the overlap between the actin and myosin filaments is reduced, which in turn reduces the number of cross bridges that can be formed. Recent work by Talbot and Morgan (104) supports this theory since greater muscle damage and decrement in strength were seen after eccentric exercise at longer muscle lengths. In addition, there is general agreement that increasing the lengthening distance of an eccentric contraction causes more muscle damage.

The second criterion measure arose from Clarkson and co-workers observations that muscles spontaneously shortened following eccentric exercise. To document this phenomenon, they measured the relaxed joint angle, which was measured as the angle at the exercised joint in the relaxed or resting position. Relaxed joint angle decreased (i.e., the joint was more flexed) immediately following lengthening contractions and continued to decrease in the next few days. The largest reduction in relaxed joint angle occurred at 3 d post-eccentric exercise. Unlike flexed joint angle, the relaxed joint angle recovered to baseline values by 10 d post-eccentric exercise.

Smith (99) suggests that the reduced range of motion may place the eccentrically damaged muscles in a position/resting fiber length that is optimal for healing. Smith refers to this idea as "voluntary " immobilization, which was first proposed by Clancy and Clarkson (20). One possible mechanism for this change is increased electrical activity in the damaged muscle. However, Bobbert et al. and Howell et al. (14, 57) found no such increase in the resting EMG activity at 24, 48, and 73 h after eccentric exercise. Therefore, the decrease in the relaxed joint angle is not the result of active muscle shortening. Another possibility is increased intracellular calcium, such that myofibrillar activity is present in the absence of electrical activity. However, there is at present no evidence that cytoplasmic calcium is sufficiently elevated in the damaged fibers to result in contraction of myofibrils. It is also possible that the decrease in the relaxed joint angle could be simply due to the mechanical effect of swelling, i.e. the muscle shortens in length to accommodate the increase in volume.

Swelling of the exercised limb is another commonly reported symptom of DMS following eccentric exercise. Numerous studies have reported an increase in the

circumference of the eccentrically exercised limb in the days following exercise (21, 22, 43, 80). Peak swelling occurred at 4-5 d post-eccentric exercise. The exercised limb circumference typically returns to baseline values by 10 d post-exercise. It is important to keep in mind that in many studies swelling was documented by measuring the circumference of the exercised limb with a tape measure. Therefore, the sensitivity of the instrument is relatively poor. In addition, this anthropometric technique was unable to localize the swelling within the exercised muscle compartment (i.e. intracellular versus extracellular fluid).

Morphological Changes Associated with DMS

In the first published report on DMS in 1902, Theodore Hough suggested that the pain and decrements in muscular performance were the result of ultrastructural damage induced by lengthening contractions (56). In order to obtain direct evidence of muscle damage following eccentric exercise, many investigators have employed light and/or electron microscopic techniques to examine changes in muscle biopsy samples. Most of the studies have reported significant morphological alterations in muscle at 24 to 48 hr following eccentric contractions but not after concentric or isometric contractions in humans and rats (8, 38, 44, 46, 47, 73, 77, 82). However, there are only a limited number of studies that have reported significant but minor cellular damage immediately following eccentric exercise (8, 47, 77, 82). Therefore, the immediate muscle damage following eccentric exercise is relatively minor compared to the damage observed in the ensuing days. Friden et al. (46) reported disorganization of myofibrillar Z-bands in human soleus m. 3 d after running down stairs. Friden and colleagues found Z-band

broadening, smearing, and in some fibers, profound Z-band disruption. Adjacent Z-bands appeared to be out of register and followed a zigzag pattern along the length of the damaged fibers. Friden and co-workers also observed that within a small percentage of damaged sarcomeres the A-bands were out of register and the thick or myosin filaments were missing.

Newham et al. (77) reported similar results in the eccentrically damaged human quadriceps muscle at 24-48 h after a 20 min step exercise. No abnormalities were reported in the human quadriceps muscle after concentric exercise. Moreover, Newham et al. presented quantitative light microscope data on human biopsies taken at various times after exercise. These researchers characterized myofibrillar damage into three distinct categories: 1) focal - areas of disruption affecting one or two adjacent myofibrils and one or two adjacent sarcomeres, 2) extensive - more than two adjacent sarcomeres and myofibrils with disruption, or more than ten focal areas within one muscle fiber, and 3) very extensive -- more than one extensive area of damage. Using these criterion, Newham and co-workers found that immediately after the eccentric exercise, 16% of the fibers counted showed focal changes, 16% had extensive damage, 8% had very extensive changes, and 58% of the total counted fibers appeared normal. In the samples collected at 30 h after eccentric exercise 6% showed focal changes, 23% had extensive disruptions, 28% had very extensive disruptions, and 45% of the total counted fibers appeared normal. Newham et al. suggested that the lesions observed immediately after the eccentric exercise were precursors of the more severe damage reported in the days following exercise. According to Newham and others, the initial injury exposes the

structural components to endogenous hydrolytic proteases, and these activated proteases autolyze the damaged muscle cell.

In another study, Friden and co-workers (47) collected muscle biopsies from human vastus lateralis m. before and at intervals after eccentric exercise on a modified bicycle ergometer. This group reported that 12% of the fibers counted showed focal disruptions of the striated band pattern at 1 h, 52% on day 3, and 32% on day 6 post-eccentric exercise. Friden et al. interpreted these results as evidence that the myofibrillar damage developed gradually in the days following eccentric exercise, and moreover, the damaged fibers had somewhat recovered by 6 d after eccentric exercise. However, some investigators have questioned whether the needle biopsy sample (approximately 50 fibers per biopsy) is representative of the damage across the entire length of the muscle. As a result, many investigators have used animal models, specifically the rat model, to systematically sample the muscle to document the predominant lesion and its distribution within the entire eccentrically exercised muscle.

Armstrong et al. (8) reported myofibril damage immediately following eccentric exercise, and progressively greater muscle degeneration at 24 and 48 h in approximately less than 5% of the muscle fibers. However, Armstrong et al. did not incorporate systematic sampling to document the different type of lesions or their predominance. This oversight was addressed in a later study by Ogilvie et al. (82). This group reported three types of lesions in rat soleus m. 30 min after running downhill: 1) focal disruptions of the A-band, 2) Z-line dissolution, and 3) clotted fibers. In addition, they reported that the 89% of lesions in the downhill running group and 97% of lesions in the level runner group were A-band disruptions. Furthermore, these investigators found that the A-band

lesion density was significantly higher in the soleus m. of the downhill runners compared to the level runners. The highest incidence of A-band lesions were found in the distal half of the soleus m. of downhill runners. On the other hand, in level runners the highest incidence of A-band lesions was reported in the proximal half of the soleus m.

These investigators suggest that the A-band lesions are the initial observable injury in muscle. However, this report failed to address the differential location of the A-band lesions between the eccentrically exercised muscle (distal region of the soleus m.) versus the concentrically exercised muscle (proximal region). This difference could be the result of differential recruitment of motor units during a lengthening contraction versus a shortening contraction. Another possibility is that the muscle fibers near the musculotendon junction experience greater active strain/stress during eccentric contractions, which, in turns, leads to greater A-band lesions.

In addition, the dominance of A-band lesions in the rat soleus m. after downhill running versus the dominance of Z-band streaming in the human vastus lateralis m. after lengthening contractions could be the result of species difference in skeletal muscle architecture. Another possibility is that the differential damage in the rat muscle could be due to the fact that the exercise intensity (downhill running) was much greater in the rat experiments compared to the human exercise protocol.

McCully and Faulkner (72) found many fibers in the rat extensor digitorum longus m. that appeared abnormal at 1 d post eccentric exercise. However, the number of fibers per cross-sectional area in the eccentrically damaged muscle was not significantly different from control. These results suggest that initial damage immediately following concentric and eccentric exercise was not different. However, two to four days after

lengthening contractions, McCully and Faulkner reported significant macrophage infiltration and muscle degeneration. Peak macrophage infiltration and muscle degeneration were reported on day 3 and by day 4. There was also evidence of muscle regeneration, such as the appearance of myoblasts and myotubes. Therefore, it appears that in a small percentage of fibers the initial damage leads to fiber necrosis.

In short, the histological evidence from human and rat studies suggest that following the initial minor injury, further degradation of the injured fibers occurs in the ensuing hours (8, 46, 47, 73, 77). The sensation of DMS appears to be related to the delayed muscle and/or connective tissue disruption that takes place in the days following lengthening contractions (7, 99). Of particular interest is the fact that peak pain correlates well with peak damage based on the histological changes; however, the strong correlation between muscular strength loss and ultrastructural damage observed 24 - 48 h post eccentric exercise begins to deteriorate after day 4 (1). Consequently, one could argue that the persistent loss in muscular strength reflects incomplete regeneration and/or re-enervation of the newly synthesized myocytes.

Biochemical Markers

In a continued attempt to characterize the initial damage immediately following eccentric exercise versus the secondary damage in the ensuing days, many investigators began to study biochemical markers of muscle damage. Serum concentrations of muscle proteins such as creatine kinase (CK), myoglobin, and slow-twitch skeletal (cardiac beta-type) myosin heavy chains (MHC) have been used as indirect markers of damage following eccentric exercise (21, 64, 78, 85, 95, 101).

The serum level of several muscle proteins has been reported to increase after exercise (21). The general consensus is that the increased serum levels of muscle proteins reflect an increase in release and/or leakage from the damaged muscle cells. CK is the most commonly studied muscle protein and is considered the "gold standard" biochemical marker of skeletal muscle damage following eccentric exercise. CK is a dimeric enzyme that catalyzes the reversible phosphorylation of ADP by creatine phosphate to form ATP and free creatine (Cr). The two isoforms of CK, M (muscle) and B (brain) combine to form three principle dimeric cytoplasmic isoenzymes: CK-MM in skeletal muscle, CK-MB in cardiac tissue, and CK-BB in the brain (55). Within skeletal muscle, the majority of the CK is in the CK-MM isoform (approximately 90% of the total CK), with the remaining activity being accounted for by the mitochondrial isozyme. Furthermore, three subisoforms of CK-MM have been identified: CK-MM1, CK-MM2, and CK-MM3. CK-MM1 is the only subisoform found within the muscle cell; the other two isoforms can be found in serum. The cleavage of either a lysine or arginine residue from the carboxyl terminus on the M monomer of CK-MM1 by carboxypeptidase N will yield CK-MM2. The additional release of either amino acid residue from the second M monomer of CK-MM1 yields CK-MM3.

Total CK activity in serum is the most commonly measured parameter for documenting muscle damage following eccentric exercise. Intensity, duration, and the type of exercise all influence the amount of CK released into the blood. After eccentric exercise, CK is released into the circulatory system and remains elevated for several days following the eccentric exercise. Generally, peak total CK activity occurs on the third or fourth day following lengthening contractions, and remains elevated for 6-7 d.

Clarkson and others (17, 21, 22, 59, 76, 78-80) have published numerous reports using CK as an indirect measure of muscle damage following eccentric exercise. This group found that, even after controlling for the exercise intensity, there was a large intersubject variability in the total CK response following exercise. Furthermore, there was no systematic relationship between the peak total CK response and the other indirect indicators of muscle damage (i.e. muscle soreness, isometric strength, relaxed arm angle, and flexed arm angle). According to Clarkson et al. the low correlation between peak CK response and other indirect indicators of damage could be due to the large variance across all indirect measures of damage. In addition, serum activity of CK depends on the rate of enzyme clearance by the reticuloendothelial system, not just on release from the damaged muscle fibers.

Hyatt et al. (59) found significant leakage of the muscle specific CK into the blood stream following eccentric exercise. In the hours following eccentric exercise, CK-MM1 is modified into the two other subisoforms: CK-MM2 and CK-MM3. These investigators studied the ratio of CK-MM1:CK-MM3 to separate CK-MM1 release from clearance. Based on previous studies, they argue that if CK-MM1 release and its clearance are equal then the ratio will be equal to one, whereas ratios greater than 1 indicate that release is larger than clearance, and ratios less than 1 indicate that clearance rate is faster than release rate. Haytt et al. reported a significant increase in the CK-MM1:CK-MM3 ratio in the hours following eccentric exercise, suggesting that release exceeded clearance. Peak CK-MM1 release occurred at 48 h post exercise. By day 4, the ratio was less than one which indicates that the rate of clearance had overtaken the rate of CK-MM1 release from muscle. This report showed that there was a significant

delayed release of CK-MM1 from the eccentrically damaged muscles and that the rate of clearance was not able to keep up with the release rate.

Myoglobin (Mb) is another biochemical marker of muscle damage. However, the profile of the Mb response after eccentric exercise is very different compared to the CK-MM response. Mb level peaks within 4 h post eccentric exercise (10, 70). This earlier appearance results from the fact that Mb is a smaller protein than CK-MM (17,200 vs. 41,300 Daltons). Thus it is able to exit the cell through smaller lesions in the sarcolemma that are present immediately following eccentric exercise. The Mb response is therefore much more sensitive to the initial damage after eccentric exercise.

Another protein that is present in very high concentration in striated muscle is myosin. Myosin is a hexameric contractile protein made up of 4 light and 2 heavy chains. After eccentric exercise, there is an increase in plasma concentration of slow-twitch skeletal myosin heavy chain (MHC) fragments (70). The temporal profile of the MHC response is similar to the CK-MM response following eccentric exercise.

Despite the availability of these other serum protein markers, neither is used nearly as often as CK in documenting exercise induced damage. There are several reasons for this: 1) there is a large amount of variability in the Mb and MHC compared to the CK response between subjects, 2) the correlation between the amount of damage and the Mb and MHC response is poor, and 3) in the exercise science literature, CK is considered the gold standard biochemical marker of muscle damage because the biochemical technique required to measure serum enzyme activity is readily available and is much easier compared to the techniques involved in measuring the specific protein levels in serum (gel electrophoresis).

Repeated Bout Effect

The foregoing discussion has described reported discrepancies in the timing of the development of muscle damage, soreness, CK release and other markers of exercise-induced trauma. Researchers have also observed differences in the timing and magnitude of the responses to subsequent bouts of eccentric exercise after an initial damaging bout. A protective effect against muscle soreness has been noted for both concentric and eccentric exercise training (12, 22); the specific protective effect produced by a single bout of eccentric exercise has been commonly referred to as the "repeated bout effect".

Nosaka et al. (80) examined the repeated bout effect by having the subjects perform two bouts of eccentric exercise of the forearm flexors. Subjects were randomly assigned to two groups. Group One, the second bout of eccentric exercise was performed 6 wk after the first bout. Subjects in Group Two performed the second bout of eccentric exercise 10 wk after the first bout. In addition, a subset of the subjects, three from each group, performed the same eccentric exercise (bout 3) 6 months after bout 1.

All the subjects in the 6 wk group reported less peak soreness after bout 2 compared to bout 1, and the soreness rapidly subsided within 3-4 d after bout 2 compared to 8-10 d after bout1. However, peak soreness reached bout 1 levels after exercise bout 2 in the 10 wk group and after bout 3 in the 6 months group. Isometric strength loss averaged about 40% after bout 1 and bout 2 in both groups. However, the 6 wk group showed a more rapid recovery in isometric strength after bout 2 compared to bout 1. On the other hand, the 10 wk group did not show any improvement in the rate of recovery between bouts 1 and 2. Similar results have been reported by others following repeated

bouts of eccentric exercise (35, 76, 79). The patterns of isometric strength loss and recovery after bout 3 (6 months after bout 1) did not differ significantly from bout 1.

Flexed joint angle and relaxed joint angle were also measured after all bouts in the above-mentioned study. These investigators found that the largest change in flexed joint angle occurred immediately after bouts 1 and 2 for both groups (6 and 10 wk). Nonetheless, the 6 wk group showed a quicker recovery compared to bout 1 but no difference between bouts in the 10 wk group. As expected, the relaxed joint angle decreased significantly immediately after the first and second bout of eccentric exercise in both groups (6 and 10 wk). However, the decrease in the relaxed joint angle was significantly less after the second bout compared to the first bout in both groups. There was no difference in the relaxed joint angle or the flexed joint angle after bout 3 (6 months after bout 1) compared to bout 1.

This group also measured total serum CK levels before, immediately after and every day for 5 days after bout 1, 2, and 3. Both groups (6 and 10 wk) showed a similar and significant increase following the first bout of eccentric exercise. The total CK increase after bout 2 was significantly less then the bout 1 change in both groups. Unlike the other indirect markers of muscle damage, the CK-MM response after bout 3 (6 months) was still significantly less than bout 1 but higher than after bout 2.

In another study, Hyatt et al. (59) separated CK-MM1 release from clearance by measuring the ratio of CK-MM1:CK-MM3 following a second bout of eccentric exercise performed 6 d after bout 1. This group reported a decrease in CK-MM1 release and an increase in the clearance rate after the second bout of eccentric exercise compared to the first bout response. These investigators interpreted the decrease in CK-MM1 release as

evidence that the injury induced by bout 2 was significantly less severe compared to bout 1. Furthermore, Hyatt and co-workers found that the increased rate of clearance contributed to the decrease in total CK-MM response after bout 2, suggesting an adaptation in the clearance process. Lastly, Hyatt et al. documented swelling by measuring the circumference of the exercised limb in the days following both bouts of eccentric exercise. As expected, swelling was significantly reduced after the second bout of eccentric exercise compared to bout 1.

Recently, Nosaka and Clarkson (79) using the same methods from their earlier study, plus ultrasound imaging, reported that repeated bouts of the same eccentric exercise performed 3 and 6 d after the first bout did not exacerbate the damage nor did it affect the rate of recovery. Ultrasound imaging is a noninvasive technique that provided these investigators with an in-vivo measurement of the exercise-induced muscle damage. Ebbeling and Clarkson (35) reported similar results after repeated bouts of the same eccentric exercise performed 5 and 14 d after the first bout.

Taken together, these results suggest that an adaptation response had taken place prior to full recovery of muscle function and ultrastructural damage after the initial bout. Moreover, this adaptive response occurred as early as 3 d after the first bout of eccentric exercise. However, researchers studying the repeated bout effect have not shown direct histological evidence of the reduced muscle damage after the subsequent bouts of eccentric exercise.

In summary, a single bout of eccentric exercise results in temporary ultrastructural muscle damage, delayed soreness, performance decrements, and an increase in serum activity of CK-MM. Complete recovery of muscle damage and

function after a single bout of eccentric exercise can take up to 10 d or longer. During the period of recovery, an adaptation or training response occurs providing a protective effect against damage from a subsequent bout of eccentric exercise. Lastly, the protective effect of a single bout of eccentric exercise, based on the reduced CK-MM response, has been reported to last up to 6 months. A review of the literature will show that several distinct hypotheses have been proposed to explain the mechanism underlying DMS and the "repeated bout effect". No single explanation shows more promise than Armstrong's model combined with Smith's acute inflammation model of DMS (6).

Mechanisms of DMS

Armstrong's model (8) divides the muscle injury process resulting from eccentric exercise into four stages: 1) initial, 2) autogenetic, 3) phagocytic, and 4) regenerative. Armstrong and co-workers have primarily focused their research on the first two stages. Conversely, Smith (99) has presented a convincing argument for acute inflammation as the underlying mechanism of DMS. However, several key studies do not support Smith's hypothesis that acute inflammation is the sole underlying mechanism of DMS (8, 47, 95). On the other hand, the acute inflammatory response could explain the phagocytic and regenerative stages proposed in Armstrong's model of DMS.

Initial and Autogenetic Stages of DMS

The most common belief in the lay exercise community is that DMS is the result of lactic acid accumulation in the exercised muscle. However, this metabolic hypothesis has been rejected by exercise scientist on the basis of two findings: 1) the metabolic cost

of lengthening contractions is significantly less than for concentric contractions at the same power output, and 2) eccentric exercise produced less lactic acid than concentric exercise at the same power output (7). These findings are not consistent with the common observation that eccentric exercise is much more likely than concentric exercise to induce DMS.

During an eccentric contraction, fewer motor units are recruited to generate the required force (13). As a result, the force generated during an eccentric contraction is distributed across a smaller cross-sectional area of muscle compared to concentric contractions. This increase in active stress/strain may be the primary cause of ultrastructural damage in the muscle. Furthermore, Armstrong et al. (8) suggest that a sub-population of fibers (<5%) nearing the end of their functional life in healthy muscles are more susceptible to this increased active strain (Δ muscle length/initial muscle length) and/or stress (force generated/cross-sectional area of the active muscle). However, McCully and Faulkner (72, 73) have shown that the eccentric exercise-induced injury is not limited to a small population of susceptible fibers. Following eccentric exercise, McCully and Faulkner reported ultrastructural damage in 34% of the total muscle cross-sectional area, of the extensor digitorum longus m. in mice, at 3 d after eccentric exercise. However, the "susceptible" fibers may be more likely to autolyze as a result of the damage, whereas other fibers recover.

