Age-related structural alterations in human skeletal muscle fibers and mitochondria are sex specific: relationship to single-fiber function

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Callahan DM, Bedrin NG, Subramanian M, Berking J, Ades PA, Toth MJ, Miller MS. Age-related structural alterations in human skeletal muscle fibers and mitochondria are sex specific: relationship to single-fiber function. J Appl Physiol 116: 1582–1592, 2014. First published May 1, 2014; doi:10.1152/japplphysiol.01362.2013.—Age-related loss of skeletal muscle mass and function is implicated in the development of disease and physical disability. However, little is known about how age affects skeletal muscle structure at the cellular and ultrastructural levels or how such alterations impact function. Thus we examined skeletal muscle structure at the tissue, cellular, and myofibrillar levels in young (21–35 yr) and older (65–75 yr) male and female volunteers, matched for habitual physical activity level. Older adults had smaller whole muscle tissue cross-sectional areas (CSAs) and mass. At the cellular level, older adults had reduced CSAs in myosin heavy chain II (MHC II) fibers, with no differences in MHC I fibers. In MHC II fibers, older men tended to have fewer fibers with large CSAs, while older women showed reduced fiber size across the CSA range. Older adults showed a decrease in intermyofibrillar mitochondrial size; however, the age effect was driven primarily by women (i.e., age by sex interaction effect). Mitochondrial size was inversely and directly related to isometric tension and myosin-actin cross-bridge kinetics, respectively. Notably, there were no intermyofibrillar or subsarcolemmal mitochondrial fractional content or myofilament ultrastructural differences in the activity-matched young and older adults. Collectively, our results indicate age-related reductions in whole muscle size do not vary by sex. However, age-related structural alterations at the cellular and subcellular levels are different between the sexes and may contribute to different functional phenotypes in ways that modulate sex-specific reductions in physical capacity with age.

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Explained, in part, by alterations in molecular and/or cellular muscle structure. Our understanding of how aging affects skeletal muscle structure at the molecular and cellular level in humans, and how these structural alterations relate to whole tissue and functional declines, however, is limited.

Aging alters human muscle structure at the tissue level, causing decreases in muscle performance. The most notable is the gross loss in muscle mass (31), but other structural alterations, such as a decrease in muscle pennation angle and fascicle length (46, 65), could contribute. These architectural changes, however, explain only a portion of the age-related decrease in whole muscle performance (65) and would not explain altered single-fiber contractile performance (28, 32, 71). Studies suggest that an age-related loss of myosin protein contributes to decreased single-fiber isometric tension (10), which may present structurally as a shorter thick-filament length and/or an altered thick-to-thin-filament stoichiometry. Moreover, our laboratory’s previous data (43) from a model system (Drosophila melanogaster) shows age-related changes in myofilament ultrastructure that may have functional consequences. To our knowledge, however, no quantitative measurements of myofilament ultrastructure have been compared between muscles of young and older humans, nor have they been evaluated for their relationship to cellular- or tissue-level functional phenotypes.

Dysregulation of cellular metabolic processes has long been suggested as an impetus for aging (16). Recent studies have suggested that mitochondrial dysfunction may contribute to muscle atrophy [reviewed in Powers et al. (55)] and alterations in cellular-level muscle structure (7, 17, 74). For instance, age-related accumulation of mitochondrial deletions has been implicated in region-specific atrophy of skeletal muscle fibers in animal systems (7). With the linking of such changes to diminished muscle performance, studies suggest that altered mitochondrial function and morphology promote intracellular oxidative stress, which can interfere with myofilament function via oxidative modification of proteins or disruptions in myofilament protein metabolism (6). The relationship of alterations in mitochondrial biology to muscle fiber/myofilament structural and functional alterations with aging in humans, however, has not been systematically examined.

