Interaction of chronic creatine depletion and muscle unloading: effects on postural and locomotor muscles

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Adams, Gregory R., Fadia Haddad, and Kenneth M. Baldwin. Interaction of chronic creatine depletion and muscle unloading: effects on postural and locomotor muscles. *J. Appl. Physiol.* 77(3): 1198–1205, 1994.—In some rodent skeletal muscles, hindlimb non-weight-bearing activity induces a shift in the expression of myosin heavy chains (MHCs) that favors the type II isoforms at the expense of type I. Chemically induced chronic creatine depletion results in isomyosin shifts favoring expression of type I MHCs. In this study, creatine depletion was induced separately and in combination with non-weight-bearing activity to determine if the response to lowering this metabolite would counter the MHC transitions expected from non-weight bearing. Creatine depletion was induced by feeding rats a diet supplemented with the creatine analogue β-guanidinopropionic acid (β-GPA). Female Sprague-Dawley rats weighing 247 ± 8 g were randomly assigned to four groups: *1*) normal diet control, *2*) β-GPA control (BC), *3*) normal diet suspended (NS), and *4*) β-GPA suspended (BS). BC and BS animals were fed a diet containing the creatine analogue for 68 days. Hindlimb non-weight bearing in BS and NS animals was accomplished by tail suspension for the final 30 days of this period. β-GPA feeding lowered the creatine content of muscles sampled by 65%. Creatine depletion resulted in a 16% increase in citrate synthase activity in the soleus (SOL) and a 24% increase in the plantaris (PLN). In two postural muscles, the SOL and vastus intermedius (VI), tail suspension resulted in large decreases in the type I MHC expression and increases in type IIx and IIb MHCs. In two locomotor muscles, the PLN and medial gastrocnemius, type I MHC declined and type IIb increased with suspension. Creatine depletion did not prevent the suspension-induced decline in type I MHC in any of these muscles. The increase in type IIb MHC was either prevented or reduced by creatine depletion before and during suspension in the SOL, VI, and PLN. Creatine depletion alone (BC group) resulted in small increases in type I and IIa MHCs in the two locomotor muscles, but it had no effect on the MHC profile of the postural muscles studied. These results indicate that, in the adult rodents used in this study, the mechanical signal generated by the hindlimb non-weight-bearing state dominated over the metabolic stimulus of creatine depletion with respect to the primary adaptation involving a reduction in type I MHC.

myosin messenger ribonucleic acid; types I, IIa, IIx, IIb isomyosin; myosin heavy chain transformation; β-guanidinopropionic acid; creatine analogue; tail suspension; hindlimb suspension

MATURE SKELETAL MUSCLE fibers can be broadly characterized as fast or slow on the basis of contractile properties. The shortening velocity of a myofiber is primarily conferred by the myosin heavy chain (MHC) isoform expressed. Adult mammalian skeletal muscle fibers have been shown to express at least four types of MHC: slow (type I) and fast (types IIa, IIx, and IIb) (32). MHCs can be identified on the basis of electrophoretic mobility and by immunohistochemistry, using isoform specific monoclonal antibodies (32). Type I MHC predominates in muscle fibers used primarily for antigravity functions. The three faster MHCs are expressed in regions of muscle thought to be used extensively during sustained locomotion (types IIa and IIx) and high-power-output activity (types IIb and IIx) (22, 25).

Some myofibers demonstrate a high degree of plasticity with respect to native myosin or MHC phenotype. Factors that have been shown to elicit changes in MHC expression include chronic alterations in mechanical activity patterns (e.g., unloading or increased loading) (1, 5, 7, 33), a change in hormone status (e.g., hypo- or hyperthyroid) (2, 3), and metabolic alterations (e.g., creatine depletion) (15, 19).

In rats, an alteration in mechanical activity pattern via tail suspension to produce hindlimb non-weight-bearing activity results in changes in the fiber-type profile of selected populations of muscle cells (5, 33). Several studies have demonstrated that non-weight-bearing activity results in a reduction in the proportion of slow native myosin or type I MHC in the isomyosin pool in a variety of rodent hindlimb muscles (5, 33).

In contrast to non-weight-bearing activity, the metabolic intervention of chronic creatine depletion has been reported to induce native isomyosin and MHC isoform shifts to a slower phenotype (18, 19). This intervention is interesting in that the MHC shifts occur in a model that does not appear to involve an alteration in load-bearing activity. One direct effect of creatine depletion involves a sharp decline in intracellular phosphocreatine levels. We speculate that some regulatory constituent within the myofiber may interpret this chronic decline in phosphocreatine as an indication of increased contractile activity.

