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Human neuromuscular aging: Sex differences revealed at the myocellular level

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Abstract

Age-related muscle loss (sarcopenia) is a major clinical problem affecting both men and women – accompanied by muscle weakness, dysfunction, disability, and impaired quality of life. Current definitions of sarcopenia do not fully encompass the age-related changes in skeletal muscle. We therefore examined the influence of aging and sex on elements of skeletal muscle health using a thorough histopathological analysis of myocellular aging and assessments of neuromuscular performance. Two-hundred and twenty-one untrained males and females were separated into four age cohorts [mean age 25 y ($n = 47$), 37 y ($n = 79$), 61 y ($n = 51$), and 72 y ($n = 44$)]. Total (–12%), leg (–17%), and arm (–21%) lean mass were lower in both 61 y and 72 y than in 25 y or 37 y ($P < 0.05$). Knee extensor strength (–34%) and power (–43%) were lower ($P < 0.05$) in the older two groups, and explosive sit-to-stand power was lower by 37 y ($P < 0.05$). At the

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histological/myocellular level, type IIx atrophy was noted by 37 y and type IIa atrophy by 61 y ($P < 0.05$). These effects were driven by females, noted by substantial and progressive type IIa and IIx atrophy across age. Aged female muscle displayed greater within-type myofiber size heterogeneity and marked type I myofiber grouping (~5-fold greater) compared to males. These findings suggest the predominant mechanisms leading to whole muscle atrophy differ between aging males and females: myofiber atrophy in females vs. myofiber loss in males. Future studies will be important to better understand the mechanisms underlying sex differences in myocellular aging and optimize exercise prescriptions and adjunctive treatments to mitigate or reverse age-related changes.

Keywords

Aging; Sarcopenia; Atrophy; Myofiber; Lean mass; Muscle power; Strength

1. Introduction

Sarcopenia affects tens of millions of adults worldwide, and reported prevalence ranges from ~10–50% in the aging population (Cruz-Jentoft et al., 2014; Janssen et al., 2000; Morley et al., 2014). Since the term was introduced in 1989 (Rosenberg, 1997), several attempts have been made to quantify and classify degrees of sarcopenia based on whole body lean mass (Janssen et al., 2004a; Janssen et al., 2002; Janssen et al., 2004b), limb muscle mass (Baumgartner et al., 1998), and whole body strength (Janssen et al., 2002; Scott et al., 2014). The health care burden attributable to sarcopenia is staggering – estimated at \$18.5 billion in 2000 in the United States alone (Janssen et al., 2004b) – and presumably rising annually. Nonetheless, there remains no consensus on what operational definition to use when classifying sarcopenia (Beaudart et al., 2014).

Studies have demonstrated that lifetime muscle mass generally peaks at ~30–40 years of age and progressively declines thereafter (Forbes and Reina, 1970; Janssen et al., 2000), with accompanying functional deficits. These findings suggest that early detection and action is necessary, since exercise may curtail the rate of decline (Cartee et al., 2016; Mikkelsen et al., 2013; Wroblewski et al., 2011). However, studies that investigate the prevalence of sarcopenia throughout the lifespan often fail to examine the muscle at a cellular level. Understanding myocellular aging and potential influences of sex, activity, hormonal regulation, etc. could help direct treatment and prevention of age-related declines.

Age-related reductions in muscle mass are thought to result from a decline in total myofiber number and/or atrophy of the remaining type II myofibers (Kosek et al., 2006; Lexell et al., 1988; Merritt et al., 2013; Nilwik et al., 2013). Given the enormous power differences among type I (slow, oxidative), type IIa (fast, oxidative), and type IIx (fast, glycolytic) myofibers (Trappe et al., 2003), type II atrophy likely contributes to a weakened and less powerful phenotype at the whole muscle level in older adults. This is especially concerning given that strength and speed may be important in avoiding falls, which are a known contributor to dependence and reduced quality of life (Rubenstein, 2006).

Muscle health throughout aging may also be affected by the systemic environment, including alterations to the hormonal milieu. A number of circulating [e.g., insulin-like growth factor 1 (IGF-1) and testosterone] and locally expressed factors are thought to be important in the regulation of muscle mass based on their involvement in muscle protein synthesis (Yarasheski, 2003) and/or activation of the normally quiescent population of skeletal muscle satellite cells (Hawke and Garry, 2001). In addition, many of these factors are considered potential targets for intervention to counteract the slow progressive decline of muscle mass with aging (Neto et al., 2015).

Because of the multifaceted nature of the age-related muscle phenotype and its numerous potential contributors, the purpose of the present human investigation was to assess neuromuscular health across a wide range of ages, and to determine whether sex influences components of muscle aging. To this end, we quantified whole limb muscle mass, strength, power, histological phenotype including myofiber type distribution, type-specific myofiber size and type I grouping, and concentrations of circulating hormones thought to influence muscle mass regulation in a cohort of 221 individuals (119F, 102 M) across four age groups.

2. Methods

2.1. Subjects

Two hundred and twenty-one adults were recruited from the Birmingham, Alabama metropolitan area and divided into four cohorts defined by age range: 20–29 y, 30–50 y, 55–64 y, and 65–81 y. All potential subjects were screened via health history questionnaire including medication history and activity history; additionally, males ≥ 45 y and females ≥ 50 y were required to complete a physical examination and pass a maximal graded exercise stress test with 12-lead ECG. Individuals with a history of strength training within the previous five years were excluded. Individuals were also excluded for obesity (BMI ≥ 30), uncontrolled hypertension, diabetes, or other uncontrolled cardiometabolic disease, cognitive impairment, movement disorder, end-stage disease, or musculoskeletal or other disorder that could affect their ability to complete testing. Individuals receiving exogenous hormones were also excluded. The Institutional Review Boards of both the University of Alabama at Birmingham (UAB) and the Birmingham Veterans Affairs Medical Center approved the study. Each subject gave written informed consent prior to participation. Methods have been published previously (Bamman et al., 2004; Kelly et al., 2018; Kim et al., 2005a; Kim et al., 2005b; Kosek et al., 2006; Petrella et al., 2005) and are presented in brief.