The present study was designed to investigate age-related changes in skeletal muscle structure at the tissue, cellular, and myofibrillar levels, including mitochondrial morphological adaptations, and to relate these parameters to skeletal muscle functional performance across the same anatomic levels. To accomplish this objective, we recruited healthy young and older men and women to have similar levels of habitual physical activity. Care was taken to account for age-related...
alterations in physical activity, as muscle use/disuse can impact whole muscle (14, 22) and single-fiber performance (10, 11), as well as mitochondrial function (3, 20, 25, 27, 29, 36). This consideration represents an advance on prior work in this area, as physical activity is not routinely objectively quantified in human aging studies. Thus some of the presumed age-related changes in skeletal muscle structure and function may be the result of reduced physical activity that frequently accompanies old age (38, 72).

METHODS

Participants. Twelve young (5 men) and twelve older (5 men) participants were enrolled in the present study. Self-reported physical activity was minimally active (≥2 sessions of ≥30 min of exercise/wk) for the young volunteers and moderately active (3–5 sessions of ≥30 min of exercise/wk) for the older volunteers. None were engaged in a structured exercise program, and self-described exercise consisted primarily of walking. These recruitment criteria were used to correct for differences in self-efficacy and lifestyle differences across age groups (39, 72) and produced young and older groups that had similar habitual physical activity levels. To confirm achievement of this goal, daily physical activity was quantified using uniaxial accelerometers, as previously described (69), worn by each participant for 9.2 ± 0.3 days (Caltrac, Hemokinetics). All volunteers were healthy, with no symptoms or signs of heart disease, hypertension, or diabetes; normal resting electrocardiogram and response to an exercise stress test; and normal thyroid function and blood cell counts and blood biochemistry values. Volunteers were not eligible to participate in the study if they had participated in a weight loss or exercise training program in the past year; a history of smoking (within 1 yr); unintentional weight loss of >2.5 kg during the last 3 mo; a body mass index >30; hospitalization longer than 3 days in the past 5 yr; active cancer, or history of unintentional weight loss as the area of the total subsarcolemmal region, to account for differences between the area of the total subsarcolemmal region, to account for differences between the area of the total subsarcolemmal region, to account for differences between MHC I and those that do not. However, in our hands (45) and others (10), fibers coexpressing both MHC I and various isoforms of MHC II are relatively rare (6% in present sample). Thus the average CSA of muscle fibers measured with this approach are referred to as MHC I and MHC II. Due to lack of tissue, IHC was not performed on two older men and one younger woman, meaning this analysis was performed on a total of 11 younger (5 men), and 9 older (3 men) adults.

Ultrastructural measurements. EM measurements were conducted on intact (i.e., unskinned) skeletal muscle fiber bundles to assess myofibrillar ultrastructure. These measures were not obtained in one younger woman due to lack of tissue, meaning this analysis was performed on a total of 11 younger (5 men), and 12 older (5 men) adults. These measures included myofibrillar fractional area, thick-to-thin-filament ratio (measured in cross sections at the A-band with full overlap of thick and thin filaments), A-band and sarcomere lengths, as well as intermyofibrillar and subsarcolemmal mitochondrial average area, average area, and number. Our intermyofibrillar mitochondrial measurements were largely similar to those described previously (45, 68) with minor modifications, including that four images (each ~152 μm² with no evidence of structural damage) at ×3,000 magnification were measured for each subject, and mitochondria were highlighted using an Intuos Graphics Pad (Wacom, Vancouver, WA). These techniques have been shown to produce similar results to conventional stereological methods (24, 77) and afforded the opportunity to further interrogate additional mitochondrial morphological characteristics. A similar approach was used for assessment of mitochondria in the subsarcolemmal space. This space was defined as the region between the sarcolemma and the first layer of myofilaments. Images were taken in random locations along the sarcolemma, where mitochondrial density was greatest, typically with nuclei or blood vessels, where mitochondria tended to localize. This approach was used to ensure consistency with more recent, relevant publications (9, 47, 53). Subsarcolemmal mitochondrial abundance was quantified in two ways. First, the sum of CSAs for visible mitochondria was divided by the total area of the image, or field of view, similar to intermyofibrillar measures. This was referred to as mitochondrial fractional area and expressed as a percent. The sum of subsarcolemmal mitochondrial CSA was also expressed relative to the length of sarcolemma visible in the image (47), as well as the area of the total subsarcolemmal region, to account for differ-
ences due to orientation of the sarcomere, or irregularity in the shape of the sarcolemma. Finally, Z-band width was measured as a proxy of fiber type (64), as described (68).

