

Reference values for vastus lateralis fiber size and type in healthy subjects over 40 years old: a systematic review and metaanalysis

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Gouzi F, Maury J, Molinari N, Pomiès P, Mercier J, Préfaut C, Hayot M. Reference values for vastus lateralis fiber size and type in healthy subjects over 40 years old: a systematic review and metaanalysis. *J Appl Physiol* 115: 346–354, 2013. First published April 4, 2013; doi:10.1152/jappphysiol.01352.2012.—Skeletal muscle atrophy is a major systemic impairment in chronic diseases. Yet its determinants have been hard to identify because a clear research definition has not been agreed upon. The reduction in muscle fiber cross-sectional area (CSA) is a widely acknowledged marker of muscle atrophy, but no reference values for the muscle fiber CSA at the age of the onset of chronic disease have ever been published. Thus, we aimed to systematically review the studies providing data on fiber CSA and fiber type proportion in the vastus lateralis of the quadriceps of healthy subjects (age >40 yr) and then to pool and analyze the data from the selected studies to determine reference values for fiber CSA. We followed the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and identified 19 studies, including 423 subjects that matched the inclusion criteria. On the basis of fiber type and gender, the mean fiber CSA and the lower limits of normal (LLNs) were (%type I*60) + 1,743 μm^2 and (%type I*60) – 718 μm^2 , respectively, for men; and (%type I*70) + 139 μm^2 and (%type I*70) – 1,485 μm^2 , respectively, for women. There was no significant heterogeneity among subgroups of fiber type and gender. The pooled type I fiber proportion was 50.3% (LLN = 32.9%). In multivariate analysis, fiber CSA was significantly correlated with $\dot{V}\text{O}_{2\text{ peak}}$ ($r = 190.92$; $P = 0.03$), and type I fiber proportion was correlated with age ($r = -0.024$; $P = 0.005$), body mass index ($r = 0.096$; $P = 0.005$), and $\dot{V}\text{O}_{2\text{ peak}}$ ($r = -0.053$; $P = 0.005$). Our metaanalysis of a homogeneous set of studies is the first to provide valuable LLNs for fiber CSA according to fiber type and gender. This analysis will be improved by prospective assessment in well-characterized healthy subjects.

skeletal muscle fiber; atrophy; chronic disease; healthy subject; elderly

SKELETAL MUSCLE ATROPHY IS a major systemic impairment in chronic diseases. In chronic obstructive pulmonary disease (COPD), this muscle atrophy has been characterized by a reduced cross-sectional area (CSA) of the myofibers on the basis of biopsies from the vastus lateralis of the quadriceps (1). Similar changes have been described in chronic heart failure [CHF (48)] and in elderly subjects (13).

In clinical practice, histopathological proof of muscle atrophy is currently not mandatory in the context of chronic disease. Similarly, in research on chronic diseases and aging, the selection of subjects with atrophic muscle is not systemat-

ically based on standardized criteria (13). Despite indirect tools such as muscle imaging (6), the lack of criteria for indisputable proof of muscle atrophy has unfortunately made it difficult for researchers to identify its biological mechanisms and determinants. Reduced muscle fiber CSA on a histopathological sample is a standardized parameter of an atrophic process and may therefore be a valuable tool for research on muscle atrophy because the fiber CSA reduction directly matches the loss in myofibrillar protein (29) and increased proteolysis (30).

Reference values for muscle fiber CSA have never been published, however, particularly for healthy subjects over 40 yr of age, which is generally the age of onset of a chronic disease (74a).

