Modulation of the dystrophin-associated protein complex in response to resistance training in young and older men

David J. Kosek and Marcas M. Bamman
Department of Physiology and Biophysics, The University of Alabama at Birmingham, and Geriatric Research, Education, and Clinical Center, Veterans Affairs Medical Center, Birmingham, Alabama

Submitted 1 July 2007; accepted in final form 20 March 2008

Kosek DJ, Bamman MM. Modulation of the dystrophin-associated protein complex in response to resistance training in young and older men. J Appl Physiol 104: 1476–1484, 2008. First published March 20, 2008; doi:10.1152/japplphysiol.00708.2007.—The dystrophin-associated protein complex (DAPC) is a scaffold of proteins linking the intracellular cytoskeleton with the extracellular matrix that is integral to structural stability and integrity, signaling and mechanotransduction, and force transmission. We hypothesized that the expression of DAPC component proteins would be altered by resistance loading during progressive resistance training (PRT)-mediated myofiber hypertrophy, and we investigated whether aging influenced these changes. Seventeen young (27 yr) and 13 older (65 yr) men completed 16 wk of PRT with muscle biopsies at baseline (T1), 24 h after bout 1 (T2), and 24 h after the final bout at week 16 (T3). Myofiber hypertrophy in the young (type I 31%, P 0.005; type II 40%, P 0.001) far exceeded hypertrophy in the old (type I only, 19.5%, P 0.005; type II 40%, P 0.01). PRT altered protein expression for caveolin-3 (decreased 24% by T2, 0.01), syntrophin (increased 16% by T3, P 0.05), α1-syntrophin linkage (14, 56), which may provide satellite cells with the necessary NO-mediated signal for activation (3, 13, 14). Caveolin-3 found within the sarcolemma is associated with the DAPC (55) and is thought to inhibit nNOS-mediated NO production (22). Dystrobrevin is a subsarcolemmal protein that has also been studied for its importance in connecting multiple other proteins for signaling and structural strength at the muscle membrane. It is a member of the dystrophin family of proteins, with the α2 isoform predominating in skeletal muscle (26).

Dystrophin has been studied extensively and is known for its subsarcolemmal role in providing a linkage between cytoskeletal actin and the extracellular matrix. Binding of β-dystrogly-
can, dystrobrevin, and the syntrophins occurs at the COOH-terminus of dystrophin, whereas α-dystroglycan, in turn, associates with β-dystroglycan intramembranously and laminin at the extracellular matrix (2). Dystrophin knockout mice are unable to localize α-syntrophin to the sarcolemma, resulting in a loss of nNOS from the muscle cell membrane (13, 16, 23). Dystrophin-deficient skeletal muscle is more susceptible to mechanically induced damage than normal muscle (45), a deleterious effect revealed most obviously in Duchenne’s muscular dystrophy. The COOH-terminal region of dystrophin also associates with α-actinin, which may provide a signaling link between the DAPC and costameric adhesion complexes (24). MacArthur and North (37) suggest that α-actinin helps to integrate several functional pathways in skeletal muscle to maintain normal contractility, signaling, and metabolism. Two isoforms predominate in skeletal muscle, encoded by two different genes (10).

While Woolstenhulme et al. (60) assessed levels of dystrophin and the intermediate filament protein desmin, the potential for modulation of complexed sarcolemmal and subsarcolemmal DAPC component proteins has not been studied during a multiweek resistance training program in humans. The primary purpose of this study was to test the hypothesis that the expression of DAPC component proteins would be altered by resistance loading during progressive resistance training (PRT)-mediated myofiber hypertrophy. In addition, because we have previously shown that the myofiber growth adaptation in young men far exceeds that in old (32), in part due to superior satellite cell recruitment (44), and others have shown that older adults are more susceptible to contraction-induced muscle damage (46), we investigated whether aging influenced load-mediated modulation of the DAPC. Extracellular signal-regulated kinase (ERK) 1/2 and p38 mitogen-activated protein kinase (MAPK) have previously been shown to associate with DAPC components and likely play important signaling roles in mechanotransduction (8, 25). We, therefore, also examined the expression and phosphorylation of ERK1/2 (primarily considered to be involved in cell division, growth, and differentiation processes) and p38 MAPK (associated with cellular stress responses) (29).

