

4 Muscle collagen accumulation is not a universal feature 5 of human aging: a systematic review

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17 ABSTRACT

18 The extracellular matrix is critical to skeletal muscle structure and function, with collagen its
19 largest component. Fibrosis, excessive collagen accumulation, disrupts muscle function. While animal
20 studies consistently report age-related intramuscular collagen accumulation, human findings are
21 inconsistent. This systematic review evaluated primary, peer-reviewed studies to assess if collagen
22 accumulation is a universal feature of human aging.

23 The review was registered on PROSPERO (CRD42024569964). Following PRISMA guidelines, five
24 databases (MEDLINE [Ovid], Web of Science, SCOPUS, CINAHL, SPORTDiscus) were searched in January
25 2026 for studies comparing intramuscular collagen/extracellular matrix content in healthy young and
26 older (> 60 years) adults. Eligible studies used histological or hydroxyproline techniques to quantify
27 collagen/extracellular matrix content. Study screening, review, data extraction, and risk of bias were
28 performed independently by two reviewers. Results were synthesized narratively.

29 Nine studies (including 122 young and 119 older adults) were included. Four reported no age-
30 related differences, four showed age-related intramuscular collagen accumulation, and one found
31 equivocal results when distinguishing perimysial from endomysial collagen. Considerable heterogeneity
32 was observed in collagen quantification methods and control of mediators including hypertension,
33 diabetes, aerobic fitness and physical activity. Studies with rigorous control generally found no age-
34 related differences, whereas those with limited control generally reported age-related collagen
35 accumulation.

36 Collagen accumulation is not an inevitable feature of human chronological aging. Observed
37 differences may instead reflect comorbidities or lifestyle factors associated with aging; thus, through
38 these mediators, muscle collagen accumulation may be elevated in older populations. Future studies
39 should control mediators and investigate mechanisms regulating collagen in skeletal muscle.
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42 **Keywords:** aging; collagen; extracellular matrix; fibrosis; skeletal muscle
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45 INTRODUCTION

46 Fibrosis is characterized by a pathological, often irreversible expansion or disorganization of the
47 extracellular matrix (ECM) that disrupts tissue function (1) and can ultimately lead to organ failure. It
48 affects many organs including, but not limited to, the lungs, liver, heart, kidneys and skeletal muscle. The
49 ECM forms the connective tissue compartment between a tissue's primary cell types. In skeletal muscle,
50 the ECM consists of three layers; the epimysium that surrounds the entire muscle, the perimysium which
51 encloses fascicles and the endomysium which fills the gaps between individual myofibres. The ECM is
52 made primarily of the fibrillar collagens type 1 (COL1) and 3 (COL3), which contribute to the tissue's
53 mechanical stability (2). Both are trimeric proteins; COL1 is a heterotrimer, composed of two different
54 polypeptide chains, whereas COL3 is a homotrimer of three identical chains (2). In bovine skeletal
55 muscle, COL1 predominates in the perimysium and epimysium with COL3 also present (3). The
56 endomysium contains a similar ratio of COL1 to COL3 as well as a small amount of collagen type 4
57 (COL4), a non-fibrillar collagen localized to the basal lamina (3). In addition to collagen, glycoproteins,
58 proteoglycans and elastin are all found in the ECM (4). The skeletal muscle ECM plays a role in lateral
59 force transmission (5) and is the site of many cell-matrix signalling pathways that influence muscle
60 adaptation and remodeling (6). Alterations in collagen content or organization; therefore, have
61 significant consequences for muscle health and function. For example, when mechanical constraints
62 mimicking collagen accumulation are introduced to myofibers, myofiber radial expansion and overall
63 shortening capacity are reduced (7). In rodents, increased total collagen content has been linked to
64 greater myofiber bundle stiffness (8) and when paired with changes to structural complexes like the
65 costamere, impairs lateral force transmission (9). Computational modeling of human muscle suggests
66 that intramuscular collagen accumulation may similarly reduce lateral force transmission (10),
67 diminishing overall muscle performance. Numerous ECM-resident cell types are implicated in collagen
68 accumulation and altered homeostasis including satellite cells (muscle stem cells that are essential for
69 muscle growth and repair) (11), fibroadipogenic progenitor (FAP) cells and fibroblasts (cells that produce
70 and maintain the ECM) (12) and immune cell populations such as macrophages (13). Further, collagen
71 accumulation may disrupt the activity and function of infiltrating cell types in response to injury,
72 mechanical loading or infection, thereby exacerbating the fibrotic phenotype.

73 Intramuscular collagen accumulation is primarily measured by histology, or hydroxyproline
74 assays. Both picosirius red and Masson's trichrome stains are acceptable methods for visualizing fibrillar
75 collagen content in skeletal muscle; however, picosirius red has been demonstrated to have better
76 reliability and reproducibility (14). Immunofluorescent staining allows for visualization of specific
77 collagen isoforms (i.e., COL1 and COL3) or the ECM as a whole rather than specific to collagen [i.e.,
78 wheat germ agglutinin (WGA)]. In humans, measures of intramuscular collagen content are derived from
79 very small muscle pieces, samples from vastus lateralis muscle biopsies typically range from 50-200mg or
80 approximately 100-300 myofibres. Analysis of such small samples; therefore, can lead to under- or
81 overinterpretation of findings.

82 In some cases of fibrosis, the underlying cause is clear, such as following acute tissue injury or in
83 genetic conditions like Duchenne muscular dystrophy (DMD). DMD results from the genetic mutation of
84 dystrophin, a key protein of the costamere complex. This leads to extreme muscle damage and fibrosis
85 and renders most individuals non-ambulatory in childhood (15). In other cases, the etiology is less well-
86 defined. Aging skeletal muscle has been described as "fibrotic" or exhibiting a "fibrotic morphology" (4,
87 16), and fibrosis is sometimes considered an intrinsic feature of aging (17-20). The degree of fibrosis in
88 aging skeletal muscle; however, is markedly different from other human models of muscle fibrosis. For
89 instance, in DMD, total muscle collagen in humans can exceed 30% of the total area analyzed (21),
90 whereas reports using the same method of analysis are closer to 20% in patients with chronic kidney
91 disease (22) and 7% in older adults (23). Moreover, several primary human studies have reported no

92 significant age-related differences in intramuscular collagen content (24–26). Our recent work also failed
93 to detect any such difference between younger and older adults free from systemic disease (27), raising
94 further questions about the universality of this phenotype.

95 These conflicting reports highlight the need for a more rigorous and systematic evaluation of the
96 human evidence. While animal models have provided valuable mechanistic insights, their findings do not
97 always translate directly to humans, particularly in the context of aging, where lifestyle, comorbidities,
98 aerobic fitness and physical activity levels vary widely. The objective of the present study was to
99 systematically review the primary, peer-reviewed human literature to evaluate whether intramuscular
100 collagen accumulation is a consistent feature of chronological aging in the absence of systemic illness.
101 Specifically, we aimed to determine the proportion of studies reporting age-related differences in
102 collagen content in skeletal muscle and to identify methodological or contextual factors that may explain
103 discrepancies across studies.