The determination of these values is a particularly complex endeavor for two reasons. First, the fiber CSA depends on the fiber type, and thus muscle fiber typing is mandatory for the assessment of fiber CSA. Second, there is a great heterogeneity of the fiber size in patients and healthy subjects, which has been observed in young subjects (62). This heterogeneity cannot be fully explained by the variability associated with the biopsy site (only 10–15% of variation within the same muscle) (7), and is also due to genetic background (61), gender (45, 67), age (58), physical activity level (45), and body weight (58). Determining reference values for fiber CSA would thus require muscle biopsies in large and well-characterized populations. Another possibility might be to combine the data from control groups of healthy subjects older than 40 yr collected from the literature. We therefore aimed to systematically review the studies providing data on fiber CSA and fiber type proportion in the vastus lateralis of the quadriceps of healthy subjects (age >40 yr) and then to pool and analyze the data from the selected studies to provide reference values and lower limits of normal for muscle fiber CSA.

MATERIALS AND METHODS

This systematic review and metaanalysis was performed according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (50). Methods of analysis and inclusion criteria were specified before the beginning of the study and documented in a protocol. The review team was composed of clinicians in pulmonary rehabilitation, academic physiologists from a university hospital, a methodologist/statistician, and researchers. The group met four times over the course of the review.

Search strategy. A computerized literature search was performed to identify the relevant trials reported in PubMed, Web of Science, Physiotherapy Evidence Database (PEDro), and Cochrane Library from January 1967 to March 2012. Congress abstracts of the European Respiratory Society and American Thoracic Society were screened between 2001 and 2012. Additional published studies were added after analysis of reviews on the topic and on the basis of references in the articles we initially retrieved. Given our study design and the low

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risk of publication bias, unpublished sources were not included. The search was also restricted to English language literature.

A combination of the following medical subject headings (MeSH) terms was used: (muscle fibers, skeletal) AND (vastus lateralis OR quadriceps OR knee-extensor OR knee-extensors OR quadriceps femoris OR muscle, quadriceps) AND (healthy subject* OR elderly OR sedentary subjects OR adult* OR aged OR patient).

To minimize information bias, the study titles and abstracts were screened by three authors (F.G., P.P., J. Maury) and the full texts of the original articles of potentially eligible studies were then retrieved to obtain complete details for inclusion. Study selection was on the basis of agreement of two authors and, in cases of disagreement, the consensus of three authors (F.G., M.H., C.P.) was sought.

Study selection. Table 1 summarizes the inclusion criteria. We included every type of clinical study (observational/comparative studies, longitudinal studies, and randomized controlled trials) in which the outcomes were fiber CSA and fiber type proportions (as at least a secondary outcome) assessed by histochemical or immunohistochemical methods. Both fiber CSA and type were mandatory because fiber CSA varies according to fiber type. The complete methodology had to be provided: biopsy site; reference for the validated technique; methodology for fiber type and size assessment; system for size measurement; and number of fibers analyzed [>100 (7)]. All the selected studies had to have been approved by a local institutional review board. The sample sizes for the CSA analysis were greater than six.

The population was restricted to healthy subjects >40 yr [to obtain values in healthy subjects potentially comparable with those of subjects with a chronic disease (74a)], with no condition susceptible to impact skeletal muscle function. Healthy subjects were defined according to the conclusion of a medical examination performed by a medical doctor. Obesity (70); diabetes (52); glucose intolerance (44); osteoarticular diseases (55); thyroid diseases (9); hormone replacement therapies (8); androgen, growth hormone, or insulin (66) supplementation; and statin use (49), all of which have demonstrated an effect on muscle morphology or function, constituted the noninclusion criteria. Thus, potential comorbidities and treatments of subjects had to be screened clinically. If these pieces of information were lacking in the full text of the published study, the authors of the study were contacted. If no answer was obtained, or if no medical examination with screening of comorbidities and treatments was performed, the study was excluded. Conversely, smoking status was not a criterion, because no definitive evidence of any effect of smoking on the quadriceps muscle has

been demonstrated (4). In addition, reduced physical activity and overweight ($25 < \text{body mass index} < 30 \text{ kg/m}^2$) were not considered as noninclusion criteria. Postoperative assessment and autopsy studies were not included because they are associated with an increased risk of bias. Studies in which CSA values were obtained on single-skinned fibers were not considered in this systematic review.

