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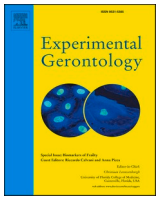

Thigh muscles are more susceptible to age-related muscle loss when compared to lower leg and pelvic muscles☆

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ABSTRACT

Background: A key hallmark of aging is the progressive loss of skeletal muscle mass. Due to limitations of the various methods typically applied to assess muscle mass, only limited information is available on age-related differences between various muscle groups. This study assessed differences in individual lower body muscle group volumes between healthy young and older males.

Methods: Lower body muscle mass assessments were performed in 10 young (age: 27 ± 4 y) and 10 older (age: 71 ± 6 y) healthy, male adults using Dual-energy X-ray Absorptiometry (DXA), single slice (thigh) Computed Tomography (CT), as well as Magnetic Resonance Imaging (MRI). Muscle volumes of all individual muscle groups in the lower body were assessed by MRI.

Results: Leg lean mass, as assessed with DXA, was not significantly different between older (9.2 ± 1.0 kg) and young (10.5 ± 2.0 kg) men ($P = 0.075$). Thigh muscle cross-sectional area, as assessed with CT, was significantly lower (by 13 %) in the older (137 ± 17 cm^2) compared to young (157 ± 24 cm^2) participants ($P = 0.044$). MRI-derived lower body muscle volume was also significantly lower (by 20 %) in older (6.7 ± 0.9 L) compared to young (8.3 ± 1.3 L) men ($P = 0.005$). This was primarily attributed to substantial differences in thigh (24 %), rather than lower leg (12 %) and pelvis (15 %) muscle volume in the older vs the young. Thigh muscle volume averaged 3.4 ± 0.5 L in older and 4.5 ± 0.7 L in young men ($P = 0.001$). Of all thigh muscle groups, the quadriceps femoris showed the most profound difference (30 %) between young (2.3 ± 0.4 L) and older (1.6 ± 0.2 L) men ($P < 0.001$).

Conclusions: The most profound differences in lower body muscle volume between young and older men are observed in the thigh. Within the thigh muscle groups, the quadriceps femoris shows the largest difference in muscle volume between young and older men. Finally, DXA appears less sensitive when compared to CT and MRI to assess age-related differences in muscle mass.

1. Introduction

Aging is associated with a progressive loss of skeletal muscle mass. The loss of muscle mass contributes to the decline in muscle strength, the loss of physical function, and increases the risk of developing chronic metabolic diseases at a more advanced age (Evans, 1995; Koopman and van Loon, 2009). Whereas skeletal muscle mass is generally reported to be lower in older adults when compared to their younger counterparts (Holloszy, 2000; Mitchell et al., 2012; Nilwik et al., 2013), there is far less information on the contribution of the individual muscle groups to

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the differences in whole-body muscle mass between the young and old. The lack of data on the contribution of different muscle groups to the observed differences in muscle mass between young and older adults is attributed to the limitations of the various methods typically applied to assess muscle mass in vivo in humans (Evans et al., 2019; Lee and Gallagher, 2008; Mijnarends et al., 2013; Heymsfield et al., 2015).

Skeletal muscle mass can be assessed in vivo in humans using various methods, which include hydrostatic weighing (densitometry), air displacement plethysmography, bioelectrical impedance analysis (BIA), dilution techniques with labeled water, ultrasound, magnetic resonance imaging (MRI), dual-energy X-ray absorptiometry (DXA), and computed tomography (CT) (Lee and Gallagher, 2008; Heymsfield et al., 2015; Rothwell et al., 2019; Franchi et al., 2018; Borga et al., 2018; van Ruijven et al., 2021; Westerterp et al., 2021). DXA and single slice CT are among the two most commonly applied techniques in research studies aiming to quantify differences in muscle mass (Nilwik et al., 2013; Holwerda et al., 2018; Snijders et al., 2015; Backx et al., 2018; Breen et al., 2013; English et al., 2016; Devries et al., 2015). Both techniques are considered reliable and accurate and can be applied with ease within a clinical and/or academic setting (Heymsfield et al., 2015; Borga et al., 2018). Nonetheless, both CT and DXA do have their limitations when assessing muscle mass. DXA provides information regarding total and/or regional (i.e. leg, arm and trunk) lean mass and, therefore, does not directly measure muscle mass. Furthermore, DXA only provides a two-dimensional (coronal) projection, rather than a three-dimensional projection, making it impossible to obtain volumetric measurements. Single slice CT is typically applied as a compromise to assess leg muscle mass, allowing a comparison of cross-sectional area of the upper leg as a proxy for total leg muscle mass. Given the lack of clinical indication and the potential harm of ionizing radiation when applying CT, whole-body or whole-leg CT are generally not performed in vivo in healthy humans. Clearly, both the application of DXA and CT do not permit us to provide a more detailed assessment of muscle volume and/or muscle mass of individual muscles or muscle groups.

MRI allows the measurement of muscle volume of individual muscle groups in a three-dimensional fashion and is generally considered the gold standard for muscle volume assessment. By using MRI, it has previously been indicated that various muscle groups may be affected differently with aging (Maden-Wilkinson et al., 2014; Ogawa et al., 2012; Maden-Wilkinson et al., 2013; Hogrel et al., 2015; Macaluso et al., 2002). However, a detailed assessment of the contribution of different muscle groups of the entire lower body (including the pelvis) to the lower muscle mass in older individuals remains lacking. A detailed assessment of such differences is important given the metabolic importance of muscle mass and the impact muscle loss can have on functional capacity and the development of chronic metabolic diseases (Evans, 1995; Koopman and van Loon, 2009). Furthermore, a greater understanding of divergent atrophy responses may aid in the development of exercise strategies to more effectively preserve muscle mass and function at a more advanced age. Therefore, we assessed all the individual lower body muscle groups in a group of healthy, young and older men.

In the present study, we recruited 10 healthy young, and 10 healthy older adult males to assess leg lean mass using DXA and muscle cross-sectional area (CSA) using single slice (thigh) CT. Furthermore, we applied MRI to allow a comparison of muscle volume of all individual muscle groups of the lower limbs (including pelvis) between young and older men. Finally, these measurements also allow a direct comparison between DXA, single slice CT, and MRI as a means to assess lower limb muscle mass in young and older men.

