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Effects of age on human skeletal muscle: A systematic review and meta-analysis of myosin heavy chain isoform protein expression, fiber size and distribution

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Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 26 June 2024 and was last updated on 26 June 2024.

INTRODUCTION

Review question / Objective The main purpose of this study was to systematically gather and review experimental evidence of relevant published literature and conduct a meta-analysis to quantify the effects of aging on the relative myosin heavy chain (MyHC) protein expression in skeletal muscle tissue (% of MyHC protein expression) in healthy adults. Additionally, we examined the effects of aging on single fiber cross-sectional area (CSA) by fiber type and fiber type distribution (% of fibers expressing MyHC isoform) as these two parameters dictate relative MyHC protein expression in skeletal muscle tissue. These three parameters were also examined to determine if there were age- and sex-related differences in their responses between measurement techniques, physical activity, and the skeletal muscle examined.

Rationale Sarcopenia, or the age-related loss of skeletal muscle mass and function, can reduce whole muscle contractile capacity and increase the likelihood of physical disability in older adults. These pathological changes can have profound consequences on physical function, leading to a greater risk for falls, frailty, and mortality. While the cause of sarcopenia is generally thought to be multifactorial, including the loss of muscle mass and contractile performance, another possible contributing factor could be an age-related shift in the amount of myosin heavy chain (MyHC) isoforms expressed. The composition of adult human skeletal muscle consists of a mixture of three distinct MyHC isoforms (I, IIA and IIX), which determine single fiber contractile velocity and power production [I < IIA < IIX] and force-generating capacity [I < II]. Thus, an age-related shift to the slower MyHC I isoform would reduce single fiber force production, contractile velocity and power output (product of force and velocity), potentially leading to similar losses at the whole muscle level as fiber type composition partially dictates whole muscle performance.

Studies examining age-related shifts in MyHC isoform composition in older adults have

produced a variety of results, finding either a shift to more slower-contracting isoforms or fast-contracting isoforms, or no change in the expression of MyHC isoforms. A potential reason for the differences in age-related responses is the variety of measurement techniques. Relative MyHC protein expression of skeletal muscle tissue can be quantified with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) where homogenized tissue is electrophoretically separated into specific bands for each MyHC isoform which are analyzed for their density, allowing for quantification of their relative amounts. Relative MyHC protein expression of skeletal muscle tissue can also be determined from thin slices of skeletal muscle tissue cross-sections. Fiber type and cross-sectional area (CSA) of individual muscle fibers are determined using fluorescence immunohistochemistry (IHC), which utilizes primary and secondary antibodies against specific MyHC isoforms, or myosin adenosine triphosphatase (mATPase), which employs differential staining from varying sensitivities to pH. Relative MyHC protein expression is calculated using the fiber type distribution and size results from IHC or mATPase, which are typically done on hundreds of fibers per subject. Instead of using skeletal muscle cross-sections, some studies manually dissect individual muscle fibers and use SDS-PAGE to determine the MyHC isoform expression of each fiber. An advantage of using this approach is that a larger amount of the muscle fiber, typically 1-3 mm in length, is used, which better represents MyHC expression throughout the fiber compared to IHC and mATPase. However, a disadvantage is that fewer fibers, commonly in ten or twenty fibers per subject, are examined compared to the hundreds per subject for IHC or mATPase. Other potential issues that could lead to inconsistent results between studies include the physical activity levels of the participants, as MyHC isoform composition may change based on how active the muscles are, and using different muscles, as muscle-specific atrophy can occur as people age. Thus, ascertaining whether relative MyHC isoform expression is directly affected by age has been challenging.

Condition being studied Systematically gather and review experimental evidence of relevant published literature and conduct a meta-analysis to quantify the effects of aging on the relative MyHC protein expression in skeletal muscle tissue in healthy adults.

