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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Abstract

Human studies examining the cellular mechanisms behind sarcopenia, or age-related loss of skeletal muscle mass and function, have produced inconsistent results. A systematic review and meta-analysis were performed to determine the aging effects on protein expression, size and distribution of fibers with various myosin heavy chain (MyHC) isoforms. Study eligibility included MyHC comparisons between young (18-49 years) and older (≥ 60 years) adults, with 27 studies identified. Relative protein expression was higher with age for the slow-contracting MyHC I fibers, with correspondingly lower fast-contracting MyHC II and IIA values. Fiber sizes were similar with age for MyHC I, while smaller for MyHC II and IIA. Fiber distributions were similar with age. When separated by sex, the few studies that examined females showed atrophy of MyHC II and IIA fibers with age, but no change in MyHC protein expression. Additional analyses by measurement technique, physical activity, and muscle biopsied provided important insights. In summary, age-related atrophy in fast-contracting fibers lead to more of the slow-contracting, lower force-producing isoform in older male muscles, which helps explain their age-related loss in whole muscle force, velocity, and power. Exercise or pharmacological interventions that shift MyHC expression towards faster isoforms and/or increase fast-contracting fiber size should decrease the prevalence of sarcopenia. Our findings also indicate that future studies need to include or focus solely on females, measure MyHC IIA and IIX isoforms separately, examine fiber type distribution, sample additional muscles to the vastus lateralis, and incorporate an objective measurement of physical activity.

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

NEW & NOTEWORTHY

Our systematic review and meta-analysis showed that older males have more of the slow-contracting, lower-force producing myosin heavy chain (MyHC) I isoform due to atrophy of the fast-contracting, higher-force producing MyHC II fibers compared to younger males. Interventions that increase MyHC II isoform expression and fiber size should decrease the age-related loss of muscle function in males. However, these results need to be verified in females due to the few studies examining this sex.

Abbreviations: Biceps brachii (BB); Confidence interval (CI); Cross-sectional area (CSA); F (Females); Gastrocnemius (Gastroc); History (Medical History); Immunohistochemistry (IHC); Males (M); Masseter (MA); Mean (M); Mean difference (MD); Minor pectoralis (MP); Myosin adenosine triphosphatase (mATPase); Myosin heavy chain (MyHC); Newcastle-Ottawa Quality Assessment Scale (NOS); Older adults (O); Phosphate (P_i); Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA); Sedentary (SED); Survey (Physical activity questionnaires); Standard deviation (SD); Standard error (SE); Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE); Vastus lateralis (VL); Young adults (Y)

INTRODUCTION

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 (1–3). These pathological changes can have profound consequences on physical function (3), leading to a greater risk for falls (4), frailty (5), and mortality (6). While the cause of sarcopenia is generally thought to be multifactorial, including the loss of muscle mass and contractile performance (7–10), another possible contributing factor could be an age-related shift in the amount of myosin heavy chain (MyHC) isoforms expressed (11, 12). 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] (13, 14) and force-generating capacity [I < II] (15, 16). 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 (17, 18).

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 (19–30) or fast-contracting isoforms (7, 11, 31), or no change in the expression of MyHC isoforms (9, 32– 42). 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 (43–45). 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 (44, 46), or myosin adenosine triphosphatase (mATPase), which employs differential staining from varying sensitivities to pH (47, 48). Relative MyHC protein expression is calculated using the fiber type distribution and size results from IHC or mATPase (for details, see Methods, Section 2.5), 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 (for examples, see Table 1). 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 (31, 49), and using different muscles, as muscle-specific atrophy can occur as people age (50). Thus, ascertaining whether relative MyHC isoform expression is directly affected by age has been challenging.

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 MyHC protein expression in skeletal muscle tissue (% of MyHC protein expression). Additionally, we examined the effects of aging on single fiber cross-sectional area (CSA, μm^2) 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-related differences in their responses between sex, measurement techniques, physical activity, and the skeletal muscle examined.

MATERIALS AND 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) standard and checklist (51) and was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY, 202460109). 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.

Study selection criteria

Articles were included if all of the following criteria were met: 1) peer-reviewed publications; 2) available in English; 3) healthy human participants free from any known disease, injury, or physical limitations; 4) assessment of MyHC isoform composition between young and older adults; 5) mean age of ≥ 60 years for older subjects; 6) young adults between the ages of 18 to 49 years; and 7) 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. Data was requested for studies where the SD could not be calculated (n = 8) and were included if the

130 authors responded and a SD could be formulated (n = 6). Control or baseline data was used if a
131 study included an intervention, such as exercise or unloading.
132
133 **Data extraction**
134 The following data were extracted from each included study: a) authors and year of
135 publication; b) participant characteristics including sample size, age (years), sex, and physical
136 activity level; c) skeletal muscle biopsied; d) the method of measurement and e) measurements
137 taken: 1) fiber cross-sectional area (μm^2), 2) MyHC fiber type distribution (% of fibers
138 expressing MyHC isoform) and/or 3) relative MyHC expression (% of MyHC protein
139 expression). Where information was not reported in text or table or not received upon request
140 from study authors, data was extracted using WebPlot Digitizer (Web Plot Digitizer, V.4.5.
141 Ankit Rohatgi, 2021) (n = 9). The quantification of physical activity levels varied by study and
142 were stratified independently by two authors (C.L. and P.C.W.) as either sedentary or active. In
143 general, sedentary was considered not regularly participating in any structured exercise training
144 or physical activity, while active was considered normally involved in recreational activities or
145 systematic training. Any disagreements were discussed between authors until a consensus was
146 reached.
147
148 **Fiber type classification**
149 Human skeletal muscle fiber types were measured in various ways, including
150 immunohistochemistry (IHC), myosin adenosine triphosphatase (mATPase), and sodium dodecyl
151 sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Human skeletal muscle contains three
152 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 CSAs, MyHC isoform distribution, and MyHC relative protein expression

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). MyHC relative protein expression (%) for each

isoform was determined by dividing the relative CSA of an MyHC isoform by the relative CSA of the entire tissue sample and multiplying by 100 (i.e., relative MyHC I expression = 100 × [relative CSA of MyHC I fibers / relative CSA of the tissue sample]).

Assessment of study quality

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.

Meta-analyses

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 I^2 statistic, which was considered to be low (< 25%), moderate (25-75%), or high (> 75%) (53). Meta-regressions were performed to determine if the measurements were moderated by age within older adults as well as 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.

Limitations of the literature search and analysis

The limitations to the literature search and analysis were: 1) publications were in English text only, potentially missing relevant studies, 2) most studies examined males, limiting our findings on females, 3) when not measured, relative protein expression was calculated from fiber CSA and fiber type distribution, and 4) meta-regressions to determine if measurements

221 moderated by age within older adults had to be performed using the mean age at the study level
222 instead of the age of each individual in the various study as this information was not available.

RESULTS

Search results and sample characteristics

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). 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. In the three studies that biopsied two different muscles, one representative muscle was selected (vastus lateralis (11,23); biceps brachii (25)) to avoid having study results be counted twice for most analyses, with the only exception being the analysis by age and muscle type where all data on muscles other than the vastus lateralis were grouped together. 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.

Measures examined by age

Relative protein expression in older adults was higher for MyHC I and lower for MyHC II and IIA isoforms compared to young adults (all $p < 0.0001$, Figure 2 and Table 2). 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 (Figure 2). 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 (Figure 3 and Table 3). Heterogeneity (I^2) was moderate to high, ranging from 58 to 92% across fiber CSA analyses for all adults (Figure 3). 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, $p = 0.40$; Figure 4 and Table 3). Heterogeneity (I^2) was low to moderate, ranging from 0 to 61% across fiber type distribution analyses for all adults (Figure 4). 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.

Measures examined by age and sex

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 (Figures 3–4). 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 (28).

Measures examined by age and measurement technique

Measurement techniques provided similar responses, in general, for relative protein expression and fiber type distribution with age but produced differences in fiber CSA (Figure 5 and Supplementary Table 1). 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 (Table 1). 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 (Table 1).

Measures examined by age and physical activity

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 (Figure 6 and Supplementary Table 2), similar to the patterns found with all studies grouped together (Figure 2). 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.

Measures examined by age and muscle type

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 (Figure 7 and Supplementary Table 3). 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.

Measures examined by age within older adults

The loss of muscle function with age increases in a non-linear fashion in older adults (55) and may be due, in part, to shifts in relative MyHC protein expression. Therefore, we performed a meta-regression to determine if average study age of older adults was a significant moderator of our outcome measures. CSA was significantly decreased with age in older adult MyHC IIA fibers ($p = 0.04$) and trended to be lower in MyHC II fibers ($p = 0.07$), while relative protein expression and fiber type distribution were not associated ($p = 0.40$-0.92, Supplementary Appendix A).

Sensitivity analyses and publication bias

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 B1-3). 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 C).