**METHODS**

**Subjects.** Thirty men (17 young, 20–35 yr; 13 older, 60–75 yr) were recruited from the Birmingham, AL metropolitan area and were free of any musculoskeletal disorders that may have affected their ability to complete the resistance training regimen. No subjects were treated with pharmacological interventions known to influence muscle mass before or during the study. Volunteers were excluded if body mass index was not <30 kg/m² or for any leg resistance training undertaken within 5 yr before the study. Each subject gave written, informed consent before participation, and the study was approved by the Institutional Review Boards of both the University of Alabama at Birmingham and Birmingham Veterans Affairs Medical Center. Subject characteristics are shown in Table 1.

**PRT program.** The PRT program utilized in this study was previously described in detail (32). Briefly, subjects trained 3 days/wk for 16 wk in a program that focused on the knee and hip extensors. The regimen consisted of a warm-up on a cycle ergometer or treadmill for 5 min, followed by three resistance training exercises, including squat, leg press, and knee extension. After two familiarization sessions and practice strength tests, baseline strength was established by one repetition maximum (1 RM) testing using established procedures (6, 32). Initial training loads were set at 80% of 1 RM, and subjects progressed to three sets of each exercise during the first three sessions. The first session of three sets per movement marked the start of the 16-wk training program. Sets of 8–12 repetitions to volitional fatigue were completed for each movement, with 90-s rest periods between sets, using weight stack resistance exercise and/or plate-loaded stations (barbell squats and linear 45° leg press). Load cell and regression procedures described previously (43) were used to calculate actual resistances for comparing training and testing loads between weight stack and free weight stations. Resistance was increased when sub-

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**Table 1. Subject characteristics by age group at baseline**

<table>
<thead>
<tr>
<th></th>
<th>Young Men</th>
<th>Older Men</th>
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<tbody>
<tr>
<td>n</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>Age, yr</td>
<td>26.5±4.8</td>
<td>64.6±3.9</td>
</tr>
<tr>
<td>Height, cm</td>
<td>178.4±7.8</td>
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<tr>
<td>Weight, kg</td>
<td>81.4±11.9</td>
<td>88.7±10.5</td>
</tr>
<tr>
<td>Body fat,* %</td>
<td>24.4±8.1</td>
<td>31.8±5.6</td>
</tr>
</tbody>
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Values are means ± SD; n, no. of subjects. *Age difference, P < 0.01.
jects completed 12 or more repetitions for at least two of the three total sets in a given exercise. This progression resulted in an average of 10–11 repetitions per set throughout the program. Subjects underwent 1 RM testing midway through training (week 8) and posttraining (week 16). We previously showed that training intensity and program adherence (~90%) were not different between age groups (32).

**Muscle biopsy and tissue preparation procedures.** All muscle biopsies were performed in the Pittman General Clinical Research Center at University of Alabama at Birmingham. Muscle samples were collected from vastus lateralis muscle by percutaneous needle biopsy using a 5-mm Bergstrom biopsy needle under suction, as previously described (21). A baseline biopsy was taken from the left leg, and an acute postexercise biopsy was taken from the right leg 24 h after the first full bilateral loading bout to avoid any residual effects of the baseline biopsy. The third 16-wk posttraining biopsy was obtained 24 h after the final training session from the left leg. At the bedside, visible connective and adipose tissues were removed with the aid of a dissecting microscope. Portions of each sample (30–40 mg) to be used for RNA and protein isolation were immediately weighed and snap-frozen in liquid nitrogen. A separate portion for immunohistochemistry was mounted cross sectionally on cork in optimum cutting temperature mounting medium mixed with tragacanth gum and frozen in liquid nitrogen-cooled isopentane. All samples were stored at −80°C until analyses.