2. Methods

2.1. Participants

Twenty healthy males participated in this study. Young ($n = 10$; age: 27 ± 4 y) and older ($n = 10$; age: 71 ± 6 y) males were recruited from the local community. The characteristics of the participants are shown in Table 1. All participants were healthy with no apparent musculoskeletal or cardiovascular disease. All participants were deemed healthy based on their responses to a medical questionnaire and were socially and physically active, but not performing any structured (≥2 times per week) exercise (training) at the time of investigation. All participants were informed of the purpose of the study, experimental procedures, and possible risks before providing written consent to participate. The procedures followed were in accordance with the ethical standards of the Medical Ethics Committee of the Maastricht University Medical Centre+ on human experimentation and in accordance with the Helsinki Declaration of 1975 as revised in October 2013.

2.2. Anthropometrics

Participants arrived into the laboratory after an overnight fast. Upon arrival in the laboratory, participants were first asked to go to the toilet to empty their bladder and bowels. Subsequently, we measured their body mass and height before the scanning procedures started.

2.3. Dual-energy X ray absorptiometry (DXA)

A DXA scan was performed on a commercially available clinical system (Discovery A; Hologic, Bedford, MA, USA) with system's software package (Hologic-Apex software, version 4.5.3, with viewer software Hologic Physician's viewer, version 7.1) to determine leg lean mass. All DXA scans and leg lean mass analyses were performed by the same technician. Each participant underwent scanning in a supine position with the arms resting next to the trunk with the hands in a pronated position. Leg lean mass was determined by applying a border through the femoral neck (Fig. 1A). The Coefficient of Variation (CV) of leg lean mass analyses was 1.3 %. Representative images of the DXA scan of a young and older man are shown in Fig. 1A.

2.4. Computed tomography (CT)

Anatomical cross-sectional area (CSA) of the upper leg was assessed via a single slice CT protocol. All CT scans were performed by the same investigator. While participants were lying supine, with their legs extended and their feet secured, a 2-mm thick axial image was taken 15 cm proximal to the top of the patella. The precise scanning position was marked with semi-permanent ink. The following scanning parameters were used on a second generation Dual-Source CT (Somatom Definition Flash; Siemens Healthineers, Forchheim, Germany): 120 kV, 300 mA, rotation time of 1 s, slice thickness 2 mm. The image was reconstructed with a medium smooth kernel (B30s, Siemens) and a field of view of 500 mm. Tissue with Hounsfield units between −29 and 150 HU was selected as muscle tissue. All CT analyses were performed by the same investigator (CV: 0.6 %). CT scans were analyzed for the CSA of the whole thigh muscles, quadriceps femoris muscle group, and all other thigh muscle groups combined (i.e. biceps femoris long head, biceps femoris short head, semitendinosus, semimembranosus, adductor magnus, gracilis, and sartorius) by manual tracing using ImageJ software (version 2.0.0; National Institutes of Health, Bethesda, MD, USA). The CSA of all other thigh muscle groups combined was assessed by

Table 1
Participants' characteristics.

	Young	Older	*P* value
Age (y)	27 ± 4	71 ± 6	<0.001
Total body mass (kg)	76.7 ± 11.9	77.4 ± 8.1	0.883
Height (m)	1.82 ± 0.07	1.75 ± 0.05	0.012
Body mass index (kg/m^2)	22.9 ± 2.5	25.4 ± 2.5	0.040
Femur length (cm)	45.9 ± 2.0	44.2 ± 2.2	0.085
Tibia length (cm)	40.4 ± 1.4	38.6 ± 1.3	0.008

Values represent means±SD, $n = 10$ per group.