Data extraction. Data were extracted blindly and independently by two independent authors using a standardized form (see supplementary materials online) (F.G., J. Maury). When discrepancy occurred, the final data record was based on consensus (F.G., J. Maury). The authors of the selected studies were all contacted to complete and verify the data. From the eligible articles, we extracted the fiber CSA (in μm^2) of all fiber types and the fiber type composition (type I/type II ratio, in %). In addition, we extracted the age, sex ratio, body mass index, physical activity level (sedentary/no structured program, 0; active, 1; trained, 2), and $\dot{V}\text{O}_{2\text{ peak}}$ if available, because these parameters have been incriminated in the variability of quadriceps fiber CSA (66).

Study quality assessment. The selected studies were both interventional and noninterventional, and study quality was assessed by the study design and the impact factor of the review in which the article was published. In addition, the methodological quality of the muscle histomorphological measurement and the population description were assessed for each article using a standardized questionnaire specifically designed for this very systematic review. The questionnaire items were devised by the experienced researchers and clinicians of the review team (see supplementary materials online). Finally, the risk of bias (selective reporting, redundancy, etc.) was assessed.

Statistical analysis. Quantitative data are presented as means \pm SD and qualitative data are presented with proportions. If there were subgroups, data were pooled into a single group and the SD was calculated according to the following formula:

$$SD = \sqrt{((n_1/\text{ntot})^2 * SD_1^2) + ((n_2/\text{ntot})^2 * SD_2^2) + \dots}$$

To estimate the pooled SD, we used a Markov chain Monte Carlo approach. Simulated data were generated according to each distribution (each study) and the pooled data set was used to obtain the pooled SD.

Forest plots were used to graphically evaluate both the variability [i.e., SD (74a)] of the data and the weight of each study, according to its population size. Heterogeneity between studies was assessed using the Q statistic and quantified using the I^2 index (36).

Metaanalytic computations were then performed on the all-fiber type CSA and the fiber type I proportion using fixed-effect or random-effect modeling if there was significant heterogeneity on the Q-tests and/or I^2 index $>50\%$. Interrater agreement was measured using a Kappa statistic.

Differences between subgroups were tested using the *t*-test. Regressions were performed for fiber CSA and type I fiber proportion with the following covariates: age; BMI; and $\dot{V}\text{O}_{2\text{ peak}}$, and using univariate and multivariate analyses with group size as the weight factor. Reference equations were established using predictors of fiber CSA and type I proportion in a stepwise, linear regression model. The models were evaluated by correlation coefficient (*r*) and standard error of the mean (SEM). The 95% confidence interval (CI) was calculated as follows: theoretical value \pm 1.64-residual standard deviation (SD). A measured CSA and type I fiber proportion lower than the lower limit of normal (LLN = theoretical value - 1.64-SD) was considered abnormal. $P < 0.05$ was considered statistically significant. Analyses were performed using R.2.13.0 software (Bell Laboratories, USA).

RESULTS

Study selection. Figure 1 details the flow of studies included in the review. A final library of 19 studies involving 423 subjects (64 ± 1 yr) was eligible. (Table 2). Subjects were recruited worldwide, mostly from Nordic European countries

Table 1. Inclusion criteria for the systematic review

Inclusion Criteria	Description
Design	Any type of clinical study: Observational studies (case-control, etc.) Quasiexperimental studies (before-and-after studies, etc.) Randomized control trials
Participants	Healthy subjects aged >40 yr No condition susceptible to impact skeletal muscle function Noninclusion criteria: Obesity Diabetes Glucose intolerance Statins Osteoarticular diseases Thyroid diseases Hormone replacement therapies/androgen, growth hormone or insulin supplementation
Interventions	NA
Comparisons	NA
Outcome measure	Fiber cross-sectional area and type in the vastus lateralis