**Myofiber cross-sectional area.** Using immunofluorescence microscopy techniques described previously (32), myofibers positive for myosin heavy chain (MHC) type I (MHC I) and negative for MHC IIa were classified as type I, fibers positive for MHC IIa and negative for MHC I were classified as type IIa, and fibers negative for both MHC I and MHC IIa were classified as type IIx. Hybrid myofibers (e.g., coexpression of IIa or IIx) that were revealed by both color and intensity using this technique were excluded from analyses. For cross-sectional area (CSA) measurements, each myofiber was manually traced along its laminin-stained border. Myofiber-type distribution and size were determined in blinded fashion by a single analyst, as described previously (31, 32).

**Protein immunoblotting.** Immunoblotting was conducted to assess muscle cell membrane protein lysate levels of nNOS, α-1-syntrophin, caveolin-3, α-dystrobrevin, dystrophin, and α-actinin. Protein lysate from the cytosolic fraction was also assessed for cytosolic nNOS content. Snap frozen muscle samples (30–40 mg) were homogenized, and supernatant assayed for total protein, as described in detail (6). Some samples taken from biopsies of subjects who entered the study in its initial stages were homogenized into whole tissue lysate, whereas samples from subjects entering the study later were divided into membrane and cytosolic fractions. Homogenization procedures were consistent within each subject across all three biopsy time points.

All samples were powdered using a liquid nitrogen-cooled mortar and pestle and homogenized in 3 μl/mg muscle of ice-cold lysis buffer specific for either whole tissue [150 mM NaCl, 50 mM Tris-HCl (pH 7.4), 0.5% Nonidet P-40, 1% deoxycholate, 0.1% SDS, 1% Triton X-100, 5 mM EDTA, 100 μM PMSF, 0.1 mM okadaic acid, 1 mM orthovanadate, and 0.5× protease inhibitor cocktail containing bestatin, leupeptin, and aprotinin (P2714, Sigma-Aldrich, St. Louis, MO)], or cytosolic and membrane fractions [whole tissue lysis buffer plus 25 mM HEPES (pH 7.4), 300 mM sucrose, and 1 mM magnesium chloride]. Using cytosolic/membrane fraction lysis buffer, supernatant (cytosolic fraction) was removed following centrifugation at 110,000 g at 4°C for 90 min. Resuspension buffer [50 mM sodium chloride, 0.1% SDS, 5 mM EDTA, 100 μM PMSF, 0.1 μM okadaic acid, 1 mM orthovanadate, 25 mM HEPES (pH 7.4), 10% sucrose, and 0.5× protease inhibitor cocktail containing bestatin, leupeptin, and aprotinin (P2714, Sigma-Aldrich, St. Louis, MO)] was then added to 2 μl/mg muscle to the remaining pellet, which was rehomogenized and centrifuged at 100,000 g at 4°C for 10 min to yield the membrane fraction. All muscle lysates (whole tissue, cytosolic, or membrane fraction) were assayed for total protein using the bicinchoninic acid technique, with BSA as a standard.