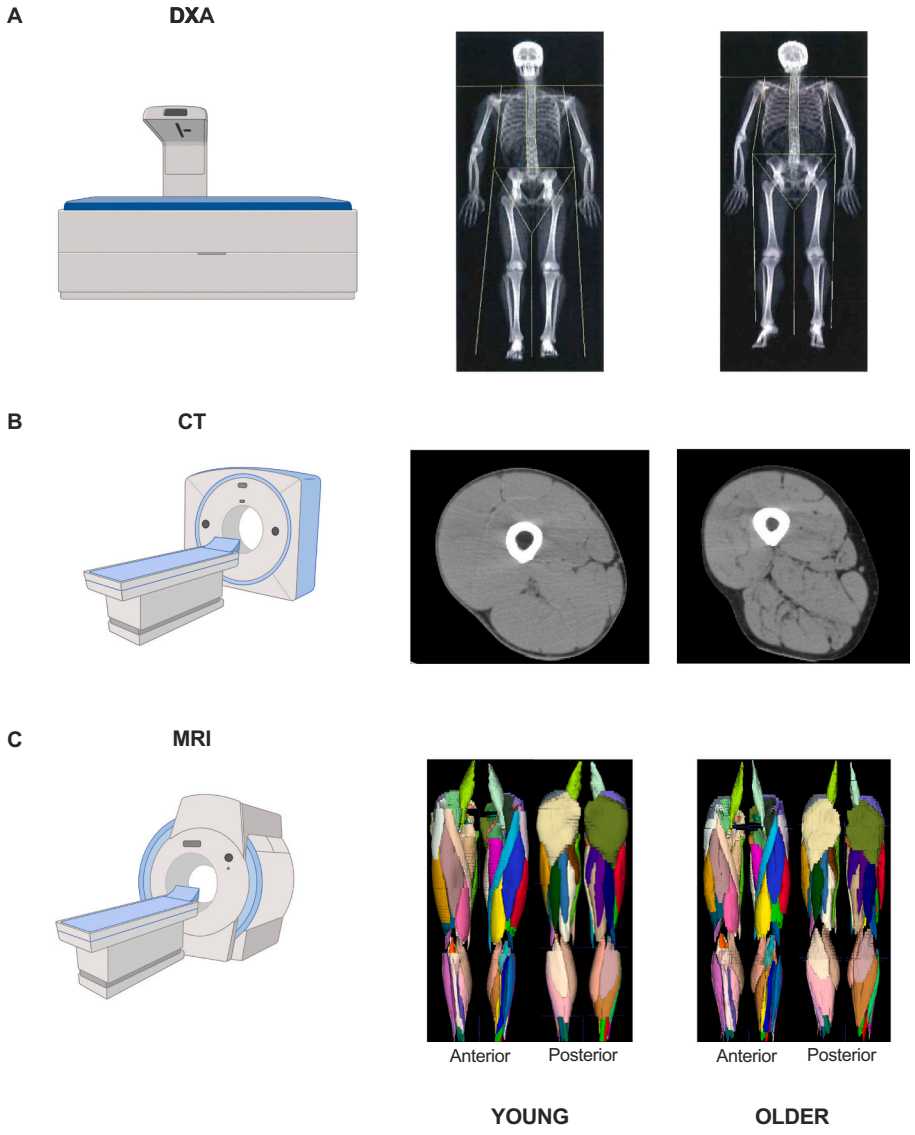

Fig. 1. A representative 2-D DXA scan image of a young and older man (A), a representative cross-sectional thigh CT scan image of a young and older man (B), a representative analyzed MRI scan image of both the anterior and posterior view of the lower body of a young and older man (C). In Panel C: for both the anterior and posterior image, different MRI segments are stacked together to provide an image of all analyzed muscles.

subtracting the quadriceps femoris muscle group from the whole thigh muscle CSA. Representative images of the CT scan of a young and older man are shown in Fig. 1B.

2.5. Magnetic resonance imaging (MRI)

A whole-body MRI scan (1.5 T, Ingenia, software release 5.4.1.1.; Philips Healthcare, Best, the Netherlands) was performed in all participants by using an integrated posterior coil, two anterior coils and one head coil. All MRI scans were performed by the same technician. Participants entered the magnet head first in a supine position. T1-weighted images were acquired and each total scanning procedure took on average 60 min. In total, 8 stacks (containing 26 slices each with a slice thickness of 10 mm and no interslice gap) were acquired containing all individual images. As we were only interested in analyzing muscle groups in the lower limbs and hips, we used 5–6 stacks for data analysis depending on the height of the participant (covering feet to abdomen). Common scanning parameters for all included stacks were: flip angle (α) = 90°, repetition time (TR) = 120 ms, echo time (TE) = 4 ms (the field of view was 256 × 144 for legs and 360 × 200 for abdomen).

Muscle volume analyses were performed manually using ITK-SNAP software (Yushkevich et al., 2006). Initially, MRI analyses were performed by three investigators (CV: 2.4 %). Subsequently, all images were carefully checked by the other (two) investigators. In case of discrepancies, images were discussed between investigators and, were appropriate, adjusted. On average, analysis of each individual participant took 2 weeks full time, which equals ~1600 h of total analysis time (including quality check). Muscle volume analysis was performed starting from the first visible lower leg muscles up towards the hips (last quantified muscle: psoas major muscle). For each participant, we analyzed all (visible) muscles in the lower legs (i.e. peroneus brevis, peroneus longus, extensor digitorum longus, extensor hallucis longus, tibialis anterior, flexor hallucis longus, flexor digitorum longus, tibialis posterior, popliteus, soleus, gastrocnemius medial head, gastrocnemius lateral head, and plantaris), the thighs (i.e. vastus lateralis, vastus intermedius, vastus medialis, rectus femoris, semimembranosus, semitendinosus, biceps femoris long head, biceps femoris short head, gracilis, adductor magnus, adductor longus, adductor brevis, pectineus, and sartorius), and the hips (i.e. gluteus maximus, gluteus medius, gluteus minimus, tensor fasciae latae, obturator externus, obturator internus,

quadratus femoris, piriformis, gemellus inferior, gemellus superior, iliacus, and psoas major). As delineation for some individual muscles in the quadriceps can be challenging, we decided to apply the manual segmentation method as proposed by Barnouin et al. (Barnouin et al., 2014).

Given that some individual muscles are often grouped together and, as such, reported in previous literature, we also expressed the combined volume of individual muscles for specific muscle compartments or groups. We further divided the lower leg into the (1) lateral compartment (i.e. peroneus brevis and peroneus longus), (2) anterior compartment (i.e. extensor digitorum longus, extensor hallucis longus, and tibialis anterior), (3) deep posterior compartment (i.e. flexor hallucis longus, flexor digitorum longus, tibialis posterior, and popliteus), and (4) superficial posterior compartment (i.e. soleus, gastrocnemius medial head, gastrocnemius lateral head, and plantaris). We further reported the following combined thigh and/or hip muscles: (1) the quadriceps femoris (i.e. vastus lateralis, vastus intermedius, vastus medialis, and rectus femoris), (2) the hamstrings (i.e. semimembranosus, semitendinosus, biceps femoris long head, and biceps femoris short head), (3) the adductors (i.e. gracilis, adductor magnus, adductor longus, adductor brevis, pectineus, and obturator externus), (4) the gemelli (i.e. gemellus inferior and gemellus superior), (5) the iliopsoas (i.e. iliacus and psoas major), and (6) the gluteal muscles (i.e. gluteus maximus, gluteus medius, and gluteus minimus). During analysis all visible fat, aponeuroses, blood vessels, nerves and bones were excluded. A representative analyzed (stacked) image of the MRI scan of a young and older man is shown in Fig. 1C.

Muscle volume (of individual muscles and/or muscle groups) was defined as the sum of the slices of the specific muscle (group). In order to be able to correct for differences in length between groups, we also measured muscle length. This was defined as the distance between the most proximal and distal images in which the specific muscle (group) was visible. By dividing (individual) muscle volume with the respective length we were able to calculate the average CSA of each (individual) muscle (group), which could be compared between groups. Femur and tibia length were also assessed from the MRI scans, for comparison between the young and older participants. Femur length was defined as the distance between the most proximal part of the greater trochanter and the most distal part of the lateral condyle of the femur. Tibia length was defined as the distance between the most proximal part of the medial condyle of the tibia and the most distal part of the medial malleolus.

To aid the comparison with the DXA scan, a representative part of leg muscle (i.e. the entire lower leg, and thigh muscle without the pectineus) volume (L) was assessed and converted into mass (kg) using a skeletal muscle density of 1.04 kg/L (Snyder et al., 1975).

