CHRISTOPHER MICHAEL GREGORY

Effects of Testosterone Replacement Therapy on Skeletal Muscle after Spinal Cord Injury.

(Under the Direction of Dr. Gary A. Dudley)

Patients with spinal cord injury (SCI) are at risk for developing several secondary pathologies likely linked to the atrophy of affected muscle. If testosterone replacement therapy (TRT) alone or as an adjunct therapy could attenuate the atrophy, these risks might be reduced. Therefore, the purpose of this study is to examine the effects of TRT on rat skeletal muscle after SCI and determine the applicability of the rat model to studies of skeletal muscle unweighting. In addition, the applicability of rat models in unweighting studies will be tested by quantifying the characteristics of anatomically similar muscles in rat and human, control and SCI muscles. The soleus, gastrocnemius, tibialis anterior, vastus lateralis and triceps brachii were taken from twelve male Charles River rats 11 weeks after complete SCI (n = 8) or sham surgery (n =4) and analyzed qualitatively for type and quantitatively for succinate dehydrogenase (SDH), α-glycerol-phosphate dehydrogenase (GPDH), and actomyosin ATPase (qATPase). Rats received either testosterone or empty capsule implantation at the time of surgery. Overall, SCI resulted in a smaller fiber size, a decrease in SDH, and an increase in GPDH activity, while the triceps brachii increased fiber size with no change in SDH or GPDH activity. Rats had smaller fibers, greater SDH and GPDH activities, an absence of slow fibers and a greater proportion of IIb/x fibers compared to humans. The reduction in SDH activity was greater in rats while atrophy and IIa → IIb/x fiber shift occurred to a greater extent in humans. TRT after SCI attenuated the fiber atrophy and the changes in SDH and GPDH

activities. There was no effect of TRT on the TRI relative to SCI alone, nor on the qATPase activity in rat or human SCI or TRT treated muscles. Thus, it is suggested that the rat is a reasonable model for studying the predominant response to SCI, atrophy. However, its high proportion of IIb/x fibers limits evaluation of the mechanical consequences of shifting to "faster" contractile machinery after SCI. It is also concluded that TRT was effective in attenuating alterations in myofibrillar proteins in affected skeletal muscles after SCI.

INDEX WORDS: muscle, spinal cord injury, enzyme, testosterone

EFFECTS OF TESTOSTERONE REPLACEMENT THERAPY ON SKELETAL MUSCLE AFTER SPINAL CORD INJURY

by

Christopher Michael Gregory

B.S.Ed., The University of Georgia, 1996

M.A., The University of Georgia, 1998

A dissertation presented to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2002

EFFECTS OF TESTOSTERONE REPLACEMENT THERAPY ON SKELETAL MUSCLE AFTER SPINAL CORD INJURY

by

Christopher Michael Gregory

Approved:

Major Professor: Gary Dudley

Committee: Kirk Cureton
Ronald Elliott
Michael Ferrara
Kevin McCully

Electronic Version Approved:

Gordhan L. Patel
Dean of the Graduate School
The University of Georgia
May 2002

To my parents:

for their unconditional love and support through all that I have ever attempted...

To my wife, Angi:

for the friendship, love and understanding that you have given to me throughout the years... I couldn't have made it through without you! I Love you!

ACKNOWLEDGEMENTS

My sincere thanks go out to:

- Dr. Gary Dudley for his guidance, both educational and personal throughout the last 5 years.
- The other members of my Doctoral Committee: Dr. Kirk Cureton, Dr. Kevin McCully, Dr. Ron Elliot and Dr. Michael Ferrara.
- Dr. Krista Vandenborne, Dr. John Ottenweller and Dr. Hosea Huang for their support of this project.
- Many fellow students with whom I have shared the ups and downs of graduate school over the years: Richard Williams, Scott Bickel, Mike Castro, Scott Stevenson, Will Calvert, Ed Mahoney, Bjossi Arngrimmsson, Mike Saunders, Ellen Evans, Greg Flowers, Jeff Allard, Bud Cooper and Connie Peterson who all made the years so special.
- Marlee Stewart, for her guidance and friendship. I couldn't have made it without your help. I cannot thank you enough!

TABLE OF CONTENTS

I. INTRODUCTION

It has been estimated that there are approximately 200,000 patients with spinal cord injury (SCI) in the Unites States alone, with roughly 10,000 new injuries occurring each year. Additionally, the relative number of new injuries is rising and expected to have increased in proportion by 20% over 1994 prevalence by the year 2010[1]. With the increasing number of people living with this condition, more emphasis is being placed on finding treatments to improve the perceived quality of life for these patients.

SCI is associated with a decrease in perceived quality of life and an increased number of health-related risk factors for developing secondary pathologies (i.e. diabetes, CVD, renal dysfunction and kidney disorders). Interventions aimed at reducing some of the health risks associated with SCI have been widely studied over the past two or so decades. As a result, the expected life span of this population has increased to approximately the same as able-bodied persons. Thus, interventions thought to potentially have a positive influence on both quality and quantity of life have become a priority for both ongoing and future research.

One type of therapy that has received little attention in the literature with respect to SCI is testosterone replacement therapy. Although the benefit to risk analysis of testosterone replacement therapy for able-bodied individuals is sometimes questioned[3], the potential ability of this type of treatment to aid patients with SCI has not yet been examined.

PURPOSE OF THE STUDY

Testosterone replacement therapy has been shown to be beneficial in maintaining strength and muscle size in the aging testosterone deficient male, even in the absence of exercise intervention [19, 20]. Thus, the potential influence of this type of therapy in the absence of traditional overload suggests the potential for a positive influence on patients with SCI, who have also been shown to be testosterone deficient [21]. Although this study as in many of the studies on the effects SCI will be done using lower mammals, the hope is that the alterations that are detected and the interventions that are developed can be applied to human subjects. The use of animal models in physiological studies has long been the basis for findings on the effects of different diseases and their treatments. However, with more recent advancements in technology, the opportunity to use humans as subjects is greater than ever. Thus, questions as to the applicability of previously accepted adaptations in animals by humans might be questioned. Thus, this study has two purposes: 1) to evaluate the effects of testosterone replacement therapy on rat skeletal muscles 11 weeks after SCI, and 2) to examine the innate characteristics of and quantify the effects of 11 weeks of SCI on rat vs. human quadriceps femoris muscle.

HYPOTHESES

1. Testosterone replacement therapy will attenuate but not ameliorate the atrophic response of skeletal muscle associated with SCI.
2. Testosterone replacement therapy will not affect the activities of enzymes representing oxidative energy supply pathways, relative to SCI alone.
3. There will be a greater relative loss of size, determined by average muscle fiber CSA, in rat vs. human vastus lateralis muscle.

4. There will be a reduction in the activities of enzymes representing oxidative energy supply pathways and an increase in the activities of enzymes estimates of energy demand in rat but not in human vastus lateralis muscle after SCI.

Attempts to address these hypotheses will be made by studying SCI affected and normal muscle with the following specific aims:

1. The fiber type specific cross-sectional area (CSA) will be measured in the soleus (SOL), gastrocnemius (GA), tibialis anterior (TA) and triceps brachii (TRI) in control (CON), SCI, and testosterone treated SCI animals (TTSCI).
2. The fiber type specific activities of succinate dehydrogenase (SDH) and α-glycerol-phosphate dehydrogenase (GPDH), enzyme estimates of oxidative and glycolytic energy supply pathways, respectively, will be measured in the SOL, GA, TA & TRI in CON, SCI, and TTSCI animals. These enzymes are used to make inferences as to the energetic capabilities of muscle. SDH activity is commonly used as an estimator of fatigability of a muscle and/or its fibers.
3. The fiber type specific CSA will be measured in CON and SCI animals as well as CON and SCI human vastus lateralis (VL) muscle 11 weeks after SCI.
4. The fiber type specific activities of SDH, GPDH and actomyosin adenosine triphosphatase (qATPase), an estimate of energy demand of contraction, will be measured in CON and SCI animals as well as CON and SCI human VL and SOL muscles 11 weeks after SCI. A ratio of SDH:qATPase activity has been shown to be a better estimate of fatigue resistance in muscle than SDH activity alone[41, 45].

LIMITATIONS

The existing concerns surrounding testosterone replacement therapy in human subjects have been noted [3]. Specifically, there are concerns as to the criteria for which to base treatment initiation. This study will not attempt to ignore or reverse such concerns but will examine another potential benefit of this type of therapy and will hopefully serve as an initial step in developing means by which some of the health risks associated with SCI can potentially be limited. In addition, the fact that an animal model is used in the present study leads to the question of applicability of the results to humans. Although this question will not be addressed with respect to testosterone replacement therapy, the second portion of the study will take steps toward examining some of the differences that inherently exist between these two species with respect to muscle morphological and enzymatic characteristics and its adaptations to unloading. This will be done in an effort to quantify innate differences that exist and make inferences as to the appropriateness of the rat model in studies of disuse in muscle physiology.

DELIMITATIONS

The ability to maintain peripheral muscle mass in SCI patients should allow for a decrease in potential risk factors associated with CVD. The potential for increased venous return, increased insulin sensitivity, increased metabolic rate with corresponding potential for more favorable body composition, and increased bone density would all have a positive influence on health related risk factors. Thus, recognizing the aforementioned issues surrounding testosterone replacement therapy, this study will hopefully serve as an introductory basis for design and administration for future therapeutic research.

II. REVIEW OF LITERATURE

Spinal cord injury (SCI) refers to any injury of the neural elements within the spinal canal. Injury of this type can result from trauma to or disease of the vertebral column or the spinal cord itself. Most cases of SCI result from trauma to the vertebral column. Trauma can result in vertebral fractures or ligament tears with associated displacement of vertebral bodies. Displacement can cause a contusion, with the potential for hemorrhage and swelling, or tearing of the spinal cord and/or its nerve roots. This damage to the spinal cord can impair both afferent and efferent control of sensory, motor, and autonomic function, depending on its ventral versus dorsal location, at and below the level of injury.

It has been shown that patients with spinal cord injury (SCI) have a 228% greater age adjusted mortality rate resulting from cardiovascular disease (CVD) than normal healthy individuals. Additionally, data indicate that one-third of SCI patients over the age of 65 and one-half of SCI patients with duration of injury exceeding 30 years die of CVD[1].

Data in able-bodied persons suggest that low physical fitness levels (VO2max) are correlated to an increased risk of CVD, with an 8-fold greater risk of CVD mortality in the lowest compared to the highest physically fit quintile. Accordingly, changes in fitness levels have been associated with decreased risk of death from CVD, with an 8% decrease in risk with each 1.75 ml/kg/min increase in VO2max[43]. This decrease in risk

factors for CVD mortality has been shown to be most dramatic for persons that are initially in the least fit groups[42]. Fitness levels in these studies categorized the least fit, or sedentary group, as those with VO2 max values < 21 ml/kg/min. In comparison, limited data on chronic SCI patients report VO2max values of approximately 19 ml/kg/min for this population[47]. These data would suggest great potential for increase and thus potential benefit with respect to CVD mortality risks. However promising, the limitations to increases in VO2max in healthy persons are not the same as in SCI. It has been reported that small peripheral muscle mass limits maximal oxygen consumption in patients with chronic SCI[7]. Thus, to help this group to reach significantly higher fitness levels, and seemingly a lesser risk of mortality resulting from CV disease, interventions aimed at increasing or, more likely, decreasing the loss of peripheral skeletal muscle mass are needed.

Along with a decrease in aerobic capacity, with its associated increased risk of CVD, there are other adverse effects associated with SCI. Loss of bone mass, insulin resistance, renal dysfunction, decreased reproductive function, pulmonary disorders, and decreased plasma androgen levels have all been associated with chronic SCI[8]. The link between the loss of muscle, loss of bone, and the decreased reproductive function in aging males has been associated with the aforementioned decreased circulating androgens, specifically testosterone[2].

Testosterone, a steroid hormone secreted by the interstitial cells of Leydig in the testes, stimulates growth of the male sex organs and promotes development of secondary sex characteristics. In the normal male, testosterone production is negligible during

childhood until the age of 10-13. Testosterone production then increases under the stimulus of anterior pituitary gonadotropic hormones at the onset of puberty. After reaching a peak around age 25-28, testosterone production begins to decline with a rapid decrease beyond the age of 50 to a level that is approximately 20-50% of peak values by age 80. Two important developmental characteristics for which testosterone is responsible are increased protein formation and increased bone density. These two potential adaptations to increased testosterone levels make it an attractive therapeutic treatment for many men.