2.2. Body composition

Total body lean mass and compartmentalized lean mass values, in addition to body fat percentage, were determined by dual energy X-ray absorptiometry (DXA) using a Lunar Prodigy (model #8743, GE Lunar Corporation, Madison, WI) and enCORE 2002 software (version 6.10.029) according to the manufacturer's instructions as described (Petrella et al., 2005). Compartmentalized DXA results were used to assess limb lean mass compartments including leg, arm, and thigh muscle mass, and skeletal muscle index (SMI) was calculated as described (Baumgartner et al., 1998; Merritt et al., 2013).

2.3. Neuromuscular testing

2.3.1. Knee extensor strength and power testing—Knee extensor power testing was conducted as detailed previously (Petrella et al., 2005). Subjects completed familiarization sessions to receive instruction on proper technique and to practice the testing protocols. To set the resistance load for the knee extensor power test, bilateral knee extension maximal voluntary isometric contraction (MVC) was first assessed as described (Petrella et al., 2005). Load-specific peak power was then determined during explosive, concentric, bilateral knee extension contractions using an external load equivalent to 40% of MVC (Petrella et al., 2005). Knee angular velocity was measured across a specific range of motion (from 0.873 rad (50°) to 0.349 (20°) of knee flexion) by 500 Hz electrogoniometry, and power in Watts was computed. Subjects were verbally encouraged to contract as fast as possible during each concentric repetition. Three repetitions were completed in succession and the repetition yielding peak power was used for analysis (Petrella et al., 2005).

2.3.2. Functional power, velocity, and motor unit activation—Sit-to-stand (STS) tests with surface electromyography (right quadriceps) and right knee electrogoniometry were performed as previously described (Petrella et al., 2007; Petrella et al., 2005). A 10-repetition test was performed at maximum speed to assess peak power and velocity. A separate 3-repetition test was performed at a standard cadence guided by an audiovisual metronome, and the quadriceps motor unit activation necessary to perform each phase (standing = concentric, sitting = eccentric) relative to maximum (recorded during unilateral knee extension MVC) was recorded as a measure of difficulty.

2.4. Muscle biopsy and tissue preparation procedures

All muscle biopsies were collected from the left *m. vastus lateralis* by percutaneous needle biopsy using a 5 mm Bergstrom biopsy needle under suction (Evans et al., 1982). At the bedside, visible connective and adipose tissues were removed with the aid of a dissecting microscope. A portion of the specimen (~50–70 mg) to be used for immunohistochemistry was mounted cross-sectionally on cork in Optimal Cutting Temperature (OCT, Tissue-Tek) mounting medium mixed with tragacanth gum, and frozen in liquid nitrogen-cooled isopentane. All samples were stored at –80 °C until analyses.

2.5. Myofiber size and type distribution

We have previously detailed our methods for myofiber typing and sizing based on myosin heavy chain (MHC) isoform immunoreactivity (Kim et al., 2005b). Briefly, the three primary myofiber types (I, IIa, IIx) and sarcolemmae were revealed on 6 µm formalin-fixed sections after a series of incubations with primary antibodies against MHCI (anti-MHCI mouse mAb NCL-MHCs, NovoCastra Laboratories, 1:100), laminin (anti-laminin mouse mAb VP-L551, NovoCastra Laboratories, 1:80), and MHCIIa (anti-MHCIIa mouse mAb, University of Iowa Hybridoma Bank, 1:80). MHC isoform specificities of these mAbs were confirmed by Western blot (Kosek et al., 2006). Each primary mAb was followed by a fluorochrome-conjugated secondary Ab (Alexa 594 following anti-MHCI, Alexa 488 following anti-laminin and anti-MHCIIa). Nuclei were identified with a Hoechst 33,258 DNA counterstain (Molecular Probes, 1:10,000).

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 blinded to age and sex of the donor. Myofiber type distribution was determined from an average $\pm$ SE of 1298 ± 58 myofibers per sample. 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. With this technique, hybrid myofibers (e.g., co-expression of I/IIa or IIa/IIx) are revealed by both color and intensity. Myofibers co-expressing more than one MHC isoform were excluded from analyses. In our hands, hybrid myofibers account for an extremely small fraction of the overall fiber distribution, particularly in specimens from untrained individuals not undergoing an intervention known to induce a fiber type shift (e.g., exercise training, thyroid hormone treatment, etc.).

Myofiber cross-sectional areas (CSAs) were determined from at least 50 randomly selected myofibers of each primary type. The average CSA was normalized to each fiber type's distribution to calculate type area distribution. The coefficient of variation for CSA within type I, IIa, and IIx myofibers was used to represent myofiber size heterogeneity within each subject for each fiber type (Kano et al., 2002). On a subset of individuals ($n = 107$), type I myofiber grouping was assessed using our recently published methods (Kelly et al., 2018). This approach takes into consideration both overall fiber type distribution and like-type myofibers neighboring a given fiber to quantify patterns beyond chance grouping.