104 **MATERIALS AND METHODS**

105 **Search Strategy**

106 This systematic review was performed in accordance with the Preferred Reporting Items for
107 Systematic Reviews and Meta-Analyses (PRISMA) standard and checklist (28) and registered on
108 PROSPERO (an International Database of Prospectively Registered Systematic Reviews;
109 CRD42024569964). The full electronic search strategy can be found in the Table 1. A systematic literature
110 search was conducted on articles published until January 28, 2026, using MEDLINE (Ovid), Web of
111 Science, SCOPUS, CINAHL and SPORTDiscus databases. Manual searches of reference lists were
112 conducted to search for manuscripts not identified by the online databases. All references were
113 imported into Covidence.org and duplicates were automatically or manually removed.

114 **Study Selection Criteria**

115 Titles and abstracts were screened, and full texts were reviewed on Covidence.org. Articles were
116 included in the systematic review if all the following criteria were met: 1) peer-reviewed, primary
117 research publications; 2) available in English; 3) participants or human cadavers free from known
118 systemic disease (to reduce mediators of intramuscular collagen accumulation); 4) comparison made
119 between any resting young and older skeletal muscle; 5) minimum age > 60 years for the older group; 6)
120 intramuscular collagen or total ECM content measured in muscle biopsies by either histological or
121 hydroxyproline assay techniques (as these are validated techniques that are used consistently in the
122 literature to report on fibrotic skeletal muscle); 7) data was reported in such a way that means and
123 standard deviations (SD) could be calculated for differences in intramuscular collagen (so that statistical
124 significance could be assessed and comparisons across studies could be made). No publication date
125 restrictions were imposed. The primary outcome of this investigation was total intramuscular
126 collagen/ECM accumulation. Other outcomes of intramuscular collagen accumulation assessed included
127 COL1 and COL3 content, pepsin-insoluble, endomysial and perimysial collagen content. Three reviewers
128 independently assessed the eligibility of titles and abstracts identified through the literature search.
129 Titles and abstracts assessed as ineligible were excluded, while those considered potentially eligible for
130 inclusion in the systematic review were retained and retrieved in full text. The full-text articles were then
131 screened for relevance by two independent reviewers using the predefined eligibility criteria. Any
132 discrepancies between reviewers were resolved through consensus.

133 **Data Extraction**

137 Data was extracted on Covidence.org (Veritas Health Innovation, Melbourne, AUS), using a
138 custom data extraction sheet. Two independent researchers extracted data from the studies deemed to
139 be included in the systematic review, in duplicate. Any disagreements were resolved by consensus. The
140 following data were extracted from each included study: *a)* study title, authors and year of publication;
141 *b)* funding sources *c)* study characteristics including aim and study design; *d)* inclusion/exclusion criteria;
142 *e)* participant characteristics including sample size, age (years), sex, body mass index (BMI) and general
143 descriptors (i.e., healthy, free from systemic illness, training status/physical activity); *f)* skeletal muscle
144 biopsied; *g)* method used to measure total intramuscular collagen/ECM content [i.e., picrosirius red stain
145 (% area), hydroxyproline content ($\mu\text{g}/\text{mg}$)]; *h)* intramuscular collagen/ECM content value; *i)* conclusions.
146 When graphical data in the manuscript was unable to be received upon requests from study authors,
147 values were extracted from a web graph digitizer (plotdigitizer.com) (23, 26, 29, 30). Figures were
148 uploaded to plotdigitizer.com and axes and datapoints were manually identified. Sample means or values
149 for individual data points were then estimated by the software. Standard deviations were calculated
150 from standard error and sample sizes where applicable. Pavan et al. (30) and Critchlow et al. (31),
151 analyzed their data by linear regression. For presentation of the data from these studies in the current
152 systematic review, the individual data points were categorized to create a young (< 60 years) and an
153 older (> 60 years) age group. An unpaired t-test was run on the grouped data (Prism 10 for macOS,
154 version 10.5.0) and significant differences were found, $p = 0.004$ (30) and $p = 0.018$ (31).
155

156 Assessment of Study Quality

157 The Revised Risk of Bias Assessment Tool for Nonrandomized Studies of Interventions (RoBANS
158 2) was used to assess the quality of the methodology used by the included studies (32). This tool
159 assesses study quality according to eight factors: 1) comparability of target group, 2) target group
160 selection, 3) confounders, 4) measurement of intervention/exposure, 5) blinding of assessors, 6)
161 outcome assessment, 7) incomplete outcome data, 8) selective outcome reporting. Factor 3) was
162 assessed based on the method reported to control for variables that have been demonstrated to affect
163 intramuscular collagen accumulation in humans (obesity, diabetes and hypertension) (12, 29, 33) or
164 significantly impact skeletal muscle physiology (aerobic fitness/resistance training/physical activity).
165 Factor 4) was removed from analysis, as the studies included in the current systematic review did not
166 have a specific intervention or exposure associated with the outcome of interest. Factor 7) was assessed
167 by matching sample sizes reported in the methods to the data presented in the graphs of the outcomes
168 of interest (intramuscular collagen content). Where sample sizes could not be assessed from graphical
169 representations (i.e., individual data points were not presented), a risk of bias score of “unclear” was
170 given. A narrative synthesis was completed for analysis of risk of bias across studies due to the small
171 number of studies included in the systematic review and the variation in methodology used. Quality
172 assessment of each paper was completed by two independent authors with no conflicts of interest in the
173 study being assessed. Conflicts were resolved through consensus.
174

175 RESULTS

176 Search Results and Sample Characteristics

177 Fig. 1 shows the flow diagram of the search and study collection process (PRISMA). The primary
178 database literature search yielded 84130 results. Manual search of reference lists retrieved an additional
179 31 citations. 45191 references were identified as duplicates, leaving 38939 titles and abstracts to be
180 screened. A total of nine cross-sectional studies were identified for inclusion in this systematic review.
181 The list of detailed study characteristics can be found in Table 2. Some data reported for Mikkelsen et al.
182 (26), was extracted from citations that assessed the same study participants (34, 35). Two studies
183 collected individual datapoints and did not group their data by age (30, 31); therefore, for presentation

184 in Table 2, means and standard deviations were calculated from the younger (< 60 years) and older (≥ 60
185 years) individual datapoints. In total, 122 young adults (females, $n = 41$; males, $n = 81$) and 119 older
186 adults were included (females, $n = 31$; males, $n = 88$). Males were included in 89% and females were
187 included in 44% of the studies. The average group size assessed by studies that did and did not favour
188 age-related collagen accumulation was 25 and 31 participants respectively. All studies ($n = 9$) examined
189 collagen content in the vastus lateralis (VL) muscle. One study also examined collagen content in the
190 rectus femoris muscle of a subset of volunteers (23). Collagen content was measured by picrosirius red
191 staining ($n = 5$), Masson's trichrome stain ($n = 1$), immunohistochemistry (IHC) ($n = 2$) and/or
192 hydroxyproline assay ($n = 2$). Some studies employed multiple measures of intramuscular collagen
193 content, and one study used WGA to quantify ECM area (31). Due to the heterogeneity of population
194 characteristics and methods of analysis used to assess intramuscular collagen content outcomes, a
195 qualitative synthesis, rather than a meta-analysis, was completed.