DISCUSSION

The primary result of this systematic review and meta-analysis shows that more of the slow-contracting, lower force-producing MyHC I isoform are present in older male muscles compared to young male muscles, which helps explain their age-related decrease in force, velocity, and power at the whole muscle level (13–16) and increased likelihood of physical disability (1–3). Additionally, our findings indicate that the relative MyHC protein expression shift to the slower isoform in older males is due to the age-related atrophy of fast-contracting MyHC II or IIA fibers and not due to changes in fiber type distribution. Females showed similar reductions in MyHC II and IIA skeletal muscle fiber size as males, but had no statistically significant reductions in relative MyHC protein expression despite smaller fast-contracting compared to slow-contracting fibers, potentially due to few studies (22%, N = 6 out of 27) including this sex making the number of subjects evaluated small compared to males (21%, n = 156 females; n = 596 males). Overall, these results suggest that exercise or pharmacological interventions that shift MyHC expression towards faster isoforms and/or increase fast-contracting fiber size (i.e., resistance training, 56) should decrease the prevalence of sarcopenia in both sexes. However, future work needs to focus more on females in order to determine if sex-specific effects are observed with age, which may require sex-specific interventions.

Individual studies examining relative MyHC protein expression in older adults produced a variety of results, finding either a shift in the expression to slower-contracting isoforms (19–30) or fast-contracting isoforms (7, 11, 31), or no change in the expression of MyHC isoforms (9, 32– 42). By performing a meta-analysis, we were able to combine these studies with smaller sample sizes into an aggregate analysis of over 750 participants that affords much greater statistical power (57). Relative MyHC protein expression was measured using three different

techniques, SDS-PAGE (N = 14 studies), mATPase (N = 7), and IHC (N = 6), which have the potential to produce different outcomes (44, 58). However, our findings indicate that at least in MyHC I and II fiber classifications the three techniques produced similar results especially in terms of mean differences, although the variation in mATPase was higher compared to SDS-PAGE and IHC (Figure 5). Relative protein expression of MyHC I isoforms increase by 10.9% and of MyHC II isoforms decrease by 10.0% in older males, based on our calculated mean difference values. This large shift in protein expression represents a significant decrease in the contractile ability of older male skeletal muscles as the force production and unloaded shortening velocity of MyHC II or IIA fibers is ~1.5- and ~3-fold greater than MyHC I fibers (9, 19, 35, 38) and the power output of MyHC II or IIA fibers is ~5- to 6-fold greater than MyHC I fibers (29, 34, 59). Thus, the age-related shift to MyHC I protein expression provides a potential mechanism to explain, at least in part, the decreased whole muscle force, velocity and power production in older males (60–62).

Our results further indicate that the shifting of MyHC protein expression to the more slow-contracting MyHC I isoform in older males is due to the age-related atrophy of fast-contracting MyHC II or IIA fibers and not due to changes in fiber type distribution. However, there are several important aspects of this data to consider. First, relative MyHC protein expression was performed in all the studies (N = 27), a few less measured fiber size (N = 22) and slightly less than half examined fiber type distribution (N = 13), so not all studies performed the same measurements which could lead to biases and fewer studies examined the important parameter of fiber type distribution. Second, the age-related response of fiber size was different based on the measurement performed (Figure 5). SDS-PAGE showed no decrease in fiber size in MyHC II or IIA fibers and IHC showed slightly smaller MyHC I fibers in older adults. This may be due to

the original focus of the studies using these various techniques. In general, the studies that use SDS-PAGE for fiber size and determination of fiber type distribution are performing these gels on single fibers that had their individual contractile performance measured. In these types of studies, researchers typically select the fibers most likely to successfully undergo calcium-activation or other specific measurements that are being performed, meaning that the larger, healthier looking fibers from young and older populations will commonly be selected. This can lead to a selection bias in that smaller, unhealthy-looking fibers are not used (63). For instance, in our previous aging work using SDS-PAGE, we observed larger MyHC I fibers in older males and smaller MyHC IIA fibers in older females compared to young (9); however, when using IHC on the same population we observed smaller fibers in both older males and females in MyHC II fibers (64), as also found in this meta-analysis. Additionally, the SDS-PAGE studies tend to test fewer fibers per subject than when using IHC or mATPase, which examine an entire portion of the biopsy, including the smaller, unhealthy-looking fibers. A recent analysis indicated that although the mean CSA remains consistent when sampling small to large numbers of fibers across multiple biopsy sites, approximately 150 fibers per fiber type are needed from a single person to reduce measurement variability (65), which is potentially achieved by a number of the IHC or mATPase studies, but not by SDS-PAGE (Table 1, see Fibers per subject). In summary, our meta-analysis results agree with reviews (63, 66, 67) that MyHC II fiber size decreases with age; however, more studies should include the measure of fiber type distribution.

A notable issue is that all IHC and half of the mATPase studies included in our analysis categorized MyHC as I or II. Older adults tend to express fibers with more mixed isoforms, such as MyHC I/IIA and IIAX (11, 49, 68), with MyHC co-expression occurring in over 50% of fibers from very old adults (69). Mass spectrometry experiments in young and older adults found

that “pure” fibers, those expressing >80% MYH7 (MyHC I or β) or MYH2 (MyHC IIA), accounted for >75% or MyHC I and IIA fibers, with a large population of MyHC I, but only few MyHC IIA fibers expressing ~100% of their respective isoform (70). These results suggest that MyHC isoform expression may be more complex, especially in MyHC IIA fibers, which may require techniques such as mass spectrometry for a complete understanding. Using IHC (44, 46) or other techniques such as mass spectrometry that measure these isoforms will be important in future work to better understand the age-related changes in fiber size and fiber type distribution.

Our results also show that MyHC II or IIA fiber size tends to decrease with age within older adults, which may help explain the increased loss of function with age in older adults (55) although other factors, such as altered neural control, may play a role as well (71). Relative protein expression and fiber type distribution were unaltered with age within older adults, potentially as these analyses had to be performed using the mean age at the study level instead of the age of each individual in the various study, which would be a better statistical approach. Additionally, a few studies were excluded from the meta-regression as the average age of older adults could not be determined. Nonetheless, the age-related loss in the size of fast-contracting fibers in older adults may exacerbate their decreased function.

Most studies biopsied the vastus lateralis (VL, N = 24), although the other muscles were sampled, specifically the biceps brachii (N = 3), gastrocnemius (N = 1), masseter (N = 1) and minor pectoralis (N = 1). Findings were mostly similar in VL and other muscles (Figure 7), with no changes in MyHC I size, but reductions in MyHC II and IIA with age and no changes in fiber type distribution. As expected with a decrease in fast-contracting size and no change in fiber type distribution, the VL muscle showed a shift towards slow MyHC expression; however, the other muscles did not. A similar observation was found in older females where their fast-contracting

fibers showed a decrease in size, but no change in relative MyHC protein expression (Figure 1). This could be due to fiber size being a more sensitive measure of age-related alterations in skeletal muscle or may be due to shifts in fiber type distribution that cause no change in relative MyHC protein expression. For example, a shift to faster contracting fibers in the fiber type distribution could counteract the greater atrophy of the faster contracting fibers. As only a few studies have examined fiber type distribution, this is an important parameter to include in future research. Future work should also examine other muscles in addition to the VL as age-related atrophy is highly variable, with the atrophy over 50 years being highest in the rectus femoris (-33%), next largest in the VL (-30%), and lowest in the soleus (-6%) muscle (50). The amount of muscle-specific atrophy in the lower limb muscles appears to be related to their percentage of MyHC II muscle fibers, with greater atrophy occurring in muscles with greater MyHC II expression (50). Thus, our finding that aging results in atrophy of the fast-contracting fibers, primarily from studies using the VL, may also occur in other lower limb muscles, but this needs to be examined in detail, especially in the upper limbs as the relationship between atrophy and fiber type was not present (50).

Physical activity level can alter relative MyHC protein expression (31, 49), so participants from the selected studies were stratified into either sedentary (SED, N = 15) or active (N = 8) to determine whether physical activity status regulates the effects of aging on the properties of MyHC I, II, and IIA fibers (Figure 6). When stratified for physical activity status, mean relative MyHC protein expression was similar between the two groups, with older adults showing an age-related shift to the slow MyHC I isoform. MyHC II and IIA fiber size decreased regardless of physical activity levels, although surprisingly, active MyHC I fibers also showed age-related atrophy while sedentary did not. The sedentary group was the only set of data that showed a

change in fiber type distribution, shifting to more MyHC I expression and less MyHC II expression, which should exacerbate the overexpression of MyHC I due to MyHC II atrophy. Thus, based on the results from this study, physical activity may not protect against the age-related atrophy of MyHC II fibers but may confer a protective mechanism against a shift in fiber type distribution to a slower overall isoform expression. Physical activity measurement can be performed in a variety of ways, with most of our selected studies using subjective measures (i.e., surveys or medical history), which have their strengths and weaknesses (72). As studies measuring cellular effects of skeletal muscle aging tend to include a smaller number of participants, all selected studies had ≤ 50 participants (Table 1), the addition of an objective measure (i.e., accelerometers or pedometers) could aid in more precise measures of physical activity levels (e.g., amount of moderate-to-vigorous activity or step counts; 72) and improve our understanding of the effects of varying doses of physical activity on aging skeletal muscle.