2.6. Serum assays

Insulin-like growth factor (IGF)-1, IGF-binding protein (IGFBP)-3, IGFBP-1, testosterone (total and free), androstenedione, and sex-hormone binding globulin (SHBG) concentrations were determined in fasted morning serum samples as previously outlined (Bamman et al., 2004). Ten mL of blood were withdrawn from an antecubital vein, and serum was aliquoted (500 μ L) and frozen at -80°C . Samples between subjects for a given hormone/binding protein were assayed in random order. All analyses were conducted by the UAB Human Hormone/Substrate Core. IGF-1, IGFBP-3, IGFBP-1, and SHBG were assessed by immunoradiometric assays (Diagnostic Systems Laboratories, Webster, TX). The inter-assay CV, average intra-assay CV, and assay sensitivity for each hormone/binding protein measured by immunoradiometric assay were as follows: IGF-1 (9.43%, 3.48%, and 4.89 ng/mL); IGFBP-3 (2.95%, 3.30%, and 200 ng/mL); IGFBP-1 (9.36%, 9.51%, and 0.42 ng/mL); and SHBG (8.21%, 7.69%, and 6.50 nM). Total testosterone and androstenedione were determined by solid-phase radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX). For total testosterone, inter-assay CV = 2.56%, average intra-assay CV = 4.92%, and the average sensitivity (90% bound) was 7.12 ng/dL. For androstenedione, inter-assay CV = 16.87%, average intra-assay CV = 7.04%, and sensitivity was 0.12 ng/mL. Free testosterone levels were computed from total testosterone and SHBG using an established formula (Sodergard et al., 1982).

2.7. Statistical analysis

Data are reported as mean $\pm$ SE. Between groups differences were tested using age x sex factorial ANOVA. For each ANOVA model with a significant main or interaction effect,

Tukey HSD tests were performed post hoc to localize the effect(s). Statistical significance was accepted at $P < 0.05$ for all tests.

3. Results

3.1. Biometric characteristics

Descriptive characteristics of each cohort are shown in Table 1. For simplicity, mean ages are used as abbreviations for the four age groups overall: 25 y, 37 y, 61 y, 72 y. Likewise, sex-specific abbreviations by age group are: 25F, 37F, 61F, and 72F for females and 25 M, 37 M, 61 M, and 72 M for males. Height and body weight did not differ across age groups. Expected sex differences in height, weight, body fat, and lean mass were confirmed ($P < 0.05$). Body composition differed across age groups, with main age group effects ($P < 0.05$) for body fat percentage and all lean mass compartments. Body fat was ~6% higher overall in 61 y and 72 y than 25 y and 37 y ($P < 0.05$). Within males, body fat percentage was ~9% higher ($P < 0.05$) in 61 M and 72 M compared to 25 M, and 61 M had 7% more body fat than 37 M ($P < 0.05$). Body fat percentage did not differ across age groups among females.

Overall age-related differences in lean mass were most pronounced in the two older age groups (Fig. 1). Because of this pattern, percent differences are represented using weighted averages of 25 y and 37 y compared to 61 y and 72 y when significance was found. The two oldest cohorts had significantly lower total (–12%), arm (–21%), leg (–17%), and thigh (–18%) lean mass than both 25 y and 37 y ($P < 0.05$). Age differences in total lean mass were not detected within either sex. Within males, lower arm (–16%) and leg (–13%) lean mass were seen in 72 M in comparison to 25 M and 37 M ($P < 0.05$), and lower thigh lean mass was seen in 72 M vs. 25 M (–15%, $P < 0.05$). In females, leg (–14%) and thigh (–16%) lean mass were lower in 61F vs. 25F and in 72F vs both 25F and 37F (leg: –15%, thigh: –18%, $P < 0.05$). Arm muscle mass was not different across age groups in females. Skeletal muscle index (SMI) was lower overall in both 61 y and 72 y than in 25 y and 37 y (–13%, $P < 0.05$).

3.2. Neuromuscular performance

Muscle strength and power are summarized in Table 2. Muscle strength, as indicated by knee extension maximal voluntary isometric contraction (MVC), was lower in 61 y (–28%, $P < 0.05$) and 72 y (–40%, $P < 0.05$) compared to the two younger age groups. Specific strength (MVC normalized to thigh lean mass) was also lower in the two older groups than in 37 y, and in 72 y than in 25 y ($P < 0.05$). Compared to the two younger groups within each sex, lower levels of MVC strength and specific strength ($P < 0.05$) were detected in 61 y females, but not until age 72 in males. Lower bilateral knee extension power was detected by 61 y in both males and females, and was 54% lower overall by 72 y ($P < 0.05$). When adjusted for thigh lean mass, specific power within each sex was lower in both 72F (–35%) and 72 M (–42%) compared to 25F and 25 M, respectively ($P < 0.05$). Additionally, compared to 25 y, explosive sit-to-stand peak power appeared to drop precipitously, with significantly lower power in 37 y (–35%), 61 y (–45%) and 72 y (–58%) ($P < 0.05$). The lower explosive power detected in 37 y was most notable among males. Relative motor unit activation, an index of sit-to-stand difficulty, was higher in both 61 y and 72 y vs the two

younger age groups, and main sex effects indicated higher difficulty among females overall in both the concentric and eccentric actions ($P < 0.05$).

3.3. Myofiber histology and histopathology

Muscle histological parameters are presented in Table 3 and Fig. 2. There were no differences across age ranges for fiber type distribution. Type I fiber distribution showed a main effect of sex such that females had a higher proportion of type I fibers than males overall (+5%, $P < 0.05$). Type IIx distribution exhibited main effects for both age and sex. Type I myofiber size (Fig. 2) was not different across age groups but exhibited a main effect for sex (10% larger in males). Substantial sex differences were noted in type II myofiber atrophy across age groups. While type IIa myofiber size was similar across male age groups, significantly smaller IIa fibers ($P < 0.05$) were seen in both 61F (−28%) and 72F (−33%) vs. 25F. Further, IIa fibers were significantly smaller in 61F (−35%) and 72F (−44%) than age-matched males ($P < 0.05$). Type IIx myofibers were also significantly smaller in females than in males across all age groups. Within males, IIx fibers were smaller in 37 M (−22%) vs. 25 M. In females, remarkably smaller IIx fibers ($P < 0.05$) were seen in both 61F (−39%) and 72F (−49%) vs. 25F.