In addition, Friden et al. (47) reported histological signs of damage in 52% of the fibers biopsied from the human vastus lateralis m., at 3 d post eccentric exercise. Histochemical analysis revealed that the ultrastructural damage occurs primarily in the type IIb fibers. Therefore, these results suggest that either more of the type IIb fibers

were near the end of their functional life, or that type IIb fibers were selectively recruited during the eccentric exercise.

The increased active strain and stress results in physical disruption of the sarcolemma, sarcoplasmic reticulum, myofibrils, and causes the release of phospholipase A_2 onto the phospholipid bi-layer causing degradation of the structural components. Moreover, the extracellular Ca^{+2} enters the cytosol down its concentration gradient through the small lesions in the sarcolemma. Armstrong (6) suggests the following as a possible sequence of events following the influx of Ca^{+2} in the initial and autogenetic stages of injury. The increase in the intracellular Ca^{+2} concentration further activates the phospholipase A_2 activity. The increased phospholipase A_2 activity results in the production of lysophospholipids, leukotrienes, and prostaglandins which, in turn, cause the degradation of contractile and non-contractile proteins. In addition, increased cytosolic Ca^{+2} concentration activates the endogenous proteases and stimulates the synthesis of free radicals. The end result is the peroxidation of lipids in the cell membrane. Further, the increase in cytosolic Ca^{+2} concentration also activates the Ca^{+2} dependent proteolytic enzymes that preferentially degrade Z-discs, troponin, and tropomyosin.

The first step in Armstrong's proposed sequence, however, is not supported by a review of the chemically-induced muscle damage literature which suggests that the increase in cytosolic Ca^{+2} concentration after eccentric exercise is well within the physiological range (7, 32, 69). In addition, when the intracellular Ca^{+2} concentration was increased using pharmacological agents, muscle cells were able to buffer the chemically induced increase in cytosolic Ca^{+2} concentration. Therefore, it appears that

the increase in intracellular Ca^{+2} concentration following eccentric exercise is within the physiological range and does not activate the endogenous Ca^{+2} mediated destructive pathway.

However, it is entirely possible that the transient changes in the cytosolic Ca^{+2} concentration could initiate the Ca^{+2} mediated destructive pathway. Therefore, a persistent increase in cytolosic Ca^{+2} may not be necessary to initiate the Ca^{+2} mediated destructive pathway. Furthermore, the time resolution of the standard measurement techniques might not be adequate to detect this rapid increase in cytosolic Ca^{+2} concentration. In addition, small lesions in the sarcolemma and the myofibrils could expose contractile and structural proteins to proteases located on the sarcotubular network. These proteases would then hydrolyze the structural proteins, resulting in the autolysis (18). The progressive deterioration of the sarcolemma leads to the diffusion of intracellular components across the interstitial space and into the plasma. Many of these intracellular components, especially prostaglandins, attract neutrophils and monocytes that convert to macrophages. Lastly, the accumulation of macrophages is required for the damaged muscle cell to proceed to the phagocytic stage (19).

Phagocytic Stage

Much of the evidence supporting the existence of a phagocytic stage was proposed by Smith's acute inflammation model of DMS (99). The following section describes the classic inflammatory response and the evidence relating this process to the phagocytic stage in Armstrong's model of DMS.

The classic model of inflammation involves two separate but related processes: 1) the vascular response, and 2) the cellular response. First, during the vascular response of inflammation, the arterioles near the site of injury vasoconstrict for approximately 5-10 min following injury. After several hours, the vasoconstriction is followed by vasodilatation. This vasodilatation results in increased blood flow and vascular permeability in the capillary bed surrounding the injury. The end result of this increase in vascular permeability is edema and pain in the injured area.

Second, the cellular response aspect of inflammation involves the activation of leukocytes. Neutrophils and monocytes are the two predominate leukocytes involved in the inflammatory response. In the hours following injury, there is a dramatic increase in the neutrophil count. The magnitude of the neutrophil response depends on the severity of the damage. Generally, peak neutrophil concentration is reported 1-4 h post-injury and declines rapidly in the ensuing hours. Neutrophils are the first phagocytic cells to arrive at the site of damage. Upon arrival, the neutorphils begin to digest necrotic cells and cellular debris. Several hours later, monocytes exit the circulatory system and enter the injured muscle cells. Monocyte concentration remains elevated for 48 h post-injury. The monocytes mature into macrophages at the site of injury and digest dead cells and damaged cell fragments.

Swelling is a cardinal symptom of acute inflammation and is the result of increased vascular permeability in the damaged tissue. Stauber et al. (102) have reported increased permeability in the rat skeletal muscle following eccentric exercise. Other studies have reported an increase in limb circumference at 24, 48, and 72 h after eccentric exercise (21, 22, 43, 80). The time course of the development of swelling/edema during

DMS parallels the development of swelling during acute inflammation. Smith suggests that the increased intramuscular pressure, due to swelling, mechanically stimulates prostaglandin E series (PGE_2) – sensitive pain receptors. However, most studies have reported that peak swelling occurs at a time when pain is recovering. For example, Foley et al. (43) reported peak swelling at 4-7 d and peak pain at 48 h after eccentric exercise. Many other studies have reported similar results (e.g., (21, 22, 80)).

Although, it is tempting to attribute the sensation of soreness to swelling, the data does not support a causal relationship between swelling and pain. Alternatively, certain chemicals can activate the pain afferents, mostly type III and IV nerve fibers; for example, histamine, acetlycholine, bradykinin, potassium, serotonin, and PGE_2. The inflammation literature suggests that the most likely candidate is PGE_2. PGE_2 per se does not stimulate the pain afferents, rather it induces a sate of hyperalgesia in the nociceptors. Smith and co-workers have reported a similar time course for the increase in PGE_2 and soreness after eccentric exercise: both peak at 24-48 h post eccentric exercise. Interleukin-1, Ca^{+2}, and macrophages have been proposed as possible candidates for the stimulation of local PGE_2 production. The exercise-induced muscle damage literature suggests that the accumulation of macrophages is most likely responsible for the increased biosynthesis of PGE_2.

Armstrong et al. (8) reported significant macrophage accumulation in rodent muscles after downhill running but found minimal neurtrophil infiltration. In contrast, Kuipers et al. (65) found significant margination and infiltration of neutrophils in the soleus m. of the rat between 0-2 h post eccentric exercise. However, Friden et al. (47) did not find invasive macrophages in muscle biopsies taken from the vastus lateralis m.

of human subjects after eccentric exercise. In contrast, Jones and others have observed the presence of invasive macrophages in the muscle of human subjects following eccentric exercise (63, 94). This discrepancy between studies is partly due to the fact that muscle biopsies require the removal of tissue from the belly of the muscle. Therefore, if the damage was initiated in the myotendinous junction, the damage will only reach the belly of the muscle at a later time (8). Furthermore, the above-mentioned studies employed a variety of different eccentric exercise protocols, which could have contributed to the discrepancy in the results. Smith et al. (100) reported significant increases in peripheral neutrophil count at 1 and 2 h after 40 minutes of downhill running. These investigators propose that the increase in peripheral white blood cell count is the result of the increased levels of circulating polymorphs (a type of neutrophil), which has been reported to increase during acute inflammation. More recently, Croisier et al. (26) reported similar increases in polymorphonuclear neutrophil activation after eccentric exercise.

Effects of Anti-Inflammatory Treatments on DMS

In a continued effort to determine the role of acute inflammation in the etiology of DMS, several investigators studied the effect of non-steriodal anti-inflammatory drugs on DMS. The data from these studies suggest that anti-inflammatory drugs reduced DMS when the eccentric exercise recruited small muscle groups for a short duration of time, i.e. lengthening contractions of the elbow flexor muscle group. On the other hand, anti-inflammatory drugs apparently do not reduce DMS following downhill running or cycling (30, 31, 50, 52, 65). However, these studies did not measure the markers of

inflammation; rather, they measured the symptoms of DMS (i.e. soreness and muscular performance).

Clinicians in sports medicine often use superficial heat and cold in the treatment of musculoskeletal injury. Heat and cold treatments have been shown to alter the circulatory response and intravascular hydrostatic pressure in injured muscles, with the overall desired effect being reduced accumulation of fluid or swelling in injured muscles (75). Immediate cold application is recommended during the acute stage of injury to decrease bleeding, capillary effusion, pain, and inflammation. On the other hand, heat application is recommend during the healing process to increase vascular and lymphatic flow. In addition, heat application improves the range of motion through increased collagen extensibility. Like therapeutic cold application, heat also reduces pain through sensory stimulation of the skin.

Most studies have found that cold application after eccentric exercise provided temporary pain relief (60, 106). Kuligowski et al. (66) recently reported that cold whirlpool and contrast therapy (alternating cycles of hot and cold), were more effective than warm whirlpool and no treatment in alleviating the signs and/or symptoms of DMS. In addition, Prentice showed that cold plus static stretching was superior to heat followed by static stretching in reducing muscle pain and increased muscle relaxation (88). The limited available evidence suggests that heat application does not alleviate the signs and/or symptoms of DMS.

To date no studies have directly tested the effectiveness of therapeutic heat application in alleviating the signs and/or symptoms of DMS. There are two main flaws in the experimental design of Kuligowski et al. and Prentice's study. First, the

application of heat immediately following the eccentric exercise has the potential to promote the injury process by increasing capillary effusion and swelling. Second, the application of heat (40°C) for 20-30 min might not be long enough to promote the clearance of extracellular fluid by the lymphatic circulatory system, which is much slower than blood flow. In addition, these studies did not include a measure of muscle damage during the treatment period. Swelling was measured using limb circumference measures, which is not sensitive to small changes in the extracellular fluid volume. Therefore, it was not possible to separate the temporary analgesic effect from the anti-inflammatory effect of the different treatments on muscle damage.

In summary, the exact mechanism of the phagocytic stage in Armstrong's model of DMS remains unclear. However, the evidence presented thus far suggests that the edema and pain are partly related to the release of PGE2. Furthermore, several key similarities exist between the proposed phagocytic stage and acute inflammation: 1) pain, swelling, and loss of function, 2) similar cellular infiltrates, specifically macrophages, 3) the presence of fibroblasts, 4) increased lysosomal activity, 4) increase in the severity of the lesions, and 5) signs of healing approximately 3 d after the initial injury. The progression of the phagocytic stage through the steps of the inflammatory process provides the only viable hypothesis to date for the delay between the initial injury and the onset of the symptoms of DMS.

Regeneration Stage

In general, the purpose of acute inflammation is to promote tissue healing, which clearly occurs in the days after eccentric exercise. Once the most severely damaged

regions of the fiber have been removed by autolysis, rapid regeneration of myofibrils begins within the existing basal lamina (19). The uninjured portions on either side of necrotic zone begin to retract. The intact myofibrils form a stump on either side of the necrotic zone, which becomes covered by a newly synthesized cell membrane. The invasion of macrophages into the necrotic area activates satellite cells, which are located in the periphery within the basement membrane of the muscle fiber. The satellite cells proliferate within the basement membrane and then migrate to the necrotic region of the muscle fiber. At the necrotic region, these satellite cells differentiate into myoblasts, these myoblasts fuse to form myotubes. Once the ends of the myotubes reach the undamaged myofibril stumps, the cell membrane on the myofibril stumps dissolve and union occurs.

However, if necrosis extends the length of the fiber, this will more than likely result in cell death. Therefore, some unknown mechanism determines if the extent of the necrosis is repairable or not. After the removal of the irreversibly damaged fiber, regeneration begins with the activation of satellite cells located within the existing basal lamina and follows a similar pathway as described above. Lastly, the newly formed myotubes are reinnervated by motor axons.

Correspondingly, Darr and Schultz (27) found peak satellite cell activation at 72 h after eccentric exercise in the rat soleus m.. These investigators reported that approximately 3% of the fibers exhibited degenerative changes under the light microscope. Furthermore, Darr and Schultz showed that the satellite cell activation was greater than expected for the repair/regeneration of the damaged fibers. Therefore, these results suggest that satellite cell activation occurred in far more cells than just those that

appeared to be damaged in the histological sections. In addition, McCully and Faulkner (73) reported the presence of myoblasts and myotubes at 4 days after eccentric exercise in the extensor digitorum longus m. of mice. These investigators found that not all fibers with local disruptions degenerated after eccentric exercise. McCully and Faulkner found that maximum isometric strength and muscle mass remained below baseline values at 30 d post eccentric exercise. These studies support Armstrong's hypothesis that skeletal muscles contain a small sub-population of muscle fibers that are vulnerable to damage during lengthening contractions.

Stress-Susceptible Fiber Theory and Repeated Bout Effect

This theory proposes that an unaccustomed bout of eccentric exercise causes irreversible ultrastructural damage to the pool of stress-susceptible fibers, which leads to degeneration of these cells in the days following the exercise. Furthermore, microinjury occurs in the "normal" or non-susceptible fibers that were recruited during the lengthening contractions but does not result in necrosis of these entire fibers. Accordingly, there is a prolonged decrease in muscle strength and range of motion, muscle soreness, and a significant increase in the serum levels of muscle proteins following the bout of eccentric exercise. During the degeneration-regeneration process, necrotic fibers are replaced with newly assembled fibers and the damaged fibers are repaired. Therefore, the process of degeneration-regeneration would result in fewer stress-susceptible fibers to be destroyed by subsequent bouts of eccentric exercise.

Histological evidence from human and rat skeletal muscle following repeated bouts of eccentric exercise supports this prediction (8, 47). These studies reported a

decrease in the percentage of damaged fibers after repeated bouts of eccentric exercise. In addition, several studies have reported that after a repeated bout of eccentric exercise, the recovery of strength and range of motion is faster compared to the first bout, soreness development is less, and serum CK-MM activity peaks much earlier and is significantly blunted (43, 59, 76, 80).

In summary, available evidence seems to support the proposal that destruction of a stres-vulnerable subpopulation of muscle fibers produces the apparent protective adaptation to a bout of eccentric exercise. After the initial damaging bout, fewer vulnerable fibers remain to be destroyed by subsequent bouts. Although the remaining fibers may again be partially damaged, the injury is less severe and should produce less inflammation and pain.

Neural Adaptations to a Bout of Eccentric Exercise

An alternative explanation for the repeated bout effect has been proposed by investigators examining alterations in neural activation after damaging eccentric exercise. A review of the relevant elctromyographic (EMG) literature will be provided followed by an outline of the neural adaptation hypothesis. Several investigators have measured EMG activity in the eccentrically damaged muscle during DMS (14, 57). These studies reported no increase in the resting EMG activity of the damaged muscle. Grabiner et al. (50) compared the maximum EMG activity after subjects performed either maximum concentric or eccentric contractions with the knee extensor muscles on an isokentic machine (30°/s). Specifically, the maximum EMG during maximal eccentric contractions averaged 84 ± 41 (SD)% of the value for the concentric contractions. This

result supports the hypothesis that muscle activation is incomplete during maximum voluntary eccentric effort, i.e., that the same force is spread over fewer fibers. In addition, Grabiner and colleagues showed that when subjects expected to perform a maximum concentric contraction, but the isokentic machine forced a lengthening contraction, the initial EMG of the unexpected eccentric contraction averaged 104 ± 40 (SD)% of that recorded in the maximum concentric effort. Grabiner and co-workers interpreted these results as evidence that eccentric contractions require a unique motor unit activation strategy from the central nervous system.

In addition, Owings and Grabiner (83) had subjects perform a single-leg exercise protocol in which the knee extensor muscles either performed maximal concentric or eccentric contractions to fatigue. Owings and Grabiner measured maximum eccentric and concentric force in the uninvolved knee extensor muscles before and after the fatiguing exercise. After fatigue, for the subjects who performed concentric exercise, the maximum concentric and eccentric forces were not different in the uninvolved knee extensor muscles compared to before the fatiguing task. On the other hand, for the subjects who performed eccentric exercise, the maximum eccentric force increased by 11% and the maximum concentric force remained unaltered in the uninvolved knee extensor muscles after fatigue compared to before. These investigators suggest that the increase in the maximum eccentric force in the non-exercised leg after fatiguing eccentric exercise supports the hypothesis that unique neural strategies are involved during repeated eccentric contractions.

Therefore, the evidence presented so far suggests that there is incomplete activation of the muscle during eccentric contractions and that eccentric contractions,

through an unknown neural circuitry, control the homologous muscles in the unexercised leg. Based on the finding that muscle activation is limited during voluntary eccentric contractions, Golden and Dudley (49) argue that the subjects "learn" to activate motor units that are not normally recruited during an eccentric contraction while recovering from the first bout. Therefore, the change in the recruitment pattern, sparing the damaged fibers, could explain the protective effect on performance and muscle damage from a subsequent bout of eccentric exercise when the muscle has not fully recovered from the initial bout.

The contention that "learning" can occur from a single bout of damage inducing eccentric exercise is not unique. Bar et al. (12) suggested that the initial damage-inducing eccentric exercise bout could be regarded as a one-trail learning session, comparable to a passive avoidance test. In the avoidance test, rats receive a shock after entering a dark cage. As a result of this unpleasant experience, rats do not reenter the dark cage. This type of avoidance behavior continues even without additional training. Bar and co-workers suggest that the central nervous system responds to the first bout of eccentric exercise, which results in pain and damage, by altering the muscle recruitment pattern, such that the muscle is more resistant to damage from subsequent bouts. However, to date there is no direct evidence to support the contention that the muscle recruitment pattern is different after a bout of eccentric exercise.

In summary, the stress-susceptible fiber theory explains the protective effect after a single bout of eccentric exercise from subsequent bouts performed several weeks later. Golden and Dudley (49) have proposed a neural adaptation, which takes place within 48 h after the first bout and alters the recruitment pattern in such a way that muscles are

resistant to damage from a repeated bout even prior to full recovery. Because of the technical limitations of standard electrophysiological methods, it has not been possible to measure the change in the recruitment pattern that Golden and Dudley have proposed.

Enoka and others (37) proposed using MR imaging to determine the distribution of activation among muscle fibers within the exercised muscle by quantify exercise-induced changes in the spin-spin (T2) relaxation time. However, the spatial resolution of MR imaging is not sensitive enough to detect the change in the recruitment pattern of individual motor units within a muscle (89). On the other hand, MR imaging is a non-invasive method that allows investigators to measure muscle damage, changes in muscle compartment cross-sectional area, and exercise intensity. Furthermore, the muscular changes that occur with DMS (i.e. edema in the extracellular compartment versus the damage to the contractile machinery) provides an unique in vivo experimental environment to study the determinants of contrast changes in MR images of muscle. The following section will review the fundamental principles of MR imaging and the literature on the application of MR imaging to study muscle damage after eccentric exercise.

Fundamental Principles of Magnetic Resonance Imaging

A complete discussion of the principles of MRI imaging is beyond the scope of this review. However, a brief description of key points is needed. Tissue contrast in MR images is based on three nuclear magnetic resonance (NMR) parameters: spin density, T1 relaxation time, and T2 relaxation time (16).

Spin density depends on the concentration of the hydrogen nuclei in the tissue. When a sample containing hydrogen compounds is placed in a strong static magnetic field, the hydrogen nuclei tend to align themselves with the external magnetic field. This alignment along the external magnetic field produces a net magnetization vector, the magnitude of which is proportional to the concentration of hydrogen nuclei in the sample. The net magnetization vector can be measured (or mapped in space to form an image) by exciting the sample with a pulse of energy at the appropriate radio frequency (RF). In the absence of relaxation effects, the "brightness" of a tissue in an MR image would simply depend on the net magnetization, and hence on hydrogen concentration, or spin density. For example, the cortex of bone is dark in MR images largely because the concentration of hydrogen nuclei in bone is less than in the surrounding soft tissues.

The effect of the excitation used to observe MRI signals is to change the orientation of the net magnetization vector. This new magnetization state is unstable because the hydrogen nuclei in the sample tend to realign themselves with the external magnetic field. This return to the original magnetization state is described by the two relaxation parameters: T1 and T2.