Several studies have indicated that the isomyosin shifts resulting from hindlimb non-weight-bearing activity can be partially blocked by the imposition of relatively short periods of weight bearing, running, or loaded contractile activity (7, 33). The possibility that creatine depletion may result in the generation of signals similar to those produced by contractile activity suggests that this intervention might also impact MHC shifts induced via a hindlimb non-weight-bearing state.

The primary aim of the current study was to examine the separate and combined effects of hindlimb non-weight-bearing activity and creatine depletion on MHC phenotype, studied at both the protein and mRNA level of analysis. We examined the working hypothesis that the metabolic alterations associated with creatine depletion would predominate over the mechanical factors with respect to MHC phenotypic shifts in the adapting myofibers. Several muscles with widely differing fiber-type profiles and functional roles were examined.

The results indicate that creatine depletion did in fact blunt the shift toward expression of the type IIb MHC in several muscles with either postural or locomotor roles.

However, creatine depletion had no effect on either the atrophy response or downregulation of type I MHC expression associated with hindlimb non-weight-bearing activity.

METHODS

Female Sprague-Dawley rats weighing 247 ± 8 g were randomly assigned to four groups: *1*) normal diet control (NC), *2*) β-guanidinopropionic acid (β-GPA) diet control (BC), *3*) normal diet suspended (NS), and *4*) β-GPA suspended (BS).

β-GPA diet. β-GPA was synthesized from β-alanine and cyanamide (Sigma Chemical, St. Louis, MO) (24). The β-GPA-supplemented diet consisted of standard rat chow in a powdered form with 1% (wt/wt) β-GPA added. Rats in the normal diet groups (NC and NS) were fed the same powdered diet without the β-GPA. All animals were housed in standard vivarium cages and were allowed food and water ad libitum.

The BC and BS groups received the β-GPA diet for 40 days to allow intracellular accumulation of the phosphorylated analogue. After this period the NS and BS animals were tail suspended for a period of 31 ± 0.4 days (see below). The BC and BS groups were maintained on the β-GPA-supplemented diet throughout this time.

Tail suspension. A bilateral hindlimb non-weight-bearing state was produced by tail suspension. Animals were suspended for 31 ± 0.4 days using the tail cast procedures described by Thomason et al. (33). Briefly, a swivel harness was attached to the tail and covered by an elastic bandage, and a casting material was applied. The most distal portion of the tail was left uncovered to facilitate thermoregulation. A swivel harness incorporated into the cast was attached to a hook on the top of the cage that was adjusted to allow only the front legs of the animal to reach the floor. Suspended animals were free to move about the cage using their front legs.

At the end of the treatment period animals from all four groups were anesthetized with pentobarbital sodium and the soleus (SOL), plantaris (PLN), medial gastrocnemius (MG), vastus intermedius (VI), and tibialis anterior (TA) muscles were excised from one leg, quick frozen, and stored at −70°C for RNA extraction. The same muscles from the opposite leg were then excised, trimmed of fat and connective tissue, weighed, placed in precooled glycerol, and stored at −20°C for subsequent myosin determination. The vastus lateralis (VL) muscle from one leg of each animal was also excised and quick frozen for the determination of creatine content.

Myofibril extraction. Muscle samples were homogenized in ~20 vol of a solution containing (in mM) 250 sucrose, 100 KCl, and 5 EDTA. The homogenate was washed successively in three solutions: *1*) 250 mM sucrose, 100 mM KCl, and 5 mM EDTA; *2*) 0.5% Triton X and 175 mM KCl; and *3*) 150 mM KCl. The final pellet was resuspended in 1 ml of 150 mM KCl. The protein concentration of this solution was determined by using the biuret method (9). An aliquot of myofibril suspension was added to a solution containing 50% vol/vol glycerol, 100 mM $Na_4P_2O_7$, and 5 mM EDTA at a concentration of 1 mg/ml and stored at −20°C.

Myosin analysis. The myofibril fraction was analyzed to determine relative content of type I, IIa, IIx and IIb MHCs in the muscles harvested. Five-microliter samples of stored myofibril solution were added to 35 μl of denaturing buffer [62.5 mM tris(hydroxymethyl)aminomethane, 20% glycerol, 1% β-mercaptoethanol, 2.3% sodium dodecyl sulfate (SDS), 0.05% bromophenol blue] and heated at 100°C for 2 min. After this treatment a 16-μl aliquot (2 μg) was withdrawn for analysis by SDS-polyacrylamide electrophoresis (SDS-PAGE).

Samples were analyzed via SDS-PAGE as described by Talmadge and Roy (30). Separating gels contained 30% vol/vol glycerol, 8% wt/vol acrylamide (2% cross-link), 1% wt/vol glycine, and 0.4% wt/vol SDS. Stacking gels contained 40% vol/vol glycerol and 4% wt/vol total acrylamide (2.7% total acrylamide). Gels were run at 275 V for ~22 h. Gels were stained for 1 h with Brilliant Blue G 250 dye (Sigma Chemical) and then were destained with 25% methanol and 5% acetic acid. MHC bands were scanned with a laser densitometer (Molecular Dynamics, Sunnyvale, CA). The peaks of interest were identified in the digitized densitometric data sets, and the area of each peak was determined by integration.