196

197 Intramuscular Collagen Outcomes Examined by Age

198 Aging is the primary mediator of collagen accumulation that was investigated in the present
199 review; the following results are from comparison between age-groups. Results of intramuscular
200 collagen/ECM outcomes are presented in Table 3. Of the studies that examined age-related differences
201 in total intramuscular collagen/ECM content ($n = 8$), 50% ($n = 4$) demonstrated no age-related differences
202 in total intramuscular collagen content (24–27) and 50% ($n = 4$) favoured increases in older adults (23,
203 30, 31, 36). In total, the number of young and older adults in the studies that showed no age-related
204 differences in total collagen content were 58 and 66 respectively. The total number of young and older
205 adults in the studies that favoured age-related total collagen accumulation were 52 and 46 respectively.
206 Gueugneau et al. (29), assessed perimysial and endomysial collagen separately and found no age-related
207 differences in endomysial collagen, but an increase in perimysial collagen in older adults. Fede et al. (23),
208 investigated age-related differences in COL1 and COL3 isoforms through IHC and found that older males
209 had more COL1 content than young males but no age-related differences in COL3 content. Mikkelsen et
210 al. (26), also found no age-related differences in COL3 content as assessed through IHC. Examining
211 intramuscular collagen accumulation during aging, without accounting for mediators, suggests findings
212 are inconclusive; however, when mediator control is addressed (below), findings suggest that
213 intramuscular collagen accumulation is not an intrinsic feature of aging.

214

215 Outcomes Examined by Age and Measurement Technique

216 Methods of intramuscular collagen content measurement are not all equivalent (14); therefore,
217 the following results compare findings from studies that used different techniques. Both studies that
218 examined total collagen content by hydroxyproline assay demonstrated no significant age-related
219 differences (24, 25). Babraj et al. (24), demonstrated a trend ($p = 0.08$) for older adults having greater
220 pepsin-insoluble collagen content. Of the studies that measured collagen content by picrosirius red, 40%
221 ($n = 2$) demonstrated an increase in total collagen associated with age (23, 30) and 40% ($n = 2$)
222 demonstrated no age-related differences in total collagen (26, 27). Findings from Gueugneau et al. (29),
223 where perimysial and endomysial collagen content were assessed independently by picrosirius red
224 demonstrated equivocal findings – an age-related increase in perimysial collagen content, but no
225 differences in endomysial collagen content. The single study that examined collagen content by Masson's
226 trichrome (36) and the single study that examined ECM area by WGA (31) demonstrated age-related
227 associations with accumulation. The one study that investigated COL1 content by IHC showed age-
228 related increases (23). Both studies that investigated COL3 content by IHC demonstrated no age-related
229 differences (23, 26). Picrosirius red staining was used preferentially and provides a relatively

230 standardized approach for estimating collagen content. The infrequent use of the other techniques in
231 human skeletal muscle limits conclusions regarding their applicability.

232

233 Outcomes Examined by Age and Population Descriptors

234 The literature has reported that intramuscular collagen accumulation in humans may be
235 influenced by diabetes, obesity, inflammation, hypertension, osteoarthritis, physical activity and aerobic
236 fitness (12, 27, 29, 33, 37, 38); therefore, the following results compare findings between studies that
237 did and did not control for these mediators (Table 4). Population descriptors varied greatly between
238 studies. Fede et al. (23), and Pavan et al. (30), examined collagen content in the muscles of volunteers
239 undergoing surgery for traumatic hip or femur fracture, and both showed greater total collagen content
240 in older muscle. Fede et al. (23), also reported age-related increases in COL1 but not COL3 content. The
241 activity level of the participants in Fede et al. (23), was not reported. The participants in Pavan et al. (30),
242 were classified as moderately active without further description. Nederveen et al. (36), recruited young
243 and older participants who, by self-report, had not participated in any structured exercise program for at
244 least four months before the study. The authors found that older adults had significantly more total
245 intramuscular collagen content than the young adults. Gueugneau et al. (29) and Critchlow et al. (31) did
246 not report any physical activity/aerobic fitness/resistance training inclusion or exclusion criteria;
247 however, Gueugneau et al. (29) reported their participant's aerobic fitness and Critchlow et al. (31)
248 reported their participant's time spent per day in moderate-to-vigorous physical activity as well as
249 estimated leg press 1RM. Gueugneau (29) found that elderly volunteers had significantly more perimysial
250 but not endomysial collagen content than the young volunteers. Critchlow et al. (31) found that ECM
251 area increased during aging.

252 Activity level was not reported in Babraj et al. (24) and the authors reported no age-related
253 differences in muscle collagen content. Mikkelsen et al. (26), compared young and older, trained and
254 untrained individuals. Trained young and older participants were matched by running distance, and
255 untrained volunteers had gone at least five years without participating in regular physical activity (34).
256 No age-related differences in total intramuscular collagen or COL3 content were demonstrated between
257 any group (26). Schweitzer et al. (27) matched the fitness level of their young and older participants
258 through age- and sex-stratified normative percentiles based on VO_{2peak} scores. The authors reported no
259 age-related differences in total intramuscular collagen content. Haus et al. (25) recruited lifelong
260 sedentary young and older adults. They interviewed their subjects to assess their lifelong history of
261 physical activity and excluded any volunteer who had ever completed a formal exercise program outside
262 of their activities of daily living (25). The authors reported no significant age-related differences in total
263 intramuscular collagen content.

264 Diabetes was controlled for in 67% of the studies included in this review (23, 25–27, 29, 36) and
265 hypertension was controlled for in 44% of the studies (25–27, 29). None of the studies that reported age-
266 related differences in collagen content reported controlling for hypertension (23, 30, 31, 36). Aging is
267 likely associated with increased intramuscular collagen content due to the higher prevalence of diabetes,
268 hypertension, obesity and osteoarthritis. When these mediators are controlled, intramuscular collagen
269 accumulation is not consistently demonstrated in older adults, suggesting these comorbidities rather
270 than aging *per se* may be responsible.

271

272 Risk of Bias

273 The results from the RoBANS 2 are presented in Table 5. All studies that reported age-related
274 increases in total intramuscular collagen content had a high risk of confounding bias (23, 30, 31, 36);
275 they did not control for at least two known mediators of intramuscular collagen accumulation or did not
276 match the physical activity or aerobic fitness of their young and older participants (Table 4). Two of the

277 three studies that found age-related increases in total collagen content had high detection bias (assessor
278 blinding) as it was not reported whether the analysis was done in a blinded fashion (23, 30). In general,
279 the studies that did not report age-related differences in intramuscular collagen content had low bias in
280 the following areas: target group comparability, confounding, assessor blinding, outcome assessment
281 and selective outcome reporting.

