Adaptations in Myosin Heavy Chain Profile in Chronically Unloaded Muscles

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Abstract

In this review, myosin heavy chain (MHC) adaptations in response to several models of decreased neuromuscular activity (i.e. electrical activation and loading of a muscle) are evaluated. In each of these "reduced-activity" models it is important to: a) quantify the changes in electrical activation of the muscle as a result of the intervention; b) quantify the forces generated by the muscle; and c) determine whether the neuromuscular junction remains normal. Most of the models, including spaceflight, hindlimb suspension, spinal cord isolation, spinal cord transection, denervation, and limb immobilization in a shortened position, result in increases in the percentage of fast MHCs (or fast MHC mRNA) in normally slow rat muscles. It also can be inferred from histochemical data that increases in fast MHCs occur with TTX application and bed rest. The only "reduced-activity" model to consistently increase slow muscle myosin mRNA, and slow fibers is limb immobilization in a stretched position; however, this model results in at least a temporary increase in tension. It appears that the most common feature of these models that might induce MHC adaptations is the modification in loading rather than a change in the neuromuscular activity.

Key words: bed rest, denervation, fiber types, hindlimb suspension, limb immobilization, myosin heavy chain isoforms, spaceflight, spinal cord isolation, spinal cord transection.

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The present paper addresses issues related to the adaptive features of MHCs and some related parameters in response to reduced neuromuscular activity. This perturbation is commonly viewed as being synonymous with "disuse". It is becoming increasingly apparent, however, that this perception of activity-dependent induction of muscle adaptation is limited with respect to understanding the specific physiological events which play a primary role in the regulation of neuromuscular adaptation. Commonly used models in which the neuromuscular system is intact and the amount of neuromuscular activity (activation and loading) is presumed to be reduced or even eliminated are bed rest, immobilization of a limb, spaceflight, and hindlimb suspension. Other models of reduced activity in which some surgical or pharmacological means is used to reduce neuromuscular function include ST, SI, denervation and the application of agents, such as TTX, which block neural transmission.

Clearly, skeletal muscle fibers are responsive to activity-related events, such as the presentation of action potentials to the muscle fibers [72, 100, 102, 113, 119, 134]. The loading characteristics associated with activation also appear to be an important factor in inducing adaptive events in skeletal muscle fibers. It also appears that the level and pattern of loading is an important factor in maintaining the expression of the original MHC composition of a fiber. Further, it remains unclear why individual muscle fibers respond differently to seemingly similar stimuli. Physiological variables other than load may also be important signals in the maintenance and induction of the expression of a specific MHC or the synthesis of additional amounts of the same MHC. Other factors which also seem to play critical roles in the control of MHC expression by skeletal muscles are hormonal growth factors, both humoral and localized inducers.

Even though it is clear that the load experienced by a muscle, the action potentials that reach a muscle fiber and the hormonal growth factor milieu of the fiber at any given time play major roles in the regulation of muscle fiber properties including MHCs, none of these factors have been quantified with respect to the amount of these stimuli required to induce a given response. Unfortunately, these factors are usually assumed to be known without any apparent reason or justification.

This review will first address the different "reduced activity" models in the context of how the modulation of MHC may be related to neuromuscular activation and loading. Secondly, the modulation of MHC expression will be related to myofibrillar and myosin ATPase activities, fiber type proportions, and to how these characteristics are linked to contractile properties. We will not address directly the adaptive responses of maximal tension, atrophy, fiber size, and fatigue. For reviews concerned with the effects of "disuse" or unloading on these properties we refer the reader to Roy et al. [113], Thomason and Booth [140] and Salmons and Vrbova [120]. Also, this review will not cover adaptations of skeletal muscle in response to increased electrical activation [72, 100, 102, 113, 119, 134].

Models of reduced neuromuscular activity

The physiological events that initiate neuromuscular adaptations in experimental models of altered use are largely undefined. For example, although bed rest, ST, limb immobilization, spaceflight and hindlimb suspension are often presumed to be models of "disuse", they are actually models of normal or decreased use (Table 1) [1, 2, 9, 43, 99]. Further, the normal level of activity for one muscle fiber can differ markedly from another fiber within the same muscle. A reduction in activity of a given magnitude may have a more dramatic effect on a normally highly active muscle fiber than on a fiber that has far less activity. In any case, for most of the physiologically intact models noted above, the amount of neuromuscular activity is not well defined.

Hindlimb suspension

There appear to be contradictory findings regarding the changes in neuromuscular activity imposed by hindlimb suspension [2, 9, 115]. Throughout a 28-day suspension period, our chronic EMG recording data suggest that the neuromuscular activation levels increase about 3-fold in an ankle flexor muscle (tibialis anterior) and markedly decrease immediately after suspension in both a slow (soleus) and fast (medial gastrocnemius) ankle extensor.

Table 1. Relative levels of neuromuscular factors associated with models of reduced activity.

Model	Muscle Activation	Muscle Force	Axoplasmic Transport	NMJ[2] Presence
Immobilization (Shortened)	+	+[1]	+++	+++
Immobilization (Neutral)	++	++[1]	+++	+++
Immobilization (Lengthened)	+++	+++++[1]	+++	+++
Bed Rest	+++	+	+++	+++
Spaceflight	+++	+	+++	+++
Hindlimb Suspension (Short-term)	+	-	+++	+++
Hindlimb Suspension (Long-term)	+++	+	+++	+++
TTX	-	-	+++	+++
Spinal Cord Transection	+	+	+++	+++
Spinal Cord Isolation	-	-	+++?	+++
Denervation	-	-	-	-
Control	+++	+++	+++	+++

[1] The muscle force changes with the immobilization models are presumed to be transient (see text).
[2] NMJ, neuromuscular junction.
The number of + or - denotes the relative change in magnitude for that parameter from +++ for control.

However, there was a progressive recovery to a control level within two weeks of continuous suspension [2]. These results demonstrate that hindlimb suspension is not a simple model of "disuse" or inactivity. On the contrary, hindlimb suspension results in a continuous process of adaptation in neuromuscular activity which could easily contribute to the magnitude and types of adaptations that occur in response to suspension [140]. Hindlimb suspension seems to be a reasonable model of hyperactivity for flexor muscles and a temporary depressor of activity in extensor muscles. There continues to be a complete void of information on the modulation of muscle loading in response to suspension. It seems reasonable to assume that the peak and mean forces generated by the extensor muscles are reduced by suspension in spite of the persistence of EMG activity after two weeks of continuous suspension. Although it would be extremely difficult to measure forces from single fibers *in vivo*, it is now possible to measure accurately the forces generated within selected tendons *in vivo* [50, 61, 151].

Spaceflight

One could predict markedly reduced muscle activation force levels in spaceflight. To date these types of data have not been reported, however. It is also important to note that conditions under which the muscles operate during spaceflight can vary considerably depending on the species. For example, with currently used spacecraft humans are free to move about so that the mobility in the various joints are largely sustained. In contrast, the movement of chaired Rhesus monkeys during flight are more restricted such that they can move their head, arms and feet, but there is relatively little mobility permitted in the hip and knees [10]. From this standpoint, Rhesus monkeys, as have flown in biosatellites, represent still another variation of a model of altered neuromuscular activity. We have found that the amount of EMG activity in the soleus and medial gastrocnemius of a Rhesus monkey during one 20 minute period when the animal was performing a motor task with the foot during flight exceeded the total amount of EMG activity recorded during a 24-hour period at 1 *g* (Hodgson JA, Aragon J, Roy RR, Day KM, Koslovskaya IB, Edgerton VR: Changing recruitment of slow and fast plantarflexors in the Rhesus during spaceflight, 1994 submitted). In addition, there appeared to be a reversal of the bias in recruitment for the soleus and medial gastrocnemius during the performance of the foot task at 0 *g* (medial gastrocnemius greater than the soleus) compared to 1*g* (soleus greater than the medial gastrocnemius) [62; Hodgson JA, Aragon J, Roy RR, Day KM, Koslovskaya IB, Edgerton VR: Changing recruitment of slow and fast plantarflexors in the Rhesus during spaceflight, 1994 submitted). Although most of the physiological and biochemical data collected during spaceflight have been from rats, no chronic muscle activity patterns during flight have been reported for rats.