METHODS

Search strategy This systematic review and meta-analysis were performed in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A systematic literature search for articles published until January 10, 2023, was conducted using PubMed, SPORTDiscus, and Web of Science online databases. The search included the following keywords: “aging,” “older adults,” “elderly,” “skeletal muscle fiber,” “fiber type composition,” “myosin heavy chain isoform distribution,” and “myosin heavy chain isoform expression.” Nonduplicate articles were independently screened by title and abstract, followed by a full-text report evaluation to determine eligibility by the authors (C.L. and P.C.W.). Additional manual searches of reference lists were conducted to identify manuscripts not revealed as part of the online database search.

Participant or population Healthy human participants free from any known disease, injury, or physical limitations; mean age of ≥ 60 years for older adults and young adults between the ages of 18 to 49 years.

Intervention Investigations into the effects of aging that examined the relative protein expression of skeletal muscle tissue between young and older adults.

Comparator Assessment of MyHC isoform composition between young and older adults with the reported unadjusted percentages of MyHC isoform distribution as a mean (M) ± standard deviation (SD) or a standard error from which a SD could be calculated. Control or baseline data was used if a study included an intervention, such as exercise or unloading.

Study designs to be included Peer-reviewed publications available in English in experimental and observational studies that included young and older adults and evaluated the effects of aging on the properties of skeletal muscle tissue.

Eligibility criteria N/A

Information sources Electronic online databases: PubMed, SPORTSDiscus, Web of Science; data was requested for studies where the SD could not be calculated (N = 8) and were included if the authors responded and a SD could be formulated (N = 6).

Main outcome(s) Relative protein expression in older adults was higher for MyHC I and lower for MyHC II and IIA isoforms compared to young adults (all $p < 0.0001$). However, there was no difference in relative protein expression for the MyHC IIX ($p = 0.41$) isoform between young and older adults. Heterogeneity (I^2) was moderate to high, ranging from 39% to 83%, across relative MyHC expression analyses for all adults. In order to determine whether the changes in relative protein expression with age were due to fiber type differences in atrophy or fiber switching, CSA and fiber type distribution were examined. CSA was smaller in older adults compared to young adults for MyHC II ($p < 0.0001$) and IIA ($p = 0.004$) fibers, but was unchanged with age in MyHC I ($p = 0.10$) fibers. Heterogeneity (I^2) was moderate to high, ranging from 58 to 92% across fiber CSA analyses for all adults. Fiber type distribution (% of fibers) for all fibers showed no difference between young and older adults (MyHC I, $p = 0.29$; MyHC II, $p = 0.30$; MyHC IIA, $p = 0.40$). Heterogeneity (I^2) was low to moderate, ranging from 0 to 61% across fiber type distribution analyses for all adults. Overall, these results suggest that the age-related changes in relative protein expression are primarily due to the greater atrophy of fast-contracting fibers, as fiber type distribution for fast- and slow-contracting fibers remained unaffected with age.

When separated by sex (males vs. females), older males and females had different results for relative protein expression (Figure 2), but similar results for fiber CSA and fiber type distribution. Relative protein expression in older males was higher for MyHC I and lower for MyHC II and IIA (all $p < 0.00001$), but was unchanged in older females for the same fiber types ($p = 0.43$–0.64). Neither sex showed differences with age in MyHC IIX protein expression ($p = 0.45$–0.72). In both sexes, fiber CSA was unchanged in MyHC I (males, $p = 0.16$; females, $p = 0.35$) and smaller in MyHC II (both $p < 0.0001$) and IIA (males, $p = 0.03$; females, $p < 0.0001$). Fiber type distribution was unchanged with age regardless of sex ($p = 0.20$–0.72). Unsurprisingly, as most studies (96%) contain males, the results of the older adult and older males are very similar. The lack of an age-related change in relative protein expression in females may be due to an actual sex-specific response or that fewer studies (22%) contain females and a large portion were from a single study.