Twenty micrograms of total protein diluted in Laemmli buffer were separated by SDS-PAGE (120-V constant) in 4–15% Tris-HCl mini-gels (Bio-Rad MP3, Bio-Rad Laboratories, Hercules, CA) for all proteins of molecular mass <120 kDa. Those proteins of molecular mass over 120 kDa (nNOS and dystrophin) were separated by SDS-PAGE (120-V constant) in 5% Tris-HCl mini-gels (Bio-Rad MP3, Bio-Rad Laboratories). Transfer from 4–15% gels was conducted at constant current (1 mA/cm² × 30 min) to polyvinylidene difluoride (PVDF) membranes using a semidyrid transfer cell (Trans-Blot SD, Bio-Rad Laboratories). Five percent gels were transferred to PVDF membranes using a wet transfer cell, 14 h 20 min at 10-V constant current in a 4°C cold room, followed by 1 h at 100 V in an ice-cold circulating water bath. Within subjects, baseline, acute postexercise, and 16-wk posttraining samples were loaded in adjacent lanes. To control for age bias, each 12-lane gel contained samples for at least one subject from each age group, with subject groups loaded in alternating fashion on each gel. Equal loading across lanes and equal transfer were verified by staining all gels (after transfer) with Coomassie blue, staining randomly selected membranes with Ponceau S, and immunoblotting for β-actin. Primary antibody specificities, optimal blocking conditions, and target antigen migration patterns (i.e., molecular masses) were confirmed in control experiments. Immunoblotting was carried out using rabbit polyclonal antibodies against α1-syntrophin (1:1,000, Sigma, S4688), caveolin-3 (1:1,000, Abcam, ab2912), α-actinin (1:2,000, Sigma, A2543), p38 MAPK (1:1,000, Cell Signaling Technology), ERK1/2 (1:1,000, Cell Signaling Technology), and phosphorylated Ser417 nNOS (1 μg/ml, Upstate Signaling Solutions, 07-544); a goat polyclonal antibody against α-dystrobrevin (1:500, Santa Cruz, sc-13812); and mouse monoclonal antibodies against dystrophin (1:250, Vector, VP-D508), nNOS (1:1,000, BD Bioscience, 610308), phosphorylated Thr180/Tyr182 p38 (1:2,000, Cell Signaling Technology), and phosphorylated Thr202/Tyr204 ERK2 (p44) (1:2,000, Cell Signaling Technology). Membranes were blocked simultaneously with primary antibody incubation, and blocking solution varied, depending on the primary antibody. Membranes treated with polyclonal primary antibodies were blocked with 2% BSA, 2% milk in PBST (1 × PBS with 0.1% Tween 20), while horseradish peroxidase-conjugated goat anti-rabbit (1:50,000) or donkey anti-goat (1:50,000) secondary antibodies were diluted in 0.5% BSA and 0.5% milk in PBST. Membranes treated with monoclonal primary antibodies were blocked with 5% goat serum in PBST, whereas horseradish peroxidase-conjugated goat anti-mouse (1:50,000) secondary antibody was diluted in 1% goat serum in PBST. The combined blocking solution/primary antibody incubation was performed overnight at 4°C under gentle agitation followed by incubation in secondary antibodies for 1 h at room temperature, with a PBST rinse protocol between treatments (one quick rinse, 1 × 10 min, 2 × 5 min).

All bands were visualized by chemiluminescence (Super West Dura kit, Pierce Biotechnologies, Rockford, IL) in a BioRad Chemidoc imaging system, and band densitometry was performed using BioRad Quantity One software, as our laboratory previously described in detail (6). For each imaging session, serial imaging ceased at the first point of saturation on the developing image. This standardization, combined with equal and random distribution of the two age groups across the gels (PVDF membranes), enabled us to accurately test for age and resistance training effects.

**Statistical analysis.** Data are reported as means ± SE. Between-groups differences in preexercise descriptive variables were tested using independent t-tests. All variables measured across 16 wk of training were analyzed using age × time repeated-measures ANOVA. Lysate levels of specific proteins were assessed across all three biopsy time points and were thus analyzed via 2 (age group) × 3 (time point) repeated-measures ANOVA. For each ANOVA model with a significant main or interaction effect, Tukey honestly significant difference tests were used to detect pairwise differences.
tests were performed post hoc to localize the effect(s). Statistical
significance was accepted at \( P < 0.05 \) for all tests. Muscle tissue
lysat was fractionated into membrane and cytosolic fractions for 17
of the 30 subjects. The nNOS analysis was limited to these 17 subjects
(11 young, 6 old), since nNOS is located in both compartments,
making the whole tissue lysate nNOS data impossible to interpret.
This restriction enabled us to test differential changes in cytosolic and
membrane-bound nNOS across training. Protein levels of caveolin-3,
\( \alpha \)-dystrobrevin, \( \alpha \)-actinin, and phosphorylated and total p38 MAPK
were determined on all 30 subjects (17 young, 13 old). Technical
problems resulted in fewer subjects for \( \alpha \)-syntrophin (13 young, 10
old), dystrophin (14 young, 10 old), and phosphorylated and total
ERK1/2 (17 young, 12 old).