While the age-related slowing of MyHC isoform expression is an important aspect of skeletal muscle performance in older adults, single fiber contractile function, such as force production and contractile velocity, may be altered within isoforms with age. A detailed narrative review on single fiber contractile function indicates that isometric force production normalized to fiber size, termed specific tension, as well as unloaded shortening velocity and power output normalized to fiber size is unchanged in MyHC I and II fibers with age, with a few important caveats (63). In MyHC II fibers, a significant proportion of the studies showed older adults had changes in specific tension, with some increasing and some decreasing, and had increased normalized power compared to young, which may be due to biological or methodological differences (63). Although a systematic review and meta-analysis in this area might provide additional insights, performing single fiber experiments under conditions that more closely simulate *in vivo* skeletal

muscle would likely provide more useful information as this approach can drastically affect contractile properties. Two experimental conditions to potentially focus on, which were also highlighted by (63), as these can have large effects on single fiber contractile function are the inorganic phosphate (P_i) concentration and temperature. In healthy, non-fatigued muscle, P_i levels are around 5 mM (73, 74) and temperature is approximately 37°C (75, 76); however, most single fiber studies are performed at P_i values at or near 0 mM and at temperatures ≤ 15°C (63). Future single fiber contractile performance studies should benefit from examining the effects of age using conditions as close to *in vivo* as methodologically and economically feasible as this would produce the most physiologically relevant results. For instance, recent unpublished results from our laboratory show that increasing temperature from 25 to 37°C in fibers from older adults modestly increases specific tension (10 to 20%) and greatly increases myosin-actin cross-bridge kinetics (4- to 9-fold), which should significantly increase contractile velocity.

There are a few other considerations that are important to put our work into the appropriate context. Although we examined the effects of physical activity, there are a number of other mechanisms that may also be responsible for MyHC II or MyHC IIA age-related atrophy via altered muscle protein synthesis and breakdown, including modified signaling pathways, increased lipid deposition, impaired amino acid sensing, as well as greater inflammation (77). Loss of muscle fibers with age, potentially due to denervation with or without reinnervation and loss of motor units, may occur along with atrophy to reduce whole muscle size, although the current evidence is equivocal on the relative importance of loss or atrophy (77). Future work examining both fiber atrophy and loss would benefit our understanding of the effects of aging on skeletal muscle structure and function (77). Another important factor to consider is that most of the included studies performed skeletal muscle biopsies instead of sampling large portions or an

entire muscle. Human skeletal muscle biopsies of the vastus lateralis have been found to have consistent MyHC I and II fiber cross-sectional area and fiber type distribution at multiple sites along the muscle and between the muscles of both legs (65, 78). However, sample variability, primarily due to biology and not due to methodology, can be high which increases measurement heterogeneity (65, 78), potentially making age-related differences difficult to find although this is reduced when examining a large number of samples, such as in our meta-analysis.

Based on the results of this systematic review and meta-analysis, relative MyHC I expression is higher in older male adults compared to young male adults, due to the atrophy of MyHC II and IIA fibers with age. This age-related shift to the slow-contracting, lower force-producing MyHC I isoform, compared to the MyHC II isoform, may explain, at least in part, the reduced whole muscle contractile capacity and increased likelihood of physical disability in older males. As females showed similar reductions in MyHC II and IIA skeletal muscle fiber size as males, exercise or pharmacological interventions that shift MyHC expression towards faster isoforms and/or increase fast-contracting fiber size should decrease the prevalence of sarcopenia in both sexes. Our findings also indicate that future studies need to include or focus solely on females, measure MyHC IIA and IIX isoforms instead of simply combining the two under MyHC II, examine fiber type distribution, sample additional muscles to the vastus lateralis, and incorporate an objective measurement of physical activity.

DATA AVAILABILITY

Data will be made available upon reasonable request.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.L., P.C.W., A.E.P and M.S.M. conceived and designed research; C.L. and P.C.W. performed systematic review and meta-analysis; C.L., P.C.W., A.E.P and M.S.M. interpreted results of systematic review and meta-analysis, C.L. prepared figures, C.L., P.C.W., A.E.P and M.S.M. drafted manuscript, C.L., P.C.W., A.E.P and M.S.M. edited and revised manuscript, C.L., P.C.W., A.E.P and M.S.M. approved final version of manuscript.

SUPPLEMENTAL MATERIAL

Supplemental figures and tables are available on Figshare, https://doi.org/10.6084/m9.figshare.27103126.

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FIGURE LEGENDS

Figure 1. Flow diagram of the search process. N = number of studies; SD = standard deviation; SE = standard error.

Figure 2. Forest plots for relative protein expression (%) with age for myosin heavy chain (MyHC) I, II, IIA, and IIX isoforms. The squares (mean difference) are colored based upon each group (males are black, females are white, both sexes together are half black and half white, all studies together are gray) and include error bars (95% confidence intervals). Each group's total results are located at the bottom of the plot along with their number of studies (N), mean difference [95% confidence interval], and heterogeneity (I^2). Table 2 contains the relative MyHC protein expression mean difference [95% confidence interval] for the individual studies.

Figure 3. Forest plots for fiber cross-sectional area (μm^2) with age for myosin heavy chain (MyHC) I, II, and IIA isoforms. The circles (mean difference) are colored based upon each group (males are black, females are white, both sexes together are half black and half white, all studies together are gray) and include error bars (95% confidence intervals). Each group's total results are located at the bottom of the plot along with their number of studies (N), mean difference [95% confidence interval], and heterogeneity (I^2). Table 3 contains the fiber cross-sectional area mean difference [95% confidence interval] for the individual studies.

Figure 4. Forest plots for fiber type distribution (%) with age for myosin heavy chain (MyHC) I, II, and IIA isoforms. The triangles (mean difference) are colored based upon each group (males are black, females are white, all studies together are gray) and include error bars (95%

confidence intervals). Each group's total results are located at the bottom of the plot along with their number of studies (N), mean difference [95% confidence interval], and heterogeneity (I^2). Table 3 contains the fiber type distribution mean difference [95% confidence interval] for the individual studies.

Figure 5. Forest plots for relative myosin heavy chain (MyHC) protein expression (%), fiber cross-sectional area (CSA, μm^2), and fiber type distribution (%) with age analyzed with various techniques. The symbols (mean difference and 95% confidence intervals) are colored based upon each method. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS) is black, myosin adenosine triphosphatase (mATPase) is white, and immunohistochemistry (IHC) is gray. The squares represent relative protein expression, the circles represent fiber CSA, and the triangles represent fiber type distribution. Each group's total results are located at the bottom of the plot along with their number of studies (N), mean difference [95% confidence interval], and heterogeneity (I^2). No studies measured MyHC IIA fiber CSA using IHC or MyHC IIA fiber type distribution.

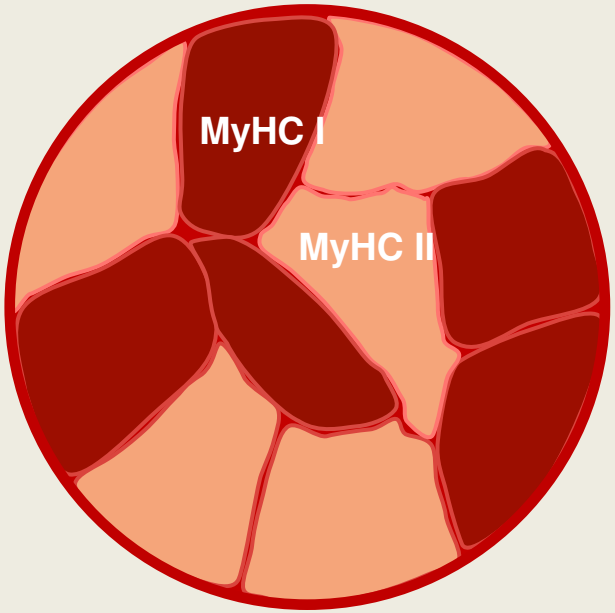
Figure 6. Forest plots for relative myosin heavy chain (MyHC) protein expression (%), fiber cross-sectional area (CSA, μm^2), and fiber type distribution (%) with age stratified by physical activity levels. The symbols (mean difference) are colored based upon each group (sedentary or SED are black, active is white) and include error bars (95% confidence intervals). The squares represent relative protein expression, the circles represent fiber CSA, and the triangles represent fiber type distribution. Each group's total results are located at the bottom of the plot along with their number of studies (N), mean difference [95% confidence interval], and heterogeneity (I^2).

Figure 7. Forest plots for relative myosin heavy chain (MyHC) protein expression (%), fiber cross-sectional area (CSA) (μm^2), and fiber type distribution (%) with age from different skeletal muscles. The symbols (mean difference) are colored based upon each muscle group (vastus lateralis or VL is black, other muscles or Other is white) and include error bars (95% confidence intervals). The squares represent relative protein expression, the circles represent fiber CSA, and the triangles represent fiber type distribution. Each group's total results are located at the bottom of the plot along with their number of studies (N), mean difference [95% confidence interval], and heterogeneity (I^2).

