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SARCOMERE NUMBER REGULATION MAINTAINED AFTER IMMOBILIZATION IN DESMIN-NULL MOUSE SKELETAL MUSCLE

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Summary

The serial sarcomere number of skeletal muscle changes in response to chronic length perturbation. The role of the intermediate filament desmin in regulating these changes was investigated by comparing the architectural adaptations of the tibialis anterior, extensor digitorum longus (EDL) and soleus from wild-type mice with those of homozygous desmin knockout mice after hindlimb immobilization. After 28 days, serial sarcomere number increased significantly in the lengthened wild-type tibialis anterior (by approximately 9 %) and EDL (by approximately 17 %). Surprisingly, muscles from desmin knockout mice also experienced significant serial remodeling, with the serial sarcomere number of the tibialis anterior increasing by approximately 10 % and that of the EDL by approximately 27 %. A consistent result was observed in the shortened soleus: a significant decrease in sarcomere number was observed in the muscles from both wild-type (approximately 26 %) and knockout (approximately 12 %) mice. Thus, although desmin is not essential for sarcomerogenesis or sarcomere subtraction in mouse hindlimb muscles, the results do suggest subtle differences in the nature of sarcomere number adaptation. We speculate that desmin may play a role in regulating the optimal arrangement of sarcomeres within the muscle or in sensing the magnitude of the immobilization effect itself.

Key words: sarcomerogenesis, atrophy, intermediate filament, muscle architecture, mouse.

Introduction

Skeletal muscle exhibits an extraordinary ability to remodel both its structural and metabolic properties in response to an altered environment. One structural property that readily adapts is serial sarcomere number. An example of such an adaptation was provided by Lynn and Morgan (Lynn and Morgan, 1994), who demonstrated that running down an incline in rats induced an increase in serial sarcomere number, presumably as a result of repeated sarcomere lengthening during running. They interpreted this result as an indication that serial sarcomere number adapted so that sarcomeres would no longer operate on the descending limb of the length/tension curve. Burkholder and Lieber (Burkholder and Lieber, 1998) demonstrated that transection of the ankle flexor retinaculum, causing a systematic decrease in muscle length, resulted in decreased tibialis anterior serial sarcomere number. Their interpretation was that the mouse muscles had a preferred resting length that was re-established by serial sarcomere number subtraction. Remodeling has also been reported in response to normal limb growth: increases in muscle length and fiber length secondary to bone growth were accompanied by increases in sarcomere number (Williams and Goldspink, 1971). These results together illustrate the highly plastic nature of sarcomere arrangement within skeletal muscle.

Sarcomere number adaptation is most dramatic under the mechanically static conditions of immobilization, in which a muscle is subjected to chronic lengthening or shortening. Immobilization, especially when the muscle is maintained in a shortened position, results in decreased protein synthesis relative to degradation and in significant reductions in cross-sectional area and mass, associated with a parallel sarcomere number adaptation (Spector et al., 1982; Thomason et al., 1989; Thomason and Booth, 1990). Serial sarcomere changes can also occur after immobilization in as little as 2 weeks, restoring optimal sarcomere length and maximizing force production at the new fiber length (Tardieu et al., 1977; Tardieu et al., 1979; Williams and Goldspink, 1973; Williams and Goldspink, 1978).

When sarcomere addition occurs, the evidence suggests that most sarcomeres are added at the muscle fiber ends *via* proliferation and subsequent migration of satellite cells

(Williams and Goldspink, 1973; Williams and Goldspink, 1971). After myoblast fusion into myotubes, myofibrils are then assembled and integrated into the existing muscle network (Mauro, 1961; Snow, 1976). However, the mechanisms that regulate these events are not clear.

Desmin, a muscle-specific intermediate filament protein, is one of the earliest known myogenic markers expressed in both cardiac and skeletal muscles (Choi et al., 1990; Hermann et al., 1989; Kaufman and Foster, 1988; Mayo et al., 1992), preceding all but one of the myogenic regulatory factors (MRFs) and all muscle proteins examined to date. In addition, unlike most other muscle-specific genes, desmin is expressed at low levels even in satellite cells and replicating myoblasts (Allen et al., 1991; Kaufman and Foster, 1988). Structurally, desmin exhibits homology to the basic helix–loop–helix regions of the critical myogenic regulatory factors MyoD (Davis et al., 1987), Myf-5 (Braun et al., 1989), myogenin (Edmonson and Olson, 1989; Wright et al., 1991) and mrf4 (Braun et al., 1990; Miner and Wold, 1990). Each MRF is a sequence-specific DNA-binding protein requiring the basic helix–loop–helix regions to bind to a consensus sequence CANNTG, known as an E-box, triggering muscle-specific gene transcription (Braun et al., 1990; Brennan et al., 1991; Davis et al., 1990). The homology between desmin and the MRFs and the very early appearance of desmin during myogenesis suggest a potential regulatory role in myogenesis.