Testosterone replacement therapy has been shown to result in favorable adaptations in measures of BMD, muscle mass and strength in the aging male[3]. For this reason, it has been referred to as a "youth hormone", with respect to its ability to reverse some of the adverse effects associated with the typical aging process. Treatment with testosterone has been reported to result in significant gains in both muscle strength and CSA in combination with resistance training. Additionally, testosterone levels themselves have been shown to be inversely correlated to incidence of coronary artery disease[48].

In addition to normal aging, a pathological condition that has been associated with a decrease in circulating testosterone, with subsequent decreases in muscle mass and bone density, is SCI. Decreased serum total and free testosterone levels have been shown in subjects with SCI, with a linear decrease in total testosterone levels with increased duration of injury[1]. The cumulative effects of this type of disuse have resulted in a need for interventions aimed at attenuating these responses.

Studies using hind limb suspended rats have shown that testosterone levels in the suspended animal are suppressed in magnitude similar to rats with SCI. Data have also shown that testosterone replacement therapy significantly lessened the loss of muscle and bone typically associated with this simulated micro-gravity environment[4]. In fact, measures of muscle CSA and BMD were no different than ground based controls after 12 days of hind-limb unweighting. Thus, testosterone replacement therapy completely ameliorated the adverse effects of unweighting on measures of muscle CSA and BMD.

However promising the results from hind-limb unweighting studies may be, the question still remains as to whether testosterone replacement therapy will be effective in maintaining muscle mass in SCI affected animals. Although hind-limb suspension and SCI are both commonly used models of unweighting, the magnitude of insult is far greater with SCI, as evident by a lower average iEMG compared to hind-limb suspension animals[5, 6]. Unlike the hind-limb unweighting model where EMG activity returns to values similar to ground based controls by 48 hours, EMG activity after SCI is negligible below the level of injury.

UNWEIGHTING AND SKELETAL MUSCLE

Data in lower mammals suggest that the alterations in muscle forces evoked after SCI are responsible for the plasticity of skeletal muscles resulting from spinal cord transection[17]. Although there does seem to be a hierarchy with respect to the magnitude of response between different muscles and between different fiber types within muscles, the result is a general atrophic response below the level of injury. In addition to smaller

muscles, a shift toward a faster myosin heavy chain (MHC) composition also occurs. Although differences are noted between muscles, based on their initial MHC composition, the faster MHC composition and accompanying increased myofibrillar adenosine triphosphatase activity, reflecting an increased energy demand of contraction, seems to be an accepted adaptation to this type of injury[18]. The consequence of such a shift would be an increased in fatigability of the muscle in the absence of a concomitant increase in activities of enzymes representing pathways for energy supply.

The differences in the atrophic response between muscles have generally followed a hierarchy with extensors > flexors and predominately slow muscles > predominately fast muscles. Thus, the composition and role of a given muscle in lower mammals seemingly dictates its response to unloading. For example, 6 months after SCI in the rat, the soleus, a slow ankle extensor, atrophies approximately 45% while the gastrocnemius, a fast ankle extensor, shows only a 15% decrease in cross-sectional area (CSA)[44]. Thus, these two muscles with similar functional roles (i.e. ankle extensors) differ in their response to disuse. This difference is thought to result from differences in the magnitude of insult between slow and fast muscles, with slow muscles undergoing a greater relative change in use.

The relative change in muscle size in humans of these same two muscles is different in both hierarchy and magnitude compared to the rat. For example, 3 months after SCI, the soleus in humans was only 12% smaller, measured by magnetic resonance imaging, than controls. In comparison, the gastrocnemius was 24% smaller than in controls over this same time period[10]. Another study using hind-limb suspension in

humans showed atrophy of similar magnitude, 17% and 26% for the soleus and gastrocnemius, respectively [46]. These differences in atrophic responses seemingly exist despite functional responsibilities in these muscles being similar to the rat. Additionally, the hierarchy with respect to fiber type percentage (i.e. predominately slow composition of the soleus relative to the gastrocnemius) is similar in the human and the rat. However, the order of magnitude of the atrophic response is reversed in the human compared to the rat. The soleus, with its approximately 70% type I fibers, shows only about one-half of the decrement in size observed in the gastrocnemius which contains about 50% type I fibers. Thus, adaptations to SCI in humans seemingly follow different hierarchical determinants compared to a rat under similar conditions.

RAT VS. HUMAN MODELS IN MUSCLE PHYSIOLOGY

Studies of skeletal muscle plasticity have often used rodents as models [25, 31]. The more stringent control, relative inexpense and apparent ease of using invasive procedures make studies of rodents quite attractive. Consequently, these studies have provided the bulk of information concerning skeletal muscle responses to acute and chronic alterations in loading history, several pathological conditions and to select therapies [25]. These studies are conducted in an effort to increase understanding of basic biological processes and hopefully, development of interventions that are successful in treating lower mammals and consequently evaluated for treating humans. However, an integrated approach of demonstrating the important role that skeletal muscle plays in human health and disease is still lacking [25].

Several observations would suggest that there is opportunity to increase our understanding of human skeletal muscle plasticity in health and disease. For example, improvements in the standard of health care have resulted in many individuals living a long lives with debilitating diseases such as multiple sclerosis and diabetes, living long after a spinal cord injury or heart attack and/or simply having a longer life span [25]. In all, deterioration of skeletal muscle compromises functional capacity and may contribute to poor health [21, 32, 34]. This information, among other things, has increased interest in the study of skeletal muscle plasticity, as evidenced by The National Institutes of Health recently creating a Skeletal Muscle Biology study section. However, it seems prudent to caution the use of animal models at the expense of human studies. Thus, an integrated approach needs to be taken.

The inherent characteristics of skeletal muscle have been shown to have a marked influence upon the nature and magnitude of its adaptive response to altered loading [17, 35]. At the same time, these characteristics can differ markedly between anatomically similar skeletal muscles between species. As a result, adaptive responses to interventions may not necessarily be comparable between humans and, for example, rats. Data showing that rat muscles are made up of predominately fast muscle fiber types are not new [24]. Additionally, previous studies on single fibers have described functional and adaptive differences to various interventions by slow and by fast fiber subtypes [17]. Thus, the fact that human muscles, unlike the rat, typically contain a mosaic of phenotypic expression would lead us to expect these species might adapt differently to disuse.

III. EFFECTS OF TESTOSTERONE REPLACEMENT THERAPY ON SKELETAL MUSCLE AFTER SPINAL CORD INJURY[1]

[1] Gregory, C.M., K. Vandenborne, J.E. Ottenweller, H.F.S. Huang and G.A. Dudley. Submitted to *Spinal Cord*, 12/27/01.

EFFECTS OF TESTOSTERONE REPLACEMENT THERAPY ON SKELETAL MUSCLE AFTER SPINAL CORD INJURY

Running Head

Testosterone after SCI

Muscle Biology Laboratory

Chris Michael Gregory

Gary Alton Dudley

Department of Exercise Science

The University of Georgia

115 Ramsey Student Center

300 River Road

Athens, GA 30602-3654

gdudley@coe.uga.edu

ABSTRACT

STUDY DESIGN: Randomized control

OBJECTIVE: To examine the effects of testosterone replacement therapy (TRT) on skeletal muscle 11 weeks after complete SCI.

SETTING: Athens, Georgia, USA.

METHODS: The soleus (SOL), gastrocnemius (GA), tibialis anterior (TA), vastus lateralis (VL) and triceps brachii (TRI) muscles were taken from twelve young male Charles River rats 11 weeks after complete SCI (T-9 transection, n = 8) or sham surgery (n =4). Rats received either testosterone (two 5 cm testosterone capsules, n = 4), or empty capsule implantation (n = 8) at the time of surgery. Muscle samples were sectioned and fibers analyzed qualitatively for myosin ATPase and quantitatively for succinate dehydrogenase (SDH), α-glycerol-phosphate dehydrogenase (GPDH) and actomyosin ATPase (qATPase) activities using standard techniques.

RESULTS: SCI decreased average fiber size (49 ± 4 %) in the affected muscles and the percentage of slow fibers from 93 ± 3 to 17 ± 2 % in the SOL. In addition, there was a decrease in SDH and an increase in GPDH and qATPase activities across the four hind-limb muscles of the SCI animals. Fiber size in the TRI was larger (31 ± 2 %) after SCI while the activities of SDH and GPDH were not different than control. TRT after SCI attenuated the fiber atrophy, the slow to fast fiber conversion as well as the changes in SDH, GPDH and qATPase activity in the hind-limb muscles. Average fiber size across these four muscles was decreased by only 30 % in testosterone treated SCI (TTSCI) animals and their soleus contained 39 ± 2 % slow fibers. There was no effect of TRT on the TRI relative to SCI alone.

CONCLUSIONS: TRT was effective in attenuating alterations in myofibrillar proteins during 11 weeks of SCI in affected skeletal muscles.

SPONSORSHIP: Supported by a grant from The National Institutes of Health (HD-33738).

INTRODUCTION

It has been estimated that there are approximately 200,000 patients with spinal cord injury (SCI) in the Unites States alone, with roughly 10,000 new injuries occurring each year. Additionally, the relative number of new injuries is rising and expected to have increased in proportion by 20% by the year 2010, compared to 1994 prevalence[1]. SCI is associated with a decrease in perceived quality of life and an increased number of health-related risk factors for cardiovascular disease (CVD). Interventions aimed at reducing some of the health risks associated with SCI have been examined over the past two or so decades. As a result of this research and the changes in care and treatment of patients with SCI, the expected life span of this population has increased to approximately the same as for able-bodied persons. However, many health problems remain for this patient group that may be related to the extensive loss of the mass of affected skeletal muscle after spinal cord injury.

One possible treatment that has received little attention with respect to SCI and skeletal muscle atrophy is testosterone replacement therapy (TRT). TRT is used to combat a variety of health problems in the aging male. The decreases in strength, muscle mass, reproductive function, and bone density commonly associated with aging have all been linked to a decrease in circulating androgens, specifically testosterone[2]. Although the benefit to risk analysis of TRT for able-bodied individuals is sometimes questioned because of the potential for increased hematopoiesis, liver toxicity and prostatic carcinogenic effects[3], the potential ability of this type of treatment to aid patients with SCI has not been examined.

Previous research has shown that TRT can ameliorate the atrophic response resulting from hind-limb unloading in rats [4], a model that also evokes decreases in plasma testosterone similar to those seen in animals with SCI. However, the fact that EMG activity of involved muscles in hind-limb suspended rats is "normal" after 48 hours of unloading while affected skeletal muscles after SCI can be electrically silent, could result in different effects of TRT in these two models of muscle atrophy [5,6]. Accordingly, TRT may attenuate but not ameliorate the atrophic response of skeletal muscle associated with SCI. It seems important to mention the lack of existing data concerning testosterone replacement on patients with this type of injury in the body of scientific literature. This being the case, assumptions of effects from other models have been required for extrapolation to this population.

In light of the aforementioned, the purpose of this study was to evaluate the effects of TRT on rat skeletal muscles after SCI. This study will not address the concerns surrounding testosterone therapy in humans [3]. However, it will hopefully serve as an initial step in assessing the efficacy of TRT for reducing several increased health risks after SCI. An increase in peripheral muscle mass in chronic SCI patients could increase exercise capacity, and thereby reduce the risk for cardiovascular disease [7]. A therapeutically induced increase in muscle mass could also result in increased insulin sensitivity, venous return, and metabolic rate with corresponding potential for more favorable body composition and increased bone density, all factors which would have a positive influence on health.

METHODS

The soleus (SOL), gastrocnemius (GA), tibialis anterior (TA), vastus lateralis (VL) and triceps brachii (TRI) muscles were taken from twelve young male Charles River rats (54 days old), 11 weeks after complete SCI (T-9 transection, n =8)) or sham surgery (n =4). A complete surgical transection of the spinal cord at T-9 with a foam barrier placed between the severed ends of the cord was performed on both SCI groups (n = 8). Rats received either testosterone (two 5 cm testosterone capsules, n = 4), or empty capsule implantation (n = 8) at the time of surgery, as described previously[8]. Rats were housed singly in hanging wire cages with Purina Rat Chow and water ad libitum. Animals were creded 3 times per day until bladder function returned after recovery from spinal shock (~ 2 weeks). There was no recovery of voluntary motor function in the hind-limbs of the rats, although some spinal reflexes do return after recovery from spinal shock.

Excised muscle tissue was mounted in an embedding medium and subsequently frozen in 2-methyl butane cooled in dry ice. Samples were stored at -70º C until analyzed.