2.6. Statistical analysis

Unless otherwise stated, all data were expressed as mean ± standard deviation (SD) (with additional individual data points being provided in all figures). Unless otherwise stated, direct comparisons of leg lean mass, muscle CSA, and muscle volume between young and older participants were performed by taking the average value of the left and right leg or body part.

Student's unpaired *t*-tests were performed to compare all values (apart from proportional differences; see below) between young and older men. To assess whether there were differences in age-related muscle volume between muscle segments, groups, and individual muscles, we calculated (1) the proportional contribution of lower body muscle segments (i.e. lower leg, thigh, and pelvis) to total lower body muscle volume, (2) the proportional contribution of thigh muscle groups (i.e. quadriceps femoris, hamstrings, and adductors) to total thigh muscle volume, and (3) the proportional contribution of individual quadriceps muscle volumes (i.e. vastus lateralis, vastus intermedius, vastus medialis, and rectus femoris) to total quadriceps muscle volume in young and older men. We analyzed age-related differences in these proportions using a repeated measures ANOVA, with the proportion of each muscle segment, group, or individual muscle as a within-participants factor and age as a between-participants factor. For determination of the relationships between measurement techniques (i.e. DXA, CT, and MRI), a simple linear regression was carried out with Pearson's product moment correlation. Here, left and right legs were considered independent of each other and, as such, there were 40 data points (i.e. 20 legs of young and 20 legs of older participants) for correlation analyses. Statistical significance was set at $P \leq 0.05$. Data were analyzed using SPSS (version 26.0, IBM Corp., Armonk, NY, USA) or Graphpad Prism Version 8.2.1 (GraphPad Software Inc., La Jolla, CA, USA).

3. Results

3.1. Participants' characteristics

Participants' characteristics are depicted in Table 1. The older participants had a lower body height compared to the young participants. No differences were observed in femur length between groups. However, tibia length was higher in the young compared with the older men. As body mass did not differ between groups, BMI was higher in the older compared with the young.

3.2. Muscle size assessment

3.2.1. DXA

Leg lean mass, as assessed with DXA, was 9.2 ± 1.0 kg in older and 10.5 ± 2.0 kg in younger men, but values did not significantly differ between groups ($P = 0.075$; Fig. 2). In contrast, MRI-derived leg muscle mass (to represent muscles included in the DXA assessment for leg lean mass) was 22 % lower in the older (4.7 ± 0.6 kg) compared to the young (6.0 ± 1.0 kg; $P = 0.003$).

3.2.2. CT

Thigh, quadriceps, and all other thigh (i.e. total thigh – quadriceps) muscle CSA as assessed with CT are depicted in Fig. 3. Thigh muscle CSA

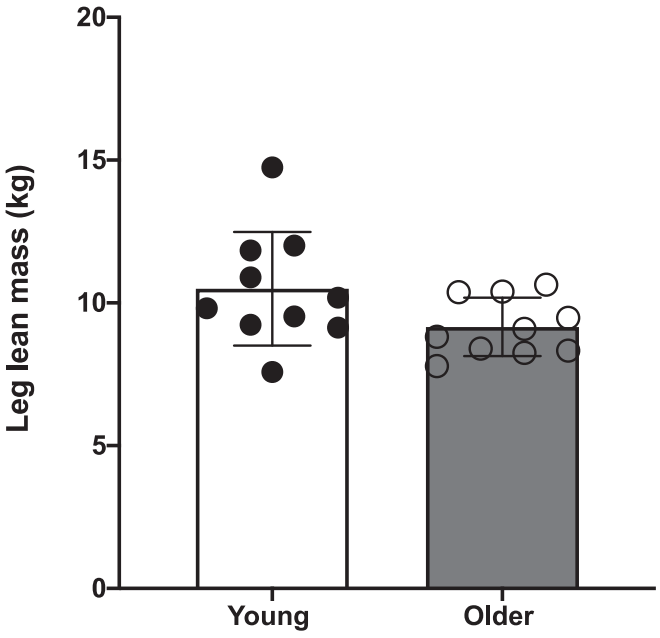

Fig. 2. DXA-derived leg lean mass in healthy, young ($n = 10$) and older ($n = 10$) men. The average of the left and right leg was taken for comparison between young and older men. Values represent means±SD and dots represent individual values. No significant differences between groups ($P = 0.075$).

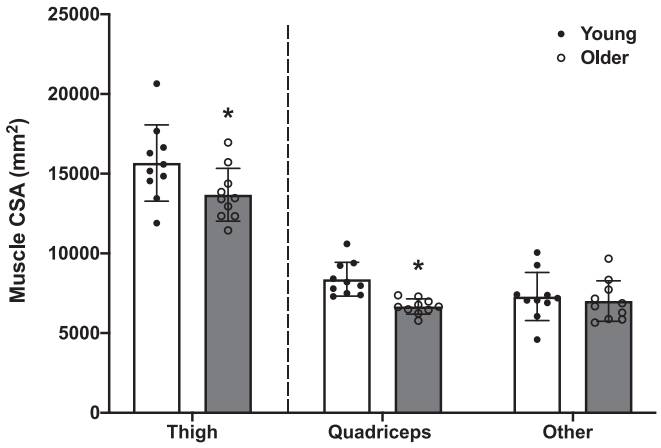

Fig. 3. CT-derived thigh muscle CSA, quadriceps muscle CSA, and other (i.e. thigh minus quadriceps) muscle CSA in healthy, young ($n = 10$) and older ($n = 10$) men. The average of the left and right leg was taken for comparison between young and older men. Values represent means±SD and dots represent individual values. CSA, cross-sectional area. *, significantly different ($P < 0.05$) between young and older men.