**Mitochondrial and nuclear DNA copy number.** DNA extraction was performed with 10–20 mg of muscle tissue per sample, and genomic DNA concentration was measured according to the manufacturer’s instructions (QIAGEN QIAamp, Hilden, Germany). Plasmid DNA controls for human beta globin and cytochrome b were used, as described previously (73). These samples were kindly provided by Dr. Phillip Nagley (Monash University, Australia).

**Whole muscle and single-fiber contractile function.** Whole muscle strength was assessed using an isokinetic dynamometer (Cybex, Computer Sports Medicine, Stoughton, MA), as described previously (69), with the change that peak torque was defined as the single repetition with the maximal value. Peak voluntary knee extensor torque was measured with the knee joint positioned in 70° of flexion (180° = full extension) and was corrected for differences in tissue size by covarying for leg FFM during statistical assessment. Measures of contractile function in single-muscle fibers were characterized by measuring maximally Ca2+-activated (pCa 4.5) tension and single-fiber mechanics, as described previously (44). Single-fiber mechanics was used to estimate specific steps of the myosin-action cross-bridge cycle, as previously described (44), including the rate of myosin force production, or the rate of transition between weakly and strongly bound states (78), and myosin attachment time (t_	ext{a}) (52).

**Statistics.** Data are presented as means ± SE. All variables of interest were assessed for normality and statistical outliers (values 2 SDs from the inclusive mean). Using this approach, no data were excluded as outliers. Two-way analysis of variance (age and sex) was used to compare measured values for age and age × sex interaction effects. In cases where multiple observations were used to characterize a single participant (e.g., single-fiber CSA), a linear mixed model was used, including a random effect to account for clustering of observations within individuals. For all analyses, if a main or interaction effect was noted (age or age by sex), post hoc contrasts were performed to identify pairwise differences. Associations between measures were determined using linear regression analysis. Statistical analyses were considered significant at \( P < 0.05 \) and were performed using IBM SPSS Statistics (version 20.0, IBM, Armonk, NY) and SAS (version 9.3, SAS Institute, Cary, NC).

**RESULTS**

**Participants.** Subject characteristics are listed in Table 1. There was a trend for young adults to be taller than older adults (\( P = 0.07 \)), and young and older participants had similar body \( (P = 0.19) \) and FFM \( (P = 0.96) \). Physical activity as determined by accelerometry was similar between age groups \( (P = 0.71) \). For comparison, sedentary to minimally active older adults produce between 200 and 300 kcal/day using identical measurement techniques (44, 67), showing that, as expected, the walking activity in our moderately active older adults increased the physical activity values from sedentary levels. When statistically corrected for differences in muscle size, voluntary knee extension strength was not different between young and older participants \( (P = 0.12) \).

**Whole body morphology.** Reduced quadriceps CSA \((P < 0.01, \text{Fig. 1A})\) and muscle attenuation \((P < 0.01, \text{Fig. 1B})\) was found in older vs. young cohorts using computerized tomography. When expressed as a percentage of body mass to adjust for the variation in body size [i.e., to account for any compensatory hypertrophy related to the reduced fractional contribution of fat-free tissue to body weight with age (Table 1)], leg FFM from DEXA was reduced in older vs. young groups \((P < 0.05, \text{Fig. 1D})\), similar to quadriceps CSA (Fig. 1A). However, expressed in absolute terms (kg), leg FFM was not different between young and older groups (Fig. 1C).