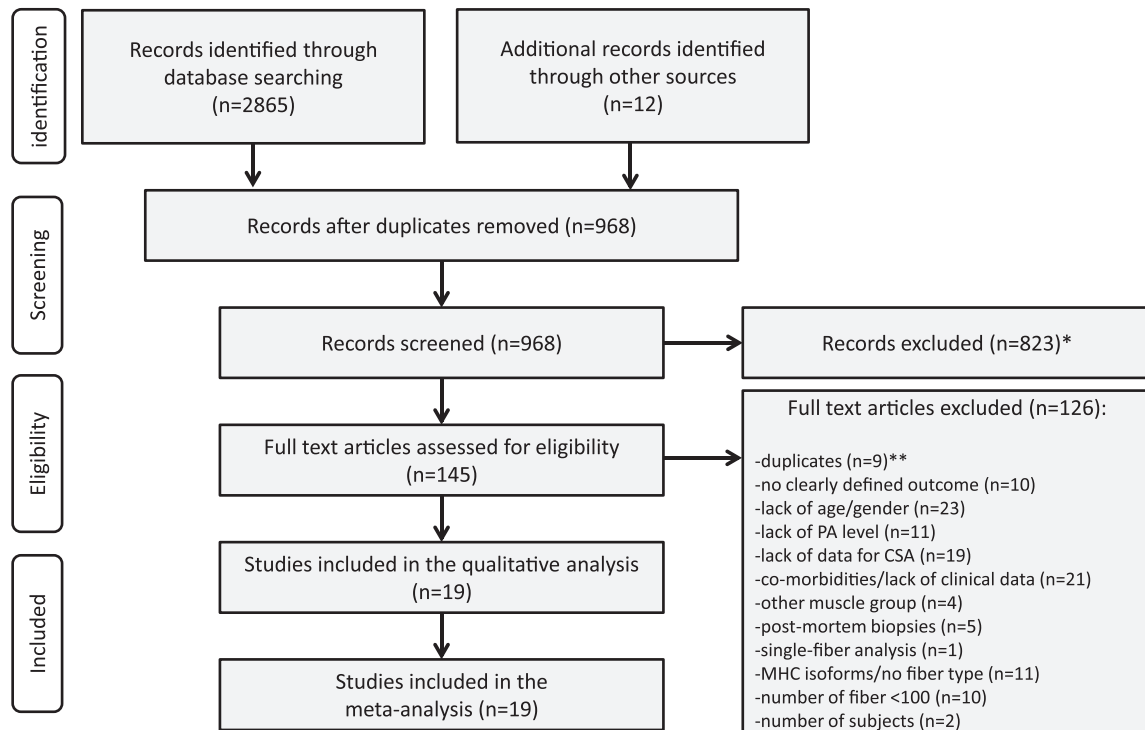


Fig. 1. PRISMA flowchart of the studies enrolled for systematic review. *Presence of a noninclusion criterion on the basis of title and abstract of the record. **Duplicates identified in the full-text article (population being part of a previous study).

and North America. Eighteen studies were identified from reference lists. To improve the precision of the retrieved data, the corresponding authors of 10 out of 31 (32.2%) studies that could potentially be included responded to our request for data that were missing from the full texts of the retrieved manuscripts. The interrater agreement for the study selection and data extraction from the included studies was 93%.

Heterogeneity and pooled results. Fiber CSA varied from $2,858 \pm 648 \mu\text{m}^2$ (11) to $5,892 \pm 1,095 \mu\text{m}^2$ (68). The forest

plot of the fiber CSA data for all the included studies revealed the heterogeneity of the studies (Fig. 2); $Q = 28.1$; $P = 0.06$. The corresponding I^2 index reached 36%. The range for the type I fiber proportion was smaller [from 44% (63) to $59\% \pm 6\%$ (34)]. The forest plot for the type I fiber proportion showed no significant heterogeneity (Fig. 3); $Q = 10.2$; $P = 0.85$.

The meta-analysis was performed using a fixed-effect model. The pooled estimates were $3,630 \pm 114 \mu\text{m}^2$ for fiber CSA (Fig. 2) and $50.3 \pm 1.9\%$ for type I fiber proportion (Fig.