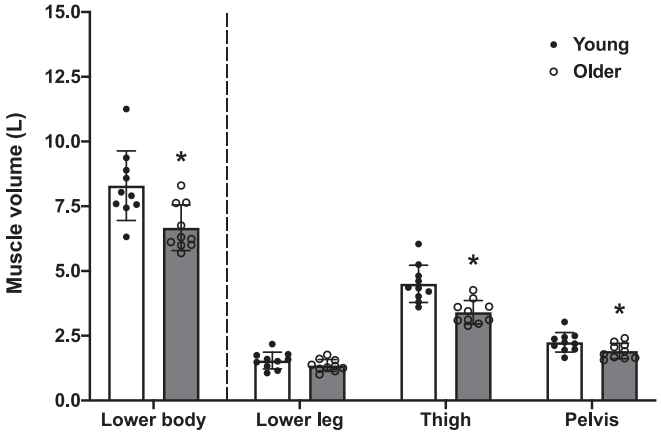

Fig. 4. MRI-derived lower body muscle volume, lower leg muscle volume, thigh muscle volume, and pelvic muscle volume in healthy, young ($n = 10$) and older ($n = 10$) men. The average of the left and right leg was taken for comparison between young and older men. Values represent means±SD and dots represent individual values. *, significantly different ($P < 0.05$) between young and older men.

was 13 % lower in the older (13,684 ± 1657 mm^2) compared to the young (15,675 ± 2392 mm^2; $P = 0.044$). Quadriceps muscle CSA was 20 % lower in the older (6669 ± 478 mm^2) compared to the young (8379 ± 1061 mm^2; $P < 0.001$). Muscle CSA of the other muscle groups in the thigh did not differ between the older (7015 ± 1269 mm^2) and young (7297 ± 1513 mm^2; $P = 0.658$).

3.2.3. MRI

Total lower body (i.e. both lower legs, both thighs, and the entire pelvis) muscle volume was lower in the older men compared with the young (13.3 ± 1.8 vs 16.6 ± 2.7 L, respectively; $P = 0.005$). Average lower body, lower leg, thigh, and pelvic (i.e. the average of the left and right leg/body part) muscle volume are depicted in Fig. 4. Average lower body muscle volume was 20 % lower in older (6.7 ± 0.9 L) compared to the young (8.3 ± 1.3 L) participants ($P = 0.005$). Average lower leg muscle volume did not differ in the older (1.4 ± 0.2 L) compared to the young (1.5 ± 0.3 L; $P = 0.150$). Average thigh (3.4 ± 0.5 vs 4.5 ± 0.7 L) and pelvic (1.9 ± 0.3 vs 2.2 ± 0.4 L) muscle volumes were 24 % and 15 % lower in older compared to young participants ($P = 0.001$ and $P = 0.038$, respectively). When assessing the proportional contribution of lower leg, thigh, and pelvic muscle volume to total lower body muscle volume in young and older men, we observed a significant interaction effect ($P = 0.005$). Further analyses revealed that the proportion of thigh to lower body muscle volume was lower in older (51 ± 2 %) compared to young (54 ± 2 %) men ($P = 0.009$), indicating more age-related muscle atrophy in the thigh muscles, compared to the other lower body parts (i.e. lower leg and pelvis).

3.2.4. MRI - Individual lower body muscle volumes

Individual lower body muscle volumes were assessed and provided for all (visible) muscle groups in the lower leg, thigh, and pelvis. For the lower leg, we only observed differences between young and older individuals for some individual muscle groups (Table 2). However, when corrected for muscle length we did not observe any significant differences in average muscle CSA of the individual muscles or for the total lower leg between the young and older participants (Supplemental Table 1).

In the thigh, the total quadriceps femoris, hamstring, and adductor volumes were all significantly lower in older compared to young men (Table 3). When assessing the proportional differences of quadriceps femoris, hamstrings, and adductors muscle volume to thigh muscle volume in young and older men, we observed a significant interaction effect ($P = 0.002$). Further analyses revealed significant differences for all thigh muscle groups ($P < 0.05$), with only the proportion of quadriceps femoris muscle volume to thigh muscle volume being lower in the

Table 2
Individual lower leg muscle volumes in healthy, young and older men.

Volume (cm^3)	Young	Older	Absolute difference	Percentage difference (%)
Lateral compartment	136 ± 46	115 ± 28	−21	−16
Peroneus brevis*	32 ± 10	21 ± 6	−11	−33
Peroneus longus	108 ± 34	94 ± 26	−14	−13
Anterior compartment	253 ± 38	219 ± 35	−34	−13
Extensor digitorum longus*	80 ± 19	67 ± 6	−14	−17
Extensor hallucis longus*	27 ± 7	21 ± 4	−7	−24
Tibialis anterior	146 ± 16	132 ± 28	−14	−9
Deep posterior compartment*	227 ± 39	188 ± 30	−39	−17
Flexor hallucis longus	75 ± 15	64 ± 13	−11	−14
Flexor digitorum longus	27 ± 7	25 ± 4	−3	−10
Tibialis posterior*	105 ± 22	83 ± 15	−22	−21
Popliteus	20 ± 6	16 ± 5	−4	−20
Superficial posterior compartment	923 ± 226	832 ± 159	−91	−10
Soleus	490 ± 127	452 ± 92	−38	−8
Gastrocnemius medial head	270 ± 76	218 ± 47	−52	−19
Gastrocnemius lateral head	150 ± 38	150 ± 30	−1	−0
Plantaris	12 ± 4	12 ± 3	−0	−2

Average values represent means±SD, $n = 10$ per group. The average muscle volume of the left and right leg was taken for comparison between young and older men.
* Significantly different ($P \leq 0.05$) between young and older men.

Table 3
Individual thigh muscle volumes in healthy, young and older men.

Volume (cm^3)	Young	Older	Absolute difference	Percentage difference (%)
Sartorius*	197 ± 36	143 ± 32	−54	−28
Total quadriceps femoris*	2336 ± 365	1629 ± 192	−706	−30
Vastus lateralis*	825 ± 164	533 ± 55	−291	−35
Vastus intermedius*	655 ± 99	464 ± 102	−191	−29
Vastus medialis*	565 ± 98	402 ± 66	−163	−29
Rectus femoris*	291 ± 42	230 ± 33	−62	−21
Total hamstrings*	800 ± 136	678 ± 74	−123	−15
Semimembranosus	259 ± 62	220 ± 43	−39	−15
Semitendinosus	204 ± 45	168 ± 35	−36	−18
Biceps femoris long head*	229 ± 47	191 ± 27	−38	−17
Biceps femoris short head	109 ± 26	99 ± 18	−10	−9
Total adductors*	1220 ± 263	1000 ± 191	−221	−18
Gracilis*	105 ± 18	83 ± 17	−22	−21
Adductor magnus	676 ± 172	559 ± 133	−116	−17
Adductor longus*	201 ± 60	144 ± 30	−58	−29
Adductor brevis	132 ± 43	110 ± 29	−21	−16
Pectineus	59 ± 10	60 ± 11	1	2

Average values represent means±SD, $n = 10$ per group. The average muscle volume of the left and right leg was taken for comparison between young and older men. It should be noted that for total adductors the values from the obturator externus (see Table 4) are also included.
* Significantly different ($P \leq 0.05$) between young and older men.