Histochemical analyses

Samples were removed from the freezer and placed in a cryostat microtome at -20º C to warm. Serial sections (6, 10 or 14 µm) were cut and placed onto cover slips for immediate assay by both qualitative and quantitative histochemical procedures. Images of the sections were acquired using a Sony xc 77 CCD camera attached to an Olympus bh-2 microscope linked to a Macintosh Quadra 800 computer. Images were saved and

analyzed using NIH image software (written by Wayne Rasband at the US National Institutes of Health, zippy.nimh.nih.gov), as done previously[9, 10].

Sections (10 µm) were assayed qualitatively for myofibrillar adenosine triphosphatase activity using the techniques of Brooke and Kaiser[11] and of Guth and Samaha[12]. Single fibers were analyzed for type by microdensitometric determination of optical density. The myosin ATPase composition, represented by the optical density, which is based on the pH lability of the fiber, is directly correlated to myosin heavy chain composition of a given fiber[13].

Quantitative histochemical determination of succinic dehydrogenase activity (SDH) and α-glycerol phosphate dehydrogenase activity (GPDH) were used as estimates of oxidative and glycolytic energy supply, respectively, essentially as done previously[9, 10]. SDH and GPDH activities were determined by the microdensitiometric technique described by Blanco et al.[14], and Martin et al[15], respectively. Images were captured using the same tools as for fiber typing with the exception that a narrow pass interference filter with peak emission of 570 nm was used so as to assess maximal absorption of Nitro-blue tetrazolium-diformazan, the in-vitro reaction end product. Enzyme activities were obtained from the difference in optical density between samples incubated in the presence and absence of substrate and are expressed as µmol fumerate/L tissue/min and µmol glycerol-3-phosphate/L tissue/min for SDH and GPDH activity, respectively. The method of Blanco and Sieck (1992) was modified and used for quantitative determination of actomyosin adenosine triphosphatase (qATPase) activity in single

fibers. Briefly, 6 µm serial sections were incubated in one of 6 solutions of different ATP concentrations (0-3mM). The use of incubations of different concentrations is necessary because, in contrast to the assays for SDH and GPDH, the qATPase reaction is non-substrate limited. The Michaelis-Menten model of enzyme kinetics was used to analyze the data and the reciprocal of the y-intercept of the Lineweaver-Burk plot was taken as the maximum velocity of the qATPase in OD/min. Enzyme activity, expressed as mmol Pi per liter of tissue per minute, was determined using the Lambert-Beer equation with a molar extinction coefficient for lead-sulfide of 1450 $M^{-1}cm^{-1}$. Images saved for SDH, GPDH and qATPase assays were matched to those saved for fiber typing, thus allowing values obtained for individual fibers in serial sections to be matched and expressed relative to fiber type and fiber size. This provides both a qualitative indicator of fiber properties via their type, and quantitative estimates of both anaerobic and aerobic energy supply via GPDH and SDH activities, respectively, and energy demand via qATPase activity.

A minimum of 100 fibers of a given type were analyzed and averaged for calculation of fiber type specific values for each animal. These averaged values were used as the dependent measures for each variable. Determination of the effect of testosterone replacement therapy on average muscle fiber CSA was assessed using a two-way (muscle x group) analysis of variance (ANOVA). Group differences in fiber type specific CSA, SDH, GPDH and qATPase activities were examined using a three-way (muscle x group x fiber type) repeated measures ANOVA. Differences were considered significant at $p < 0.05$.

RESULTS

Muscle Characteristics in Control Rats

The CSA of individual fibers followed an overall hierarchy with type IIx > IIa > I ($p < 0.01$). Additionally, values for fiber type percentage (FT%) showed that type IIx fibers accounted for a significantly greater proportion of the fibers in the muscles studied than type IIa or type I fibers ($p < 0.01$). Accordingly, calculation results for the relative area of these muscles followed the same hierarchy, as did FT%. With respect to individual muscles, the hierarchy for the SOL in both FT% and relative area differed from the group data, with type I > IIa > IIx. The other muscles all resembled the group data with type IIx fibers occupying more of the muscle than either type I or type IIa ($p < 0.01$).

The SDH, GPDH and qATPase activities of individual fibers studied also followed hierarchies. The GPDH and qATPase activities followed the same hierarchy as did CSA with type IIx > IIa > I ($p < 0.01$), while the SDH activities of type I and IIa fibers were significantly higher than type IIx fibers ($p < 0.01$). Muscle effects for SDH in the hind-limb muscles followed a hierarchy with SOL > GA > VL = TRI > TA ($p \leq 0.01$), expectedly almost opposite of that for GPDH and qATPase with TA, TRI and VL > GA > SOL ($p < 0.02$).

Effects of SCI on rat muscle

The overall effect of SCI on skeletal muscle is a general atrophic response below the level of injury. In the present study, affected muscle fibers in the spinal cord injured animals were approximately 49% smaller ($p < 0.01$, figure 1) than those in control

animals. A muscle x fiber type interaction showed the response to be different among muscles, with fibers in the SOL showing the greatest and the fibers in the TA showing the least reduction in size ($p < 0.01$). Fibers in the TRI were 31 ± 2 % bigger than in the CON animals.

With respect to fiber type percentage, the effects of inactivity is a general reduction in the percentage and proportion of type I fibers. This was the case in the present study. The percentage of type I fibers was lower in the SCI compared to the CON animals ($p < 0.01$) and, as expected, the type I fibers occupied a significantly smaller area of the affected muscle ($p < 0.01$). Muscle x fiber type x group interaction showed that differences in FT% were significant only for the SOL. This was most likely the result of a low percentage of slow fibers in the other muscles studied.

Activities of enzymes representing pathways for both oxidative and glycolytic energy supply also change as a result of SCI. Average SDH activity was lower in hind-limb muscles while average GPDH and qATPase activity was higher in the SOL of SCI affected animals ($p < 0.01$). There were no differences in the activities of these enzymes in the TRI of CON and SCI animals. Fiber type specific results indicate the greatest reduction in SDH activity resulting from SCI in the fiber type with the highest initial activity, the type IIa fibers ($p < 0.01$). There was not significant group x fiber type interaction with respect to GPDH or qATPase activity ($p \geq 0.17$).

Effects of testosterone replacement therapy

The resulting plasma testosterone concentrations were 3.83 ± 1.11 ng/ml in the sham surgery rats, 1.34 ± 0.31 in the SCI rats without testosterone capsules and $5.67 \pm$

00.83 in SCI rats with testosterone capsules. Plasma testosterone levels were higher in the SCI rats receiving testosterone than in those that did not ($p < 0.01$), but they were not significantly different from those in sham SCI rats. With respect to the health of the animals, there was an initial loss of body weight after SCI, but the rats continued to gain weight over the 11 weeks without becoming obese. There were no other side effects of the treatment in this group of rats, although we have noticed prostate enlargement in other groups of testosterone treated rats.

The effect of TRT on average fiber CSA in the hind-limb of TTSCI animals was an attenuation of the atrophic response resulting from SCI. Average fiber CSA in the TTSCI animals was higher and lower compared to SCI and CON animals, respectively ($p < 0.01$, figure 1). This attenuation in fiber atrophy was demonstrated across all fiber types ($p = 0.002$), and in all muscles except the TA ($p < 0.01$). The most dramatic effect was observed in the SOL, with fibers in the TTSCI animals being 58% larger than in SCI animals (figure 1). Additionally, the decrease in the percentage of slow fibers observed in the SCI animals was also attenuated in the TTSCI animals, with TTSCI animals retaining 152% more slow fibers in the SOL than SCI animals ($p < 0.01$, figure 2). There was no additional increase in size resulting from TRT in the TRI, compared to SCI alone (figure 1).

Testosterone therapy also affected the activity of enzymes representing pathways for both oxidative and glycolytic energy supply. The SCI animals had higher values for average GPDH and qATPase activity than CON and testosterone treated SCI animals ($p = 0.02$). The increased GPDH activity in the SCI animals was significant across all four muscles ($p = 0.01$). Another effect of testosterone therapy was an attenuation in the

decrease in SDH activity relative to SCI animals. This resulted in a hierarchy for this measure with CON > TTSCI > SCI ($p < 0.01$, figure 3).

DISCUSSION

The results of this study reveal some new and important findings with respect to the effects of TRT on skeletal muscle after SCI. Additionally, many of the findings in the present work examining normal as well as SCI affected muscles are consistent with previous literature and lend to the validity of this work. First, the expected hierarchies with respect to fiber size and fiber type percentage agree with existing literature [16]. Accordingly, the reduction in fiber size, SDH activity and percentage of type I fibers as well as the increase in GPDH activity resulting from SCI are within the range of reported values for animals of similar age and duration of injury [16, 17, 18].

The most important finding of this study is that TRT ameliorated the decrease in fiber CSA resulting from SCI. TRT also attenuated the slow to fast fiber type shift as well as the decrease in oxidative enzyme activity. To our knowledge, this is the first study to investigate the potential of TRT to prevent atrophy in SCI. Therefore the novelty of our findings necessitate comparison with studies performed on other populations. The use of TRT in aging models of sarcopenia as well as other models associated with muscle wasting (i.e. AIDS) result in favorable effects on bone, muscle size and strength that have been demonstrated in both low-average and hypogonadal men [19]. These studies seem to be the most comparable given the magnitude of reduction in serum and circulating free testosterone levels. The most telling comparison may be with studies using both exercise and TRT as interventions, where increases in muscle CSA were equal, if not greater in TRT only groups than in exercise groups without TRT [20].

These data are important in that they demonstrate the effects of TRT to be equal to and independent of exercise intervention. These two models both demonstrate a positive effect of TRT on muscle size without traditional overloading. The mechanisms responsible for the increased muscle size resulting from TRT remain unclear. In addition, the positive responses to TRT after SCI demonstrated in this study most likely resulted from preservation of existing muscle proteins and not the hypertrophic responses thought to occur in other androgen treated populations. Nonetheless, whether the positive responses to TRT stem from a testosterone deficiency, a tissue-specific or relative resistance to androgen replacement in these populations, or some other systemic response, TRT has been shown to have beneficial effects in populations with below average testosterone levels. This is promising for patients with SCI who show an inverse correlation between duration of injury and testosterone levels [21].

Although the data in the present work are promising, TRT did not completely ameliorate the atrophic response associated with this type of injury. Thus, additional treatment interventions, beyond TRT, aimed at further attenuating loss and/or producing skeletal muscle hypertrophy in this population are needed. The potential for these types of results already exists. Although previous studies using electrically assisted cycling or walking in an SCI population reported minimal muscle growth, data generated using electrical stimulation protocols designed to mimic resistance training have been extremely successful [22]. Patients acutely affected with SCI using high intensity electrical stimulation have been able to realize tremendous muscle growth. These results bear similarity to training protocols in healthy populations comparing traditional resistance training and endurance training. As a result, similar to a healthy population, a

hypertrophic response, unlike the atrophic response typically associated with SCI, is seemingly modulated by more than just activity of the muscle, or lack there of. Thus, the plasticity in muscle size is seemingly affected by much the same stimuli in healthy and SCI affected muscle, at least in the first year after injury. This is an important finding with respect to interventions aimed at this population.

Another interesting finding of this work is the lack of additional increase in muscle size of the TRI after testosterone therapy in this population. The apparent ceiling effect of muscle hypertrophy and the effectiveness of this anabolic hormone is informative for development of therapeutic intervention and potential application of TRT results to other populations. The results suggest a potential lack of benefit to the combination of TRT and overload in healthy muscle. However, given the magnitude of the hypertrophic response resulting from SCI (~30%), limitations resulting from this apparent ceiling effect may not be realized in traditional models of skeletal muscle hypertrophy.

The results of this paper indicate several potential benefits of TRT in patients with SCI, namely maintenance of muscle size and slow fiber percentage. These benefits include the potential for increased venous return, increased insulin sensitivity, and increased metabolic rate, all resulting from the maintenance of skeletal muscle characteristics after SCI. Although a myriad of health related problems occur as a result of SCI, the potential to reduce the severity of these problems with TRT is an exciting possibility and one that demands future research.

Figure Legend:

Figure 3.1: Average Fiber Size

- Values are expressed in μm^2. Mean data ± S.E. are given for control (CON), spinal cord injured (SCI) and testosterone treated spinal cord injured (TTSCI) groups in the soleus (SOL) gastrocnemius (GA), tibialis anterior (TA), and vastus lateralis (VL) muscles. * Indicates values significantly greater than values for the same muscles in other groups. # Indicates values significantly greater than values for the same muscles in SCI group.