Table 2. Fiber CSA and type I proportion in the included studies

Study No.	Study	N	Age	BMI	M/F	Peak VO ₂	Physical Activity Level	Proportion Type I	All Fiber CSA
1	Essen-Gustavsson et al. 1986 (19)	45	40–80	24.3	23/22		Active/sedentary	0.54 ± 0.05	3749 ± 223
2	Cress et al. 1991 (11)	21	72 ± 6	25.6	0/21	19.95	All levels	0.47	2858 ± 648
3	Hepple et al. 1997 (34)	9	65–73		9/0	27.9 ± 4.5	No structured program	0.59 ± 0.06	3874 ± 942
4	Ferretich et al. 1998 (21)	15	68.1 ± 1.3	24.9	0/21		No structured program	0.49 ± 0.05	4087 ± 488
5	Hakkinen et al. 1998 (31)	10	61 ± 4	27.3	10/0	27.9 ± 1.5	Active	0.48 ± 0.09	4039 ± 700
6	Esmarck et al. 2001 (18)	13	74 ± 3.6	25 ± 3.6	13/0		No structured program	0.54 ± 0.10	4164 ± 815
7	Widrick et al. 2003 (74)	17	52.5 ± 2.1	24.3	0/17		Active/sedentary	0.38 ± 0.06	3061 ± 391
8	Toft et al. 2003 (66)	51	60.7 ± 2.2	26.8 ± 2.8	36/15		All levels	0.58 ± 0.11	5154 ± 1196
9	Whitman et al. 2005 (73)	21	73 ± 8	26.9	11/10		Sedentary	0.54 ± 0.10	4826 ± 851
10	Sinha-Hikim et al. 2006 (63)	36	65 ± 5	27 ± 4	36/0		No structured program	0.44	3339 ± 243
11	Martel et al. 2006 (46)	18	68.6 ± 3.1	26.3 ± 3.1	11/7	22.4 ± 1.3	No structured program	0.49 ± 0.07	2917 ± 356
12	Cristea et al. 2008 (12)	11	67.8 ± 6.2	23.8	11/0		Trained	0.42 ± 0.07	4572 ± 642
13	Green et al. 2009 (28)	8	68 ± 14	27 ± 5.7	0/8		No structured program	0.58 ± 0.13	3372 ± 421
14	Verdijk et al. 2010 (68)	59	72 ± 5	26.8 ± 3.2	59/0		No structured program	0.52 ± 0.13	5892 ± 1095
15	Hvid et al. 2010 (38)	9	67.3 ± 3.9	26.6	9/0		Active	0.56 ± 0.11	4973 ± 1035
16	Vogiatzis et al. 2011 (69)	8	60 ± 5.7	25.6 ± 1.4	8/0	25.1 ± 2.6	No structured PA program	0.49 ± 0.10	3942 ± 973
17	Torres et al. 2011 (67)	26	58 ± 8	26.7 ± 2.7	9/17		No structured PA program	0.53 ± 0.08	5588 ± 1097
18	Flueck et al. 2011 (23)	17	67 ± 2.1	26.9 ± 2.9	11/6		Active	0.53 ± 0.13	5218 ± 1296
19	Gouzi et al. 2012 (27)	23	61.5 ± 5.7	25.9 ± 2.8	11/12	25.7 ± 6	Sedentary	0.45 ± 0.11	4409 ± 1679

BMI, body mass index; M/F, male/female ratio; CSA, cross-sectional area.