Table 4
Individual pelvic muscle volumes in healthy, young and older men.

Volume (cm^3)	Young	Older	Absolute difference	Percentage difference (%)
Tensor fasciae latae	81 ± 17	75 ± 22	−6	−7
Obturator externus	48 ± 11	43 ± 9	−5	−10
Obturator internus*	51 ± 13	35 ± 10	−17	−33
Quadratus femoris*	29 ± 4	23 ± 6	−6	−21
Piriformis	37 ± 10	32 ± 10	−5	−14
Gluteal muscles	1505 ± 279	1308 ± 220	−197	−13
Gluteus maximus	1036 ± 205	901 ± 155	−135	−13
Gluteus medius	357 ± 64	325 ± 67	−32	−9
Gluteus minimus*	112 ± 26	82 ± 23	−30	−27
Gemelli	28 ± 7	28 ± 8	−0	−1
Gemellus inferior	13 ± 3	13 ± 3	−0	−1
Gemellus superior	15 ± 4	15 ± 6	−0	−1
Iliopsoas*	469 ± 56	365 ± 55	−104	−22
Iliacus*	208 ± 28	178 ± 29	−30	−14
Psoas major*	262 ± 41	187 ± 39	−74	−28

Average values represent means±SD, $n = 10$ per group. The average muscle volume of the left and right body part was taken for comparison between young and older men.
* Significantly different ($P \leq 0.05$) between young and older men.

older (48 ± 2 %) compared with the young (52 ± 2 %) group. This indicates that quadriceps femoris muscle volume is more affected (i.e. lowered) by age when compared to the other thigh muscle groups (i.e. hamstrings and adductors). To further investigate whether there were age-related differences in muscle volume of individual quadriceps femoris muscles, we assessed the proportional differences in individual quadriceps femoris muscle volume to total quadriceps femoris muscle volume in young and older men, but no interaction effect was observed ($P = 0.260$). This indicates that there were no differences in age-related atrophy between the four parts of the quadriceps femoris muscle. In line with the overall quadriceps volume, all individual quadriceps femoris muscle volumes were significantly lower in older compared to young participants (Table 3). Whereas the sartorius muscle volume was also lower in older compared to young participants, only few individual muscles of the adductors and hamstrings differed between groups. When volumes were corrected for muscle length, similar findings were observed, with all thigh muscle groups (i.e. quadriceps femoris, adductors, and hamstrings) as well as total thigh muscle remaining lower in the older compared to the young men (Supplemental Table 2).

For the pelvic muscles, only some individual muscle volumes were lower in the older compared to the young (Table 4). When corrected for muscle length, some of these differences were no longer significant (i.e. obturator internus and iliacus) (Supplemental Table 3). Total pelvic average muscle CSA did not differ between groups.

3.3. Correlations between DXA, single slice CT, and MRI

Strong correlations were observed between all values of DXA, CT, and MRI (Fig. 5). A Pearson r value of 0.91 was observed between DXA-derived leg lean mass and MRI-derived leg muscle mass (Fig. 5A). The correlation between DXA-derived leg lean mass and CT-derived thigh muscle CSA (Fig. 5B) as well as between CT-derived thigh muscle CSA and MRI-derived thigh muscle volume (Fig. 5C) showed a similar Pearson r value of 0.84 and 0.83, respectively. The strongest correlation was observed between CT-derived quadriceps muscle CSA and MRI-derived quadriceps muscle volume ($r = 0.94$; Fig. 5D). A Pearson r value of 0.72 was observed for the correlation between CT-derived thigh muscle (−quadriceps) CSA and MRI-derived thigh muscle (−quadriceps) volume (*data not shown*).

4. Discussion

In the present study we observed that the most profound difference in lower body muscle volume between young and older males were