**RESULTS**

**Myofiber CSA.** Figure 2 illustrates the results that 16 wk of
PRT had on myofiber growth in young and older men. Data are
displayed as magnitudes of myofiber growth (measured in
\( \mu m^2 \)) for type I and type II myofibers in young and older men.
Due to the well-known shift of MHC IIx to MHC IIa expression
induced by resistance training, an insufficient number of
IIx myofibers for CSA assessment were found at the 16-wk
point. Training-induced changes in myofiber size were,
therefore, restricted to type I and type II myofibers, with type
II CSA being a weighted average based on the relative distri-
butions of IIa and IIx myofibers, as we have described (32).

A main time effect \( (P < 0.001) \) and age group \( \times \) time
interaction \( (P < 0.005) \) were found for type I myofiber size. No
pretreatment differences were noted between young and older
men. However, PRT resulted in an overall 17% increase \( (P < 0.0005) \)
in CSA that was driven by young men, who gained
over 1,300 \( \mu m^2 \) in type I myofiber area (31\% increase, \( P < 0.0005) \).
Older men showed no significant training-induced
type I myofiber growth.

PRT elicited significant growth of type II myofibers. A main
age effect was observed \( (P < 0.05) \), as young men possessed
19\% larger type II fibers overall compared with their older
counterparts. A main time effect \( (P < 0.0001) \) revealed a 32\% increase in type II CSA. Post hoc analysis showed that young
men enhanced CSA of type II myofibers by 1,992 \( \mu m^2 \) (over
40\%, \( P < 0.001) \), more than twice the type II hypertrophy seen
in old men (888 \( \mu m^2 \) or 19.5\%, \( P < 0.05) \). The age difference
in hypertrophy response was revealed by an age group \( \times \) time
interaction \( (P < 0.05) \), despite the significant type II myofiber
growth found in each age group.

**Components of the DAPC.** A schematic of the DAPC in
Fig. 1 shows the associations among DAPC proteins bound to
one another, the contractile apparatus, and other costameric
proteins. DAPC protein results are presented in Figs. 3 and 4.
Levels of the transmembrane protein, caveolin-3, are shown in
Fig. 3A. A main time effect was found, as caveolin-3 levels dropped by week 16 \( (P < 0.005) \). Post hoc Tukey analysis revealed a 20\% decline from 0 to 16 wk
\( (P < 0.05) \) and a 24\% reduction from the acute time point to
week 16 \( (P < 0.01) \). Because caveolin-3 inhibits nNOS
(22, 55), we also analyzed the ratio of caveolin-3 to mem-
brane nNOS; however, no significant changes across time
were noted for either age group (data not shown). Older men
tended to express 34\% more caveolin-3 \( (P = 0.051) \) than their young counterparts before training.

\( \alpha \)-Syntrophin protein levels are shown in Fig. 3B. No
significant difference in expression existed between young and
older men, but a main time effect \( (P < 0.05) \) was found, as
\( \alpha \)-syntrophin expression increased 16\% from baseline to week 16.
Because caveolin-3 has been shown to inhibit nNOS
function, while \( \alpha \)-syntrophin is necessary for localizing this
enzyme to the sarclemma, we analyzed the ratio of caveolin-3 to
\( \alpha \)-syntrophin as a method for indirectly determining any
modulation of the DAPC that would contribute to nNOS
inhibition or potential enhancement as a result of training.
Results illustrated a main time effect \( (P < 0.001) \), whereby
subjects decreased the ratio of caveolin-3 to \( \alpha \)-syntrophin by
39\% by week 16. The effect was driven primarily by older men,
who decreased this ratio 48\% \( (P < 0.05) \).