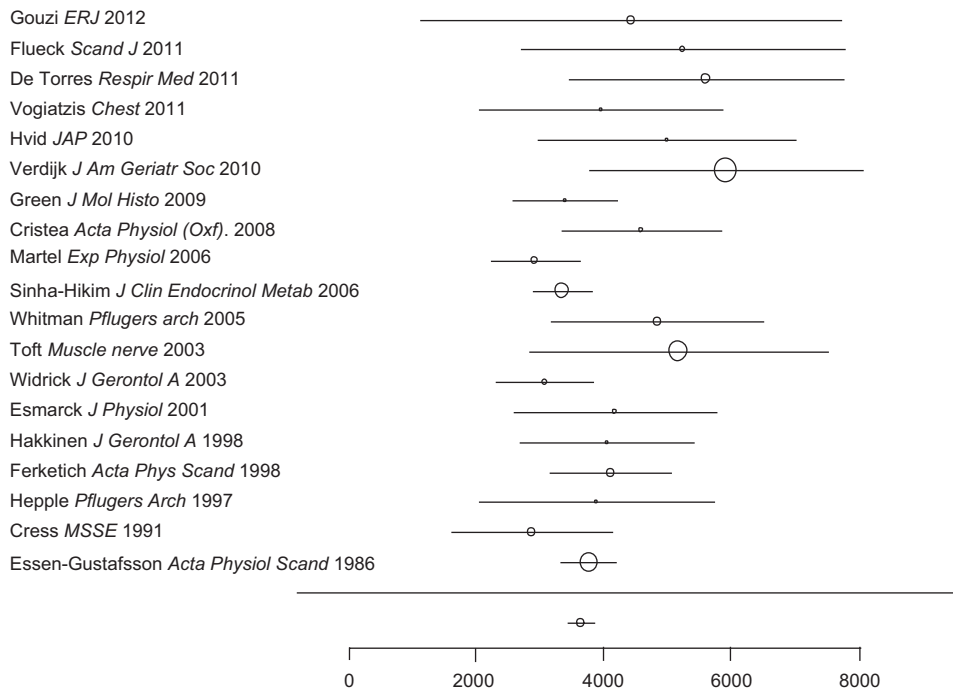


Fig. 2. Forest plot of studies reporting the fiber cross-sectional area (mean and SD, in μm^2) for the 19 studies included in the systematic review.

3). Based on the variability of the selected studies, the simulated SDs were $1,352 \mu\text{m}^2$ for fiber CSA and 10.6% for type I fiber proportion.

Post hoc subgroup comparisons showed significant differences in fiber CSA between men and women: $4,329 \pm 193$ and $3,447 \pm 155 \mu\text{m}^2$, respectively; $P < 0.01$. The simulated SDs of the pooled fiber CSA in men and women were $1,501 \mu\text{m}^2$ and $985 \mu\text{m}^2$, respectively.

Significant differences in fiber CSA between type I and type II fibers were also found: $4,351 \pm 203$ vs. $3,628 \pm 245 \mu\text{m}^2$, $P < 0.001$ in men; and $3,829 \pm 180$ vs. $2,652 \pm 196 \mu\text{m}^2$, $P < 0.001$ in women. There was no significant heterogeneity in CSA per gender or fiber type (in men, type I $Q = 19$, $P = 0.1$;

type II $Q = 11$, $P = 0.54$; in women, type I $Q = 4.62$, $P = 0.87$; type II, $Q = 5.9$, $P = 0.75$). Only 10 and 8 studies of men and women, respectively, provided results per each fiber type ($n = 266$). The resulting means and simulated SDs per gender and fiber type were as follows: men ($n = 141$) type I, $4,652 \pm 1,391$, type IIa, $4,167 \pm 1,630$, type IIx, $3,697 \pm 1,577$; women ($n = 125$) type I, $3,840 \pm 1,587$, type IIa, $3,056 \pm 1,598$; type IIx, $2,033 \pm 1,519$.