Limb immobilization

The effects of limb immobilization on neuromuscular activation depend on the position of the muscles being immobilized, i.e. fixed at a lengthened, neutral or shortened position. Analyses of 24-hour recordings of integrated EMG performed 28 days after immobilization in the rat reveal that compared to pre-immobilization: 1) immobilization in a shortened position results in a 77 and 55% decrease in activity for the soleus and medial gastrocnemius, respectively; 2) immobilization in a neutral position results in a 50% decrease in soleus activity and no change for the medial gastrocnemius; and 3) immobilization in a lengthened position results in no change for either the soleus or medial gastrocnemius [43]. The EMG of the tibialis anterior of rabbits immobilized in a lengthened position for 6 weeks also revealed no significant change from control (pre-immobilized recordings) [99]. These studies demonstrate the effect of a chronically imposed length on a muscle, as well as, joint immobilization.

Fixation of the limb would also appear to be a simple technique for the manipulation of the load on a muscle in which a high, moderate, or low force is statically imposed when immobilized in a lengthened, neutral or shortened position. However, the actual forces imposed or removed under these conditions remain undefined. The force that is imposed initially may not persist, however, because the number of sarcomeres arranged in series in the chronically "stretched" muscle is increased and in the chronically "shortened" muscle is decreased [125]. Thus, within a matter of days the forces chronically imposed on the immobilized muscles may be normal. It is quite possible that the critical variable imposed with this model is the elimination of the changes in length and perhaps the dynamic (concentric and eccentric) forces on the immobilized muscles. For example, the largest forces recorded during dynamic testing either *in situ*, i.e. during the determination of the force-velocity properties of a muscle [154], or *in vivo*, i.e. during locomotion [50, 61, 151], occur when the activated muscle is being lengthened. When the muscle is immobilized in a shortened position it is unable to generate normal dynamic forces and will atrophy more rapidly if it is active than if it is inactive [150].

Spinal cord transection

A significant decrease in the amount of EMG activity in muscles innervated below the level of the lesion occurs after complete low thoracic transection of the spinal cord [1]. The relative EMG activity was decreased ~75% of control in a fast (lateral gastrocnemius) and a slow (soleus) ankle extensor in young adult cats spinalized at two weeks of age and maintained for 6 months. The absolute decrease in activity was much greater in the slow than the fast extensor. In fact, the EMG activity remained higher in the slow extensor of the spinal animal compared to the control (pre-spinalization) level for the fast extensor. Although the evidence is not complete, most measures of activity that reflect a relative activation level for motor pools suggest

that recruitment order may be similar before and after spinalization. For example, the soleus is more readily recruited than the medial gastrocnemius in control animals and in spinal animals after they have been trained to step on a treadmill [82]. In spite of the considerable level of EMG activity remaining in chronic spinal cats, the level of spontaneous weight-supporting function is almost absent after spinalization due principally to an inability to maintain balance in the hindquarters. However, the tendon forces of the medial gastrocnemius and soleus muscles in the chronic spinal cat stepping on a treadmill are similar to control cats stepping under similar conditions [82]. Thus, it appears that the primary factors imposed by chronic spinalization are a reduction in neuromuscular activity and a more substantial decrease in forces generated due to the absence of weight-bearing function, particularly when the animals are not trained to step. The motoneurons below the level of the transection and their muscle units have remarkably normal neurophysiological, morphological and metabolic properties [23, 84, 93].

Spinal cord isolation

A more extensive reduction in the activity of the neural networks that regulate the execution of motor tasks such as stepping and standing occurs after SI. This model was first described by Tower [144] and consists of complete spinal cord transections at low thoracic and high sacral levels and complete bilateral deafferentation between the transection sites. This surgery induces complete flaccidity of the musculature of the hindlimbs, and a virtual absence of activity in all associated motoneurons as measured by EMG activity [105, 127]. The animals can be sustained for months if their health is maintained properly [38, 39, 118]. This preparation is very important because it is the only model of reduced activity that, for all practical purposes, can result in a known level of neuromuscular activity, i.e., "electrical silence" or zero activity, for months. It is critically different from denervation in that the connections between the motoneuron and the muscle fibers remain physically intact. In fact, the distribution of muscle fibers within motor units in the tibialis anterior of the cat after 6 months of SI are similar to control, suggesting that there has been no reorganization of nerve-muscle connections following chronic inactivity [105]. SI also differs from complete inactivation by TTX administration, because with SI the motoneurons receive no peripheral afferent, supraspinal, or infraspinal input.

Denervation

Denervation continues to be used occasionally and inappropriately as a model of neuromuscular "disuse" [15, 37, 157]. Although neural influences on skeletal muscle fibers cannot be accounted for by activity alone [16, 39], denervation provides little or no insight into neuromuscular induced plasticity that is activity dependent. One of the clearest and most dramatic demonstrations of a neural influence that is not activity dependent is the marked loss of muscle fiber mass in fast flexor muscles in response to denervation [74, 94], while SI results in little or no atrophy of fast flexors after up to six months [105]. Denervation also results in a series of events characterized by dedifferentiation of the neuromuscular unit [121] and the reappearance of several molecules which are normally present only during development, including: 1) extrajunctional acetylcholine receptors [139]; 2) the gamma subunit of the acetylcholine receptor [52, 53]; 3) TTX-resistant action potentials [59, 139]; 4) apamin-sensitive Ca^{2+} activated K^{+} channels [122]; 5) the neural cell adhesion molecule and its mRNA [24, 25]; 6) the Thy-1 glycoprotein at the sarcolemma [11]; and 7) the myogenic regulatory proteins [15, 37]. Thus, denervation is clearly different from other models of reduced neuromuscular activation and loading, most likely due to the absence of a nerve-muscle connection (Table 1).

Tetrodotoxin

The chronic application of TTX to a peripheral nerve blocks neural conductance in the efferent axons distal to the block while allowing the motoneurons proximal to the block to continue to generate action potentials and receive synaptic input as occurs normally. On the other hand, TTX eliminates the action potential induced transmittance of all proteins and other molecules at the neuromuscular synapse, including acetylcholine. In this model, the sensory end organs such as the spindles, tendon organs and sensory endings in the joints and skin that are distal to the block can be activated and generate action potentials, but these action potentials will be conducted only up to the site of the TTX block. The motoneurons would most likely receive less synaptic input from the muscles that have been blocked, although each motoneuron receives thousands of synapses, most of which originate from sources other than its own muscle, i.e. heteronomous. Therefore, this loss of synaptic input may be insignificant. Thus, the persistence of a significant level of synaptic input could be important, if the non-activity mechanisms of influencing muscle are dependent on the persistence of synaptic input to the dendrites and soma of the motoneuron. These factors theoretically could become important if they influence any mechanism that the motoneuron has to regulate muscle proteins via non-activity dependent means.

In the chronic administration of a nerve block such as TTX, it is important to know whether or not the nerve escapes the block at any time. Further, if a nerve cuff is used to administer the drug, one must demonstrate that the cuff has not caused any trauma to the nerve. If some trauma does occur the effects of denervation should be considered in the interpretation of the results. Another complicating factor in comparing results across different laboratories using slightly different techniques may be in the nerve selected to block. For example, a more extensive change in the normal oscillations of muscle length may occur if the entire sciatic nerve is anesthetized than if only a small muscle nerve, such as the soleus nerve, is blocked.