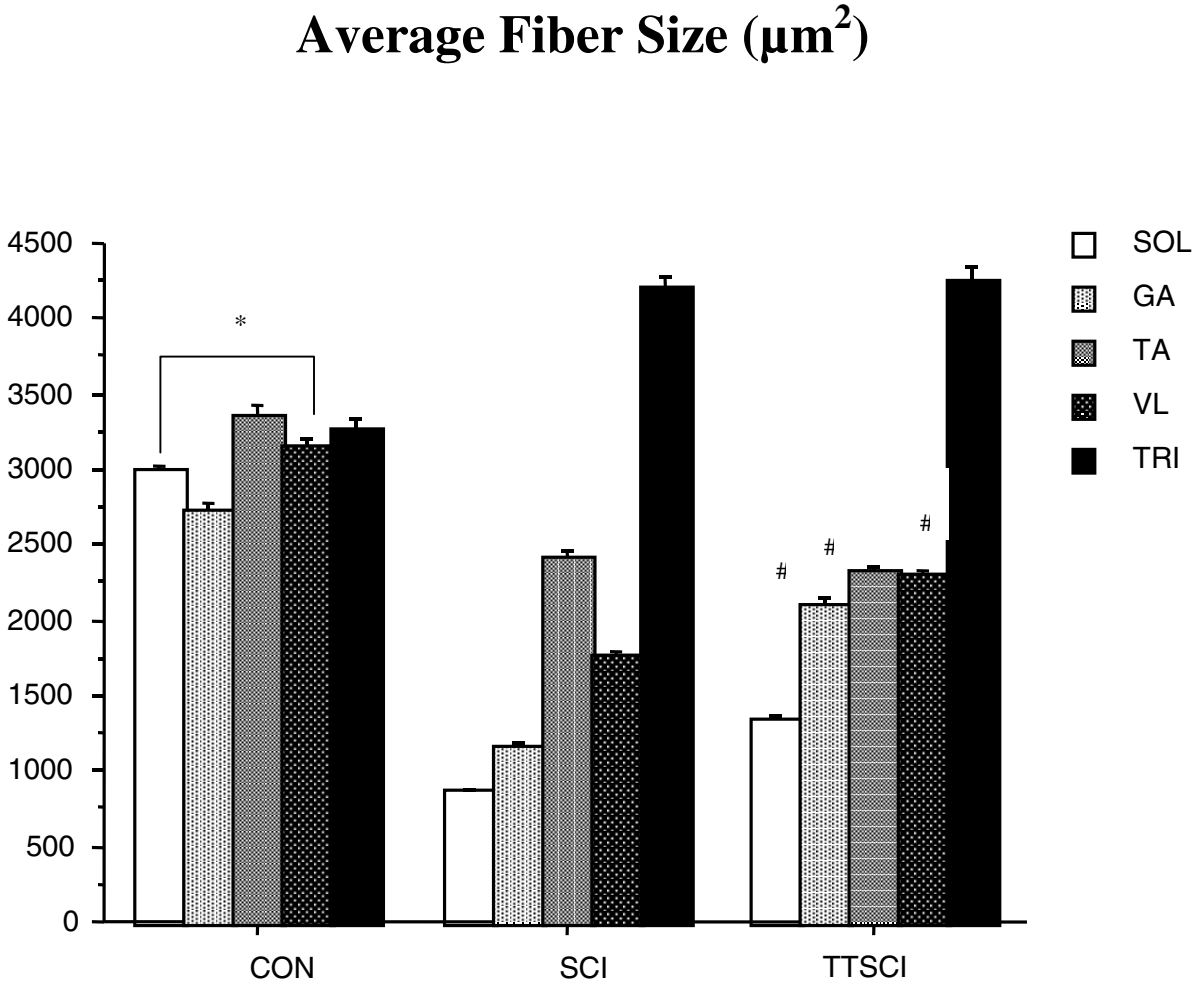
Figure 3.2:

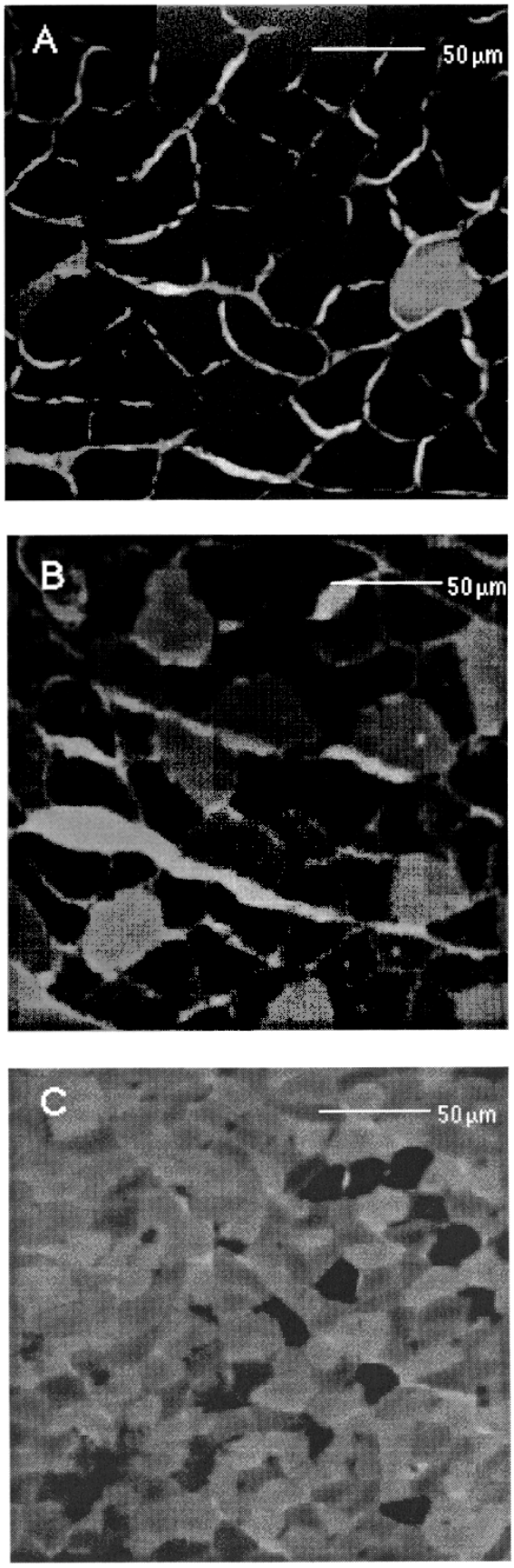
- Images taken from the soleus muscle stained histochemically for myofibrillar adenosine triphosphatase (hATPase) CON (panel A), TTSCI (panel B) and SCI (panel C).

Figure 3.3: Changes in Enzyme Activities

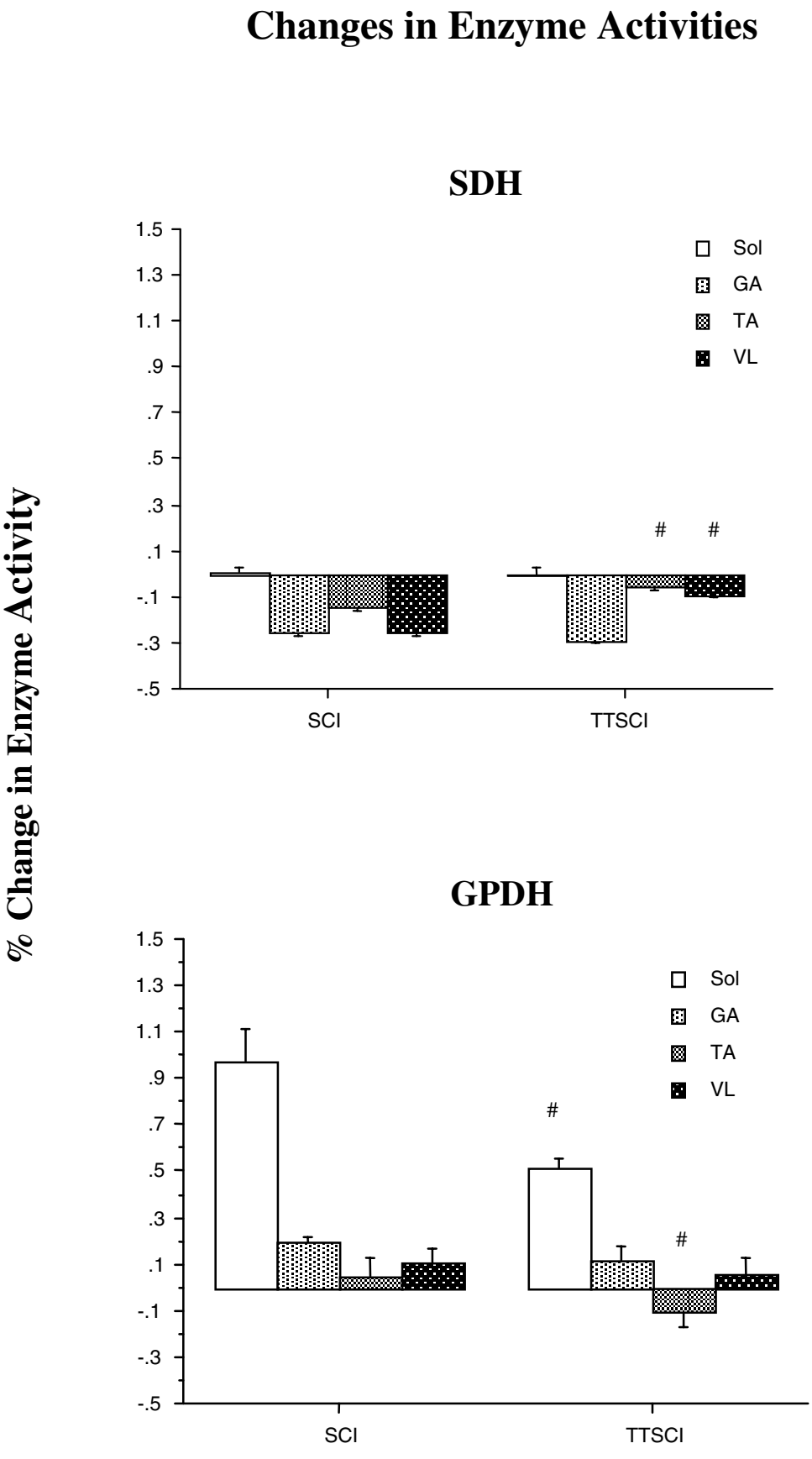
- Values are expressed as percent difference from control (CON) values. Mean data ± S.E. are given for SCI and TTSCI groups in the SOL, GA, TA, and VL muscles. # Indicates values significantly different than values for the same muscles in SCI animals.

(Figure 3.1)


Average Fiber Size (µm²)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
*
#
#
#
CON
SCI
TTSCI
SOL
GA
TA
VL
TRI


A
50 µm
B
50 µm
C
50 µm

(Figure 3.3)


Changes in Enzyme Activities
SDH
% Change in Enzyme Activity
1.5
1.3
1.1
.9
.7
.5
.3
.1
-.1
-.3
-.5
Sol
GA
TA
VL
#
#
SCI
TTSCI
GPDH
1.5
1.3
1.1
.9
.7
.5
.3
.1
-.1
-.3
-.5
Sol
GA
TA
VL
#
#
SCI
TTSCI

REFERENCES

1. Phillips, W.T., Kiratli, B.J., Sarkarati, M., & Weraarchakul, G. Effects of Spinal Cord Injury on the Heart and Cardiovascular Fitness. Current Problems in Cardiology 1998; 23 (11): 644-717.
2. Wimalawansa, S.M., and S.J. Wimalawansa. Simulated weightlessness-induced attenuation of testosterone production may be responsible for bone loss. Endocrine 1999; 10(3): 253-260.
3. Basaria, S. & A.S. Dobs. Risk versus benefit of testosterone therapy in elderly men. Drugs and Aging 1999; 15 (2): 131-142.
4. Wimalawansa, S.M., M.T. Chapa, J.N. Wei, K.N. Westlund, M.J. Quast, and S.J. Wimalawansa. Reversal of weightlessness-induced musculoskeletal losses with androgens: quantification by MRI. Journal of Applied Physiology 1999; 86 (6): 1841-1846.
5. Alford, E.K., R.R. Roy, J.A. Hodgson, & V.R. Edgerton. Electromyography of rat soleus, medial gastrocnemius, and tibialis anterior during hind limb suspension. Experimental Neurology 1987; 96:635-649.
6. Alaimo, M.A., J.L. Smith, R.R. Roy, and V.R. Edgerton. EMG activity of slow and fast ankle extensors following spinal cord transection. Journal of Applied Physiology 1984; 56: 1608-1613.
7. Hopman, M.T.E., C. Dueck, M. Monroe, W.T. Phillips, & J.S. Skinner. Limits to maximal performance in individuals with spinal cord injury. International Journal of Sports Medicine 1998; 19: 98-103.