RNA isolation and blotting. Total RNA was isolated from skeletal muscles using the RNAzol method (TEL-TEST, Friendswood, TX), which is based on the method described by Chomczynski (4). After extraction the RNA pellet was suspended in 0.5% SDS. RNA concentration was measured by optical density at 260 nm, and samples were stored at −20°C until later analyses. In this study, slot blots were used to analyze the relative expression of each of the MHC mRNA using synthetic oligonucleotide probes. RNA (4–5 μg) was denatured and loaded directly on a nylon membrane (GeneScreen Plus, New England Nuclear, Boston, MA) using a filtration manifold apparatus (Schleicher and Schuell, Keene, NH) and then fixed by ultraviolet irradiation. Blots were dried at 80°C for 30 min and then stored dry at 4°C until subsequent hybridization.

RNA analysis using synthetic probes. Four different synthetic oligonucleotides, 20 bases in length, were purchased from Chemgene (Waltham, MA). These oligonucleotides are complementary to the 3′ nontranslated sequences of rat skeletal muscle MHC mRNA isoforms and are highly specific for the various MHC isoforms expressed in skeletal muscle. The sequences for the oligonucleotides used for MHC types I, IIa, and IIb were as reported by Gustafson et al. (10). For MHC type IIx, we used a 20-base oligonucleotide complementary to a portion of the 3′ nontranslated sequence of a cDNA clone isolated by S. Schiaffino (University of Padua, Padua, Italy) from rat diaphragm muscle (12; B. Russell, personal communication). The sequence of this probe is 5′ GGTCACTTTCCTGCTTTGGA 3′. Probes were labeled at the 5′ end to a high specific activity, 1–2 × 10^9 counts · min^{-1} · μg^{-1}, using polynucleotide kinase and [γ-^{32}P]ATP (16). Labeled probes were separated from unincorporated ATP by gel filtration through a Sephadex G_{25} minicolumn (Quick spin G_{25}, Boehringer Mannheim, Indianapolis, IN). RNA blots were prehybridized and hybridized with the MHC probes as described previously (6). After autoradiography, the probes were stripped from the blots by boiling 10–15 min in 1% SDS. These same blots were then prehybridized and hybridized with a ^{32}P-labeled oligo(dT) probe, which hybridizes to poly(A) RNA (mRNA). Autoradiogram band intensity was quantitated using laser scanning densitometry (Molecular Dynamics), and each specific MHC absorbance was normalized to its corresponding oligo(dT) signal.

Creatine assay. Frozen VL muscles were pulverized in liquid N_2 and extracted in perchloric acid (13). Creatine was assayed by using standard enzymatic techniques (13).

Citrate synthase (CS) assay. To obtain tissue for CS analysis, a separate group of 18 rats was divided into two groups. All rats from each group were housed in standard vivarium cages and allowed food and water ad libitum for 70 ± 2 days. During this time the diet of one group of nine animals was supplemented with 1% β-GPA. The SOL and PLN muscles of these animals were removed, weighed, and placed in precooled glycerol and stored at −20°C. Muscle samples for CS determination were homogenized in a solution containing 175 mM KCl, 10 mM glutathione, and 2 mM EDTA at a pH of 7.4. CS activity was measured as described by Srere (27).

Statistical analyses. All values are reported as means ± SE. Group and treatment effects were determined by multivariate analysis of variance (ANOVA) and Student-Newman-Kuels

TABLE 1. *Effect of metabolic and mechanical manipulations on body weights, muscle weights, creatine content, and citrate synthase activity*

	NC	BC	NS	BS
Body weight, g	314±8	283±3*	286±8*	254±4*
Muscle weight, mg				
SOL	141±5.9	122±5.3*	61±2.8*†	48±4.2*†
VI	89±3.4	92±3.8	61±4.9*†	58±4.7*†
PLN	283±6.8	253±7.8*	252±10.5	207±10.6*†‡
MG	676±15.7	592±7.9*	595±33.1*	485±13.6*‡
TA	537±12.1	469±13.4*	479±19.7*	386±15.0*‡
Muscle weight, mg/g body wt				
SOL	0.45±0.01	0.43±0.02	0.21±0.01*†	0.19±0.02*†
VI	0.29±0.02	0.33±0.01	0.21±0.02*†	0.23±0.02*†
PLN	0.90±0.03	0.90±0.02	0.88±0.03	0.82±0.04
MG	2.15±0.05	2.09±0.04	2.07±0.09	1.91±0.04*
TA	1.71±0.02	1.66±0.05	1.67±0.05	1.52±0.04*
Creatine content, μmol/g wet wt				
VL	9.6±0.9	3.2±0.2*	9.0±0.5†	3.3±0.2*‡
Citrate synthase activity, $\mu mol \cdot g^{-1} \cdot min^{-1}$				
SOL	36±1	42±2*		
PLN	33±1	41±2*		

