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Department of Physiology
Robert C. Byrd Health Sciences Center
West Virginia University
Morgantown, WV 26506-9229

Cumulative Trauma Disorder: Skeletal Muscle Dysfunction

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William T. Stauber, Ph.D., Principal Investigator

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List of Abbreviations

BAPN = β-aminoproprionitrile

b.w. = body weight

C = control

CTD = cumulative trauma disorder

deg = degrees

E-C = excitation-contraction

ELISA = enzyme-linked immunoassay

F = females

HPLC = high pressure liquid chromatography

Hz = Hertz

i.p. = intraperitoneal

M = males

mRNA = messenger RNA

ms = milliseconds

SEM = scanning electron microscopy

TGF-β1 = transforming growth factor beta 1

TGF-β2 = transforming growth factor beta 2

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1. Final Performance Report:

A. Abstract:

Chronic pain originating from the musculoskeletal system is a dominant cause of sick-leave in modern industry and often a very disabling and troublesome condition for the individual. Although the cause of this problem in skeletal muscle is unknown, one of the most frequent situations in which muscle pain is experienced is in industrial workers who have to move repeatedly and/or forcibly. The cumulative trauma disorder (CTD) which results from repetitive movements is of special interest because these repeat-motion injuries are one of the most difficult to anticipate and prevent.

Our studies in humans have shown that exposure to a single bout of repeated strains at moderate can lead to myofiber and fascial rupture without bleeding but accompanied by muscle pain, restricted motion, and loss of strength and power. Little is known about the effect of repeated strains on muscles or the dynamic components of repeated use such as velocity and acceleration which produce injury resulting in CTD or CTD risk. Since variations in human exposure and response, together with the necessity for repeated tissue sampling, make man unsuitable as a research subject, we have developed a rat model of repeated strain injury (CTD).

The remarkable similarity of our injured rat muscles and the extensor carpi radialis brevis taken from humans with long standing lateral epicondylitis requiring surgery (Appendix I)(8) provides support for the rat as a good research model and muscles as important tissues in the development of pain and dysfunction. Since the extensor carpi radialis brevis is also very susceptible to strain injury (7), we believe that more studies using our chronic strain injury protocol in rats will reveal why repeated strain injury results in pain at the attachment sites of human muscles.

Using our rat model of repeated strains, the present study was designed: 1) to determine the dynamic factors (velocity, acceleration and dose) which produce dysfunctional versus adaptive muscles, 2) to document changes in the extracellular matrix and myofibers which lead to a pathologic muscle, and 3) to study the functional outcome and reversibility of repeated injury at different speeds and accelerations commonly experienced by hand-intensive industrial jobs. This research consisted of experiments in which muscles were chronically injured by mechanical overloading in deeply anesthetized rats. The tissues were surveyed at various time intervals by biochemical, immunohistochemical and histological techniques for specific cellular markers, components and mediators involved in tissue injury and repair. The functional outcome of repeated injury was assessed by in vivo dynamometry of muscle performance.

Insight into the dynamic factors producing muscle injury should provide a better understanding of the healing (adaptive) or failed-healing (pathologic) processes of muscle and aid in the design of preventative regimens for individuals in specific industrial settings. The long range goals are to determine: 1) if diminished muscle shock absorption due to increased stiffness from connective tissue proliferation is important in the development of clinical CTD; 2) if prevention of CTD can be implemented by behavioral alterations (work/rest intervals, etc.).

B. Significant Findings:

It is the focus of this work to provide sufficient understanding of the mechanisms and etiology of the development of muscle dysfunction from repeated strain injury to establish early non-invasive monitoring, effective preventative programs and/or work-site modifications. Chronic strain injury dramatically increased collagen and collagen cross-links (fibrosis) in rat muscles (20). Although some of the collagen was degraded during a recovery period of 3 months without specific rehabilitation, increased collagen remained which contained an elevated amount of collagen cross-links. Increased cross-links could increase muscle stiffness (4) and reduce the shock absorption capabilities of muscles. Any major decrease in shock absorption by fibrotic muscles could transfer excessive force to attachments sites creating pain and additional shearing of tendons. Increased collagen cross-links also make collagen resistant to degradation (22) and the remaining fibrosis or muscle scarring may be permanent. This negative outcome of muscles experiencing chronic strain injuries, emphasizes the need for prevention of muscle strain injury. However, further insight into the factors (e.g. energy balance, repetition number, and rest intervals) leading to fibrotic and not adaptive outcomes is required from experimental models such as rat chronic strain injury (18) and chronic overload (12;19) to identify critical parameters to measure in occupational settings.

The remarkable similarity of our injured rat muscles and the extensor carpi radialis brevis taken from humans with long standing lateral epicondylitis requiring surgery (Appendix I)(8) provides support for the rat as a good research model and muscles as important tissues in the development of pain and dysfunction. Since the extensor carpi radialis brevis is also very susceptible to strain injury (7), we believe that more studies using our chronic strain injury protocol in rats will reveal why repeated strain injury results in pain at the attachment sites of human muscles. We have observed changes in the tendons from chronically strained muscles and intermuscular connections but did not perform comprehensive evaluations.

From our studies on the dynamic parameters that produce muscle injury, the magnitude of the force was the major factor in producing strain injury (a non-recoverable force deficit) whereas variations in velocity and acceleration only produced small differences in strain injuries. In addition, it appears that most of the damage to the muscle occurs during the first few stretches after which an injury limit appears (Fig.) such that increasing the number of strains does not produce further injury.

In very old rats, the plantar flexor muscles were <u>not</u> more susceptible to injury, only weaker during isometric tests. The muscles from old rats were able to produce similar large peak stretch forces as muscles from young rats. Similarly, female rats and male rats had equivalent force deficits following a single bout of repeated strains. Therefore, strain injuries appear to be independent of age and gender.