T1 relaxation involves the dissipation of the energy that was absorbed from the RF pulse. This energy must be lost to the surrounding "lattice" as the net magnetization vector reorients with the static magnetic field. On the other hand, T2 relaxation describes the loss of phase coherence of the excited hydrogen nuclei following the exciting pulse. Typically, this loss of phase coherence is faster than the energy transfer associated with T1 relaxation. Therefore, T2 is typically much shorter than T1. Two processes contribute to the loss in phase-coherence: 1) as the nuclei begin to interact with each

other, this creates local distortions in the magnetic field around each nucleus, and 2) heterogeneity of the static magnetic field produces local differences in field strength. These two processes combined together contribute to the loss in phase-coherence. However, generating "spin-echoes" can minimize the effect of static field heterogeneity. With heterogeneity effects minimized, measurement of the T2 time provides insight into the molecular interactions of the observed nuclei. For example, if the sample contains only freely mobile hydrogen nuclei, then the random interactions of the nuclei occur so fast that they average each other out, thus preventing local distortions from persisting long enough to affect phase coherence. In contrast, if the sample contains hydrogen nuclei bound to other molecules (i.e. proteins), the random interactions occur at much lower speed resulting in the faster loss of phase-coherence.

Image, contrast between tissues can be generated either using T1 or T2 relaxation time differences. The details of various MRI pulse sequences are also beyond the scope of this review. However, in brief, in a T1-weighted image (i.e., an image acquired with short repetition time, TR), tissues with long T1 relaxation times will appear dark and tissues with short T1 relaxation times will appear bright. In contrast, in a T2-weighted image (i.e., long echo time, or TE), tissues with short T2 relaxation times will appear dark and tissue with long T2 relaxation times will appear bright. For example, in a T2-weighted image of the brain, cerebrospinal fluid appears bright compared to the surrounding, protein- and lipid-filled regions of brain tissue. Similarly, skeletal muscle tissue (T2=30 ms) appears darker than adipose tissue (T2=50ms) in a T2-weighted image.

Exercise-Induced Acute T2 Increase

Over a decade ago, Fleckenstein et al. (41) first reported an increase in T1 and T2 in exercised muscle. In addition, these investigators found a weak correlation between exercise intensity and the signal intensity in T2-weighted images. More recent studies, using a stronger external magnetic field, found that the change in T2 was much greater compared to the change in T1 in exercised muscle (2, 23). Fisher et al. (40) reported that the acute increase in the T2 occurs during exercise and decays exponentially after the exercise at a relatively slow rate (half time, $t_{1/2}$ = 10 min). In this study, the exercise intensity was altered by increasing the target force while maintaining a constant contraction rate. These investigators reported a strong correlation between the increase in T2 and muscle force generated during the exercise.

In a similar study, Jenner et al. (62) varied the exercise intensity by altering the rate of contractions while maintaining a constant target force for each contraction. Results again showed a strong correlation between exercise intensity and the increase in signal intensity in T2-weighted images. Moreover, Jenner et al. reported an exponential increase in image intensity at each exercise intensity and that the T2 change reached a plateau after 3-5 min at all exercise intensities.

Shellock et al. (96) compared the exercise-induced acute T2 increase after concentric versus eccentric exercise. They reported a larger increase in T2 of the elbow flexor muscles immediately after concentric compared to eccentric exercise. Since the energy cost of an eccentric contraction is significantly less compared to a concentric contraction at the same load, and previous studies have shown that the increase in T2 is

related to exercise intensity, it is not surprising that Shellock et al. found significantly lower T2 values after eccentric exercise compared to concentric exercise.

In a continued effort to determine the relationship between muscle T2 and exercise intensity, Adams et al. (2) compared the increase in T2 with EMG data after graded concentric and eccentric exercise. These investigators found that at any given exercise intensity the integrated root mean squared EMG (IEMG) of the biceps brachii m. was significantly less after eccentric contractions compared to concentric contractions. Both IEMG and T2 increased as a function of relative exercise intensity. Furthermore, linear regression analysis revealed a significant correlation ($r = 0.99$, $p < 0.05$) between IEMG and the increase in T2 after both concentric and eccentric exercise.

Based on these studies, several investigators suggested that the increase in T2 could be used as a method for estimating the relative intensity of muscle recruitment. Furthermore, recent studies have employed the exercise-induced T2 increase to map regional variations in motor unit recruitment within a given muscle (3, 86). However, in a recent study, Prior et al. (89) showed that the exercise-induced T2 increase on a pixel-by-pixel basis could not be used to map recruitment of individual motor units within a muscle. Nonetheless, Prior et al. reported a linear relationship between the mean T2 of the entire exercised muscle and exercise intensity.

In summary, several studies have consistently reported a linear relationship between exercise intensity and the acute T2 increase across the entire exercised muscle. Therefore, the muscle T2 averaged across the whole muscle could serve as an index of gross muscle activity.

The exact mechanism of the exercise-induced T2 increase remains unclear. Several physiological changes associated with exercise could the cause in muscle T2, including decreased intracellular pH, increased perfusion, and changes in the extracellular and intracellular fluid compartments.

Proposed Mechanism of the Acute T2 Increase After Exercise

An increase in the extracellular water (H_20_e) volume during exercise has been well documented in the exercise literature (98). This increase in the extracelluar and/or vascular fluid volume is the most commonly cited explanation for the increase in muscle T2 during exercise. Several studies have shown that the T2 relaxation is multiexponential in isolated muscle preparations (24, 36, 48, 54, 67). Generally, three T2 components are resolved in isolated muscle: the first component has a short T2 relaxation time of less than 5ms, the second component has an intermediate T2 relaxation time in the range of 25 to 40 ms, and the third component has a long T2 relaxation time greater than 80ms. The components of T2 relaxation were resolved through the application of a complex non-negative least squares (NNLS) algorithm on T2 relaxation curves collected using echo planar imaging. Echo-planar imaging is a rapid imaging technique that is able to collect the T2 relaxation or decay signal at several hundred echo times.

The short T2 component, which arises from interactions of water with intracellular proteins, has been calculated to account for less than 5% of the total T2 signal intensity. On the other hand, the second component with an intermediate T2 accounts for more than 70% of the total T2 signal intensity and the long component accounts for 10-20% of the total T2 signal intensity. The intermediate component of the

T2 relaxation has been suggested to represent the bulk of intracellular fluid and the long component has been proposed to represent the extracellular and/or vascular fluid. Therefore, the contribution of the slow-relaxing long component should increase after exercise and other conditions that increase the extracellular and/or vascular component.

Ploutz-Snyder et al. (87) tested this hypothesis by comparing the T2 response to exercise versus pressure-induced edema in the lower leg muscles. The temporary edema in the lower leg muscles was produced by applying external negative leg pressure; suction in this study was applied from the waist down. This type of negative leg pressure has been shown to result in blood pooling in the compliant vessels of the legs. Continued exposure to negative pressure results in increased capillary filtration, which leads to increased interstitial fluid volume. The NNLS algorithm was used to resolve the different components of the T2 response during exercise and edema.

Ploutz-Snyder et al. showed significant swelling of the lower leg in all subjects during negative leg pressure. Furthermore, these investigators showed that the T2 response during exercise was different compared to the T2 response during edema. First, during edema, a slow-relaxing long T2 component (>80ms) was resolved which was not present during rest or exercise. Second, appearance of the long T2 component did not result in the shift of the faster relaxing component toward the longer component, which occurs during exercise. Lastly, Ploutz-Snyder et al. estimated that the extracellular fluid volume would have to increase to over 30% to account for the T2 change during exercise. However, extracellular fluid volume has been measured in human muscle to increase from 12% at rest to 18% during intense exercise (98). Therefore, these results suggest

that the increase in T2 during exercise is more than likely related to changes in the intracellular fluid.

Another well-documented physiological change that occurs with exercise is the decrease in intracellular pH. However, pH recovers much faster than the T2 increase after exercise (84). Nonetheless, there is a significant increase in metabolic osmolites during exercise due to the increase in the metabolic rate (i.e. inorganic phosphate and lactate). Therefore, the accumulation of these metabolites could draw water into the intercellular compartment and this increase in the intercellular fluid volume could result in altered T2 relaxation within the active muscle cell. In addition, the accumulation of metabolites at a given rate of stimulation will be significantly less in muscles with a high aerobic capacity compared to muscles with low aerobic capacity. Recently, experiments conducted in our laboratory found that the increase in muscle T2 of the human knee extensor muscles depend on the exercise intensity relative to peak aerobic power and not absolute power output (92).

In another set of experiments conducted in our laboratory, Prior et al. (91) studied the T2 response in different muscles of the rat after three treatments that have been documented to alter the net metabolite accumulation which, in turn, would alter the intracellular fluid volume. The three treatments are as follows: ischemia, inhibition of lactic acid production with iodoacetate, and depletion of total creatine by feeding a creatine analog, β-guanidinopropinate. Prior et al. showed that the increase in T2 during exercise was significantly less in muscles with a high aerobic capacity (triceps surare m.) compared to muscles with a low aerobic capacity (white gastrocnemius m). Furthermore, the replacement of phosphocreatine with the less labile analog diminished the increase in

T2 during exercise. The inhibition of lactic acid production by idoacetate also decreased the T2 response during exercise. However, this decrease in the T2 response was not significant across all muscle groups. Prior et al. suggest that inhibiting glycolysis during exercise caused an increase in the accumulation of phosphomonoesters which, in turn, increased the osmotic load. This effect would counter balance the reduced osmotic load caused by inhibiting lactate production.

During ischemic stimulation, Prior et al. reported an increase in the T2 response with no change in the total muscle volume. Prior et al. interpreted these results as evidence that the increase in T2 during exercise does not simply depend on the intracellular volume. These investigators proposed that the increase in T2 during ischemic stimulation could be the result of fluid shits between intracellular sub-compartments, i.e. the hydration layer surrounding the myofibrillls. In these same experiments, Prior et al. found an immediate increase in T2 when blood flow was reestablished.

In summary, the results of this recent study suggest that the T2 increase during exercise is related to intracellular fluid changes. Specifically, the accumulation of metabolic osmolites could cause a shift in intracellular fluid into a sub-compartment within the muscle cell. This redistribution of the intracellular fluid is hypothesized to be primarily responsible for the T2 increase during exercise. In addition, the aerobic capacity of the muscle determines the extent of the T2 increase during exercise and the recovery of T2 following exercise.

Delayed T2 Increase after Eccentric Exercise

During eccentric exercise there is an immediate increase in muscle T2, as described above, and this increase in muscle T2 returns to baseline values 1-2 h after the exercise (61). However, a delayed increase in muscle T2 following eccentric exercise has also been reported, beginning at about 12 h post exercise (103). This delayed increase in muscle T2 is not observed following concentric exercise (96).

Fleckenstein and colleagues were the first to report on the delayed T2 increase following eccentric exercise (42). These investigators measured MRI parameters, plasma CK activity and subjective muscle soreness before, immediately after, and 24, 36, 48, and 72 h (n = 5) post-exercise and at 13, 23, and 48 (n=2) days after exercise. Pain and T2 signal intensity peaked at 24-48 h post-exercise. As expected, the CK release lagged behind peak pain and T2 signal intensity. Peak CK release was reported at 72 h after the eccentric exercise. This group reported a significant positive correlation between CK release and T2 during the first 72 h post-exercise. They suggest that the underlying mechanism of the delayed increase in T2 is related to muscle damage that develops with DMS in the phagocytic stage of Armstrong's model of DMS.

Nurenberg et al. (81) collected muscle biopsy specimens from the soleus m., lateral gastrocnemius m., medial gastrocnemius m., tibialis anterior m., and peroneus longus m. at 48 h following downhill running. These investigators collected muscle biopsy samples from muscle regions that showed an elevated T2 at 48 h post-exercise. Nurenberg et al. reported a strong correlation ($r = -0.88$, $p < 0.05$) between areas of high T2 signal intensity and ultrastructural damage. In addition, Nurenberg et al. reported poor correlation between the following pairs of variables: soreness and mean T2 increase,

soreness in the region of the biopsy and the degree of ultrastructural damage, and peak soreness and peak CK levels. Furthermore, Nurenberg et al. showed direct histological evidence correlating the increase in T2 signal intensity and ultrastructural muscle damage after eccentric exercise.

Proposed Mechanism of the Delayed T2

In a partial report of the results to be described in this thesis, Foley et al. (43) examined the long-term time course of the delayed T2 increase and the effect of a repeated bout of eccentric exercise on the delayed T2 increase. These researchers acquired MR images, CK release data, and subjective pain ratings before, immediately after, and 1, 2, 4, 7, 14, 21, and 56 d after the initial bout of eccentric exercise and at 2, 4, 7, and 14 d after bout 2. The delayed T2 increase peaked at day 7 (>60% increase compared to rest) after bout 1 and remained elevated at day 56 (>35%). After bout 2, peak T2 (<25% increase) was significantly less compared to bout 1and T2 peaked earlier after bout 2 (2 – 4 d), T2 recovered more rapidly after bout 2 but still remained above baseline values at day 14 (≈ 5%).

Overall arm swelling peaked at day 7 (≈13%) after bout 1 and decreased to below pre-exercise values at 56 d (≈ -7.0%). After bout 2, there was less swelling of the upper arm, approximately a 5% increase, and swelling peaked earlier, at day 2, compared to bout 1. Peak CK released was reported on day 7 (100-fold increase) and returned to baseline values by day 14 after bout 1. After bout 2, CK release peaked at day 2 and was negligible compared to bout 1. Peak pain was reported on day 2 after both bouts of

eccentric exercise. However, there was significantly less pain reported after bout 2 compared to bout 1.

These results are consistent with pervious studies on the repeated bout effect. However, Foley et al. were the first to document the repeated bout effect on the delayed T2 increase. In addition to overall arm swelling, Foley et al. measured the volume of the elbow flexor muscles in the imaged region. These results showed a decrease in muscle volume, approximately 7%, at day 56 after bout 1. Muscle volume remained below pre-exercise values at day 14 after bout 2. Foley et al. interpreted these results as evidence supporting the stress-susceptible fiber model of the repeated bout effect (see earlier discussion). If this reported decrease in the elbow flexor muscle compartment is truly the result to muscle loss, then there should be a decrease in MVC. However, Foley et al. did not measure isometric strength loss in the eccentrically damaged muscle. On the other hand, several studies have reported similar muscle loss in human and rat muscles after eccentric exercise (69, 73).

In addition, these investigators proposed that the persistent elevation in muscle T2 at 56 d after bout 1 and 14 d after bout 2 might reflect a more permanent change in the muscle. Based on the fact that overall upper arm swelling had subsided and elbow flexor muscle volume had actually decreased, it is very unlikely that residual edema caused the elevated T2. Therefore, the persistent increase in T2 after a bout of eccentric exercise is more than likely the result of changes in the intracellular water chemistry and/or redistribution of the intracellular fluid into sub-compartments within the cell.

Conclusions

This review of the DMS literature shows that the MR technique has already contributed unique observation to the elucidation of the mechanisms underlying DMS. In particular, the result of recent studies, including pilot work for this dissertation, support the theory that destruction of stress-susceptible fibers underlines both the symptoms resulting from an initial bout of eccentric exercise and the reduction of symptoms induced by subsequent bouts.

The review also reveals two major unanswered questions related to the outcomes of these recently reported MR studies. First, how does the anatomical muscle volume loss relate to changes in functional capacity of the muscle? In the subsequent chapter we propose experiments to answer this question by determining how well muscle strength loss correlates with muscle volume loss during the extended recovery period after damaging eccentric exercise. Secondly, this review shows that no studies to date have examined the separate components of the multiexponential T2 decay in eccentrically damaged muscle. We propose additional studies employing echo-planar imaging and NNLS analysis to resolve the short, intermediate, and long components of T2 relaxation in such muscle. This work will allow testing of the hypothesis that the persistent elevation in muscle T2 long after apparent recovery from eccentric-induced DMS is due to changes in the intracellular compartment of the repaired and/or newly-synthesized muscle cells.

CHAPTER III

Effects of Exercise-Induced Damage on T2 Relaxation in Skeletal Muscle

Abstract.

The purposes of this study were, first, to compare T2 relaxation computed by the non-negative least squares algorithm (NNLS) from muscles after two bouts of damaging eccentric exercise (each 5 sets of 10 at 110% of 1RM) separated by 8 wk, and second, to examine the effect of eccentric exercise-induced muscle damage on the acute T2 response to bouts of milder concentric exercise (each 3 sets of 10 at 20% of 1 RM) performed before [Con 1] and at 2 [Con 2] and 21 d [Con 3] after the first eccentric exercise. Conventional T2-weighted axial spin-echo MRI images (TE=30,60 ms) and echo-planar images (64 TE's from 20 to 272 ms) of the upper arm were obtained before, and 1, 2, 4, 7, 12, 21, and 56 days after eccentric bout 1 (Ecc 1), and at 2,4,7, and 15 days after eccentric bout 2 (Ecc 2). Conventional spin-echo images were also obtained immediately before and after each concentric exercise bout. The prolonged T2 increase measured by conventional imaging after Ecc 1 (from 27.6 ± 0.2 ms before to 46.7 ± 3.8 ms at 2 days, $p < 0.05$, mean ± SE, n=6) was correlated with the presence of slow-relaxing, long T2 components (60-300 ms) in the NNLS T2 spectra. However, T2 remained elevated by 2.5 ms even after 56 days, when distinct slow-relaxing components were no longer detectable. The magnitude of slow-relaxing components was less after Ecc 2 compared to Ecc 1, consistent with the previously-reported protective effect of prior eccentric exercise. The acute T2 increase after Con 2 (1.5 ± 0.5 ms, mean ± SE, n=6) was significantly less compared to Con 1 (3.2 ± 0.5 ms) and Con 3 (5.0 ± 0.4 ms).

The results confirm that the prolonged T2 increase observed by conventional imaging methods after eccentric exercise is primarily caused by edema, and show that this edema diminishes the acute T2 change measured during exercise using conventional spin-echo imaging methods.

Introduction.

Exercise causes an increase in the ^{1}H-NMR transverse relaxation time (T2) of muscle water in active muscle (40, 41). This increase in T2 has been reported during the first few seconds of dynamic exercise (62), and continues to rise exponentially toward a plateau that depends on exercise intensity. The acute increase in T2 decays relatively quickly ($t_{1/2}$ <20 min) once the exercise is discontinued. Adams et al. (2) reported a strong correlation between mean T2 of exercised muscle and the relative intensity of muscle recruitment measured using electromyography. Based on these and similar results, the relationship between muscle T2 and exercise intensity is increasingly exploited by "functional" muscle MRI (fMRI) studies in order to map the recruitment pattern (3, 86) and the relative intensity of muscle recruitment across different muscle groups during a variety of exercises (86).

The biophysical mechanism underlying the acute T2 increase during exercise is not entirely clear. Although muscle T2 relaxation is typically assumed to be a simple monoexponential process in fMRI studies, it is well-known that the T2 relaxation decay of intact muscle can be decomposed into multiple components (24, 36). Generally, two major T2 components are resolved in isolated muscles: a large component with intermediate T2 (20-50 ms) and a typically smaller component with longer T2 (>60 ms).

The intermediate T2 component corresponds to the bulk of the intracellular fluid while the long component corresponds to the extracellular and/or vascular fluids. Although an increase in the extracellular water (H_20_e) volume during exercise has been well documented in the exercise literature (98), and is the most commonly cited explanation for the increase in muscle T2 during exercise (24, 36, 48, 54, 67), more recent studies show that the T2 response to pressure-induced edema was different compared to the exercise-induced T2 increase (25, 87). Furthermore, Prior et al. (91) showed that the exercise-induced T2 increase in stimulated rat muscle is related to osmotically driven changes in the distribution of intracellular fluid within the cellular sub-compartments, and does not simply depend on total tissue fluid volume.

In addition to the acute T2 increase during exercise, MRI studies have reported a more prolonged, delayed T2 increase in eccentrically damaged muscles. This delayed T2 increase coincides roughly with the onset of delayed muscle soreness (DMS), a syndrome of pain or discomfort following unaccustomed eccentric exercise (42, 43, 61, 81, 96, 103). Numerous studies have documented the exercise-induced muscle damage following eccentric contractions by tracking different indices, including serum levels of muscle enzymes, inflammation markers, swelling, and decrements in muscle performance (7, 12, 17, 21, 22, 44, 53, 64, 76, 78, 80, 85, 99, 101). Several recent studies have shown that performance of a repeated bout of the same exercise 1-6 wk later produced more modest changes in the indicators of damage and muscular performance (10, 17, 21, 22, 35, 64, 76, 79, 80). This protective effect is commonly referred to in the exercise literature as the "repeated bout effect". Foley et al. (43) reported a persistent elevation in muscle T2 at 56 d post-eccentric exercise and showed that the repeated bout

effect extends to the delayed T2 increase, which is significantly reduced following the second bout of eccentric exercise.