Overall, type I percent area distribution was higher in 61 y (+3%) and 72 y (+3%) than in 37 y ($P < 0.05$). Type IIa percent area distribution showed a main effect of sex (higher in males), and type IIx percent area distribution showed main effects for both age and sex ($P < 0.05$). The 61 y group had significantly less area occupied by IIx fibers than 37 y (−7%, $P < 0.05$). Within-type myofiber size heterogeneity demonstrated a main effect of age across all three fiber types, and this parameter was additionally influenced by sex for both type IIa and IIx fibers. Type I fiber size heterogeneity was greater in 61 y than 37 y (+7%, $P < 0.05$), driven primarily by the difference in 61F vs. 37F (+8%, $P < 0.05$). Type IIa and IIx fiber sizes were more heterogeneous in 61 y and 72 y vs. 25 y and 37 y, primarily driven by differences in the female subgroup (Table 4) ($P < 0.05$). Analysis of abnormal type I myofiber grouping showed an effect of aging on the percentage of grouped type I myofibers (Table 4): 61 y (+16%) and 72 y (+30%) had more grouped type I fibers than 25 y. While the number of type I myofiber groups was not different across the age groups or between the sexes, type I group size (i.e. number of type I myofibers per abnormal group) exhibited main effects for both age and sex, with a strong trend ($P = 0.05$) toward an interaction effect. These patterns were driven by 72F, with ~5-fold more fibers per type I group than any of the other female or male age groups.

3.4. Serum hormone concentrations

Circulating hormone concentrations are shown in Table 4. IGF-1 was lower in 37 y than in 25 y and in both 61 y and 72 y vs 25 y or 37 y ($P < 0.05$). IGFBP-3 was lower in 37 y vs 25 y (−14%, $P < 0.05$), but no other age differences were found. IGFBP-1 was higher in 72 y than any of the other three age groups (~69%, $P < 0.05$). This difference was primarily due to higher IGFBP-1 in 72F (+113%). Significantly lower androstenedione was seen in 37 y (−23%, $P < 0.05$) and 61 y (−36%, $P < 0.05$) vs. 25 y, and 72 y had lower levels than both 25 y and 37 y (−35%, $P < 0.05$). Within males, this age-related difference was seen only in the oldest group, whereas in females, lower androstenedione levels (compared to 25 y) were

seen in all other age groups ($P < 0.05$). Sex hormone binding globulin (SHBG) was lower in 37 y than in 25 y (-49% , $P < 0.05$) but higher in 72 y vs. 37 y ($+102\%$, $P < 0.05$). Among females, lower SHBG was seen in both 37F (-55%) and 61F (-45%) vs. 25F ($P < 0.05$). No age-related differences were seen in males. Total and free testosterone showed the obvious main effects of sex (not shown). Total testosterone was not different across age groups in either sex; however, free testosterone was lower in 61 M vs. 37 M (-40% , $P < 0.05$) and lower in 72 M vs both 25 M and 37 M (-48% , $P < 0.05$).

4. Discussion

The roles of skeletal muscle in mobility, disability, independence, and quality of life have made age-related muscle loss a primary concern in health care and aging research. The primary purpose of this cross-sectional aging study of 221 adults was to advance our understanding of aging beyond measures of whole limb muscle mass by interrogating potential sex differences in myocellular aging coupled with assessments of muscle performance. By comparing four age groups with means of 25 y, 37 y, 61 y, and 72 y, we report novel, sex-specific findings. Despite similar magnitudes and time courses of whole limb muscle atrophy and declines in muscle performance among females and males, effects of aging at the histological and myocellular levels were markedly different by sex.

The results in aging females suggest the declines in whole muscle mass are largely due to type II myofiber atrophy. In fact, type IIX myofibers among the oldest females were half the size of IIX fibers in young females. In stark contrast, while similar whole muscle mass declines were seen in aging males, type IIA and IIX fibers were generally not atrophic, and type I fibers were $\sim 15\%$ larger in both 61 M and 72 M compared to the youngest male cohort. These data combined strongly suggest total fiber number may be reduced with aging in males (Lexell et al., 1988), and a subset of surviving myofibers undergoes hypertrophy to compensate for fiber loss (Aniansson et al., 1992; Frontera et al., 2008). In further support of this concept, we qualitatively observed a spread in the CSA of type IIA and IIX myofibers in males across the age groups (Fig. 3), suggesting that atrophy and hypertrophy may be occurring simultaneously in these fiber types (Lexell, 1997). While a significant difference was only detected in CSA CV% for type IIA myofibers in 61 M vs. 37 M, there was a general trend for higher myofiber size heterogeneity in the two older male groups. It is possible that these trends are more pronounced with advancing age (> 80 y), which might contribute to the precipitous declines in muscle function and adaptability previously observed (Aniansson et al., 1992; Morley et al., 2001; Slivka et al., 2008).

In both sexes, age-related performance deficits persisted after adjusting for thigh muscle mass, suggesting that changes in muscle quality (e.g., fat infiltration, fibrosis) not detectable by DXA scan, and/or changes in non-muscle components (e.g., neural drive) are involved with functional decline. Thus, factors that influence motor unit organization, fiber size changes, and fiber loss all likely play a role in age-related declines in muscle mass and performance, although their relative contributions and importance are difficult to quantify (Aniansson et al., 1992).