If fatigue is prevented in rats, repeated strain injury still occurs but without muscle pathology. The complex interaction of strain injury and fatigue to produce pathology leading to muscle dysfunction is a novel finding of this research and has required many years of labor intensive work to finally emerge. This complex interaction of fatigue and cellular injury, verified in our rat models, could explain why in groups of people doing identical work some individuals do not report any musculoskeletal problems. Thus, it may not be individual variations in the biomechanics of the task that lead to dysfunction (CTD) but inherent differences in fatiguability due to divergences in energy balance (2).

Using a quick, isotonic testing and submaximal loading protocol (16), we have demonstrated surprising differences in fatigability in humans which appear to be related to differences in energy balance. The test requires only 15 minutes to perform and reveals information on both fatigue and recovery reflecting differences in energy utilization and resynthesis. Once a clear understanding of the relationship between fatigue and injury emerges from our animal studies, we are in a unique position to test those industrial workers who while performing identical jobs do so with and without musculoskeletal pain.

C. Usefulness of Findings:

- Large forces produce the greatest force deficits and potential for pathology so that reductions in loads during movements that can produce muscle strains seems appropriate.

- Even though prevalence rates of CTD are 3 times higher in female workers, no differences were found between male and female rats or between old and young rats so all workers would be expected to have similar relative thresholds for and outcomes from strain injury.

- Isometric force deficits (weakness) following stretches were greater at an ankle position of 90 degrees than at 40 deg (i.e. dorsiflexed) revealing that position may be critical in the way people perform their tasks (i.e. ergonomics) and in assessment of injury using isometric testing.

- Isometric force deficits (weakness) appearing in standard strength tests of humans would not in themselves be indicative of strain injuries that would become pathologic and lead to myofiber necrosis and fibrosis.

- Fatigue may play an intricate role in producing a pathological outcome from strain injuries. So defining susceptible individuals would allow job placements that would decrease the incidence of lost work and reduce potential negative outcomes from repeated strain injuries. If these inherent differences in fatigability are not immutable, specific training protocols could be implemented to prevent some chronic strain injuries in some individuals.

- The appearance of bradykinin around injured muscle fibers as a putative algesic compound would explain why non-steroidal anti-inflammatory drugs do not reduce muscle pain.

- The marked increase in collagen and collagen crosslinks remaining in muscles following chronic injury and long-term recovery could explain why some individuals get recurrence of symptoms almost immediately after returning to work following adequate rest and successful rehabilitation. The increased connective tissue could serve as a barrier to nutrient flow resulting in an energy crisis during repeated use or physically entrap the mechanoceptors producing pain once a force threshold has been reached.

D. Scientific Report:

We studied the effects of cumulative microtrauma (CTD) on the soleus muscles of female rats (n = 350) with special concern for the development of muscle pathology - fibrosis and impaired muscle function. Female rats were used because as said earlier the prevalence rates of CTD are three times higher in female workers (13). The study was functional because physiological measurements were made with our dynamometer. Cellular changes were followed using specific tissue markers (quantitative pathology) to document structural damage and pathology leading to the development of CTD.

We have shown that in humans a 50% force deficit can be produced in the elbow flexor muscles in untrained volunteers using only 50% of their maximal torque capability (15) which is close to the deficit we see for repeated strains in rats (Fig. 3). In human and rat muscles, similar structural damage was observed using our immunohistochemical tests providing verification of muscle damage resulting from repeated strains. Although our rat model of multiple strains uses a maximally activated muscle to exaggerate the responses for clarity of study, the myopathic changes were similar to those reported for human wrist extensor muscles in patients with chronic lateral epicondylitis requiring surgery (Appendix I)(8). Muscle fibers subjected to repeated strains, even during submaximal activation levels and required for most occupational tasks, could produce pathology in the population of over-used fibers (6). The entire rat soleus muscle, in our experiments, could be considered as one large, low threshold (tonic) motor unit recruited during submaximal activities in humans. Even during submaximal activities, the firing rates of some tonic motor units could be at high frequencies especially as fatigue progresses. These tonic motor units do not increase their firing rates much as total muscle force increases (ramp and plateau force-frequency relationship) (5) but still would be expected to be injured by repeated strains if the strain exceeded the threshold for injury. We have used lower frequencies of muscle activation producing less strain injury with less pathology in the soleus muscle of rats. We are in the process of defining the threshold for producing pathology in rat muscles and level of activation is one consideration.

We have developed a model of chronic overload by creating a chronic hindlimb spasm that requires the rat to walk on three legs and the muscle spasm supports the outstretched leg during functional activities (19). The rat can rest the overloaded leg volitionally by lying down but all other activities require overloading the non-spastic leg. Using this model, the plantar flexor muscles of the overloaded limb were morphologically similar to those from chronic strain injury revealing a common endpoint for repeated over-use and repeated strain. From these and other studies, skeletal muscle has only a limited number of responses to chronic overload and overactivity: 1) muscle adaptation (hypertrophy; increased energy stores, increased mitochondria, regeneration, repair) and 2) muscle pathology (atrophy, fibrosis, necrotic fibers). Repeated pathologic responses of muscles eventually lead to dysfunction.