Changes in myosin heavy chain and related properties in response to reductions in neuromuscular activity

Hindlimb suspension

A large number of studies using a variety of histochemical, immunohistochemical and biochemical techniques have clearly demonstrated that hindlimb suspension is accompanied by a progressive increase in the percentage of fast fibers in predominantly slow muscles (e.g., soleus, adductor longus, vastus intermedius) whereas there appears to be less of a change in fiber type composition of predominantly fast extensor (e.g., medial gastrocnemius, plantaris, vastus lateralis) or flexor (tibialis anterior, extensor digitorum longus) muscles [32, 33, 113, 140]. The shift towards faster MHC isoforms may involve all three known fast isoforms present in rodent muscle, with *de novo* expression of some of the MHCs. Campione et al. [20] reported a marked increase in the relative percentage of Type IIa-IIx MHC in the soleus after 21 days of hindlimb suspension: relative Type I and IIa-IIx MHC was 99 and 1% for control and 67 and 33% for suspended rats. Oishi et al. [97], using SDS PAGE analyses, showed the following myosin composition for the soleus of control and 4-week suspended rats: 87:13:0 vs 76:17:7 for Type I, IIa and IId MHC isoforms, respectively. Type IId MHC was also observed in the soleus after 21 and 28 days of suspension by Takahashi et al. [132]. Preliminary data from our laboratory using immunohistochemical techniques show an increased expression of type IIa and IIx MHCs in the rat soleus after 14 days of suspension (Talmadge, Roy and Edgerton, unpublished observations, 1994). We observed similar results in the rostral portion of the adductor longus muscle which is normally comprised of only Type I and IIa fibers and showed an increased percentage of fibers expressing IIa, and de novo expression of IIx and even IIb MHCs following 10 days of suspension of hypophysectomized rats (Talmadge, Roy, Grindeland, Grossman and Edgerton, unpublished observations, 1994). There was a concomitant decrease in the percentage of fibers expressing only Type I MHCs.

Hindlimb suspension is accompanied also by a progressive decrease in the concentrations of myofibrillar and myosin protein in the soleus muscle [141, 142, 143, 145, 146, 147]. There is a relative shift in myosin isoform expression towards faster forms [140, 142, 143, 146] and an increase in the fast MHC composition [107]. For example, a 10 [143], 12 [29] and 33% [143] decrease in relative native slow myosin content in the rat soleus has been reported after 14, 18 and 56 days of suspension. The presence of minimal "degeneration-regeneration" changes in the muscle cross-section [138] and the maintenance of fiber number [137] indicate a transformation of fibers from slow to fast rather than de novo formation of new fast fibers. Immunohistochemical data demonstrate that only selected fibers expressed fast MHC following suspension that did not before suspension. Therefore, the increase in fast myosin is likely quite significant in a select population of soleus muscle fibers. There is a marked remodelling of the soleus muscle consisting of an up and a down regulation of fast and slow myosin isoforms, respectively, with some of the fibers that expressed only the slow isoforms before suspension expressing fast isoforms after suspension.

Although the results are quite variable, it appears that myofibrillar ATPase activities are elevated in slow muscles and in some slow fibers following suspension [29, 142, 143]. For example, at the single fiber level, McDonald and Fitts [89] have reported a 55, 63 and 279% increase in mean ATPase activity after 1, 2 and 3 weeks of suspension in the rat soleus, although some fibers seemed to have been unaffected. At the whole muscle level, Diffee et al. [29] showed only a 22% increase in myosin ATPase activity in the soleus after 28 days of suspension. Thomason et al. [143] reported no change in ATPase activity in the soleus after 56 days of suspension, although these authors suggested that the "fast" myosin in the suspended rats may have been an embryonic or intermediate form with lower specific ATPase activities than adult fast myosin. Adaptations in ATPase activities of fast muscles or fast muscle fibers in response to suspension have been minimal [29, 45]. Other contractile-related proteins adapt to hindlimb suspension. For example, myosin light chain isoform composition shift to faster forms in soleus muscle homogenates and single fibers following suspension [42, 45, 54, 107, 143]. In addition, fast isoforms of troponin-T and -I which are not observed in control rat soleus muscles were expressed after 21 days of suspension [20]. These adaptations are not observed or are markedly less in predominantly fast muscles [29, 54].

In general, the adaptation in the mRNA for a protein is consistent with the adaptation in the protein following hindlimb suspension. However some exceptions have been observed: 1) Thomason et al. [141] reported a decrease in Type I MHC protein and no change in the mRNA in soleus after 7 days of suspension; and 2) Diffee et al. [29] reported an increase in Type IIa MHC with no change in the corresponding mRNA and an increase in Type IIb mRNA with no change in the corresponding MHC in the soleus and vastus intermedius of suspended rats after 14 days of suspension. Although these authors indicate that some of these discrepancies could be due to their inability to distinguish between the Type IIa and IIx MHC pools at that time, this explanation could not account for the discrepancy found between the Type I message and protein. Diffee et al. [30] suggested that these differential responses reflect either some translational or posttranslational regulatory mechanism or simply a temporal phenomenon.

In general, hindlimb suspension results in the predominantly slow soleus becoming "faster", whereas the mixed fast muscles are relatively unaffected [32, 33, 113, 140]. In the soleus, the isometric twitch contraction and half-relaxation times are consistently shorter and the tension response to various stimulation frequencies is shifted towards that observed in "faster" muscles following suspen-

sion. These changes most likely reflect adaptations in the sarcoplasmic reticulum calcium kinetics (see [73] for a discussion of possible mechanisms). In contrast, the extent and the consistency of the change in either the maximum rate of shortening or the velocity of unloaded contractions of the soleus are unclear. The maximum rate of shortening of the soleus under *in situ* conditions has been reported to increase by ~40% [42] after one week and by 125% [42] after 2 weeks or to not change significantly (a 4-10% increase, [59, 103, 154]) or increase by ~35% [29] up to 4 weeks following suspension. A large increase in the shortening velocity would not be expected based on the reported minor changes (0-22%) in whole muscle myosin ATPase activity [29, 142, 143], a measure highly correlated with the maximum rate of shortening [7, 117]. The mean velocity of unloaded contraction in soleus single fibers increases progressively following suspension. For example, McDonald and Fitts [89] report a 46, 51 and 123% increase in the velocity of unloaded contraction after 1, 2 and 3 weeks of suspension and these increases in shortening velocities were highly correlated with the fiber ATPase activities. Other reports have shown a much smaller increase in mean fiber maximum rate of shortening following 14 (29% [45]) and 28 (31% [107]) days of suspension. Despite the variability in the magnitude of adaptation, it is clear that at least some fibers are becoming faster following suspension.

Studies using hindlimb suspension in conjunction with *in vivo* contractile stimulation against controlled loads have provided unique insights into the role of loading in regulating muscle properties and protein expression. For example, as few as 24 loaded contractions every 4 days were sufficient to cause 13 to 18% increases in muscle mass of rat ankle plantarflexors, provided that the muscles contracted against a substantial load [156]. Eccentric exercise, 24 contractions every 4 days, was effective in attenuating 80% of the soleus atrophy associated with hindlimb suspension [76]. Using a similar stimulation protocol Diffee et al. [31] demonstrated that 40 isometric contractions performed against a load equivalent to 90-95% of maximal tetanic force, performed every other day could attenuate the increase in type IIa MHC observed with hindlimb suspension. Interestingly, the same stimulation protocol (i.e., the same number of stimulated contractions) performed under isovelocity conditions (i.e. producing lower loads) did not attenuate the increase in type IIa MHC associated with suspension. Thus, load as opposed to electrical activation appears to play a primary role in regulating the MHC isoform composition of a muscle. These data taken collectively point to a strong role for loading as a mediator for neuromuscular activity induced muscle adaptation.

In summary, these data clearly show that the reduced load associated with hindlimb suspension results in a muscle with increased proportions of fast (type II) MHCs. This increase in fast MHCs is more apparent in the slow "anti-gravity" muscles normally used to maintain the body in an upright posture, i.e. the soleus, vastus intermedius, and adductor longus of the hindlimb.

Lower limb suspension

Lower limb suspension in humans is a relatively new model of unloading that induces a significant atrophy and a loss of strength within 4 weeks. However, there was no evidence that the types of MHC expressed in the vastus lateralis are affected by this unloading procedure. Based on the categorization of fibers according to myofibrillar ATPase staining [8], the percentage of type I, IIa, and IIb fibers in biopsies from the vastus lateralis were unchanged as a result of the chronic suspension of one leg.