Additional outcome(s) Measurement techniques provided similar responses, in general, for relative protein expression and fiber type distribution with age but produced differences in fiber CSA. Relative MyHC protein expression responded similarly when examined by measurement technique (SDS-PAGE vs. mATPase vs. IHC) with an increase for MyHC I and a decrease for MyHC II, except for mATPase data for MyHC II which showed a similar average decrease, but greater variation, leading to a non-significant difference ($p = 0.12$). Relative MyHC protein expression in MyHC IIA was decreased with age using SDS-PAGE ($p < 0.0001$), but unchanged for mATPase ($p = 0.87$) potentially due to few studies ($N = 3$), whereas IHC did not evaluate this isoform. Fiber CSA with age was unchanged, regardless of fiber type, when measured using SDS-PAGE, but was smaller with IHC in MyHC I ($p = 0.008$) and II ($p < 0.00001$) fibers and with mATPase in MyHC II ($p < 0.00001$) and IIA ($p = 0.009$) fibers. The lack of differences with SDS-PAGE may be due to the fewer number of fibers analyzed per subject using this technique. Fiber type distribution was unchanged with age regardless of measurement technique. Notably, no studies have used IHC to look at fiber CSA and SDS-PAGE has not been used to examine fiber type distribution, again most likely and understandably due to the fewer number of fibers analyzed per subject using this technique.

When accounting for physical activity (Sedentary or SED vs. Active), the relative protein expression in older adults was higher for MyHC I (SED: $p < 0.0001$; Active: $p = 0.04$) and lower for MyHC II (SED: $p < 0.0001$; Active: $p = 0.04$) and IIA (SED: $p = 0.004$; Active: $p = 0.01$) isoforms compared to young adults, similar to the patterns found with all studies grouped together. However, fiber CSA for MyHC I fibers and fiber type distribution for MyHC I and IIA fibers responded differently to aging when separated by physical activity. MyHC I fiber CSA was smaller with age ($p = 0.002$) in physically active older adults, whereas MyHC I fiber CSA was similar in sedentary older adults ($p = 0.29$). Physically active older adults showed no change in MyHC I ($p = 0.91$) or II ($p = 0.89$) fiber distribution compared to young adults, while sedentary older adults had increased MyHC I ($p = 0.006$) and reduced MyHC II ($p = 0.007$) fiber type distribution. The remaining measures responded similarly to physical activity, where MyHC II and IIA fiber CSA (SED: MyHC II, $p < 0.0001$, MyHC IIA, $p = 0.04$; Active: MyHC II, $p = 0.002$, MyHC IIA, $p = 0.0006$) decreased with age and MyHC IIA fiber type distribution was similar with age (SED: $p = 0.93$; Active: $p = 0.97$). As a whole, these findings indicate that older adults who are physically active, may partially, reverse the effects of aging on these various parameters.

When examined by muscle type (vastus lateralis or VL vs. other muscles), the only differences in the age-related results were for relative protein expression in MyHC I, II and IIA

fibers. The vastus lateralis showed greater protein expression with age in MyHC I ($p < 0.001$) isoforms and reduced protein expression in MyHC II ($p < 0.00001$) and IIA ($p < 0.0001$) isoforms, while the other muscles showed no change with age. The fiber CSA and fiber type distribution were similar between VL and other muscles.

Data management All data was managed and shared through Microsoft OneDrive

Quality assessment / Risk of bias analysis The Newcastle-Ottawa Quality Assessment Scale (NOS) modified for cross-sectional studies was utilized to assess the methodological quality of the included studies (52). In this scale, study quality is evaluated according to six items: 1) sample representative, 2) sample size, 3) health assessment, 4) group comparability with confounding factors controlled, 5) outcome assessment, and 6) statistical analysis, classified into three categories: 1) selection, 2) comparability, and 3) outcome. Total NOS score can range from 0 to 9, and methodological qualities of the studies were considered "good" with ≥ 7, "fair" with 4 – 6, and "poor" with ≤ 3. Quality assessment was performed independently by two authors (C.L. and P.C.W.), with disagreements discussed until a consensus was reached.