1. Experiments for Specific Aim 1

To define the dynamic factors (velocity and acceleration) of muscle strain overload which produce injury (material fatigue). Intermittent stretching will be compared to oscillatory stretching at velocities known to produce damage to indicate if interrupted work is more damaging than cyclic.

a. Muscle strain injury

Overload (strain) injury ((lengthening an active muscle by stretching) was produced using our rat dynamometer to stretch muscles activated by nerve stimulation. The dynamometer was designed as a multiparameter testing device (isometric and isokinetic) similar to that used to test humans (e.g., Kin-Com, (1)). Because oscillatory stretching proved to be more fatiguing due to the long stimulation times (n=4), we shifted focus to intermittent stretching and velocity testing to avoid physiological fatigue. First, dynamic and static force development in rat plantar flexor muscles in vivo was measured (n=16) (24). The maximal isometric force (19.2 N) of the rat plantar flexors was nearly constant at all ankle positions (range of motion (90 deg) indicating that the muscles are probably operating on the plateau of their length-tension relationship [in agreement with published reports on medial gastrocnemius tested in situ, (3)]. During slow concentric muscle actions, the force decreased progressively (23%) throughout the range of movement (muscle shortening) which could not be explained by fatigue (6.3%). Thus, preloaded slow concentric muscle actions do not parallel the isometric force-position relationship of the rat plantar-flexors in vivo; in the future, dynamic measurements such as reported above must be included in all functional testing to fully understand changes in muscle performance resulting from repeated strain injury. Our dynamometer measures important in vivo muscle parameters similar to those used for human testing and has performed up to expectations on all tests.

Multiple strains result from stretching the plantar flexor muscles at different speeds by controlling the angular rotation of the ankle (isokinetic)(n=18). Surprisingly, the magnitude of injury was not altered by velocity (Fig. 2) except at very slow speeds. It appears that high forces resisting the stretches were similar at all constant angular velocities tested. Similar high forces during the stretches produced about the same amount of muscle injury (i.e. a non-recoverable force deficit). The magnitude of the force which strains a muscle is the major factor in producing strain injury (force deficit) as reported earlier by other researchers (10).

Subtle effects of velocity could be observed during the relaxation phase (stress-relaxation) following the stretch but before the stimulation was turned off (n=12). Repeated fast stretches but not slow stretches resulted in an increase in stress-relaxation. This alteration in stress-relaxation probably resulted from internal structure damage (21;25;25) leading to sarcomere heterogeneity (11); some sarcomeres are operating on the ascending limb of their length-tension relationship while others are over-stretched on the descending limb. If sarcomere homogeneity exists, the force decay following the stretch is small. Thus, sarcomeres are damaged with repeated strains, more so at higher velocities, and we can detect this damage by testing the stress-relaxation properties of the muscles. Damaged sarcomeres may require intracellular remodeling for restoration of function which may take days but may not necessarily lead to pathology.

As with most viscoelastic materials, loading history is important. If a series of muscle strains (n=6) was preceded by a maximal isometric contraction at long, but not short lengths, there was a decrease in the relative amount of force deficit (i.e. injury). Thus, if maximal isometric exercise with the muscle fully stretched preceded an activity which would strain a muscle, the damage would be less albeit not prevented. This observation supports the use of our methodological approach with rat muscles to study muscle responses and the potential for the development of prevention strategies for humans. Although this technique did not result in

a dramatic reduction in injury resulting in weakness (force deficits), it encourages us to continue this avenue of research.

b. Role of age

Because the workforce is also composed of older people who continue to work beyond current retirement age, we tested a few very old rats (>22 months)(n=5). In general, the plantar flexor muscles were not more susceptible to strain injury (lengthening an active muscle by stretching) but did produce less isometric force (weaker) and demonstrated a small increase in passive force (i.e. increased stiffness). The increase in muscle stiffness was accounted for by an increased amount of collagen and collagen cross-links as measured by HPLC analysis in these muscle from old animals. Studies on aging have revealed that increased connective tissue could have functional consequences by decreasing the efficiency of movements.

c. Role of acceleration

We began testing a few rats (n=4) at different accelerations. Large force deficits (>50%) were produced when acceleration was controlled. The accelerations (>3,000 deg/sec^2) and peak velocities (450 deg/sec) used to produce such large force deficits were similar to those we recorded for our manual injury protocol and are within the ranges reported by Marras and coworkers as high risk CTD jobs (9;14;14). However, these protocols produced pathology only if also accompanied by fatigue (n=2). Isometric force deficits were not in themselves indicative of strain injuries that would become pathologic and lead to myofiber necrosis and ECM proliferation.

Comparisons were made between constant acceleration (3,000 deg/sec^2) and constant velocity (300 deg/sec) stretches keeping the area under the velocity time curve equivalent (i.e. constant total time of the stretches) (n=6). Similar force deficits (i.e. injury) followed the controlled acceleration compared to constant velocity. Using the acceleration profile, a substantial decrease in force deficit was observed when the stimulation frequency was reduced from 80 Hz to 20 Hz but injury was nevertheless still evident. Force deficits (muscle injury) can be produced even at low frequencies of activation in rat muscles.

d. Role of gender

Susceptibility to strain injury (lengthening an active muscle by stretching) was compared in male and female rats. Stretches of plantar flexor muscles, imposed on isometric contractions, were produced by dorsiflexion (a = 3000°/s^2, ankle position 90° to 40°) in weight-matched female (F, n = 6, b.w. 273 ± 18 g, age 141 ± 23 days, mean ± SD) and male (M, n = 6, b.w. 285 ± 25 g, age 62 ± 10 days) rats. No gender differences were observed for isometric forces at different stimulation frequencies (force-frequency relationship) before the stretch protocol. During the stretch protocol, the relative declines in isometric force and stretch force were similar for female and male rats. At the end of the stretch protocol, deficits in isometric force were 49.9 ± 5.1% (F) and 45.9 ± 5.3% (M) ($P = 0.2$) and deficits in peak stretch force were 32.2 ± 5.5% (F) and 35.0 ± 4.4% (M) ($P = 0.4$). One hour after the stretches, the remaining isometric force deficits (weakness) at 5, 10, and 20 Hz were not different. However, isometric force deficits at 40, 60, and 80 Hz were 18.6% ($P < 0.05$) (F: 50.4 ± 4.9%; M: 42.5 ± 5.8%), 13.7% ($P < 0.05$) (F: 51.0 ± 4.3%; M: 44.9 ± 4.4%), and 16.3% ($P < 0.05$)

