J Appl Physiol 101: 531–544, 2006.
First published April 13, 2006; doi:10.1152/japplphysiol.01474.2005.

Efficacy of 3 days/wk resistance training on myofiber hypertrophy and myogenic mechanisms in young vs. older adults

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Submitted 22 November 2005; accepted in final form 4 April 2006

Kosek, David J., Jeong-su Kim, John K. Petrella, James M. Cross, and Marcas M. Bamman. Efficacy of 3 days/wk resistance training on myofiber hypertrophy and myogenic mechanisms in young vs. older adults. *J Appl Physiol* 101: 531–544, 2006. First published April 13, 2006; doi:10.1152/japplphysiol.01474.2005.—Resistance training (RT) has shown the most promise in reducing/reversing effects of sarcopenia, although the optimum regime specific for older adults remains unclear. We hypothesized myofiber hypertrophy resulting from frequent (3 days/wk, 16 wk) RT would be impaired in older (O; 60–75 yr; 12 women, 13 men), sarcopenic adults compared with young (Y; 20–35 yr; 11 women, 13 men) due to slowed repair/regeneration processes. Myofiber-type distribution and cross-sectional area (CSA) were determined at 0 and 16 wk. Transcript and protein levels of myogenic regulatory factors (MRFs) were assessed as markers of regeneration at 0 and 24 h postexercise, and after 16 wk. Only Y increased type I CSA 18% ($P < 0.001$). O showed smaller type IIa (−16%) and type IIx (−24%) myofibers before training ($P < 0.05$), with differences most notable in women. Both age groups increased type IIa (O, 16%; Y, 25%) and mean type II (O, 23%; Y, 32%) size ($P < 0.05$). Growth was generally most favorable in young men. Percent change scores on fiber size revealed an age × gender interaction for type I fibers ($P < 0.05$) as growth among Y (25%) exceeded that of O (4%) men. Myogenin and myogenic differentiation factor D (MyoD) mRNAs increased ($P < 0.05$) in Y and O, whereas myogenic factor (myf)-5 mRNA increased in Y only ($P < 0.05$). Myf-6 protein increased ($P < 0.05$) in both Y and O. The results generally support our hypothesis as 3 days/wk training led to more robust hypertrophy in Y vs. O, particularly among men. However, this differential hypertrophy adaptation was not explained by age variation in MRF expression.

sarcopenia; myogenin; MyoD; myosin heavy chain

IT IS WELL ESTABLISHED THAT muscle mass declines with age (termed sarcopenia). In the knee extensors (e.g., vastus lateralis), which are important for ambulation and weight-bearing function, a 30% decrease in whole muscle size occurs between the ages of 50 and 80 yr (15, 40). This whole muscle atrophy results from atrophy of type II myofibers (34) and apparent loss of both type I and type II motor units as evidence from cadaveric studies of vastus lateralis indicate the number of myofibers, regardless of fiber type, declines substantially between the sixth and eighth decades (39). In the United States, $18.5 billion of total direct health care costs in 2000 were attributable to sarcopenia (31), and this will undoubtedly increase because the percentage of American adults 65 yr and older is expected to increase from one in nine to 20% of the adult population by 2030 (National Institute on Aging statistics). Resistance training has shown the most promise among interventions aimed to decrease the effects of sarcopenia, as it enhances strength, power, and mobility function and induces varying degrees of skeletal muscle hypertrophy (29). Based on this recent review of findings in older adults, it is not clear whether resistance training prescriptions currently recommended for young adults provide the most effective hypertrophy stimulus for aging muscle (29). We suggest differences in the state of the muscle may be a factor (i.e., the regenerative/recovery rate), as older, sarcopenic adults (vs. young) may benefit from prolonged recovery periods between loading bouts.

Repair/regeneration and growth of skeletal muscle is largely dependent on the addition of myonuclei to existing, terminally differentiated myofibers, by activation of satellite cells (28). With injury or stress to muscle fibers, satellite cells are induced to proliferate and differentiate, ending with their incorporation into mature myofibers as myonuclei. These events are regulated by several local processes that appear to respond in a load-dependent manner. Myogenic regulatory factors (MRFs) are basic helix-loop-helix transcription factors specific to skeletal muscle that regulate satellite cell differentiation and induce transcription of skeletal muscle-specific genes such as creatine kinase, myosin light chains, myosin heavy chains (MHC), troponin I, and desmin (10, 44, 49, 53). Expression of the "early" MRFs, myogenic factor (myf)-5 and myogenic differentiation factor D (MyoD), commit somatic cells to the myogenic lineage, whereas the "late" MRFs, myf-6 and myogenin, terminally differentiate proliferative myoblasts toward formation of multinucleated myotubes in developing muscle (58), or into nuclear donors to developed myofibers for repair/regeneration and growth. Myogenin and MyoD are also implicated in regulating MHC transitions (16, 47, 48).

Basal levels of some MRFs are upregulated in aging, sarcopenic muscle (7, 34, 49) and, based on recent findings in rodents, the magnitude of upregulation appears to be directly linked to the degree of sarcopenia (17), suggesting that sarcopenic muscles remain in a state of failing compensatory effort in an attempt to stave off degeneration and atrophy. Resistance loading upregulates MRF mRNA and protein concentrations (34, 53), presumably facilitating growth/regeneration mechanisms. However, our laboratory's previous findings indicate that acute mRNA responses to loading are generally most favorable in young men (compared with other age/gender groups) whereas the muscles of older, sarcopenic women are less responsive (33, 34). Load-mediated satellite cell activation

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is impaired in aged muscle in vivo (8, 13, 21, 51), and it has been shown that older adults possess a decreased basal rate of mixed muscle protein synthesis (4, 66), which may (65) or may not (63) improve with resistance training. Recent insight from the overload (ablation) model in rodents demonstrates that impaired type II hypertrophy in old age is highly associated with elevated AMP-activated protein kinase (AMPK) phosphorylation, indicative of inhibited protein synthesis (62). Because myofiber hypertrophy is dependent on both net muscle protein synthesis and satellite cell recruitment, overall these findings suggest resistance training-induced myofiber hypertrophy may be impaired in older vs. young adults.

The age-related slowing of regenerative and recovery processes in aged muscle after mechanical loading and/or damage are well documented in animal models and are typically linked to blunted or slowed satellite cell activation (8, 12, 21, 43). Existing data in humans are sparse, but the 3 days/wk resistance training findings of Roth et al. (55) indicate that older women are more susceptible to training-induced myofiber damage than their young counterparts (56), whereas older men (vs. young men) are not. Although these data point to a possible gender difference, we suggest damage susceptibility may be more a function of the state of the muscle than gender per se. For example, in these two studies, myofiber size data were not provided, but because older men possessed pretraining strength levels similar to young men (no significant difference) (55) and older women were 31% weaker than young (56), it is quite possible that the older women were "sarcopenic" within gender, whereas the older men were not. Based on the combined findings from animal and human models, we suggest a 3 days/wk program of sufficient loading intensity to activate regenerative pathways may be effective in young beginners but may not allow sufficient recovery between loading bouts to optimize the growth adaptation in older, sarcopenic muscles. Although limited, there are some published findings in support of this concept after 3 mo of resistance training (64). In a study of young and older men and women, Roth et al. (57) found significant thigh muscle hypertrophy (determined via MRI) in all four age $\times$ gender groups after 6 mo of training. The magnitudes of increase were not statistically different among the four groups and were quite similar in young and older men, whereas young women gained nearly twice the muscle area of older women.

The current recommendations set forth by the American College of Sports Medicine do not differentiate between young and older adults regarding frequency of resistance training, recommending 2–3 days/wk for all beginning adults (36). Further research is needed to better define optimal training frequencies for specific age groups. In our view, an important first step is to establish whether age influences the hypertrophic adaptation to a standardized frequency regimen while controlling for as many potential confounders between young and older subject cohorts as possible (e.g., initial training status, gender composition, etc.).

The primary aim of this study was to test the hypothesis that myofiber hypertrophy as a result of frequent (3 days/wk) resistance training would be impaired in older, sarcopenic adults compared with young subjects. Young (20–35 yr) and older (60–75 yr) untrained men and women completed 16 wk of 3 days/wk leg resistance training. To effectively test this hypothesis, care was taken to ensure that the training program was of the same relative intensity and volume for all subjects in the four age-gender groups. Considering that age-dependent differences in hypertrophy may be mediated by a blunted regenerative capacity and/or slowed recovery rate in older muscle, we also evaluated MRF expression both early and late in the training program. Muscle biopsies were collected at three time points hereafter referred to as baseline preexercise, acute postexercise, and 16-wk posttraining. The acute sample was collected 24 h after each subject's first full exercise bout, whereas the 16-wk sample was obtained 24 h after each subject's final training bout.

Of clinical significance, we report that, after only 16 wk of resistance training, older adults were capable of restoring the size of type II myofibers to the pretraining sizes found in adults ~35 yr younger. However, the findings generally support our overarching hypothesis, because more robust hypertrophy was found in the younger cohort (particularly young men). Although these data demonstrate that adults ~64 yr of age obviously retain the ability to undergo load-mediated hypertrophy, we suggest alternate, age-specific training programs should be pursued to maximize the efficacy of this adaptation.