**Single-muscle fiber morphology.** CSA results from single-muscle fibers measured using IHC are presented in Fig. 2. The CSAs from MHC I fibers were not different between groups \((P = 0.56, \text{Fig. 2A})\), while older adults had reduced CSAs in MHC II fibers \((P = 0.04, \text{Fig. 2B})\) compared with young adults. The CSA histograms for MHC II fibers in men (Fig. 2C) and women (Fig. 2D) illustrate the age-related differences between the sexes in their fiber-size distribution. Older men tend to have a loss of fibers with large CSAs (>2,500 \( \mu \text{m}^2 \)) compared with young men, while older women show a leftward shift to smaller fibers across the entire range of values compared with young women.

**Ultrastructural measurements.** Subcellular measures of skeletal muscle structure revealed no differences in our cohort of young and older men and women (Fig. 3). Cross-sectional EM measures found no difference in myofibrillar fractional area (Fig. 3A) or thick-to-thin-filament ratio (Fig. 3C) between groups. Analysis of longitudinal EM showed A-band length (Fig. 3B), or thick-filament length, was similar between groups. There was no age-related difference found in the average number of intermyofibrillar mitochondria observed in our samples (Fig. 4A). However, the average size of intermyofibrillar mitochondria decreased with age \((P < 0.01, \text{Fig. 4B})\), and there was a trend for an age \( \times \) sex interaction \((P = 0.08)\). Pairwise comparisons showed the smaller mitochondrial size with age was significant in women \((P < 0.01)\), but not in men \((P = 0.43)\). Fractional area of intermyofibrillar mitochondria (Fig. 4C) was not different with age. These findings were recapitulated by the assessment of mitochondrial and nuclear DNA copy number (Fig. 4D). Analyses of the images used to

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Values are means ± SE; \( n \), no. of subjects. NS, nonsignificant. No differences were found between young and older groups for measures apart from age, including isometric torque after statistical correction for leg lean tissue mass. \( *P < 0.01 \).
measure intermyofibrillar mitochondrial morphology showed a uniform distribution and no differences between groups for Z-band width, indicating no fiber-type differences between groups. Furthermore, no associations were found between Z-band width and other measures of myofibrillar or mitochondrial morphology.

Mitochondria in the subsarcolemmal region were not morphologically different (number or size) between groups. Mitochondria [A] and [B]: quadriceps cross-sectional area (CSA). [C] and [D]: leg fat-free mass in kg (C) and %body weight (%BW; D). Values are means ± SE. Text indicates significant difference ("P ≤ 0.05 or "P < 0.01) between young and older groups (Age).

Fig. 1. Whole muscle morphology was characterized using computerized tomography (CT; A and B) and dual-energy X-ray absorptiometry (DEXA; C and D). A: quadriceps cross-sectional area (CSA). B: attenuation in Hounsfield units (HU). C and D: leg fat-free mass in kg (C) and %body weight (%BW; D). Values are means ± SE. Text indicates significant difference ("P ≤ 0.05 or "P < 0.01) between young and older groups (Age).

Fig. 2. Single-fiber CSA from myosin heavy chain (MHC) I (A) and MHC II (B) fiber types determined using immunohistochemical analysis. Text indicates significant difference ("P ≤ 0.05) between young and older groups (Age). Nos. in bars in A and B indicate the no. of measured fibers. Values are means ± SE. The relative frequency of muscle fiber CSA in MHC II fibers is presented for men (C) and women (D).
Mitochondrial density in this region was also not different between groups, regardless of whether the sum of mitochondrial area was normalized to the total field of view (Fig. 5C) or the length of visible sarcolemma (Fig. 5D).