In an *in vitro* study, Li et al. (Li et al., 1994) demonstrated that antisense desmin transcripts exhibited strong inhibition of MyoD and differential inhibition of myogenin. This suggested a potential myogenic regulatory role for desmin during development. Subsequent experiments have also supported the idea that MyoD plays a regulatory role in myogenesis during regeneration on the basis of the increased and prolonged expression of MyoD observed during satellite-cell-induced myogenesis (Megeney et al., 1996; Yablonka-Reuveni and Rivera, 1994). The important role of MyoD during cellular regeneration may, in turn, suggest an important role for desmin during periods of extensive satellite cell proliferation such as those occurring in response to the altered use conditions described above.

In the light of the data supporting a possible myogenic role for desmin, it was somewhat surprising that expression of cDNA encoding a truncated form of desmin in fibroblasts, post-mitotic myoblasts and myotubes did not prevent the transcription of sarcomere components (Schultheiss et al., 1991). In addition, desmin-null mice created *via* homologous recombination are viable (Li et al., 1997; Milner et al., 1996), further demonstrating that desmin is not essential for developmental myogenesis.

In addition to any possible interactions between desmin and MRFs, the structural role of desmin in maintaining sarcomere organization could also permit it to play a role in mediating signal transduction during sarcomerogenesis. In mature striated muscle, desmin interconnects Z-disks both laterally and, to a lesser extent, longitudinally, as well as connecting them to other intracellular structures including costameres, mitochondria and nuclei (Georgatos et al., 1987; Granger and Lazarides, 1979; Granger and Lazarides, 1982; Lazarides, 1982; Milner et al., 2000; Richardson et al., 1981; Tokuyasu et al., 1982; Wang and Ramirez-Mitchell, 1983). Because of the numerous mechanical connections between desmin and critical elements within the muscle fiber, it is believed that the intermediate filament network maintains the proper alignment of cellular structures during loading and remodeling of muscle (Lazarides, 1980; Milner et al., 1996). The highly organized filamentous network may also mediate the strain responses of the muscle fiber in a manner similar to that demonstrated in endothelial cells (Maniotis et al., 1997).

To resolve whether desmin is required for sarcomerogenesis and/or sarcomere deletion during induced remodeling, we compared the effects of immobilization of three different muscles on sarcomere number adaptation in series and in parallel between wild-type and desmin-null mice. A brief version of this work has been presented previously (Shah et al., 2000).

Materials and methods

Animal subjects

Experiments were performed on two groups of young adult mice: wild-type 129/Sv (*N*=8, 8–12 weeks; Taconic Farms, Germantown, NY, USA) and desmin homozygous knockout 129/Sv (*N*=9, aged 8–12 weeks; Milner et al., 1996). All procedures were performed in accordance with the NIH Guide for the Use and Care of Laboratory Animals and were approved by the University of California and Department of Veteran's Affairs Committees on the Use of Animal Subjects.

Hindlimb immobilization

The right lower hindlimb of each mouse was immobilized for 28 days by wrapping fast-setting plaster strips (Johnson and Johnson Medical 7372; Arlington, TX, USA) over a layer of Webril undercast padding (Kendall 1418; Boston, MA, USA). The contralateral limb of each animal served as its own control. The immobilized ankle was maximally plantarflexed and the knee was maximally flexed (within the constraints of the cast thickness), yielding a mean ankle joint angle of 179.8±1.3° and a mean knee joint angle of 103.4±11.5° (means ± S.D., *N*=17) as measured from lateral radiographs. This configuration (Fig. 1) resulted in lengthening of the EDL and tibialis anterior and in shortening of the soleus. Casts were inspected daily for fraying or loosening and were repaired or replaced when damage was observed. All casting and cast repair were performed while the animal was anesthetized by inhalation of 2% halothane (Halocarbon Laboratories, River Edge, NJ, USA) delivered by an Ohmeda anesthesia system (model VMS; UK).