METHODS

Subjects

Forty-nine adults were recruited from the Birmingham, Alabama metropolitan area into two age groups. Age ranges were 60–75 yr for the older group (12 women, 13 men) and 20–35 yr for the younger group (11 women, 13 men). Subjects were free of any musculoskeletal or other disorders that might have affected their ability to complete testing and/or resistance training. Subjects were not obese (body mass index <30 kg/m^2) nor had any leg resistance training experience within the past 5 yr. None of the subjects were being treated with exogenous testosterone or other pharmacological interventions known to influence muscle mass. The study was approved by the Institutional Review Boards of both the University of Alabama at Birmingham and the Birmingham Veterans Affairs Medical Center. Each subject gave written, informed consent before participation.

Progressive Resistance Training Program and Dynamic Strength Testing

The resistance training program focused on the knee extensors. Subjects trained 3 days/wk for 16 wk. Subjects warmed up on a cycle ergometer or treadmill for ~5 min or until warm (light sweat) before each training session. Resistance training consisted of three exercises, including knee extension, leg press, and squats. Each exercise was performed for 3 sets at 8–12 repetitions using resistance exercise stations or plate-loaded stations (barbell squats and linear 45° leg press). To accurately compare training and testing loads between weight-stack and free-weight stations, actual resistances were determined for each weight stack using a load cell and regression procedures as our laboratory has described previously (52). The amount of rest between sets was standardized for all subjects at 90 s.

Subjects completed two familiarization sessions to receive instruction on proper technique and to practice the dynamic strength testing protocols (52). Subjects returned to the laboratory 2–3 days after the second familiarization session for knee extension, leg press, and squat one-repetition maximum (1 RM) assessments using established methods. Attempts of 1 RM with progressively increasing load were performed with each attempt separated by 90- to 120-s rest intervals. 1 RM was defined as the highest load lifted through a full range of motion before two failed attempts at a given load. Verbal encouragement was provided during all 1-RM attempts.

After dynamic strength testing to establish 1 RM, subjects initiated the training protocol by performing two sets of each exercise for two to three sessions. Once completed, subjects began the full training protocol of three sets per exercise, and this session was considered the start of the 16-wk training period. Initially, training loads were based on 80% of baseline 1-RM strength. As training progressed, resistance was incremented when a subject completed 12 repetitions for at least two of the three total sets at a given resistance while maintaining proper form. The typical increase in resistance was 2.3 kg for knee extension, and it was ~5% for leg press and squat. The goal of this progression was to induce volitional fatigue in the 8- to 12-repetition range for each subject throughout the training program. 1-RM testing was repeated at midtraining (*week 8*) and posttraining (*week 16*). Actual load intensities and repetitions per set were statistically evaluated and are summarized in RESULTS.

Body Composition

Thigh lean mass, total body lean mass, and percent body fat were determined by dual-energy X-ray absorptiometry (DEXA) using a Lunar Prodigy (model 8743, GE Lunar, Madison, WI) and enCORE 2002 software (version 6.10.029) according to the manufacturer's instructions.

Muscle Biopsy and Tissue Preparation Procedures

All muscle biopsies were performed in the Pittman General Clinical Research Center at the University of Alabama at Birmingham. Muscle samples were collected from the vastus lateralis muscle by percutaneous needle biopsy using a 5-mm Bergstrom biopsy needle under suction as previously described (18). The baseline biopsy was taken from the left leg, the 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, and the third, 16-wk posttraining biopsy was obtained 24 h after the final training session. At the bedside, visible connective and adipose tissues were removed with the aid of a dissecting microscope. Portions of each sample 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 it was frozen in liquid nitrogen-cooled isopentane. All samples were stored at −80° C until analyses.

Total RNA Isolation

The procedure of RNA isolation has been described in detail previously (33). Briefly, frozen muscle samples (average 35 mg) were homogenized, and total RNA was extracted using the TRI Reagent (Molecular Research Center, Cincinnati, OH), followed by precipitation with isopropanol, two ethanol washes, drying, and suspension in nuclease-free water at a ratio of 0.8 μl/mg muscle. Fluorometric analysis (TD-700, Turner Designs, Sunnyvale, CA) was performed to determine RNA concentration using the RiboGreen RNA Quantitation Kit (Molecular Probes, Eugene, OR) as described previously (33). RNA samples were stored at −80°C.

RT-PCR

One microgram of RNA was reverse transcribed in a total volume of 20 μl using SuperScript II Reverse Transcriptase (Invitrogen, GIBCO-BRL, Carlsbad, CA) with a mix of oligo(dT) (100 ng/reaction) and random primers (200 ng/reaction). After 50-min incubation at 45°C, the RT reaction mixtures were heated at 90°C for 5 min to discontinue the reaction and then stored at −80°C for subsequent PCR analyses.

A relative RT-PCR method using 18S ribosomal RNA as an internal standard (Invitrogen, Life Technology, GIBCO-BRL) was used to determine relative expression levels of the target mRNAs. The specific primer sets used to amplify myogenin, MyoD, myf-5, and myf-6 mRNAs were published previously (34). Primers were designed using the Primer Select computer program (DNAStar, Madison, WI) and custom-made by Invitrogen (GIBCO). Interaction tests between target mRNA primers and the alternate 18S primers were conducted and primer sets were selected for use after confirming no interaction.

For each PCR reaction, 18S (with a 324-bp product) was coamplified with each target cDNA (mRNA) to express each as a ratio of target mRNA/18S. The 18S primers were mixed with competimers to ensure that 18S and each target mRNA coamplified in the linear range. The ratio of this primer-competimer mixture was optimized in preliminary experiments and ranged from 1:15 to 1:50 depending on the abundance of the target mRNA.

Based on the number of PCR reactions, a PCR premix was prepared as described previously (33). For each PCR, 1 μl of RT product (cDNA) was added into 24 μl of premix and topped with 50 μl mineral oil (Sigma-Aldrich, St. Louis, MO). PCR was carried out in a DNA Engine (PTC-200) Peltier Thermal Cycler (MJ Research, Waltham, MA) with an initial denaturing step of 3 min at 96°C, followed by specific cycles (30–34 cycles depending on the results of linearity tests for each target mRNA and 18S) of 1 min at 96°C, 45 s at specific annealing temperatures (57–62°C depending on primers), 45 s at 75°C, and a final step of 3 min at 72°C. Immediately after PCR, 25 μl of PCR product (22 μl of the reaction mixture diluted with 3 μl of loading buffer) were separated by electrophoresis (100 V constant) in a 2% agarose gel for 1.5 or 2 h [depending on the band separation between 18S (324 bp) and the specific target gene due to the size of each mRNA product]. Gels were run with molecular weight markers (100 bp Hyper Ladder IV, Genesee Scientific, San Diego, CA) to confirm the expected size of each mRNA. To eliminate age group or gender bias, each 20-well gel contained samples for subjects within each group (i.e., young men, young women, older men, older women) with the different subject groups loaded in random order on each gel. Ethidium bromide (0.1 μg/ml) was premixed in the 2% agarose gel, and images were captured under UV light in a Bio-Rad ChemiDoc imaging system (Hercules, CA). Band densitometry was performed using Bio-Rad Quantity One software. Parameters for image development were described in detail previously (33).

Protein Immunoblotting

Immunoblotting was conducted to assess muscle protein lysate concentrations of MyoD, myogenin, and myf-6. In pilot experiments, we were unable to verify a reliable primary antibody against myf-5. Frozen muscle samples (30–40 mg) were homogenized and supernatant assayed for total protein as described in detail (7). Briefly, samples were powdered using a liquid nitrogen-cooled mortar and pestle and homogenized in 3 μl/mg muscle of ice-cold lysis buffer [150 mM NaCl, 50 mM Tris·HCl (pH 7.4), 0.5% NP-40, 1% deoxycholate, 0.1% SDS, 1% Triton X-100, 5 mM EDTA, 100 μM PMSF, and 0.5× protease inhibitor cocktail containing bestatin, leupeptin, aprotinin (P2714, Sigma-Aldrich, St. Louis, MO)]. After two centrifugation steps (15,000 *g* for 20 min at 4°C), supernatant samples were assayed for total protein using the bicinchoninic acid technique with BSA as a standard (5). Twenty micrograms of total protein diluted in Laemmli buffer were separated by SDS-PAGE (120 V constant) in 12% minigels (Bio-Rad MP3, Bio-Rad Laboratories). Proteins were transferred at constant current (1 mA/cm^2 × 30 min) to polyvinylidene difluoride membranes using a semidry transfer cell (Trans-Blot SD, Bio-Rad Laboratories). Within subjects, baseline, acute postexercise, and 16-wk posttraining samples were loaded in adjacent lanes. To control for age or gender bias, each 10- or 12-lane gel contained samples for one subject from each of three different age-gender groups, with the different subject groups loaded in random order on each gel. Equal loading across lanes and equal transfer were verified

by staining all gels (after transfer) with Coomassie blue and staining randomly selected membranes with Ponceau-S.