Values are means ± SE. NC, normal diet control; BC, β-guanidinopropionic acid (β-GPA) control; NS, normal diet suspended; BS, β-GPA suspended; SOL, soleus; VI, vastus intermedius; PLN, plantaris; MG, medial gastrocnemius; TA, tibialis anterior; VL, vastus lateralis. * $P < 0.05$ vs NC. † $P < 0.05$ vs. BC. ‡ $P < 0.05$ vs. NS.

post hoc tests using the Instat software package (Graphpad Software, San Diego, CA). This approach tested for differences between the means of all four groups and thus examined the efficacy of the entire β-GPA feeding treatment (pretreatment plus during tail suspension) in countering MHC shifts resulting from non-weight-bearing activity. In addition, two-by-two factor ANOVAs were conducted to elucidate direct load (ambulatory vs. non-weight-bearing activity), diet (standard vs. β-GPA supplemented), and diet-load interactions. These tests block the β-GPA groups (BC, BS) and the normal diet group (NC, NS). Using this test, significant diet vs. load interactions should be found only when nonparallel changes (e.g., from BC to BS) in MHC percentage occur. Applied together, these two tests indirectly indicate when changes seen in the BS group were most probably a result of MHC shifts starting in the prefeeding period (see RESULTS). The 0.05 level of confidence was accepted for statistical significance. Analyses of all percent data were performed on arcsine-transformed values to correct for nonnormal distribution. Percent data presented represent the nontransformed values.

RESULTS

Creatine depletion (BC vs. NC). As reported in similar studies, β-GPA feeding resulted in a significant reduction in the creatine content of VL muscles relative to control values (Table 1) (14, 17). Creatine depletion induced a 16% increase in CS activity in the SOL and a 24% increase in the PLN muscles (Table 1). As previously reported, the β-GPA-fed animals (BC and BS) tended to have lower body weights (≈10%) than the respective control groups (Table 1) (14, 17, 19). Collectively these data are consistent with the known effects of β-GPA feeding.

With regard to the MHC profile, the results can be separated into distinct patterns apparently corresponding to the functional roles of the muscles involved. In the two weight-bearing locomotor muscles (PLN and MG), β-GPA feeding resulted in small but significant increases in types I and IIa MHCs (Table 2, Figs. 1 and 2). In contrast, the distribution of MHCs in the two postural muscles (SOL and VI) was unaffected by creatine depletion (Table 2, Figs. 1 and 2). With the exception of a small increase in the type I MHC in the BS group, the MHC profile of the TA, a non-weight-bearing ankle flexor, was unaffected by any of the interventions employed in this study. In each case significance of the diet effect was found using either a fully factorial ANOVA (Table 2) or the two-by-two blocked design (not shown).

Type IIb MHC mRNA was significantly decreased by

TABLE 2. *Effect of metabolic and mechanical manipulations on MHC isoform distribution*

	Type I	Type IIa		Type IIx	Type IIb
VI					
NC	75.9±1.3	17.0±1.0		7.1±0.7	ND
BC	75.3±1.7	18.3±1.7		6.4±0.4	ND
NS	45.3±2.2*†	15.3±2.4		25.3±1.8*†	14.1±1.4*†
BS	49.6±2.2*†	19.4±1.7		22.8±1.5*†	8.2±1.7‡
SOL					
NC	93.6±1.3	6.2±1.3	§	0.24±0.22	ND
BC	93.6±3.5	6.3±2.4		0.08±0.08	ND
NS	73.2±3.4*	11.8±1.8		11.1±1.3*†	4.0±1.9*†
BS	70.4±3.5*†	12.3±1.6		16.6±1.8*†‡	0.7±0.5‡
PLN					
NC	3.7±0.3	14.4±1.0		43.5±2.7	38.4±3.4
BC	5.4±0.4*	17.6±1.0*		44.7±1.1	32.3±2.1
NS	2.5±0.3*†	10.9±0.6*†		38.4±1.4	48.1±1.8*
BS	2.8±0.2*†	13.2±1.7†		42.6±1.0	41.4±1.4†‡
MG					
NC	6.5±0.3	7.2±0.5		25.2±1.4	61.2±1.9
BC	8.5±0.5*	9.2±0.5*		25.3±0.6	57.1±0.8
NS	3.3±0.2*†	7.4±0.6†		21.0±1.4	68.4±1.6*†
BS	3.7±0.3*†	7.6±0.3†		22.7±1.5	66.0±1.2*†
TA					
NC	0.6±0.2	6.1±1.8		22.2±0.6	71.1±2.0
BC	0.4±0.2	5.6±1.7		23.1±1.3	70.9±1.7
NS	1.2±0.3	7.8±0.3		20.2±0.7	70.8±0.7
BS	2.2±0.5*†‡	7.4±1.4		24.1±1.7	66.3±1.6

Values are means ± SE. MHC, myosin heavy chain; ND, none detected. * $P < 0.05$ vs. NC. † $P < 0.05$ vs. BC. ‡ $P < 0.05$ vs. NS. § Significant load effect, $P < 0.05$ (2×2 analysis of variance).