Muscle fixation and harvesting

After cast removal under halothane anesthesia, each mouse was anesthetized with a cocktail composed of (in mg kg^{-1}) 10 ketamine, 5 rompum and 1 acepromazine delivered by

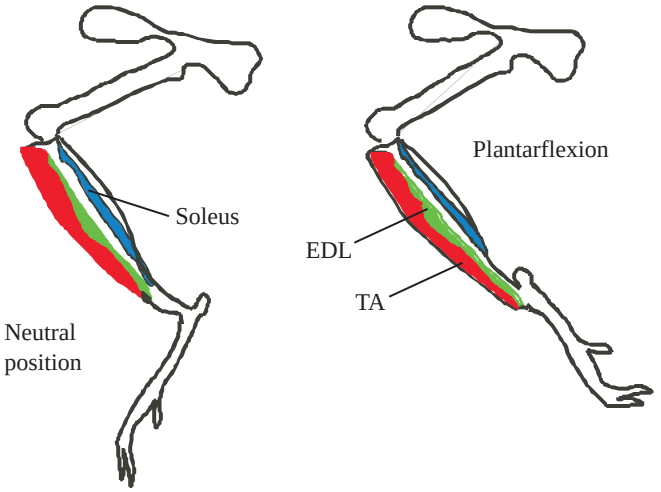

Fig. 1. Schematic diagram of the mouse hindlimb in both the neutral and plantarflexed positions. As the joint moves into plantarflexion, the tibialis anterior (TA) and extensor digitorum longus (EDL) are elongated while the soleus is shortened. This chronic length change results in changes in sarcomere number.

intraperitoneal injection, followed by intracardiac injection of concentrated sodium pentobarbital. Each limb was skinned and transected below the hip, leaving the entire knee joint intact, and placed (within 30 min of death) in low-odor 10 % formalin (Fisher SF93-4; Fair Lawn, NJ, USA). Limbs were fixed with the knee and ankle joints at 90 °. After overnight fixation, the limbs were rinsed three times in phosphate-buffered saline to remove residual fixative and stored in buffer for subsequent dissection.

Muscle architecture and serial sarcomere number measurements

Methods for architecture measurements were slightly modified from procedures described previously in detail (Burkholder et al., 1994). Briefly, individual muscles were dissected from origin to insertion and blotted dry, and muscle mass (M) was determined. Muscle length (L_m), defined as the distance from the origin of the most proximal muscle fibers to the insertion of the most distal muscle fibers, was measured using digital calipers (Mitutoyo Corporation CD-6CS, Japan) under a dissecting microscope (Wild model M3; Wild, Inc., Heerbrugg, Switzerland). Muscles were partially digested in 15 % H_2SO_4 for approximately 0.5 h to dissolve connective tissue and facilitate fiber dissection.

Three to six small fiber bundles (1–10 fibers) were then teased from the medial, lateral, superficial and deep regions of the tibialis anterior, the fourth and fifth toe muscles of the EDL and the entire soleus. No significant regional differences were detected within the tibialis anterior or soleus, and data from these muscles were therefore combined. Data from the EDL fourth and fifth toe muscles, making up the bulk of the EDL (Chleboun et al., 1988), were also combined to reflect the remodeling of the entire muscle. Bundle dissection was performed at 50× magnification, allowing identification of single fibers running completely from origin to insertion. Fiber bundles were then mounted on slides. Fiber length (L_f) was measured using a calibrated digital filar eyepiece (Lasico model 112983; Los Angeles, CA, USA) mounted on the dissecting microscope. Slides were illuminated from below to observe the relative opacity of tendon compared with muscle and, thus, accurately identify that an intact fiber was indeed obtained from tendon to tendon.