We observed a disparate histological pattern in muscle of aging males and aging females including type II myofiber atrophy, greater size variance, and type I myofiber grouping in women. In the oldest female cohort, fiber CSA spanned the range of a full order of magnitude (Fig. 3), and abnormal groups of type I myofibers were ~5-fold larger than any other cohort. Type I fiber grouping is thought to be the outcome of denervation and reinnervation events (Lexell and Downham, 1991), and our recent findings suggest peripheral changes in neuromuscular junction integrity may play an important role (Kelly et al., 2017). It is well accepted that aging leads to increased type I myofiber grouping; an oft qualitative assessment that we recently quantified (Kelly et al., 2018). Further, we recently found that a high degree of grouping is associated with a greater motor unit activation requirement during a sit-to-stand task, suggesting inefficient motor unit recruitment and thus greater physiologic difficulty to complete the task (Kelly et al., 2017). Thus, while denervation and reinnervation cycling might be protective against complete fiber loss, the consequent motor unit reorganization is likely not functionally advantageous.

The novel sex differences in myocellular aging identified here may be important considerations in designing interventions to prevent or combat sarcopenia. For males, type II atrophy appears not to be a major contributor, suggesting whole muscle atrophy is driven primarily by myofiber loss. This is supported by relatively low levels of type I grouping among the males, suggesting a low success rate of reinnervation. If this is indeed the case, reversal of sarcopenia would require substantial hypertrophy of a relatively small number of remaining myofibers. It is therefore a high priority to identify the mechanisms and time course of myofiber loss among males to optimize interventions for both prevention and reversal. By stark contrast, among females, our cumulative data (severe type II atrophy, increased size heterogeneity, and type I myofiber grouping) suggest aging females may more successfully retain myofiber number (perhaps via more effective reinnervation), but are unable to retain type II myofiber size. These sex differences may present unique challenges for interventions designed to prevent sarcopenia. We have previously demonstrated no major sex differences among older adults in adaptability to resistance exercise training as a sarcopenia reversal strategy (Kosek et al., 2006; Petrella et al., 2006; Stec et al., 2017); however, there may be substantial sex differences in optimal *prevention* strategies at younger ages.

Because there has been a long-standing interest in the role that systemic anabolic hormones may play in sarcopenia progression, we quantified a few key serum hormones and binding proteins. For example, “andropause” among aging men has become popular as a potential contributor to aging muscle atrophy; thus testosterone replacement has become a fairly common therapeutic. Indeed, the existing data indicate androgen replacement therapy increases muscle mass in elderly males (Neto et al., 2015). In our cohort, total testosterone levels among the older men, on average, would not warrant replacement therapy but one might argue the declines in estimated free (bioactive) testosterone could play a role in aging atrophy. While we did not assess estrogen levels in females, the well-established, precipitous drop with menopause (Vitale et al., 2016) may partially contribute to the decline in muscle size and strength among women (Horstman et al., 2012). Androstenedione, a known precursor to estrogen and other androgens, was lower with aging in our cohort. We found

sex differences in androstenedione in addition to an overall decline with age yet no sex difference with IGF-1. Overall, our results generally agree with others who have found systemic hormones such as IGF-1, GH, and testosterone are reduced with age (Budui et al., 2015; Evans et al., 1982; Horstman et al., 2012; Vitale et al., 2016). It is possible that the aging hormonal milieu in combination with chronic systemic or local inflammation (Merritt et al., 2013; Mikkelsen et al., 2013; Schaap et al., 2006; Visser et al., 2002) could contribute to the decline in muscle mass with age but there are no definitive studies as of yet.

The use of different operational definitions of sarcopenia in aging research has led to variation in estimates of its prevalence (Baumgartner et al., 1999; Beaudart et al., 2015; Morley et al., 2014; von Haehling et al., 2010). Highlighting this, we applied the most established criteria (Beaudart et al., 2014) to the current study cohorts and found appreciable differences in the percent considered “sarcopenic” (Table 5), ranging from 12 to 35% in older males and 0–25% in older females. Some criteria even classified 4–8% of the youngest female group as sarcopenic. The ideal definition of sarcopenia should be in accordance with the progressive nature of the declines seen in muscle mass. In the current male cohort, this was best achieved by the Baumgartner criteria (Baumgartner et al., 1998), which defines sarcopenia as a skeletal muscle index (SMI, appendicular lean mass normalized to height in meters squared) more than two standard deviations below that of a population aged 20–30 y. In women, the optimal definition appeared to be that established by the European Working Group on Sarcopenia in Older People, which applies an absolute cutoff for SMI rather than comparing values to young adults. While this might be due to larger variability of SMI in the youngest female group, it certainly raises the question as to whether the operational definition of sarcopenia should be different for males and females. Further sex comparisons are necessary to validate the proposed criteria and to determine their relationship with functional and health outcomes.

A strength of this study is our comprehensive data set including neuromuscular performance, histological analyses, body composition, and circulating hormones in a fairly large cohort of healthy males and females. Important histopathological indices, such as myofiber grouping and myofiber size heterogeneity, were quantified in the context of aging in a sex-specific manner for the first time. However, we recognize some weaknesses of the study. First, this study was necessarily cross-sectional in design; thus longitudinal (within-subjects) changes in any of the outcomes studied can only be inferred. In an effort to best characterize the effects of aging in a between-groups design, we were careful to standardize key inclusion and exclusion criteria. Second, we did not study subjects in the “oldest-old” age bracket (85+ years old), which would be a valuable future pursuit. Third, while we assessed muscle health on multiple levels, additional measurements not included [MRI, satellite cell abundance, molecular profiling (e.g., genomics, transcriptomics)] could add substantial knowledge to the field. Finally, because of the single-site design, caution should be taken when extending findings to the population outside of the study area or to non-healthy adults.