Inasmuch as edema has been reported to occur during DMS, it is plausible that the delayed T2 increase after eccentric exercise is related to changes in extracellular and/or vascular fluid compartments in muscle. The appearance of a slow-relaxing long T2 component in NNLS computed T2 spectra would confirm this increase in extracellular and/or vascular fluid (87). However, to date, no studies have directly tested the hypothesis that the delayed T2 increase during DMS is the result of edema, or the hypothesis that the smaller increase in the delayed T2 after a repeated bout of eccentric exercise is due to reduced edema development after bout 2. Furthermore, the mechanism underlying the recently reported persistent elevation in muscle T2 at 56 d post-eccentric exercise remains unresolved.

The purposes of this study were: 1) to determine if the delayed T2 increase that develops during DMS is associated with the accumulation of the slow-relaxing T2 components that are characteristic of muscle edema, 2) to test whether these slow relaxing components persist for up to 8 weeks, and thus explain the elevated muscle T2 previously reported 8 weeks after eccentric exercise, 3) to determine if the protective effect of a first bout of eccentric exercise on the response to a second bout 8 weeks later is also manifest by decreased magnitude of slow-relaxing T2 components, and 4) to examine if the increase in muscle T2 caused by damaging eccentric exercise alters the acute T2 response to a subsequent bout of mild concentric exercise.

Methods.

Subjects. Six male subjects (age = 22.3 ± 1.5 years, mean ± SD, height = 178 ± 5 cm, weight = 84 ± 5 kg, 1 RM = 20 ± 3 kg) were recruited from the population at Michigan State University. Subjects were screened for any medical and/or orthopedic conditions that might preclude severe leg exercise or MR imaging procedures. Furthermore, subjects who engaged in heavy lifting as part of their daily activities were excluded from the study. The study was approved by the University Committee on Research Involving Human Subjects and each subject gave informed, written consent before participation.

Exercise Protocol. Each subject was tested for the one repetition maximum (1RM) for the standing concentric biceps curl of the right arm using a single weighted dumbbell. The 1RM lift was performed with the right elbow, starting from full extension and proceeding to full flexion. A trainer demonstrated the lift using proper technique and subjects were coached to maintain proper form during the 1RM testing. During these tests, the trainer lowered the dumbbell on each repetition so subjects performed no eccentric exercise. The initial weight load was set at 15% of body weight and was increased in 2.5 lb. increments on subsequent trials until the subject failed to attain full flexion of the elbow with proper form (43). There were 90 seconds of rest between trials. The 1RM value was defined as the final load with which the subject was able to perform, unassisted, a complete biceps curl. Approximately 4 to 5 trials were needed to reach the 1RM value. Following the 1RM test, subjects were introduced to a muscle soreness scale (21), wherein they were asked to rate their perceptions of overall soreness in the fully

extended and relaxed biceps brachii m. on a scale of 1 (no soreness) to 10 (extremely sore).

Following a preliminary MRI scan, each subject performed concentric exercise (Con 1) with the left arm at 20% of the 1RM (43). The concentric lift started with the left elbow fully extended. A trainer placed the weighted dumbbell in the subject's grip; the subject then slowly flexed the elbow to full flexion to a 3 second count. A trainer removed the weight at the top of the curl, and the subject returned the unweighted arm to full elbow extension. Subjects repeated this movement for 3 sets of 10 repetitions at approximately 1 repetition every 5 seconds. A 90 s rest period separated each set of 10 repetitions. MR images were acquired immediately (< 2 min) following the completion of the concentric exercise bout.

Later in the same day (3-4hr after Con1), muscle injury was induced by eccentric biceps curls (Ecc 1) at 110% of the concentric 1 RM (43). The eccentric contraction began with the left elbow in the fully flexed position. The trainer placed the weighted dumbbell in the subject's grip; the subject then smoothly extended the elbow to full extension. Subjects repeated this movement for 5 sets of 10 repetitions at approximately 1 repetition every 5 seconds. Subjects were verbally encouraged to follow strict form (standing erect with feet shoulder-width apart) during each repetition. If the subject was unable to complete the exercise protocol, the trainer provided minimal effort to help the subject complete the exercise set. Muscle soreness measures and MRI scans were repeated at the same time of day 2, 4, 7, 14, 21 d after Ecc 1. At 2 d (Con 2) and 21 d (Con 3) post-eccentric exercise, each subject was imaged before and after performing the same concentric exercise bout (3 sets of 10 repetitions at 20% of 1RM). At 56 d post-Ecc

1, each subject was imaged before and after performing the same eccentric exercise protocol (Ecc 2). Pain ratings and MRI scans were taken 2, 4, 7, and 14 days after Ecc 2. Subjects were instructed to refrain from taking any anti-inflammatory medications or other therapeutic treatments to relieve pain.

MRI methods. Images were acquired using a linear extremity coil and a 1.5 T Signa whole body imager (General Electric, Milwaukee, WI). Subjects were positioned head first and prone, with the left arm extended overhead. The coil housing was centered on a mark inked 1/3 of the distance along a line from the middle of the antecubital fossa to the acromion process of the scapula. Eight spin echo axial images 10mm thick and 5mm apart were acquired from a 12cm long region centered on the landmark. A 16 x 16 cm field of view was used, with 2 echoes (TR/TE = 1500/30, 60 ms), a 256 x 128 acquisition matrix and 1 NEX. Echo-planar images were acquired at 64 TEs in sequential order from 20-272 ms. The first image (TE = 20 ms) was excluded from the analysis to minimize saturation effects. The pre-exercise and post-exercise relaxation curves at the different time points were analyzed using the non-negative least squares (NNLS) algorithm with minimum energy constraints as described previously (87).

Mean T2 values were determined for the entire elbow flexor muscle group in each of the 8 axial images collected with the spin-echo pulse sequence. Data from the two echoes of the fast spin echo imaging sequence were fitted to a monoexponential decay algorithm using the analysis software package "x-vessel" (40). Specifically, a region of interest was defined by manually tracing around the biceps brachii m. and brachialis m., excluding subcutaneous fat and skeletal tissues. Once the region of interest was established, the software automatically calculated both the number of pixels within the

region and the mean T2 of those pixels. Volume weighted average T2 was calculated as follows:

$$\text{Volume weighted average T2} = \frac{\Sigma\,(\text{Pixel count} * \text{mean T2 in each slice})}{\Sigma\,(\text{Pixel count in each slice})}$$

In addition, "x-vessel" was used to measure the volumes of the whole imaged arm region as well as the exercised (elbow flexors) and unexercised (elbow extensors) muscle compartments by the same manual tracing method. The volume data was calculated by multiplying the pixel counts within the defined region by the pixel area (field of view/matrix) and then multiplying by the interslice interval (2 cm). All MRI data analysis was done by the same person, working with unmarked images which were later decoded.

Statistical Analysis. To test the hypothesis that the mechanism underlying the delayed T2 increase during DMS is edema, NNLS analysis was applied to the echo-planar imaging data to separate the components of the multiexponential T2 decay in eccentrically damaged muscle. The presence of a slow-relaxing long T2 component at any time point confirmed the contribution of edema to the elevated T2 value at that time. Pearson product moment correlation coefficients (r) were calculated to assess the relationship between the peak slow-relaxing long T2 component and peak increase in exercise-induced edema (i.e., swelling).

To test the hypothesis that the reported lower peak T2 elevation after Ecc 2 is the result of reduced edema (i.e., repeated bout), paired t-tests were used to test for the difference in the contribution of the slow-relaxing long T2 component to the overall mean muscle T2 on the day peak swelling occurred after Ecc 1 versus Ecc 2. Finally, our hypothesis that the edema-induced elevation in muscle T2 after eccentric exercise will

not abolish the acute exercise-induced T2 increase was tested using the t-statistic for a one-tailed test on paired differences. Specifically, paired t-tests were used to test the acute T2 increase, relative to the pre-exercise value, after a bout of light concentric exercise performed before, 2 d and 21 d after eccentric exercise. Significance levels for all tests were set at $p < 0.05$. Data are reported as mean ± SE, $n = 6$. A sample size of six was determined to be required in order to detect a 10% difference in the contribution of slow relaxing long T2 component to the overall T2 signal after Ecc2 versus Ecc 1 for a paired t-test with alpha set at 0.05, beta set at 0.20, and an estimated standard deviation of 5%. To detect an acute T2 increase of > 3ms after exercise using a paired t-test, a sample size of five was required for alpha set at 0.05, beta set at 0.20 and an estimated standard deviation of 1ms.

Results.

The image series in Figure 1 shows the progression of swelling and T2 in the elbow flexor muscles of one subject during the 8 wk period after Ecc 1. At the start of the experiment, NNLS analysis of the pre-exercise echo-planar data showed that the T2 relaxation is characterized by a single component of < 60 ms in all subjects. In contrast to pre-exercise, there were components with T2 above 60 ms in the NNLS spectra in five out of the six subjects at 2, 4, 7, 14, and 21 d after Ecc 1 (Figure 2A). The T2 spectrum computed by NNLS at 56 d post-Ecc 1 showed that the T2 relaxation had reverted back to a single component model in all subjects. On the other hand, after Ecc 2, components with T2 above 60 ms in the NNLS spectra were only observed on days 2 and 4 post-exercise in all subjects (Figure 2B). The shape and peak T2 of these components varied

widely between different subjects. The magnitude plotted at 1000ms is the background parameter, and is equal to the mean magnitude of the noise measured in a large region of interest outside the arm.

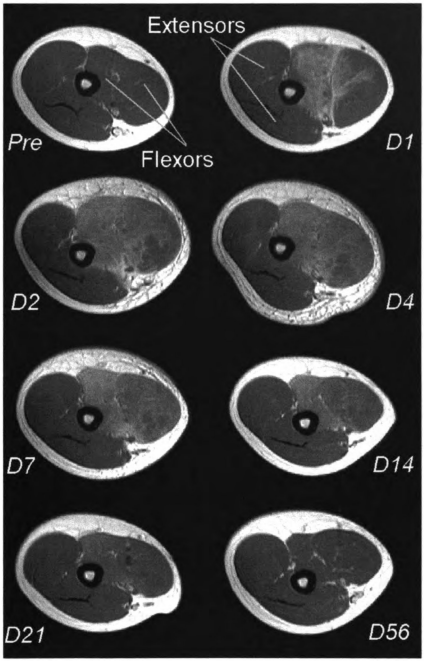

Figure 1. Representative mid-brachial axial plane MR images from one subject before (top left image; pre-exercise) and at each test interval during the 8 wk after Ecc 1. Images are oriented such that the anterior direction is to the right and the lateral direction is upward.

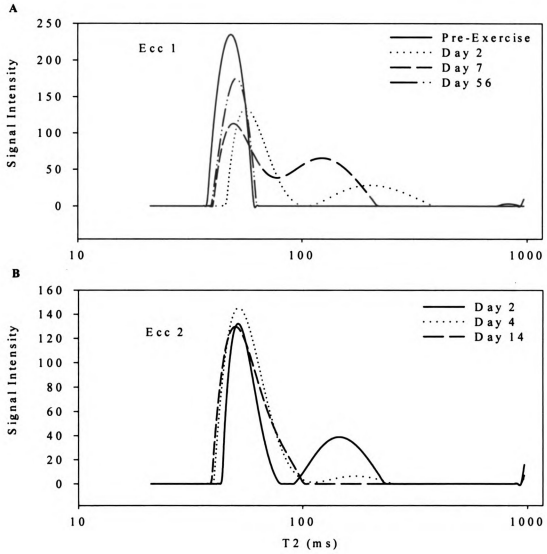

Figure 2. NNLS spectra of one subject before and in the days following Ecc 1 (A) and Ecc 2 (B). The T2 relaxation is characterized by a single component at pre-exercise (A). Note on days 2 and 7 after Ecc 1, the NNLS spectra is more complex, with variable components at much higher T2. In comparison, the NNLS spectrum in the days following Ecc 2 shows that the fraction of the overall T2 attributable to the slow relaxing component is less on days 2 and 4.

Subjects reported peak pain on day 2 after both bouts of eccentric exercise.

However, subjective pain ratings were lower after Ecc 2 (5.6 ± 0.5, soreness rating on a

1-10 scale) compared to Ecc 1 (8.0 ± 0.4). Figure 3 shows the change in muscle volume (Figure 3A) and the contribution of the long component to the overall T2 (Figure 3B) in the days following Ecc 1 and Ecc 2. Muscle swelling peaked on day 2 (39 ± 11%) and remained swollen by nearly 23 ± 6% on day 7 after Ecc 1 (Figure 3A). During the second week after Ecc 1, muscle volume decreased to a value < 90% of the baseline volume (217 ± 7 cm^3 at 14 d vs. 245 ± 10 cm^3 preexercise; $p < 0.05$). This decrease in muscle volume was maintained during the period from 3 to 8 wk after Ecc 1 (227 ± 6 cm^3 at 56 d). In contrast, peak swelling was only about one-third as much after Ecc 2 (14 ± 3%) on day 2 vs. Ecc 1. During recovery from Ecc 2, the muscle volume reverted to the reduced size that had been maintained at 2 to 8 wk after Ecc 1.

The delayed T2 increase peaked on day 7 after Ecc 1 (46.7 ± 3.8 ms) and remained above pre-exercise values on day 56 (29.9 ± 0.3 ms at 56 d vs. 27.6 ± 0.2 ms pre-exercise; $p < 0.05$). Peak delayed T2 increase after Ecc 2 was less and occurred earlier compared to Ecc 1 (37.6 ± 3.9 ms at 2 d, $p < 0.05$). The peak percent of the overall T2 attributed to the long component (> 60 ms) after Ecc 1 occurred several days after the peak swelling, 32 ± 8% at 7 d (Figure 3B). The correlation between peak muscle swelling on day 2 after Ecc 1 and % total signal intensity attributed to the long component on the same day was not significant ($r = 0.741$, $p = 0.092$). However, Pearson's correlation test revealed a significant strong correlation between peak % total signal intensity attributed to the long component on day 7 and muscle swelling ($r = 0.895$, $p < 0.05$) on the same day. Peak % of the total signal intensity attributed to the long component occurred earlier and was significantly less after Ecc 2, (13 ± 6% at 2 d, $p < 0.05$). The correlation between muscle swelling and % total signal intensity attributed

to the long component was significant on day 2 ($r = 0.884$, $p < 0.05$) and 4 ($r = 0.977$, $p < 0.05$) after Ecc 2.

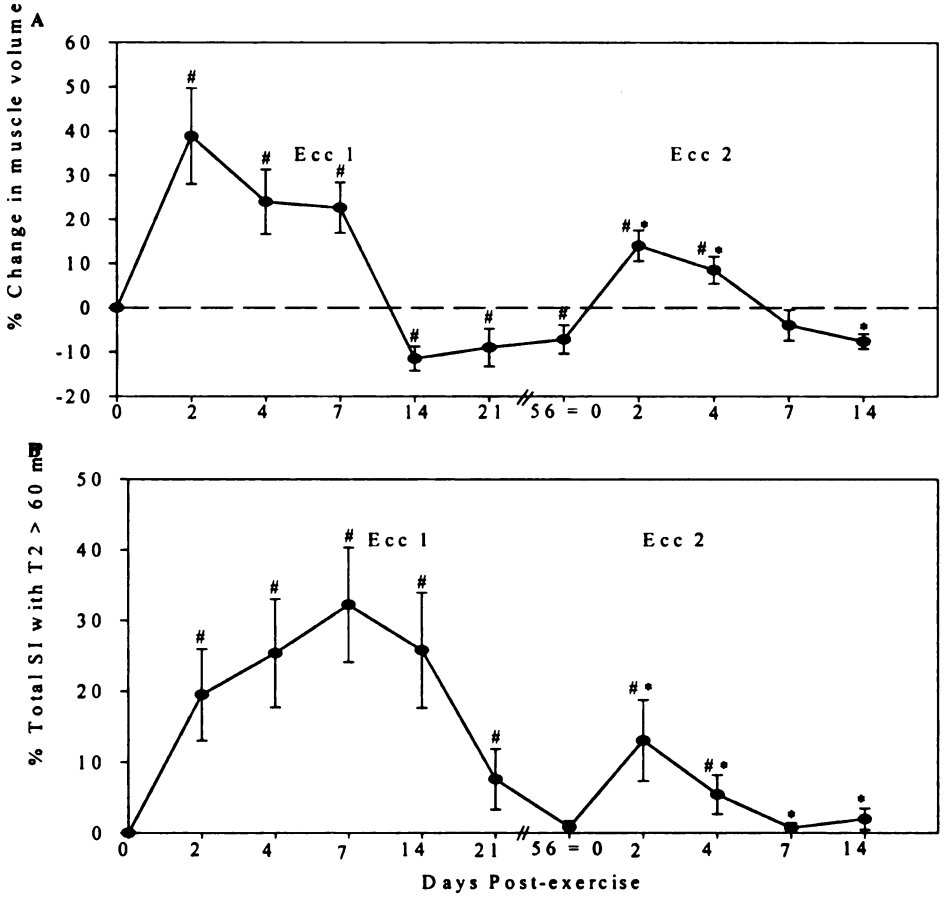

Figure 3. Time courses of knee extensor muscle volume and percent of the total signal intensity attributable to components with T2 above 60 ms in 6 subjects for 8 wk after eccentric exercise bout 1 (Ecc 1) and 2 wk after eccentric exercise bout 2 (Ecc 2) in 5 subjects. Ecc 2 was performed after the scan done 56 d after Ecc 1. All values are given as percent change from baseline or pre-exercise values. Values are means ± SEM.
Significantly different from pre-exercise or baseline, $p < 0.05$ (Ecc 1, pre-exercise = day 0; Ecc 2, pre-exercise = day 56).
* Significantly different from Ecc 1, $p < 0.05$.

A transient or acute T2 increase was observed in MR images taken immediately after Con 1, Con 2, and Con 3 (Table 1). An acute $\Delta T2$ of 3.2 ± 0.5 ms (change in T2

from pre-exercise) was observed immediately after Con 1, the pre-injury condition. The acute ΔT2 after Con 2 (1.5 ± 0.5 ms, $p < 0.05$; 2 d after Ecc1) was significantly reduced compared to Con 1. The acute ΔT2 after Con 3, performed 21 d after Ecc 2 (5.0 ± 0.4 ms), was somewhat higher compared to Con 1 and significantly higher compared to Con 2.

Table 1. Acute T2 increase after concentric exercise.

	Pre-exercise T2 (ms)	Post-exercise T2 (ms)	Acute ΔT2 (ms)
Con 1	27.8 ± 0.1	31.0 ± 0.5*	3.2 ± 0.5
Con 2	43.7 ± 3.3	45.2 ± 2.9*	1.5 ± 0.5#
Con 3	34.0 ± 1.4	39.0 ± 1.6*	5.0 ± 0.4

Values are means ± SE; n = 6 subjects.
* Significantly different from pre-exercise, $p < 0.05$.
# Significantly different from Con 1 and Con 3, $p < 0.05$.

Discussion.

The main results of this study are that the increase in muscle T2 during DMS is primarily due to the eccentric exercise-induced edema, and that the persistent elevation in T2 long after the apparent recovery from eccentric-induced DMS is not due to residual edema. It is well known that eccentric exercise causes structural damage to the muscles and it is this damage to muscles that results in the sensation of pain or soreness and swelling that gradually develops in the ensuing days (7, 12, 17, 21, 22, 44, 53, 64, 77, 80, 85, 99, 101). In our study, the T2 spectra computed by NNLS at 2, 4, 7, 14, and 21 d

after Ecc1 showed components with T2 above 60 ms. This result is consistent with the hypothesis that the T2 increase observed during DMS is caused by the exercise induced edema (42, 43, 61, 81, 96, 103). Furthermore, the T2 spectra computed by NNLS at 56 d post-Ecc 1 showed that the T2 relaxation had reverted back to a single component in all subjects. Accordingly, this result is consistent with the hypothesis that the persistent elevation in T2 long after recovery from eccentric exercise induced muscle damage may be related to more permanent changes in the intracellular water chemistry and/or redistribution of the intracellular fluid into sub-compartments within the muscle cells (43, 91).

Peak swelling occurred on day 2 after Ecc 1 and peak percent of the overall T2 signal attributed to the slow relaxing long T2 component occurred on day 7 after Ecc 1 (Figure 3). Consequently, the correlation between muscle swelling and the contribution of the slow-relaxing long T2 component to the overall T2 on day 2 after Ecc 1 was poor. However, on day 7 there was a strong significant correlation between muscle swelling and the contribution of the long component to the overall T2 signal intensity. Note that although peak swelling occurred on day 2, there was still significant swelling on days 4 and 7 after Ecc 1. The late peak in the slow relaxing long T2 component could be due to the delayed gross heterogeneity of the extracellular fluid (i.e., accumulation of muscle proteins and circulating polymorphs) following eccentric exercise induced muscle damage.