282

283 **DISCUSSION**

284 This systematic review synthesized findings from nine studies comparing intramuscular collagen
285 content between young and older adults. Overall, the evidence indicates that collagen accumulation
286 with aging is not consistent or universal. Four studies reported no age-related differences in total
287 intramuscular collagen content (24–27), four observed greater total collagen content in older adults (23,
288 20, 31, 36), and one reported higher perimysial, but not endomysial collagen content in older muscle
289 (29). These findings challenge the prevailing narrative, often reported in literature reviews (4, 16–20)
290 that collagen accumulation and a fibrotic morphology are intrinsic features of aging skeletal muscle. The
291 discrepancy between prior reviews and the present findings underscores several sources of
292 inconsistency and points to three central issues: 1) insufficient control or acknowledgement of age-
293 related mediators in human research 2) failure to distinguish between preclinical and human data in
294 review articles, and 3) inconsistent terminology and failure to distinguish between different collagen
295 outcomes.

296 Two of the four studies that observed greater total collagen content in older adults recruited
297 participants undergoing surgery for traumatic hip or femur fracture, and the cause of fracture was not
298 reported (23, 30). In older adults, hip fractures are commonly the result of a fall, whereas in young
299 individuals, they are more likely the result of high-energy trauma (i.e., motor vehicle accidents) (39). The
300 potential presence of frailty in the older groups was not reported, nor were key health variables such as
301 obesity and diabetes (30), osteoarthritis or hypertension (23, 30) – which have been associated with
302 increased muscle collagen across all age groups (12, 29, 33, 37, 38).

303 Nederveen et al. (36), did not control for obesity, but controlled for diabetes and major
304 orthopedic disability and observed age-related increases in intramuscular collagen. The mean difference
305 in collagen area between age groups was 1.6% (36), compared to 3–4% in the other three studies (23, 30,
306 31). The smaller difference reported by Nederveen et al. (36) suggests that controlling for diabetes
307 and/or major orthopaedic disability, which did not occur in the other three studies (23, 30, 31), may
308 have contributed to these differences. Another explanation, however, is the variation in staining
309 techniques used (Masson’s trichrome compared to picrosirius red and WGA). Masson’s trichrome has
310 been demonstrated to be less sensitive to collagen fibres than picrosirius red (14, 40) and WGA stains
311 the entire ECM rather than collagen specifically.

312 Three of the four studies that reported greater total intramuscular collagen content in older
313 adults omitted a report of objective levels of physical fitness or activity. Fede et al. (23) did not report the
314 activity levels of their participants, and Pavan et al. (30) described their participants as “moderately
315 active” with no additional descriptors. Nederveen et al. (36) reported that their participants did not
316 participate in structured training for at least four months prior to participating in the study but did not
317 provide any objective measures. Critchlow et al. (31) reported time spent per day in moderate-to-
318 vigorous level physical activity in their participants by accelerometer data over a minimum four-day
319 period. The reported averages suggest the participants were meeting or exceeding the World Health
320 Organization’s physical activity guidelines of 150 minutes/week of moderate activity (41); however, the
321 large standard deviations suggest a very heterogenous dataset (31). In comparison, three of the four
322 studies that observed no differences in total collagen reported objectively measuring physical fitness or
323 activity (25–27, 34). Schweitzer et al. (27), reported a mean VO_{2peak} of 24.2 mL/min/kg for the older

324 adults, placing them in the 70th (males) or 90th (females) percentile for their age of 69 years or older (42).
325 The mean VO_{2max} for the older adults in Mikkelsen et al. (26), was 30.1 mL/min/kg for the untrained
326 males and 48.3 mL/min/kg for the trained males (34). This puts these groups into the 80th and 90th
327 percentiles, respectively, for their age (42). The high reported aerobic fitness in these participants
328 highlight their health status, as higher cardiorespiratory fitness is associated with reduced risk of all-
329 cause mortality, cardiovascular and coronary heart disease (43). In contrast, Haus et al. (25) did not
330 recruit fit older adults and instead recruited lifelong sedentary young and older adults with no history of
331 exercise training or physical activity and saw no differences in muscle hydroxyproline content.
332 Participants in this study were excluded if they had diabetes, hypertension or arthritis and on average
333 the older adults had 32% body fat (*n* = 10 males and 12 females) and the young adults had 25% body fat
334 (*n* = 10 males and 10 females) (25). Despite the volunteers being sedentary and having body fat
335 percentages that might classify them as overweight or obese (depending on their sex), the rigorous
336 exclusion criteria ensured that a population free of systemic disease was recruited. Therefore, even
337 though physical activity levels and body fat percentages can give an indication of health status, and likely
338 mediate the processes involved in intramuscular collagen accumulation (such as increased risk of
339 diabetes or hypertension), being free of systemic illness appears to be more important for preserving
340 muscle collagen homeostasis.

341 Findings from Gueugneau et al. (29) support that chronic illness mediates intramuscular collagen
342 accumulation by demonstrating that elderly volunteers with hypertension and elderly volunteers with
343 metabolic syndrome have greater endomysial collagen content than normotensive elderly volunteers. In
344 contrast, however, all elderly participant groups (normotensive, hypertensive and metabolic syndrome)
345 demonstrated similar perimysial collagen content which was elevated compared to young adults. These
346 differences could be interpreted as suggesting that the endomysium and perimysium are regulated
347 differently during aging and disease, however, they may also be related to the author's method of
348 analysis. There is ongoing debate whether endomysial and perimysial collagen can be reliably
349 distinguished in skeletal muscle. Lieber and Binder-Markey (44) argue that these connective tissue layers
350 are defined more by anatomical location than by distinct composition or function, making it unlikely that
351 endomysium can be measured independently of perimysium in histological samples. Under the
352 microscope, regions of dense collagen surrounding fascicles may appear perimysial, but could equally
353 represent aggregated endomysium. Thus, findings such as those of Gueugneau et al. (29) may be better
354 interpreted as showing expanded or thickened ECM, rather than specifically increased perimysial
355 collagen. Such changes could reflect altered regulation of the connective tissue layers but may also result
356 from scar formation or older adults having smaller muscle fibers (45) and; therefore, more fascicles in
357 each biopsy sample. Given the absence of clear physiological distinctions and reliance on visual criteria,
358 separate analysis of the endomysium and perimysium remains problematic.

359 Inflammation has been implicated in fibrotic processes in numerous organs (46) and in
360 pathological skeletal muscle remodeling, such as DMD (21). However, its role in age-related muscle
361 collagen accumulation remains unclear. Three studies included in this review measured inflammatory
362 markers in their participants (26, 27, 35), yet none reported a link between inflammation and
363 intramuscular collagen content. Schweitzer et al. (27) reported no significant age-related differences in
364 systemic C-reactive protein content (CRP). The older participants in Mikkelsen et al. (26), had greater
365 systemic CRP and interleukin-6 (IL-6) (30), and Babraj et al. (24), reported that older adults had increased
366 NFκB protein content and TNF-α and IL-6 mRNA expression in their muscles (as was reported in their
367 discussion as unpublished data). Despite inflammation being higher in two of the three studies, none of
368 these authors reported age-related differences in intramuscular collagen content. These findings suggest
369 that inflammation alone likely does not drive collagen accumulation in the muscle. This is supported
370 further by findings in individuals with chronic kidney disease. Abramowitz et al. (22) found that
371 individuals with chronic kidney disease had greater total collagen content in their muscles compared to

372 controls, but that muscle gene expression of TNF- α , CCL5, CD68 and CCL2 were lower. Furthermore, in
373 both controls and participants with chronic kidney disease, intramuscular collagen content was inversely
374 related to muscle TNF- α gene expression.