Protein levels of nNOS were determined in cytosolic and
membrane-bound fractions separately. Membrane fraction nNOS protein levels are shown in Fig. 3C. There was a strong trend
toward an age \( \times \) time interaction \( (P = 0.054) \), as membrane
nNOS tended to increase by week 16 among older men, while
remaining relatively stable in the young. Cytosolic nNOS
expression did not differ by age group and was unaltered by
resistance training (data not shown). We also examined the
phosphorylation state of an activating phosphorylation site on
nNOS. Phosphorylation of nNOS at Ser1417 upregulates NO
synthesis in the kidney (42); thus we examined this activation
site in skeletal muscle (Fig. 3D). We found a striking main time
effect \( (P < 0.005) \), as nNOS Ser1417 phosphorylation was 70\%
lower at week 16 compared with baseline. Among older men,
Ser1417 phosphorylation dropped 81\% by week 16 \( (P < 0.05) \),
while young men showed no significant changes.

\( \alpha \)-Dystrobrevin protein data are shown in Fig. 4A. A main
time effect \( (P < 0.05) \) was found, as expression tended to rise
acutely (not significant), followed by a significant 23\% decline
by week 16 \( (P < 0.01) \). Post hoc analysis revealed that a
significant 28\% decrease from the acute time point to 16 wk in
young men \( (P < 0.05) \) drove the main effect. Young men
tended to express more \( \alpha \)-dystrobrevin before training (37\%,
\( P = 0.088 \)). Dystrophin results are presented in Fig. 4B.
Immunoblotting using an antibody specific for the rod domain of dystrophin detected two bands that may result from the presence of two dystrophin isoforms, but this was not confirmed. The densitometry results presented in Fig. 4 represent both bands combined (analyses of each band individually yielded the same statistical results as when the bands were combined). A main time effect (P<0.01) was brought on by a 30% acute increase (P<0.05) followed by a significant decrease back to near baseline levels by 16 wk (P<0.05). A trend toward an age group x time interaction (P<0.07) was noted, as only young men significantly decreased dystrophin levels from the acute time point to week 16 (P<0.05). Figure 4C illustrates the stability of α-actinin in response to 16 wk of resistance training, as well as the similarity in expression between age groups. No significant changes were found for α-actinin in either age group across the training regimen.

*Intracellular MAPK signaling.* Total and phosphorylated levels of ERK1/2 (p42/44) and p38 MAPK were assessed, since these kinases have shown activation in response to mechanical stretch (41) and exercise (1) and associate with DAPC components (8, 25). Figure 5, A and B, displays the ratios of phosphorylated to total ERK and p38 MAPK, respectively. Representative Western blots are shown in Fig. 5C for each age group across the three time points. β-Actin served as a loading control. β-Actin levels were quite stable, as no age differences or time effects were found. ERK1/2 results revealed no significant age differences or time effects in phosphorylated or total protein levels. However, results for p38 MAPK illustrated a main time effect for total protein (P<0.05), as p38 MAPK expression fell roughly 22% from the acute time point through week 16 (P<0.05). A main time (P<0.01) effect and age group x time interaction (P<0.05) were found for the phosphorylation state of p38 MAPK. Post hoc analysis revealed an overall 65% increase in the phosphorylation state from the acute time point through week 16 (P<0.01). The interaction was caused by a marked increase among older men, who doubled the relative amount of phosphorylated
p38 MAPK by week 16 compared with both the baseline ($P < 0.01$) and acute response ($P < 0.05$) time points.

**DISCUSSION**

Overall, these findings generally support the hypothesis that resistance training alters the skeletal muscle protein content of specific DAPC components. Aging had limited influence over DAPC modulation, but markedly affected stress-response signaling (p38 MAPK phosphorylation), which may have impaired the myofiber hypertrophy adaptation in old men. Young men were capable of inducing two times the growth of their older counterparts in type II myofibers, and only young men experienced type I myofiber growth. Several components of the DAPC were altered in response to a single exercise bout and/or after 16 wk of resistance training (caveolin-3, α1-syntrophin, α-dystrobrevin, and dystrophin).