The difference in physical activity level was also significantly different (sedentary/no structured physical activity program vs. active: $3,552 \pm 154 \mu\text{m}^2$ vs. $4,517 \pm 408 \mu\text{m}^2$; $P < 0.001$), but the sex ratio was higher in the sedentary subgroup than in the active subgroup (men/women: 52/6 vs. 167/75),

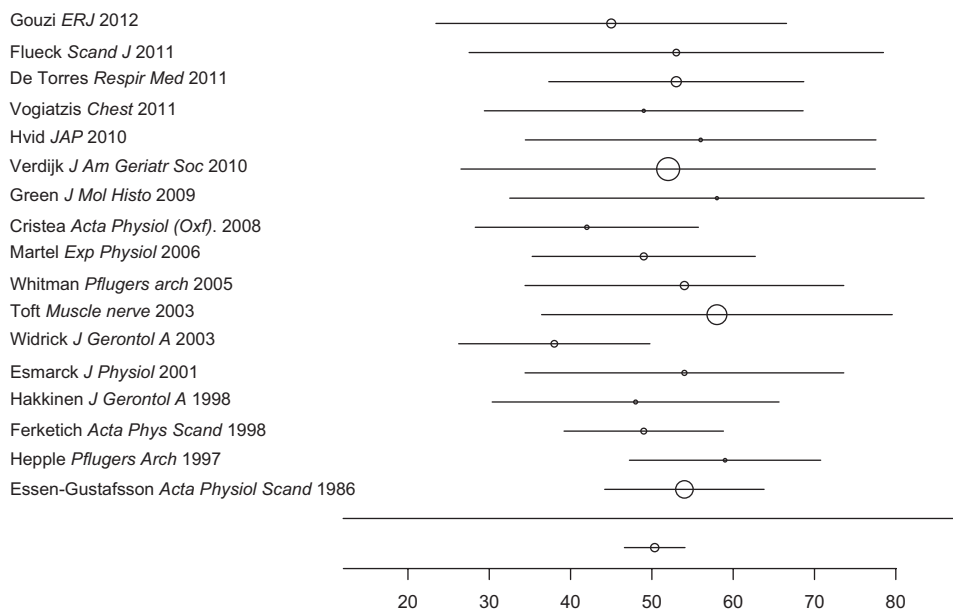


Fig. 3. Forest plot of studies reporting the type I fiber proportion (mean and SD, in %) for the 19 studies included in the systematic review.

suggesting that the increased fiber CSA in the active group may have been the effect of the higher proportion of men in this latter group. There was no significant difference between men and women in type I fiber proportion: 53.9 ± 2.8 and 48.1 ± 2.6 , respectively, $P = 0.33$; nor between sedentary/no structured physical activity program and active: 53.2 ± 2.3 and 47.4 ± 4.6 , respectively, $P = 0.41$.

Regression analyses. The univariate analysis showed significant correlations between fiber CSA and body mass index ($P = 0.057$), $\dot{V}O_{2\text{ peak}}$ ($P = 0.035$), and type I fibers ($P = 0.035$). In multivariate analysis, only $\dot{V}O_{2\text{ peak}}$ remained significant ($\beta = 190.92$, $P = 0.03$; see Table 3). In multivariate analysis (see Table 4 and Fig. 4), we found significant correlations between type I fiber proportion and age ($\beta = -0.024$; $P = 0.005$), body mass index ($\beta = 0.096$; $P = 0.005$), and $\dot{V}O_{2\text{ peak}}$ ($\beta = -0.053$; $P = 0.005$).

Quality assessment of the studies. The interobserver agreement between F.G. and J. Maury was $>95\%$. Quality assessment is summarized in the online supplementary materials. The main discrepancies between the studies concerned study outcomes, reporting on the management of potential pathologies and treatments, exercise capacity assessment, and number of fibers analyzed (from 104 to 387). We observed very little description of ethnic origin (in no study), physical activity level (in 9 studies), and smoking status (in 6 studies).