Age-related Isoform Shift Due to Differential Atrophy

Young Adult Skeletal Muscle

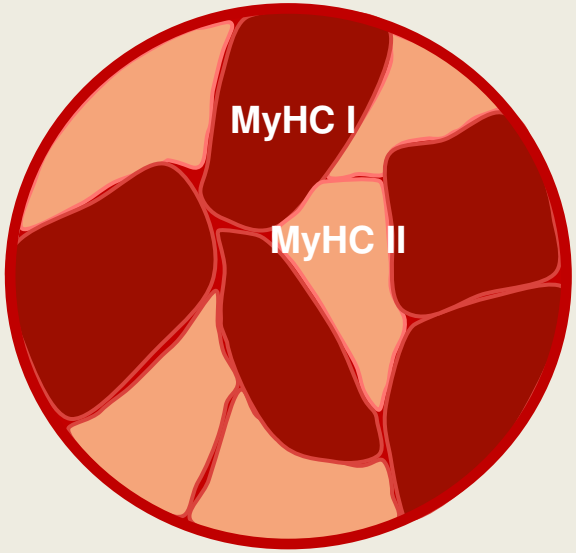
Males n = 303 Females n = 67



Aging

Older Adult Skeletal Muscle

Males n = 293 Females n = 89



	MyHC I	MyHC II
Relative Protein Expression	↑ Increased	↓ Decreased
Fiber Cross-sectional Area	↔ No change	↓ Decreased
Fiber Type Distribution	↔ No change	↔ No change

Conclusion: Relative MyHC I (slow-contracting) expression increases with age due to the atrophy of MyHC II (fast-contracting) fibers.

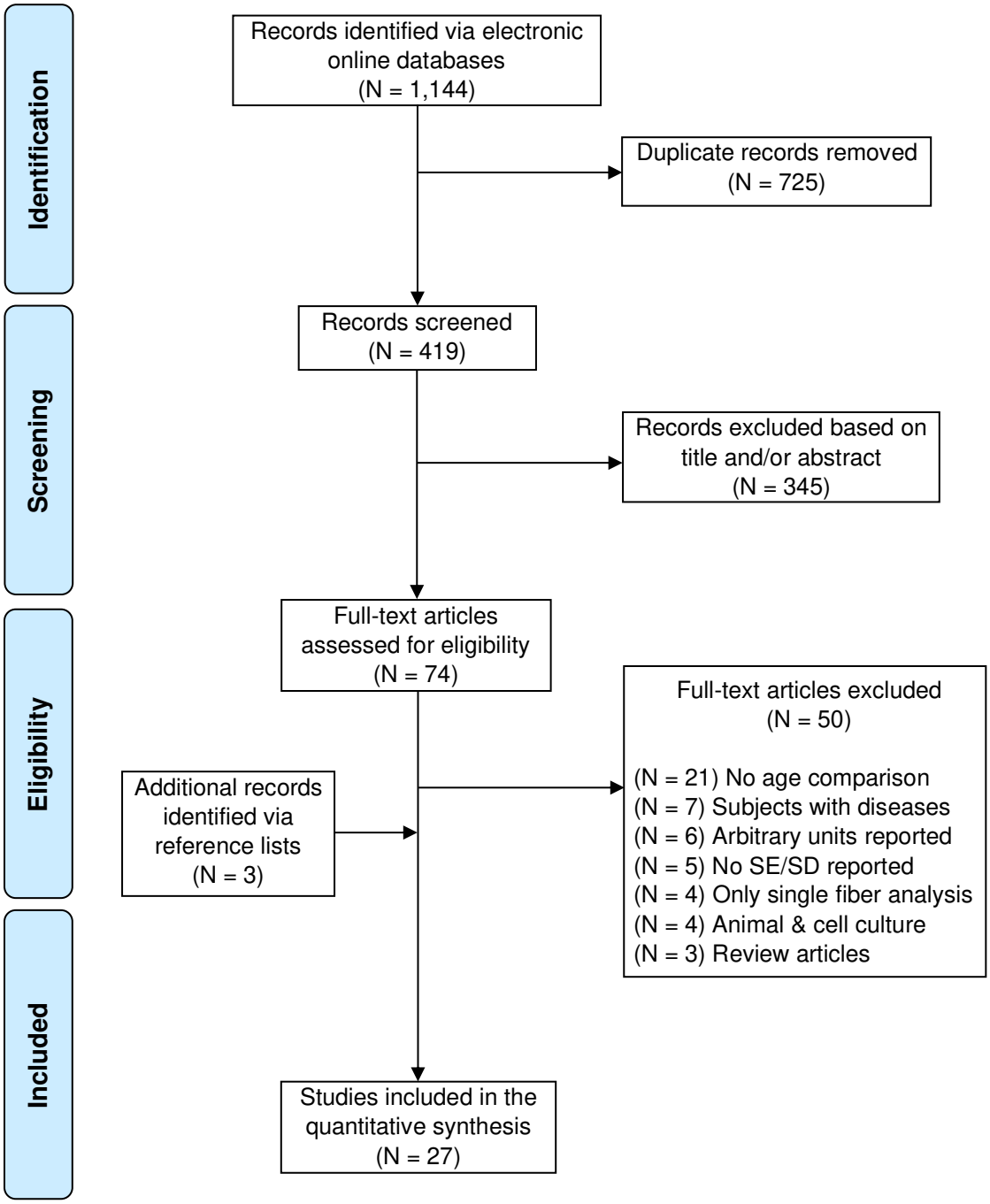


Figure 1.

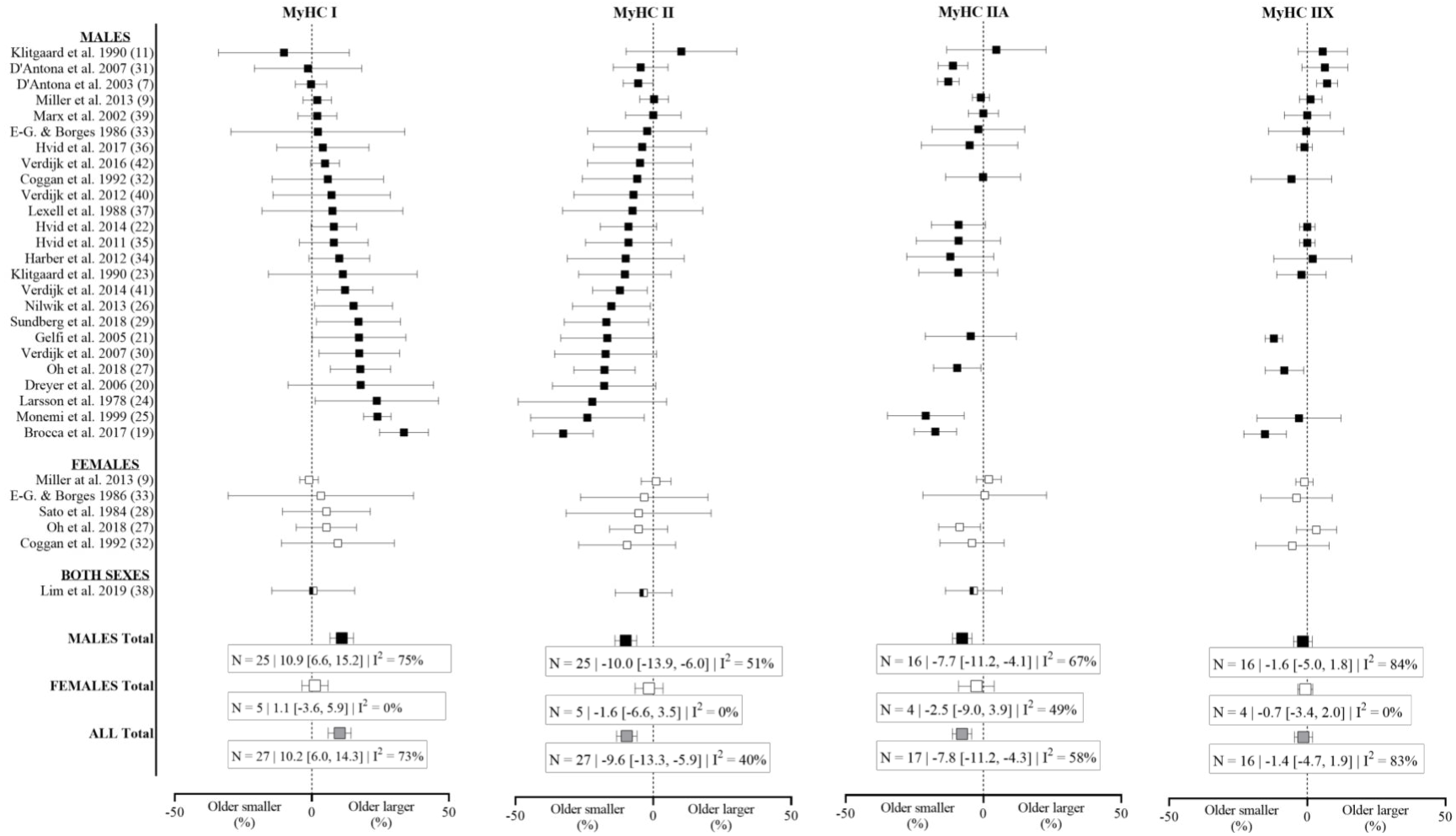


Figure 2.