As changes in mitochondrial structure and/or function may be linked to alterations in single-fiber structure (58, 74), we examined the relationship between the intermyofibrillar mitochondrial CSA and indexes of fiber structure and function (Fig. 6). The average CSA of mitochondria in the intermyofibrillar space was not correlated to single-fiber CSA in MHC I (R² = 0.16, P = 0.11) or IIA fibers (P = 0.11). However, average intermyofibrillar mitochondria CSA was inversely related to isometric tension in MHC I (R² = 0.33, P < 0.01; Fig. 6A), as well as myosin attachment time in MHC IIA fibers (R² = 0.28, P = 0.02), and directly related to rate of myosin force production (R² = 0.32, P < 0.01; Fig. 6B) in MHC IIA fibers. Although these coefficient of determination (R²) values are moderate to low, these results show that the average intermyofibrillar mitochondrial CSA explains 28–33% of the variation in isometric tension and cross-bridge kinetics in MHC IIA fibers, a potentially relevant finding considering the variety of ways tension and cross-bridge kinetics can be modified. The coefficient of determination values were lower in MHC I fibers, explaining only 21% of the variation in tension. Exploring these relationships in the sexes independently, most remained significant in women, but not in men, suggesting these correlations were primarily driven by the women. Specifically, average mitochondrial CSA was inversely associated with MHC I (R² = 0.32, P = 0.05) and MHC IIA tension (R² = 0.43, P = 0.02) in women, but not in men (P = 0.57 and 0.21, respectively). Average intermyofibrillar mitochondria CSA was directly related to MHC IIA rate of myosin force production in men (R² = 0.53, P = 0.04), while a trend for this relationship was observed in women (R² = 0.31, P = 0.06). Myosin attachment time in MHC IIA fibers was negatively associated with average mitochondrial CSA in women (R² = 0.36, P = 0.04) but not men (P = 0.39). No significant associations were found between these measures and the morphology of subsarcolemmal mitochondria. To explore the potential that enhanced mitochondrial volume leads...
to reduced tension via reduced myofibrillar density, we examined the relationship between mitochondrial fractional area and measures of myofibrillar density and single-fiber tension. Mitochondrial fractional area was inversely correlated to myofibrillar fractional areas ($R^2 = 0.21$, $P = 0.03$), but no such relationship existed between mitochondrial fractional area and tension in MHC I or MHC IIA fibers ($P = 0.69–0.88$).

**DISCUSSION**

Aging human skeletal muscle is typically characterized by reductions in size and alterations in contractile performance from the tissue to the single-fiber level. While reports in the literature describe these measures in older humans (61), we are not aware of any studies that have comprehensively evaluated the role of age-related changes in cellular or subcellular (i.e., myofilament) structure. Furthermore, although studies have long considered a link between mitochondrial biology and sarcopenia, only a few studies have investigated the effects of age on mitochondrial morphology in humans (9, 47, 51, 54), and none has sought to relate mitochondrial adaptations to age-related changes in single-fiber function. Although our sample size was relatively modest, it is fairly comparable with the range in previous investigations, which studied between 10 (47) and 56 adults (51). In contrast, none of the aforementioned studies have controlled for habitual physical activity, health status, and medication use, as we have in the present study, to attempt to define primary aging effects and minimize the significant variability in physical activity observed across the human lifespan (38, 72). Our results show no effects of age on myofilament ultrastructure. However, we did observe that older adults had smaller CSAs in MHC II fibers, and, in women, aging reduced the size of intermyofibrillar mitochondria.
Smaller mitochondrial size was related to increased isometric tension and reduced myosin-actin cross-bridge kinetics, contractile measures that were also altered primarily in older women (42). Collectively, these results highlight unique age-related structural adaptations in skeletal muscle that may partially contribute to sex-specific adaptations in muscle performance with age.

The effects of gross changes in skeletal muscle tissue mass or CSA with age to reduce functional capacity is well-accepted (33), but we know little about how other alterations in muscle structure at the subcellular and molecular level may contribute because few studies have performed such assessments, and we know of no study that has assessed muscle size/structure at all of these anatomic levels in the same cohort. To our knowledge, this is the first study to unambiguously assess the effects of age on myofilament ultrastructure in human skeletal muscle. Unlike variability noted at the cellular level, however, we found no effect of age on myofilament quantity or structure. Specifically, thick-filament length (A-band length), thick-to-thin-filament ratio, and myofibrillar fractional area were not different between young and older men and women, in contrast to our laboratory’s previous findings in a model system (43).

Parenthetically, this discrepancy between an animal model of aging and human aging should be a cautionary note for extrapolation of results about muscle morphology across species.