8. Ottenweller, J.E., M. Li, W. Giglio, R. Anesetti, L.M. Pogach, & H.F.S. Huang. Alteration of Follicle-Stimulating Hormone and Testosterone regulation of messenger Ribonucleic Acid for Sertoli Cell proteins in the rat during the acute phase of spinal cord injury. Biology of Reproduction 2000; 63: 730-735.
9. Gregory, C.M., K. Vandenborne, & G.A. Dudley. Metabolic enzymes and phenotypic expression among human locomotor muscles. Muscle & Nerve 2001; 24: 387-393.
10. Castro, M.J., D.F. Apple, R.S. Staron, G.E.R. Campos & G.A. Dudley. Influence of complete spinal cord injury on skeletal muscle within 6 mo of injury. Journal of Applied Physiology 1999; 86 (1): 350-358.
11. Brooke MH & Kaiser KK. Three "Myosin Adenosine Triphosphatase" systems: The nature of their pH lability and sulfhydryl dependence. Journal of Histochemistry & Cytochemistry 1970; 18: 670-672.
12. Guth L & Samaha J. Qualitative differences between Actomyosin ATPase of slow and fast mammalian muscle. Experimental Neurology 1969; 25: 138-152.
13. Staron RS & Pette D. Correlation between myofibrillar ATPase activity and myosin heavy chain composition in rabbit muscle fibers. Histochemistry 1986; 86: 19-23.
14. Blanco CE, Sieck GC & Edgerton VR. Quantitative histochemical determination of succinic dehydrogenase activity in skeletal muscle fibers. Histochemistry Journal 1988; 20: 230-243.
15. Martin TP, Vailas AC, Durivage JB, Edgerton VR & Castleman KR. Quantitative histochemical determination of muscle enzymes: biochemical verification. Journal of Histochemistry & Cytochemistry 1985; 33: 1053-1059.

16. Grossman EJ, Roy RR, Talmadge RJ, Zhong H, & Edgerton VR. Effects of inactivity on myosin heavy chain composition and size of rat soleus fibers. Muscle & Nerve 1998; 21: 375-389.

17. Roy, R.R., K.M. Baldwin, & V.R. Edgerton. The plasticity of skeletal muscle: Effects of neuromuscular activity. Exercise & Sport Sciences Reviews 1991; 19:269-312.

18. Gordon, T., and M.C. Pattullo. Plasticity of muscle fiber types and motor unit types. Exercise & Sport Sciences Reviews 1990; 21: 331-362.

19. Tenover JS. Effects of testosterone supplementation in the aging male. Journal of Clinical Endocrinology & Metabolism 1992; 75: 1092-1098.

20. Bhasin S, Storer TW, Berman N, Callegari C, Clevenger BA, Phillips J, Bunnell T, Tricker R, Shirazi A & Casabari R. The effects of supraphysiological doses of testosterone on muscle size & strength in men. New England Journal of Medicine 1996; 335:1-7.

21. Bauman, W.A., A.M. Spungen, R.H. Adkins, & B.J. Kemp. Metabolic and Endocrine changes in persons with spinal cord injury. Asst. Technologies 1999; 11: 88-96.

22. Dudley GA, Castro MJ, Rogers S, Apple DF Jr. A simple means of increasing muscle size after spinal cord injury. European Journal of Applied Physiology 1999; 80:394-396.

IV. HUMAN AND RAT SKELETAL MUSCLE ADAPTATIONS TO SPINAL CORD INJURY[2]

[2] Gregory C.M., K. Vandenborne, M.J. Castro and G.A. Dudley. Submitted to *Canadian Journal of Applied Physiology*, 3/18/02.

HUMAN AND RAT SKELETAL MUSCLE ADAPTATIONS TO SPINAL CORD INJURY

Running Head

Skeletal Muscle After SCI

Muscle Biology Laboratory

Chris Michael Gregory

Gary Alton Dudley

Department of Exercise Science

The University of Georgia

115 Ramsey Student Center

300 River Road

Athens, GA 30602-3654

gdudley@coe.uga.edu

ABSTRACT

Results of studies of rodent skeletal muscle plasticity are often extrapolated to humans. However, responses to "disuse" may be species specific, in part, because of different inherent properties of anatomically similar muscles. Thus, this study quantified human and rat *m*. vastus lateralis (VL) fiber adaptation prior and subsequent to 11 wk of spinal cord injury (SCI). The VL was taken from 8 young male Charles River rats after complete SCI (T-9 transection (n = 4)) or sham surgery (n =4). In addition, the *m*. VL was biopsied in 7 able-bodied and in 7 SCI humans. Samples were sectioned and fibers analyzed for type (I, IIa, IIb/x), cross-sectional area (CSA), succinate dehydrogenase (SDH), α-glycerol-phosphate dehydrogenase (GPDH), and actomyosin adenosine triphosphatase (qATPase) activities. Rat fibers had 1.5- to 2-fold greater SDH and GPDH activities while their fibers were 60 % the size of those in the human. The most striking differences, however, were the absence of slow fibers in the rat and its 4-fold greater proportion of IIb/x fibers (80 versus 16 % of the CSA) compared to the human. SCI decreased SDH activity more in rats while atrophy and IIa to IIb/x fiber shift occurred to a greater extent in humans. It is suggested that the rat is a reasonable model for studying the predominant response to SCI, atrophy. However, its high proportion of IIb/x fibers limits evaluation of the mechanical consequences of shifting to "faster" contractile machinery after SCI. Because human skeletal muscle eventually takes on the inherent characteristics of the rat hind limb after injury, it seems worthy to ascertain how rats function with the fastest of fibers occupying most of their hind limb.

INTRODUCTION

Studies of skeletal muscle plasticity have often used rodents as models (Baldwin, 2000. Holloszy, 1983). The stringent control, relative inexpense and apparent ease of using invasive procedures make studies of rodents quite attractive. Consequently, these important studies have provided the bulk of information concerning skeletal muscle responses to acute and chronic alterations in loading history, several "pathological" conditions and to select therapies (Baldwin, 2000). Most of these studies are conducted in an effort to increase understanding of a basic biological process. The results of such work may result in development of an intervention that is successful in lower mammals and consequently evaluated for treating humans. The work of Edgerton's laboratory concerning treadmill locomotion after spinal cord injury (SCI), first in cats to assess spinal neural circuit plasticity and now in humans to enhance ambulation after incomplete injury, is an excellent example in this regard. However, as noted by Holloszy, this would be viewed as the exception (Holloszy, 1983). Almost twenty years later, marked strides have been made in basic biology, however, an integrated approach of demonstrating the important role that skeletal muscle plays in human health and disease is still lacking (Baldwin, 2000).

Several observations suggest that there is opportunity, and in fact a frank need, to increase our understanding of human skeletal muscle plasticity in health and disease. For example, the standard of health care in the modern world has resulted in many individuals living a long time with a debilitating disease such as multiple sclerosis, living long after a spinal cord injury or heart attack and/or simply having a longer life span (Baldwin, 2000). In all, deterioration of skeletal muscle probably compromises

functional capacity and may contribute to poor health (Massie et al, 1996, Kent-Braun et al, 1997, Bauman et al, 1999). This information, among other things, has increased interest in the study of skeletal muscle plasticity as evidenced by The National Institutes of Health recently creating a Skeletal Muscle Biology study section. However, it seems prudent to caution the use of animal models at the expense of human studies, an integrated approach needs to be taken. For example, the inherent characteristics of skeletal muscle have a marked influence upon the nature and magnitude of its adaptive response to altered loading (Pette and Staron, 2000, Roy et al, 1991). At the same time, they can differ markedly between anatomically similar skeletal muscles in humans and, for example, rats. As a result, adaptive responses to an intervention may not necessarily be comparable between species. Accordingly, this study tested the hypothesis that m. vastus lateralis (VL) of humans and rats would show divergent responses to 11 weeks of SCI.

METHODS

The m. VL was taken from eight young male Charles River rats 11 weeks after complete SCI (T-9 transection, n = 4) or sham surgery (n = 4). Additionally, percutaneous needle biopsy samples were taken 11 weeks post injury from *m*. VL of 7 patients with complete SCI as well as 7 recreationally active, able-bodied controls. Excised muscle tissue was mounted in an embedding medium and subsequently frozen in 2-methyl butane cooled to its freezing point in liquid nitrogen. Samples were stored at -70º C until analyzed. Subjects were informed of the protocol and signed an informed consent prior to participation in the study.

Histochemical analyses

Samples were removed from the freezer and placed in a cryostat microtome at -20º C to warm. Serial sections (6, 10 or 14 µm) were cut and placed onto cover slips for immediate assay by both qualitative and quantitative histochemical techniques. Images were acquired using a Sony xc 77 CCD camera attached to an Olympus bh-2 microscope linked to a Macintosh Quadra 800 computer. Images were saved and analyzed using NIH image software (written by Wayne Rasband at the US National Institutes of Health, zippy.nimh.nih.gov), as done previously (Castro et al., 1999, Castro et al., 2000, Gregory et al., 2001, Kent-Braun et al., 1997, Levine et al., 2002).

Sections (10 µm) were assayed qualitatively for myofibrillar adenosine triphosphatase activity (hATPase) using the techniques of Brooke and Kaiser (1970) and of Guth and Samaha (1969). Single fibers were analyzed for type by microdensitometric determination of optical density. The myosin ATPase composition, represented by the optical density of the fiber and based on its pH lability, is directly correlated to the myosin heavy chain (MHC) composition of the given fiber (Staron & Pette, 1986). In an attempt to simplify comparisons between species, fibers were classified as either type I, IIa, or IIb/x, as done previously (Castro et al., 1999, Castro et al., 2000, Gregory et al., 2001, Levine et al., 2002).

Quantitative histochemical determination of succinic dehydrogenase activity (SDH) and alpha-glycerol phosphate dehydrogenase (GPDH) activity were determined by the microdensitometric technique described by Blanco et al. (1988), and Martin et al. (1985), respectively. Images were captured using the same tools as for hATPase with the exception that a narrow pass interference filter with peak emission of 570 nm was used so

as to assess maximal absorption of Nitro-blue tetrazolium-diformazan (NBT-dfz), the in-vitro reaction end product. Enzyme activities were obtained from the difference in optical density between samples incubated in the presence and absence of substrate and are expressed as µmol fumerate/L tissue/min and µmol glycerol-3-phosphate/L tissue/min for SDH and GPDH activities, respectively.

The method of Blanco and Sieck (1992) was modified and used for quantitative determination of actomyosin adenosine triphosphatase (qATPase) activity in single fibers. Briefly, 6 µm serial sections were incubated in one of 6 solutions of different ATP concentrations (0-3mM). The use of incubations of different concentrations is necessary because, in contrast to the assays for SDH and GPDH, the qATPase reaction is non-substrate limited. The Michaelis-Menten model of enzyme kinetics was used to analyze the data and the reciprocal of the y-intercept of the Lineweaver-Burk plot was taken as the maximum velocity (Vmax) of the qATPase in OD/min. Enzyme activity, expressed as mmol Pi per liter of tissue per minute, was determined using the Lambert-Beer equation with a molar extinction coefficient for lead-sulfide of 1450 $M^{-1}cm^{-1}$.

Images saved for SDH, GPDH, and qATPase assays were matched to those saved for hATPase, thus allowing enzyme activities obtained for individual fibers in serial sections to be matched and expressed relative to fiber type and cross sectional area. This provides a qualitative indicator of fiber properties via hATPase, quantitative estimates of both glycolytic and oxidative energy supply via GPDH and SDH activities, respectively, and a quantitative estimate of energy demand via qATPase activity.

Statistics

Statistical analyses were done using SPSS 10.0 software. Comparisons made between species in control muscles were done using a planned comparison from a one-within/one-between analysis of variance (ANOVA). Comparisons made between species for adaptations over time were made using the interaction term from a one-within/one-between ANOVA. Inter-species changes in fiber type percentage were tested using t-tests. Differences were considered significant with $p < 0.05$.