Primary antibody specificities, optimal blocking conditions, and target antigen migration patterns (i.e., molecular weights) were confirmed in control experiments and in our laboratory's previous work (7). Immunoblotting was carried out using rabbit polyclonal antibodies against MyoD (1:1,000, sc-760), myogenin (1:1,000, sc-576), and myf-6 (1:1,000, sc-301). Membranes were blocked with 2% BSA and 2% milk in PBST (PBS and 1% Tween 20), while primary and horseradish peroxidase-conjugated goat anti-rabbit secondary (1:50,000) antibodies were diluted in 0.5% BSA and 0.5% milk in PBST. Membranes were blocked overnight at 4°C under gentle agitation followed by incubation in primary and secondary antibodies for 1 h each at room temperature, with a PBST-rinse protocol (1 quick rinse, 1 × 15 min, 2 × 5 min) after each treatment. All bands were visualized by chemiluminescence (Super West Dura kit, Pierce Biotechnologies, Rockford, IL) in a Bio-Rad ChemiDoc imaging system, and band densitometry was performed using Bio-Rad Quantity One software as our laboratory previously described in detail (7). 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 four age-gender groups across the membranes, enabled us to accurately test for age, gender, and resistance training effects.

Immunofluorescence Microscopy

Our laboratory recently detailed the methods used for myofiber typing based on MHC isoform immunoreactivity (34). Briefly, 6-μm sections were fixed for 45 min at room temperature in 3% neutral-buffered formalin. After fixation, sections were washed 2 × 5 min with 1× PBS (all subsequent PBS wash steps were 3 × 5 min). Sections were blocked with 5% goat serum in PBS for 20 min at room temperature followed by a wash step. Primary and secondary antibodies were diluted in 1% goat serum in PBS. Anti-MHC I primary antibody [mouse monoclonal antibody (MAb) NCL-MHCs, NovoCastra Laboratories, 1:100] was applied for 30 min at 37°C. After a wash step, sections were incubated with ALEXA 594-conjugated goat anti-mouse secondary antibody (Ab) (Pierce Biotechnologies, 1:200) for 30 min at 37°C. Sections were washed and again blocked (5% goat serum in PBS) for 20 min at room temperature. To locate sarcolemmae for myofiber sizing, a wash step was followed by incubation with anti-laminin mouse MAb (VP-L551, NovoCastra Laboratories, 1:80) for 30 min at 37°C, a wash step, and incubation with ALEXA 488-conjugated goat anti-mouse secondary Ab (Pierce Biotechnologies, 1:200) for 30 min at 37°C. Slides were then washed and subjected to a third and final block (5% goat serum in PBS) for 20 min at room temperature. After a wash step, sections were incubated with the final primary antibody (anti-MHC IIa mouse MAb, University of Iowa Hybridoma Bank, 1:80) for 30 min at 37°C, washed, and incubated with ALEXA 488-conjugated goat anti-mouse secondary Ab (Pierce Biotechnologies, 1:200) for 30 min at 37°C. Nuclei were revealed by a Hoecsht 33258 DNA counterstain (Molecular Probes, 1:10,000 in PBS) for 2 min at room temperature. Slides were mounted with 1% paraphenylene diamine, 90% glycerol in PBS. Slides and coverslips were bound together by use of nail polish, and they were stored protected from light at −20°C.

High-resolution (48-bit TIFF) fluorescent images were captured at ×10 and ×20, and image analysis was performed using Image-Pro Plus 5.0 software. All analyses were conducted by a single analyst blinded to age, gender, and time point (before or after 16 wk of training) of each sample. Myofiber type distribution was determined from 928 ± 37 myofibers per sample before training and 815 ± 33 after training. We confirmed MHC isoform specificities of these MAbs by Western blot (Fig. 1). Myofibers positive for 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 (Fig. 1). With this technique, hybrid myofibers (e.g., coexpression of I/IIa or IIa/IIx) are revealed by both color and intensity. Myofibers coexpressing more than one MHC isoform were excluded from analyses. For cross-sectional area (CSA) measurements, each myofiber was manually traced along its laminin-stained border. CSA (in μm^2) was calibrated using a stage micrometer, and only those fibers determined to be cross sectional based on a roundness factor <1.639 were included in the analysis (roundness = $perimeter^2/4\pi area$; perfect circle = 1.0, pentagon = 1.163, square = 1.266, equilateral triangle = 0.639). The number of randomly selected myofibers by type included in CSA analyses were 60 ± 2 type I, 63 ± 2 type IIa, and 49 ± 2 type IIx (pretraining only). Three subjects did not have sufficient type IIx distribution to obtain IIx CSA or IIx area distribution before training. Because of the well-documented MHC IIx to MHC IIa shift induced by resistance training, this increased to 41 subjects after training who lacked a sufficient number of IIx myofibers for CSA assessment. Training-induced changes in myofiber size were therefore restricted to type I, type IIa, and type II myofibers, with type II CSA being a weighted average based on the relative distributions of IIa and IIx myofibers.

Statistical Analysis

Data are reported as means ± SE. Between groups differences in preexercise descriptive variables were tested using age × gender ANOVA. All variables measured before and after training were

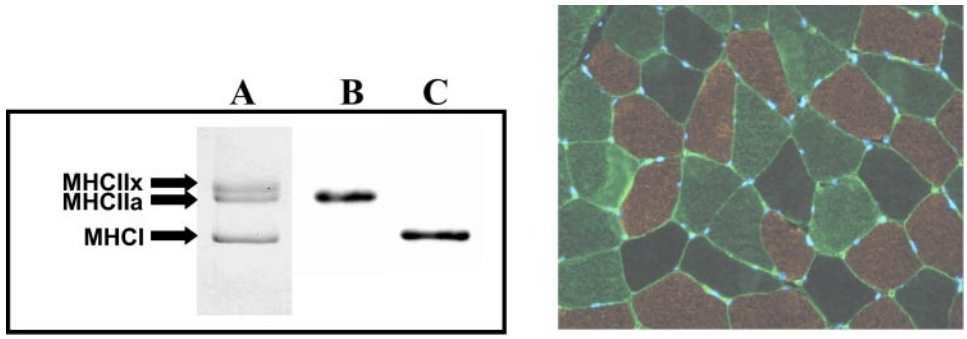

Fig. 1. Myosin heavy chain (MHC) immunoblot and immunostain. MHC isoform specificities of monoclonal antibodies used for myofiber typing were confirmed by immunoblot (*left*). *Lane A*: 8% SDS-PAGE (50:1 acrylamide-bis) of myofibrillar protein stained with rapid Coomassie blue (*top* band, MHC IIx; *middle* band; MHC IIa, *bottom* band, MHC I). *Lane B*: MHC IIa-specific monoclonal antibody (MAb) A4.74 (University of Iowa Hybridoma Bank). *Lane C*: MHC I-specific MAb NCL-MHCs (Novocastra Laboratories). Sample immunostain (*right*) displays MHC I-positive myofibers in rust and MHC IIa-positive myofibers in green, and MHC IIx myofibers are dark (negative for both MHCI and MHC IIa). Sarcolemmae were revealed by anti-laminin MAb (VP-L551, Novocastra Laboratories) and nuclei via Hoechst DNA counterstain.

analyzed using age × gender × training repeated-measures ANOVA. MRF protein and mRNA concentrations were assessed across all three biopsy time points and were thus analyzed via two × two × three repeated-measures ANOVA. Myofiber size and distribution were assessed at baseline and posttraining and were thus tested via two × two × two repeated-measures ANOVA. For each ANOVA model with a significant main or interaction effect, Tukey's honestly significant difference tests were performed post hoc to localize the effect(s). Statistical significance was accepted at $P < 0.05$ for all tests.

RESULTS

Subject Characteristics

Body composition and strength results for each of the 4 age-gender groups are shown in Table 1. Within age group, men and women were of similar age. Typical gender differences ($P < 0.05$) were noted for height, weight, lean mass, and strength, with higher values among the men. Significant main effects confirmed the expected age and gender differences in percent body fat (lower in men, lower in young; $P < 0.05$), but these were accompanied by an age × gender interaction ($P < 0.05$), because percent body fat among young women was relatively high and not different from older women. Main age effects ($P < 0.05$) were noted for all three dynamic strength measures. Across the three strength tests, 1-RM strength at baseline was 29% lower in older vs. young men and 19% lower in older vs. young women.

During resistance training, a minimum adherence rate of 83.3% (5 of 6 sessions) was required, and the average adherence rate was 90%. There were no differences in adherence rates between groups based on age or gender (young women 91%, young men 90%, older women 92%, older men 89%). We conducted analyses of training program design variables post hoc to test whether the program differed by age or gender in volume or intensity (data not displayed). No differences by age or gender were found in the number of repetitions performed per set for any of the three exercises, with overall averages of 10.6, 10.9, and 10.2 repetitions per set for the squat, leg press, and knee extension, respectively. Squat training intensity did not differ by age or gender and averaged 74% 1 RM at 8 wk and 75% 1 RM at 16 wk. Leg press training intensity was unexpectedly different by gender (higher in women, $P < 0.01$), averaging 84% 1 RM at 8 wk in women vs. 78% in men. This gender difference remained at 16 wk; 83% in women vs. 76% in men ($P < 0.01$). Knee extension training intensity did not differ by age or gender at 8 wk but was significantly higher in older adults (68% 1 RM) vs. young (63% 1 RM) by 16 wk ($P < 0.05$). We presume subjects required relatively lower loads to achieve the target number of full range of motion repetitions during knee extension training due to fatigue because this movement was always performed last.