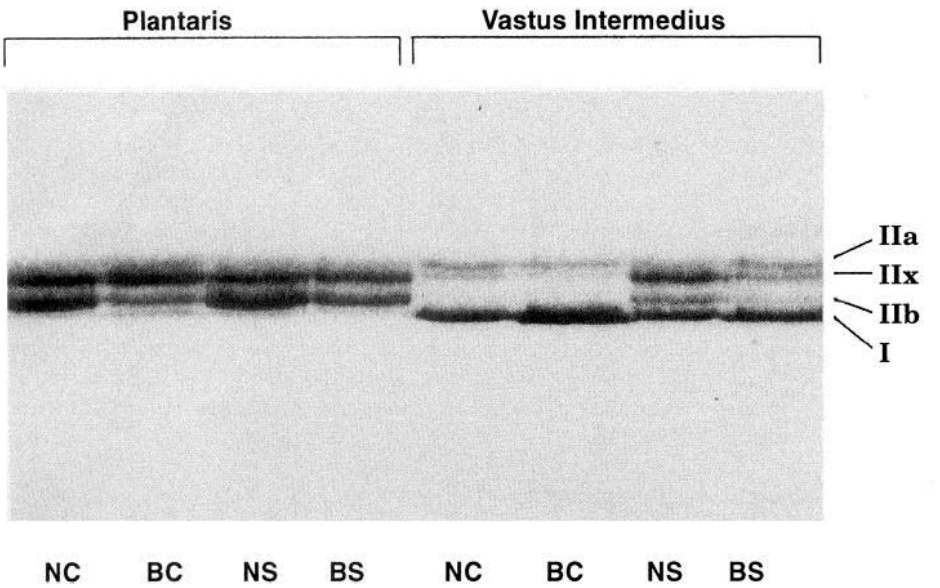

FIG. 1. Electrophoretograph showing sets of myosin heavy chain (MHC) bands from representative plantaris and vastus intermedius muscles by sodium dodecyl sulfide-polyacrylamide gel electrophoresis. NC, normal diet control; BC, β-guanidinopropionic acid (β-GPA) diet control; NS, normal diet suspended; BS, β-GPA diet suspended. MHCs by increasing electrophoretic mobility: IIa, IIx, IIb, I.

creatine depletion in the PLN and MG muscles (Fig. 2 and data not shown). In the postural muscles, type I MHC mRNA increased in the SOL (4.3 ± 0.3 vs. 2.6 ± 0.7 units), whereas type IIx MHC mRNA declined in the VI (Fig. 2).

Hindlimb non-weight-bearing activity (NC vs. NS). Hindlimb non-weight-bearing activity resulted in a significant reduction in the absolute and relative weights of the VI and SOL muscles (Table 1). However, the normalized wet weights of the faster locomotor muscles were not