As previously reported, there was a 7-10% loss in muscle volume on days 14 and 21 after Ecc 1. Others have also reported similar loss in muscle mass in the days following eccentric exercise (63, 70). The NNLS computed T2 spectra on days 14 and

21 after Ecc 1 consistently resolved a slow relaxing long T2 component. Consequently, the MRI-determined muscle volume may have under-estimated the true loss in muscle volume because of the residual edema present on days 14 and 21 after Ecc 1. The T2 spectra computed by NNLS on day 56 after Ecc 1 and 14 d after Ecc2 showed that the T2 relaxation had reverted back to a single component model. However, one cannot completely rule out the presence of residual edema at these later time points purely based on the absence of the slow relaxing long T2 component. For example, although an increase in extracellular water (H_2O_e) volume during exercise has been well-documented in the exercise literature (98), Ploutz-Snyder et al. failed to resolve a slow relaxing long T2 component in the tibialis anterior muscle after exercise (87). This result suggests that small changes in the extracellular and/or vascular fluid compartments (i.e., < 8-10% increase in the extracellular fluid volume) do not result in the appearance of long T2 components. However, there is no evidence, to date, that would suggest that the eccentric exercise induced edema might be present at 8 wk post-exercise. Therefore, the absence of the slow relaxing long T2 combined with the fact that this volume loss was observed on two separate occasions, further increases the credibility of the MRI-determined muscle volume loss observed in this study.

The time course of changes in the magnitude of the slow relaxing long T2 component did not lag behind the changes in muscle swelling after Ecc 2, as they did after Ecc 1 (Figure 3). On the average, the magnitude of the slow relaxing long T2 component and muscle swelling was less after Ecc 2 compared to Ecc 1. The peak increase in swelling and the slow relaxing long T2 component occurred earlier after Ecc 2, on day 2, and there was a strong significant correlation between the two variables.

These results are consistent with the hypothesis that the repeated bout effect extends to the delayed T2 increase, recently proposed by Foley and colleagues in 1999 (43). The results from NNLS computed T2 spectra in the present study represent the first quantitative evidence that the smaller increase in the delayed T2 after the second bout of eccentric exercise is the result of more modest changes in the extracellular and/or vascular fluid compartments in the eccentrically damaged muscle.

Furthermore, the observed muscle volume loss is also consistent with the existence of a small population of "susceptible" or mechanically weak fibers within a muscle, a hypothesis originally proposed by Armstrong and colleagues (8). These investigators reported necrosis in approximately 5% of fibers in muscles of rats after down hill running. Other groups have also reported similar decreases in muscle mass in humans after eccentric exercise (63, 70). Since the MRI-determined muscle volume data was taken from only a portion of the entire muscle, it is possible that this decrease in muscle volume was due to a partial destruction of fibers and/or decrease in muscle diameter rather than a decrease in the number of muscle fibers.

Despite the exercise induced edema, we observed a significant increase in the acute T2 immediately following Con 2, performed at 2 d after Ecc 1. This result is consistent with the hypothesis that the exercise induced acute T2 increase is mainly related to the osmotically driven changes in the distribution of intracellular fluid within the cellular sub-compartments (91). However, the acute ΔT2 after Con 2 (1.5 ± 0.5 ms) was lower compared to Con 1 (3.2 ± 0.5 ms) and Con 3 (5.0 ± 0.4 ms). Since the subjects performed the same concentric exercise during Con 1, Con 2, and Con 3, the results of the current study suggests that in the presence of exercise induced edema (i.e.,

> ~20% of the total signal intensity attributable to the slow-relaxing long T2 component), T2 calculated assuming a monoexponential decay from spin-echo images does not fully reflect the degree of muscle activation during exercise.

Although the somewhat larger increase in acute ΔT2 after Con 3, compared to Con 1, was not significant, it is possible that this increase in acute ΔT2 might be due to increased activation of the uninjured muscle fibers in an attempt to counter the loss in force production from the damaged and/or destroyed fibers (i.e., decreased muscle volume). However, in the absence of echo-planar data after Con 3, this conclusion could be criticized on the grounds that the residual edema might have contributed to the somewhat larger increase in acute ΔT2 calculated assuming a monoexponential decay from spin-echo images acquired immediately after Con 3 rather than increased muscle activation.

In summary, the main result of this study is that the delayed T2 observed in eccentrically damaged muscles during DMS is mostly due to edema. On the other hand, the persistent elevation in eccentrically damaged muscles long after recovery from eccentric exercise is not due to residual edema. Therefore, the long-term elevation in muscle T2 may reflect a more permanent change in the intracellular water chemistry and/or redistribution of the intracellular fluid into the sub-compartments within the eccentrically exercised muscle. NNLS computed T2 spectra revealed that the smaller increase in muscle T2 after Ecc 2 compared to Ecc 1 is the result of more modest changes in the extracellular and/or vascular fluid compartments in the eccentrically damaged muscle. This result is consistent with the hypothesis that the repeated bout effect extends to the delayed T2 increase (43). Lastly, the eccentric exercise-induced edema diminishes

the acute T2 change measured during exercise using conventional spin-echo imaging methods; therefore, this parameter should not be used an index of muscle activity in such cases.

CHAPTER IV

MRI Evaluation of Heat and Stretch as Treatments for Muscle Damage After Eccentric Exercise.

Abstract

The initial aim of this study was to monitor the effects of topical heat and/or static stretch treatments on the recovery of muscle damage by eccentric exercise. For this purpose, 32 untrained male subjects performed intense eccentric knee extension exercise, followed by two weeks of treatment (heat, stretch, heat plus stretch) or no treatment (control, n =8/group). Isometric strength testing, pain ratings, and multi-echo magnetic resonance imaging of the thigh were performed before and at 2, 3, 4, 8, and 15 d following the exercise. Increased T2 relaxation time, muscle swelling, pain ratings, and strength loss confirmed significant muscle damage during the post-exercise period. Pain ratings and muscle volume recovered to baseline by 15 d, although muscle strength remained lower (77 ± 4 vs. 95 ± 3 kg pre-exercise, $p < 0.05$, mean ± SE) and T2 values higher (32.2 ± 0.8 vs. 28.6 ± 0.2 ms pre-exercise).

Treatment modality showed no effect on extent of recovery in any measured parameter, allowing pooling of data for a follow-up study in which a subset of nine subjects returned for testing 6 wk post exercise. Results showed a persistent elevation in T2 (31.0 ± 1.3 at 6 wk vs. 29.2 ± 0.3 ms pre-exercise) and a small but significant muscle volume loss (1340 ± 180 at 6 wk vs. 1540 ± 90 cm^3 pre-exercise), confirming a previous report on arm muscle. These changes were accompanied by a persistence of muscle

weakness (17 ± 4% below baseline strength). These long term changes are consistent with the hypothesis that intense eccentric exercise partially or totally destroys a small subpopulation of mechanically weak fibers. The data also indicate that heat and/or static stretching treatments are not effective in preventing or reversing this damage.

Introduction.

Delayed muscle soreness (DMS) is the sensation of pain and/or discomfort experienced 24 - 48 hours after unaccustomed exercise (6-8, 21, 45, 58, 77). DMS occurs most commonly in individuals who engage in sporadic, strenuous exercise or abruptly increase the intensity of the training. Research has shown that eccentric or lengthening contractions cause structural damage to the muscles, and it is this damage to muscles that results in the sensation of soreness in the days following eccentric exercise (6, 8, 9, 12, 46, 47, 77). Numerous studies have documented the exercise-induced muscle damage following lengthening contractions by tracking different indices, including serum levels of muscle enzymes, markers of inflammation, swelling, and decrements in muscle performance (7, 12, 17, 21, 22, 44, 53, 64, 76, 78, 80, 85, 99, 101).

In the lay exercise community, post-exercise stretching is often recommended as a preventive measure against the development of DMS. However, there is little experimental evidence to support these claims. Early studies by deVries and Abraham reported that static or stationary stretching after eccentric exercise reduced muscle soreness (1, 28). Recently, Buroker and Schwane found that post-exercise static stretching did not alleviate the signs and/or symptoms of DMS (15).

Sports medicine practitioners often use superficial heat and cold to treat musculoskeletal injury. Immediate cold application is recommended during the acute stage of injury to decrease bleeding, capillary effusion, pain, and inflammation (75). On the other hand, heat application is recommended during the healing process to increase vascular and lymphatic flow. Heat application also improves the range of motion through increased collagen extensibility. Like therapeutic cold application, heat also reduces pain through sensory stimulation of the skin.

Most studies have found that cold application after eccentric exercise provided temporary pain relief (60, 106). Kuligowski et al. (66) recently reported that cold whirlpool and contrast therapy (alternating cycles of hot and cold), were more effective than warm whirlpool and no treatment in alleviating the signs and/or symptoms of DMS. In addition, Prentice showed that cold plus static stretching was superior to heat followed by static stretching in reducing muscle pain and increasing muscle relaxation (88). The limited available evidence suggests that heat application does not alleviate the signs and/or symptoms of DMS.

To date, no studies have tested directly the effectiveness of therapeutic heat application in alleviating the signs and/or symptoms of DMS. There are two main flaws in the experimental design of Kuligowski et al. and Prentice's study. First, the application of heat immediately following the eccentric exercise has the potential to promote the injury process by increasing capillary effusion and swelling. Second, the application of heat (40°C) for 20-30 min might not be long enough to promote the clearance of extracellular fluid by the lymphatic circulatory system, which is much slower than blood flow. In addition, these studies did not include a measure of muscle

damage during the treatment period. Swelling was measured using limb circumference measures, which is not sensitive to small changes in the extracellular fluid volume. Therefore, it was not possible to separate the temporary analgesic effect from the anti-inflammatory effect of the different treatments on muscle damage.

Recent advances in the field of magnetic resonance imaging (MRI) have allowed investigators to measure in vivo the extent of muscle damage and changes in muscle compartment cross-sectional area during DMS (42, 43, 61, 81, 97, 103). In addition to the acute, temporary T2 increase which is observed in muscles even after non-damaging exercise (e.g. Prior et al.(91)), damaging eccentric exercise results in the appearance of a delayed and much longer lasting T2 increase in muscle (42, 43, 61, 81, 96, 103). This delayed T2 increase is correlated with the appearance of very slow relaxing T2 components in high resolution T2 relaxation spectra of the muscles (ref. Expt. 1), and therefore is likely due to the accumulation of slow relaxing extracellular fluid in the damaged regions. Thus, the damaged regions of the muscles appear brighter in T2-weighted MR images compared to undamaged or normal muscles. Several MRI studies have exploited this increase in muscle T2 to document the time course of muscle damage after eccentric exercise and the subsequent recovery in days following (42, 43, 61, 97, 103).

Recently, Foley et al. (43) reported a persistent elevation in T2 up to 8 wk after an eccentric exercise bout. In addition, these investigators reported a 7-10% muscle volume loss at 2 to 8 wk after the damaging eccentric exercise. To date no MR studies have been published to confirm the enduring muscle volume loss reported by Foley et al. (43). Furthermore, if this reported decrease is truly the result of muscle loss, then a parallel

decrease in maximal isometric strength would be expected. However, Foley et al. did not measure isometric strength loss in the eccentrically damaged muscle, an omission that will be addressed in the current study.

The purposes of this study were: (1) to examine the effects of long term heat application and/or static stretch on the recovery of muscle damage as indicated by the delayed T2 response, recovery of isometric strength, dissipation of pain, and swelling, (2) to test whether the volume loss reported by Foley et al. in the elbow flexor muscle group can be repeated in a different subject group using a different muscle group (knee extensor muscle group) and exercise, and (3) if muscle volume loss occurs in our current study, to test whether this is reflected by strength loss.

Methods.

Subjects. 32 non-weight trained male subjects were recruited from the university community. Subjects were screened for any medical and/or orthopedic conditions that might preclude severe leg exercise or MR imaging procedures. Furthermore, subjects who engaged in strenuous leg exercise as part of their daily activity were excluded from the study. The University Committee for Research Involving Human Subjects approved procedures for the study, and all subjects gave informed written consent before participation.

Subjects were randomly assigned to one of four groups. The three treatment groups were heat (n=8), static stretching (n=8), and heat plus static stretching (n=8). The fourth group (control, n=8) received no treatment. A randomly selected subset of twelve subjects was asked to return at 6 wk after eccentric exercise for additional MR scans and

single-leg isometric maximal voluntary contraction force (MVC) testing. Out of these twelve subjects, three were unable to return for additional testing, giving an n of nine for the 6 wk tests.

Exercise Protocol. Following a preliminary MRI scan, subjects performed three MVC trials with the quadriceps femoris muscle group in the seated position (hip at ≈ 90° and the knee at ≈ 135°) using a standard cable tensiometer. Subjects were given 3 min rest between each contraction and were verbally encouraged to give maximal effort on each trial. Isometric strength was determined by averaging the MVC force from the three trials. Upon completion of the MVC testing, there was a short rest period during which a trainer demonstrated the single-leg eccentric knee extension exercise. The starting load for the eccentric exercise was set at 100% of the MVC force. A trainer lifted the weight to the starting position in which the knee was fully extended. The weight was lowered smoothly to a 4s count to the finish position (≈ 60° knee angle). Subjects performed eccentric knee extensions with maximal load in each set to failure (unable to lower the weight in a controlled manner), with 3 min of rest between sets. On average, subjects performed 6-8 sets of 5-10 repetitions. If a subject was unable to perform a minimum of 5 repetitions, the load was lowered by 5 kg so that he could complete the set. This reduced load was used for the next set. The eccentric exercise continued until the eccentric exercise load was below 50% of the MVC force.

Subjects were asked to rate perception of overall soreness in the quadriceps femoris muscle by drawing a line on a linear pain scale of 1 (no soreness) to 100 (extremely sore)(33). The subjects were seated with the hip at ≈ 90° and the test leg bent

at ≈ 90° before rating perception of pain. Pain ratings and MVC force measurements were repeated at the same time of day 2, 3, 4, 6, 8, 15 d and after eccentric exercise.

MRI methods. Subjects were positioned feet first and supine in a 1.5 T Signa whole body imager (General Electric, Milwaukee, WI). The body coil was centered on a mark inked 1/3 of the distance along a line from the top of the patella to the anterior iliac crest. Twelve to fourteen T2-weighted spin-echo axial MR images (TR/TE = 1500/30,60; 256x192 acquisition matrix; 10 mm thick; 10 mm gap; 32 cm FOV) of the thigh region were acquired. The MR images were acquired at rest prior to MVC testing and again at 2, 3, 4, 6, 8, 15 d after eccentric exercise. Images, pain ratings and MVC measurements were also acquired in a subset of nine subjects 6 weeks after eccentric exercise.

Mean T2 values were determined for the quadriceps muscle group from the first axial image below the scrotum and continuing through to the last image not showing the quadriceps tendon. This criterion was used in order to measure the changes in the belly of quadriceps muscle group across all subjects with varying heights. T2 images were calculated from the images acquired at the two echo times assuming a monoexponential decay (40). A region of interest in each image was defined by manually tracing around the entire quadriceps muscle group, excluding subcutaneous fat and skeletal tissues, using the software package Xvessel (43). Once the region of interest was established, the software automatically calculates the number of pixels within the region and the mean T2 according to the equation:

$$\text{Volume weighted average T2} = \frac{\Sigma\,(\text{Pixel count} * \text{mean T2 in each slice})}{\Sigma\,(\text{Pixel count in each slice})}$$

In addition, "x-vessel" was used to measure the volume of quadriceps muscle compartment by the same manual tracing method. Muscle volume was estimated by multiplying the pixel counts within the defined region by the pixel area (field of view/matrix) and then by the slice thickness plus interslice interval (2 cm). All MRI data analysis was done by the same person, who was blinded to subject identity and treatment group.

Treatment Protocol. At 24 h post-eccentric exercise, pain ratings, MRI scans, and MVC force measurements were recorded in order to measure muscle status prior to beginning any treatment. Subjects were then separated according to group assignments and were given a brief training session on their specific treatment. In addition, subjects were required to fill out a daily log verifying treatment start and stop times. The subjects were asked to refrain from discussing the treatment assignment with other subjects and also with the investigators.

The heat treatment was applied using a heating pad (GAYMAR, Orchard Park, NY) set at 41°C for 2 h starting at 36 h post-eccentric exercise to avoid the detrimental effects of heat during the acute injury stage. The temperature and duration were chosen to maximize the increase in vascular and lymphatic flow that has been suggested to occur with heat application, and to minimize any discomfort that might occur with prolonged local hyperthermia (75). The heating units were calibrated and the temperature was set at 41°C prior to distribution to the subjects. The heating pad (18"x24") was placed around the leg to cover the belly of the quadriceps muscle group. The subjects were instructed to apply heat for 2 hours every day at the same time of day until the soreness completely subsided.

The static stretching treatment began at 36 hours post-eccentric exercise. Stretches for the major muscle groups of the thigh were selected from two popular texts (4, 5). A criterion for selection was that the stretches were easy and safe for an untrained individual. Subjects warmed up the exercised leg by performing alternating leg lifts while lying supine, 15 times without stopping. Two static stretches were performed for each muscle group of the thigh: standing quadriceps stretch, prone quadriceps stretch, standing hamstring stretch, and seated hamstring stretch. In addition, subjects performed two additional static stretches: standing calf stretch and wall squat stretch. Subjects were instructed to hold each static stretch for a 20 s count and each static stretch was performed 3 times with a 30 s rest period. Subjects were instructed to perform the stretching program at the same time of the day until soreness subsided.

Subjects in the heat plus static stretching group were also instructed on the proper use of the heating apparatus and the proper technique of the different stretches. Like the other groups, heat plus stretching treatment began at 36 h post-eccentric exercise. The subjects were instructed to first apply the heat, which was then followed by the stretching program.

All the subjects were instructed to refrain from taking any anti-inflammatory medications and using other forms of therapeutic treatments (i.e. ice or message), other than their assigned treatment.

Statistical Analysis. Data were analyzed using a repeated measures two way analysis of variance (ANOVA) [heat (df =1) x stretch (df =1)] to assess the difference between treatments in muscle soreness, muscle T2 relaxation time, MVC, and muscle volume at 2, 3, 4, 6, 8, and 15 d following eccentric exercise. This analysis tests main

effects for treatment and time, and the interaction terms for heat x stretch and treatment-by-time. If there appeared to be a difference in baseline values for a specific parameter, a repeated measures two way analysis of covariance (ANCOVA) was performed on the time points after exercise, with the baseline preexercise values as a covariate to correct for this difference. This type of statistical analysis allowed us to test the treatment effect and the interaction between the two treatments in promoting recovery of the damaged muscles.

Our hypothesis that the volume of the exercised muscle compartment will be reduced after recovering from damaging eccentric exercise was tested using a two-tailed paired t-test on the values for the control group for day 15 versus pre-exercise. If a volume loss was detected, Pearson's product moment correlation coefficient (r) was calculated to assess the relationship between muscle volume loss and the decrease in MVC in the control group at day 15. Initially, analysis of just the control group was planned to test the previously reported persistent elevation in muscle T2 and muscle volume loss, independent of treatment. However, our finding of no treatment effect allowed pooling of the data and use of a repeated measures one-way ANOVA to assess the difference across time on the data from the subset of subjects who returned for additional testing at 42 days after the eccentric exercise. Significance levels for all tests were set at $P < 0.05$.

Results.

Figure 4 shows a representative series of MRI images of the thigh muscles of one subject during the 6 wk following eccentric exercise. These images provided both

muscle volume and T2 relaxation time data (lighter contrast corresponds to longer T2 values). Analysis of pre-exercise images showed no significant differences in muscle volume or muscle T2 between subject groups (Table 2). Likewise, no between-group differences were noted on any other recorded parameters prior to exercise.

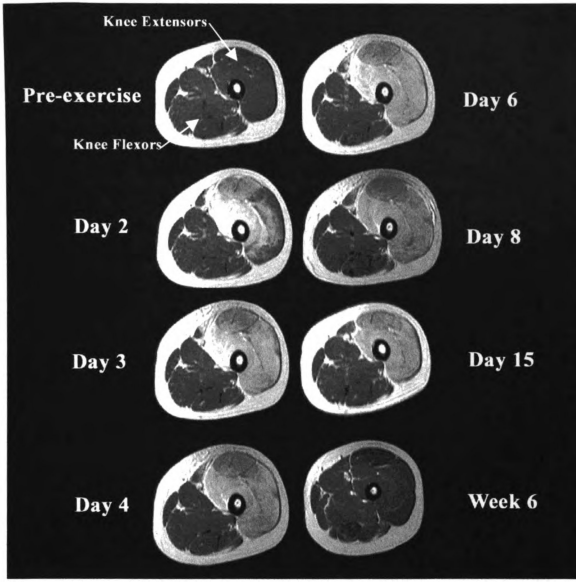

Figure 4. Representative mid-thigh axial plane MR images from one subject before (top left image; pre-exercise) and at each test interval during the 6 wk after the eccentric exercise bout. Images are oriented such that the anterior direction is to the right and the medial direction is upward.