Bed rest

Although there have been a number of studies using bed rest as a model of reduced activity in humans, there have been no direct measures of adaptations in the types of MHCs in skeletal muscles under these conditions. Most of the focus in these studies has been on muscle cross-sectional area, volume, and strength measures. Some indirect measures of the types of MHC expressed in muscle have been made using myofibrillar ATPase histochemistry. For example, after 30 days of bed rest (6° head-down tilt), the percentage of fibers categorized as slow (type I) was 57% compared to a pre-bed rest value of 66% in the vastus lateralis, and 75% compared to 81% in the soleus [60]. Neither of these differences were significant, but the values in both muscles changed in the expected direction, i.e., assuming that unloading enabled some fibers that expressed only type I myosin to express type II myosin.

Spaceflight

The response of a muscle to microgravity appears to be related, in part, to the function of the muscle and the fiber type composition. In general, predominantly slow muscles, such as the soleus, adductor longus and vastus intermedius, show a greater response to microgravity than predominantly fast muscles such as the medial gastrocnemius, tibialis anterior and extensor digitorum longus. In a 7-day spaceflight (Space-lab 3), Martin et al. [85] found a significant increase in the percentage of fast fibers in the adductor longus (20% to 46%) and the soleus (39% to 50%) of young (50 days old, mean body weight 248 g) male rats as determined by myofibrillar ATPase staining. An increase (76% to 84%) in fast fibers was also observed in the deep, but not the superficial region of the medial gastrocnemius. Further, no changes were observed in predominantly fast extensor (plantaris) or flexor (tibialis anterior or extensor digitorum longus) muscles. Interestingly, Riley et al. [108] found no fiber type changes in the soleus of older male rats (85 days old, mean body weight of 382 g) flown aboard this same mission. An increase in the percentage of fast fibers in the soleus (11% to 21%) was also found by Desplanches et al. [28] after a 7-day spaceflight aboard a Russian biosatellite (Cosmos 1667). After only 4 days of flight, Jiang et al. [68], observed an increase in the percentage of fibers expressing both fast and slow

MHCs in the soleus. Thus, changes in the expression of MHC genes appear to occur relatively soon after exposure to microgravity with the primary changes being a down-regulation in slow MHC and an up-regulation in fast MHC.

In spaceflights of 12 to 14 days duration similar changes in MHC expression have been observed. Using antibodies which recognize the slow (type I) and fast (all type II) MHCs, Miu et al. [92] found an 18% decrease in the number of fibers expressing only the slow MHC and a 23% increase in the number of fibers that co-expressed slow and fast MHCs in the soleus after a 12.5 day flight (Cosmos 1887). No fiber type changes were found in the medial gastrocnemius. In the same flight using non-denaturing gel electrophoresis, Baldwin et al. [4] found an 18% decrease in the relative content of the slow myosin isoform (SM) and a 27% increase in the three fast isoforms (FM1, FM2 and FM3) of the vastus intermedius. No changes were observed in the relative amounts of slow and fast isoforms in the predominantly fast vastus lateralis. After 14 days of spaceflight (Cosmos 2044), the percentage of fibers expressing both fast and slow MHCs increased from < 1% to 14% in the soleus [96] and increased ~4-fold in the medial gastrocnemius [70]. Based on myofibrillar ATPase staining after acid and alkaline preincubations, Riley et al. [109] reported a 20% decrease in the number of slow fibers and a corresponding increase in the number of intermediate (perhaps slow/fast) fibers in the predominantly slow adductor longus, and no changes in the predominantly fast extensor digitorum longus or plantaris muscles in Cosmos 1887 rats. Similar findings were subsequently found after a 14-day Russian biosatellite flight (Cosmos 2044) [110]. We have also identified an increase in the percentage of soleus fibers that are labelled by an antibody specific for types IIx and IIb after a 14-day spaceflight (Cosmos 2044); however, no labelling was observed with an antibody specific for only type IIb, suggesting an increase in the percentage of fibers expressing type IIx MHC during flight (Talmadge, Roy and Edgerton, unpublished observations, 1994). Curiously, several of the fibers labelling for type IIx also labelled for type I MHC, but not IIa. Therefore, some type I fibers appear to transform directly to type IIx, bypassing type IIa, as a result of spaceflight.

Our understanding of how muscles respond to spaceflight has primarily come from data collected on hindlimb muscles of the rat. Recently, however, biopsies have been obtained from the soleus, medial gastrocnemius and tibialis anterior muscles of young male Rhesus monkeys (body weights of 3.5 to 4.5 kg) prior to and after 12- (Cosmos 2229) and 14-day (Cosmos 2044) spaceflights. In contrast to the findings in the rat, significant atrophy was observed in the tibialis anterior, but not in the soleus or medial gastrocnemius in the monkey. The expression of fast MHCs was significantly increased in the soleus and no change was observed in the medial gastrocnemius or tibialis anterior (Fig. 1) (Bodine-Fowler SC, Pierotti DJ, Talmadge RJ: Preferential atrophy of flexor muscles of the monkey after spaceflight, 1994, submitted).

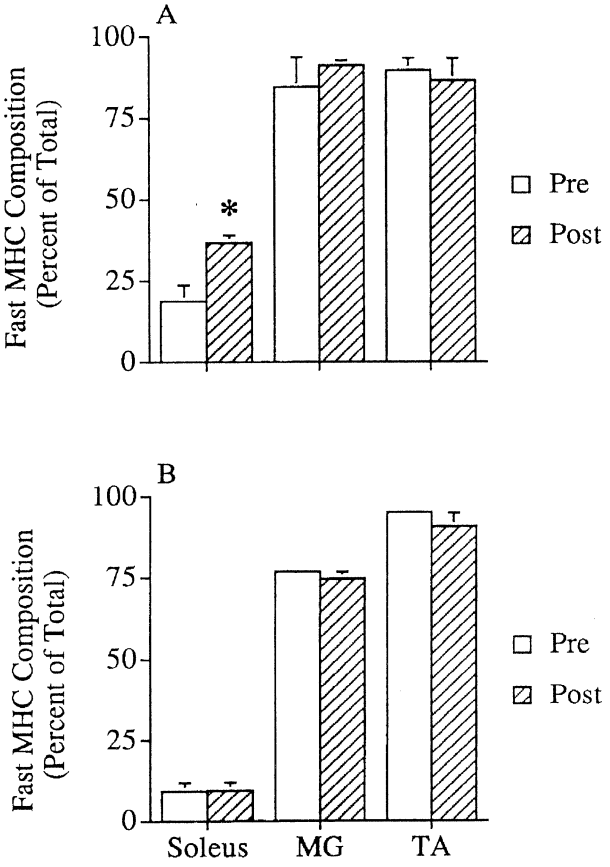

*Figure 1. MHC percentage composition of selected hindlimb muscles of Rhesus monkeys flown on Cosmos 2229, a 12-day spaceflight. Muscle biopsies were obtained 91 days prior to flight (Pre, preflight biopsies) and 3 to 5 days after return to earth (Post, postflight biopsies). The n = 2 flight animals (A) and n = 3 synchronous ground based controls (B). *, statistically different from preflight and ground based controls at p < 0.10. Abbreviations: MG, medial gastrocnemius; TA, tibialis anterior. Note the increase in fast MHC in postflight soleus muscle biopsies for the flight animals. No other significant differences were observed.*

In the vastus lateralis of astronauts following 5-11 days of flight, there was an increase in the number of fibers expressing fast MHCs and a higher percentage of fibers that co-expressed fast and slow MHCs in biopsies taken post-flight compared to pre-flight [36]. Thus, in humans [158], the type of myosin expressed in individual fibers can change as rapidly as that observed in rats [68]. In addition, the fiber myofibrillar ATPase activity in type II fibers was increased slightly [36]. These data are consistent with the torque-velocity results following longer duration flights indicating that cosmonauts were more efficient in producing force at the high velocities post-flight compared to pre-flight [77].