In summary, we have identified novel sex differences in the aging human muscle phenotype that may prove to be important, including more severe type IIx myofiber atrophy, higher type I myofiber grouping, and greater myofiber size heterogeneity among aging females vs

males. Studies of muscle and/or motor unit remodeling would be useful to better understand concurrent atrophy and hypertrophy of like-type myofibers during aging. Interventions or preventative measures such as resistance training that improve the overall integrity of skeletal muscle and target type II myofibers continue to be an important pursuit and should be optimized. We made progress toward dose optimization in our recently published resistance training clinical trial, but more work is warranted (Stec et al., 2017). The remarkable sex differences observed highlight the need to better understand the mechanistic basis for what appears to be whole muscle atrophy by fiber loss among males vs. fiber atrophy among females. Further research into these differences and possible intervention strategies is warranted.

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Fig. 1.

Lower total, leg, and arm lean body mass in older adults. Lean mass values for each individual are expressed relative to the mean of the 25 y group. †Main effect of age. ‡Main effect of sex; ¹ $P < 0.05$ vs. 25 y; ² $P < 0.05$ vs. 37 y.

Fig. 2.

Differences in myofiber CSA across age groups. †Main effect of age. ‡Main effect of sex;

*Within-group difference in males vs. females; ¹ $P < 0.05$ vs. 25 y; ² $P < 0.05$ vs. 37 y.

Fig. 3.

Color histogram representing percentage of fibers of a given size within each group as a qualitative assessment of age-related atrophy and compensatory hypertrophy.

Table 1

Biometric data.³

<i>n</i>	Age (y)	Height (cm) ^b	Weight (kg) ^b	Body fat (%) ^{a,b}	Lean Mass (kg) ^{a,b}	Arm Lean Mass (kg) ^{a,b}	Leg Lean Mass (kg) ^{a,b}	Thigh Lean Mass (kg) ^{a,b}	Skeletal Muscle Index (kg/m ²) ^{a,b}
Overall									
25 y	47	25 ± 0	171 ± 1	29.4 ± 1.6	48.1 ± 1.7	6.0 ± 0.3	17.3 ± 0.7	12.0 ± 0.5	7.9 ± 0.2
37 y	79	37 ± 1	173 ± 1	30.1 ± 1.1	50.3 ± 1.3	6.5 ± 0.2 ¹	17.6 ± 0.5	12.0 ± 0.3	7.9 ± 0.2
61 y	51	61 ± 0	168 ± 1	36.3 ± 1.0 ^{1,2}	43.9 ± 1.4 ^{1,2}	5.0 ± 0.2 ^{1,2}	14.7 ± 0.5 ^{1,2}	10.1 ± 0.4 ^{1,2}	6.9 ± 0.2 ^{1,2}
72 y	44	72 ± 1	167 ± 1	35.6 ± 1.0 ^{1,2}	43.5 ± 1.5 ^{1,2}	5.0 ± 0.2 ^{1,2}	14.4 ± 0.6 ^{1,2}	9.7 ± 0.4 ^{1,2}	6.8 ± 0.2 ^{1,2}
Males									
25M	21	25 ± 1	176 ± 2	21.8 ± 2.2	58.4 ± 1.7	7.7 ± 0.3	21.2 ± 0.7	14.8 ± 0.5	9.2 ± 0.2
37M	45	38 ± 1	180 ± 1	24.6 ± 1.2	58.7 ± 0.9	8.1 ± 0.2	20.6 ± 0.3	13.9 ± 0.3	8.9 ± 0.1
61M	19	60 ± 1	177 ± 2	31.4 ± 1.5 ^{1,2}	55.4 ± 1.3	7.0 ± 0.2 ²	19.1 ± 0.5	13.2 ± 0.4	8.3 ± 0.2 ¹
72M	17	72 ± 1	176 ± 2	30.3 ± 1.2 ¹	54.3 ± 1.4	6.8 ± 0.2 ^{1,2}	18.1 ± 0.6 ^{1,2}	12.3 ± 0.4 ^{1,2}	8.0 ± 0.2 ^{1,2}
Females									
25F	26	26 ± 0	166 ± 2	35.4 ± 1.4	40.0 ± 1.1	4.6 ± 0.2	14.3 ± 0.5	9.9 ± 0.4	6.8 ± 0.2
37F	34	36 ± 1	164 ± 1	37.3 ± 1.3	39.4 ± 0.6	4.3 ± 0.1	13.7 ± 0.3	9.5 ± 0.2	6.7 ± 0.1
61F	32	62 ± 0	163 ± 1	39.0 ± 1.1	37.6 ± 0.6	3.9 ± 0.1	12.4 ± 0.3 ¹	8.4 ± 0.2 ¹	6.1 ± 0.1 ¹
72F	27	72 ± 1	162 ± 1	39.1 ± 1.1	36.5 ± 0.8	3.8 ± 0.1	11.9 ± 0.4 ^{1,2}	8.0 ± 0.2 ^{1,2}	6.0 ± 0.2 ^{1,2}

^aMain effect of age.^bMain effect of sex.¹*P* < 0.05 vs. 25 y.²*P* < 0.05 vs. 37 y.³*P* < 0.05 vs. 61 y.

Table 2
Neuromuscular Performance.