Table 2. Subject characteristics before exercise.

Group	Age (yr)	Height (cm)	Weight (kg)	Muscle T2 (ms)	Knee extensor Volume (cm^3)	MVC (kg)	Pain (0 - 100mm)
Control	20.8 ± 0.8	179 ± 1	79 ± 5	28.7 ± 0.3	1500 ± 90	89.3 ± 6.9	0.75 ± 0.2
Heat	20.5 ± 0.8	179 ± 2	85 ± 7	28.7 ± 0.4	1700 ± 90	96.1 ± 4.4	1.7 ± 0.6
Stretch	20.8 ± 0.4	180 ± 3	83 ± 4	28.5 ± 0.4	1660 ± 90	97.3 ± 4.5	4.9 ± 1.8
Heat + Stretch	22.3 ± 1.1	178 ± 1.4	80 ± 4	28.4 ± 0.3	1450 ± 60	98.1 ± 6.5	1.9 ± 0.9
Pooled Mean	21.1 ± 0.4	179 ± 6	82 ± 2	28.6 ± 0.2	1580 ± 40	95.2 ± 2.8	1.8 ± 0.5

Values are means ± SE.

Muscle T2 progressively increased in all groups in the days following eccentric exercise and remained significantly elevated at 15 d post eccentric exercise (Figure 5). A significant main effect for time was found in the ANOVA ($p < 0.001$), but the main effects for heat ($p = 0.829$) and stretch ($p = 0.911$) were not significant. Neither the heat-by-stretch interaction ($p = 0.745$) nor the treatment-by-time interaction ($p = 0.143$) was significant.

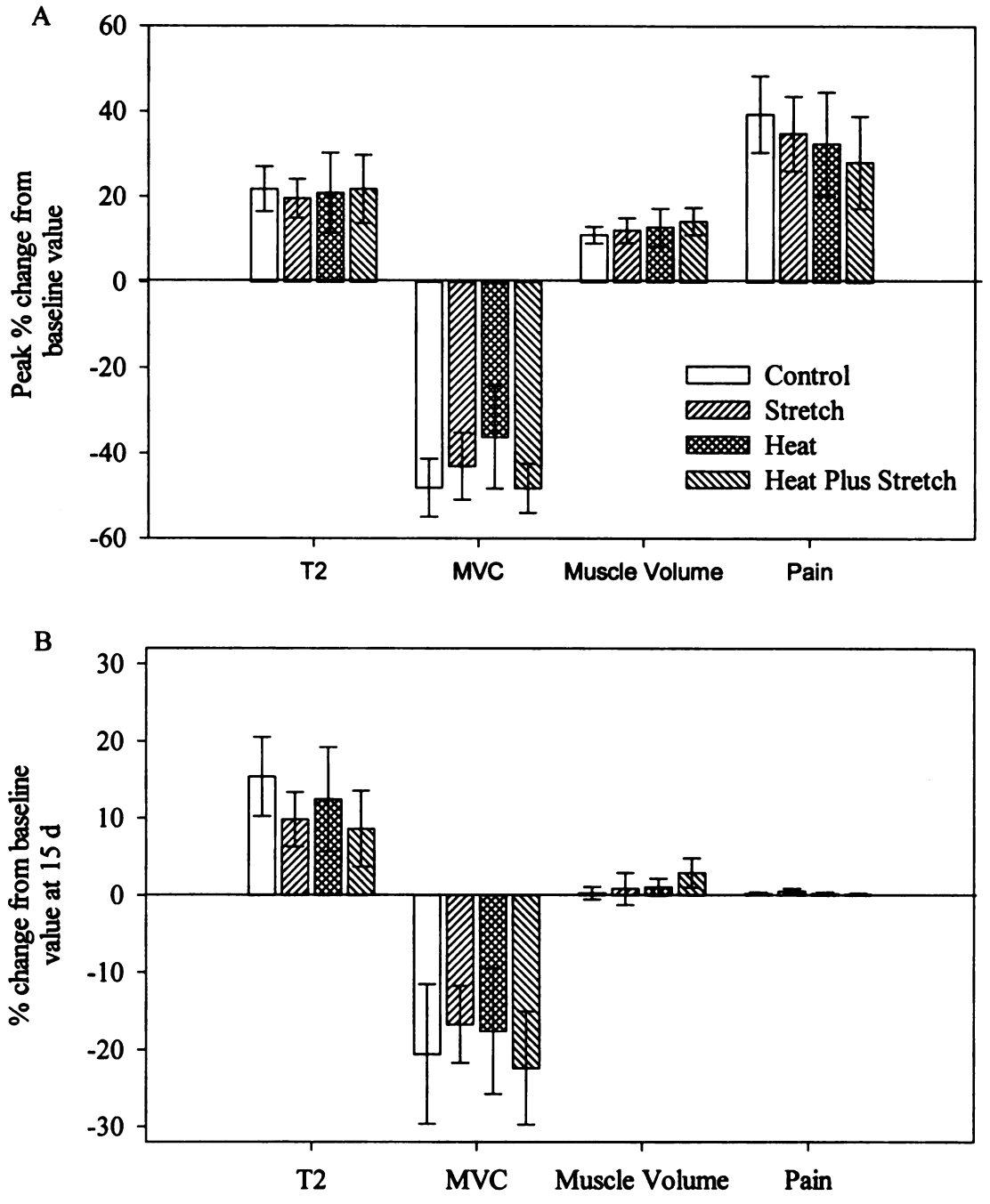

Figure 5. Peak percent change from baseline value (A) and percent change from baseline value at 15 d post-eccentric exercise (B) in T2, MVC, muscle volume, and pain across the different treatment groups.

Knee extensor muscle volume increased in the days following eccentric exercise in all groups and returned to baseline values on day 15 (Figure 5). The main effect of time ($p < 0.001$) and the heat-by-stretch interaction ($p < 0.05$) were found to be significant, but the main effect for treatment (heat, $p = 0.605$, stretch, $p = 0.979$) and the treatment-by-time interaction ($p = 0.888$) were not significant. Since the treatment-by-time interaction was not significant, we concluded that there was only a small heat-by-stretch interaction across time.

In the days following eccentric exercise there was a significant decrease in MVC in all groups (Figure 5). A significant main effect for time was found in the ANOVA ($p < 0.001$), but the main effects for heat ($p = 0.561$) and stretch ($p = 0.872$) were not significant. Neither the heat-by-stretch interaction ($p = 0.278$) nor the treatment-by-time interaction ($p = 0.153$) was significant. MVC remained below baseline values on day 15 in all groups ($p < 0.05$).

All subjects reported an increase in pain in the days following eccentric exercise (Figure 5). There was a significant main effect of time ($p < 0.001$), but the main effect for treatment (heat, $p = 0.501$, stretch, $p = 0.712$), treatment interaction ($p = 0.719$) and the treatment-by-time interaction ($p = 0.346$) were not significant. Pain ratings returned to baseline values on day 6 in all groups.

A randomly selected subset of the subjects ($n = 9$) returned for additional testing 42 d after the eccentric exercise. Since neither main effect of treatment nor treatment-by-time interaction was found in the repeated measures two way ANOVA, the data from this subset of subjects was analyzed using a repeated measures one-way ANOVA to test for significance across time in the dependent variables.

Figure 6 shows the time course of the change in T2 in these nine subjects as a percent change from pre-exercise or baseline. T2 relaxation times were significantly elevated compared to baseline at each time point after the eccentric exercise ($p < 0.05$). Peak increase in muscle T2 occurred on day 8 (40.9 ± 1.8 ms, mean ± SE) and remained significantly elevated from baseline on day 42 (31.0 ± 0.4 ms vs. 29.2 ± 0.3, $p < 0.05$).

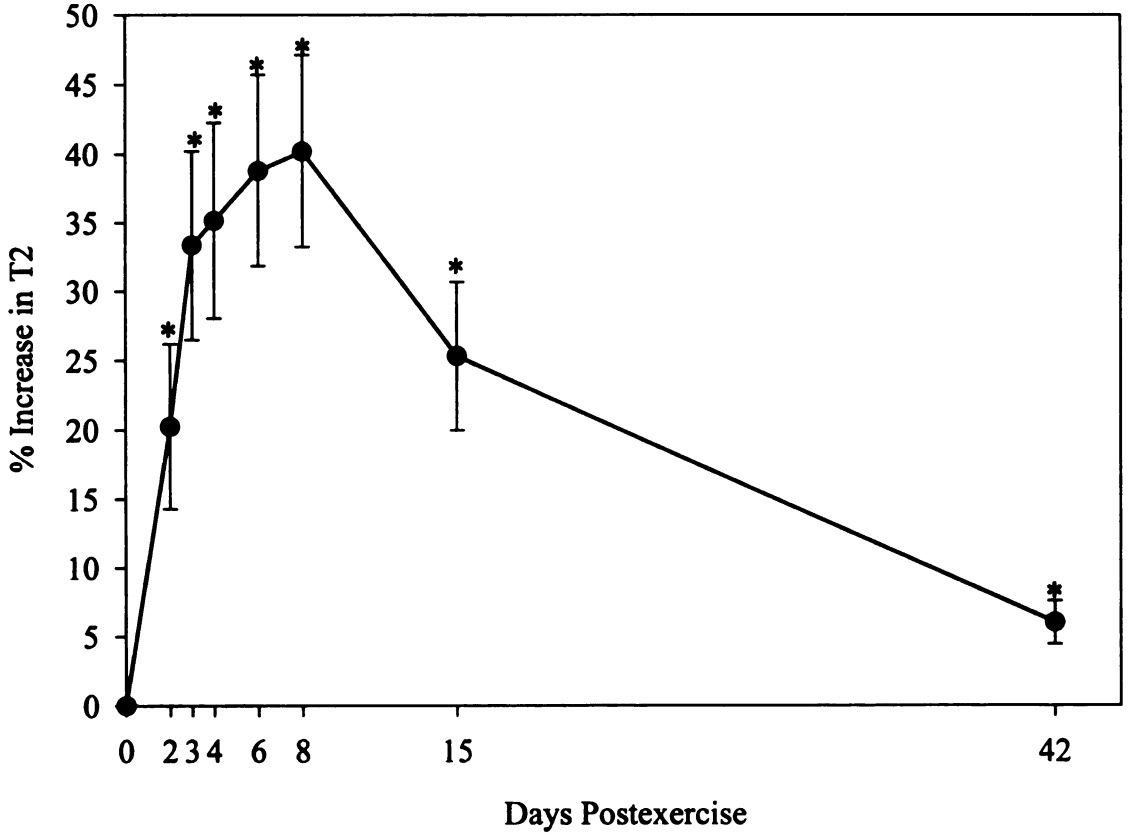

Figure 6. Time course of muscle T2 relaxation times for 6 wk after eccentric exercise (n = 9). All values are given as percent change from baseline muscle T2 measured before the eccentric exercise bout. * Significantly different from baseline values, $P < 0.05$; values are means ± SE.

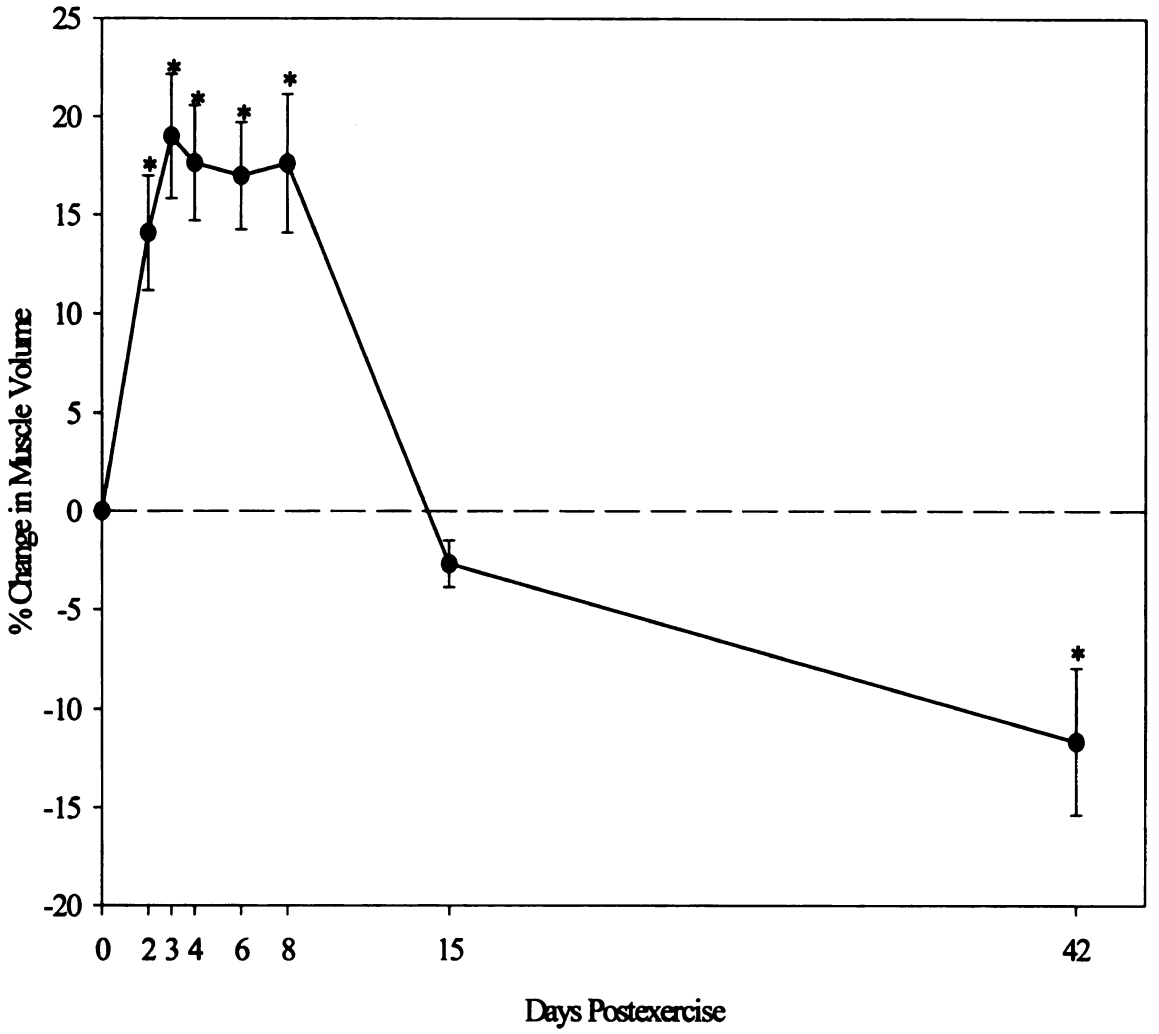

Figure 7. Time course of muscle volume changes for 6 wk after eccentric exercise (n = 9). All values are given as percent change from baseline muscle volume measured before the eccentric exercise bout. * Significantly different from baseline values, $P < 0.05$; values are means ± SE.

A significant increase in the muscle volume was observed on days 2 through 8 ($p < 0.05$) and peak swelling occurred on day 3 (17.2 ± 3.5%) following eccentric exercise (Figure 7). By day 15, muscle volume had not only returned to baseline, but also showed a trend towards a slight volume loss (3 ± 1%, $p = 0.057$). Muscle volume continued to decrease in the weeks after eccentric exercise to a value approximately 10% below

baseline volume (1536 ± 89 cm^3 at 42 days vs. 1339 ± 60 cm^3 baseline; $p < 0.05$). Both T2 and muscle volume showed a rapid rate of recovery towards baseline values between days 8 and 15, followed by a more gradual rate of recovery between days 15 and 42.

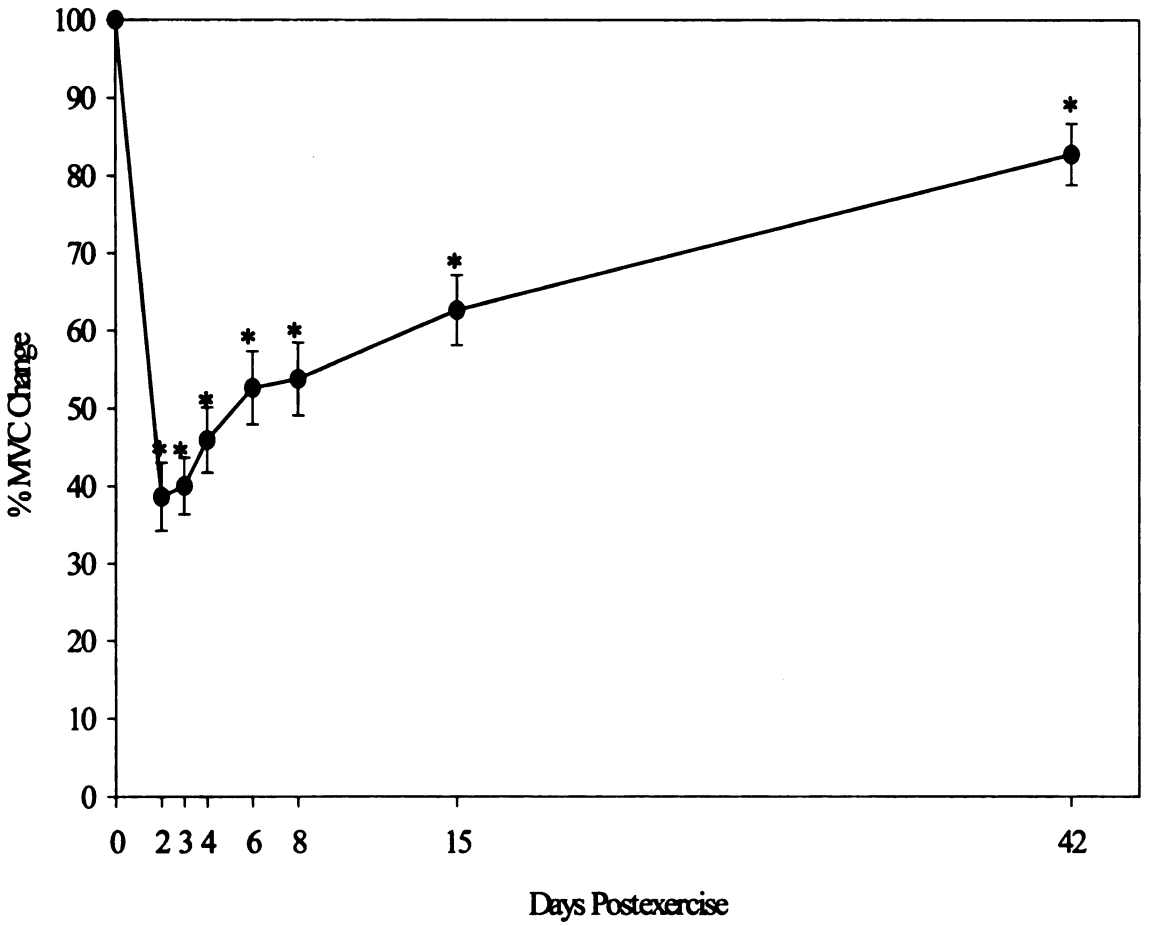

Figure 8. Time course of MVC changes for 6 wk after eccentric exercise (n = 9). All values are given as percent change from baseline MVC measured before the eccentric exercise bout. * Significantly different from baseline values, $P < 0.05$; values are means ± SE.

MVC values were significantly lower compared to baseline at each time point after the eccentric exercise ($p < 0.05$). Peak decrease in MVC (39 ± 4.4% loss in isometric strength) was observed on day 2 (Figure 8). Despite the rapid recovery in

isometric strength from day 3 to 8, there was still a significant deficit in strength on days 15 and 42 (-37.4 ± 5.0% at 15 days and 17 ± 4.0% at 42 days, $p < 0.05$). Pearson's correlation test revealed a significant moderately high correlation between mean baseline muscle volume and MVC ($r = 0.719$, $p < 0.05$). The correlation between muscle volume and MVC on day 42 was not significant ($r = .511$, $p = .160$). On the other hand, there was a significant moderate correlation between the change in muscle volume and the change in MVC from baseline on day 42 ($r = .683$, $p < 0.05$).