Strength levels for each of the 1-RM tests improved substantially after 16 wk of RT in all four groups ($P < 0.05$). Across groups, strength increases averaged 38–49% (leg press), 34–44% (knee extension), and 28–37% (squat). Relative strength gains were similar among men and women. On absolute strength data, gender × training interactions for each strength test ($P < 0.05$) were driven by larger absolute gains in men.

Myofiber-Type Distribution and Size

Myofibers were classified based on MHC isoform expression as type I (MHC I only), IIa (MHC IIa only), or IIx (negative for both MHC I and MHC IIa). Hybrid myofibers (I/IIa or IIa/IIx) were excluded from distribution and size measurements. Fiber-type distribution results as shown in Table 2 were determined on 928 ± 37 myofibers per sample before training and 815 ± 33 fibers per sample after training. The distribution of type I myofibers did not differ among the

Table 1. *Descriptive characteristics and effects of training on body composition and strength*

	Young Women (n = 11)	Young Men (n = 13)	Older Women (n = 12)	Older Men (n = 13)
Age, yr	27.9±1.1	26.2±1.4	63.3±0.9	64.5±1.1
Height,[d] cm	164.5±2.4	179.7±2.0	161.8±2.2	178.5±1.9
Weight,[d] kg				
Baseline	67.6±3.4	79.1±2.4	65.5±3.0	87.9±3.0
16 wk	68.2±3.4	80.3±1.8	64.8±3.3	87.7±3.1
Body fat,[b,c,d,f] %				
Baseline	36.8±1.8	21.8±2.5	39.1±1.4	31.7±1.6
16 wk	36.3±2.1	20.8±2.3	37.7±1.7	30.4±1.6
Total LM,[b,d] kg				
Baseline	40.1±1.1	59.0±1.3	37.5±1.6	57.3±1.6
16 wk	40.8±1.1	60.0±1.3	38.1±1.6	58.1±1.7
		Bilateral 1-RM strength assessments, N		
Knee extension[b,c,d,e]				
Baseline	397±27	637±35	302±14	423±27
16 wk	547±33[a]	853±50[a]	415±31[a]	609±29[a]
Squat[b,c,d,e,f]				
Baseline	592±34	921±54	503±22	695±19
16 wk	757±28[a]	1,265±50[a]	683±30[a]	927±40[a]
Leg press[b,c,d,e]				
Baseline	928±47	1,538±90	754±45	1,181±55
16 wk	1,361±65[a]	2,121±99[a]	1,122±63[a]	1,758±91[a]

Values are means ± SE; n, no. of subjects. LM, lean mass; TLM, thigh lean mass; 1 RM, one-repetition maximum voluntary strength. [a]Within-groups training effect, $P < 0.05$. [b]Main training effect, $P < 0.05$. [c]Main age effect, $P < 0.05$. [d]Main gender effect, $P < 0.05$. [e]Gender × training interaction, $P < 0.05$. [f]Age × gender interaction, $P < 0.05$.

Table 2. *Myofiber cross-sectional area, type distribution, and type area distribution*

	All Young (n = 23)	All Older (n = 24)	Young Women (n = 10)	Young Men (n = 13)	Older Women (n = 11)	Older Men (n = 13)
			Myofiber CSA, μm^2			
Type I[b,e]						
Baseline	4,277±199	4,754±271	4,346±375	4,225±216	4,100±311	5,308±368
16 wk	5,055±247[a]	5,105±227	4,728±258	5,306±384[a]	4,773±353	5,385±284
Type IIa[b,c,d]						
Baseline	4,726±232	3,980±297	4,083±244	5,220±305	2,979±186	4,827±398
16 wk	5,905±307[a]	4,613±319[a]	4,853±265	6,714±374[a]	3,611±201	5,461±449
Type II[b,c,d]						
Baseline	4,450±238	3,701±282	3,788±225	4,960±324	2,713±174	4,537±365
16 wk	5,869±307[a]	4,552±323[a]	4,823±270[a]	6,673±373[a]	3,544±202	5,406±457[a]
Type IIx[c,d]						
Baseline	3,481±209	2,632±285	2,934±197	3,845±281	1,642±135	3,470±382
			Myofiber type distribution, %			
Type I						
Baseline	37.8±2.9	35.0±1.8	38.8±2.9	37.0±4.8	38.5±2.4	32.1±2.3
16 wk	35.8±2.8	35.9±2.2	38.4±4.2	33.8±3.8	38.2±2.8	33.4±3.0
Type IIa[b]						
Baseline	47.2±2.4	50.2±1.7	45.8±2.6	48.2±4.0	49.1±2.3	51.3±2.3
16 wk	63.2±2.8[a]	62.8±2.2[a]	61.1±4.1[a]	64.7±4.0[a]	60.0±2.6	65.8±3.1[a]
Type IIx[b]						
Baseline	15.1±1.9	14.8±2.0	15.4±3.0	14.8±2.6	12.4±2.4	16.5±2.9
16 wk	1.0±0.5[a]	1.3±0.5[a]	0.5±0.2[a]	1.5±0.8[a]	1.9±0.9[a]	0.8±0.4[a]
			Myofiber type area distribution, %			
Type I[b,d]						
Baseline	37.6±2.8	41.4±2.2	41.5±2.4	34.5±4.5	48.1±3.0	35.7±2.2
16 wk	32.7±2.6	39.4±2.6	37.7±3.8	28.9±3.4	45.7±3.5	34.0±3.1
Type IIa[b,d]						
Baseline	50.5±2.4	48.5±1.8	47.2±1.9	53.0±4.0	44.9±2.2	51.7±2.4
16 wk	66.5±2.6[a]	60.3±2.5[a]	62.3±3.8[a]	69.8±3.4[a]	53.4±3.1	66.1±3.1[a]
Type IIx[b]						
Baseline	12.0±1.8	10.0±1.6	11.3±2.5	12.5±2.6	7.0±1.8	12.6±2.5
16 wk	0.8±0.5[a]	0.4±0.3[a]	0.0±0.0[a]	1.4±0.9[a]	0.8±0.6	0.0±0.0[a]

Values are means ± SE; n, no. of subjects. CSA, cross-sectional area. [a]Within-groups training effect, $P < 0.05$. [b]Main training effect, $P < 0.05$. [c]Main age effect, $P < 0.05$. [d]Main gender effect, $P < 0.05$. [e]Age × gender × training interaction, $P < 0.05$.

groups and did not significantly change with training, as the overall average distribution was 36.4 ± 1.7% at baseline and 35.9 ± 1.7% after training. However, taking into account changes in fiber size, a main training effect revealed an overall decline in the area distribution of type I myofibers after training (39.5 ± 1.8% pretraining to 36.1 ± 1.9% posttraining; $P < 0.05$). A main gender effect ($P < 0.005$) revealed that women had a substantially higher type I area distribution than men both before (45.0 ± 2.0% vs. 35.1 ± 2.5%) and after (41.9 ± 2.7% vs. 31.4 ± 2.3%) training.

The resistance training program resulted in the IIx to IIa MHC shift typically found after exercise training. The distribution of MHC IIa (type IIa) myofibers increased markedly after training ($P < 0.001$) in both women (47.4 ± 1.7 to 60.1 ± 2.3%, $P < 0.001$) and men (49.8 ± 2.3 to 65.3 ± 2.5%, $P < 0.001$). Similar increases were also noted by age ($P < 0.001$). No significant age or gender effects in type IIa distribution were found. Post hoc tests within groups showed that older women were the only age-gender group not to significantly increase the distribution of MHC IIa myofibers ($P = 0.118$) Overall, the area distribution of type IIa myofibers increased robustly with training (49.5 ± 1.5% pretraining to 63.3 ± 1.9% posttraining; $P < 0.001$). A main gender effect ($P < 0.005$) was driven primarily by a significantly higher posttraining value in men (67.9 ± 2.3%) vs. women (57.7 ± 2.6%). Within age-gender groups, only elderly women did not significantly increase type IIa area distribution, whereas young men exhibited the most pronounced change.

The distribution of MHC IIx myofibers (MHC IIa and MHC I negative fibers) fell markedly in all groups after training with an overall drop from 14.9 ± 1.4 to 1.2 ± 0.3% (main training effect, $P < 0.001$). All four groups responded similarly to training with a robust decline in these IIx myofibers as no age or gender differences were found. As expected, a main training effect was also observed for the area distribution of type IIx myofibers ($P < 0.001$). Within groups, the area distribution of MHC IIx myofibers declined significantly in all but older women ($P = 0.158$). Presumably, this lack of effect was due in part to the extremely small type IIx myofibers among older women.