Spaceflight appears to result in the mechanical properties of rat soleus muscle and muscle fibers becoming more like those observed in a fast muscle. For example, recently Caiozzo et al. [19] reported the first study of mechanical properties *in situ* of whole muscles of flight animals. The soleus from 6-day spaceflight (NASA, PARE-02) rats were tested 3 to 9 hours after landing (thus minimizing any effects of recovery at 1 *g*). Compared to control rats, the adaptations in the isometric twitch times, frequency-tension response and force-velocity relationship of the flight rats were consistent with that observed following hindlimb suspension. Contraction and half-relaxation times were decreased by ~10%, the frequency-tension response was shifted to the right and the maximum rate of shortening was increased by ~15%. In addition, based on classical qualitative histochemical staining (myofibrillar ATPase pH sensitivity), the type I, IIa and IIc fiber type distribution was 70%, 27% and 3%, respectively, for controls; and 64%, 36% and < 1%, respectively, for flight rats. The muscles from the flight rats showed a slight decrease in type I and IIa MHC isoforms and a *de novo* expression of type IIx MHC. In addition, the relative contents of fast myosin light chains-1 and -2 increased as a result of flight. Similar preliminary results have been presented for a 14-day flight (STS-58) [18]. The data on the mechanical properties of the whole muscle are consistent with single fiber results from Cosmos flights of a similar duration (Cosmos 1667, 7 days and Cosmos 1514, 5 days) [63, 106] and up to 22 days of flight [see 95 for a review). For example, using skinned fiber preparations, Holy and Mounier [63] reported that the contraction times of soleus fibers were faster and that the response to changes in the calcium concentration of the medium became more similar to that observed in fast muscle fibers after than before a 7-day flight. Together, all of these data indicate that the soleus muscle becomes "faster" following exposure to microgravity.

Thus spaceflight, a condition presumed to unload the musculature, increases the expression of fast MHC isoforms, particularly in slow postural muscles, i.e. the soleus. In the soleus muscle the increase in fast myosin appears to be predominantly of the type IIx MHC isoform. This adaptation in MHC expression is associated with faster contractile properties, and higher myosin and myofibrillar ATPase activities.

Limb immobilization

After immobilization in a neutral position, isometric twitch contraction and 1/2 relaxation times of the rat soleus were decreased [12, 42, 149, 155] and the percentage of fibers defined as fast oxidative based on histochemical techniques was increased [12]. In addition, an increase in the percentage of fast, low oxidative (presumably type IIB or fast glycolytic) fibers was observed in the fast rectus femoris muscle. Increases in maximal velocities of shortening for the rat soleus after immobilization in a neutral position also have been observed [149, 155]. Similarly, increases in the percentage of fast oxidative fibers were observed in the soleus of the rabbit after immobilization in a shortened position [87] and in the soleus of the *Galego senegalensis*, a non-human primate, when the hindlimb was immobilized in a neutral position at the ankle and knee [35]. These data are consistent with a muscle fiber transformation from slower to faster fiber types when a muscle is immobilized in either a shortened or a neutral position.

Immobilizing muscles in a lengthened position (and thereby placing a load or tension on the muscle) can cause the opposite effect to that observed when fixed in either a neutral or a shortened position. For example, when the rabbit tibialis anterior was immobilized in a stretched or lengthened position there was a rapid increase in the percentage of slow oxidative fibers, such that after 2 weeks the number of slow fibers was doubled and after 6 weeks the number of slow fibers was 5 times that observed in control muscles [99]. These data are consistent with a fast to slow fiber type transformation. Since, there was no change in the amounts of neuromuscular activation as measured by integrated EMG analyses [99], the increase in slow fibers was most likely related to an increase in tension placed on the muscle, at least initially, after immobilization in the lengthened position.

Rapid alterations in mRNA levels for the various MHCs can occur after immobilization [81]. Type IIb MHC mRNA increased in the soleus (control soleus contained no type IIb MHC mRNA) after only 3 days of immobilization in a shortened position. However, if the soleus was immobilized in a stretched position no IIb MHC mRNA was detected. Curiously, immobilization in a stretched position resulted in a decrease in types I and IIa MHC mRNA and an increase in embryonic MHC mRNA in the soleus. In fast muscles (gastrocnemius and plantaris) a pattern of MHC mRNA expression similar to that seen in the soleus was observed after immobilization in a shortened position (i.e., maintenance of a high level of type IIb MHC mRNA, with reductions in types I and IIa MHC mRNA), but a different pattern of MHC mRNA expression was observed after immobilization in a stretched position (i.e., increases in types I, IIa, and embryonic MHC mRNAs and a decrease in type IIb MHC mRNA). No mRNA data were available for the type IIx MHC isoform.

Despite the rapid alterations in MHC mRNA, changes in the MHC content of immobilized muscles were much less dramatic. For example, rat soleus and gastrocnemius muscles immobilized in either a neutral or shortened position for 4 days showed no changes in types I, IIa or IIb MHC content [131]. No data were available for the type IIx MHC isoform. However, as described above, alterations in the fiber type distribution consistent with the short-term changes in MHC mRNA occurred with long-term immobilization. Thus, not unexpectedly, the time course for alterations in MHC mRNA levels were faster than the time course for changes in the MHC protein. Finally, despite the quantitative and temporal differences in the adaptation in MHC mRNA, MHC protein, fiber type composition and contractile properties associated with this

model, immobilization in either a shortened or neutral position results in increases in fast fiber characteristics, whereas immobilization in a lengthened position results in increases in slow fiber characteristics.

Collectively the data on immobilized muscles indicate a major role for loading in determining muscle fiber properties. Immobilization of a muscle in a lengthened position (increased loading) rapidly reduces expression of type IIb MHC mRNA and increases expression of type I and IIa MHC mRNAs in fast muscles (plantaris and medial gastrocnemius) [81]. These adaptations apparently occur with minimal changes in the total amount of electrical activation of the muscle [43, 99]. In contrast, decreased loading induced by immobilization in a shortened position clearly results in a muscle with faster contractile properties, and increases the expression of the fast MHC genes as determined by mRNA analyses [81]. Thus, with limb immobilization the direction of MHC adaptations of the immobilized muscles is correlated with whether or not the muscle is loaded or unloaded. Unloaded muscles show increased expression of fast MHCs and loaded muscles show increased expression of slow MHCs.

Spinal cord transection

In adult cats subjected to a complete ST at a low thoracic level, isometric twitch contraction times for the soleus were 45% [88], 38% [71], and 31% [23] shorter than in control cats. However, the isometric twitch times for the soleus after ST do not approach the twitch times normally observed for fast muscles [23, 71, 88]. Isometric twitch contraction times for the fast medial gastrocnemius after ST in adult cats were 27% shorter [88], or not different from control [71]. Also, the maximal shortening velocity and the myosin ATPase activity of the cat soleus muscle are 52% and 67% higher, respectively, 6 months after ST [5]. Similarly, in the medial gastrocnemius the shortening velocity increased 20%, and myosin ATPase activity increased 30% 6 months after ST in the adult cat [5].

Mayer et al. [88] demonstrated a significant increase in the percentage of fast, fatigue intermediate motor units in the medial gastrocnemius of adult ST cats at the expense of fast fatigue-resistant and slow motor units. Similarly, the mean contraction time of single motor units isolated from the soleus of adult ST cats were 30% [23], or 35% [84] shorter than in controls. The increase in the percentage of fast motor units after ST appears to be related to an increase in the percentage of fast fiber types. Mayer et al. [88] reported increases in the percentages of fibers classified as type IIb (46% to 65%) and IIab (3% to 7%) in the adult cat medial gastrocnemius after 5 months of ST. Similarly, Jiang et al. [66, 67] showed an increase in the percentage of type II fibers, based on myofibrillar ATPase histochemistry and immunohistochemistry, in the adult cat soleus (0% to 45%) and in the deep portion of the medial gastrocnemius (60% to 85%) muscles 6 months after ST.