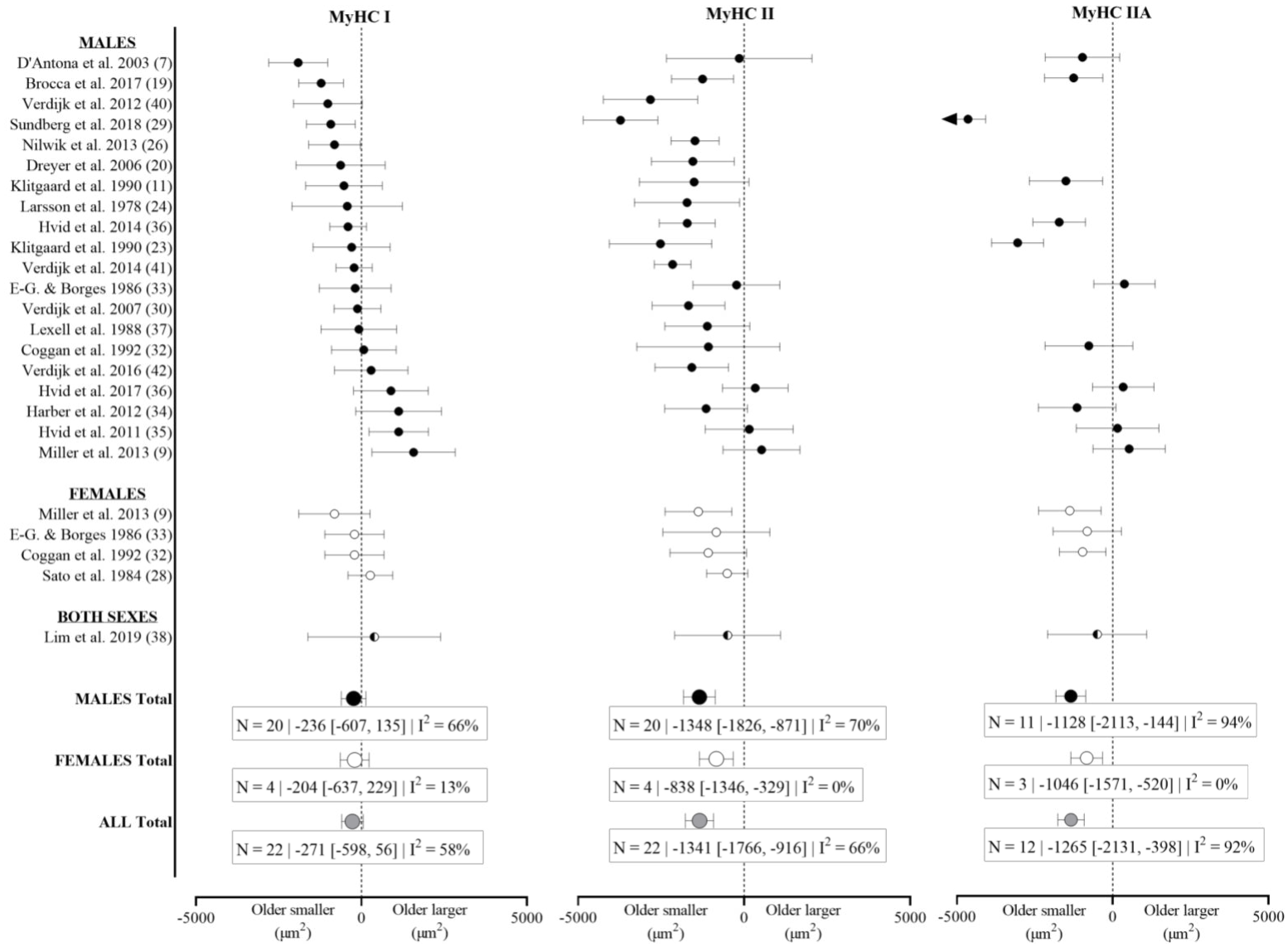


Figure 3.

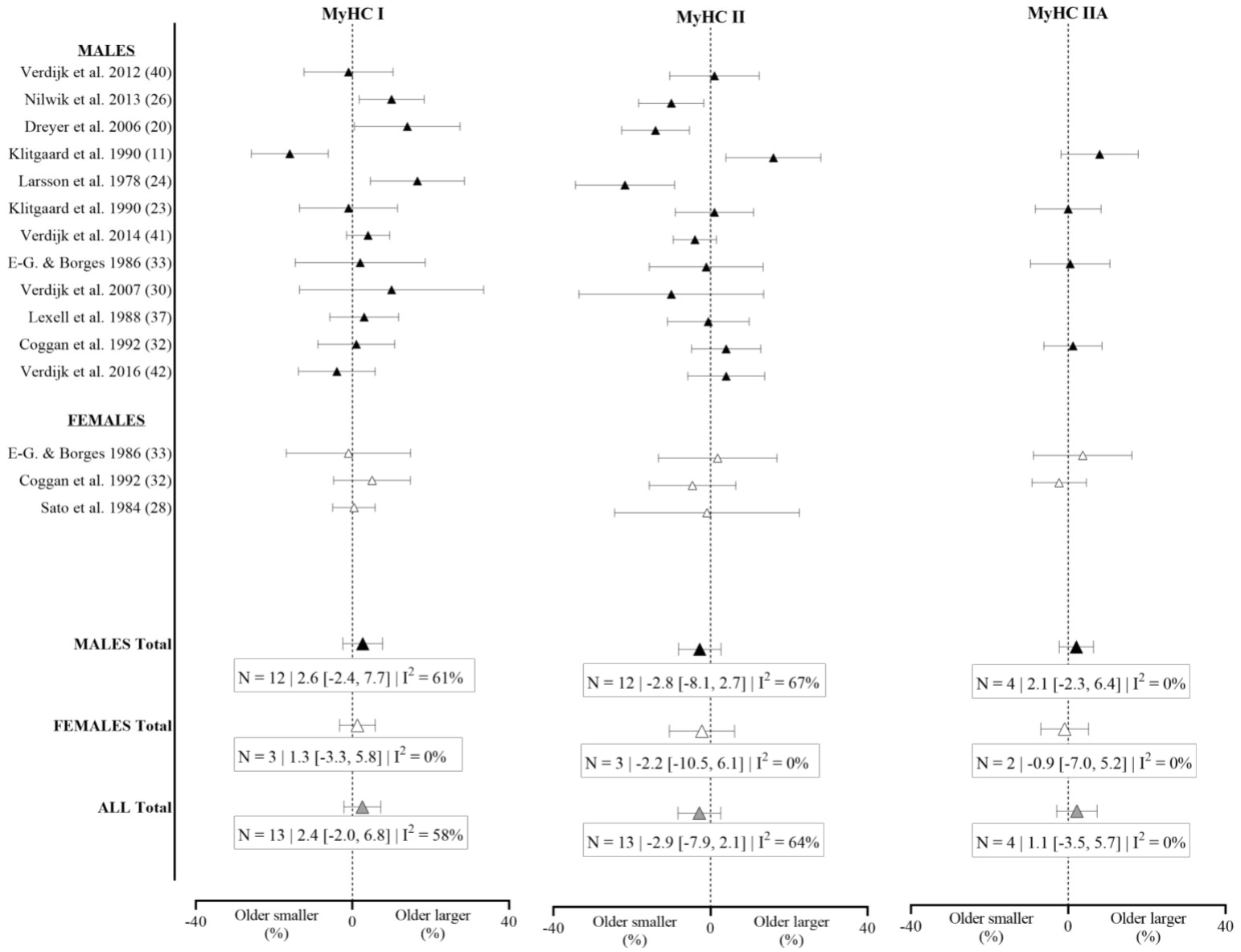


Figure 4.