<i>n</i>	Knee extension				3-Repetition Sit-to-Stand Test		10-Repetition Sit-to-Stand Test	
	Unilateral MVC (kg) ^{a,b}	Unilateral specific strength (kg MVC/kg TLM) ^{a,b}	Bilateral power (W) ^{a,b}	Specific power (W/kg TLM) ^{a,b}	Concentric relative motor unit activation ^{a,b}	Eccentric relative motor unit activation ^{a,b}	Explosive peak power (W) ^{a,b}	Explosive peak velocity (m/s) ^{a,b}
Overall								
25 y 44	54.2 ± 3.8	8.5 ± 0.5	439 ± 33	33.6 ± 1.7	0.38 ± 0.02	0.33 ± 0.02	1232 ± 100	1.31 ± 0.07
37 y 72	59.5 ± 2.4	9.9 ± 0.3	416 ± 20	34.1 ± 1.3	0.43 ± 0.02	0.37 ± 0.02	800 ± 88 ¹	1.26 ± 0.04
61 y 50	41.6 ± 3.0 ^{1,2}	7.8 ± 0.4 ²	271 ± 20 ^{1,2}	25.2 ± 1.2 ^{1,2}	0.60 ± 0.04 ^{1,2}	0.50 ± 0.02 ^{1,2}	675 ± 78 ¹	1.08 ± 0.07 ¹
72 y 41	33.6 ± 2.7 ^{1,2}	6.8 ± 0.4 ^{1,2}	205 ± 15 ^{1,2}	20.4 ± 0.9 ^{1,2}	0.66 ± 0.04 ^{1,2}	0.55 ± 0.03 ^{1,2}	515 ± 78 ¹	0.96 ± 0.05 ^{1,2}
Males								
25M 20	73.7 ± 3.5	10.0 ± 0.5	587 ± 37	39.0 ± 2.3	0.35 ± 0.03	0.32 ± 0.03	1524 ± 158	1.43 ± 0.09
37M 40	72.2 ± 2.7	10.4 ± 0.5	520 ± 23	37.7 ± 1.6	0.37 ± 0.02	0.33 ± 0.02	901 ± 132 ¹	1.33 ± 0.07
61M 19	60.4 ± 4.6	9.1 ± 0.8	405 ± 31 ^{1,2,3}	30.7 ± 2.3	0.48 ± 0.04 ^{1,2}	0.42 ± 0.04 ¹	887 ± 144 ¹	1.30 ± 0.14
72M 16	47.9 ± 3.9 ^{1,2}	7.6 ± 0.6 ²	291 ± 23 ^{1,2,3}	23.1 ± 1.5 ^{1,2}	0.62 ± 0.06 ^{1,2}	0.52 ± 0.06 ¹	824 ± 153 ¹	1.18 ± 0.07
Females								
25F 24	37.9 ± 4.0	7.4 ± 0.7	284 ± 21	28.5 ± 1.8	0.41 ± 0.03	0.34 ± 0.02	975 ± 92	1.19 ± 0.10
37F 32	43.7 ± 1.7	9.2 ± 0.4	277 ± 15	29.2 ± 1.7	0.49 ± 0.03	0.44 ± 0.03	692 ± 112	1.17 ± 0.06
61F 31	30.7 ± 2.0 ^{1,2}	7.2 ± 0.4 ²	186 ± 11 ²	22.0 ± 1.1 ²	0.68 ± 0.06	0.56 ± 0.03	550 ± 84	0.97 ± 0.07
72F 25	24.0 ± 2.1 ^{1,2}	6.2 ± 0.6 ²	147 ± 7 ^{1,2}	18.6 ± 1.0 ^{1,2}	0.69 ± 0.06 ^{1,2}	0.57 ± 0.04 ^{1,2}	308 ± 49 ¹	0.82 ± 0.05 ^{1,2}

MVC: Maximum voluntary contraction; TLM: thigh lean mass.

^aMain effect of age.

^bMain effect of sex.

¹*P* < 0.05 vs. 25 y.

²*P* < 0.05 vs. 37 y.

³*P* < 0.05 vs. 61 y.

Table 3

Myofiber profile.

n	No. fibers analyzed per sample	Myofiber type distribution			Myofiber type area distribution			Myofiber size heterogeneity			Type I myofiber grouping characteristics ^c		
		Type I (%) ^a	Type IIa (%)	Type IIx (%) ^{a,b}	Type I (%) ^{a,b}	Type IIa (%) ^b	Type IIx (%) ^{a,b}	Type I (CV%) ^a	Type IIa (CV%) ^{a,b}	Type IIx (CV%) ^{a,b}	Grouped Type I (%) ^a	# Type I Groups	Mean # Type I Fibers/Group ^{a,b}
Overall													
25 y	36	1066 ± 59	47 ± 2	14 ± 2	39 ± 2	50 ± 2	11 ± 2	28 ± 1	22 ± 1	24 ± 1	34 ± 4	9 ± 1	18 ± 12
37 y	43	1484 ± 90	47 ± 2	19 ± 2	35 ± 2	51 ± 2	14 ± 1	26 ± 1	20 ± 1	28 ± 1	32 ± 4	12 ± 1	16 ± 12
61 y	36	1259 ± 115	51 ± 1	13 ± 2	43 ± 2 ²	49 ± 2	8 ± 1 ²	32 ± 2 ²	31 ± 1 ^{1,2}	36 ± 2 ^{1,2}	49 ± 4 ²	12 ± 1	22 ± 12
72 y	25	1365 ± 206	49 ± 3	14 ± 2	44 ± 4 ²	47 ± 3	9 ± 2	30 ± 2	30 ± 2 ^{1,2}	34 ± 2 ^{1,2}	61 ± 5 ^{1,2}	11 ± 2	67 ± 14
Males													
25M	19	1054 ± 79	48 ± 3	14 ± 2	35 ± 4	51 ± 3	13 ± 3	28 ± 2	20 ± 1	23 ± 1	26 ± 6	8 ± 2	17 ± 16
37M	23	1474 ± 131	48 ± 3	20 ± 2	31 ± 2	53 ± 3	16 ± 2	25 ± 1	19 ± 1	24 ± 1	29 ± 6	12 ± 2	9 ± 17
61M	14	1082 ± 196	49 ± 2	18 ± 3	36 ± 3	50 ± 2	13 ± 3	28 ± 1	26 ± 2 ²	28 ± 3	49 ± 6	12 ± 2	18 ± 18
72M	13	918 ± 96	50 ± 5	18 ± 3	35 ± 5	52 ± 5	12 ± 3	29 ± 2	26 ± 2	32 ± 3	55 ± 7 ¹	11 ± 2	20 ± 19
Females													
25F	17	1078 ± 90	46 ± 2	13 ± 2	42 ± 3	48 ± 3	9 ± 2	29 ± 1	23 ± 2	25 ± 2	41 ± 6	9 ± 2	20 ± 16
37F	20	1496 ± 127	46 ± 2	19 ± 2	39 ± 3	48 ± 3	12 ± 2	27 ± 2	22 ± 1	32 ± 2	35 ± 6	11 ± 2	22 ± 16
61F	22	1372 ± 139	52 ± 2	10 ± 2	47 ± 3	48 ± 2	5 ± 1	35 ± 3 ²	35 ± 2 ^{1,2}	40 ± 2 ^{1,2}	48 ± 6	12 ± 2	26 ± 17
72F	12	1850 ± 375	47 ± 2	10 ± 2	53 ± 4	42 ± 4	5 ± 1	31 ± 3	35 ± 3 ^{1,2}	36 ± 3 ¹	68 ± 7 ²	12 ± 2	114 ± 20 ^{1,2,3}