375 Hypertension was also identified as a possible mediator of intramuscular collagen accumulation
376 by this systematic review. None of the studies that reported age-related increases in intramuscular
377 collagen content reported controlling for hypertension in their participants (23, 30, 31, 36), whereas
378 three of four studies that showed no differences did (25–27). One mechanism linking hypertension to
379 intramuscular collagen accumulation is the renin-angiotensin-system (RAS). Altered regulation of this
380 system has well-known implication in the pathogenesis of hypertension. Angiotensin II, a primary active
381 product of the RAS, induces transforming growth factor- β (TGF- β) and connective tissue growth factor
382 (CTGF) expression from skeletal muscle cells (47). Both TGF- β and CTGF have potent profibrotic effects.
383 Evidence for RAS dysregulation is present in the muscles of patients with muscular dystrophies, a
384 pathology hallmarked by muscle fibrosis (48). Therefore, altered RAS activity, due to hypertension, may
385 lead to alterations in ECM homeostasis and intramuscular collagen accumulation.

386 Cadaveric human studies assessing intramuscular collagen content yield mixed results and are
387 especially susceptible to mediators of intramuscular collagen content. Inokuchi et al. (49) observed
388 increases in rectus abdominis total collagen content from age 20-50, but this is followed by a plateau or
389 decline, depending on sex and parity and statistical analyses were not provided. Calvi et al. (40), found
390 no age-related differences in rectus abdominis collagen using trichrome staining, but observed greater
391 collagen in the younger group when staining with picrosirius red. The average age of the younger group
392 assessed by Calvi et al. (40), was 23.3 years and the older group had an average age of 46.2 years;
393 however, this underscores the methodological sensitivity of collagen quantification. McKelvie et al. (50)
394 reported increased endomysial fibrous tissue in extraocular muscles with age but included only
395 qualitative and no statistical analyses.

396 Numerous literature reviews cite that muscle fibrosis is a hallmark of aging; however, a critical
397 omission is whether the muscle sample originated from a human or preclinical specimen (4, 16, 17).
398 When human studies are referenced, findings that contradict the aging-collagen narrative are often
399 omitted (i.e., publication bias). For example, Wohlgemuth et al. (20), cite rodent hydroxyproline data to
400 support age-related intramuscular collagen accumulation but do not mention that human studies using
401 the same method found no such effect (24, 25). Preclinical research is indispensable for advancing
402 scientific knowledge, but findings from these models often do not translate directly to humans. This is
403 largely due to the differences in rodent and human physiology and the extreme variability in human
404 lifestyles compared to preclinical lab models. Results from preclinical experiments should be
405 extrapolated to humans with caution, and the species from which the muscle sample came should be
406 clearly acknowledged. For example, most laboratory rodents live highly sedentary lives without access to
407 voluntary exercise, whereas even sedentary humans typically engage in varied levels of incidental
408 activity. Endurance and resistance exercise activate collagen turnover in human muscle; it stimulates
409 both collagen synthesis (51–53) and increases markers of collagen breakdown (27, 54). Thus, the stark
410 contrast in physical activity levels may partially explain why rodent models are more likely to show
411 collagen accumulation with age. A study by Kim et al. (55), exemplifies this point; no differences in total
412 intramuscular collagen content were observed between young, sedentary rats allowed to eat ad libitum
413 and old, calorie-restricted rats that had access to a voluntary running wheel. Furthermore, the old,
414 calorie-restricted rats with access to a voluntary running wheel had less collagen in their muscles than
415 the old, calorie-restricted rats that did not have access to exercise (55). The old rats that were allowed to
416 eat ad libitum and were not given access to a running wheel had significantly more collagen in their
417 muscles than all groups (55). These findings highlight the mediating effect of physical activity on
418 intramuscular collagen content and identify the role of diet as well.

419 Ambiguous terminology may further contribute to discrepancies between this review and earlier
420 reports. For instance, the term “fibrotic morphology” encompasses a wide range of ECM alterations,
421 including changes in total collagen content, cross-link abundance and solubility, isoform expression,
422 organization, and density. Given the inconsistency in reported findings of these variables, grouping them
423 under a single term can be misleading. Nederveen et al. (36) reported age-related increases in total
424 intramuscular collagen, but comparison of basal lamina thickness demonstrated age-related increases
425 around type II myofibers only. Fede et al. (23), reported increases in total collagen and COL1 protein in
426 aged human skeletal muscle, but not COL3 protein. Haus et al. (25) reported no differences in total
427 collagen or pyridinoline collagen cross-linking but did show increases in the advanced glycation end-
428 product, pentosidine, a non-enzymatic cross-link. Babraj et al. (24) found no differences in total collagen,
429 but an increase in pepsin-insoluble collagen in the elderly muscle, suggesting greater collagen cross-
430 linking. Equivocal findings in collagen outcomes have also been demonstrated in preclinical studies. The
431 mouse study ran by Fede et al. (23) showed that aged mice had greater total collagen and COL1, but not
432 COL3 content (measured by IHC) than younger mice. Abbott et al. (56) showed an increase in total
433 collagen in the gastrocnemii of old mice compared to young (measured by hydroxyproline), but not in
434 the tibialis anterior (measured by picosirius red) and Rahman et al. (57) reported age-related increases
435 in COL1 but not COL4 protein in mice by immunofluorescent staining. Failure to acknowledge these
436 differences in collagen outcomes when describing skeletal muscle’s fibrotic morphology omits important
437 information that may alter general conclusions and understanding of the mechanisms of collagen
438 accumulation.

439 A prospective cohort study with yearly sampling would greatly contribute to understanding the
440 relationship between aging and intramuscular collagen accumulation; however, is not likely a feasible
441 option. Therefore, a large-scale cross-sectional human study of approximately 50-100 participants per
442 age group that examines skeletal muscle ECM morphology would clarify the extent to which collagen
443 accumulation is an intrinsic feature of human aging. This sample size should identify differences with an
444 effect size of 0.5 or greater. To accurately isolate the effects of age, this study must rigorously control for
445 age-related comorbidities like hypertension, diabetes, major orthopaedic disability, cardiorespiratory
446 fitness and resistance training status, which we hypothesize from the present systematic review’s
447 findings, are mediating factors of intramuscular collagen accumulation. Further investigation into the
448 mechanisms underlying collagen accumulation in the presence of comorbidities would also be valuable
449 for understanding its implications for muscle health. Collagen accumulation is likely shaped by nuanced,
450 cell-specific contributions. For example, satellite cells, fibroblasts and various immune cell populations
451 differentially express collagen isoforms as well as proteins involved in ECM degradation (58), suggesting
452 multiple potential cellular drivers of ECM remodeling. Assessing the expression and activity of these cell
453 types in muscle with intramuscular collagen accumulation would provide valuable mechanistic insight.
454 Existing evidence already points to comorbidity-specific changes; FAP cells are differentially expressed in
455 type 2 diabetic human muscle (12). Hypertension also warrants closer examination, as it emerged as a
456 key mediator in the current review; none of the studies reporting age-related collagen increases
457 controlled for it.