Resistance training consists of repeated bouts of high-intensity contractions separated by lengthy rest periods. As is the case with any exercise treatment, the long-term “training” adaptations can be viewed as the cumulative result of acute responses to each loading bout. Acute resistance loading stimulates muscle transcript (30, 31, 47) and protein (59) expression and transient activation of intracellular protein kinase cascades (e.g., ERK, MAPK) (58). However, the acute responses to a single bout of resistance loading can differ markedly in trained vs. untrained muscle (7, 27, 32). It is generally understood that mechanical load-mediated hypertrophy of skeletal myofibers requires upregulation of the protein synthesis machinery (57, 61) and is facilitated by myonuclear addition (28, 44), yet the mechanotransduction events sparking these processes are unclear. The DAPC is a mechanosensitive structure that may, in fact, be responsible for providing the spark, but it has received little attention in resistance training studies. Although the myotendinous junction, neuromuscular junction, DAPC, and other costameric proteins probably all play important roles in mechanotransduction (23, 54), the DAPC was examined due to the suggested necessity of nNOS for stretch-mediated satellite cell activation (4, 5) and the importance of other DAPC components for structural stability, force transmission, and additional intracellular processes. Reductions in caveolin-3 promote nNOS enzyme activity (22, 55), as caveolin-3 expression disrupts NO synthesis (22) without affecting nNOS expression (50). It is attractive to speculate that the decrease in caveolin-3 expression reported here may have enhanced nNOS function, which appears to play a key role in satellite cell activation (3, 52). Initially, we predicted α1-syntrophin expression levels would increase in response to training, enhancing signaling via direct syntrophin phosphorylation (25, 38), offering more structural stability to growing myofibers, and providing increased linkage for nNOS at the sarcolemma. Although subtle, levels of α1-syntrophin did increase in response to resistance training, which may have enhanced both structural strength and signaling capacity of the DAPC. On the other hand, the rise may have been in response to membrane overstress and heightened p38 activity, as p38-γ
binds the PDZ domain of α1-syntrophin, resulting in phosphorylation (25).

Dystrophin levels increased acutely after the initial resistance loading bout. In stark contrast, a single exposure to damage-inducing, high-force (9), or extremely high-velocity (36) eccentric (i.e., lengthening) contractions without prior adaptation (i.e., unaccustomed) has been shown to reduce dystrophin levels in injured myofibers. The discrepancy may be explained by marked differences in the contraction paradigms used. Our conventional resistance exercise paradigm involved concentric-eccentric actions with identical external resistances; thus only submaximal eccentric forces were generated as limited by concentric strength. The severity of membrane wounding was, therefore, likely much lower in our model. Regardless, the initial elevation in dystrophin may have been an overcompensation, as lack of a similar response at week 16 suggests acclimation to the repetitive stimulus and indicates additional dystrophin is not necessary during training-mediated myofiber hypertrophy.

It is apparent that absence of nNOS from the sarcolemma (13) and pharmacological inhibition of NO (3) result in dysregulation of satellite cell activation. Anderson and colleagues (4, 5) proposed that the primary source of NO for such a task is membrane-bound nNOS, due to its proximity with satellite cells, as well as for the possibility of NO production in response to stretch on the sarcolemma during contraction. NO has been shown to enhance hepatocyte growth factor-mediated satellite cell activation (51, 52). We predicted that membrane-bound nNOS protein content would increase in young men in response to training, based on the idea that this would provide enhanced NO production for the purposes of activating satellite cells (51, 52) and contributing to other processes. We also expected this effect to be less robust in older men, affording some explanation for their reduced ability to activate these quiescent stem cells. Contrary to our hypothesis, membrane-bound nNOS protein content did not change. To test whether nNOS function may have been altered, we assessed the phosphorylation state of Ser1417. Phosphorylation of this residue has been shown to induce NO production in the kidney (42), and Chen et al. (17) assessed this phosphorylation site in human muscle homogenates following a bout of high-intensity cycling. We found no change in nNOS phosphorylation following the initial resistance loading bout, and, in fact, a substantial reduction was noted in response to resistance exercise after week 16 of training. This finding was unexpected, but the functional importance of nNOS phosphorylation at this and alternative sites, at least in skeletal muscle, is not fully understood.