(F: 47.8 ± 4.1%; M: 41.1 ± 4.2%) larger for female than for male rats. In conclusion, susceptibility of skeletal muscles to force deficits 1 hour following the stretches is larger for female rats than for male rats of comparable body weight but not immediately following a repeated strain protocol. It would appear that females do not recover as rapidly as males for the reversible component of the force deficit.

e. Role of collagen crosslinks of the extracellular matrix

The role of the stiffness of the extracellular matrix was indirectly assessed as a factor in susceptibility to strain injury by producing muscles with reduced collagen crosslinks. Rats (n = 6, age 87 days) were injected 2x daily for 43 days with β-aminopropionitrile (BAPN, 333 mg·kg-1·day-1 i.p.) which inhibits the enzyme, lysyl oxidase, responsible for collagen cross-links. Age-matched saline-injected rats served as controls (C, n = 6). Isometric forces at 40° were measured before repeated stretches (20) of active plantar flexor muscles at 80 Hz. Isometric forces were measured at 90°, before and 1 hr after the stretches at 5, 10, 20, 40, 60 and 80 Hz (force-frequency relationship). Relative weights of the muscles were not different between groups. Significant reductions in collagen cross-links, pyridinoline, (mol/mol collagen) were found in tendon (22.9%), plantaris (17.1%), and soleus (7.4%) with no changes in collagen content (hydroxyproline). In gastrocnemius medialis, cross-links were 4.4% lower but were not significant (P = 0.2). Groups had similar isometric forces at all stimulation frequencies before the stretches and isometric force deficits following the stretches; deficits were 51.1 ± 2.4% © and 54.7 ± 4.6% (BAPN). After 1 hr of rest, deficits were similar at 80, 60, 40 and 20 Hz but were 26% and 29% larger at 10 and 5 Hz in BAPN-rats ($P < 0.05$). Isometric force deficits (weakness) after stretches of rat plantar flexor muscles with reduced collagen cross-links were larger only at low stimulation frequencies. Isometric force deficits are produced by intracellular processes residing within the myofibers and are not influenced substantially by collagen crosslinking or the composition of the extracellular matrix.

2. Experiments for Specific Aim 2.

To quantify the dose-response relationship of the specific damaging factors (i.e. number of repetitions).

a. Role of repetition number

The effect of repetition (Figures 1&2) was tested for slow (50 deg/sec) and fast (600 deg/sec) velocities for thirty repeated strains (lengthening an active muscle by stretching). This number of repetitions was not excessive for a rat as rats will run 4-5 km/day if provided a running wheel. Other researchers ran rats downhill to produce damage for a couple of hours or have used 500-1800 strains. Because physiological fatigue complicates those studies, we feel our stimulation protocol allows us to observe the process of mechanically-induced injury within a time-frame suitable for research purposes.

Isometric force at two different ankle positions, work, and half-contraction times were measured - all parameters were altered with repetition (n=18). The force deficits (weakness) following stretches were greater at an ankle position of 90 degrees that at 40 deg (i.e. dorsiflexed) revealing that position may be critical in assessment of injury and in the way people perform their tasks (i.e. ergonomics). One half of the nonlinear force deficit (injury) of 30%

occurred by the sixth stretch for both slow and fast stretches (Figure 2). From stretch number 18-30, slow stretches produced slightly greater force deficits but a fatigue limit was evident for both groups by the 30th stretch. Work also decreased more with each stretch as more force was lost. In humans, such a decrease in work capacity of injured muscles with time would require a greater sense of effort as more motor units supplying uninjured muscle fibers are recruited to produce the same submaximal work.

Force deficits can be produced by failure of excitation-contraction (E-C) coupling processes disrupted by muscle injury. Half-contraction times, the time it takes for the muscle to reach 50% of its maximal force, increased linearly for the stretches and were much larger than during the same number of isometric contractions. Thus, repeated fast and slow stretching resulted in similar amounts of injury as revealed by comparable non-recoverable decreases in muscle performance. Damage to cellular membranes resulting in failure of E-C coupling and sarcomere disruption most likely accounts for the weakness during and following repeated strain injury.

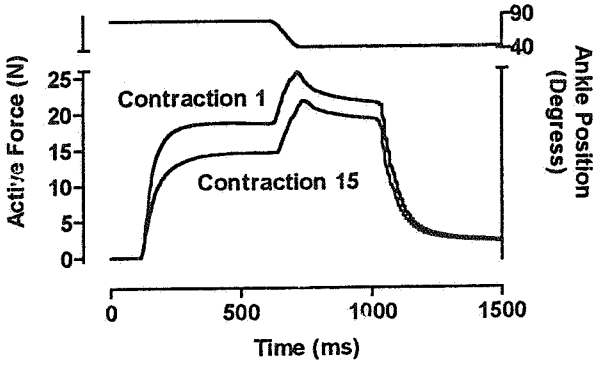

Figure 1. Force vs. Time

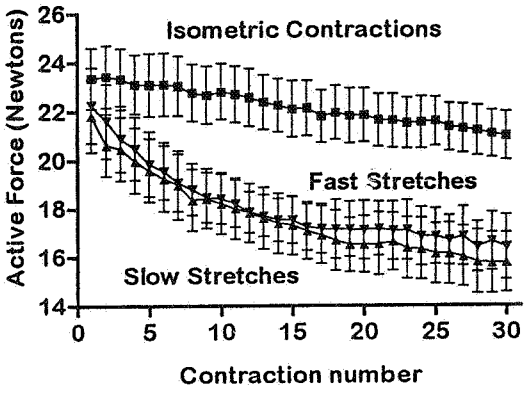

Figure 2. Repeated strains and force deficit

b. Role of long rest periods

The effect of a long rest period on the injury susceptibility was tested by repeating our stretching protocol after a 2 hour rest (n=6). Additional force deficits (weakness) were produced indicating that extra damage could result in an injured but rested muscle. This series of experiments revealed that the fatigue limit seen during repeated stretches does not remain following a long rest period. Unfortunately, it is not practical to repeat these experiments more than twice daily to mimic some workplace activities as the necessity for rest intervals makes these experiments very long (close to six hours) with the animal under continuous anesthesia.