Using high resolution SDS-PAGE [133], we found both type IIa (17%) and IIb (15%) MHC in the cat soleus 6 months after ST [136]. Thus, even 6 months after ST in the cat, the primary MHC type expressed by the soleus is the slow type I isoform (68%) [136]. These data compare to ~100% type I MHC in control cat soleus. Histochemical fiber type analyses, based on myofibrillar ATPase pH sensitivity, of other slow muscles are in agreement with these results. For example, the cat vastus intermedius (normally 90% slow fibers) had 63% slow fibers 6 months after ST. The capsularis (normally 93% slow fibers), and the quadratus femoris (normally 98% slow fibers) had 80 and 70% slow fibers, respectively, 6 months after ST [112]. Not all of the slow fibers within predominantly fast muscles convert to a fast phenotype after ST. Based on immunohistochemistry, 15% of the fibers in the cat medial gastrocnemius 6 months after ST express slow MHC, compared to 38% in controls [67]. In the deep regions of the rectus femoris, and the proximal and distal portions of the semitendinosus, at least 50% of the slow fibers are maintained 6 months after ST [112]. However, the percentage of fibers expressing slow and fast MHC was not determined. Thus, while neuromuscular activation is a factor in regulating fiber type, MHC type, and motor unit type composition of a muscle, it is not the sole mechanism for regulation, since all slow fibers in the soleus and medial gastrocnemius did not convert to a fast phenotype. Perhaps, some slow fibers are more resistant to adaptation to a faster phenotype or the small amount of neuromuscular activation that remains after ST is sufficient to prevent conversion of some slow fibers to a faster phenotype. It is also possible that the time-frame (~6 months in most cases) for all fibers to transform from slow to fast is too short for the cat. Similar results have been observed in the cat when the spinal cord was transected at an early age, i.e. at two weeks of age [6, 117, 152] or at 2 days of age in the rat [90]. However, these data are more difficult to interpret because of the confounding effects of development on the contractile and metabolic properties of skeletal muscle.

A more complete adaptation to a fast phenotype occurs after ST in adult rats. For example, 12 months after ST surgery the twitch contraction times for the rat soleus (normally predominantly slow) is decreased by approximately 50% and becomes indistinguishable from that of the extensor digitorum longus (a fast muscle) [80]. The percentage of soleus muscle fibers identified as type II (fast) based on alkaline preincubation myofibrillar ATPase histochemistry increased from 13% in control rats to 98% in rats 12 months after ST [79]. The extensor digitorum longus muscle showed no change in either the contractile properties or the percentage of type II fibers (95% in control, 99% 12 months after ST) [79, 80]. It should be noted however, that the specific types of MHC expressed in the soleus or extensor digitorum longus after ST were not determined. The use of myofibrillar ATPase histochemistry to identify fibers as type II can be problematic. For example, fibers that co-express type II and type I MHCs and fibers that express developmental MHCs will stain as adult type II fibers with myofibrillar ATPase at an alkaline preincubation.

In order to determine which MHC isoforms are expressed in the rat soleus after ST, we have used SDS-PAGE and immunohistochemistry [114, 135]. Fifteen days after ST there was a significant increase in the percentage of type IIx MHC at the expense of type IIa MHC with no change in the relative proportion of type I MHC (Table 2). Based on immunohistochemistry, it appeared that some type IIa fibers began to express type IIx MHC. One month after ST there was a decrease in the percentage of type I MHC, a return to the control level of type IIa MHC, a further increase in type IIx MHC, and some type IIb MHC expressed in the soleus muscle (Table 2). Immunohistochemistry revealed significant co-labelling for types I and IIa, or types I and IIx, or types I and IIa and IIx in single fibers. Nearly 60% of all fibers expressed some type I MHC with at least one type II MHC isoform. These findings suggest that some of the fibers that originally expressed type I MHC also expressed fast (type II) MHCs after ST. Type IIb MHC was coexpressed with either type IIx or with IIx and IIa MHCs, but never with type I MHC. Thus, muscle fiber transformation could have proceeded sequentially I > IIa > IIx > IIb as previously suggested [101]. However, some type I fibers appeared to have the capacity to transform directly to type IIx MHC, bypassing type IIa. This observation is similar to that found following spaceflight (see above). The potential for type I fibers to transform directly to type IIa and IIx, but not IIb seems reasonable physiologically, for type IIa and IIx fibers have similar contractile velocities which are intermediate between type I and type IIb, whereas type IIb fibers are significantly faster than types I, IIa and IIx fibers [13, 14, 44]. The fact that type IIb MHC was found in the soleus one month after ST suggests that at least some of the myonuclei were not restricted in adaptational ability as was previously suggested by Ausoni et al. [3]. These results also are consistent with an incomplete fiber type shift from type I toward type IIb one month following ST in the rat. No studies to date have identified the MHC composition of fast or slow rodent muscles after long-term ST. Therefore, at present, it is not known whether a complete shift to type IIb will occur in the rat soleus after prolonged periods, e.g. after several months, following ST.

To identify the long-term effects of ST on muscle phenotypic properties, one must refer to the studies performed on human SCI patients. However, only a few studies have attempted to identify the fiber type changes that occur. Grimby et al. [51] demonstrated that in the soleus, gastrocnemius and vastus lateralis muscles of 7 subjects suffering from a complete spinal cord lesion at the cervical or thoracic levels nearly 90% of all fibers stained histochemically as type II compared to only 40% in the nonaffected deltoid muscle of the same patients (2 - 10 years post-injury). Normal values for type II fiber type percentages in the human soleus, gastrocnemius and vastus lateralis are 20%, 40%, and 50%, respectively [34, 48]. A study by Round et al. [111] recently demonstrated that long-term paralysis (11 months - 9 years) in humans results in a near complete conversion of type I fibers to type II (based on alkaline preincubation myofibrillar ATPase) in the vastus lateralis and that the degree of conversion was dependent upon the duration of time following the SCI. For example, one year following SCI there was no change in the percentage of fibers identified as fast, but after 2 or more years the muscles were comprised of nearly 100% fast fibers. Also, Martin et al. [86] demonstrated a reduction in the percentage of type I fibers (from 70 to 10%) and a commensurate increase in type II fibers in the tibialis anterior muscle after SCI in humans (2 - 11 years post-injury). A reduction in the oxidative capacity in type II fibers after SCI to about one third of that of normal was observed [86], suggesting a conversion to the fast glycolytic fiber type (most likely type IIb fibers). It appears that most fibers in SCI humans can completely transform to the type IIb phenotype, and that the completeness of the transformation is dependent on the duration of time following the SCI. On the other hand, no data that directly demonstrate MHC types after SCI in humans have been reported.

In summary, all of these data suggest that species specific differences exist regarding the time-course for MHC adaptations following ST. Figure 2 shows that rats have a much faster rate of adaptation than do cats or humans. In fact, although limited data are available, humans show no change in fiber type content during the first year after SCI [111]. Clearly, the smaller the animal, the faster is the rate of fiber type transformation. Thus, it is speculated that the

Table 2. MHC percentage composition of soleus muscle from control and 15-, and 30-day spinal cord transected (ST) rats.

Group	Type I	Type IIa	Type IIx/d	Type IIb
Control	90 ± 2	9 ± 1	1 ± 1	0
ST (15 days)	87 ± 2	1 ± 1*	12 ± 2*	0
ST (30 days)	59 ± 8*	7 ± 1	30 ± 6*	4 ± 2*

* significantly different from control, $p < 0.05$.

observed scaling factor relating metabolic rates with body size may play a role in determining the time-course for MHC adaptation. This hypothesis is reasonable since muscle fiber transformation would likely depend upon the absolute rates for catabolism and anabolism of myofibrillar components. However, regardless of the differences in time-frame for adaptations across different animal species, it is clear that some muscle fibers in slow muscles eventually adopt characteristics of fast muscle fibers after ST or SCI, including the accumulation of fast MHC at the expense of slow MHC.