Results of CSA measurements by fiber type are displayed in Table 2. As a consequence of the IIx-to-IIa MHC shift, the numbers of MHC IIx myofibers posttraining were not sufficient to quantify type IIx CSA after training. Training effects for CSA were therefore evaluated for type I, type IIa, and a combined measure of all type II myofibers (weighted by percent distribution of MHC IIa and MHC IIx fibers). For type I myofiber CSA, a main training effect ($P < 0.001$) and an age × gender × training interaction ($P < 0.05$) were observed. Overall, type I CSA increased 12% from 4,521 ± 171 μm^2 to

5,080 ± 166 μm^2. This was driven by a significant 18% (778 μm^2) increase ($P < 0.001$) in young adults, as type I hypertrophy was not found among older subjects. Within age-gender groups, type I hypertrophy was found only in young men (26% or 1,081 μm^2; $P < 0.005$). No significant main effects of age or gender were noted.

Main age and gender effects ($P < 0.005$) indicated that type IIa myofibers were overall 19% smaller in older adults and, across age, 31% smaller among women compared with men. The IIa fibers of older women were significantly smaller than all other groups. Resistance training resulted in an overall main training effect ($P < 0.001$) indicating 21% type IIa hypertrophy (4,345 ± 195 to 5,245 ± 239 μm^2). Collapsed across gender, young adults realized 25% (1,179 μm^2; $P < 0.001$) type IIa hypertrophy, whereas significant but somewhat lesser growth (16% or 633 μm^2; $P < 0.05$) was noted in older adults, resulting in a trend toward age group × training interaction ($P = 0.10$). However, it is noteworthy that type IIa size after training in older adults was not significantly different from young before training and, in fact, the two values were nearly matched ($P = 0.98$). Within groups, only young men significantly increased type IIa myofiber size (1,494 μm^2 or 29%; $P < 0.001$).

Analysis of type II CSA (weighted by percent distribution of MHC IIa and MHC IIx fibers) yielded significant main effects for age ($P < 0.005$), gender ($P < 0.001$), and training ($P < 0.001$) and a tendency for an age × training interaction ($P = 0.084$). Type II fibers were smaller in older vs. young adults and in women compared with men. Overall, type II CSA increased 28% from 4,068 ± 191 to 5,197 ± 241 μm^2. Young adults realized 32% (1,419 μm^2; $P < 0.001$) type II hypertrophy compared with 23% (851 μm^2; $P < 0.005$) among older subjects. However, type II fiber size after training in older adults was restored to the young value before training ($P = 0.99$). Within age-gender groups, resistance training induced type II hypertrophy was found in young men and young women, as well as in older men ($P < 0.05$). Young men realized the largest absolute (1,713 μm^2) and relative (35%) increases in type II CSA, which represented roughly twice the growth seen among older men (869 μm^2 or 19%). Type II myofiber CSA in young women increased 1,035 μm^2 (27%). The numerical change of 831 μm^2 among older women did not reach significance ($P = 0.14$) even though the small average type II fiber size at baseline appeared to expand 31%.

In a separate analysis, we tested whether individual percent change scores in fiber size differed among the four groups. We found an age × gender interaction for type I fibers ($P < 0.05$) as the mean percent growth among individual young men (25%) exceeded that of older men (4%). No significant group differences were noted for percent change scores in type II fiber size.

Between-groups ANOVA was tested on pretraining CSA data to assess age-related atrophy in MHC IIx fibers (Table 2). Marked group differences were noted for the CSA of type IIx myofibers, as main age ($P < 0.01$) and gender ($P < 0.001$) effects were found. Overall, type IIx myofibers were 24% smaller in older compared with young subjects and 40% smaller in women (2,186 ± 187 μm^2) vs. men (3,650 ± 238 μm^2). Age-related type IIx atrophy among older women was indicated by a striking 44% smaller IIx CSA compared with young women. In contrast, type IIx myofiber size did not differ significantly between young and older men.

mRNA and Protein Levels of Myogenic Regulatory Factors

MRF mRNA and protein results by age and gender are shown in Figs. 2–5. mRNA data are plotted by age in Fig. 2 and by gender in Fig. 3, and protein data are plotted by age in Fig. 4 and by gender in Fig. 5 (age and gender were the independent variables in the repeated-measures ANOVA models). We did not plot results for each age-gender within-groups cohort separately because no three-way interactions (age × gender × training) were found. We recognize that transcript and protein levels vary over time and likely in response to each loading bout; thus there are no lines connecting the biopsy time points on each figure.

MRF mRNA levels. These results are shown by age group in Fig. 2 and by gender in Fig. 3. A main training effect for myogenin mRNA concentration indicated an overall increase of 52% from baseline to *week 16* ($P < 0.001$), with the bulk of the increase occurring in response to the initial loading bout (48% acute response, $P < 0.001$). No main age or gender effects were noted in the overall ANOVA model for myogenin mRNA levels. However, as shown in Fig. 2, the acute response was not significant among older adults as post hoc tests revealed that myogenin mRNA expression increased at this early time point in young subjects only (55%; $P < 0.001$). Myogenin mRNA levels did not increase significantly above baseline in elderly subjects until the 16-wk time point (47%; $P < 0.05$). When collapsed across age and displayed by gender, men and women responded as shown in Fig. 3. A significant, early acute elevation of myogenin mRNA occurred only among men (66%; $P < 0.005$), whereas in women a significant increase was not detected until after 16 wk of training (49%; $P < 0.005$). These differential responses by age (Fig. 2) and gender (Fig. 3) were primarily driven by young men as they saw the largest load-mediated elevation of myogenin mRNA and, in fact, were the only age-gender cohort to show significant within-groups increases (71% acute response, $P < 0.01$; 68% overall increase from baseline to wk 16, $P < 0.05$).

No main effect of the training program was found for myf-6 mRNA concentration. However, myf-6 expression tended to be higher in older adults ($P = 0.078$) across the three time points (Fig. 2). A gender × training interaction ($P = 0.05$) was noted, as myf-6 mRNA levels tended to fall in women by wk 16 ($P = 0.051$) but did not change among the men (Fig. 3). A main training effect for MyoD mRNA levels resulted from an overall increase of 69% from baseline to wk 16 ($P < 0.001$). MyoD expression significantly rose 90% in young adults ($P < 0.005$) and 54% in older subjects ($P < 0.05$) (Fig. 2). Parsed by gender (Fig. 3), a significant increment in MyoD mRNA was noted only among women from baseline to 16 wk ($P < 0.001$) and from the acute time point to 16 wk ($P < 0.01$). This effect was primarily attributed to young women because they exhibited remarkably low levels of MyoD mRNA before training and were the only age-gender cohort to show a significant within-groups increase after 16 wk of training (5-fold; $P < 0.005$). Young women were also responsible for a significant

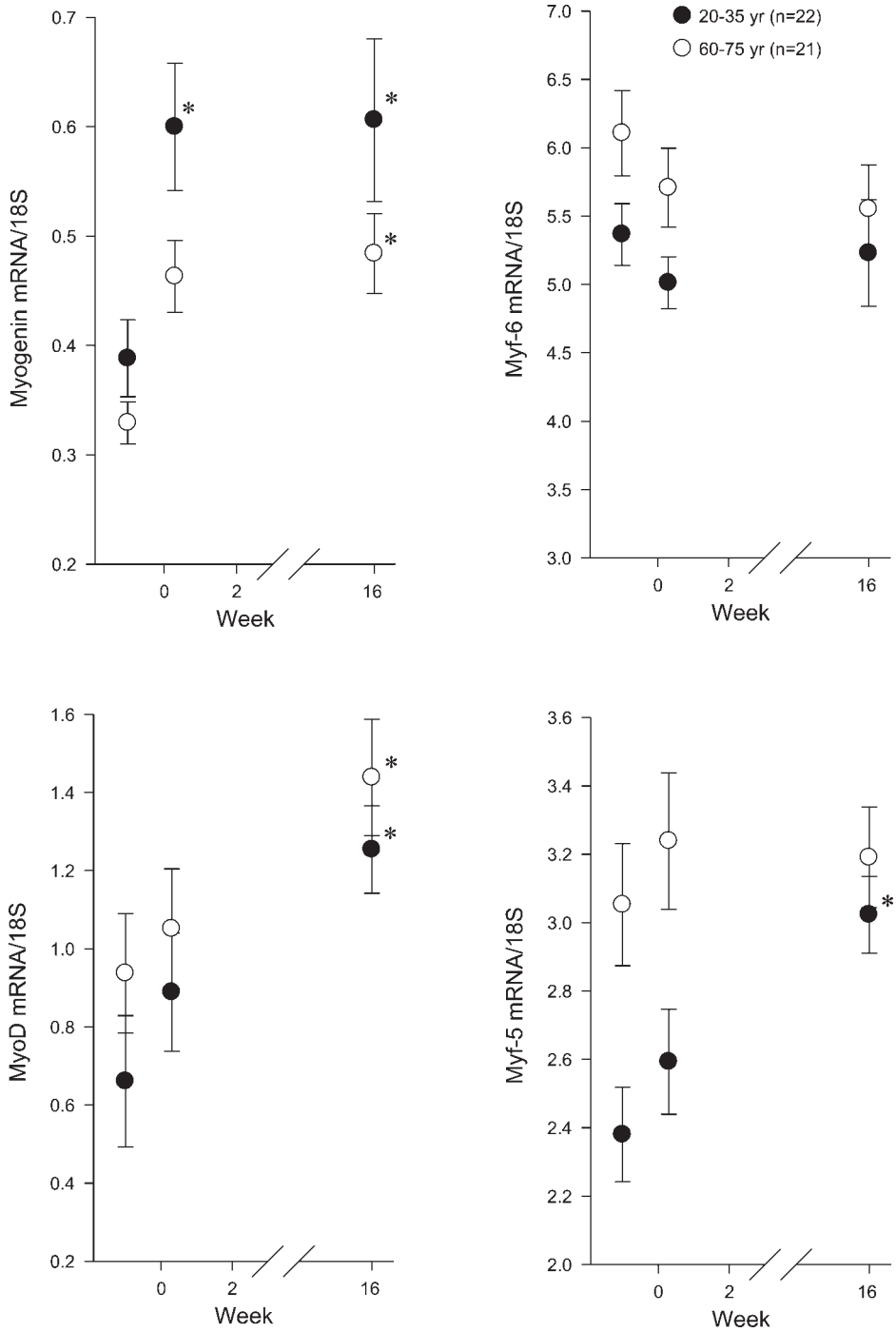

Fig. 2. Myogenic regulatory factor mRNA levels by age group. Relative RT-PCR results for myogenin, myogenic factor (myf)-6, myogenic differentiation factor (MyoD), and myf-5 mRNA levels by age across 3 time points (baseline, acute loading, 16-wk training) are shown. Values are means ± SE. *Significant change from baseline within group, $P < 0.05$. Overall main and interaction effects from the age × gender × training ANOVA models are described in RESULTS.