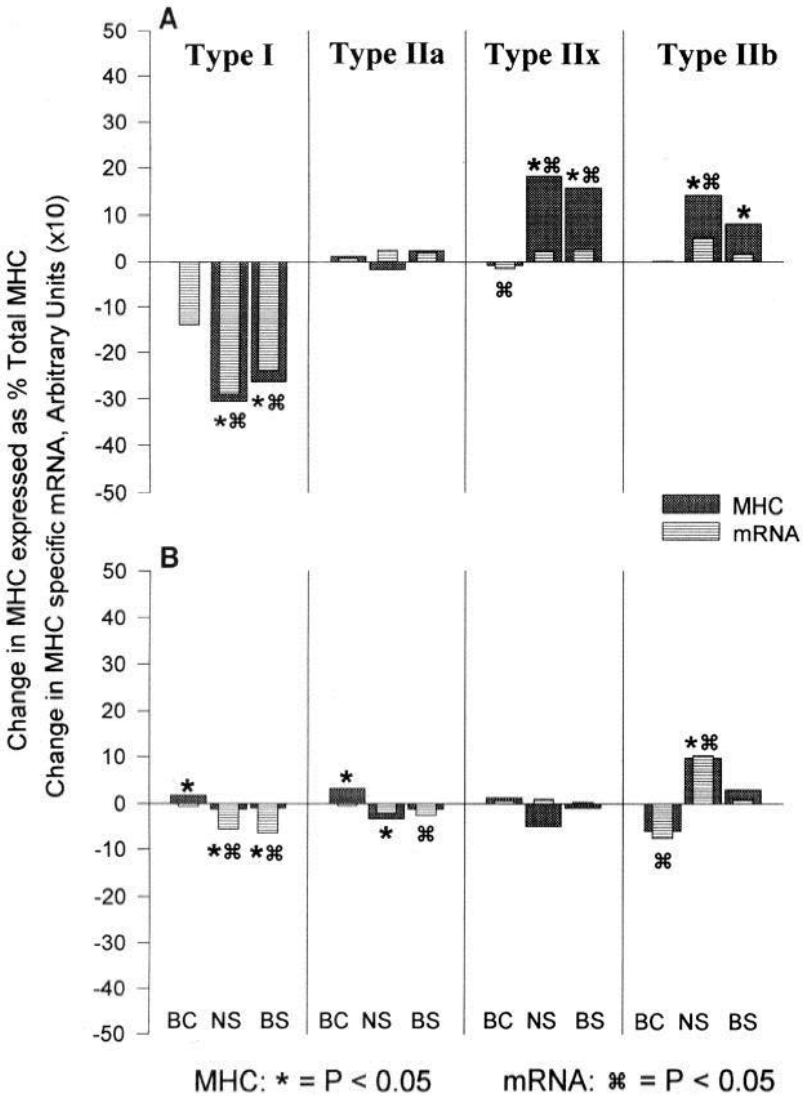

FIG. 2. Absolute change from NC group in MHC type expressed as percentage of total MHC and MHC-specific mRNA normalized to poly(A) RNA for vastus intermedius (*A*) and plantaris (*B*).

significantly altered by the non-weight-bearing activity protocol (Table 1).

In non-weight-bearing PLN and MG muscles the normally low proportion of type I MHC was further suppressed as was type IIa MHC in the PLN (Table 2, Figs. 1 and 2). These changes were accompanied by increases in type IIb MHC (Table 2, Fig. 1). In the SOL and VI, non-weight-bearing activity caused type I MHC to decline by 22 and 40%, respectively (Table 2, Figs. 1 and 2). In the VI the decline in type I MHC was offset by increases that were distributed fairly evenly between the type IIb and IIx MHCs (Table 2, Figs. 1 and 2). In the SOL the decline in type I MHC was reflected by an increase in type IIb, IIx, and IIa MHC (Table 2, Figs. 1 and 2). In each case significant shifts in MHC induced by non-weight-bearing activity were mirrored by changes in the corresponding MHC-specific mRNA levels (Fig. 2 and data not shown).

Creatine depletion vs. non-weight-bearing activity (BS vs. NS). The SOL and VI muscles of the BS group experienced the same degree of atrophy as did those from the NS group (Table 1). The MG and TA muscles from the BS group experienced a small degree of atrophy compared with the NC group, but these values were not statistically different in normalized weight from the NS group (Table 1).

The decrease in type I MHC induced by non-weight-bearing activity was similar in both the NS and BS groups across the muscles analyzed. Thus creatine depletion did not alter the primary MHC shift resulting from this intervention in either the postural or locomotor muscles studied. However, in SOL and VI, β-GPA feeding partially counteracted the increase in IIb MHC seen in the NS group (Table 2, Fig. 2). This result was a direct effect of β-GPA feeding in that a significant diet vs. load interaction was also seen. In the SOL and VI muscles the bulk of the shift from type I MHC expression was accounted for by increases in either the type IIx or IIa MHCs (Table 2, Fig. 2). In contrast to the SOL and VI, type IIx MHC proportions were unaffected in the faster PLN and MG muscles of both suspension groups (Table 2, Fig. 2). In a result similar to that seen in the slower postural muscles, the suspension-induced increase in IIb MHC of the PLN was apparently opposed by the creatine depletion protocol (Table 2, Fig. 2). This effect was probably a result of MHC shifts that occurred during the 40-day prefeeding period, in that no significant diet vs. load interaction was found using the blocked ANOVA (note that type IIb appeared to be trending down in the BC group; Table 2). A similar result can be seen with type IIa MHC in the PLN where prefeeding with the β-GPA diet shifted the isoform profile (BC) such that the BS group was not different from NC using comparison of means in the factorial ANOVA, whereas a blocked ANOVA revealed no diet vs. load interaction.

As with the NS group, significant changes in MHC were mirrored by changes in MHC-specific mRNA signals (e.g., Fig. 2).