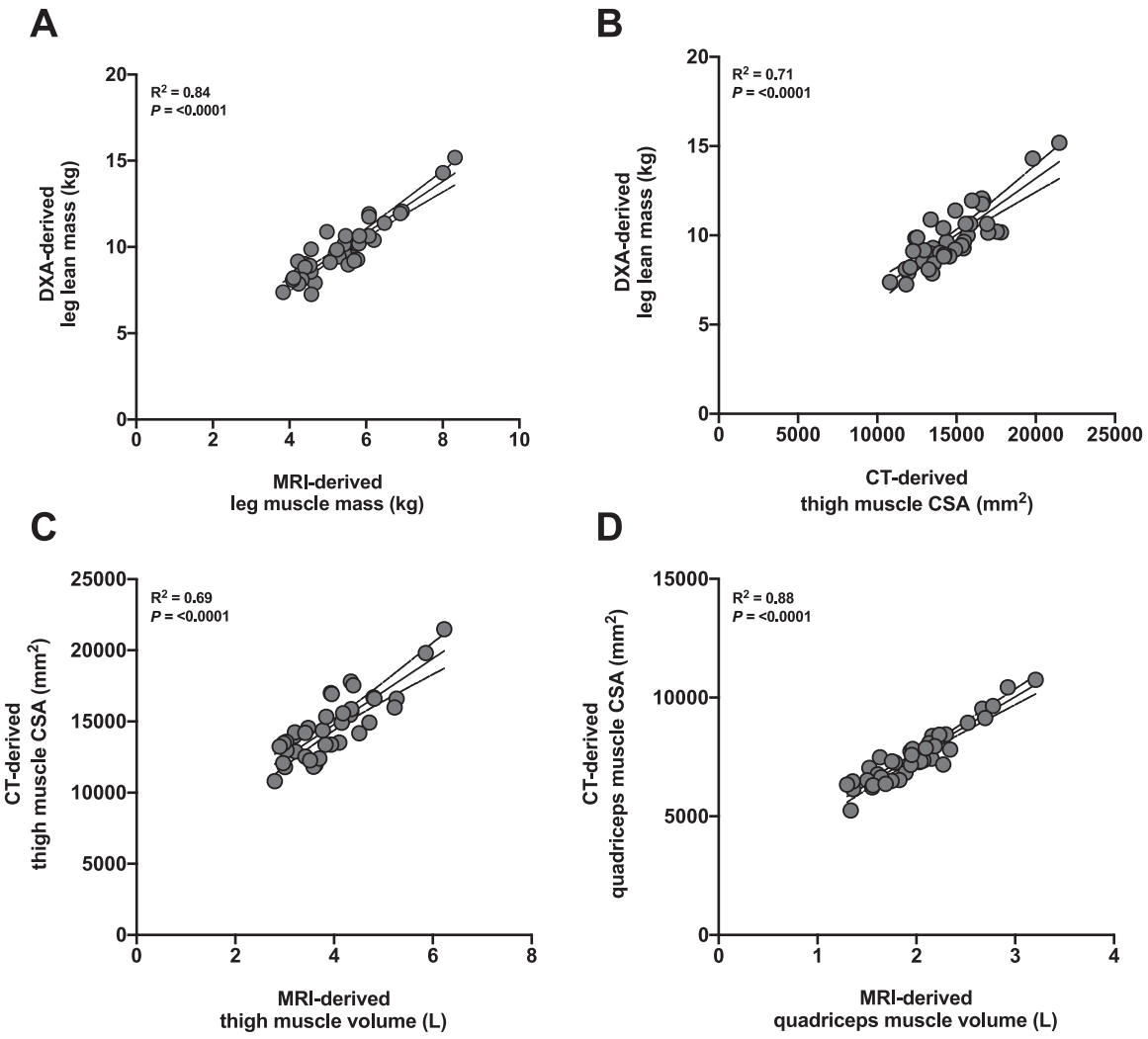

Fig. 5. Scatter plots for comparisons between DXA-derived leg lean mass and MRI-derived leg muscle mass (A), DXA-derived leg lean mass and CT-derived thigh muscle CSA (B), CT-derived thigh muscle CSA and MRI-derived thigh muscle volume (C), and CT-derived quadriceps muscle CSA and MRI-derived quadriceps muscle volume (D) from both legs of healthy, young ($n = 10$) and older ($n = 10$) men. The best fit line with 95 % confidence bands together with the R^2 and P value are provided in all figures. CSA, cross-sectional area.

observed in the thigh. Within the thigh, the quadriceps femoris muscle volume is much lower compared to other muscle groups in healthy older when compared to young men. Though muscle mass as assessed by DXA, CT, and MRI were strongly correlated, differences in muscle mass between young and older adults were easier to detect when applying CT and MRI when compared to the use of DXA.

It has been well-established that muscle mass is generally lower in older when compared to young individuals (Forbes and Reina, 1970; Melton et al., 2000; Baumgartner et al., 1998; Short and Nair, 2000). The age-related decline in skeletal muscle mass is accompanied by impairments in functional capacity and imposes an increased risk of developing chronic metabolic diseases at a more advanced age. The loss of muscle mass with aging has been reported to be most evident in the lower body (Janssen et al., 2000) and is of key concern because of the importance for proper ambulation. Consequently, from both an academic as well as a practical perspective it is imperative to accurately assess leg muscle mass. We first applied DXA (Fig. 1A) to assess total leg lean mass in the healthy, young and older participants (Fig. 2). We observed a trend for a 13 % lower leg lean mass in the older when compared with the young participants ($P = 0.075$). An alternative approach to assess leg muscle mass is to assess the cross-sectional area of the thigh muscles using a single slice CT (Fig. 1B). Using this approach, we confirmed the observations using DXA, with a significant 13 % lower thigh muscle CSA in the older compared to the young group ($P = 0.044$; Fig. 3).

Besides being more sensitive for the assessment of muscle mass compared to DXA, the use of a single slice CT scan also allows the assessment of the cross-sectional area of the various muscle groups in the upper leg. This CSA can be used as a proxy for the actual volume of the various individual muscle groups visible within the cross-sectional area of the single slice CT scan (Fig. 1B). When looking at muscle group specific CSA differences, we found that differences in quadriceps muscle CSA between groups are mainly responsible for the observed differences in total upper leg muscle CSA between the young and the older groups (Fig. 3). Apart from the substantial 20 % lower quadriceps muscle CSA in the older compared to the young adults, no significant differences were observed in muscle CSA for the other thigh muscles (i.e. sartorius, hamstrings, and adductors). The absence of significant differences in cross-sectional area of the various muscle groups between the young and older group may simply be attributed to the inability of the single slice CT approach to properly assess muscle group volume or to the greater susceptibility of the quadriceps muscle to age-related atrophy when compared to other thigh muscle groups (Frontera et al., 2008; Abe et al., 2011; Overend et al., 1992a; Overend et al., 1992b; Abe et al., 2014). As the age-related decline in skeletal muscle volume may be attributed to repeated and accumulative periods of muscle disuse over the lifespan (English and Paddon-Jones, 2010; Oikawa et al., 2019), this greater

susceptibility of the quadriceps muscle to age-related atrophy may (at least in part) be explained by the fact that with muscle disuse also greater declines in quadriceps muscle volume have been observed when compared to the changes in volume of other thigh muscle groups (Belavy et al., 2009; Kilroe et al., 2020).