age × gender interaction ($P < 0.05$) because levels of MyoD mRNA expression were significantly lower in young vs. older women (no age difference was noted in men). The underlying cause of these low MyoD transcript levels in young women is not known, but the finding has been consistent in our laboratory (34). For myf-5 mRNA concentration, a main age effect ($P < 0.01$) was noted as older adults expressed 28% more myf-5 at baseline (Fig. 2). An overall training effect for myf-5 mRNA levels (15% increase; $P < 0.005$) was driven almost entirely by young adults, as post hoc tests revealed a significant increase by *week 16* (27%, $P < 0.005$) for myf-5 mRNA expression among young subjects only (Fig. 2). Furthermore, this response was driven more by young men than young women; of the four age-gender groups, only young men significantly enhanced transcript levels of myf-5 from 0 to 16 wk (34%; $P < 0.05$). Parsed by gender (Fig. 3), young men were responsible for the significant overall increase seen among men only (19%; $P < 0.05$).

MRF protein levels. These results are shown by age in Fig. 4 and by gender in Fig. 5. For myogenin protein concentration, a main training effect ($P < 0.01$) was found. Post hoc testing localized this to an overall increase from the acute time point

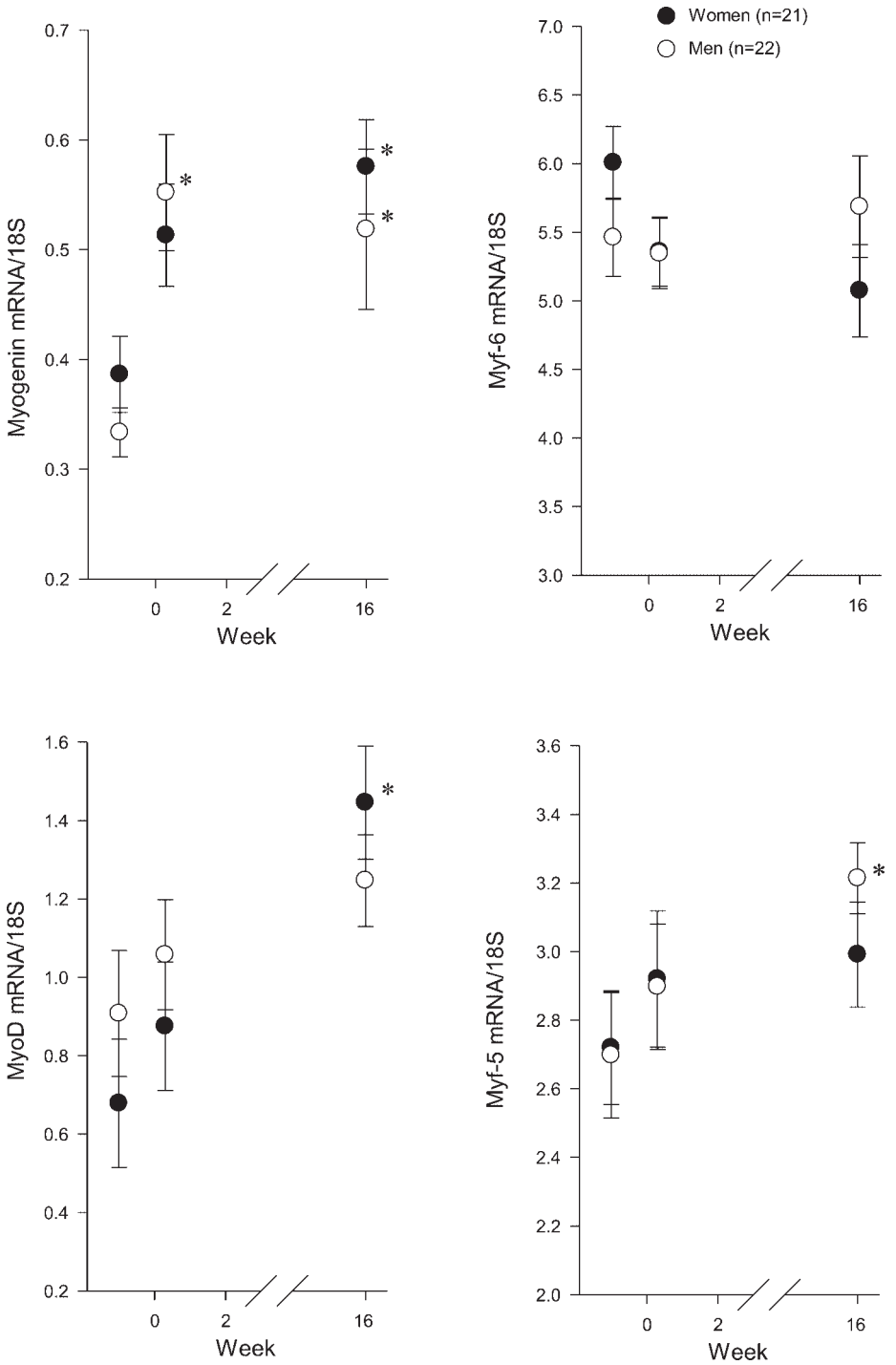

Fig. 3. Myogenic regulatory factor mRNA levels by gender. Relative RT-PCR results for myogenin, myf-6, MyoD, and myf-5 mRNA levels by gender across 3 time points (baseline, acute loading, 16-wk training) are shown. Values are means ± SE. *Significant change from baseline within group, $P < 0.05$. Overall main and interaction effects from the age × gender × training ANOVA models are described in RESULTS.

to 16 wk (14%; $P < 0.005$). No significant differences by age were noted (Fig. 4). The rise from the second time point to 16 wk was driven by women (23%; $P < 0.05$) after a tendency for myogenin protein concentration to fall acutely (Fig. 5). A robust overall increase (77%) in myf-6 protein concentration was found (main training effect; $P < 0.001$). Post hoc analyses showed significant increases in myf-6 protein from baseline to 16 wk and from the acute time point to 16 wk ($P < 0.001$). As shown in Fig. 4, both age groups responded similarly with rather large increases in myf-6 protein concentration (64% in young; 93% in older adults). A main gender effect was observed ($P < 0.01$), as myf-6 protein levels were on average 43% higher among the men (Fig. 5). Furthermore, the training response was mainly driven by men with nearly a twofold increase in myf-6 protein concentration by 16 wk ($P < 0.005$) (Fig. 5). Despite the findings at the transcript level, MyoD protein concentrations were not influenced by age or gender and remained unchanged across the three time points (Figs. 4 and 5). We were unable to assess myf-5 protein concentrations because no Ab could be validated.