Sarcomere length (L_s) was measured from three regions of each dissected bundle using laser diffraction (Lieber et al., 1984) and averaged to provide the mean sarcomere length for that fiber. In several cases, where diffraction patterns were unusually weak, sarcomere lengths were measured optically using a phase-contrast microscope (Nikon Optiphot, Tokyo, Japan). The distance between 25–30 serial Z-bands at three sites along the bundle was measured with the filar eyepiece and divided by the corresponding number of sarcomeres to determine mean sarcomere length. To compare techniques quantitatively, sarcomere length from 10 randomly selected bundles that did generate diffraction patterns ($L_{s,LD}$) was also measured using phase-contrast microscopy ($L_{s,PC}$). Sarcomere lengths (ranging from approximately 1.9 μm to approximately 2.9 μm) were analyzed by linear regression and, in addition a one-sample t-test was used to compare the ratio $L_{s,LD}$:$L_{s,PC}$ with 1. Since sarcomere lengths obtained from the same fibers were highly correlated ($L_{s,PC}$=0.17+0.92$L_{s,LD}$, r^2=0.96, P<0.0001) and their ratio was not significantly different from 1 (P>0.2), the two methods were deemed equivalent. It should be noted that fiber bundles that did not yield strong diffraction patterns still possessed clear striations and were, therefore, adequately fixed. The basis for the weak diffraction patterns is not understood. All muscle and sarcomere lengths were normalized to a resting sarcomere length of 2.5 μm to permit comparisons among groups independent of the precise angle of muscle fixation.

Based on measured mass (M; g), fiber length (L_f; mm), sarcomere length (L_s; μm) and values in the literature for muscle density (ρ; g mm^{-3}) (ρ=0.001056 g mm^{-3}; Mendez and Keys, 1960) and pennation angle (θ) for the tibialis anterior (θ_{TA}=11.7 °), EDL (θ_{EDL}=8.3 °) and soleus (θ_{soleus}=8.5 °) (Burkholder et al., 1994), serial sarcomere number (SN) and physiological cross-sectional area (PCSA) were calculated using the following equations:

$$SN = L_f/L_s, \quad (1)$$

$$PCSA = M\cos\theta/\rho L_f. \quad (2)$$

Statistical analyses

Sample size (N) was calculated using an iterative formula based on the ratio of population standard deviation (σ) to the difference desired to detect (δ), number of groups (a=2), degrees of freedom [ν=a(N–1)], desired significance level (α=0.05), and desired type II error rate (β=0.1) using standard statistical equations (Sokal and Rohlf, 1981). A sample size of seven was calculated on the basis of a relatively conservative ratio of σ/δ=4/7, and a sample size of eight was calculated on

the basis of a ratio of 1/2; therefore, 7–9 animals were used per group. A sample size of seven was used only when muscle damage occurred during harvesting. Two-way analysis of variance (ANOVA) was used for each muscle to compare mean sarcomere number between immobilized and non-immobilized legs and between wild-type and knockout types (Statview 5.0; Abacus Concepts, Inc., Berkeley, CA, USA). Fisher's least-square-difference (LSD) test was used to make paired comparisons between specific functional groups following significant one-way ANOVAs.

Results

The mass of wild-type control and immobilized muscles was greater than that of their desmin-null counterparts for both the tibialis anterior and the EDL (P<0.02), but not for the soleus (P>0.3). Muscle mass in the tibialis anterior and EDL was significantly affected both by immobilization (P<0.001) and by the presence or absence of desmin (P<0.05), the latter finding implying an inherent difference in mass due to the knockout procedure. Immobilization also significantly affected muscle mass in the soleus (P<0.0001), although the presence of desmin did not (P>0.5). For all three muscles, there was no interaction between the grouping factors (P>0.2), indicating that the dramatic loss of muscle mass due to immobilization was independent of the presence of desmin. As expected, immobilization also had a significant effect on fiber length, causing lengthening of the tibialis anterior and EDL and shortening of the soleus (two-way ANOVA; P<0.003). The presence of desmin, however, did not affect fiber length in the tibialis anterior or soleus (P>0.3), but an almost significant effect was recorded in the EDL (P=0.098). These measurements of muscle mass and length are summarized in Table 1.