458 The current review focused primarily on total collagen content, but it’s acknowledged that other
459 ECM characteristics, such as organization, density, and collagen isoform distribution may be altered with
460 aging and have important impacts on muscle function. To further understand if differences in muscle
461 quality exist in aging, is it essential these nuanced measures are investigated in future studies. Functional
462 measures of muscle quality, such as force produced per unit of muscle mass, are also important and
463 should be measured in relation to ECM composition. It is also acknowledged that methodological
464 limitations challenge directly linking human aging to muscle fibrosis and that only English-language
465 publications were included, potentially omitting relevant data. Females represented 30% of the study
466 participants, limiting sex-specific conclusions while highlighting the need for more comprehensive

467 analysis of sex-based differences in muscle collagen content. Parker et al. (59) demonstrate sex-related
468 differences in fibrosis-related gene expression in muscle from older adults; however, further research is
469 needed in this area. To draw stronger conclusions, large-scale studies with analysis of protein content
470 and activity are needed.

471 Inconsistencies in methodology and reporting of the papers included was a key limitation of this
472 systematic review. Pavan et al., (30) and Critchlow et al. (31) did not recruit defined young and older
473 participant groups. To compare the findings of this study to the others, we therefore grouped the
474 participants based on age (< 60 years for young and > 60 years for older). The mean age of the young
475 groups; however, was not reflective of what is consistently reported as young in the literature (< 30
476 years). The proximity of the ages of the oldest young adult and the youngest older adult, reduces
477 heterogeneity in these compared samples.

478 This systematic review highlights that age-related differences in intramuscular collagen are not
479 universally observed in humans; instead, collagen accumulation is likely driven by comorbidities that
480 accompany aging in some populations. The findings from this review underscore the need for rigorous
481 control of mediators in primary human research, careful distinction between human and animal findings
482 in reporting, and greater precision in the use of certain terminology. Future research should also aim for
483 greater female sex representation and nuanced interpretation of collagen-related outcomes in skeletal
484 muscle aging.

485

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488

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492

493 **DISCLOSURES**

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

495

496 **AUTHOR CONTRIBUTIONS**

497 Allyson M. Schweitzer conceived and designed the research, conducted the review process, analyzed
498 data, interpreted results, prepared figures, drafted, edited and revised and approved the final version of
499 the manuscript. Max J. Abercrombie conducted the review process and approved the final version of the
500 manuscript. Justin M. Losciale conceived and designed the research, edited, revised and approved the
501 final version of the manuscript. Cameron J. Mitchell conceived and designed the research, edited,
502 revised and approved the final version of the manuscript.

503

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Figure 1. Flow diagram of search processes. n, number of studies.

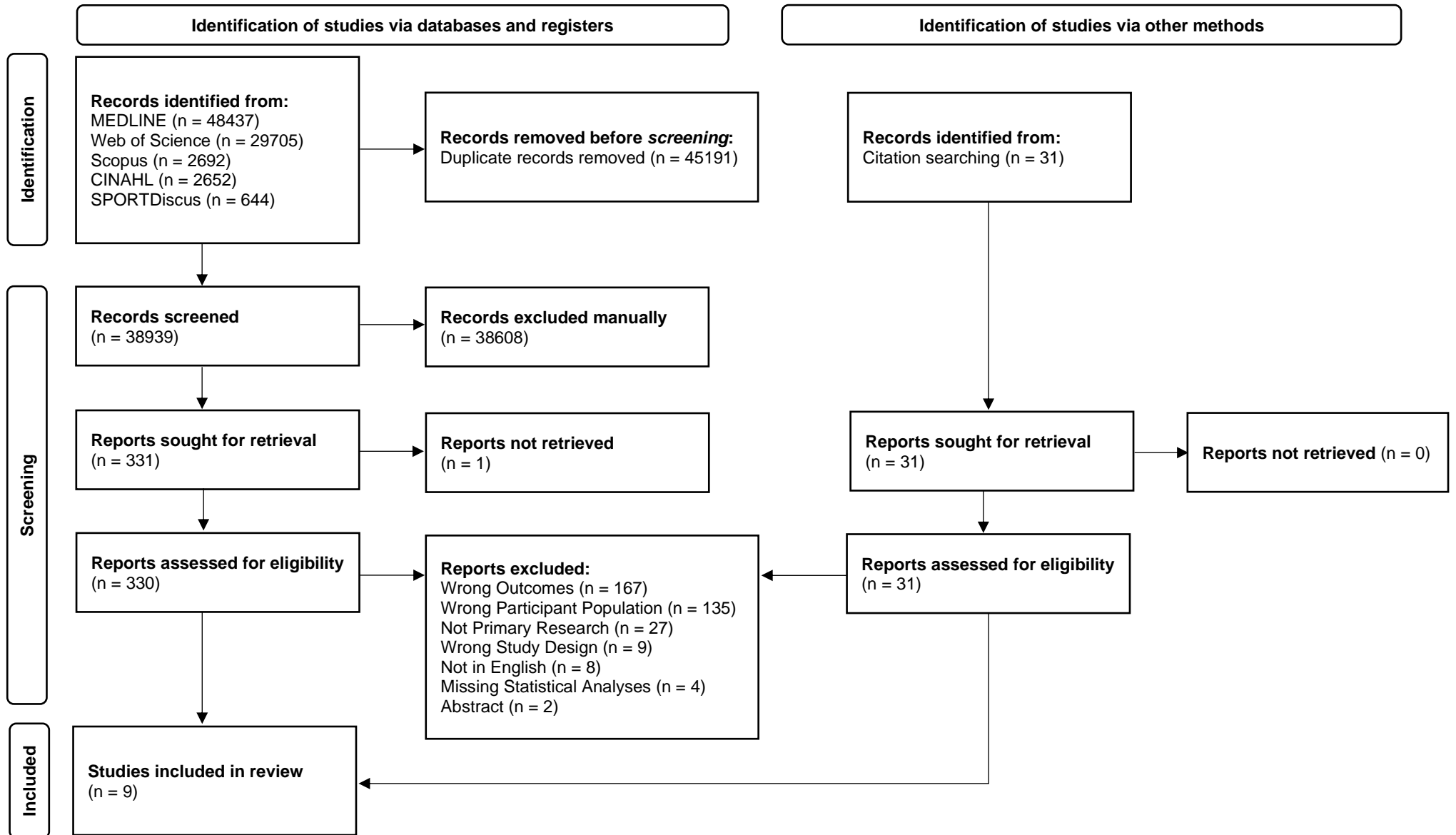


Table 1. Search strategy for MEDLINE (Ovid), Web of Science, Scopus, CINAHL and Sport Discus databases

Term 1		Term 2		Term 3		Term 4
Human* or man or men or woman or women or female* or male* or people* or population* or person* or adult* or individual* or cadaver* or anatomical specimen* or anatomical specimens*or	<i>AND</i>	Collagen* or extracellular matrix* or interstitial or matrix or ECM or fibro* or COL1 or COL3 or COL4 or COLI or COLIII or COLIV or fibroblast* or hydroxyproline	<i>AND</i>	Muscle* or myofibre* or myofiber* or contractile tissue* or myocyte* or myofibril* or muscle morphology or muscle composition or muscle structure	<i>AND</i>	Old or older or aged or ageing or aging* or elder or senior* or veteran* or geriatric* or frail* or senec* or mature*
anatomical donation or anatomical donations or anatomy donation or anatomy donations* or body or bodies or embalmed or unembalmed or subject or participant* or volunteer*						

The search was conducted on January 28, 2026. * Denotes truncation.