c. Strain injury and fatigue produces pathology

Strain injury (lengthening an active muscle by stretching) was produced by our dynamometer with nerve activation. For the strains, the plantar flexors were activated at an ankle position of 1.57 rad (i.e. isometric preload) and 600 ms later the ankle rotated to 0.70 rad at an angular velocity of 5.2 rad/sec (300 deg/sec) (i.e. stretch or eccentric contraction). Total stimulation time for such a contraction was 1.9 s. Two hundred and forty repeated stretches were performed (n=2) with rest periods between contractions of 45 sec. which resulted in force

deficits due to both injury and fatigue (duty cycle (i.e. contraction time / rest period) is 0.042)(Figure 3). Total time for the protocol is 3 hours. We have also tried 160 stretches using this protocol with almost identical results. There may be a critical threshold of injury and fatigue that results in muscle pathology and dysfunction instead of muscle adaptation and growth (e.g. exercise training). Our studies on chronic slow and fast stretching that produced adaptive or fibrotic muscles (18) first(17) revealed different outcomes could result from different stretching protocols.

d. Strain injury without fatigue does not produce pathology

Sixty repeated stretches (see protocol above) were performed with rest periods of 3 min between stretches which resulted in force deficits (n=1) mainly due to injury (duty cycle is 0.011). Total time for the protocol is 3 hours (Figure 3). These muscles did not show evidence of pathology but the force deficits could be up to 50% of the initial values and not recover over a one-hour period, a time normally sufficient for complete recovery from similar isometric (i.e. non-damaging) contraction protocols.

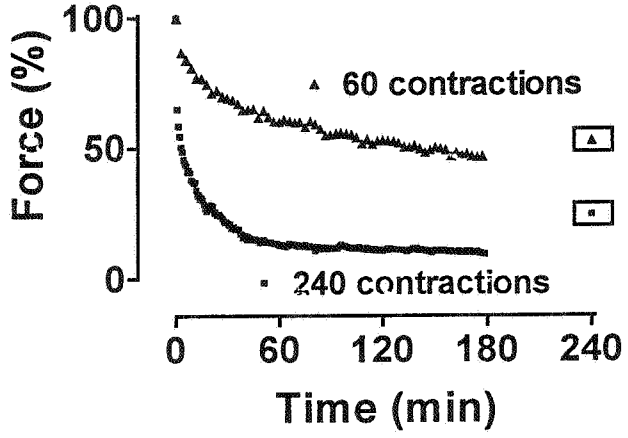

Figure 3. Force deficit as a function of time

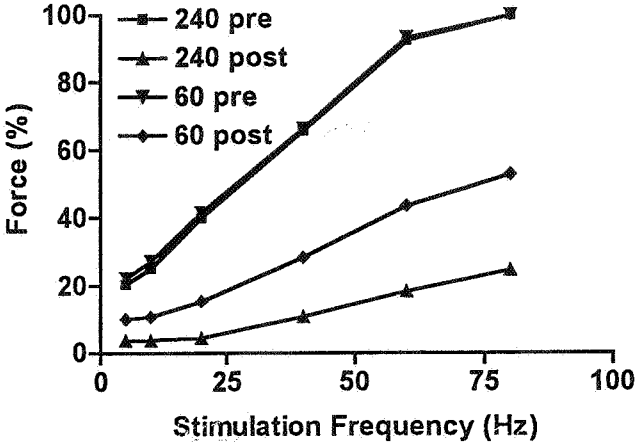

Figure 4. Force-Frequency Testing

Muscle damage by immunohistochemical tests revealed that little correlation between force deficits and morphology, as has been reported by others (23), could be made unless fatigue was also present. This lack of direct correlation demonstrates the multiplicity of the sites of injury (many of which we have no markers for) and the complex nature of the strain injury. We have no plan to take biopsies to evaluate CTD in humans but the remarkable similarity of our strain injured rat muscles and human muscles from long standing lateral epicondylitis (Appendix I;(8)) warrants continued research with rats to reveal the type of cellular damage which leads to pathology and fibrosis.

The ability to produce muscle fibrosis following repeated strain injury has led to a search for indicators of damage in the blood such as has been reported for lung and liver. We believe that the development of ELISA assays for TGF betas in muscle and blood will be good indicators for ongoing pathology in humans as we have detected marked elevations in respective mRNA levels in injured rat muscle samples.

3. Experiments for Specific Aim 3.

To measure the functional outcome of a protocol for cumulative muscle microtrauma (CTD) which is known to produce muscle pathology (17;18) in the rat and to assess if it is reversible (Recovery Studies). Muscle weakness will be assessed by isokinetic evaluation of the concentric muscle action. Dynamic muscle fatigue will be determined by the drop in concentric muscle force as a function of time. Muscle stiffness will be evaluated using our dynamometer to produce small amplitude cyclic deformations. Morphological and histochemical techniques will be used to define the specific tissue changes (e.g. myofiber atrophy or hypertrophy; connective tissue proliferation, etc.) which are responsible for variations in functional outcome.