DISCUSSION

Creatine depletion model. The function of muscles from chronically creatine-depleted animals has been reported to be either impaired (21), unaltered (14), or enhanced (17, 20) depending on the type of muscle, the type of measurement, and the conditions under which the data were collected. In the most complete characterization published to date, Petrofsky and Fitch (21) reported that the SOL muscles of rats fed β-GPA exhibited slower twitches, decreased maximal velocity of shortening, and attained maximal isometric tetanic tension at lower stimulation frequencies (21). The PLN muscles from the same study were relatively unaffected by creatine depletion with respect to their force-velocity, force-frequency, and force-length relationships. Several studies have reported that SOL muscles from creatine-depleted animals demonstrate some measure of increased endurance (17, 20).

Creatine depletion has also been reported to alter the metabolic profile of muscles (17, 26). Shoubridge et al. (26) reported that CS activity was increased, whereas the activities of several glycolytic enzymes were decreased in the PLN and MG muscles of rats fed a diet containing β-GPA. Ren et al. (23) have recently reported similar results in the PLN, including the observation of increased cytochrome *c* activity after β-GPA feeding.

Histochemical analysis of SOL muscles from creatine-depleted rats has indicated a shift to 100% type I fibers vs. 85 (26) or 96% (15) in control animals. Assuming some level of correspondence between fiber type as determined by myosin adenosinetriphosphatase (ATPase) histochemistry and the predominate MHC expressed by a myofiber (1), these analyses suggest that the muscles from these studies were shifted toward expression of 100% type I MHC. In mice, Moerland et al. (19) reported that β-GPA feeding induced a shift toward a slower native myosin isoform profile in SOL muscles. Matoba et al. (15) also reported a shift to 100% type I fibers in SOL muscles resulting from hindlimb non-weight bearing in rats fed a β-GPA-supplemented diet.

With regard to the SOL muscle, the results of the current study (i.e., no change in MHC expression in the BC group) appear to be at odds with previously reported findings, indicating rather large MHC shifts in SOL muscles of rats. On examination of the methods employed in these studies, we can find only one consistent difference with those reported herein, namely the age of the animals. Studies reporting significant effects of creatine depletion on the fiber-type profile of SOL muscles have uniformly employed very young (e.g., weanling) animals (15, 19, 26). The animals used in the current study were young adults (e.g., body weight $\approx$250 g). This leads us to speculate that the imposition of creatine depletion at a time when animals are rapidly growing may have a profound effect on some aspect of myosin production and/or isoform expression that is absent in older animals. In support of this theory, Ingwall et al. (11) reported that creatine is a positive effector of the rate of myosin synthesis in cultured muscle cells and muscle explants (11). Interestingly, this effect was only demonstrated when cells were already differentiated and producing myosin. A differential effect between young and mature animals would suggest that creatine depletion during development may be an attractive model for the manipulation of muscle phenotype. It would be of partic-

ular interest to determine whether muscles from rats subjected to creatine depletion from a very young age retain an altered phenotype in adulthood after withdrawal of the β-GPA diet.

A discrepancy also exists between the current study and the results reported by Park et al. (20), who found that creatine depletion for 12–15 wk did not affect the fiber-type profile of PLN muscles from male rats. In the current study, using electrophoretic analysis, we found small but significant increases in type I and IIa MHCs in this muscle. Although it does appear that there is a general correspondence between histochemical fiber typing (myosin ATPase) and the MHC profile of fibers as determined by electrophoretic separation (1, 28), the two may not always be interchangeable. For example, without methodological modifications, type IIx fibers generally will not be distinguished using traditional myosin ATPase histochemistry. Further, electrophoretic determination, which involves homogenization of tissues, will not distinguish MHCs, which might be coexpressed in single myofibers (29, 31). Either of these differences or a combination to the two could produce the discrepancy between these two reports. In apparent agreement with the current study, Park et al. reported an increase of 25% in CS activity in creatine-depleted PLN muscles, a value less than the 40% increase reported by Soubridge et al. (26) but similar to the 24% reported here.

Creatine depletion and muscle plasticity models. Several paradigms have been used to produce fast to slow shifts in either the fiber-type or MHC profile of skeletal muscles. Of those interventions, several alter MHC distributions in a manner qualitatively similar to that seen with creatine depletion. For example, Diffee et al. (6) reported increased type I and decreased type IIb MHC expression in the PLN muscles of hypothyroid rats, a result similar to that reported here. However, hypothyroidism also altered the proportions of type I and IIa MHCs in the SOL and VI, whereas in the current study creatine depletion had no effect in these muscles. Another experimental intervention that has a potent effect on MHC distribution is functional overload. In the PLN, this intervention can result in increases in the proportion of type I and IIa MHCs and a corresponding decrease in type IIb MHC (7). Although the direction of these changes are similar to those seen with creatine depletion, the shift in type I expression in the compensatory hypertrophy model is almost three times as great as that reported here for the BC group.