Taking into account the varying effects of the presence of desmin on mass and fiber length, physiological cross-sectional area was calculated as a collective measure of muscle size based on fiber organization and mass (Table 1). This calculation served as a more reliable indicator of atrophy, was a better predictor of maximum force potential than mass and provided a quantitative measure of parallel adaptation within each muscle. Each of the three muscles, both wild-type and desmin-null, experienced a significant reduction in physiological cross-sectional area as a result of immobilization (P<0.0001), with the magnitude of the reduction being dependent on the muscle. The reduction in physiological cross-sectional area was most dramatic in the soleus, with the wild-type decreasing by approximately 25 % and the knockout by approximately 44 %. The physiological cross-sectional area of the wild-type EDL was reduced by approximately 29 % and that of the knockout by approximately 36 %, while those of the tibialis anterior in both wild-type and desmin-null muscles decreased by approximately 25 %. A significant effect of the presence of desmin on physiological cross-sectional area was revealed in the tibialis anterior and EDL, although not in the soleus (tibialis anterior, P<0.0001; EDL, P<0.01; soleus, P>0.5). For all three muscles, there was no interaction between the grouping factors (tibialis anterior, P>0.4; EDL, P>0.1; soleus, P>0.3), indicating that the decrease in physiological cross-sectional area due to immobilization was independent of the presence of desmin. These results are similar to those observed in the analysis of muscle mass, suggesting that mass, rather than fiber length or fiber organization, dominated the calculation of physiological cross-sectional area. The findings also suggest that, although inherent differences in size in the larger tibialis anterior and EDL were related to the presence of desmin, the absence of the desmin gene did not seem to affect the ability of the muscle to undergo parallel remodeling.

Serial sarcomere number provided a measure of stretch-induced remodeling, or serial adaptation within each muscle. As expected, the wild-type tibialis anterior and EDL added sarcomeres serially upon immobilization in a lengthened position. Surprisingly, chronically lengthened desmin-null muscles also added sarcomeres serially (Fig. 2A,B). The

Table 1. *Architectural changes in muscles from wild-type and desmin knockout mice*

		Wild-type		Desmin knockout	
Muscle	Variable	Control	Immobilized	Control	Immobilized
Tibialis anterior	Mass (g)	39.7±2.6†,*	32.1±4.5*	32.4±3.5†	27.2±1.9
	L_f (mm)	8.1±1.0†	8.9±0.6	8.3±0.7†	9.1±0.5
	PCSA (mm^2)	4.60±0.54†,*	3.40±0.62*	3.64±0.45†	2.76±0.19
Extensor digitorum longus	Mass (g)	7.9±0.6†,*	6.6±0.8*	7.1±0.6†	5.7±0.5
	L_f (mm)	5.5±0.6†	6.4±0.4*	5.5±0.5†	6.9±0.6
	PCSA (mm^2)	1.37±0.17†	0.97±0.13*	1.21±0.16†	0.78±0.11
Soleus	Mass (g)	6.3±1.1†	3.5±0.6	6.8±0.9†	3.3±0.7
	L_f (mm)	6.2±0.5†	4.6±0.3	6.1±0.9†	5.2±0.4
	PCSA (mm^2)	0.95±0.17†	0.71±0.16	1.07±0.19†	0.60±0.17

Fiber lengths (L_f) are normalized to a resting length of 2.5 μm. These values were then used for all calculations of physiological cross-sectional area (PCSA). Data are presented as means ± S.D. (N=7–9 per group).

†Control significantly different from immobilized (P<0.05).

*Wild-type significantly different from knockout (P<0.05).

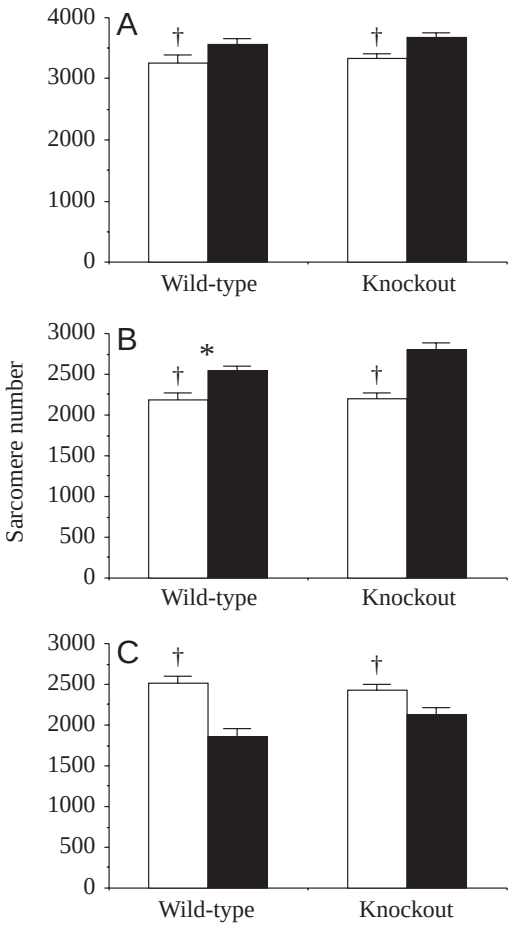

Fig. 2. Serial sarcomere number in (A) tibialis anterior, (B) extensor digitorum longus and (C) soleus muscles from wild-type and desmin knockout mice. Open columns represent control (contralateral) muscles, while filled columns represent muscles immobilized with the ankle plantarflexed. Data are plotted as means + S.E.M. (N=7–8). Symbols above the columns represent statistically significant differences (P<0.05): † control significantly different from immobilized; *wild-type significantly different from knockout.