Our findings suggest maintenance of structural proteins within the myofilament lattice and are supported by our finding of no age effect on MHC protein content (42). Our data in healthy, older adults suggest alterations in skeletal muscle myofilament content/architecture do not play a role in the atrophy process beyond an assumed overall loss of parallel sarcomeres due to reduced CSA, nor do they appear to mediate age-related differences in contractile function. This is in contrast with some reports (10), but apparent conflict in these results may be explained by physical activity between groups (11), a caveat that we attempted to control in the present study.

The mitochondrial morphological data reported here are generally in agreement with previous studies that describe reductions in the average size of skeletal muscle mitochondria with age (51, 54). However, this observation is not universal (9, 47) and appears to be at least somewhat dependent on cellular localization (intermyofibrillar vs. subsarcolemmal) (9, 47) and sex, as we found alterations only in older women. Our findings are most appropriately compared with those of Crane et al. (9), who report maintenance of intermyofibrillar mitochondrial size, but decreased fractional area, in healthy older adults compared with young. However, the participants described in Crane et al. (9) were significantly more active than our relatively sedentary population, with their participants engaged in alternate modes of physical activity; young per-
formed primarily aerobic exercise, while older adults had just completed a twice weekly strength training intervention. Although we recently reported that resistance training does not alter mitochondrial content/morphology (68), aerobic exercise is a well-known modulator of mitochondrial biology (4), suggesting differences in the type of activity of the populations may explain the discrepancy with our findings. Of further interest is the apparent greater durability of subsarcolemmal mitochondria compared with the effects of the ultrastructural changes in the subsarcolemmal fraction may be obscured by the relatively fewer physical constraints placed on those mitochondria compared with the effects of the ultrastructure of the contractile machinery on intermyofibrillar mitochondria.

Although controversial, mitochondria are frequently identified as causal agents in the process of cellular aging (16, 63). Multiple investigations have suggested or implied causal relationships between mitochondrial dynamics and atrophic processes in skeletal muscle (58, 59, 76). A recent animal study suggests mitochondrial fractionation occurs as a primary consequence of healthy aging in rats (23), and others have suggested this may be the case in humans (2). Potential relationships between mitochondrial dynamics and atrophic processes are supported by studies that demonstrate a reduction in muscle atrophy (58) or protection of ischemia-reperfusion injury (50) by blocking mitochondrial fractionation. In the present study, older women had reduced individual intermyofibrillar mitochondrial size, which correlated with increased tension and slower myosin-actin cross-bridge kinetics, measures that were also primarily altered in older women (42). We observed age-related alterations in mitochondrial morphology that are linked, either directly or as an epiphenomenon, with age-related changes in skeletal muscle function, perhaps via signaling pathways involving peroxisome proliferator-activated receptor-α/β coactivator-1 (60). Here again, we are cautious not to imply cause-effect from our cross-sectional associations, but they are nonetheless compelling in supporting a long-held link between mitochondrial biology and age-related alterations in skeletal muscle. It is tempting to speculate that mitochondrial fractionation, as evidenced by reduced mitochondrial size, contributes to an intracellular environment characterized by enhanced reactive oxygen species (76) that may alter contractile function via posttranslational modification of myofilament proteins (56).

We have also identified morphological distinctions at the whole tissue level of young and older adults. We found age-related muscle atrophy, evidenced by reduced quadriceps CSA (cm²) and leg FFM (%body weight). However, absolute leg FFM (kg) was not different between young and older groups, despite similar height, weight, and %FFM in young and older individuals. Clearly, our cohort did not fit a clinical definition of sarcopenia, consistent with our efforts to ensure the older cohort in this study was well-matched to the young in health and physical activity. Despite this, we still found tissue and cellular level evidence for age-related morphological adaptations consistent with sarcopenia. The implication of this finding being that the phenotypes we have identified were robust enough to be detectable in an aged cohort that does not show manifestation of clinically apparent muscle wasting. However, we acknowledge that whole tissue imaging techniques may suffer from imprecision related to the measurement itself and confounding factors inherent to a between-subject design in human subjects (8). In contrast, muscle atrophy was apparent at the single-fiber level. Specifically, age-related atrophy was found in MHC II fibers, consistent with other reports in the literature showing age-related muscle atrophy is typically more prominent in MHC II fibers (34, 35). On closer examination between these mitochondrial subfractions than previously thought (53). Indeed, the mitochondria are increasingly appreciated as a dynamic reticulum, and it is possible that morphological changes in the subsarcolemmal fraction may be obscured by the relatively fewer physical constraints placed on those mitochondria compared with the effects of the ultrastructure of the contractile machinery on intermyofibrillar mitochondria.