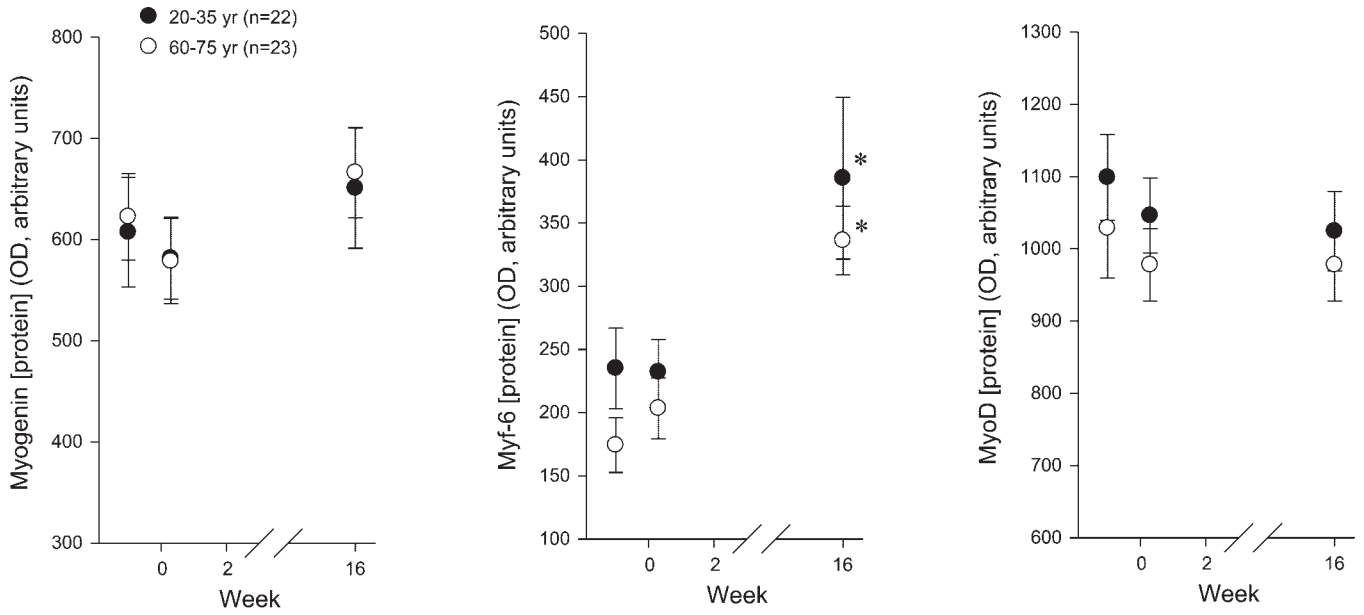

Fig. 4. Myogenic regulatory factor protein levels by age group. Immunoblot results for myogenin, myf-6, and MyoD protein levels by age across 3 time points (baseline, acute loading, 16-wk training) are shown. Values are means ± SE. *Significant change from baseline within group, $P < 0.05$. Overall main and interaction effects from the age × gender × training ANOVA models are described in RESULTS.

DISCUSSION

Age Differences in Resistance Training-Induced Myofiber Hypertrophy

A primary finding was that frequent 3 days/wk resistance training at a relatively high intensity (8–12 RM) and volume (9 sets for knee extensors) induced greater myofiber hypertrophy in young vs. older adults. We found no significant increase in type I myofiber CSA among the older adults compared with 18% growth in young, and type II hypertrophy was less robust (851 μm^2 or 23%) compared with that seen among young subjects (1,419 μm^2 or 32%). However, the fact that, after only 16 wk of resistance training, older adults restored type II fibers to the size of young subjects before training should not be overlooked. This is a clinically significant finding because age-related myofiber atrophy is known to be localized to type II myofibers (as supported by our pretraining data). Preferential type II hypertrophy (exceeding type I hypertrophy) is a typical resistance training adaptation and likely results from the relatively greater increase in type II motor unit recruitment. Nom-

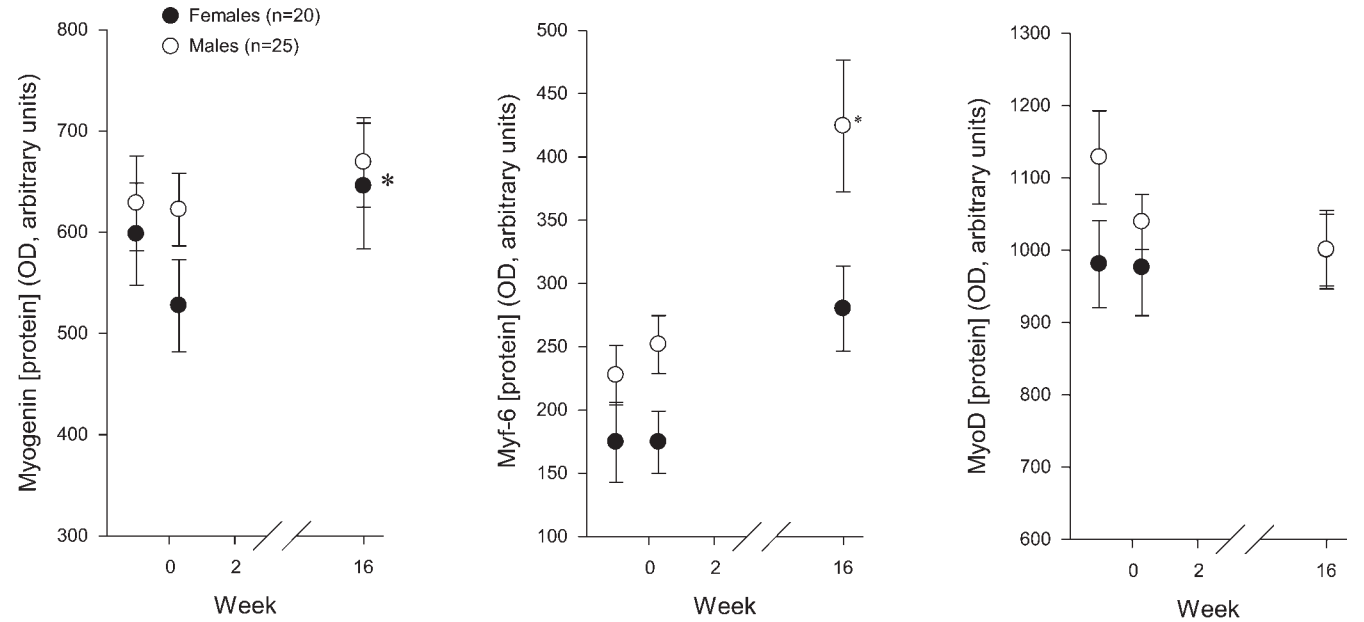

Fig. 5. Myogenic regulatory factor protein levels by gender. Immunoblot results for myogenin, myf-6, and MyoD protein levels by gender across 3 time points (baseline, acute loading, 16-wk training) are shown. Values are means ± SE. OD, optical density. *Significant change from acute time point (myogenin) or from baseline (myf-6) within group, $P < 0.05$. Overall main and interaction effects from the age × gender × training ANOVA models are described in RESULTS.

inal daily activities are accomplished primarily by recruitment of the smaller, type I motor units (lower recruitment threshold), whereas type II motor units are typically not recruited during low-intensity daily activities but are rapidly recruited when contraction intensity increases (higher recruitment threshold). Thus the type II myofibers realize the greatest relative increase in activity (compared with type I) during high-intensity contractions such as resistance exercise.

Young men clearly experienced the most marked adaptation as the only age-gender group with significant hypertrophy of both type I and type IIa myofibers and with the largest increase in the size of type II myofibers. By contrast, we did not detect significant growth of either fiber type within older women. Again though, we would argue that the trends toward myofiber growth in these older women hold clinical relevance, as type II myofiber size posttraining was nearly restored to the baseline type II fiber size recorded in women 35 yr younger. The muscles of older adults are clearly capable of hypertrophy in response to resistance training as shown in several prior studies (reviewed in Ref. 29); however, because we found the response to be less robust compared with young subjects using this specific 3 days/wk program, we suggest alternate training programs for older adults should be pursued to improve the efficacy of myofiber growth. Maximizing growth of each myofiber is particularly important considering that some myofiber loss (~20–25%) occurs by the seventh decade (38). This compounds the degree of atrophy at the whole muscle level, and, obviously, resistance training can only enhance myofiber size (not number).

One of two possible reasons must account for the noted age difference in 3 days/wk resistance training-mediated hypertrophy: *1*) the training program design that is most effective for hypertrophy in older novice participants differs from what works well in young; or *2*) irrespective of training program design, young novice trainers will always adapt with superior hypertrophy compared with their older counterparts. Although the latter cannot be ruled out based on the current shortage of published findings, we suggest the former explanation has merit. Several animal studies have demonstrated slowed repair/regeneration in older vs. young adult animals (8, 12, 21, 43, 61). If recovery time is rate limiting in older muscle, one might expect a lesser hypertrophy adaptation in older vs. young adults subjected to the same fairly high loading frequency. There is some indirect evidence to support this concept in aging humans, as substantial myofiber hypertrophy has been detected in older women (26) at a reduced training frequency (2 days/wk). Furthermore, in a recent survey of the literature we noted that older men and women both appear to adapt more favorably to 2 days/wk loading (29). Hakkinen et al. (24) studied both young and older men with ages similar to our cohort and found comparable gains in myofiber size between the two age groups; however, the program was periodized weekly, resulting in only 1 day/wk of "hypertrophy training" at an intensity similar to our 3 days/wk program (8–10 RM), suggesting less frequent loading for hypertrophy might be beneficial.

Whereas our overarching hypothesis focused on frequency of loading, another program design variable that should be considered is volume within each loading bout. It is certainly reasonable to expect the volume of loading within each exercise bout to influence the repair/regeneration time course as well. For example, Roth et al. (57) found no age differences in MRI-determined muscle size gains after 6 mo of 3 days/wk training. Their subjects completed only four sets of knee extensor work per exercise bout compared with nine total sets in the present study. In another study of older men, three total sets of knee extensor training per bout led to significant myofiber hypertrophy on a 3 days/wk program (19). Furthermore, our laboratory previously found that 4 sets of loading per bout induced robust myofiber hypertrophy in older men on a 3 days/wk training frequency (although growth remained impaired among the older women) (6). Based on these combined findings, the older adults in the present study may have realized greater myofiber growth by reducing loading volume even if frequency was maintained at 3 days/wk.