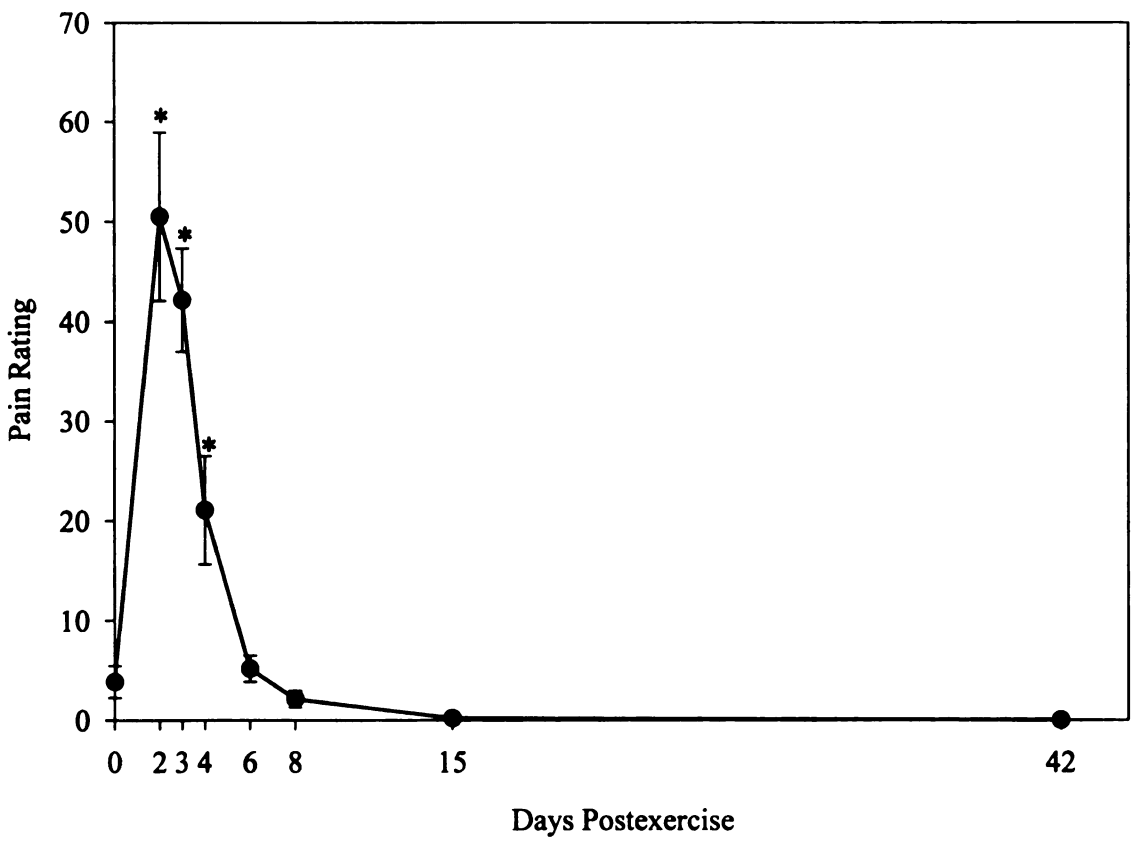

Figure 6. Time course of the pain ratings for 6 wk after eccentric exercise (n = 9). Subjects rated their perception of pain by drawing a line on a linear pain scale of 1 (no soreness) to 100 (extremely sore) in the seated position. * Significantly different from baseline values, $P < 0.05$; values are means ± SE.

On average, subjects reported peak pain on day 2 following eccentric exercise with recovery by day 6 (Figure 6). The time courses of changes in the indexes of muscle damage varied widely, with pain and isometric strength loss peaking earliest at 2 days postexercise, followed by muscle swelling on day 3 and finally by T2 which peaked on day 8.

Discussion.

Treatment effect. The main result of this study is that therapeutic application of superficial heat and/or static stretching after intense eccentric exercise did not promote the recovery from muscle damage as indicated by the similar time course and magnitude of the T2 changes after the eccentric exercise. Additionally, swelling, pain, and the deficit in strength on day 15 were not different between the treatment groups. It is well known that eccentric contractions cause structural damage to the muscles, and it is this damage to muscles that ultimately results in the sensation of soreness and the decrements in muscular performance in the days following eccentric exercise. (6, 8, 9, 46, 47, 77). The effectiveness of the eccentric exercise protocol in producing muscle damage is evident from the large increase in T2 and muscle swelling, and from the decrease in MVC in the first few days after the exercise (Tables 1 through 4). Previous studies have reported similar results in the days following eccentric exercise (21, 42, 43, 78, 97).

Although the exact mechanism of DMS is unknown at present, DMS ultimately arises from a sequence of events that occur after the eccentric exercise. Armstrong's model (7) divides the muscle injury process in DMS into four distinct stages: 1) initial, 2) autogenetic, 3) phagocytic and 4) regenerative. Therapeutic application of heat and/or

static stretching could potentially affect the latter two stages of the model. Application of topical heat increases local vascular and lymphatic flow, which, in turn, may enhance the removal and repair aspects of the healing process (75). Possible mechanisms by which static stretching post-eccentric exercise could alleviate the sensation of pain are as follows: 1) disperse the excess fluid that accumulates from muscle damage (14), 2) reduce muscle spasms that may occur during DMS (28, 56), and 3) alter the response of the afferent group IV nerve fibers, which conduct pain impulses to central nervous system (7). Accordingly, heat combined with static stretching should be effective in reducing the sensation of pain as well as enhancing the healing process.

In our study, the peak increase in T2 and the persistent elevation in T2 at day 15 were similar across the different groups, which suggests that heat and/or static stretching did not enhance the healing process. As expected, there was significant swelling in the exercised muscle compartment during the first week after the eccentric exercise (21, 22, 43, 58, 78, 80). Heat and/or static stretching did not reduce the extent of swelling in the exercised muscle compartment, nor did any treatment expedite the recovery from edema.

Accompanying the increase in T2 and muscle swelling, there was a significant decrease in MVC in the days following eccentric exercise in all groups. The decrease in MVC and subjective pain ratings were not different between the treatment groups across time. The decrease in MVC on days 2 and 3 may be due at least in part to the reluctance of subjects to voluntarily produce maximal force in sore muscles. However, soreness cannot explain the long term decrements in strength, because strength was still reduced when soreness was no longer apparent.

Our study results are consistent with other reports that have shown that post-exercise static stretching did not alleviate the signs and/or symptoms of DMS (15, 74, 105). On the other hand, deVries (29) and Abraham (28) reported a significant chronic reduction in soreness with repeated stretching after eccentric exercise. However, a comparison of deVries findings with ours may not be valid because of the wide variety of muscle disorders among his subjects.

A more appropriate comparison can be drawn between our results and the findings of Buroker and Schwane. Specifically, Buroker and Schwane found that post-exercise static stretching did not alleviate the signs and/or symptoms of DMS as indicated by the creatine kinase (CK) release (an indirect marker of muscle damage), muscle strength and limb girth (an indirect measure of muscle swelling) (15). The lack of a difference in the T2 response from our study confirms the interpretation from Buroker and Schwane's study that the cellular processes of muscle recovery were not affected by the static stretching regimen.

Therapists often use superficial heating modalities to increase tissue extensibility to allow for increased efficacy of stretching techniques (75). Furthermore, this decreased tension, along with increased clearance of metabolites could hypothetically aid recovery in the later stages of DMS (phagocytic and regenerative stages of Armstrong's model of DMS), during which the muscles are stiff and painful, but not acutely inflamed. Our results show that neither heat alone nor heat plus static stretching reduced the sensation of pain. These results are consistent with previous reports that have shown that heat by itself and heat combined with static stretching were not effective in alleviating the subjective symptoms of DMS (51, 66, 88).

The MRI technique employed in the present study provides the first objective evidence that heat and/or static stretching does not enhance the recovery of damaged muscle fibers after eccentric exercise. Despite the widespread belief that heat and/or static stretching alleviates DMS, our results indicate that heat and/or static stretching does not consistently reduce soreness, swelling or muscle damage. The practical implication of these results is that clinicians should be aware that prescribing heat and/or static stretching following intense eccentric exercise will not enhance the recovery of damaged muscles.

Long term time courses of muscle damage. In the subset of subjects who returned for additional testing, T2 increased significantly in the days following eccentric exercise and peaked on day 8, (40.9 ± 1.8 ms, Figure 6). Although there was a gradual decline in T2 over time, T2 remained elevated by ~6% on day 42 in the present study. Based on the fact that muscle volume had decreased, it is unlikely that residual edema caused the elevated T2 on day 42. Therefore, the persistent increase in T2 after a bout of eccentric exercise is more than likely the result of more permanent changes in the intracellular water chemistry and/or redistribution of the intracellular fluid into sub-compartments within the cell.

Foley and co-workers reported a much larger mean peak T2 value of 46.7 ± 3.8 ms on day 7 in the elbow flexor muscles, which suggests that the exercise induced muscle damage was more severe in their study, despite similar intensity of the eccentric exercise protocols between the two studies. Unlike the arm muscles, the leg muscles are more likely to perform lengthening contractions during daily activities (i.e., walking downhill or stairs). Therefore, it is possible that subjects in the current study may have

been somewhat "pre-adapted" which, in turn, could make them less susceptible to the eccentric exercise induced muscle damage (21).

During the first week after eccentric exercise, we observed significant swelling in the exercised muscle compartment (Figures 4, 7). These results are consistent with previous reports in which the investigators tracked swelling by measuring the change in the circumference of the exercised limb (21, 58, 93). In particular, Foley et al. recently reported a similar time course of changes in the MR measurements of the elbow flexor muscle group after eccentric exercise (43). Foley et al. reported a ~40% increase in the exercised muscle compartment on day 2 following eccentric exercise. We observed peak swelling on day 3 but this increase (19.0 ± 3.2%) was not nearly as high as the previous report. This is consistent with the smaller peak T2 increase noted above, again suggesting some degree of pre-adaptation in the leg muscles examined in the current study.

During the second week after the eccentric exercise, swelling subsided and a trend towards a small decrease in muscle volume was observed on day 15 (2.7 ± 1.2%) (Figure 7). Muscle volume continued to decrease in the weeks after the exercise to a value <90% of baseline volume on day 42 (Figure 7). Other groups have also reported a decrease in muscle mass after eccentric exercise in humans (63, 70). Recently, Foley et al. assessed muscle volume in MR images after eccentric exercise and found a 7-10% decrease in the elbow flexor muscle compartment 2 to 8 wk post-exercise (43). The MRI-determined muscle volume loss found in our study confirms the volume loss previously reported by Foley and co-workers. Since the MRI-determined muscle volume data was taken from only a portion of the entire muscle, it is possible that this decrease in

muscle volume was due to a partial destruction of fibers and/or decrease in muscle diameter rather than a decrease in the number of muscle fibers.

A unique aspect of this study was that we measured both MVC force and muscle volume in the days following eccentric exercise (Figure 8). Pearson's product moment correlation test revealed a significant moderate correlation between mean muscle volume and MVC ($r = 0.719$, $p < 0.05$) before the start of the eccentric exercise. Others have reported similar correlations between uninjured muscle mass/volume and MVC (34, 71). Recently, Bamman et al. reported that the correlation between MRI-determined muscle volume of the uninjured triceps surae muscle and MVC ($r = 0.649$) was better than whole limb antrhopometric ($r = 0.584$) and dual-energy x-ray absoprtiometry (DEXA) ($r = 0.381$) estimates of muscle size (11).

In the present study, the correlation between mean muscle volume and MVC 42 days after the eccentric exercise was not significant, mostly due to the large variance in these measures. In an attempt to reduce the variance in these measures, we calculated delta (Δ) scores for muscle volume and MVC (i.e., the change in muscle volume and MVC from baseline values) which, in turn, resulted in a significant correlation between Δ muscle volume and Δ MVC on day 42 ($r = 0.683$, $p < 0.05$). To date no studies have examined the relationship between MVC and MRI-determined volume of eccentrically damaged muscle. This persistent deficit in MVC weeks after the eccentric exercise is mostly likely the functional consequence of the chronic loss in muscle volume observed in this study.

The persistent decrease in muscle volume on day 42 is consistent with the "stress susceptible" fiber theory proposed by Armstrong and co-workers (8). Armstrong et al.

suggest that a sub-population of fibers (~5%) nearing the end of their functional life in healthy muscles are more susceptible to the increased active strain and/or stress produced during lengthening contractions. This increase in active stress/strain is the primary cause of the irreversible ultrastructural damage to the pool of stress-susceptible fibers, which leads to degeneration of these cells in the days following the exercise. Accordingly, there is prolonged decrease in muscle strength and mass, and a significant increase in muscle soreness and serum levels of muscle proteins after eccentric exercise. We interpret the persistent loss in muscle volume and the decrease in MVC observed in this study weeks after eccentric exercise as evidence in favor of the existence of a "susceptible" or "vulnerable" subpopulation of muscle fibers and that these fibers may fail to repair and subsequently undergo degeneration.

In summary, we interpret the lack of a treatment effect across time as evidence that heat and/or static stretching did not enhance the cellular processes involved in the phagocytic and regeneration stages of DMS. Despite the widespread belief in the lay exercise community that heat and/or stretching post-eccentric exercise alleviates DMS, our results indicate that these modalities do not consistently reduce soreness. The 11% loss in MRI-determined muscle volume in the weeks after the eccentric exercise confirms the previously reported results in arm muscle by Foley and co-workers (43). This volume loss combined with the persistent deficit in MVC may be related to the partial or total destruction of a small subpopulation of mechanically weak fibers in days following intense eccentric exercise.

CHAPTER V

SUMMARY AND FUTURE STUDIES

The first set of experiments in this study demonstrated that the delayed T2 increase in eccentrically damaged muscles during DMS is primarily due to edema in the early post-exercise period, as indicated by the presence of the slow relaxing long T2 component and muscle swelling. However, the T2 parameter remains elevated long after recovery from edema. This persistent elevation in muscle T2 may thus reflect a more permanent change in the intracellular water chemistry and/or redistribution of the intracellular fluid into the sub-compartments within the eccentrically exercised muscle.

The application of the echo planar MRI method in the current study also demonstrated that the attenuation of the acute T2 increase after a second eccentric bout is the result of more modest changes in the extracellular and/or vascular fluid compartments in the previously damaged muscle. This result confirms and extends a previous report that the repeated bout effect extends to the delayed T2 increase (43).

Although several investigators have suggested that the increase in T2 could be used as a method for estimating the relative intensity of muscle recruitment (2, 3, 40, 86, 87), the current study shows that this is not true under all circumstances. In the current study, the acute ΔT2 after repeated bouts of the same concentric exercise performed before and 2 and 21 d after eccentric exercise was different. This result suggests that the acute T2 change calculated from standard spin-echo images in the presence of eccentric exercise-induced edema might not accurately reflect the relative intensity of muscle

recruitment during exercise. This finding also supports a recommendation that caution be used in applying the T2-based method of estimating muscle recruitment in other populations with damaged or abnormal muscle tissue.

The initial aim of the second set of experiments was to monitor the effects of topical heat and/or static stretch treatments on the recovery of muscle damage from intense eccentric exercise. Increased T2 relaxation time, muscle swelling, pain ratings, and strength loss confirmed significant muscle damage during the post-exercise period. Pain ratings and muscle volume recovered to baseline by 15 d, although muscle strength remained lower and T2 values higher. Treatment modality showed no effect on extent of recovery in any measured parameter, allowing pooling of data for a follow-up study in which a subset of nine subjects returned for testing 6 wk post exercise. Results showed a persistent elevation in T2 and a small but significant muscle volume loss, confirming a previous report on arm muscle. These changes were accompanied by a persistence of muscle weakness. These long term changes are consistent with the hypothesis that intense eccentric exercise partially or totally destroys a small subpopulation of mechanically weak fibers.

In general, the current study shows that MRI provides investigators a new technique to study, in vivo, the time course of the development and recovery of muscle damage and swelling during DMS. Furthermore, MRI promises to be a valuable tool in clinical rehabilitation research in evaluating the efficacy of different treatment modalities in promoting the recovery of damaged muscles.

Future Studies. The following are offered as potential experiments that could build on the findings of our current study applying MRI to the study of muscle damage.

A review of the MRI literature shows that all of the MR studies documenting muscle damage following eccentric exercise have used healthy, young subjects. To date, no in vivo measurements of muscle damage have been reported following eccentric exercise in the elderly population. This may be due to the fact that previously used methods of measuring damage are not very sensitive, requiring the induction of significant eccentric damage to produce a reliably measurable change. The sensitivity of the MR T2 measurement may allow studies of more moderate eccentric injury, suitable to application in an elderly population.

Faulkner et al. (39) suggest that age-related development of muscle atrophy, weakness, and loss of power in the elderly are due to intrinsic age-related changes in the muscles that render them mechanically weak. Furthermore, these investigators suggest that the repeated exposure to lengthening contractions from daily activities could cause selective atrophy and/or degeneration of the large fast twitch fibers in the old, thus explaining the age-related decrease in muscle mass and power. However, no studies to date have examined the time course of the exercise-induced muscle damage and swelling during DMS at the start of a strength training program in the elderly population. Furthermore, no studies have examined the long term effects of strength training on muscle volume in the elderly.

We propose to use MRI to document the muscle damage and swelling, and measure isometric strength loss after a bout of light eccentric knee extension exercise in older subjects. Based on the findings of our current study and the available evidence on the effects of aging on skeletal muscle, we expect that the muscle volume loss, swelling, and strength loss will be greater in the older subjects compared to the young. Moreover,

due to the decrease in the ability of muscle fibers to regenerate in older hosts, we expect to observe deficits in strength and muscle volume for a longer period of time following eccentric exercise in the older individuals. The practical implication of this study is that it will provide insight into the design of strength training programs for the elderly that minimize the negative effects of eccentric contractions while maximizing the positive, hypertrophic effects of eccentric contractions, by manipulating the rest period between training sessions and the intensity of the eccentric or lengthening contractions.

In another set of experiments, we propose to follow up on our finding that the acute $\Delta T2$ response to identical bouts of concentric exercise is altered by the presence of edema or stress-induced adaptations in the muscle. In the current study, the T2 change was calculated using the standard method of fitting a monoexponential decay to data from several spin-echo images. Our echo-planar results on damaged muscles at rest have shown that this assumption of monoexponential T2 decay does not apply in acutely damaged muscle. However, in the current study we did not collect echo-planar images in the concentrically exercised muscles. Repeating this experiment using the echo planar method should clarify the source of the apparent differences in the acute T2 response in normal, damaged, and recovered muscle.

Our hypothesis is that echo-planar analysis will show that the T2 decay in edematous muscle follows a double rather than single exponential time course. If this hypothesis holds up, then application of a double exponential model to standard spin echo data should result in a T2 estimate that more accurately reflects the extent of muscle activity during the exercise.

REFERENCES

1. Abraham, W. M. Factors in delayed muscle soreness. *Med Sci Sports* 9: 11-20, 1977.

2. Adams, G. R., M. R. Duvoisin, and G. A. Dudley. Magnetic resonance imaging and elctromyography as indexes of muscle function. *J. Appl. Physiol.* 73: 1578-1583, 1992.

3. Adams, G. R., R. T. Harris, D. Woodard, and G. A. Dudley. Mapping of electrical muscle stimulation using MRI. *J. Appl. Physiol.* 74: 532-537, 1993.

4. Alter, M. J. *Science of stretching*. Champaign, IL.: Human Kinetics, 1988.

5. Anderson, B. *Stretching*. Bolinas, CA: Shelter Publications, 1980.

6. Armstrong, R. B. Initial events in exercise-induced muscular injury. *Med. Sci. Sports Exerc.* 22: 429-435, 1990.

7. Armstrong, R. B. Mechanisms of exercise-induced delayed onset muscular soreness: a brief review. *Med. Sci. Sports Exerc.* 16: 529-538, 1984.

8. Armstrong, R. B., R. W. Ogilvie, and J. A. Schwane. Eccentric exercise-induced injury to rat skeletal muscle. *J. Appl. Physiol: Respirat. Environ. Exercise Physiol.* 54: 80-93, 1983.

9. Asmussen, E. Observation on experimental muscular soreness. *Acta Rheum. Scand.* 2: 109-116, 1956.

10. Balnave, C. D., and M. W. Thompson. Effect of training on eccentric exercise-induced muscle damage. *J. Appl. Physiol.* 75: 1545-1551, 1993.

11. Bamman, M. M., B. R. Newcomer, E. Larson-Meyer, L. Weinsier, and G. R. Hunter. Evaluation of the strength-size relationship in vivo using various muscle size indices. *Med. Sci. Sports Exerc.* 32: 1307-1313, 200.