![Graph](http://example.com/graph.png)

**Fig. 6.** Mitochondrial morphology (average mitochondrial CSA) was significantly correlated with average single-fiber Ca²⁺ activated tension (A) and rate of force development (B) in MHC IIA fibers.
examination, the age-related atrophy appears different between the sexes, as the distribution of fiber sizes was largely similar in young and older men, with younger men simply having slightly more very large (CSA > 2,850 μm²) fibers. In contrast, older women had a noticeable shift in fiber size distribution with a skew toward smaller fiber sizes. Together, these findings highlight the overall loss of muscle in cross section with age and suggest a greater contribution of MHC II fibers to that loss, with the most dramatic differences observed in older women. We also observed sex specificity in associations between intermyofibrillar mitochondrial size and indexes of single-fiber function (Fig. 6), wherein significant associations observed in the entire cohort persisted when assessed in women alone, but trended or were not significant in men. These sex-specific associations are consistent with observations in this cohort of age-related differences in skeletal muscle morphology being more pronounced in women than men. A few important caveats to our interpretation of the data presented here are warranted. Worth noting is the potential effect of oral contraceptives on physiological outcomes in the younger women in this study (taken by 4 of the 7 volunteers). While some studies performed in animals (41) and humans (1) demonstrate a net anabolic effect of estrogen, evidence that hormone replacement supplementation increases muscle size and strength in younger adult women is limited (12). At least one study has shown decreased myofibrillar synthesis rates in young women taking oral contraceptives (15), while studies in postmenopausal women by our laboratory and others suggest no effect of hormone replacement therapy on skeletal muscle size and function (70, 75). Regarding physical activity assessment, our measures relied on a single summary value that was relatively insensitive to variations in the intensity of physical activity performed by our participants. However, our participants were not engaged in strenuous exercise by self-report and referred to walking as their primary form of physical activity, minimizing the potential effects of variations in intensity between age groups. Finally, we acknowledge the inherent limitations associated with performing correlation analyses with small sample sizes in a cross-sectional study. These types of analyses are prone to type I statistical error and, despite our careful matching for health status and habitual physical activity levels in study volunteers, it is possible, as with any correlation analysis, that we have uncovered false-positive associations. Nonetheless, the variables used in the present correlation analyses were chosen based on prior evidence and classical physiological relationships between muscle protein structure and function. While these data are perhaps not definitive, they provide compelling support for future research involving larger cohorts.

Conclusions. Our findings illustrate the complexity of physiological properties inherent in the age-related phenotype of skeletal muscle and the potential sex-specific nature of these adaptations at the cellular and subcellular levels. Despite the atrophic effects of age and sex at the cellular level, subcellular myofilament ultrastructure (thick-filament length, thick-to-thin-filament ratio, and myofibrillar fractional area) remained unchanged. In addition, mitochondrial fractional area and number were maintained, regardless of cellular location, suggesting maintenance of oxidative capacity across age groups (62) with similar physical activity levels (27). However, here again, sex specificity in the aging response was noted in average inter-myofibrillar mitochondrial size, with greater reductions in older women. In these healthy adults, matched for physical activity level, it is unlikely that overall oxidative capacity was different between groups (3, 25, 27, 29, 30, 57), but associations between mitochondrial morphology and muscle function at the fiber level suggest coordination between cellular metabolism and single-fiber function. Future studies should be directed at characterizing the functional metabolic consequences of altered mitochondrial morphology with age and sex, and specific perturbations to contractile proteins that might be responsible for the structural and functional phenotypes observed.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
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