Spinal cord isolation

SI for 6 to 9 months decreased the isometric twitch contraction time of the slow soleus muscle, but not the fast medial gastrocnemius muscle of the cat [39, 113]. On the other hand, the maximal velocity of shortening was increased in both the soleus (75%) and the medial gastrocnemius (15%), and was highly correlated with increases in myosin ATPase activity [5]. In contrast, the cat soleus contraction time after short term SI (less than 50 days) was normal [27]. Following SI, the fast myosin light chain complement increased and the slow myosin light chain complement decreased in the cat soleus, such that 2.5 years

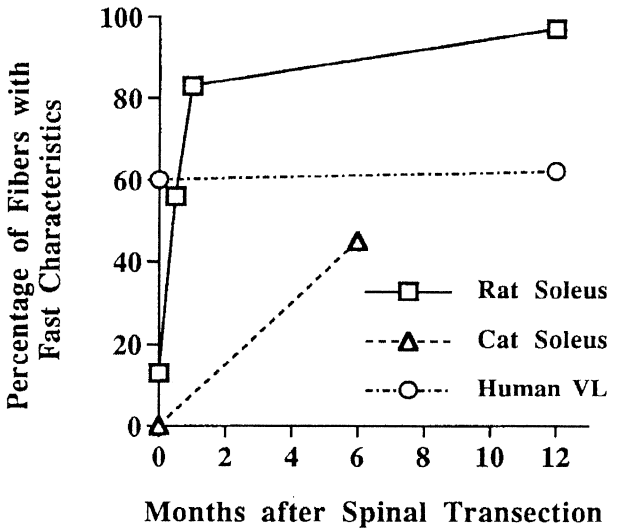

Figure 2. Time course for the appearance of fast fiber characteristics in muscles during the first year after spinal transection in three mammalian species. Fast fiber characteristics are defined as some distinguishing characteristic normally associated with fast fibers, i.e., immunoreactivity with monoclonal antibodies specific for fast MHC and/or dark staining after alkaline preincubation myofibrillar ATPase. The rat soleus data (squares) are from Roy et al. [114] for the first three time points and Lieber et al. [79] for the 1 year time point. The cat soleus data (triangles) are from Talmadge et al. [136] and Jiang et al. [67]. The human vastus lateralis (VL) data (circles) are from Round et al. [111].

after SI the myosin light chain pattern in the soleus was indistinguishable from that of the fast flexor hallucis longus muscle [127]. In addition, the percentage of fibers labelled with an antibody specific for fast troponin-I increased from < 1% to ~60% in the cat soleus after 8 months of SI [40]. The flexor hallicus longus showed no change in its complement of myosin light chains after 2.5 years of SI [127], while the percentage of fibers expressing fast troponin-I increased from 86% to 100% after 8 months of SI [40]. Thus, both fast and slow extensor muscles appear to get faster after SI; however, there is a greater effect on the slow muscles.

Graham et al. [48] have reported that 45% of the fibers in the soleus stained darkly after alkaline preincubation myofibrillar ATPase histochemistry in cats 6 months after SI, whereas the soleus in control cats contained no darkly stained fibers. The apparent conversion of the ATPase types was reinforced by the finding that the dark ATPase fibers reacted with monoclonal antibodies for fast MHC. One of the critical findings in this study was that while all the fibers were inactive, only half of the fibers in the soleus converted from a slow to a fast myosin type after six months of inactivity. This observation is very similar to the situation in cats subjected to ST; however, in the case of SI, the activity of all fibers is zero, whereas with ST some fibers remain active. Thus, some non-activity related factor(s) maintained the original MHC type of some inactive soleus fibers. An increase in the percentage of fast fibers also has been observed for the medial gastrocnemius of cats after six months of inactivity [69]. In fact, all fibers in the medial gastrocnemius expressed some fast myosin after 6 months of SI. The transitions in MHC isoform content from slow to fast of only some of the fibers in the cat soleus after SI are similar to the fiber type changes observed in several other muscles of the hindlimb (including the vastus intermedius, rectus femoris, plantaris, quadratus femoris, semitendinosus, and capsularis) after SI, where reductions in the percentage of slow oxidative fibers are matched by increases in the percentage of fast glycolytic and fast oxidative glycolytic fibers in predominantly fast and predominantly slow muscles, respectively [116]. Expression of types IIa and IIb MHC isoforms, as well as developmental MHC isoforms, are increased in cat soleus 6 months after SI [136]. However, developmental MHC isoforms are not observed in the cat tibialis anterior (a predominantly fast flexor muscle), despite uniform absence of contractile activity, i.e., electrical silence [104].

Thus, as with spinal transection, 6 months of SI resulted in the conversion of only a select population of muscle fibers from slow to fast in the slow muscles of the cat. It is possible that species specificity accounted for the maintenance of some slow fibers. In other species, notably humans and rats, almost all fibers transform from slow to fast in normally slow or mixed muscles after long-term ST. However, no animals other than cats have been evaluated for muscle MHC content after SI. Alternatively, there may be a sub-population of fibers in the cat soleus that is non-trans-

formable and thus the phenotype of these fibers would be independent of neural activity.

To establish if load is a factor in regulating the MHC isoform transitions associated with SI, experiments have been performed in which the soleus muscles of SI cats were stimulated to contract against a controlled load. Preliminary data from our laboratory [57] indicate that as little as 30 minutes of intermittent electrical activation (mimicking that observed in the cat locomoting on a treadmill) of the soleus muscle under loaded conditions in SI cats ameliorates the atrophy associated with this model of inactivity. Further, it appears that stimulation and loading during either the isometric, shortening or lengthening phase of an oscillating cycle is effective, with the isometric regime apparently being the most effective. It also appears that as little as 30 minutes of loaded contractions per day attenuate the increase in fast MHC profiles and the shift towards faster mechanical properties normally associated with SI [57; Roy, Talmadge, Hodgson and Edgerton, unpublished observations). Thus, load and not the total number of stimulated contractions appears to play a major role in determining the MHC profile of cat muscle.

In summary, SI clearly results in an increased expression of fast MHCs. This shift towards a faster phenotype is observed in both fast and slow muscles, although the proportional increase in fast MHC expression is greater for the slow postural than fast muscles. The fast MHCs expressed in the soleus muscle of cats in response to chronic inactivity are both types IIa and IIb.

Denervation

Although denervation is not a good model for studying activity-dependent plasticity of the neuromuscular system, it is relevant to many clinical conditions such as direct trauma to peripheral nerves. Some studies on the effects of denervation on skeletal muscle contractile properties showed a trend towards a slowing of the muscle. For example, Kean et al. [75] demonstrated an increase in the twitch contraction times and a decrease in the shortening velocities for both fast (flexor digitorum longus) and slow (soleus) cat muscles 28-35 days following denervation. Similar results have been reported for rat muscles [41, 55]. Gutmann et al. [56] demonstrated that although the twitch contraction time for the fast extensor digitorum longus was increased 75 days after denervation, it was decreased for the slow soleus following denervation in rats. Syrovy et al. [130] demonstrated a similar differential response to denervation for rabbit fast and slow muscles for both contraction time and myosin ATPase activity. In short-term (7 days) denervated rat muscles, however, no changes were observed in myosin ATPase activities for fast or slow muscles [83]. Spector [124] showed a prolongation of the isometric twitch contraction time in the rat soleus after denervation (consistent with the muscle becoming slower), but an increase in the maximum shortening velocity (consistent with the muscle becoming faster) 4 weeks after denervation. One explanation for these results could be that the proteins associated with excitation-contraction coupling were altered resulting in a "slowing" of twitch properties, while the myofibrillar proteins changed toward the "faster" isoforms, resulting in faster shortening velocities. Recently, however, d'Albis [26] showed that chronic denervation of rabbit gastrocnemius and soleus at 8 days of age resulted in both muscles having slow twitch contraction times and maximal velocities of shortening. Thus, the denervation data show very few consistencies regarding contractile physiology, which may be related to species- and muscle-specific responses.

Virtually all fibers in the neonatally denervated rat soleus and extensor digitorum longus muscles stain histochemically as type II (69% IIb and 31% IIc in extensor digitorum longus, and 58% IIb and 42% IIc in soleus) at 30 days of age compared to age-matched controls [98]. It should be pointed out, however, that fibers categorized as type IIc can occur based on the presence of developmental (embryonic and/or neonatal) MHCs, which may be slower than adult fast MHCs, or based on the coexpression of types I and II MHC in the same fiber [126]. Spector [124] observed a reduction in type I fibers in the rat soleus 2 and 4 weeks after denervation in young adult rats. d'Albis [26] demonstrated that the rabbit soleus and gastrocnemius muscles contained primarily large slow fibers 2 months after denervation at 8 days of age. Thus, as observed for contractile physiology, the data regarding fiber type composition of muscles after denervation are inconsistent.