magnitude of sarcomere addition was muscle-specific, with the wild-type tibialis anterior exhibiting an increase of approximately 9% compared with an increase of approximately 17% in serial sarcomere number in the wild-type EDL, and the knockout tibialis anterior displaying an approximately 10% increase compared with an approximately 27% increase in the knockout EDL. Thus, immobilization had a significant effect on sarcomere number for lengthened muscles (tibialis anterior, P<0.005; EDL, P<0.0001), although there was no effect of the presence of desmin on serial sarcomere number (tibialis anterior, P>0.3; EDL, P<0.05). A consistent result was observed in the soleus, where a significant decrease in sarcomere number was observed in both wild-type (approximately 26%) and knockout (approximately 12%) muscles as a result of immobilization (Fig. 2C; P<0.0001). As with the lengthened muscles, the presence of desmin did not significantly affect sarcomere number in the soleus (P>0.3). No significant interaction between the effects of immobilization and the presence of desmin on the addition of sarcomeres was observed in the tibialis anterior and the EDL (tibialis anterior, P>0.7; EDL, P>0.1). However, a marginally significant interaction (P=0.048) between the effects of grouping factors on deleting sarcomeres was observed in the soleus, suggesting that the presence or absence of the desmin gene produced a differential response of the soleus to immobilization in a shortened position.

Discussion

The serial and parallel arrangement of sarcomeres is the primary influence on the force/length and force/velocity characteristics of a muscle. This arrangement is tightly regulated, as shown by the relative lack of variation in fiber length and arrangement within a particular muscle of a given species and the corresponding association between architecture and functional muscle groups (Burkholder et al., 1994; Friederich and Brand, 1990; Lieber and Blevins, 1989). Despite the wealth of descriptions of muscle plasticity, molecular mechanisms underlying sarcomere remodeling are not well understood. The use of the highly reproducible model of immobilization in conjunction with a gene-knockout system allowed us to test the hypothesis that desmin plays a critical role in sarcomere number regulation. This hypothesis was proposed in the light of the important structural and organizational role played by desmin and the conflicting data published regarding its regulatory role during myogenesis (Li et al., 1994; Li et al., 1997; Milner et al., 1996; Schultheiss et al., 1991).

The validity of our experimental model was determined by comparing the effects of immobilization with those described in the existing immobilization and desmin-null literature. General trends of sarcomere number addition and subtraction were similar to those observed by Williams and Goldspink (Williams and Goldspink, 1973; Williams and Goldspink, 1971) in the mouse soleus and by Tabary et al. (Tabary et al., 1972) in the cat soleus. Quantitative differences between the results for the mouse soleus and cat soleus probably reflect differences between species. Parallel remodeling responses were also compared with reports in the literature, and magnitudes of reductions in muscle cross-sectional area were comparable with those reported by Spector et al. (Spector et al., 1982) in the rat tibialis anterior and soleus. Our data obtained from contralateral wild-type and desmin-null muscles were also consistent with the differences in mass and physiological cross-sectional area reported in our previous functional study of this same knockout strain (Sam et al., 2000).

Calculations of sarcomere numbers may be subject to error as a result of morphological abnormalities in the desmin knockout system. Serial sarcomere number was calculated by dividing fiber length by mean sarcomere length measured in selected regions along the fiber. These calculations assume that sarcomeres are consistent along the fiber length. On the basis

of available desmin knockout morphological data, it is likely that errors in sarcomere number resulting from this limitation would occur primarily in the soleus, rather than in the faster EDL or tibialis anterior muscles. This result would indicate that, although Z-band striations in the knockout may not be as sharp as in the wild-type muscle, they would reflect an accurate regional mean sarcomere length under the measurement protocols utilized in this study and, thus, would not significantly affect serial sarcomere number results.