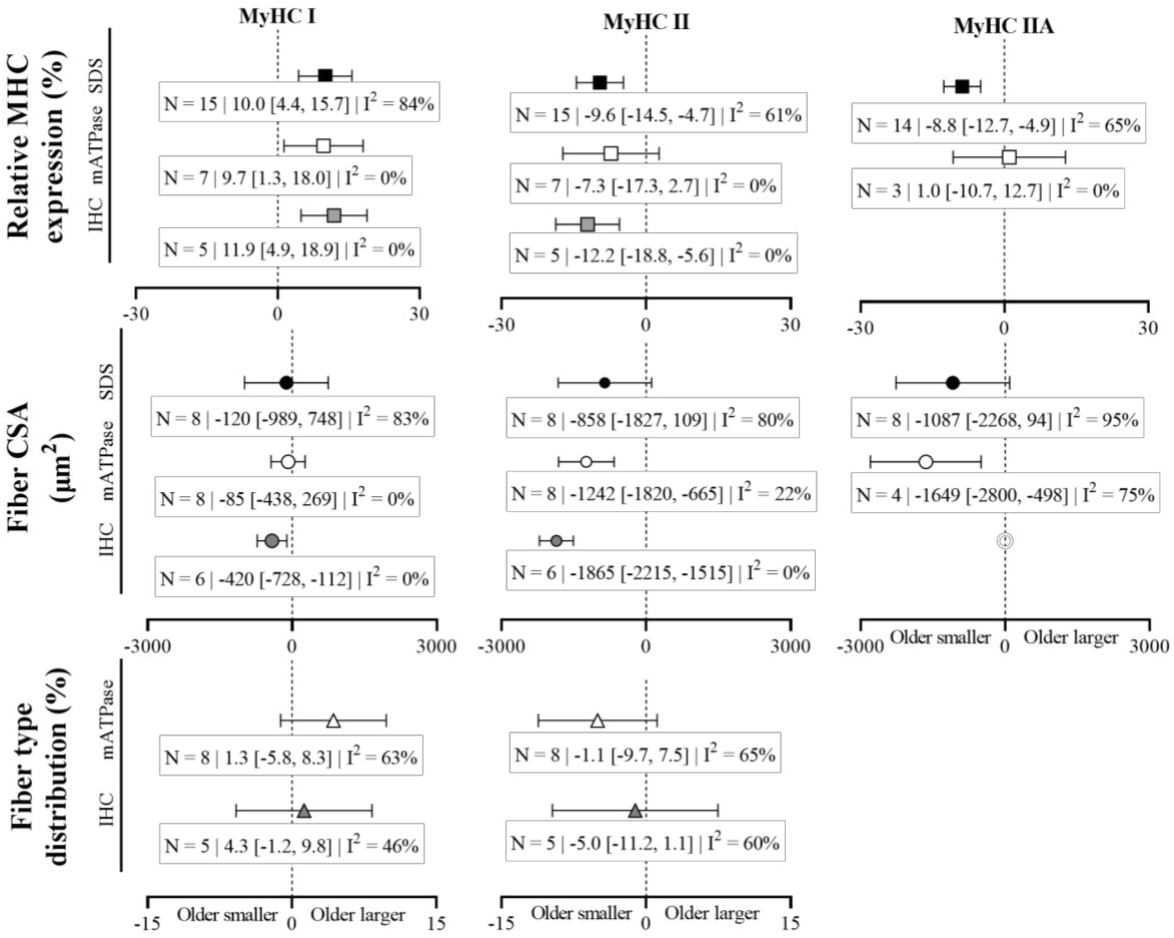


Figure 5.

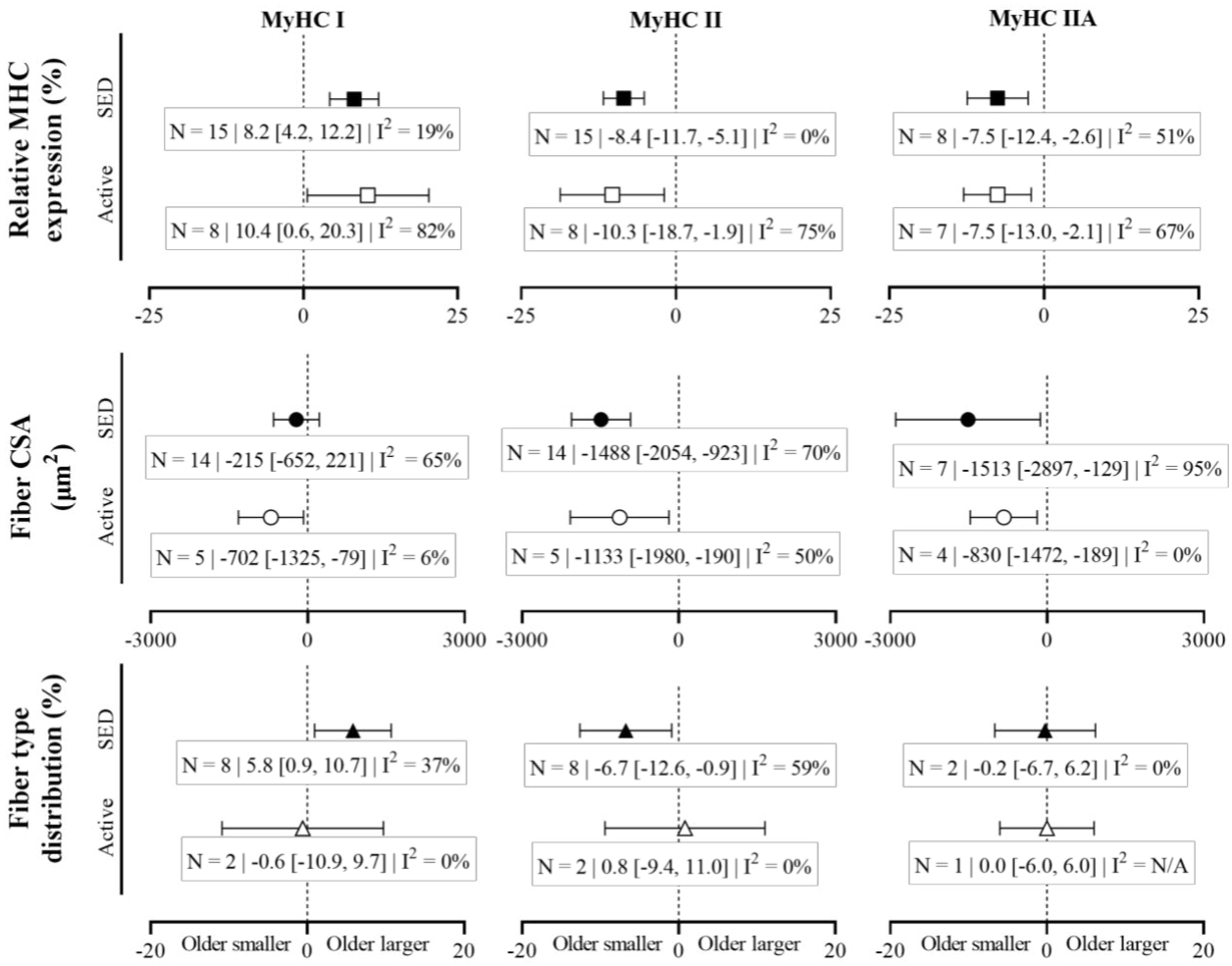


Figure 6.

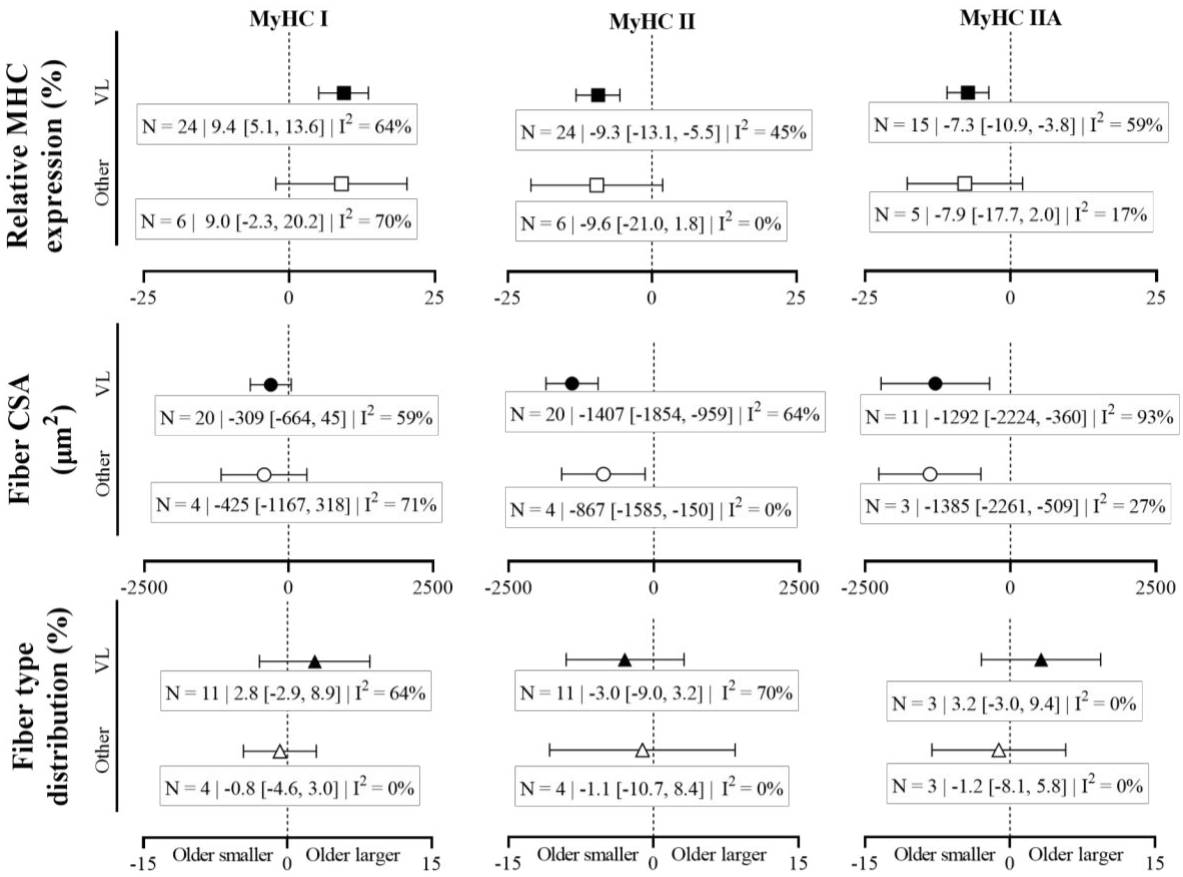


Figure 7.