Effects of Age on Load-Mediated Changes in Regenerative Markers

MRFs are commonly studied markers of myogenic activities including transcription of muscle genes (41), differentiation of satellite cells (50), and transitions in myofiber phenotype (32). There are several reports of MRF upregulation in response to various muscle perturbations including stretch overload (11, 42), ablation (2, 3), denervation (35), myotoxicity (43), and acute resistance loading (9, 22, 34, 53, 61). In a number of these studies, MRF responses and/or muscle repair mechanisms were found to be limited by old age (34, 41–43, 61). This may result from heightened basal levels of MRFs in the aging muscle (7, 34, 35, 49) because MRF expression tends to increase as the degree of sarcopenia advances (17).

Elevated resting levels of MRFs suggest sarcopenic muscles remain in a state of failing regenerative effort; thus it stands to reason that MRF responses to a growth/regeneration stimulus such as resistance loading could be impaired. The studied group of older adults were, however, fairly healthy (based on the stringent exclusion criteria applied for this training study) and were "young-old" in light of the growing population of octogenarians. Type II atrophy indicative of sarcopenia was noted (driven primarily by older women) but, compared with animal models of senescent muscle, we do not consider the stage of sarcopenia in the ~64-yr-old human muscles studied to be advanced. In fact, one could argue that older men were only in the beginning stages of decline (while older women had regressed further). This may explain why myf-5 mRNA was only one of seven MRF transcript and protein measurements found to be significantly higher in the older muscles at baseline.

Our overarching hypothesis was that 3 days/wk resistance training would not provide sufficient recovery between bouts for older adults to adapt appropriately. We generally noted a lesser hypertrophy adaptation among older adults and followed that with a series of experiments to evaluate whether there was an associated age difference in regenerative drive based on the load-mediated expression of MRFs. At the transcript level, young adults responded with greater increases in myogenin and myf-5, whereas the MyoD response was similar in the two age groups. The responses were not remarkably different by gender. Barring protein level analyses, one might argue that the MRF-based myogenic potential tended to improve with training more in young than old. However, no distinct age differences in responsiveness were found at the protein level. In fact,

we report a general lack of coordinated change between mRNAs and their translated protein products.

Because of a number of posttranscriptional controls (e.g., RNA splicing, RNA editing, blocked nuclear export, subcellular localization, negative translational control), a close relationship between mRNA and protein would not be expected. As reviewed recently by Moore (45), a number of factors, including small noncoding micro-RNAs and proteins that complex with a given mRNA to form a messenger ribonucleoprotein particle, largely dictate its fate. These components offer myriad posttranscriptional control mechanisms that can lead to large variation in the levels of agreement between mRNA and protein abundances (45). As an example, it has previously been shown that an age-related 80-fold elevation of myogenin mRNA corresponded to only a 6-fold elevation at the protein level (35). Our reported differences by age and/or training in MRF levels were on a much smaller scale (less than 1-fold), which would likely limit the ability to detect coordinate mRNA and protein levels. However, similar general trends between the two seemed plausible, particularly at the later time point after several repeated exposures to loading (16 wk of training). In our view, these findings underscore the strength in assessing both mRNA and protein whenever possible.

Both myogenin and MyoD mRNA concentrations increased over time with little to no change at the protein level. This suggests increased transcription of myogenin and MyoD was largely futile, overridden perhaps by negative translational control or some other form of feedback. The opposite occurred for myf-6 because we found robust increases in myf-6 protein with no change in transcript. Based on the numerous control mechanisms reviewed recently (45), it is possible that myf-6 mRNA existed in sufficient quantities in a sequestered cytosolic location and was transported to the ribosomal machinery for translation in response to training without requiring additional transcription. Why only myf-6 protein and not the other MRF proteins accumulated with resistance training is unknown. Of interest, however, is the finding in a recent resistance training study that changes in myf-6 protein expression correlated with changes in myofiber size, whereas changes in myogenin and MyoD did not (27). The bulk of the increase in myf-6 protein was localized to men in the present study, and young men experienced the greatest myofiber hypertrophy. These data combined suggest myf-6 may play an important role in load-mediated myofiber growth.

Strength vs. Hypertrophy; Non-Muscle-Mass Adaptations

A notable finding was that strength gains among the older subjects were sufficient to meet or exceed the pretraining strength levels recorded in subjects ~35 yr younger. It is generally accepted that increases in strength during the first few weeks of RT result from neural adaptations, including enhanced motor unit recruitment and firing frequency of agonists and/or reduced cocontraction of antagonists (23–25, 46). Based on relative gains in strength vs. myofiber size, the observed increases in strength appear to have been largely driven by neural adaptations. However, because relative strength improvements were similar across groups, our data indicate that older adults relied more heavily on neural or other non-muscle-mass adaptations than their younger counterparts. Combining older men and women, gains in 1-RM strength ranged 33–49% across the three movements whereas type I myofibers did not hypertrophy (nonsignificant 7%) and type II CSA increased by only 23%. Young adults combined increased 1-RM strength by 28–47% accompanied by type I and type II hypertrophy of 18 and 32%, whereas young men alone improved strength 34–38% matched by myofiber growth of 26–35%. Young men appear to have gained strength almost entirely as a consequence of hypertrophy, while in older subjects hypertrophy accounted for a lesser fraction of the improved strength.

A common criticism of RT-induced strength improvements among older subjects is that changes may be largely driven by factors other than physiological adaptations such as increased confidence, leading to 1-RM strength values before training that are underestimated. This is a valid criticism if familiarization is not sufficient before testing baseline strength. We standardized familiarization procedures across all subjects in an effort to avoid this problem and therefore obtain accurate baseline strength levels. With these procedures in place, our laboratory has previously shown similar specific strength estimates among untrained men and women in the two age groups (52). Furthermore, others have shown, with adequate familiarization, that voluntary vs. electrically evoked contractile force does not differ by age and it has been reported that both old and young can achieve 95–100% of electrically activated maximal force with voluntary effort (14, 30, 37, 59). It is therefore unlikely that strength gains among the older subjects were biased by underestimated baseline strength, leading us to presume that neural or other non-muscle mass adaptations improved with time more in old than young.

All subject groups showed a typical compensatory shift in MHC isoform expression that is generally accepted to occur in response to both endurance (54) and resistance (1, 6, 20, 60) training. There was a marked decrease in the number of histologically determined myofibers expressing exclusively MHC IIx in all groups, whereas the number of MHC IIa expressing myofibers significantly increased in all but elderly women. A sparse distribution of IIa/IIx and I/IIa coexpressing myofibers were observed but remained excluded from analyses. Type I myofiber distribution did not significantly change in any subject group. Because both young and older muscles underwent a similar degree of fiber-type transition (yet differed significantly in hypertrophy), this provides further support that the mechanisms controlling muscle mass and myosin isoform expression are distinctly different (16).

Finally, we identified a subset of three older men with extremely large myofibers. Without these 3 men, we would have reported significant age-related type II atrophy in the 10 remaining older men compared with the young group before training. Compared with the remaining 10 older men, these 3 subjects had substantially larger type I (52%, 7,209 vs. 4,738 μm^2), type IIa (45%, 6,349 vs. 4,370 μm^2), and type IIx (72%, 5,125 vs. 2,973 μm^2) myofibers before training. Their apparent changes with training followed trends similar to the other 10, because type II CSA increased whereas type I CSA did not. We spent a great deal of time discussing whether or not these three men constitute a true representation of ~65 yr men in the population at large but we found no compelling reason to exclude their data. Based on DEXA results, thigh muscle mass for these 3 men (13.66 kg) did not appear different from the remaining 10 older men (13.22 kg). Although the DEXA

results cannot be compartmentalized into specific muscles, the striking difference in fiber sizes leads us to speculate perhaps a lower vastus lateralis total myofiber number in these three men. These three men made up almost one-quarter of our older male cohort; however, we do not know whether this high a fraction of ~65 yr men actually possess such large myofibers.

In conclusion, the functional benefits of resistance training for older adults cannot be overlooked. Furthermore, we report that, after only 16 wk of resistance training, older adults were capable of restoring myofibers to the pretraining fiber sizes found in adults ~35 yr younger. Certainly these data support the work of others in demonstrating that adults ~64 yr of age retain the ability to undergo load-mediated hypertrophy. However, in general we noted more robust hypertrophy in the younger cohort (particularly young men). We suggest that alternate, age-specific training programs should be evaluated to maximize enhancements in both function and muscle mass to counteract sarcopenia. This might include direct comparisons between various manipulations of training frequency and/or volume to affect rest/recovery rates between training bouts to meet this goal.

ACKNOWLEDGMENTS

We are indebted to the research subjects for their effort and dedication throughout the study. We thank S. C. Tuggle and S. Hall for conducting training sessions and administering performance tests, C. Benton and V. Hill for project coordination, and Drs. R. Allman, C. Brown, and R. Sims for conducting geriatric physical examinations.

GRANTS

Funding for this work was provided by National Institute on Aging Grant R01 AG-17896 (to M. M. Bamman), Department of Veterans Affairs Merit grant (to M. M. Bamman), and General Clinical Research Center Grant M01 RR-00032.

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