Table 2. Characteristics and summary of results of included trials comparing intramuscular collagen content between age groups

Study	Population	Sample Size	General Descriptors	Age (years)	Sex (#F, #M)	BMI (kg/m ²)	Muscle Analyzed	Measure of Intramuscular Collagen Content	Difference in Intramuscular Collagen Outcome Older vs. Young
Babraj et al. (24)	Young	4	Healthy	28 ± 6	0, 4	24 ± 3	VL	<u>Total and Pepsin Insoluble Collagen</u> Hydroxyproline assay (µg/mg muscle wet weight)	Total collagen ↔
	Elderly	4		70 ± 6	0, 4	26 ± 4			Pepsin insoluble collagen ↔
Critchlow et al. (31)	Young Females	23	Healthy	39.4 ±	23, 0	18-35	VL	<u>Total ECM Area</u> Wheat Germ Agglutinin	↑
	Older Females	11		13.5 70.6 ± 6.2	11, 0				
Fede et al. (23)	Young Males	10	Healthy, undergoing surgery for femur fracture	37.5 ± 9.5	0, 10	23.9 ± 1.5	VL (n = 6), RF (n = 4)	<u>Total Collagen</u> Picrosirius red (% area)	Total collagen ↑
	Elderly Males	12		79.0 ± 12.4	0, 12	24.8 ± 3.1	VL (n = 8), RF (n = 4)	<u>COL1/COL3</u> IHC, DAB (average optical density)	COL1 ↑ COL3 ↔
Gueugneau et al. (29)	Young Males	12	Healthy	21.6 ± 2.4	0, 12	22.9 ± 2.8	VL	<u>Total Endomysial and Perimysial Collagen</u> Sirius Red (% area)	Endomysial collagen ↔
	Elderly Males	7		73.5 ± 0.8	0, 7	23.6 ± 2.1			Perimysial collagen ↑
Haus et al. (25)	Young	20	Life-long sedentary, healthy	22 ± 3	10, 10	Body Fat % = 25 (8.9)	VL	<u>Total Collagen</u> Hydroxyproline assay (µg/mg muscle wet weight)	↔
	Older	22		78 ± 6	12, 10	Body Fat % = 32 (9.4)			
Mikkelsen et al. (26)	Young Untrained	12	Healthy, sedentary for at least 5 years	24 ± 3	0, 12	22 ± 2	VL	<u>Total Collagen</u> Sirius red (raw integrated density)	Total collagen ↔

	Young Trained	10	Healthy, lifelong runners	26 ± 4	0, 10	23 ± 2		<u>COL3</u> IHC, DAB (raw integrated density)	COL3 ↔
	Older Untrained	12	Healthy, sedentary for at least 5 years	66 ± 4	0, 12	25 ± 2			
	Older Trained	15	Healthy, lifelong runners	64 ± 4	0, 15	23 ± 2			
Nederveen et al. (36)	Young Males	10	Healthy	21 ± 3.2	0, 10	25.8 ± 3.5	VL	<u>Total Collagen</u> Masson's Trichrome (% area)	↑
	Older Males	16		68 ± 6.3	0, 16	28.8 ± 6.1			
Pavan et al. (30)	Young	9	Healthy, undergoing surgery for fracture of hip or femur	38.7 ± 11.4	2, 7	Not reported	VL	<u>Total Collagen</u> Picrosirius red (% area)	↑
	Older	7		77.7 ± 13.2	1, 6				
Schweitzer et al. (27)	Young	12	Healthy	21.8 ± 2.8	6, 6	22.4 ± 2.3	VL	<u>Total Collagen</u> Picrosirius red (% area)	↔
	Older	13		73.2 ± 3.9	7, 6	24.1 ± 2.1			

Values are shown as mean ± standard deviation. Standard deviations were calculated from standard error where applicable. Mikkelsen et al. (26), participant characteristics were obtained in conjunction with data reported in Mackey et al. (34). Mean differences between age groups were extracted from graphs using plotdigitizer.com for Fede et al. (23), Gueugneau et al. (29), Mikkelsen et al. (26), and Pavan et al. (30). AOD, average optical density; BMI, body mass index; COL1, collagen type one; COL3, collagen type three; ECM, extracellular matrix; RF, rectus femoris; RID, raw integrated density; VL, vastus lateralis.

Table 3. Results of intramuscular collagen outcomes

	Population	Total Collagen	Pepsin Insoluble Collagen	COL1	COL3	Endomysial Collagen	Perimysial Collagen	Total ECM (WGA)
Babraj et al. (24)	Young Males	9.5 ± 2.0 µg/mg muscle wet weight	2.3 ± 1.0 µg/mg muscle wet weight					
	Elderly Males	9.9 ± 1.1 µg/mg muscle wet weight	3.9 ± 0.7 µg/mg muscle wet weight					
Critchlow et al. (31)	Young Females							8.3 ± 2.5%
	Older Females							11.2 ± 3.1%*
Fede et al. (23)	Young Males	2.9% area		0.3 AOD	0.3 AOD			
	Elderly Males	6.8% area*		0.4 AOD*	0.3 AOD			
Gueugneau et al. (29)	Young Males					4.6% area	2.0% area	
	Elderly Males					3.8% area	3.3% area*	
Haus et al. (25)	Young Adults	9.6 ± 4.9 µg/mg muscle wet weight						
	Older Adults	10.2 ± 5.6 µg/mg muscle wet weight						
Mikkelsen et al. (26)	Young Untrained Males	1.0 RID			1.0 RID			
	Young Trained Males	0.9 RID			1.0 RID			
	Older Untrained Males	0.9 RID			0.9 RID			
	Older Trained Males	0.9 RID			0.9 RID			
Nederveen et al. (36)	Young Males	2.0 ± 1.6% area						
	Older Males	3.6 ± 2.0% area*						
Pavan et al. (30)	Young Adults	5.4% area						
	Older Adults	9.4% area*						
Schweitzer et al. (27)	Young Adults	5.4 ± 2.1% area						
	Older Adults	5.1 ± 3.1% area						

Data are presented as mean ± standard deviation. Standard deviations were calculated from standard error where applicable. For Pavan et al. (30) and Critchlow et al. (31), individual datapoints from linear regression analysis were grouped into a young (< 60 years) and an older (≥ 60 years) age group and an

unpaired t-test was conducted on the grouped data. Data from Pavan et al. (30) was extrapolated using plotdigitizer.com. * Denotes statistical significance from young adult value. Squares are left blank if that intramuscular collagen outcome was not reported. Data for Fede et al. (23), Gueugneau et al. (29), Mikkelsen et al. (26), and Pavan et al. (30), were extracted from graphs using plotdigitizer.com. AOD, average optical density; COL1, collagen type one; COL3, collagen type three; RID, raw integrated density; WGA, wheat germ agglutin.