Table 1. Characteristics of the included studies

Study	Year	Young Adults		Older Adults		Muscle	Fiber Cross-sectional Area				Relative MyHC Expression		Physical Activity		NOS Score
		M/F	Age	M/F	Age		Method	Isoforms	Fibers per subject	Fiber Type Distribution	Method	Isoforms	Level	Method	
Dreyer et al. (20)*	2006	10/0	23-35	9/0	60-75	VL	IHC	I, II	118	Yes	Calculated	I, II	Sedentary	History	7
Hvid et al. (22)*	2014	11/0	24	11/0	67	VL	IHC	I, II	50-200		SDS-PAGE	I, IIA, IIX	Sedentary	Survey	7
Nilwik et al. (26)	2013	25/0	23	26/0	70	VL	IHC	I, II	385	Yes	Calculated	I, II	Sedentary	Survey	7
Verdijk et al. (40)#	2012	8/0	31	8/0	75	VL	IHC	I, II	308	Yes	Calculated	I, II	Active	Survey	7
Verdijk et al. (41)	2014	49/0	18-49	50/0	70-86	VL	IHC	I, II	422	Yes	Calculated	I, II	Sedentary	Survey	5
Verdijk et al. (42)	2016	14/0	26	16/0	72	VL	IHC	I, II	175	Yes	Calculated	I, II	Sedentary	Survey	7
Coggan et al. (32)^	1992	10/10	26/23	10/10	64/63	Gastroc	mATPase	I, IIA, IIX	480	Yes	Calculated	I, IIA, IIX	Sedentary	History	8
E-G. & Borges (33)^	1986	10/9	20-30	12/10	60-70	VL	mATPase	I, IIA, IIX	300	Yes	Calculated	I, IIA, IIX	Active	Survey	7
Klitgaard et al. (11)#	1990	7/0	28	8/0	69	VL,(BB)	mATPase	I, IIA, IIX	250	Yes	SDS-PAGE	I, IIA, IIX	Sedentary	Survey	6
Klitgaard et al. (23)	1990	5/0	27	5/0	69	VL,(BB)	mATPase	I, IIA, IIX	272	Yes	Calculated	I, IIA, IIX	n/s	n/s	4
Larsson et al. (24)^	1978	23/0	31	10/0	62	VL	mATPase	I, II	301	Yes	Calculated	I, II	Sedentary	Survey	4
Lexell et al. (37)^	1988	18/0	28	17/0	77	VL	mATPase	I, II	10,045	Yes	Calculated	I, II	n/s	n/s	5
Sato et al. (28)^	1984	0/26	26-39	0/50	60-80	MP	mATPase	I, II	135	Yes	Calculated	I, II	n/s	n/s	3
Verdijk et al. (30)	2007	8/0	20	8/0	76	VL	mATPase	I, II	454	Yes	Calculated	I, II	Sedentary	History	7
Brocca et al. (19)	2017	10/0	23	10/0	71	VL	SDS-PAGE	I, IIA	10		SDS-PAGE	I, IIA, IIX	Active	Survey	7
D'Antona et al. (7)#	2003	7/0	30	7/0	73	VL	SDS-PAGE	I, IIA, IIX	10		SDS-PAGE	I, IIA, IIX	Sedentary	Survey	6
Harber et al. (34)*	2012	7/0	20	6/0	74	VL	SDS-PAGE	I, IIA	26		SDS-PAGE	I, IIA, IIX	Sedentary	History	7
Hvid et al. (35)*	2011	9/0	24	8/0	67	VL	SDS-PAGE	I, IIA	11		SDS-PAGE	I, IIA, IIX	Sedentary	Survey	7
Hvid et al. (36)*	2017	6/0	24	6/0	68	VL	SDS-PAGE	I, IIA	11		SDS-PAGE	I, IIA, IIX	Sedentary	Survey	6
Lim et al. (38)^	2019	6/4	26	5/2	79	VL	SDS-PAGE	I, IIA	16		SDS-PAGE	I, IIA	Active	Survey	8
Miller et al. (9)^	2013	5/7	26	5/7	69	VL	SDS-PAGE	I, IIA	12		SDS-PAGE	I, IIA, IIX	Active	Accel.	8
Sundberg et al.(29)	2018	6/0	23	6/0	82	VL	SDS-PAGE	I, IIA, IIAX	10		SDS-PAGE	I, II	Sedentary	Accel.	7
D'Antona et al. (31)#	2007	5/0	30	7/0	73	VL					SDS-PAGE	I, IIA, IIX	Active	Survey	6
Gelfi et al. (21)	2005	6/0	20-25	6/0	70-76	VL					SDS-PAGE	I, IIA, IIX	Active	Survey	6
Marx et al. (39)	2002	16/0	22	16/0	74	VL					SDS-PAGE	I, IIA, IIX	Sedentary	Survey	7
Monemi et al. (25)	1999	5/0	22	6/0	74	BB,(MA)					SDS-PAGE	I, IIA, IIX	n/s	n/s	4
Oh et al. (27)	2018	17/11	29	15/10	70	VL					SDS-PAGE	I, IIA, IIX	Active	Survey	8

Age in years is reported as the mean, if available, or the age range of participants. *Baseline data only; #Control data only; ^Males and females pooled within study; (), muscle only included in the secondary analysis examining age and muscle type, NOS (Newcastle-Ottawa Scale) score: good ≥ 7, fair 4 – 6, poor ≤ 3; Accel, accelerometry; BB, biceps brachii; Calculated, determined from fiber CSA and type distribution as explained in Methods section, CSA, cross-sectional area; F, females; Gastroc, gastrocnemius; History, medical history; IHC, immunohistochemistry; M, males; mATPase, myosin adenosine triphosphatase; MyHC, myosin heavy chain; n/s, not specified; MP, minor pectoralis; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SED, sedentary; Survey, physical activity questionnaires; VL, vastus lateralis.