RESULTS

Comparisons between rat and human control VL

Average fiber size was 63% greater in humans than in rats ($p \leq 0.0001$, table 1). The magnitude of this difference was most dramatic in type IIa fibers with human fibers being 131% larger (table 1). Rats had no type I fibers and a greater proportion of type IIb/x fibers (table 1). This differed greatly from the mosaic of human phenotypes [1] (figure 1). As a result, the relative cross-sectional area (CSA) occupied by fast and by IIb/x fibers was greater in the rat ($p \leq 0.0001$, table 1). A two- and a three-fold greater average fiber SDH and GPDH activity, respectively, was also found in the rat ($p \leq 0.0001$, table 1). Like fiber CSA, species differences in enzyme activities were greatest in type IIa fibers. Average fiber qATPase activity was 30 % lower in rats than in humans ($p \leq 0.0001$, table 1).

Effects of SCI on rat and human VL

In both species, average fiber CSA and SDH activity were lower and there was an apparent shift in fast fiber subtype proportions, IIa $\rightarrow$ IIb/x after SCI. However, the

decrease in fiber CSA was greater in humans ($p \leq 0.0001$) while the opposite was the case for SDH activity ($p = 0.026$, table 1). The 77% increase in the proportion of type IIb/x fibers in humans was five times the 14% increase noted for rats ($p \leq 0.0001$, figure 1). No changes in GPDH or qATPase activities, or fast fiber percentages were noted with injury (table 1).

DISCUSSION

The unique aspect of this study was the comparison of adaptations of fibers of the same major locomotor muscle, m. VL, to complete SCI in humans and in the rat. Of the variables assessed, the results indicate that fiber atrophy is the major adaptation of m. VL to 11 weeks of SCI. The loss of fiber size occurred to a greater extent in humans while the opposite was the case for the decline in SDH activity. Neither response appeared to be particularly fiber type dependent, thus the high proportion of IIb/x fibers in the rat would not be overly problematic. However, the predominance of fibers of this type would seemingly limit conversion to faster muscle, as appeared to be the case. Before addressing these issues, a few comments concerning inherent characteristics of m. VL are warranted.

The small size and high SDH activity of rat compared to human fibers of *m*. VL in combination with the slightly lower qATPase activity in this rodent are characteristics of muscle with a substantial resistance to fatigue. The smaller fibers provide a shorter diffusion distance, while the activities of SDH and qATPase, used as estimates of energy supply and energy demand, respectively, are determinants of several mechanical characteristics of skeletal muscle. In addition, resistance to fatigue is strongly related to their ratio (SDH: qATPase) (Tesch et al., 1985, Van Der Laarse et al., 1991). The rat *m*.

VL has no slow fibers, unlike the human *m*. VL that is about 1/3 slow contractile machinery. This would suggest a high energy demand of contraction, noting qATPase activity of fast fibers is 1.5 to 3 times that of slow (Barany, 1967). However, the high proportion of fast fibers in the rat *m.* VL, and in fact in its hind limb, is countered by the unique hierarchy of its fibers with respect to markers of aerobic-oxidative metabolism. This hierarchy in the rat followed IIa > I > IIb/x (Rivero et al., 1998) versus that of humans being I > IIa > IIb/x (Gregory et al., 2001). Thus, the impact of the high, inherent proportion of fast fibers in the rat is seemingly lessened.

Data showing that rat muscles are made up of predominately fast muscle fiber types are not new (Armstrong & Phelps, 1984). Additionally, previous work on single fibers has described functional and adaptive differences to various interventions by slow and by fast fiber subtypes (Roy et al., 1991). Thus, the fact that human muscles, unlike the rat, typically contain a mosaic of phenotypic expression would lead us to expect these species might adapt differently to disuse. Nonetheless, the overall decrease in fiber size and SDH activity combined with a shift between fast fiber subtypes (IIa $\rightarrow$ IIb/x) was common to both species. However, the magnitude of change in these variables was different. Humans showed the greatest reduction in size and percentage of type IIa fibers, given their approximate 63% larger fibers and 1.5 times greater proportion of this fast subtype in able-bodied individuals. Rat fibers showed a greater reduction in SDH activity, given their approximate 150% higher inherent activity for this enzyme.

Given the described changes resulting from SCI, the response with the greatest impact is most likely fiber atrophy. The atrophic response that typically accompanies an injury of this type has been linked to many of the secondary pathologies associated with

SCI and has been shown to be the limiting factor in exercise capacity in such patients (Bauman et al., 1999, Hopman et al., 1998). Thus, the greater decrease in size, both absolute and relative, in the human suggests a greater overall insult from SCI compared to the rat.

The most obvious inherent difference between these two species was in the percentage of fast fibers. Conventional wisdom suggests slow to fast fiber conversion after SCI (Roy et al., 1991, Pette & Staron, 2000). However, this could have little impact in the rat given nearly all of its' hind limb inherently consists of fast fibers. The one exception is the *m*. soleus, which occupies about 5% of the hind limb and contains a high percentage of slow fibers (Armstrong et al., 1984). Interestingly, this muscle converted to a predominantly fast muscle after the 11 weeks of SCI in these same animals (Gregory & Dudley unpublished observations). In contrast, we did not find a decrease in the percentage of slow fibers 11 weeks after SCI in human *m*. VL in this study, nor in these same subjects 24 weeks after injury (Castro et al., 1999, Talmadge et al., 2002). Evaluation of affected muscles that inherently contain slow fibers in long term SCI patients, nonetheless, suggests that this slow → fast shift does eventually occur in humans (Martin et al., 1992). Clearly, when this occurs, it would be expected to have a much greater impact on human than rat muscle (i.e. an increase in contraction speed (Shields et al., 1997, Gerrits et al., 1999).

The IIa → IIb/x shift was the major phenotypic adaptation that occurred in *m*. VL in humans and rats. The potential for change in the rat, however, was minimal because of its high inherent proportion of type IIb/x fibers. The fact that only ~ 15% of the CSA of *m.* VL in able-bodied humans is occupied by the "fastest" fibers, in contrast, leaves room

for greater phenotypic adaptations. Thus, humans would be expected to show greater "speeding" of and energy demand owing to contraction, especially if slow to fast fiber transformation eventually occurred (Martin et al., 1992, Gerrits et al., 1999, Shields et al., 1997). This may partly account for the extreme fatigability evident in human m. VL in long term SCI individuals. On the other hand, the minimal increase in type IIb/x fibers that can occur in rats could be viewed to counter its greater decline in SDH activity with regards to fatigue resistance. The potential influence of conversion to a faster muscle upon contraction mechanics and fatigue resistance is based in part on a lack of plasticity in qATPase activity after SCI. This does appear to be the case after short-term injury in humans (Castro et al., 2000) while long term patients may actually show a decline (Martin et al., 1992). However, it is not so clear that force loss during contraction reflects fatigue in the classical sense because SDH activity has been reported to explain little of the performance decrement during electrical stimulation in long term SCI patients (Martin et al., 1992, Rochester et al., 1995).

The results of this study suggest that the major adaptation of *m*. VL to 11 weeks of SCI is fiber atrophy in both humans and rats. Neither this response, nor the decline in SDH activity was shown to be fiber type dependent. Accordingly, it is suggested that the rat is a reasonable model for evaluation of mechanistic aspects of and probable therapies for these responses to short term SCI. Such studies should not be conducted at the expense of human work, however, realizing that human experiments are the ultimate testing ground. On the other hand, it is suggested that studies of phenotypic expression in rats be conducted with caution. Most importantly, the inherent slow phenotype of human skeletal muscle is extremely resistant to change. Even when altered by such an extreme

incident as chronic SCI, human skeletal muscle takes on the inherent characteristics of the rat hind-limb. Accordingly, the importance of figuring out how the rat functions with such a high proportion of fast fibers in its hind limb could be valuable given that it has been difficult to increase the proportion of slow fibers in paralyzed human muscle (Anderson et al., 1996).

REFERENCES

1. Anderson, J.L., Mohr, T., Biering-Sorensen, F., Galbo, H., & Kjær, M. (1996). Myosin heavy chain isoform transformation in single fibres from *m.* vastus lateralis in spinal cord injured individuals: Effects of long-term functional electrical stimulation (FES). Pflugers Archives; 431:513-518.
2. Armstrong, R.B. and Phelps, R.O. (1984). Muscle fiber type composition of the rat hindlimb. The American Journal of Anatomy; 171: 259-272.
3. Baldwin, K.M. (2000). Research in the exercise sciences: Where do we go from here? Journal of Applied Physiology; 1:332-336.
4. Barany, M. (1967). ATPase activity of myosin correlated with speed of muscle shortening. Journal of Physiology; 50: 197-216.
5. Bauman, W.A., Adkins, R.H., Spungen, R.M. and Waters, R.L. (1999). The effect of residual neurological deficit on oral glucose tolerance in persons with chronic spinal cord injury. Spinal Cord; 37:765-771.
6. Blanco, C.E., Sieck, G.C. & Edgerton, V.R. (1988). Quantitative histochemical determination of succinic dehydrogenase activity in skeletal muscle fibers. Histochemistry Journal; 20: 230-243.
7. Blanco, C.E. & Sieck, G.C. (1992). Quantitative determination of calcium activated myosin adenosine triphosphatase activity in rat skeletal muscle fibers. Histochemistry Journal; 24: 431-44.
8. Brooke, M.H. & Kaiser, K.K. (1970). Three "Myosin Adenosine Triphosphatase" systems: The nature of their pH lability and sulfhydryl dependence. Journal of Histochemistry & Cytochemistry 18: 670-672.

9. Castro, M.J., Apple, D.F., Staron, R.S., Campos, G.E.R. & Dudley, G.A. (1999). Influence of complete spinal cord injury on skeletal muscle within 6 mo. of injury. Journal of Applied Physiology; 86(1): 350-358.
10. Castro, M.J., Apple, D.F. Jr, Melton-Rogers, S., Dudley, G.A. (2000). Muscle fiber type-specific myofibrillar Ca (2+) ATPase activity after spinal cord injury. Muscle & Nerve; 23(1): 119-21.
11. Gerrits, H.L., De Haan, A., Hopman, M.T., van Der Woude, L.H., Jones, D.A. & Sargeant, A.J. (1999). Contractile properties of the quadriceps muscle in individuals with spinal cord injury. Muscle Nerve; 22(9): 1249-56.
12. Gregory, C.M., Vandenborne, K. & Dudley, G.A. (2001). Metabolic enzymes and phenotypic expression among human locomotor muscles. Muscle & Nerve; 24: 387-393.
13. Guth, L. & Samaha, J. (1969). Qualitative differences between Actomyosin ATPase of slow and fast mammalian muscle. Experimental Neurology; 25: 138-152.
14. Holloszy, J.O. (1983). Exercise, health, and aging: a need for more information. Medicine and Science in Sports & Exercise; 15(1): 1-5.
15. Hopman, M.T.E., Dueck, C., Monroe, M., Phillips, W.T. & Skinner, J.S. (1998). Limits to maximal performance in individuals with spinal cord injury. International Journal of Sports Medicine; 19: 98-103.
16. Kent-Braun, J.A., Ng, A.V., Castro, M., Weiner, M.W., Gelinas, D., Dudley, G.A. & Miller, R.G. (1997). Strength, skeletal muscle composition, and enzyme activity in multiple sclerosis. Journal of Applied Physiology; 83(6): 1998-2004.

17. Levine, S., Gregory, C.M., Nguyen, T., Shrager, J.B., Kaiser, L.R., Rubinstein, N.A., and Dudley, G.A. Bioenergetic adaptation of individual human diaphragmatic myofibers to severe chronic obstructive pulmonary disease. Journal of Applied Physiology; 92(3): 1205-13.

18. Martin, T.P., Vailas, A.C., Durivage, J.B., Edgerton, V.R. & Castleman, K.R. (1985). Quantitative histochemical determination of muscle enzymes: biochemical verification. Journal of Histochemistry & Cytochemistry; 33: 1053-1059.

19. Martin, T.P., R.B. Stein, P.H. Hoeppner, & D.C. Reid. (1992). Influence of electrical stimulation on the morphological and metabolic properties of paralyzed muscle. Journal of Applied Physiology; 72:1401-1406.

20. Massie BM, Simonini A, Sahgal P, Wells L, Dudley GA. (1996) Relation of systemic and local muscle exercise capacity to skeletal muscle characteristics in men with congestive heart failure. Journal of American College of Cardiology; 27(1): 140-5.

21. Pette, D., Staron, R.S. (2000). Myosin isoforms, muscle fiber types, and transitions. Microsc Research Technology; 50(6): 500-9.