a. Muscle weakness, dynamic muscle fatigue, and muscle stiffness

For a variety of logistical and technical reasons, the functional outcome of the chronically injured muscles were not tested rigorously during this study. First, the production of muscle pathology and the time course of restoration of muscle morphology required definition (see below) before selected time points for functional measurement could be established. The chronic injury experiments were labor intensive and we were hesitant to perform testing which itself might produce pathologic changes in muscles. Second, studies on muscle stiffness had to be mostly abandoned because additional time would be needed to modify our dynamometer to increase its sensitivity to detect small differences in passive forces. This loss of time would not allow us to finish our chronic injury studies and would decrease the productivity of our group. Third, we had an unexpected failure of our departmental tissue storage freezer, not due to technical errors by our research staff, but due to a custodial accident. We and other research groups in our department lost valuable tissue samples that required us to repeat 1 year's worth of experiments. However, considering the results of this project, we feel the most important aims were completed and novel findings uncovered.

b. Recovery of muscle pathology and connective tissue proliferation

The recovery of myofibers and ECM from 6 weeks of repeated strains (50 strains daily, 5x/wk) was followed for 3 months (n=48) (Figures 5 & 6, C=control). Histochemistry and image analysis were used to evaluate fiber areas and ECM content. After 6 weeks of repeated strains, loss of mass by 63% was accompanied by a marked reduction in fiber area by 87% and an expansion of the ECM by 221%. Over the 3-month recovery period, the muscles never fully returned to normal. Although muscle mass increased over time, there remained a significant deficit in mass of 22% after 3 months of normal caged activity. Muscle mass corresponded to changes in myofiber area, which increased throughout the 3-month recovery period, but remained atrophic by 20% (Figure 6). Likewise, the content of non-contractile tissue (ECM) was still elevated by 18% even after 3 months of normal ambulation. Thus, recovery of rat soleus muscles from chronic strain injury appears to be a very slow process.

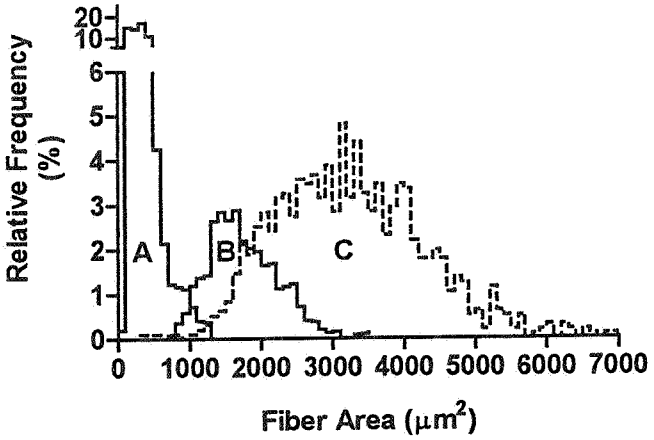

Figure 5. One month recovery

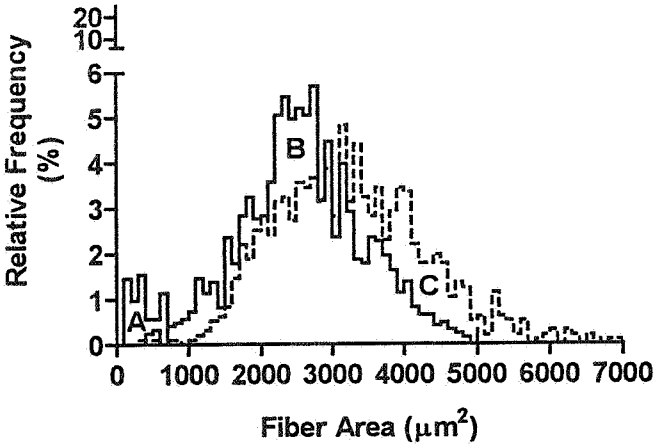

Figure 6. Three months recovery

The expansion of non-contractile tissue was not due to swelling from chronic inflammation but increased amounts of collagen (Figure 7). Both SEM and HPLC showed that matrix expansion (i.e. fibrosis) had occurred that did not return to control levels after 3 months of normal activity. The amount of collagen crosslinks also increased and the molar ratio of crosslinks to collagen actually increased during recovery (Figure 8). Increased amounts of crosslinks has been observed to result in stiffer muscles (4). Our work on reduced crosslinks by inhibition of lysyl oxidase did decrease muscle stiffness but not injury susceptibility supporting the role of collagen crosslinks in muscle stiffness but not in injury susceptibility.

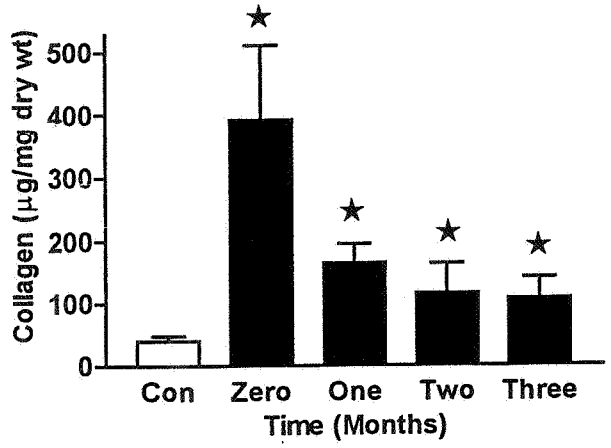

Figure 7. Collagen content during recovery

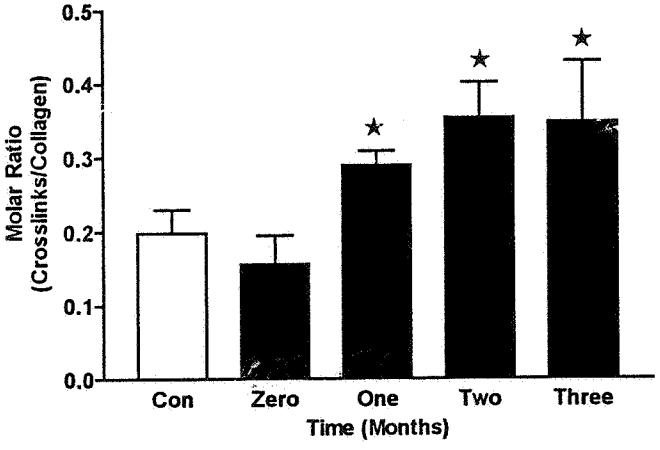

Figure 8. Ratio of crosslinks to collagen

c. Biomarker detection

Since the rat can not report pain and musculoskeletal pain does not inhibit rats from eating, even if noxious material is injected into their muscles, a marker for muscle pain would be useful. Recently, we have localized bradykinin, a known algesic compound, in areas with muscle pathology but not in adjacent areas without pathology or in muscles with marked force deficits (injury) albeit without fatigue. These bradykinin positive areas were also positive for