Strategy of data synthesis The following data were extracted from each included study: a) authors and year of publication; b) participant characteristics including sample size, age (years), sex, and physical activity level; c) skeletal muscle biopsied; d) the method of measurement and e) measurements taken: 1) fiber cross-sectional area (μm^2), 2) MyHC fiber type distribution (% of fibers expressing MyHC isoform) and/or 3) relative MyHC expression (% of MyHC protein expression). Where information was not reported in text or table or not received upon request from study authors, data was extracted using WebPlot Digitizer (Web Plot Digitizer, V.4.5. Ankit Rohatgi, 2021) (N = 9). The quantification of physical activity levels varied by study and were stratified independently by two authors (C.L. and P.C.W.) as either sedentary or active. In general, sedentary was considered not regularly participating in any structured exercise training or physical activity, while active was considered normally involved in recreational activities or systematic training. Any disagreements were discussed between authors until a consensus was reached.

Human skeletal muscle fiber types were measured in various ways, including immunohistochemistry (IHC), myosin adenosine triphosphatase (mATPase), and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Human skeletal muscle contains three MyHC isoforms that are expressed in pure fiber types (I, IIA, and IIX) and mixed fiber types (I/IIA, IIAX, and I/IIA/IIX), which were measured using IHC or SDS-PAGE. mATPase produces pure fiber types (I, IIA and IIB) and mixed fiber types (IIC and IIAB). For this work, MyHC IIB and IIAB was labeled as MyHC IIX and IIAX to have consistent terminology between the various fiber typing techniques. As IHC and mATPase studies commonly grouped all II isoforms into a single group (N = 10), we defined IIA as II for the studies that only identified IIA isoforms (N = 6) and calculated II fiber type distribution for the studies that identified multiple II isoforms (N = 6) by adding these isoforms together, i.e. MyHC II (%) = MyHC IIA (%) + MyHC IIAX (%) + MyHC IIX (%). Only three studies examined the IIC isoform and the expression was very low (<1%), so this isoform was not included in any calculations.

Fiber cross-sectional areas (CSAs) were from IHC or mATPase performed on muscle bundles or SDS-PAGE performed on single fibers. MyHC isoform distributions were determined in most IHC (N = 5) and all mATPase (N = 8) studies. Relative MyHC protein expression was determined from SDS-PAGE using relative band intensity from densitometry and was performed on a muscle bundle or sample. Relative MyHC protein expression for this study was calculated for most studies that used IHC and mATPase for fiber CSAs. First, the relative CSA of each MyHC isoform in the tissue sample was calculated by multiplying the MyHC fiber type distribution (% of fibers) by the mean fiber CSA for each isoform (i.e., relative CSA of MyHC I fibers). Second, the relative CSA of the entire tissue sample was calculated by summing the values for each MyHC isoform (i.e., relative CSA of tissue sample = relative CSA of MyHC I fibers + relative CSA of MyHC II fibers). Relative MyHC protein expression (%) for each isoform was determined by dividing the relative CSA of a MyHC isoform by the relative CSA of the entire tissue sample and multiplying by 100 (i.e., relative MyHC I expression = 100 x [relative CSA of MyHC I fibers / relative CSA of the tissue sample]).

Subgroup analysis Relative MyHC protein expression, single fiber cross-sectional area, and fiber type distribution for each MyHC isoform in M ± SD for young and older adults were analyzed using R (v4.3.0, RStudio Team, 2023). Values were extracted, calculated and entered independently by the authors (C.L. and P.C.W.) and cross-checked for any errors. When M ± SD values within age groups were reported separately by sex, a total value pooling both males and females of the