22. Rivero, J.L., Talmadge, R.J. & Edgerton, V.R. (1998). Fiber size and metabolic properties of myosin heavy chain based fiber types in rat skeletal muscle. Journal of Muscle Research and Cell Motility; 19: 733-742.

23. Rochester, L., Barron, M.J., Chandler, C.S., Sutton, R.A., Miller, S. & Johnson, M.A. (1995). Influence of electrical stimulation of the tibialis anterior muscle in paraplegic subjects; morphological and histochemical properties. Paraplegia; 33:514-522.

24. Roy, R.R., Baldwin, K.M. & Edgerton, V.R. (1991). The plasticity of skeletal muscle: Effects of neuromuscular activity. Exercise and Sport Science Reviews; 19: 269-312.

25. Shields, R.K., Law, L.F., Reiling, B., Sass, K. & Wilwert, J. (1997). Effects of electrically induced fatigue on the twitch and tetanus of paralyzed soleus muscle in humans. Journal of Applied Physiology; May; 82(5): 1499-507.

26. Staron, R.S. & Pette, D. (1986). Correlation between myofibrillar ATPase activity and myosin heavy chain composition in rabbit muscle fibers. Histochemistry*;* 86: 19-23.

27. Talmadge, R.J., Castro, M.J., Apple, D.F. Jr. & Dudley, G.A. (2002). Phenotypic adaptations in human muscle fibers 6 and 24 wk after spinal cord injury. Journal of Applied Physiology; 92(1): 147-54.

28. Tesch, P.A., Wright, J.E., Vogel, J.A., Sharp, D.S. & Sjodin, B. (1985). The influence of muscle metabolic characteristics on physical performance. European Journal of Applied Physiology; 54: 237-245.

29. Van Der Laarse, W.J., Lannergren, J. & Diegenbach, P.C. (1991). Resistance to fatigue of single muscle fibers from Xenopus related to succinate dehydrogenase and myofibrillar ATPase activities. Histochemistry Journal; 16: 529-541.

Acknowledgements: The authors would like to thank everyone involved in the collection and analysis of the samples from the University of Pennsylvania, UNDML - New Jersey Medical School, Shepherd Center, & The University of Georgia. In addition, we would like to thank Dr. Steve Olejnik for his help with statistical analyses. Supported in part by

the National Institutes of Health (HD-33738 to KV and GAD, HD 39676 to GAD) and The Paralyzed Veterans of America: Spinal Cord Research Foundation (GAD).

Table 4.1:

Data are presented as Mean ± S.E. Data are given for fiber type percentage (FT%), succinate dehydrogenase activity (SDH), glycerol phosphate dehydrogenase activity (GPDH), actomyosin adenosine triphosphatase activity (qATPase), and percent cross-sectional area (%CSA). $^{\#}$ denotes value > control rat, $^{+}$ denotes value > control human, $^{\#\#}$ denotes value > SCI rat, $^{++}$ denotes value > SCI human ($p < 0.05$).

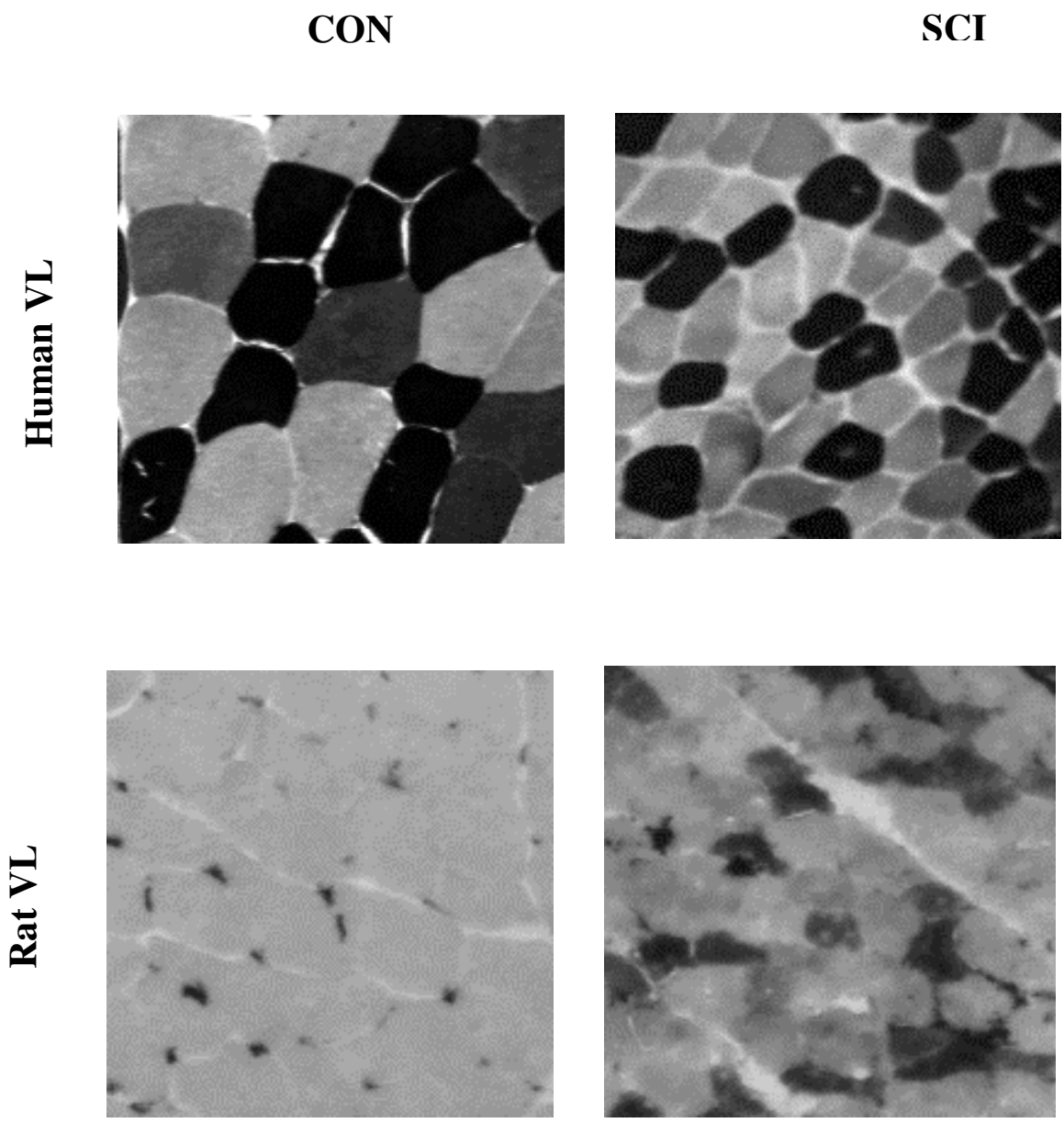
Figure 4.1:

hATPase images captured of human m. VL in control (upper left) and SCI (upper right) subjects, and rat VL in control (lower left) and SCI (lower right) animals.

Table 4.1

	FT	CSA	SDH	GPDH	qATPase	%CSA
	I	-	-	-	-	-
CON Rat	**IIa**	2599 ± 26	1944 ± 32^{+}	181.1 ± 4.7^{+}	26.1 ± 2.1	20.7 ± 1.9
	IIb/x	3446 ± 43	1567 ± 22^{+}	200.6 ± 2.7^{+}	31.3 ± 1.7	79.3 ± 3.0^{+}
	I	4765 ± 323	859 ± 83	37.3 ± 3.1	20.6 ± 2.9	34.6 ± 2.6
CON Human	**IIa**	6012 ± 512 $^{\#}$	720 ± 75	54.2 ± 3.4	35.8 ± 3.2 $^{\#}$	50.2 ± 3.9 $^{\#}$
	IIb/x	5111 ± 421 $^{\#}$	618 ± 60	62.9 ± 3.1	40.1 ± 3.8 $^{\#}$	15.2 ± 0.5
	I	-	-	-	-	-
SCI Rat	**IIa**	1254 ± 30	1608 ± 47^{++}	197.4 ± 3.9^{++}	27.9 ± 2.0	9.5 ± 0.6
	IIb/x	1833 ± 42	1141 ± 26^{++}	213.3 ± 5.4^{++}	33.4 ± 2.3	90.5 ± 1.1^{++}
	I	2545 ± 202	688 ± 67	45.9 ± 3.3	21.7 ± 3.1	39.7 ± 4.6
SCI Human	**IIa**	2633 ± 354$^{\#\#}$	525 ± 42	60.2 ± 3.7	36.2 ± 3.3$^{\#\#}$	33.4 ± 6.0$^{\#\#}$
	IIb/x	2569 ± 347$^{\#\#}$	428 ± 45	63.9 ± 2.9	39.7 ± 3.2$^{\#\#}$	26.9 ± 5.1

Figure 4.1



V. CONCLUSIONS

In testing the effectiveness of TRT in maintaining skeletal muscle mass after complete SCI, the hypothesis of an attenuation in muscle CSA relative to SCI was not disproved. However, the null hypothesis dealing with the effect of TRT on activities of enzymes representing pathways for oxidative energy supply was rejected. In the case of the second hypothesis, TRT resulted in an attenuation of the decline in SDH activity in the TTSCI animals. Thus, the benefits of TRT on rat skeletal muscle after complete SCI are more promising than originally thought.

In regards to the applicability of the results of studies on rat skeletal muscle after SCI to human subjects, the hypotheses that there would be greater overall loss in size and a subsequent increase and decrease in activities of SDH and GPDH, respectively, solely in the rat were disproved. The exaggerated atrophic response of human muscle, relative to the rat, and the similar tendency of a decrease in SDH and increase in GPDH activity in both species resulted in the rejection of these original hypotheses.

Given the described results, it is concluded that there is a beneficial effect of TRT on rat skeletal muscle protein expression after complete SCI. However, although these results are promising for the potential treatment of SCI, these results should be viewed with caution until further studies can be performed using human subjects.

BIBLIOGRAPHY

1. Phillips, W.T., Kiratli, B.J., Sarkarati, M., & Weraarchakul, G. Effects of Spinal Cord Injury on the Heart and Cardiovascular Fitness. *Current Problems in Cardiology* 1998; 23 (11): 644-717.
2. Wimalawansa, S.M., and S.J. Wimalawansa. Simulated weightlessness-induced attenuation of testosterone production may be responsible for bone loss. *Endocrine* 1999; 10(3): 253-260.
3. Basaria, S. & A.S. Dobs. Risk versus benefit of testosterone therapy in elderly men. *Drugs and Aging* 1999; 15 (2): 131-142.
4. Wimalawansa, S.M., M.T. Chapan, J.N. Wei, K.N. Westlund, M.J. Quast, and S.J. Wimalawansa. Reversal of weightlessness-induced musculoskeletal losses with androgens: quantification by MRI. *Journal of Applied Physiology* 1999; 86 (6): 1841-1846.
5. Alford, E.K., R.R. Roy, J.A. Hodgson, & V.R. Edgerton. Electromyography of rat soleus, medial gastrocnemius, and tibialis anterior during hind limb suspension. *Experimental Neurology* 1987; 96:635-649.
6. Alaimo, M.A., J.L. Smith, R.R. Roy, and V.R. Edgerton. EMG activity of slow and fast ankle extensors following spinal cord transection. *Journal of Applied Physiology* 1984; 56: 1608-1613.

7. Hopman, M.T.E., C. Dueck, M. Monroe, W.T. Phillips, & J.S. Skinner. Limits to maximal performance in individuals with spinal cord injury. International Journal of Sports Medicine 1998; 19: 98-103.
8. Ottenweller, J.E., M. Li, W. Giglio, R. Anesetti, L.M. Pogach, & H.F.S. Huang. Alteration of Follicle-Stimulating Hormone and Testosterone regulation of messenger Ribonucleic Acid for Sertoli Cell proteins in the rat during the acute phase of spinal cord injury. *Biology of Reproduction* 2000; 63: 730-735.
9. Gregory, C.M., K. Vandenborne, & G.A. Dudley. Metabolic enzymes and phenotypic expression among human locomotor muscles. *Muscle & Nerve* 2001; 24: 387-393.
10. Castro, M.J., D.F. Apple, R.S. Staron, G.E.R. Campos & G.A. Dudley. Influence of complete spinal cord injury on skeletal muscle within 6 mo. of injury. *Journal of Applied Physiology* 1999; 86 (1): 350-358.
11. Brooke MH & Kaiser KK. Three "Myosin Adenosine Triphosphatase" systems: The nature of their pH lability and sulfhydryl dependence. *Journal of Histochemistry & Cytochemistry* 1970; 18: 670-672.
12. Guth L & Samaha J. Qualitative differences between Actomyosin ATPase of slow and fast mammalian muscle. *Experimental Neurology* 1969; 25: 138-152.
13. Staron RS & Pette D. Correlation between myofibrillar ATPase activity and myosin heavy chain composition in rabbit muscle fibers. *Histochemistry* 1986; 86: 19-23.
14. Blanco CE, Sieck GC & Edgerton VR. Quantitative histochemical determination of succinic dehydrogenase activity in skeletal muscle fibers. *Histochemistry Journal* 1988; 20: 230-243.