TGF-β1 and TGF-β2 - known fibrogenic cytokines. These observations provide an exciting link between muscle injury, pain and pathology with the possibility of providing a mechanism for how muscle fibrosis can occur in some, but not all, muscles subjected to repeated strain protocols.

In summary, the discovery of a link between strain injury and metabolic fatigue in the development of pathology would explain why exercise training does not produce pathology - the long rests between exercise bouts minimizes metabolic fatigue. Therefore, the importance of rest periods for different occupational tasks with repeated strains will likely emerge from our studies and prove significant in prevention of muscle pathology, pain, and dysfunction.

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H. Publications: Future (Submitted)

1. Willems, M. E. T., Miller, G. R. & W.T. Stauber. Force deficits after active stretches of rat skeletal muscles with reduced collagen cross-links. Experimental Physiology (Submitted).

2. Willems, M. E. T. & W.T. Stauber. Force deficits after repeated stretches of activated skeletal muscles in female and male rats. Acta Physiologica Scandinavica (Submitted).
3. Willems, M. E. T. & W.T. Stauber. Effect of isometric contractions and recovery on torque deficits by stretches of active rat skeletal muscle. Archives of Physiology and Biochemistry (Submitted).
4. Willems, M. E. T., Barill, E.R., Miller, G. R. & W.T. Stauber. Individual variation in loss of power of knee extensor muscles with maximum isotonic contractions. Eur. J. Appl. Physiol. (Submitted).

I. **Publications: Future - In preparation.**

1. Willems, M. E. T. & W.T. Stauber. Muscle length related fatigue and recovery of resistance trained rat plantar flexor muscles (In preparation).
2. Willems, M. E. T. & W.T. Stauber. Loss of function by stretches of rat plantar flexor muscles skeletal muscles with constant velocity or acceleration of ankle rotation (In preparation).
3. Willems, M. E. T., Miller, G. R. & W.T. Stauber. Changes in collagen and collagen cross-links in rat plantar flexor muscles skeletal muscles after resistance training (In preparation).

J. **Abstracts:**

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Appendix I.

Comparison of Human Muscle Biopsies and Strain Injured Rat Soleus Muscles.

Although the techniques and the magnification of the photomicrographs were different, striking similarities were apparent when comparing our strain injured rat muscles with fatigue to the extensor carpi radialis brevis from patients with longstanding lateral epicondylitis requiring surgery (2). Figures A (human) & B (rat) illustrate necrotic fibers with infiltrating cells. When dual localization of laminin, a cell boundary marker (Figs. C & E), and desmin, an intracellular cytoskeletal marker (Figs. D & F), were performed, some myofibers which were positive for laminin were negative for desmin (arrows) indicating degradation of intracellular proteins. Lieber, et al. (1) demonstrated that, in rabbits subjected to repeated strain injuries, the loss of desmin was accompanied by cell injury because fibronectin, an extracellular protein, was found within the desmin negative fibers. We have demonstrated similar results using albumin which can enter injured myofibers that are also desmin negative. Taken together, it can be argued that the cellular responses of muscles from humans with lateral epicondylitis were similar to rats subjected to repeated strain injuries and support the use of rats as appropriate subjects for research into the cause and outcome of repeated strain injuries leading to pathology.

It can be seen from the laminin staining (Fig. C) that the extracellular matrix (ECM) was probably expanded as well, although the authors did not measure it. We are beginning a collaboration to evaluate the non-myofiber changes in their samples.