α-Actinin levels remained unchanged. α-Actinin binds the COOH terminus of dystrophin, providing a linkage that associates the DAPC with the actin cytoskeleton and other costameric adhesion complex proteins, such as vinculin, talin, and β1-integrin (24). Not only does this provide structural support to myofibers but also it allows for signaling between two complexes thought to be important in mechanotransduction from the contractile machinery outwardly to the cell membrane [DAPC and α/β-integrin complex (15)]. Our findings suggest adequate levels of α-actinin exist; however, we did not differentiate α-actinin isoforms.

Intracellular signaling events, such as protein kinase cascades, may “prime” the myofiber’s transcriptional and translational machinery, and there is evidence that MAPK signaling is

Fig. 5. Immunoblot results for ratios of phosphorylated to total ERK1/2 (A) and p38 MAPK (B). Values are means ± SE for all subjects. Closed and open circles indicate mean values for young and older men, respectively; statistical results shown are for all subjects. C: representative immunoblots of each protein from each age group across the 3 time points. ▲Main time effect, P < 0.05. †Age group × time interaction, P < 0.05. Post hoc results on main time effects are shown on bars: #different from bout 1, P < 0.05.
upregulated in response to exercise in skeletal (1, 62) and cardiac (27) muscle and in response to skeletal muscle stretch (41). Such signaling provides the muscle with a contraction-mediated method for regulating a host of intracellular processes, including transcription of muscle-specific genes, the initiation of growth processes, as well as the activation of protein degradation. ERK-MAPK signaling responses are largely rapid and short lived following mechanical load (58).

Based on the transient nature of the normal signaling time course, a prolonged or delayed MAPK activation (i.e., detected 24 h after mechanical load) suggests an abnormal response that may be indicative of a failing compensatory effort. This might be expected in the old, as older animals clearly demonstrate an impaired and prolonged regeneration and/or recovery time course following muscle injury (19, 39, 40). We found a twofold elevation in the phosphorylation state of p38 MAPK only among the old, 24 h after resistance exercise at the conclusion of the 16-wk training program. Phosphorylation of p38 MAPK in skeletal muscle is induced by mechanical stress and damage (8, 12) and is associated with muscle atrophy (18). In fact, p38 phosphorylation has been shown to be required for cytokine-mediated expression of the muscle-specific E3 ubiquitin ligase muscle atrophy F-box/atrogen-1 (35), which is associated with increased proteasome activity. We have previously reported robust increases in protein abundance of the myogenic transcription factor, myf-6, during resistance training in both young (64%) and older (93%) adults, even though myofiber hypertrophy was blunted in the old (32). Heightened activation of p38 MAPK has been shown to suppress myogenesis via phosphorylation (inhibition) of myf-6 (49). These data combined suggest the impaired rate of hypertrophy among older men may have been at least partially mediated by the deleterious effects of overstress.

Conclusions. Overall, we report that acute resistance loading and/or long-term resistance training modules the levels of some component proteins comprising the mechanosensitive DAPC, with limited age differences in DAPC responsiveness. Additionally, the marked 24-h postexercise elevation in p38 MAPK phosphorylation among the old after 16 wk of 3 day/wk high-intensity resistance training points to the possibility that the muscles of older men experienced an overstress throughout training and hence an impaired growth rate. Identifying the specific signaling process(es) that drives myofiber growth in the young, and slows the rate of hypertrophy among older adults, will be an important step toward designing the most efficacious, age-specific resistance training prescriptions.

ACKNOWLEDGMENTS

We are indebted to the research subjects for invaluable contributions to this work. We thank S. C. Tuggle for administering the resistance training program.

GRANTS

Funding for this work was provided by National Institute on Aging Grant R01 AG017896 (M. M. Bamman), Veterans Affairs Merit Grant (M. M. Bamman), and General Clinical Research Center Grant M01 RR 00032.

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