Table 2. Relative MyHC protein expression of the included studies

Study	Year	Sex (Y/O)	Muscle	Relative MyHC Protein Expression (%)			
				MyHC I	MyHC II	MyHC IIA	MyHC IIX
				MD [95% CI]	MD [95% CI]	MD [95% CI]	MD [95% CI]
Klitgaard et al. (11)	1990	M (5/5)	VL	-10.2 [-34.1, 13.7]	10.2 [-9.9, 30.3]	4.7 [-13.3, 22.7]	5.6 [-3.3, 14.5]
D'Antona et al. (31)	2007	M (5/7)	VL	-1.4 [-21.0, 18.2]	-4.6 [-14.5, 5.3]	-11.0 [-16.4, -5.6]	6.4 [-1.9, 14.7]
D'Antona et al. (7)	2003	M (7/7)	VL	-0.3 [-6.0, 5.4]	-5.5 [-10.9, -0.1]	-12.7 [-16.6, -8.8]	7.2 [3.4, 11.0]
Miller et al. (9)	2013	M (5/5)	VL	0.2 [-3.2, 7.2]	0.3 [-4.9, 5.5]	-0.9 [-4.0, 2.2]	1.2 [-2.9, 5.3]
Marx et al. (39)	2002	M (16/16)	VL	2.0 [-5.1, 9.1]	0.0 [-10.0, 10.0]	0.0 [-5.5, 5.5]	0.0 [-8.3, 8.3]
E-G. & Borges (33)	1986	M (10/12)	VL	2.5 [-29.5, 33.9]	-2.2 [-23.8, 19.4]	-1.8 [-18.6, 15.0]	-0.4 [-14.0, 13.2]
Hvid et al. (36)	2017	M (6/6)	VL	4.0 [-12.8, 20.8]	-4.0 [-21.7, 13.7]	-5.0 [-22.5, 12.5]	-1.0 [-3.8, 1.8]
Verdijk et al. (42)	2016	M (14/16)	VL	4.8 [-15.7, 25.3]	-4.8 [-23.9, 14.3]		
Coggan et al. (32)	1992	M (10/10)	Gastroc	5.8 [-14.5, 26.1]	-5.8 [-25.8, 14.2]	-0.1 [-13.7, 13.5]	-5.7 [-20.3, 8.9]
Verdijk et al. (40)	2012	M (8/8)	VL	7.2 [-14.2, 28.6]	-7.2 [-28.8, 14.4]		
Lexell et al. (37)	1988	M (18/17)	VL	7.5 [-18.2, 33.2]	-7.5 [-32.9, 17.9]		
Hvid et al. (22)	2014	M (11/11)	VL	8.0 [-0.3, 16.3]	-9.0 [-19.2, 1.2]	-9.0 [-18.8, 0.8]	0.0 [-2.8, 2.8]
Hvid et al. (35)	2011	M (9/8)	VL	8.0 [-4.5, 20.5]	-9.0 [-24.6, 6.6]	-9.0 [-24.3, 6.3]	0.0 [-2.8, 2.8]
Harber et al. (34)	2012	M (7/6)	VL	10.0 [-1.1, 21.1]	-10.0 [-31.2, 11.2]	-12.0 [-27.8, 3.8]	2.0 [-12.1, 16.1]
Klitgaard et al. (23)	1990	M (7/8)	VL	11.3 [-15.8, 38.4]	-10.3 [-27.1, 6.5]	-9.1 [-23.4, 5.2]	-2.1 [-11.0, 6.8]
Verdijk et al. (41)	2014	M (50/49)	VL	12.1 [1.9, 22.3]	-12.1 [-22.0, -2.2]		
Nilwik et al. (26)	2013	M (25/26)	VL	15.2 [1.0, 29.4]	-15.2 [-29.3, -1.1]		
Sundberg et al. (29)	2018	M (6/6)	VL	17.0 [1.7, 32.3]	-17.0 [-32.3, -1.7]		
Gelfi et al. (21)	2005	M (6/6)	VL	17.2 [0.1, 34.3]	-16.7 [-33.5, 0.1]	-4.6 [-21.1, 11.9]	-12.1 [-15.2, -9.0]
Verdijk et al. (30)	2007	M (8/8)	VL	17.3 [2.6, 32.0]	-17.3 [-35.8, 1.2]		
Oh et al. (27)	2018	M (17/15)	VL	17.7 [6.7, 28.7]	-17.7 [-28.8, -6.6]	-9.5 [-18.1, -0.9]	-8.3 [-15.2, -1.3]
Dreyer et al. (20)	2006	M (10/9)	VL	17.8 [-8.7, 44.4]	-17.8 [-36.6, 0.9]		
Larsson et al. (24)	1978	M (23/10)	VL	23.7 [1.2, 46.2]	-22.1 [-49.0, 4.8]		
Monemi et al. (25)	1999	M (5/6)	BB	23.9 [18.9, 28.9]	-23.9 [-44.5, -3.3]	-20.9 [-34.8, -7.0]	-3.0 [-18.2, 12.2]
Brocca et al. (19)	2017	M (10/10)	VL	33.6 [24.7, 42.5]	-32.7 [-43.6, -21.8]	-17.4 [-25.1, -9.7]	-15.3 [-23.0, -7.6]
Miller et al. (9)	2013	F (7/7)	VL	-1.0 [-4.4, 2.4]	1.0 [-4.4, 6.4]	2.0 [-2.5, 6.5]	-1.0 [-4.1, 2.1]
E-G. & Borges (33)	1986	F (9/10)	VL	3.3 [-30.5, 37.1]	-3.3 [-26.4, 19.8]	0.5 [-21.9, 22.9]	-3.9 [-16.8, 9.0]
Sato et al. (28)	1984	F (26/50)	MP	5.3 [-10.7, 21.3]	-5.3 [-31.6, 21.0]		
Oh et al. (27)	2018	F (11/10)	VL	5.3 [-5.7, 16.3]	-5.3 [-15.8, 5.2]	-8.6 [-16.2, -1.0]	3.3 [-3.9, 10.6]
Coggan et al. (32)	1992	F (10/10)	Gastroc	9.5 [-11.1, 30.1]	-9.5 [-27.1, 8.1]	-4.1 [-15.7, 7.5]	-5.4 [-18.7, 7.9]
Lim et al. (38)	2019	M & F (7/10)	VL	0.5 [-14.6, 15.6]	-3.5 [-13.8, 6.8]	-3.5 [-13.8, 6.8]	

BB, biceps brachii; CI, confidence interval; F, females; Gastroc, gastrocnemius; M, males; MD, mean difference; MyHC, myosin heavy chain; MP, minor pectoralis; O, older adults; VL, vastus lateralis; Y, young adults.

Table 3. Fiber cross-sectional area and fiber type distribution of the included studies

Study	Year	Sex (Y/O)	Muscle	Fiber Cross-sectional Area (μm²)			Fiber Type Distribution (%)		
				MyHC I	MyHC II	MyHC IIA	MyHC I	MyHC II	MyHC IIA
				MD [95% CI]	MD [95% CI]	MD [95% CI]	MD [95% CI]	MD [95% CI]	MD [95% CI]
D'Antona et al. (7)	2003	M (7/7)	VL	-1911 [-2802, -1019]	-150 [-2340, 2040]	-971 [-2165, 223]			
Brocca et al. (19)	2017	M (10/10)	VL	-1218 [-1894, -542]	-1253 [-2188, -318]	-1253 [-2188, -318]			
Verdijk et al. (40)	2012	M (8/8)	VL	-1017 [-2057, 23]	-2821 [-4240, -1402]		-1.0 [-12.4, 10.4]	1.0 [-10.4, 12.4]	
Sundberg et al. (29)	2018	M (6/6)	VL	-925 [-1662, -188]	-3722 [-4844, -2600]	-4646 [-5210, -4082]			
Nilwik et al. (26)	2013	M (25/26)	VL	-812 [-1593, -31]	-1479 [-2200, -758]		10.0 [1.7, 18.3]	-10.0 [-18.3, -1.7]	
Dreyer et al. (20)	2006	M (10/9)	VL	-627 [-1974, 720]	-1543 [-2789, -297]		14.0 [0.5, 27.5]	-14.0 [-22.6, -5.4]	
Klitgaard et al. (11)	1990	M (5/5)	VL	-527 [-1683, 629]	-1506 [-3155, 143]	-1503 [-2682, -324]	-16.0 [-25.8, -6.2]	16.0 [3.9, 28.1]	8.0 [-1.8, 17.8]
Larsson et al. (24)	1978	M (23/10)	VL	-429 [-2095, 1237]	-1721 [-3304, -138]				
Hvid et al. (22)	2014	M (11/11)	VL	-404 [-958, 150]	-1716 [-2558, -874]		16.6 [4.6, 28.6]	-21.8 [-34.4, -9.2]	
Klitgaard et al. (23)	1990	M (7/8)	VL	-296 [-1460, 868]	-2518 [-4061, -975]	-3055 [-3887, -2223]	-1.0 [-13.5, 11.5]	1.0 [-9.0, 11.0]	0.0 [-8.3, 8.3]
Verdijk et al. (41)	2014	M (50/49)	VL	-219 [-765, 327]	-2153 [-2703, -1603]		4.0 [-1.5, 9.5]	-4.0 [-9.5, 1.5]	
E-G. & Borges (33)	1986	M (10/12)	VL	-187 [-1273, 899]	-230 [-1538, 1078]	373 [-610, 1356]	2.0 [-14.6, 18.6]	-1.1 [-15.6, 13.4]	0.5 [-9.6, 10.6]
Verdijk et al. (30)	2007	M (8/8)	VL	-118 [-826, 590]	-1675 [-2769, -581]		10.0 [-13.5, 33.5]	-10.0 [-33.5, 13.5]	
Lexell et al. (37)	1988	M (18/17)	VL	-78 [-1220, 1064]	-1109 [-2388, 170]		3.0 [-5.8, 11.8]	4.0 [-4.8, 12.8]	
Coggan et al. (32)	1992	M (10/10)	Gastroc	74 [-905, 1053]	-1078 [-3231, 1075]	-767 [-2178, 644]	1.0 [-8.8, 10.8]	-0.6 [-11.0, 9.8]	1.2 [-6.2, 8.6]
Verdijk et al. (42)	2016	M (14/16)	VL	296 [-814, 1406]	-1577 [-2684, -470]		-4.0 [-13.8, 5.8]	4.0 [-5.8, 13.8]	
Hvid et al. (36)	2017	M (6/6)	VL	893 [-236, 2022]	335 [-651, 1321]	335 [-651, 1321]			
Harber et al. (34)	2012	M (7/6)	VL	1125 [-170, 2420]	-1145 [-2390, 100]	-1145 [-2390, 100]			
Hvid et al. (35)	2011	M (9/8)	VL	1129 [229, 2029]	152 [-1170, 1473]	152 [-1170, 1473]			
Miller et al. (9)	2013	M (5/5)	VL	1578 [320, 2836]	524 [-634, 1682]	524 [-634, 1682]			
Miller et al. (9)	2013	F (7/7)	VL	-816 [-1898, 266]	-1377 [-2381, -374]	-1377 [-2381, -374]			
E-G. & Borges (33)	1986	F (9/10)	VL	-212 [-1105, 681]	-838 [-2448, 772]	-818 [-1916, 280]	-1.0 [-16.9, 14.9]	1.8 [-13.3, 16.9]	3.7 [-8.8, 16.2]
Coggan et al. (32)	1992	F (10/10)	Gastroc	-396 [-1068, 276]	-1080 [-2235, 75]	-968 [-1713, -223]	5.0 [-4.8, 14.8]	-4.6 [-15.6, 6.4]	-2.3 [-9.2, 4.6]
Sato et al. (28)	1984	F (26/50)	MP	270 [-406, 946]	-508 [-1128, 112]		0.4 [-5.0, 5.8]	-0.9 [-24.4, 22.6]	
Lim et al. (38)	2019	11/6	VL	390 [-1612, 2392]	-500 [-2091, 1091]	-500 [-2091, 1091]			

CI, confidence interval; F, females; Gastroc, gastrocnemius; M, males; MD, mean difference; MyHC, myosin heavy chain; MP, minor pectoralis; O, older adults; VL, vastus lateralis; Y, young adults.