¹ $P < 0.05$ vs. 25 y.² $P < 0.05$ vs. 37 y.³ $P < 0.05$ vs. 61 y.^a Main effect of age.^b Main effect of sex.^c Sample sizes for grouping analysis: 25 y ($n = 30$), 37 y ($n = 29$), 61 y ($n = 27$), 72 y ($n = 21$).

Table 4

Serum hormone concentrations.

	<i>n</i>	IGF-1 (ng/mL) ^a	IGFBP-3 (ng/mL) ^a	IGFBP-1 (ng/mL) ^{a,b}	Androstene-dione (ng/mL) ^{a,b}	SHBG (nM) ^{a,b}	Total Testosterone (ng/dL) ^b	Free Testosterone (nM) ^{a,b}
Overall								
25 y	36	382 ± 20	4238 ± 180	31 ± 5	2.2 ± 0.1	142 ± 26		
37 y	45	296 ± 16 ¹	3624 ± 107 ¹	25 ± 3	1.7 ± 0.1 ¹	73 ± 11 ¹		
61 y	37	195 ± 13 ^{1,2}	4085 ± 120	34 ± 4	1.4 ± 0.1 ¹	112 ± 14		
72 y	24	173 ± 18 ^{1,2}	3829 ± 170	51 ± 9 ^{1,2,3}	1.2 ± 0.1 ^{1,2}	151 ± 29 ²		
Males								
25M	19	397 ± 27	4093 ± 308	17 ± 3	2.3 ± 0.2	62 ± 7	503 ± 35	0.299 ± 0.040
37M	25	297 ± 23 ¹	3498 ± 153	22 ± 3	1.9 ± 0.1	49 ± 8	496 ± 30	0.343 ± 0.036
61M	14	227 ± 23 ¹	4088 ± 169	33 ± 8	1.8 ± 0.2	88 ± 7	462 ± 31	0.204 ± 0.029 ²
72M	13	182 ± 22 ^{1,2}	3864 ± 263	35 ± 11	1.6 ± 0.1 ¹	98 ± 13	439 ± 30	0.170 ± 0.017 ^{1,2}
Females								
25F	17	366 ± 29	4400 ± 168	47 ± 8	2.2 ± 0.2	231 ± 47	32 ± 5	0.008 ± 0.002
37F	20	294 ± 21	3781 ± 144	29 ± 4	1.5 ± 0.1 ¹	103 ± 22 ¹	27 ± 4	0.010 ± 0.002
61F	23	176 ± 14 ^{1,2}	4083 ± 166	35 ± 4	1.2 ± 0.1 ¹	127 ± 21 ¹	24 ± 3	0.009 ± 0.002
72F	11	161 ± 31 ^{1,2}	3787 ± 214	69 ± 13 ^{2,3}	0.9 ± 0.1 ¹	209 ± 54	23 ± 7	0.006 ± 0.002

IGF: insulin-like growth factor I; IGFBP: IGF binding protein; SHBG: serum hormone binding globulin.

^aMain effect of age.^bMain effect of sex.¹*P* < 0.05 vs. 25 y.²*P* < 0.05 vs. 37 y.³*P* < 0.05 vs. 61 y.

Table 5
Prevalence of sarcopenia in current study cohorts.

		“Sarcopenia” criterion		
	EWGSOP/IWGS	Baumgartner Criteria (Baumgartner et al., 1998)	Society of Sarcopenia, Cachexia, and Wasting Disorders	Foundation of NIH Sarcopenia Project
Males	% SMI < 7.25	% SMI 2SD < 20–30 y (7.65)	% SMI 2SD < 20–39 y (7.43)	% ALM/ BMI < 0.789
25M	0.0	0.0	0.0	0.0
37M	0.0	4.4	2.2	0.0
61M	0.0	13.0	0.0	4.3
72M	17.6	35.3	23.5	11.8
Females	% SMI < 5.67	% SMI 2SD < 20–30 y (4.89)	% SMI 2SD < 20–39 y (5.06)	% ALM/ BMI < 0.512
25F	7.7	3.8	3.8	0.0
37F	8.8	0.0	0.0	0.0
61F	19.4	0.0	0.0	0.0
72F	25.0	7.1	10.7	0.0

EWGSOP: European Working Group on Sarcopenia in Older People; IWGS: International Working Group on Sarcopenia; SMI: skeletal muscle index (kg/m^2); ALM: appendicular lean mass (kg); BMI: body mass index (kg/m^2).