No statistically significant difference in serial sarcomere addition was observed in the tibialis anterior or EDL between wild-type and knockout muscles. If anything, lengthened knockout muscles added more sarcomeres than the corresponding wild-type muscles, suggesting an alteration in factors that control sarcomere addition (Fig. 2A,B). These findings are consistent with the results from soleus muscles that lost sarcomeres as a result of chronic shortening. Significant serial deletion of sarcomeres after immobilization was observed in both wild-type and desmin-null muscles in the soleus, indicating the ability of both to adapt to the shortened length (Fig. 2C). However, there was a marginally significant difference in the degree of adaptation between the knockout and wild-type soleus muscles, the former tending to delete fewer sarcomeres than the latter. These results suggest subtle differences in the nature of serial sarcomere number adaptation. A disparity in the regulation of sarcomere organization between wild-type and desmin-null muscles was also suggested by results of parallel adaptation because knockout muscles in the soleus and EDL demonstrated a greater reduction in physiological cross-sectional area compared with the respective wild-type muscles (Table 1). Thus, while desmin is not essential for sarcomere addition or subtraction, it may play a role in regulating the optimal arrangement of sarcomeres within the muscle or in sensing the magnitude of the immobilization effect itself. A tightly regulated scheme of sarcomere organization in wild-type muscles can be envisioned on the basis of the well-defined structural role of desmin. Ingber and colleagues describe a role for cytoskeletal elements in a tensegrity model, a scheme in which each mechanical linkage is assumed to be optimally stretched and stressed, providing well-defined force and displacement boundary conditions (Ingber, 1994; Wang and Ingber, 1994). Disruption of cytoskeletal elements could lead to an altered loading scheme for elements within a cell, such as the nucleus (Maniotis et al., 1997), possibly affecting transcriptional regulation.

Given the relationship between sarcomere arrangement and muscle performance, confirmation of a role for desmin in regulating sarcomere organization or sensing the magnitude of strain in a muscle would also be relevant in characterizing post-immobilization muscle function. Several reports have shown that, under typical loading conditions, not only does the desmin knockout mouse exhibit significant myofibrillar misalignment at the electron microscope level but also a loss in force-generating ability and a reduction in endurance (Milner et al., 1996; Li et al., 1997; Sam et al., 2000). Thus, subtle differences in sarcomere organization in the absence of desmin could further influence the mechanical properties of desmin-null muscle upon completion of remodeling.

The fact that desmin is not essential for sarcomerogenesis in this immobilization model may indicate that its early appearance during muscle development and its localization within satellite cells simply reflect a correlation in time rather than a causative event. However, because of redundancy in the expression of myogenic regulatory factors (for reviews, see Arnold and Braun, 2000; Megeney and Rudnicki, 1995), the possibility exists that an association between desmin and myogenic regulatory factor could be masked or compensated for by over-expression of another myogenic regulatory factor. For example, if loss of desmin inhibited the expression of MyoD or myogenin (Li et al., 1994), it is conceivable that Myf-5 or mrf4 expression could increase in compensation. The overlap in function between MyoD and Myf-5 and between myogenin and mrf4 has been well documented in MyoD-null mice, in which Myf-5 expression is compensatorily upregulated (Rudnicki et al., 1992), and in mrf4-null mice, in which myogenin expression is compensatorily upregulated (Zhang et al., 1995). A potential relationship between desmin and the myogenic regulatory factors is also consistent with the recent observations of Zador et al. (Zador et al., 1999), who detected both an increase in desmin expression and myogenic regulatory factor transcript expression within 3 days of immobilization in the rat.

Although our results reveal a non-essential role for desmin in sarcomerogenesis, our model offers a reliable system in which to make a further assessment of the role of desmin in muscle plasticity. For example, analysis of myogenic regulatory factor expression at various time points after immobilization may clarify which, if any, myogenic regulatory factors are affected by desmin. In addition, while no known intermediate filaments are implicated in a compensatory structural role in the unperturbed desmin-null model (Milner et al., 1996), differences in expression of various structural proteins in response to an atypical loading environment may be assessed. The ability to gauge, in one animal, the response of multiple muscles differing in size, function and composition provides the potential to dissect the effects of a variety of experimental factors on the remodeling response. Furthermore, our experimental model may be readily adapted for use with existing knockout mice lacking various combinations of myogenic regulatory factors, helping to clarify their role in the largely uninvestigated field of sarcomerogenic control.

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