We also applied MRI to assess muscle volume of all individual muscle groups present in the lower body in both the young and older males (Fig. 1C). Here we confirm our CT data, with quadriceps muscle volume being mainly responsible for the observed differences in total thigh muscle volume between young and older men (Table 3). In addition, our observation of a 30 % lower muscle volume in the quadriceps in older compared to young men agrees with previous work showing a~20–30 % lower quadriceps muscle volume in older compared to the young (Maden-Wilkinson et al., 2014; Ogawa et al., 2012; Maden-Wilkinson et al., 2013; Hogrel et al., 2015; Macaluso et al., 2002; Letocart et al., 2021). Within the quadriceps femoris, we observed that all individual quadriceps muscles were significantly different between young and older men and that there were no (clear) indications of age-related differences between the individual quadriceps femoris muscles (Table 3). Although previous work has shown contrasting findings on the contribution of the individual quadriceps femoris muscles to overall age-related quadriceps atrophy (Maden-Wilkinson et al., 2013; Hogrel et al., 2015; Trappe et al., 2001), our findings support previous findings by Trappe et al. *(Trappe et al., 2001)* (in men and women) and Maden-Wilkinson et al. (Maden-Wilkinson et al., 2013) (in men only), suggesting that all individual quadriceps femoris muscles atrophy to a similar extent.

By using MRI, we also determined muscle volume in the lower leg (Table 2) and hip region (Table 4). Although lower muscle volumes in old vs young were observed for some of the individual muscles in these compartments, we clearly show that the obvious 20 % difference in total lower body muscle volume between young and older men is primarily attributed to differences in thigh muscle volume (Fig. 4). This finding clearly implies that thigh muscles are more susceptible to age-related atrophy when compared to lower leg and pelvic muscles and, thus, when assessing lower body skeletal muscle atrophy and function with aging, the primary focus should be on the thigh muscles.

In the present study the older men were on average shorter than the younger men (Table 1). It could be argued that the difference in height may have influenced the muscle volume results. Therefore, we additionally corrected muscle volume for muscle length to assess average muscle CSA. Importantly, even when correcting for muscle length it was still evident that thigh muscles appear more susceptible to age-related atrophy compared to lower leg and pelvic muscles (Supplemental Tables 1–3).

In the present study we used three different techniques (i.e. DXA, CT, and MRI) that are often applied to assess muscle mass in healthy young and older men. We observed strong correlations between all measurement techniques (Fig. 5). However, only with CT and MRI we were able to assess statistically significant muscle mass differences between groups. For a more direct comparison between DXA and MRI, we also assessed muscle mass with MRI that included the muscles that are being examined in the DXA-derived leg lean mass measurement. Here we observed a significant 22 % lower MRI-derived leg muscle mass in older compared to young men. Our data show that DXA is less sensitive to pick up age-related differences in muscle mass when compared to the use of CT or MRI. This agrees with previous work showing that DXA underestimates the age-related loss in (thigh) muscle mass compared to MRI (Maden-Wilkinson et al., 2013). In addition, this reinforces the concept that the use of only DXA may lead to erroneous conclusions about the importance of muscle mass in health and disease (Evans et al., 2019). Therefore, the present data does not support the contention that DXA should be applied as a reference standard to assess (age-related differences in) muscle mass (as proposed by some (Buckinx et al., 2018), but not others (Clark et al., 2018; Scafoglieri and Clarys, 2018)). We would argue that the advantages of DXA (i.e. wide availability, modest scan costs, low (ionizing) radiation exposure, short scan time, and the provision of extensive body composition information) make it a suitable technique to provide valuable information in large cohort studies where body composition information is warranted. When more sensitivity is warranted for the assessment of muscle mass in particular, a single slice CT scan can be applied. The advantages of (single slice) CT are its (very) short scan time and low costs. However, a clear disadvantage of CT is that only limited information is acquired. Besides the fact that MRI does not expose individuals to ionizing radiation, more information can be acquired on the exact location of differences in skeletal muscle mass between individuals. However, its high costs and time-consuming analyses need to be considered. Therefore, the appropriate methodological approach for assessing muscle mass should be selected based on the scientific question(s) that need to be answered, thereby considering both the advantages and disadvantages of each individual technique (Erlandson et al., 2016).

The present study provides an extensive overview of individual muscle (group) volumes in healthy young and older men. We observed clear heterogenous differences in individual muscle group volumes between young and older males. Aging is generally accompanied by lower physical activity levels (Milanovic et al., 2013) and changes in movement patterns (e.g., arm push-off to stand up in older vs young individuals) (van der Kruk et al., 2022), which are likely explanations for some of the heterogenic differences in muscle volume observed between young and older men. Here, muscle groups that are predominately recruited for locomotion and movement (e.g., quadriceps femoris) demonstrated greater age-related differences compared to other individual muscle groups. Whether other (biological) mechanisms (e.g., neuromuscular, histological, hormonal) also contribute to the observed heterogenous volume differences of individual muscle group volumes between young and older men remains to be elucidated. Irrespective of the mechanisms, the present study provides relevant information that may be relevant when designing a more targeted approach on what muscle groups (e.g., quadriceps femoris) should be prioritized with exercise strategies aimed to preserve muscle mass and/or function over the lifespan.

In conclusion, the most profound differences in lower body muscle volume between healthy young and older males are observed in the thigh. Within the thigh, the quadriceps femoris muscle shows the largest difference in muscle volume between young and older men. DXA is less sensitive to assess age-related differences in muscle mass when compared to the application of CT and MRI.

CRediT authorship contribution statement

Cas J. Fuchs: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing-Original draft, Visualization, Project administration. Remco Kuipers: Validation, Formal analysis, Writing-Reviewing and Editing. Jan A. Rombouts: Validation, Formal analysis, Visualization, Writing-Reviewing and Editing. Kim Brouwers: Methodology, Formal analysis, Investigation, Writing-Reviewing and Editing. Vera B. Schrauwen-Hinderling: Conceptualization, Formal analysis, Writing-Reviewing and Editing. Joachim E. Wildberger: Conceptualization, Resources, Writing-Reviewing and Editing. Lex B. Verdijk: Formal analysis, Writing-Reviewing and Editing. Luc J.C. van Loon: Conceptualization, Writing-Reviewing and Editing, Supervision.

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Declaration of competing interest

Cas J. Fuchs, Remco Kuipers, Jan A. Rombouts, Kim Brouwers, Vera B. Schrauwen-Hinderling, Joachim E. Wildberger, Lex B. Verdijk, and Luc J.C. van Loon declare no conflicts of interest related to this study.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exger.2023.112159.

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