Neonatal denervation did not prevent the appearance of adult fast myosins identified immunohistochemically in the rat medial gastrocnemius; however, denervation at this age did result in a maintenance of neonatal myosins [17]. Embryonic and neonatal MHCs also are expressed in rat skeletal muscles when denervation is performed on adult animals [121], and the developmental MHC isoforms are found preferentially in the type IIa fiber population. Six months after denervation, the rat medial gastrocnemius and diaphragm express only adult fast MHCs based on tryptic mapping [21, 22]. After denervation of adult rat diaphragm all fibers were labelled by an antibody against fast myosin, and some fibers were also reactive with an antibody against slow myosin [47].

Jakubiec-Puka et al. [64] used SDS-PAGE to analyze MHC adaptations in the denervated rat soleus. A reduction in type I MHC and an increase in type IIa MHC was observed after denervation. The percentage of type IIa MHC increased following denervation of the rat soleus, when combined with the administration of procainamide (an antifibrillatory drug) [91]. These adaptations in the relative content of MHC isoforms are inconsistent with denervation resulting in a slowing of the muscle. Using immunoblotting techniques, however, it was determined that at least some of the MHC present in the type IIa band was indeed embryonic MHC that co-migrated with type IIa MHC in the electrophoretic technique used [64]. Thus, one explanation for the apparent discrepancy between a physiological "slowing" of the muscle and an increased percentage of type II fibers as determined histochemically

may be the observation that denervated muscles express some developmental MHC isoforms. In contrast to these studies are the observations that following long-term denervation of neonatal rabbit soleus and gastrocnemius the only MHC observed using high resolution SDS-PAGE is type I (slow) MHC [65].

In summary, the data concerning the effects of denervation on the MHC profile of muscles are rather inconsistent. These inconsistencies, however, may be due to species, age, and muscle specific responses. Another factor that could potentially contribute to the observed inconsistencies is the purity of the denervations, i.e., the possibility for reinnervation of the muscles during the denervation period. Many studies did not verify the completeness of the denervation. Regardless, denervation is not an appropriate model to study the effects of reduced load or activity on muscle properties, for elimination of the motoneuron-muscle connection may have effects beyond merely reducing the electrical activity and force production of the affected muscle(s).

Tetrodotoxin

Another method to achieve reductions in neuromuscular activity is to eliminate the capacity for the motor nerve to conduct action potentials to the muscle. This can be accomplished by blocking the action potential conduction at a site on the motor nerve axon with agents that block ion channels. One agent commonly used is TTX, a Na^{2+} channel blocker.

As with denervation, the speed-related contractile properties of muscles following TTX-induced paralysis are divergent. LaPointe and Gardiner [78] and St.-Pierre and Gardiner [128] observed no effect of short-term (7 - 9 days) TTX sciatic nerve block on the rat gastrocnemius muscle time to peak twitch tension. There was a lengthened 1/2 relaxation time, an elevated twitch:tetanus ratio, and an elevated relative maximal tension at 25 Hz stimulation frequency, suggesting that the muscle contractile properties became slower. However, the rate of rise of tetanic tension also was elevated, suggesting a "faster" muscle. After a more prolonged period of TTX-induced paralysis (28-days) significant increases in time to peak tension and 1/2 relaxation time were observed for adult rat medial gastrocnemius [129], suggesting a "slowing" of muscle contractile properties.

Spector [123, 124], observed an increase in the velocity of unloaded shortening and no change in time to peak tension and 1/2 relaxation times in adult rat soleus after 2 and 4 weeks of TTX administration. In addition, there was an increase in the percentage of type II fibers (based on alkaline preincubation myofibrillar ATPase) compared to control muscles. In contrast, the medial gastrocnemius (deep region) showed no change in fiber type proportion, a reduction in succinate dehydrogenase activity in both type I and type II fibers [46], and a decrease in total myofibrillar ATPase activity [148] after 2 weeks of TTX administration. Thus, these data may be interpreted to mean that fast (medial gastrocnemius) and slow (soleus) muscles show different responses to TTX administration, with the soleus acquiring more fast muscle characteristics, and the medial gastrocnemius acquiring some slow muscle characteristics.

Other similarities between denervation and TTX administration exist. For example, both TTX [46] and denervation [79, 94] are accompanied by a pronounced type II fiber atrophy in fast muscles, and both conditions also result in the expression of developmental MHC isoforms which appear preferentially in the type IIa fibers [121]. The similarities between the effects of TTX induced paralysis and denervation on skeletal muscle have been interpreted to mean that the adaptations associated with denervation are a result of the lack of neuromuscular activity. Neurotrophic factors however, cannot be excluded, since it is not known whether TTX effects certain aspects of axoplasmic transport, in particular, slow and retrograde axoplasmic transport. It is known however, that TTX administration does not affect the fast axoplasmic transport of acetylcholinesterase.

At this point in time the effects of TTX induced paralysis on the MHC composition of fast and slow muscles are unknown. While increases in histochemically classified type II (fast) fibers have been observed, the conflicting data regarding the mechanical properties of the muscles make it difficult to predict what, if any, adaptations are occurring at the level of the MHC.

Summary

In the majority of the models designed to reduce neuromuscular activity, including ST, SI, TTX application, hindlimb suspension, spaceflight, and limb immobilization in either a shortened or neutral position, the percentage of fast fibers increases in predominantly slow muscles of all mammals studied to date, as assayed by alkaline preincubation myofibrillar ATPase. These findings suggest that an increase in fast (type II) MHCs, most likely resulting from more fibers expressing type II MHCs. For virtually all of the models, a reduction in type I MHC expression has been observed in the rat soleus (Table 3). A corresponding increase in at least one type II MHC isoform usually has been observed. However, there is little data for type IIx MHC or for MHC mRNA expression. Also, unfortunately no MHC data are available for TTX application, and the data for denervation are complicated by the presence of developmental MHCs in some fibers.

Thus, the majority of these models of reduced neuromuscular activity result in an increased expression of "fast" MHC isoforms. These models differ greatly with respect to the levels of electrical activation, motor end-plate function and motoneuron properties. However, one common element is the unloading of the muscles produced by each of these models (Table 1), suggesting that the loading characteristics of a muscle are important regulatory factors for the expression of specific MHC isoforms.

Table 3. Effects of reduced activity models on MHC isoforms in rat soleus muscle.

Model	Reference	Type I	Type IIa	Type IIx/d	Type IIb	Emb/Neo	Duration
Hindlimb Suspension	Protein						
	25	↓	↑	NA	0	NA	14 days
	18	↓	↑	↑	0	NA	21 days
	118	↓	↑	↑	0	NA	21, 28 days
	86	↓	↑	↑	0	NA	28 days
	mRNA						
	126	↔	NA	NA	NA	NA	5 hrs, 7 days
	25	↓	↓	NA	↑	NA	14 days
Spaceflight	Protein						
	17	↔	↓	↑	0	NA	7 days
	81	↓	NA	NA	NA	NA	12 days
	85	↓	NA	NA	NA	NA	14 days
LI-Short	Protein						
	117	↔	↔	NA	0	NA	4 days
	mRNA						
	71	↓	↓	NA	↑	NA	3-5 days
Spinal Cord Transection	Protein						
	102	↓	↔	↑	↑	0	30 days
	79	↓	NA	NA	NA	NA	2-3 months
Denervation	Protein						
	108	NA	NA	NA	NA	↑	3-7 days
	80	↓	↑	NA	0	NA	21 days
	54	↓	↑	NA	0	↑	25 days
TTX	Protein						
	108	NA	NA	NA	NA	↑	3-7 days

Abbreviations and symbols:
LI - Short = limb immobilization with muscle in a shortened position; Emb/Neo = embryonic and neonatal MHCs; ↑ = increase; ↓ =decrease; ↔ = no change; 0 = none detected; NA = data not available.

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Abbreviations

Adenosine triphosphatase, ATPase; Electromyogram, EMG; Messenger ribonucleic acid, mRNA; Myosin heavy chain, MHC; Sodium dodecyl sulfate polyacrylamide gel electrophoresis, SDS-PAGE; Spinal cord injury, SCI; Spinal cord isolation, SI; Spinal cord transection, ST; Tetrodotoxin, TTX.

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