Table 4. Mediators of intramuscular collagen accumulation and method of control or report used in each included study

Study	Physical Activity and Fitness	Diabetes	Obesity	Osteoarthritis	Hypertension	Inflammation (Old vs. Young)
						<u>Muscle Analysis</u>
Babraj et al. (24)	<i>Not reported</i>	<i>Not reported</i>	Young BMI < 30 Older BMI ≤ 30	<i>Not reported</i>	<i>Not reported</i>	NFκB Protein ↑ TNF-α mRNA ↑ IL-6 mRNA ↑
Critchlow et al. (31)	<u>Moderate-vigorous physical activity minutes per day, mean (SD)</u> 18-29 years = 35.5 (14) 30-39 years = 48.8 (21) 40-49 years = 54.8 (60.5) 50-59 years = 38 (16.5) 60-69 = 48 (47.4) 70-80 = 26 (36.3)	<i>Not reported</i>	BMI < 35 for all participants	<i>Not reported</i>	<i>Not reported</i>	<i>Not reported</i>
Fede et al. (23)	<i>Not reported</i>	Volunteers with diabetes mellitus were excluded	BMI < 30 for all participants	<i>Not reported</i>	<i>Not reported</i>	<i>Not reported</i>
Gueugneau et al. (29)	<u>VO_{2peak} (mL/min/kg), mean (SD)</u> Young = 40.0 (2.7) Older = 31.1 (2.4)	Volunteers with type 2 diabetes were excluded	BMI < 30 for all participants	<i>Not reported</i>	Healthy participants were non-hypertensive	<i>Not reported</i>
Haus et al. (25)	Lifelong sedentary individuals with no history of formal exercise training or physical activity apart from activities of daily living	Volunteers were excluded if they had insulin- or noninsulin-dependent diabetes	<u>Body Fat %, mean (SD)</u> Young = 25 (8.9) Older = 32 (9.4)	Volunteers excluded if they had arthritis	Participants were excluded if they had uncontrolled hypertension	<i>Not reported</i>

	<u>Running Distance</u> (km/week), mean (SD) Young trained = 43 (5) Older trained = 49 (3)					<u>Systemic Markers (ELISA)</u> CRP ↑ IL-6 ↑ SuPAR ↑ sTNFR1 ↑ sTNFR2 ↑
Mikkelsen et al. (26)	<u>VO_{2max} (mL/kg/min), mean (SD)</u> Young trained = 59.5 (3.1) Young untrained = 44.6 (4.3) Older trained = 48.3 (5.6) Older untrained = 30.1 (3.5)	Volunteers were screened for diabetes by an oral glucose test and were excluded if there were any signs of pre-diabetes or diabetes	BMI < 30 for all participants	Volunteers excluded if they had any joint pathology	All subjects were normotensive	
Nederveen et al. (36)	Self-reported not participating in any structured exercise for at least four months before the study	Volunteers with diabetes were excluded	BMI < 35 for all participants	Volunteers were excluded if they had any major orthopedic disability	<i>Not reported</i>	<i>Not reported</i>
Pavan et al. (30)	Moderately active	<i>Not reported</i>	<i>Not reported</i>	<i>Not reported</i>	<i>Not reported</i>	<i>Not reported</i>
Schweitzer et al. (27)	Self-reported having not participated in structured, lower-body resistance or aerobic training in the past six months and were completing less than two hours per week of lower body exercise Required to fall between the 25 th -75 th age and sex-stratified percentile for aerobic fitness as measured by VO _{2peak}	Volunteers were excluded if they had type 1 or type 2 diabetes	BMI < 30 for all participants	<i>Not reported</i>	Participants were non-hypertensive	Systemic CRP (ELISA) ↔

VO_{2peak} (mL/min/kg), mean

(SD)

Young = 39.1 (5.0)

Older = 24.2 (5.1)

Terminology for physical activity and fitness in this table mimics closely that used by the respective authors in their reports. Mikkelsen et al. (26), participant characteristics were retrieved from Mackey et al. (34), and Mikkelsen et al. (35). Muscle inflammatory analysis from Babraj et al. (24), was reported in the discussion only as unpublished data. BMI, body mass index; CRP, C-reactive protein; ELISA, enzyme-linked immunosorbent assay; IL-6, interleukin-6; NFκB, nuclear factor kappa-B; SD, standard deviation; SuPAR, soluble urokinase plasminogen activator receptor; SD, standard deviation; sTNFR1, soluble tumor necrosis receptor-I; sTNFII, soluble tumor necrosis factor-II.

Table 5. Risk of bias assessment

Study	Target Group Comparability (selection bias)	Target Group Selection (selection bias)	Confounders (selection bias)	Assessor Blinding (detection bias)	Outcome Assessment (detection bias)	Incomplete Outcome Data (attrition bias)	Selective Outcome Reporting (reporting bias)
Babraj et al. (24)	Unclear	Unclear	High	Unclear	Low	High	Low
Critchlow et al. (31)	Low	Low	High	Low	Unclear	Low	Low
Fede et al. (23)	Unclear	High	High	High	Low	Unclear	Low
Gueugneau et al. (29)	Low	Unclear	Low	Low	Low	Unclear	Low
Haus et al. (25)	Low	Low	Low	Low	Low	Unclear	Low
Mikkelsen et al. (26)	Low	Unclear	Low	Low	Low	Low	Unclear
Nederveen et al. (36)	Low	Low	High	Low	Low	Unclear	Low
Pavan et al. (30)	High	High	High	High	Low	Low	Low
Schweitzer et al. (27)	Low	Low	Low	Low	Low	Low	Low

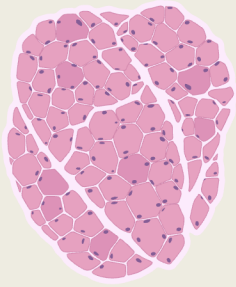
Risk of bias was assessed using the Revised Risk of Bias Assessment Tool for Nonrandomized Studies of Interventions (RoBANS 2), Seo et al. (32).

Muscle Collagen Accumulation is not an Inevitable Feature of Human Chronological Aging

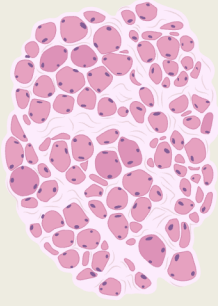
Systematic Search
(January 2026)

Age-related Comparison of Human
Skeletal Muscle Collagen Content

→ 9 Studies Identified



No age-related differences in collagen content (n = 4)



Age-related increase in collagen content (n = 4)

Equivocal findings (n = 1)

Possible Mediators of Collagen Accumulation During Aging

- Hypertension
- Diabetes
- Obesity
- Osteoarthritis
- Physical inactivity/low aerobic fitness

Conclusion: Observed differences in collagen content with aging may reflect comorbidities or lifestyle factors rather than aging *per se*.