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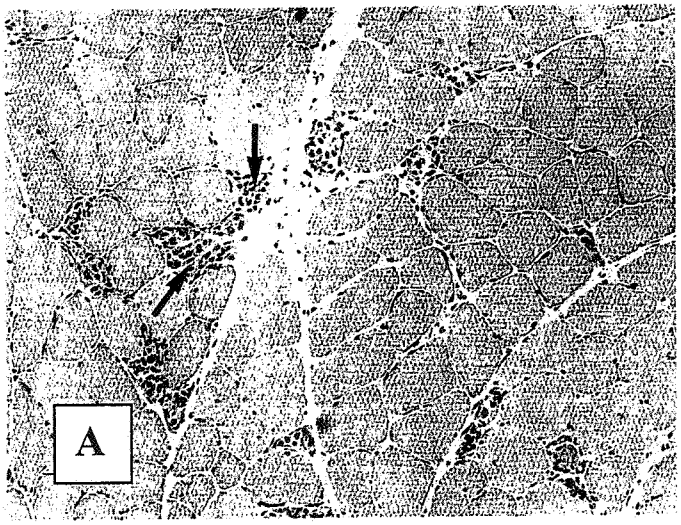
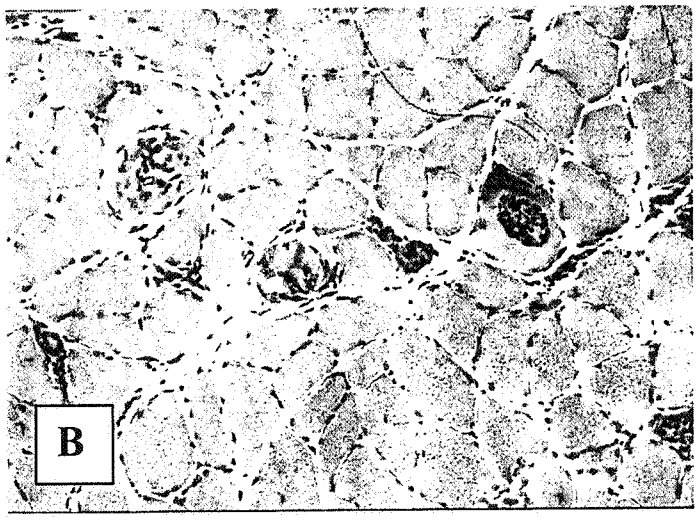
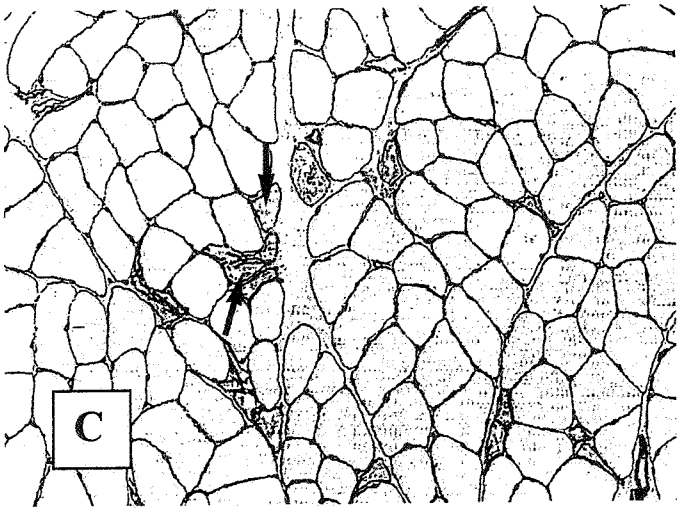
Human Muscle (Epicondylitis)



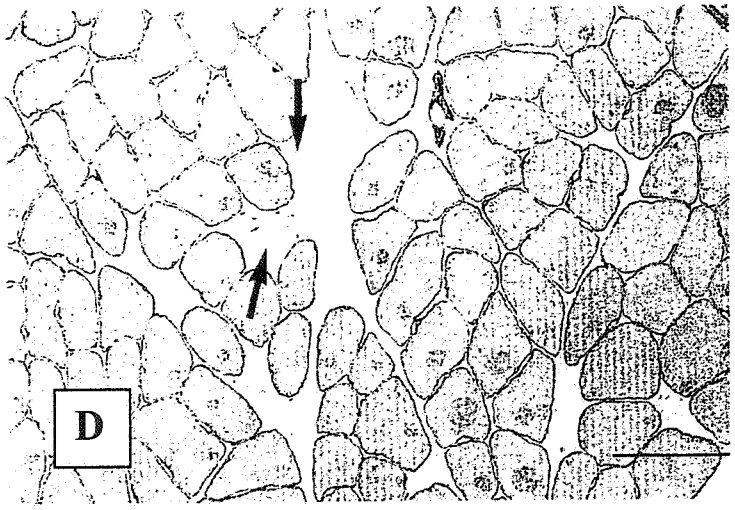
Rat Muscle (Strain Injury)



Human Muscle (Epicondylitis)

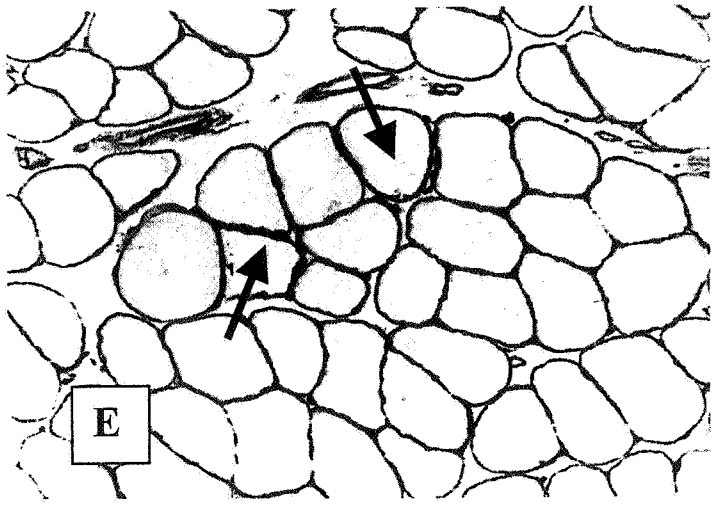


Laminin

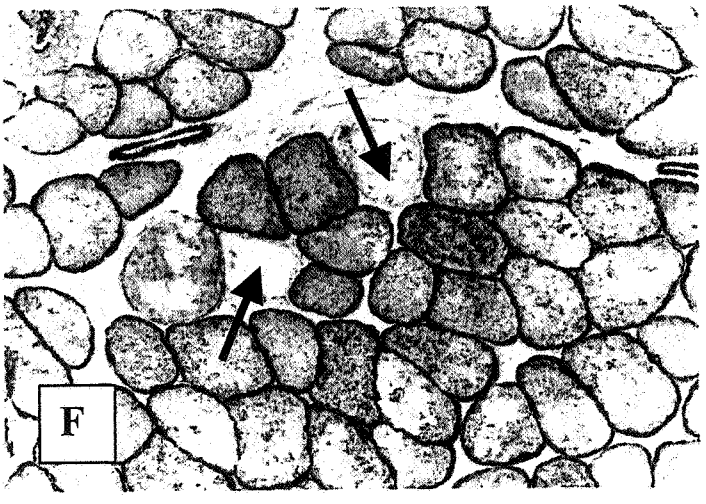


Desmin

Rat Muscle (Strain Injury)



Laminin



Desmin