12. Bar, D. P. R., A. J. B. Rodenburg, R. W. Koot, and A. H. G.J. Exercise-induced muscle damage: recent developments. *BAM* 4: 5-16, 1994.

13. Bigland-Ritchie, B., and J. J. Woods. Integrated electromyogram and oxygen uptake during positive and negative work. *J. Physiol.* 260: 267-277, 1976.

14. Bobbert, M. F., A. P. Hollander, and P. A. Huijing. Factors in delayed onset muscular soreness of man. *Med. Sci. Sports Exerc.* 18: 75-81, 1986.

15. Buroker, K. C., and J. A. Schwane. Does postexercise static stretching alleviate delayed muscle soreness? *Physician Sportsmed* 17: 318-322, 1989.

16. Bushong, S. C. *Magnetic Resonance Imaging--Physical and Biological Principles.* St. Louis: Mosby Company, 1988.

17. Byrnes, W. C., P. M. Clarkson, J. S. White, S. S. Hsieh, P. N. Frykman, and R. J. Maughan. Delayed onset muscle soreness following repeated bouts of downhill running. *J. Appl. Physiol.* 59: 710-715, 1985.

18. Canonico, P. G., and J. W. C. Bird. Lysosomes in skeletal muscle tissue. *Am. J. Anat.* 137: 119-150, 1970.

19. Carlson, B. M., and J. A. Faulkner. The generation of skeletal muscle fibers following injury: a review. *Med. Sci. Sports Exerc.* 15: 187-198, 1983.

20. Clancy, S., and P. M. Clarkson. Immobilization during recovery from eccentric exercise. *Med. Sci. Sports Exerc.* 22: S37, 1990.

21. Clarkson, P. M., K. Nosaka, and B. Braun. Muscle function after exercise-induced muscle damage and rapid adaptation. *Med. Sci. Sports Exerc.* 24: 512-520, 1992.

22. Clarkson, P. M., and I. Tremblay. Exercise-induced muscle damage, repair, and adaptation in humans. *J. Appl. Physiol.* 65: 1-6, 1988.

23. Cohen, M. S., F. K. A. Shellock, J. Nadeau, J. Odershaw, R. Boxerman, R. Weisskoff, and T. J. Brady. Acute muscle T2 changes during exercise. *Soc. Magn. Reson. Med.*, 12th Ann. Meeting, 1991, p. 107.

24. Cole, W. C., A. D. LaBlanc, and S. G. Jhingran. The origin of biexponential T2 relaxation in muscle water. *Magn. Reson. Med.* 28: 19-24, 1993.

25. Conley, M. S., J. M. Foley, R. A. Ploutz-Snyder, and G. A. Dudley. Effect of acute head-down tilt on skeletal muscle cross-sectional area and proton transverse relaxation time. *J. Appl. Physiol.* 81: 1572-1577, 1996.

26. Croisier, J. L., G. Camus, G. Deby-Dupont, F. Bertrand, C. Lhermerout, J. M. Crielaard, A. Juchmes-Ferir, C. Deby, A. Albert, and M. Lamy. Myocellular enzyme leakage, polymorphonuclear neutrophil activation and delayed onset muscle soreness induced by isokinetic eccentric exercise. *Archives of Physioloy and Biochemistry* 104: 322-329, 1996.

27. Darr, K. C., and E. Schultz. Exercise-induced satellite cell activation in growing and mature skeletal muscle. *J. Appl. Physiol.* 63: 1816-1821, 1987.

28. deVries, H. A. Prevention of muscular distress after exercise. *Res Q* 32: 177-185, 1961.

29. deVries, H. A. quantitative electromyographic investigation of the spasm theory of muscle pain. *Am J Phys Med* 45: 119-134, 1966.

30. Donnelly, A. E., R. J. Maughan, and P. H. Whiting. Effects of ibuprofen on exercise-induced muscle soreness and indices of muscle damage. *Br. J. Sp. Med.* 24: 191-195, 1990.

31. Donnelly, A. E., K. McCormick, R. J. Maughan, P. H. Whiting, and P. M. Clarkson. Effects of a non-steroidal anti-inflammatory drug on delayed onset muscle soreness and indices of damage. *Brit. J. Sports Med.* 22: 35-38, 1988.

32. Duan, C., M. D. Delp, D. A. Hayes, P. D. Delp, and R. B. Armstrong. Rat skeletal muscle mitochondrial [Ca+2] and injury from downhill walking. *J. Appl. Physiol.* 68: 1241-1251, 1990.

33. Dudley, G. A., J. Czerkawski, A. Meinrod, G. Gillis, A. Baldwin, and M. Scarpone. Efficacy of naproxen sodium for exercise-induced dysfunction muscle injury and soreness. *Clin. J. Sport Med.* 7: 3-10, 1997.

34. Dudley, G. A., M. R. Duvoisin, G. R. Adams, R. A. Meyer, A. H. Belew, and P. Buchanan. Adaptations to unilateral lower limb suspension in humans. *Aviat. Space Environ. Med.* 63: 678-83, 1992.

35. Ebbeling, C. B., and P. M. Clarkson. Muscle adaptation prior to recovery following eccentric exercise. *Eur. J. Appl. Physiol.* 60: 26-31, 1990.

36. English, A. E., M. L. G. Joy, and R. M. Henkelman. Pulsed NMR relaxometry of striated muscle fibers. *Magn. Reson. Med.* 21: 264-281, 1991.

37. Enoka, R. M. Eccentric contractions require unique activation strategies by the nervous system. *J. Appl. Physiol.* 81: 2339-2346, 1996.

38. Faulkner, J. A., S. V. Brooks, and J. A. Opiteck. Injury to skeletal muscle fibers during contractions: conditions of occurance and prevention. *Phys. Ther.* 73: 911-921, 1993.

39. Faulkner, J. A., S. V. Brooks, and E. Zerba. Muscle atrophy and weakness with aging: contraction-induced injury as an underlying mechanism. *The Journal of Gerontology* 50A: 124-132, 1995.

40. Fisher, J. M., R. Meyer, A., G. R. Adams, J. M. Foley, and E. J. Potchen. Direct relationship between proton T2 and exercise intensity in skeletal muscle MR images. *Invest. Radiol.* 25: 480-485, 1990.

41. Fleckenstein, J. L., R. C. Canby, R. W. Parkey, and R. M. Peshock. Acute effects of exercise on MR imaging of skeletal muscle in normal volunteers. *AJR* 151: 231-237, 1988.

42. Fleckenstein, J. L., P. T. Weatherall, R. W. Parkey, J. A. Payne, and R. M. Peshock. Sports-related muscle injuries: evaluation with MR imaging. *Radiology* 172: 793-798, 1989.

43. Foley, J. M., R. C. Jayaraman, B. M. Prior, J. M. Pivarnik, and R. A. Meyer. MR measurements of muscle damage and adaptation after eccentric exercise. *J. Appl. Physiol.* 87: 2311-2318, 1999.

44. Friden, J., and R. L. Lieber. Structural and mechanical basis of exercise-induced muscle injury. *Med. Sci. Sports Exerc* 24: 521-530, 1992.

45. Friden, J., P. N. Sfakianos, and A. R. Hargens. Muscle soreness and intramuscular fluid pressure: comparison between eccentric and concentric load. *J. Appl. Physiol.* 61: 2175-2179, 1986.

46. Friden, J., M. Sjostrom, and B. Ekblom. A morphological study of delayed muscle soreness. *Experientia* 37: 506-507, 1981.

47. Friden, J., M. Sjostrom, and B. Ekblom. Myofibrillar damage following intense eccentric exercise in man. *Int. J. Sports Med.* 4: 170-176, 1983.

48. Fung, B. M., and P. S. Puon. Nuclear magnetic resonance transverse relaxation in muscle water. *Biophy. J.* 33: 27-38, 1981.

49. Golden, C. L., and G. A. Dudley. Strength after bouts of eccentric or concentric actions. *Med. Sci. Sports Exerc.* 24: 926-933, 1992.

50. Grabiner, M. D., T. M. Owings, M. R. George, and R. M. Enoka. Eccentric contractions are specified a priori by the CNS. *Proc. VXth Congr. Int. Soc. Biomech. Jyvaskyla Finland* July 2-6: 521-530, 1992.

51. Gulick, D. T., I. F. Kimura, M. Sitler, A. Paolone, and J. D. Kelly IV. Various treatment techniques on signs and symptoms of delayed onset muscle soreness. *Journal of Athetic Training* 31: 145-152, 1996.

52. Hasson, S. M., J. C. Daniels, J. G. Divine, B. R. Niebuhr, S. Richmond, P. G. Stein, and J. H. Williams. Effect of ibuprofen use on muscle soreness, damage, and performance: a preliminary investigation. *Med. Sci. Sports Exerc.* 25: 9-17, 1993.

53. Hather, B. M., P. A. Teschi, P. Buchanan, and G. A. Dudley. Influence of eccentric actions on skeletal muscle adaptations to resistance training. *Acta Physiol. Scand.* 143: 177-185, 1991.

54. Hazlewood, C. F., D. C. Chang, B. L. Nichols, and D. E. Woesner. Nuclear magnetic resonance transverse relaxation times of water protons in skeletal muscle. *Biphys. J.* 14: 583-606, 1974.

55. Hortobagyi, T., and T. Denaham. Variability in creatine kinase: methodological, exercise, and clinically related factors. *Int. J. Sports Med.* 10: 69-80, 1990.

56. Hough, T. Ergographic studies in muscular soreness. *Am. J. Physiol.* 7: 76-92, 1902.
57. Howell, J. N., A. G. Chila, G. Ford, D. David, and T. Gates. An electromyographic study of elbow motion duirng postexercise muscle soreness. *J. Appl. Physiol.* 58: 1713-1718, 1985.

58. Howell, J. N., G. Chleboun, and R. Conatser. Muscle stiffness, strength loss, swelling and soreness following exercise-induced injury in humans. *J. Physiol.* 464: 183-196, 1993.

59. Hyatt, J.-P. K., and P. M. Clarkson. Creatine kinase release and clearance using MM variants following repeated bouts of eccentric exercise. *Med. Sci. Sports Exerc.* 30: 1059-1065, 1998.

60. Isabell, W. K., E. Durrant, W. Myer, and S. Anderson. The effects of ice massage, ice massage with exercise, and exercise on the prevention and treatment of delayed onset muscle soreness. *J. Athl. Tran.* 27: 208-217, 1992.

61. Jayaraman, R. C. The use of functional magnetic resonance imaging in the study of delayed muscle soreness. In: *Department of Physical Education and Exercise Science*. East Lansing: Michigan State University, 1997, p. 131.

62. Jenner, G., J. M. Foley, T. G. Cooper, E. J. Potchen, and R. A. Meyer. Changes in magnetic resonance images of muscle depend on exercise intensity and duration, not work. *J. Appl. Physiol.* 76: 2119-2124, 1994.

63. Jones, D. A., D. J. Newham, J. M. Round, and S. E. J. Tolgree. Experimental human muscle damage: morphological changes in relation to other indices of damage. *J. Physiol.* 375, 1986.

64. Kuipers, H. Exercise-induced muscle damage. *Int. J. Sports Med.* 15: 132-135, 1994.
65. Kuipers, H., H. A. Keizer, T. J. Vestappen, and D. L. Costill. Influence of a prostaglandin-inhibiting drug on muscle soreness after eccentric work. *Int. J. Sports Med.* 6: 336-339, 1985.

66. Kuligowski, L. A., S. M. Lephart, F. P. Giannantonio, and R. O. Blanc. Effect of whirpool therapy on the signs and symptoms of delayed-onset muscle soreness. *Journal of Athletic Training* 33: 222-228, 1998.

67. Le Rumeur, E. C., J. De Certaines, P. Toulouse, and P. Rochcongan. Water phases in rat striated muscles as determined by T2 proton NMR relaxation times. *Magn. Reson. Imaging* 5: 267-272, 1987.

68. Lieber, R. L., T. M. Woodbrun, and J. Friden. Muscle damage induced by eccentric contractions of 25% strain. *J. Appl. Physiol.* 30: 86-92, 1991.

69. Lowe, D. A., G. L. Warren, D. A. Hayes, M. A. Farmer, and R. B. Armstrong. Eccentric contraction-induced injury of mouse soleus muscle: effect of varying [Ca+2]o. *J. Appl. Physiol.* 76: 1445-1453, 1994.

70. Mair, J., M. Mayr, E. Muller, A. Koller, C. Haid, E. Artner-Dworzak, C. Calzolari, C. Larue, and B. Puschendorf. Rapid adaptation to eccentric exercise-induced muscle damage. *Int. J. Sports Med.* 16: 352-356, 1995.

71. Maughan, R. J., R. B. Stein, and R. G. Lee. Relationship between muscle strength and muscle cross-sectional area implications for training. *Sports Med.* 1: 263-269, 1984.

72. McCully, K. K., and J. A. Faulkner. Characteristics of lengthening contractions associated with injury to skeletal muscle fibers. *J. Appl. Physiol.* 61: 293-299, 1986.

73. McCully, K. K., and J. A. Faulkner. Injury to skeletal muscle fibers of mice following lengthening contractions. *J. Appl. Physiol.* 59: 119-126, 1985.

74. McGlynn, G. H., N. T. Laughlin, and V. Rowe. Effect of electromyographic feedback and static stretching on artificially induced muscle soreness. *Am J Phys Med* 58: 139-148, 1979.

75. Nanneman, D. Thermal modalities- heat and cold: a review of physiologic effects with clinical applications. *AAOHN Journal* 39: 70-75, 1991.

76. Newham, D. J., D. A. Jones, and P. M. Clarkson. Repeated high-force eccentric exercise effects on muscle pain and damage. *J. Appl. Physiol.* 63: 1381-1386, 1987.

77. Newham, D. J., K. R. McPhail, K. R. Mills, and R. H. T. Edwards. Ultrastructural changes after concentric and eccentric contractions of human muscle. *J. Neurol. Sci.* 61: 109-122, 1983.

78. Nosaka, K., and P. M. Clarkson. Changes in indicators of inflammation after eccentric exercise of the elbow flexors. *Med. Sci. Sports Exerc.* 28: 953-961, 1996.

79. Nosaka, K., and P. M. Clarkson. Muscle damage following repeated bouts of high force eccentric exercise. *Med. Sci. Sports Exerc.* 27: 1263-1269, 1995.

80. Nosaka, K., P. M. Clarkson, M. E. McGuiggin, and J. M. Byrne. Time course of muscle adaptation after high force eccentric exercise. *Eur. J. Appl. Physiol.* 63: 70-76, 1991.

81. Nurenberg, P., C. J. Giddings, J. Stray-Gundersen, J. L. Fleckenstein, W. J. Gonyea, and R. M. Peshock. MR Imaging - guided muscle biopsy for correlation of increased signal intensity with ultrastructural change and delayed-onset muscle soreness after exercise. *Radiology* 184: 865-869, 1992.

82. Ogilvie, R. W., R. B. Armstrong, K. E. Baird, and C. L. Bottoms. Lessions in the rat soleus muscle following eccentrically biased exercise. *Am. J. Anat.* 182: 335-345, 1988.

83. Owings, T. M., and M. D. Grabiner. Disparate ipsi- and contralateral effects on concentrically vs. eccentrically-induced fatigue. *Med. Sci. Sports Exercise* 28: S141, 1996.

84. Pan, J. W., J. R. Hamm, D. L. Rothman, and R. G. Shulman. Intracellular pH in human skeletal muscle by 1H NMR. *Proc. Natl. Acad. Sci. USA* 85: 7836-7839, 1988.

85. Pizza, F. X., J. B. Mitchell, B. H. Davis, R. D. Straling, R. W. Holtz, and N. Bigelow. Exercise-induced muscle damage: effect on circulating leukocyte and lymphocyte subsets. *Med. Sci. Sports Exerc.* 27: 363-370, 1995.

86. Ploutz, L. L., P. A. Tesch, R. L. Biro, and G. A. Dudley. Effect of resistance training on muscle use during exercise. *J. Appl. Physiol.* 76: 1675-1681, 1994.

87. Ploutz-Snyder, L. L., S. Nyren, T. G. Cooper, E. J. Potchen, and R. A. Meyer. Different effects of exercise and edema on T2 relaxation in skeletal muscle. *Magn. Reson. Med.* 37: 676-682, 1997.

88. Prentice, W. E. An elctromyographic analysis of the effectiveness of heat or cold and stretching for inducing relaxation in injured muscle. *The Journal of Orthopaedic and Sports Physical Therapy* 3: 133-140, 1982.

89. Prior, B. M., J. M. Foley, R. C. Jayaraman, and R. A. Meyer. Pixel T2 distribution in functional magnetic resonance images of muscle. *J. Appl. Physiol.* 87: 2107-2114, 1999.

90. Prior, B. M., R. C. Jayaraman, R. W. Reid, T. G. Cooper, J. M. Foley, G. A. Dudley, and R. A. Meyer. Biarticular and monoarticular muscle activation and injury in human quadriceps muscle. *Eur. Journal Applied Physiology* In Press, 2000.

91. Prior, B. M., L. L. Ploutz-Snyder, T. Cooper, G., and R. A. Meyer. Fiber type and metabolic dependence of T2 inreases in stimulated rat muscles. *J. Appl. Physiol.* 90: 615 -- 623, 2001.

92. Reid, R. W., J. M. Foley, R. C. Jayaraman, B. M. Prior, and R. A. Meyer. Effect of aerobic capacity on the T2 increase in exercised skeletal muscle. *J. Appl. Physol.* 90: 897-902, 2000.

93. Rodenburg, J. B., R. W. de Boer, P. Schiereck, C. J. A. van Echteld, and P. R. Bar. Changes in phosphorus compounds and water content in skeletal muscle due to eccentric exercise. *Eur J Appl. Physiol.* 68: 205-213, 1994.

94. Round, J. M., D. A. Jones, and G. Cambridge. Cellular infiltrates in human skeletal muscle: exercise induced damage as a model for inflammatory muscle disease? *J. Neurol. Sci.* 82: 1-11, 1987.

95. Schwane, J. A., S. R. Johnson, C. B. Vandenakker, and R. B. Armstrong. Delayed-onset muscular soreness and plasma CPK and LDH activities after downhill runing. *Med. Sci. Sports Exerc.* 13: 51-56, 1983.

96. Shellock, F. G., T. Fukunaga, J. H. Mink, and V. R. Edgerton. Acute effects of exercise on MR imaging of skeletal muscle: concentric vs eccentric actions. *AJR* 156: 765-768, 1991.

97. Shellock, F. G., T. Fukunaga, J. H. Mink, and V. R. Edgerton. Exertional muscle injury: evaluation of concentric versus eccentric actions with serial MR imaging. *Radiology* 179: 659-664, 1991.

98. Sjogaard, G., and B. Saltin. Extra- and intracellular water spaces in muscles of man at rest and with dynamic exercise. *Am. J. Phsiol.* 243: R271-R280, 1982.

99. Smith, L. L. Acute inflammation: the underlying mechanism in delayed onset muscle soreness? *Med. Sci. Sports Exerc.* 23: 542-551, 1991.

100. Smith, L. L., M. McCammon, S. Smith, M. Chamness, and K. O'Brien. White blood cell response to uphill walking and down-hill jogging at similar metabolic loads. *Eur. J. Appl. Physiol.* 58: 833-837, 1989.

101. Sorichter, S., A. Koller, C. H. Haid, K. Wicke, W. Judmaier, P. Werner, and E. Raas. Light concentric exercise and heavy eccentric muscle loading: effects on CK, MRI, and makers of inflammation. *Int. J. Sports Med.* 16: 288-292, 1994.

102. Stauber, W. T., P. M. Clarkson, V. K. Fritz, and W. J. Evans. Extracellular matrix disruption and pain after eccentric muscle action. *J. Appl. Physiol.* 69: 868-874, 1990.

103. Takahashi, H., S.-y. Kuno, T. Miyamoto, H. Yoshioka, M. Inaki, H. Akima, S. Katsuta, I. Anno, and Y. Itai. Changes in magnetic resonance images in human skeletal muscle after eccentric exercise. *Eur. J. Appl. Physiol.* 69: 408-413, 1994.

104. Talbot, J. A., and D. L. Morgan. The effects of stretch parameters on eccentric exercise-induced damage to totad skeletal muscle. *Journal of Muscle Research and Cell Motility* 19: 237-245, 1998.

105. Wessel, J., and A. Wan. Effect of stretching on the intensity of delayed-onset muscle soreness. *Clinical Journal of Sport Medicine* 4: 83-87, 1994.

106. Yackzan, L., C. Adams, and K. T. Francis. The effects of ice message on delayed muscle soreness. *Am J. Sports Med.* 12: 159-165, 1984.