Fitzsimons et al. (8) reported that the proportions of slow and intermediate native myosin of rat PLN muscle were increased by 50 and 28%, respectively, after a 10-wk running program involving adult female rats (8). These native myosins are known to contain type I and IIa MHCs, respectively (35). In the current study, type I and IIa MHCs were increased 46 and 29%, respectively, in creatine-depleted PLN muscles. In the Fitzsimons study, endurance training had no effect on the distribution of slow and intermediate isomyosins in either the SOL or VI, again consistent with the MHC profiles of these muscles after the creatine depletion treatment in the current study. With respect to the direction and magnitude of MHC phenotype shifts, the similarity of response between creatine depletion and endurance training supports the hypothesis that the creatine depletion mimics some aspects of the stimulus provided by endurance training.

Creatine depletion and hindlimb non-weight-bearing activity. Hindlimb non-weight-bearing activity has been consistently shown to have the greatest effect on muscles with a high proportion of slow-twitch fibers (5, 34). The response of such muscles to non-weight-bearing activity generally includes a loss of muscle mass and a shift in MHC phenotype toward the expression of greater proportions of type II MHCs (5–7). The results reported here are in accord with previous findings indicating a significant impact of non-weight-bearing activity on the SOL and VI muscles with regard to both muscle mass and MHC distribution. In addition, the SOL and VI muscles from the NS group were found to contain small amounts of type IIb MHC. This finding is of particular interest in that it suggests that some myofibers in these muscles may be capable of expressing the full range of MHC isoforms.

Thyroid deficiency has been demonstrated to prevent the slow to fast shift in MHC phenotype resulting from non-weight-bearing activity (6). As indicated above, the effects of creatine depletion on MHC distribution bear at least a qualitative resemblance to those resulting from thyroid deficiency in fast skeletal muscles. As part of our initial hypothesis we speculated that creatine depletion might have a similar predominating effect vs. non-weight-bearing activity with regard to MHC expression. However, we found that creatine depletion had no sparing effect on the loss of type I MHC either in fast or slow muscles.

Creatine depletion and loading state. Type I MHC is the predominant isoform expressed in SOL and VI muscles. This factor would seem to render these muscles best suited to perform the constant tonic type of contractile activity associated with postural maintenance. PLN and MG muscles are composed of myofibers expressing primarily type II MHCs. This suggests that these muscles would be best suited to more dynamic short-term contractile activity such as that associated with locomotion.

The unique roles of these postural and locomotor muscles may be reflected in their differential response to both non-weight-bearing activity and creatine depletion. For instance, the distribution of MHC isoforms in postural muscles depleted of creatine was maintained by the normal weight-bearing activity of rats maintained in cages while, at the same time, the MHC profile of the PLN and MG changed.

In contrast to their creatine depletion response, postural muscles (NS and BS) demonstrated a much greater magnitude of non-weight-bearing activity-induced MHC plasticity compared with the locomotor muscles. In this context, the differential in sensitivity to creatine depletion between postural and locomotor muscles might suggest that some myofibers in the locomotor muscles of sedentary rats are primed to respond to increased contractile activity such as running. Because creatine depletion appears to mimic some aspects of the metabolic state associated with repeated bouts of increased contractile activity, this stimulus might be sufficient to trig-

ger shifts in MHC expression in a population of infrequently recruited myofibers. The increase in CS activity seen in the PLN of the sedentary creatine-depleted rats is consistent with this notion. Further, Park et al. (20) recently reported that the combined interventions of creatine depletion and interval training had a synergistic effect on fiber-type transformations in the PLN. The latter result clearly supports the concept of a permissive or interactive function of loading state with the myoplastic effects of creatine depletion.

Summary. Hindlimb non-weight-bearing activity resulted in increases in fast MHC isoforms in SOL, VI, PLN, and MG muscles. MHC isoform shifts were of much greater magnitude in SOL and VI muscles. In three of these muscles, the SOL, VI, and PLN, chronic creatine depletion counteracted the shift from slower isoforms to type IIb MHC but did not interfere with the reduction in the proportion of type I MHC that accompanied non-weight-bearing activity. Creatine depletion in rats that were allowed normal weight-bearing activity resulted in small increases in the relative amounts of type I and IIa MHC in PLN and MG muscles, whereas SOL, VI, and TA muscle MHC profiles were unaffected. In each case the observed shifts in MHC expression appeared to be the result of pretranslational events.

These results indicate that the plastic response of a myofiber to some change in loading status may depend on the functional role of the muscle. Further, the plastic response of some myofibers can be modified by factors such as creatine depletion that appear to impinge only on metabolic parameters rather than mechanical factors. The similarity of response between the stimulus of creatine depletion and that of endurance training suggests that the former intervention may produce intracellular effects that are interpreted by the cell as a chronic increase in contractile activity.

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