15. Martin TP, Vailas AC, Durivage JB, Edgerton VR & Castleman KR. Quantitative histochemical determination of muscle enzymes: biochemical verification. *Journal of Histochemistry & Cytochemistry* 1985; 33: 1053-1059.

16. Grossman EJ, Roy RR, Talmadge RJ, Zhong H, & Edgerton VR. Effects of inactivity on myosin heavy chain composition and size of rat soleus fibers. *Muscle & Nerve* 1998; 21: 375-389.

17. Roy, R.R., K.M. Baldwin, & V.R. Edgerton. The plasticity of skeletal muscle: Effects of neuromuscular activity. *Exercise & Sport Sciences Reviews* 1991; 19:269-312.

18. Gordon, T., and M.C. Pattullo. Plasticity of muscle fiber types and motor unit types. *Exercise & Sport Sciences Reviews* 1990; 21: 331-362.

19. Tenover JS. Effects of testosterone supplementation in the aging male. Journal of *Clinical Endocrinology & Metabolism* 1992; 75: 1092-1098.

20. Bhasin S, Storer TW, Berman N, Callegari C, Clevenger BA, Phillips J, Bunnell T, Tricker R, Shirazi A & Casabari R. The effects of supraphysiological doses of testosterone on muscle size & strength in men. *New England Journal of Medicine* 1996; 335:1-7.

21. Bauman, W.A., A.M. Spungen, R.H. Adkins, & B.J. Kemp. Metabolic and Endocrine changes in persons with spinal cord injury. *Asst. Technologies* 1999; 11: 88-96.

22. Dudley GA, Castro MJ, Rogers S, Apple DF Jr. A simple means of increasing muscle size after spinal cord injury. *European Journal of Applied Physiology* 1999; 80:394-396.

23. Anderson, J.L., Mohr, T., Biering-Sorensen, F., Galbo, H., & Kjær, M. Myosin heavy chain isoform transformation in single fibres from *m.* vastus lateralis in spinal cord injured individuals: Effects of long-term functional electrical stimulation (FES). *Pflugers Archives* 1996; 431:513-518.

24. Armstrong, R.B. and Phelps, R.O. Muscle fiber type composition of the rat hindlimb. *The American Journal of Anatomy* 1984; 171: 259-272.

25. Baldwin, K.M. Research in the exercise sciences: Where do we go from here? *Journal of Applied Physiology* 2000; 1:332-336.

26. Barany, M. ATPase activity of myosin correlated with speed of muscle shortening. *Journal of Physiology* 1967; 50: 197-216.

27. Bauman, W.A., Adkins, R.H., Spungen, R.M. and Waters, R.L. The effect of residual neurological deficit on oral glucose tolerance in persons with chronic spinal cord injury. *Spinal Cord* 1999; 37:765-771.

28. Blanco, C.E. & Sieck, G.C. Quantitative determination of calcium activated myosin adenosine triphosphatase activity in rat skeletal muscle fibers. *Histochemistry Journal* 1992; 24: 431-44.

29. Castro, M.J., Apple, D.F. Jr, Melton-Rogers, S., Dudley, G.A. Muscle fiber type-specific myofibrillar Ca (2+) ATPase activity after spinal cord injury. *Muscle & Nerve* 2000; 23(1): 119-21.

30. Gerrits, H.L., De Haan, A., Hopman, M.T., van Der Woude, L.H., Jones, D.A. & Sargeant, A.J. Contractile properties of the quadriceps muscle in individuals with spinal cord injury. *Muscle Nerve* 1999; 22(9): 1249-56.

31. Holloszy, J.O. Exercise, health, and aging: a need for more information. *Medicine and Science in Sports & Exercise* 1983; 15(1): 1-5.

32. Kent-Braun, J.A., Ng, A.V., Castro, M., Weiner, M.W., Gelinas, D., Dudley, G.A. & Miller, R.G. Strength, skeletal muscle composition, and enzyme activity in multiple sclerosis. *Journal of Applied Physiology* 1997; 83(6): 1998-2004.

33. Martin, T.P., R.B. Stein, P.H. Hoeppner, & D.C. Reid. Influence of electrical stimulation on the morphological and metabolic properties of paralyzed muscle. *Journal of Applied Physiology* 1992; 72:1401-1406.

34. Massie BM, Simonini A, Sahgal P, Wells L, Dudley GA. Relation of systemic and local muscle exercise capacity to skeletal muscle characteristics in men with congestive heart failure. *Journal of American College of Cardiology* 1996; 27(1): 140-5.

35. Pette, D., Staron, R.S. Myosin isoforms, muscle fiber types, and transitions. *Microsc Research Technology* 2000; 50(6): 500-9.

36. Rivero, J.L., Talmadge, R.J. & Edgerton, V.R. Fiber size and metabolic properties of myosin heavy chain based fiber types in rat skeletal muscle. *Journal of Muscle Research and Cell Motility* 1998; 19: 733-742.

37. Rochester, L., Barron, M.J., Chandler, C.S., Sutton, R.A., Miller, S. & Johnson, M.A. Influence of electrical stimulation of the tibialis anterior muscle in paraplegic subjects; morphological and histochemical properties. *Paraplegia* 1995; 33:514-522.

38. Shields, R.K., Law, L.F., Reiling, B., Sass, K. & Wilwert, J. Effects of electrically induced fatigue on the twitch and tetanus of paralyzed soleus muscle in humans. *Journal of Applied Physiology* 1997; May; 82(5): 1499-507.

39. Talmadge, R.J., Castro, M.J., Apple, D.F. Jr. & Dudley, G.A. Phenotypic adaptations in human muscle fibers 6 and 24 wk after spinal cord injury. *Journal of Applied Physiology* 2002; 92(1): 147-54.

40. Tesch, P.A., Wright, J.E., Vogel, J.A., Sharp, D.S. & Sjodin, B. The influence of muscle metabolic characteristics on physical performance. *European Journal of Applied Physiology* 1985; 54: 237-245.

41. Van Der Laarse, W.J., Lannergren, J. & Diegenbach, P.C. Resistance to fatigue of single muscle fibers from Xenopus related to succinate dehydrogenase and myofibrillar ATPase activities. *Histochemistry Journal* 1991; 16: 529-541.

42. Haskell, W.L. Health consequences of physical activity: understanding and challenges regarding dose-response. *Med. Sci. Sports Exerc*. 1994; 26(6): 649-660.

43. Blair, S.N., H.W. Kohl III, C.E. Barlow, R.S. Paffenberger, L.W. Gibbons, & C.A. Macera. Changes in physical activity and all-cause mortality: A prospective study of healthy and unhealthy men. *J.A.M.A.* 1995; 273(14): 1093-1098.

44. Thomason, D.B., and F.W. Booth. Atrophy of the soleus muscle by hindlimb unweighting. *J. Appl. Physiol* 1996*;* 68:1-12.

45. Grimby L., A. Tollback, U. Muller, L. Larsson. Fatigue of chronically overused motor units in prior polio patients. *Muscle Nerve* 1996; 19: 728-737.

46. Hather B.M., G.R. Adams, P.A. Tesch, & G.A. Dudley. Skeletal muscle responses to lower limb suspension in humans. *J. Appl. Physiol* 1992*;* 72(4): 1493-1498.

47. Hooker S.P., and C.L. Wells. Effects of low- and moderate-intensity training in spinal cord-injured persons. *Med. Sci. Sports Exerc* 1989; 21(10: 18-22.

48. Morley J.E. Testosterone replacement and the physiologic aspects of aging in men. *Mayo Clin Proc* 2000*;* 75: S83-S87.

49. Levine, S., Gregory, C.M., Nguyen, T., Shrager, J.B., Kaiser, L.R., Rubinstein, N.A., and Dudley, G.A.. Bioenergetic adaptation of individual human diaphragmatic myofibers to severe chronic obstructive pulmonary disease. *Journal of Applied Physiology* 2002; 92(3): 1205-13.

APPENDIX

ABSTRACT: AMERICAN COLLEGE OF SPORTS MEDICINE ANNUAL MEETING, 2002 (ST. LOUIS, MO.)

Gregory C.M., K. Vandenborne and G.A. Dudley. Effects of testosterone replacement therapy on rat skeletal muscle after spinal cord injury.

Spinal cord injury (SCI) is associated with increased risks for development of several diseases that are probably linked to the extreme atrophy of skeletal muscle. If testosterone replacement therapy (TRT) alone or as an adjunct to other therapies could attenuate the atrophy, these risks might be reduced. **PURPOSE:** To examine the effects of TRT on rat skeletal muscle after SCI. **METHODS:** The soleus (SOL) was taken from twelve young male Charles River rats 11 weeks after complete SCI (T-9 transection, n = 8) or sham surgery (n =4). Rats received either testosterone (two 5 cm testosterone capsules, n = 4), or empty capsule implantation (n = 8) at the time of surgery. Muscle samples were sectioned analyzed qualitatively for myosin ATPase and quantitatively for succinate dehydrogenase (SDH) and α-glycerol-phosphate dehydrogenase (GPDH) activities using standard techniques. **RESULTS:** SCI decreased ($P < 0.05$) average fiber size 49 ± 4 % and the percentage of slow fibers from 93 ± 3 to 17 ± 2 %. These responses were accompanied by a decrease in SDH and an increase in GPDH activities. TRT after SCI attenuated the fiber atrophy, the slow to fast fiber conversion as well as the changes in SDH and GPDH activity. Average fiber size was decreased by only 30 % in TRT SCI animals and their soleus had 39 ± 2 % slow fibers. **CONCLUSIONS:** TRT was effective in attenuating alterations in myofibrillar proteins during 11 weeks of SCI.

Supported by NIH HD-33738

ABSTRACT: TEXAS CHAPTER, AMERICAN COLLEGE OF SPORTS MEDICINE ANNUAL MEETING, 2002 (GEORGETOWN, TX.)

Gregory C.M., K. Vandenborne, H.F.S. Huang, J.E. Ottenweller, G.S. Morris & G.A. Dudley. Effects of testosterone replacement therapy on hind-limb and forelimb muscles after spinal cord injury.

Spinal cord injury (SCI) is associated with increased risk for developing several diseases, including diabetes, that are possibly linked to the extreme atrophy of skeletal muscles. It is possible that the risk for developing these secondary pathologies could be reduced by attenuating this muscle atrophy. Because testosterone replacement therapy (TRT) can attenuate muscle atrophy, treatment with TRT might reduce these risks following SCI. **PURPOSE**: Because the impact of TRT on muscle atrophy in SCI animals has not been investigated, the purpose of this study was to determine if TRT could positively impact skeletal muscle after SCI. **METHODS:** Three hind-limb muscles, the gastrocnemius (GA), vastus lateralis (VL), and tibialis anterior (TA), and one fore-limb muscle, the triceps brachii (TRI), were taken from twelve young male Charles River rats 11 weeks after complete SCI (T-9 laminectomy, n = 8) or sham surgery (n =4). Rats received either testosterone (2 x 5 cm testosterone capsules, n = 4), or empty capsule implantation (n = 8) at the time of surgery. Muscles were collected, sectioned and fibers analyzed qualitatively for myosin ATPase and quantitatively for succinate dehydrogenase (SDH) and α-glycerol-phosphate dehydrogenase (GPDH) activities using standard histochemical techniques. **RESULTS:** SCI reduced the average fiber size**,** the percentage of slow fibers, and SDH activity in the hindlimb muscles while increasing the GPDH activity. The TRI demonstrated an increase in fiber size following

SCI with no change in fiber type percentage or in the activities of SDH or GPDH. TRT after SCI attenuated the fiber atrophy, the slow to fast fiber conversion as well as the changes in SDH and GPDH activities in the three hind-limb muscles. Interestingly, there was no effect of TRT on the TRI relative to SCI alone. **CONCLUSIONS:** TRT was effective in attenuating SCI induced changes in myofibrillar and metabolic proteins, thus potentially reducing the likelihood of some of the secondary pathologies associated with this type of injury.

Supported by NIH HD-33738