M and SD were calculated according to the Cochrane Handbook for Systematic Reviews of Interventions (53) and the American Community Survey (54). Random-effects meta-analyses were performed in R for relative protein expression for MyHC I, II, IIA and IIX isoforms, whereas MyHC I, II and IIA fibers were examined for fiber CSA and fiber type distribution, if available, as there were too few MyHC IIX isoforms for these measures (N < 6). Mixed fiber types were excluded from the analysis as only two studies examined MyHC IIAX fibers for single fiber type distribution (7) and fiber cross-sectional area (29). All outcomes were continuous, and effect sizes were presented as the mean difference (MD) and 95% confidence intervals (95% CI). A priori stratified analyses by sex (males vs. females), measurement technique (SDS-PAGE vs. mATPase vs. IHC), physical activity (sedentary vs. active), and skeletal muscle (vastus lateralis vs. other muscles) were also conducted. The degree of heterogeneity of the effect sizes was quantified with the I2 statistic, which was considered to be low (< 25%), moderate (25-75%), or high (> 75%) (53). Meta-regressions were performed to determine if the various measurement techniques were significant moderators. Funnel plots were performed to visually assess publication bias. To determine whether the results were not influenced due to one large study or a study with an extreme result, leave-one-out sensitivity analyses were conducted. Significance was set at a $p < 0.05$ for all analyses.

Sensitivity analysis Sensitivity analyses using leave-one-out procedure revealed evidence of influential studies for only MyHC I fiber distribution (N = 1) and MyHC I CSA (N = 2) to suggest an age effect. No studies influenced the difference in means or significance in relative protein expression for MyHC I, II, IIA and IIX isoforms, and fiber CSA and distribution for MyHC II and IIA fibers. There was no evidence of publication bias for relative MyHC protein expression for MyHC I, II, IIA and IIX isoforms, and fiber CSA and distribution for MyHC I, II and IIA fibers through visual inspections of funnel plots and tests for funnel plot asymmetry (Supplementary Appendix A). The various measurement techniques to analyze relative MyHC protein expression, fiber CSA and fiber type distribution were not significant moderators in the meta-regression models (Supplementary Appendix B).

Language restriction None.

Country(ies) involved United States.

Other relevant information The primary search resulted in 1,144 potentially relevant references, with 27 studies identified as fulfilling the inclusion criteria (Figure 1). Study-level characteristics are presented in Table 1. Altogether, these studies examined 370 young adults (males, n = 303; females, n = 67) and 382 older adults (males, n = 293; females, n = 89). Some studies biopsied 36 adult males (young males, n = 17; older males, n = 19) for two different muscles (11, 23, 25). Males were included in almost all studies (96%, N = 26), while females were included in less than a quarter of the studies (22%, N = 6). Notably, a large portion of the female adults came from a single study (young females, n = 26 or 39%; older females, n = 50 or 56%) that examined the minor pectoralis (28). Relative MyHC expression (N = 27 studies) was measured using SDS-PAGE (N = 15) and calculated for this study using IHC (N = 5) and mATPase (N = 7). Fiber CSA (N = 22 studies) was measured using IHC (N = 6), mATPase (N = 8) and SDS-PAGE (N = 8), with more fibers measured per subject using IHC and mATPase compared to SDS-PAGE (Table 1). Fiber type distribution (% of fibers expressing MyHC isoform, N = 13 studies) was measured in most IHC (N = 5) and all mATPase (N = 8) studies. Biopsies were most commonly performed on the vastus lateralis (N = 24), although the biceps brachii (N = 3), gastrocnemius (N = 1), masseter (N = 1) and minor pectoralis (N = 1) were also used. The Newcastle-Ottawa Quality Assessment Scale (0-9) scores ranged from 3 (poor) to 8 (good). Physical activity was not specified (N = 4), sedentary (N = 15), or active (N = 8), with studies using surveys (N = 17), medical history (n = 4), and accelerometry (n = 2) for quantification.

Keywords aging, sarcopenia, sex, physical activity, MyHC.

Dissemination plans All authors conceived and designed the research. The systematic review and meta-analysis was performed by Christopher Lee and Philip C. Woods, and the results were interpreted by all authors. The figures were prepared by Christopher Lee. All authors were involved in drafting the manuscript, editing and revising, and approving the final version of the manuscript.

Contributions of each author

Author 1 – Christopher Lee. Conceived and designed research; performed systematic review and meta-analysis; interpreted results of systematic review and meta-analysis; prepared figures; drafted manuscript; edited and revised

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