Prediction equations and lower/upper limits of normal. The number of studies ($n = 10$ and $n = 8$ for men and women, respectively) and subjects ($n = 141$ and $n = 125$ for men and women, respectively) providing data per each fiber type was not enough to determine valid LLNs, in particular for type IIx fiber in women. According to type I and type II fibers, the LLNs were $1,642 \mu\text{m}^2$ and $593 \mu\text{m}^2$, respectively, in men ($n = 247$); and $2,084 \mu\text{m}^2$ and $338 \mu\text{m}^2$, respectively, in women ($n = 148$). We also provide prediction equations and LLNs for the pooled fiber CSA per each gender and according to the type I fiber proportion, expressed in % ($n = 267$ and $n = 156$ for men and women, respectively). Given the β of the type I fiber proportion (in %) in the regression analysis, the prediction equations for the fiber CSA were as follows: male fiber CSA = (%type I-60) + $1,743 \mu\text{m}^2$; female fiber CSA = (%type I-70) + $139 \mu\text{m}^2$.

Therefore, the LLNs for fiber CSA were (%type I-60) - $718 \mu\text{m}^2$ and (%type I-70) - $1,485 \mu\text{m}^2$, in men and women, respectively.

DISCUSSION

The major finding of our metaanalysis is that the vastus lateralis of the quadriceps of a healthy subject over 40 yr old is characterized by a mean fiber CSA of (%type I-60) + $1,743 \mu\text{m}^2$ and (%type I-70) + $139 \mu\text{m}^2$ in men and women, respectively. The variability in the fiber CSA was explained by gender, fiber type proportion, and $\dot{V}O_{2\text{ peak}}$. However, this variability remained substantial in subgroups. The LLNs were thus (%type I-60) - $718 \mu\text{m}^2$ and (%type I-70) - $1,485 \mu\text{m}^2$ in men and women, respectively.

Table 3. Multivariate regression analysis for fiber CSA

	Estimate (β)	SEM	<i>t</i>	<i>P</i>
Intercept	-996	1,467	-0.68	0.53
Peak $\dot{V}O_2$	191	60	3.16	0.034

Table 4. Multivariate regression analysis for type I fiber proportion

	Estimate (β)	SEM	<i>t</i>	<i>P</i>
Intercept	0.834	0.009	92.74	0.007
Age	-0.025	0.0002	-118.19	0.005
Body mass index	0.096	0.001	138.27	0.005
Peak $\dot{V}O_2$	-0.053	0.0004	-125.57	0.005

Variability and physiological factors. Our analyses revealed that the fiber CSA depended on gender, fiber type proportion, and $\dot{V}O_{2\text{ peak}}$. The physical activity level was also associated with fiber CSA in the univariate analysis. These findings are consistent with basic muscle physiology (19, 67) and highlight the validity of the present systematic review. Interestingly, in our multivariate regression, age was inversely correlated with type I fiber proportion, whereas a type I fiber predominance and a decrease in type II fibers has been classically reported (58). Moreover, in contrast with former studies showing reduction in all (19) and type II fiber size (54, 58), age did not alter fiber CSA in our >40 yr old subjects. However, over an 8-yr prospective study of healthy elderly [older than the subjects of the previous studies (19, 54, 58)] with stable physical activity levels, no significant change in fiber type proportion or size occurred (24). These discrepancies may thus be explained by the progressive physical activity reduction associated with aging. Therefore, taking physical activity level into account in the multivariate analysis, the independent relationship between type I fiber proportion and age would be inverse. Similarly, the type I fiber proportion was positively correlated with body mass index. An increase in fiber CSA (42, 70) and a reduction in type I fiber proportion (35, 65) have been reported in obesity. Here again, taking into account the physical activity reduction (which occurs with increases in body mass index) and multivariate analysis did not reveal a correlation between body mass index and fiber CSA in our nonobese subjects.

Variability and quality assessment. The quality assessment indicated little discrepancy in the study designs and methodologies, but a low quality in reporting of populations (i.e., potential pathologies and treatments, ethnic origin, and physical activity level/exercise capacity of the subjects). In all but 1 of the 19 studies included in the review, muscle sampling was performed using the percutaneous biopsy needle technique described by Bergström (5) and modified by Evans et al. (20). Only Flueck et al. (23) used a conchotome technique, which appears to be satisfactory for most histochemical analyses (16). Thus, the fiber CSA in the study by Flueck et al. did not appear discrepant with the others. The transverse sections of the fibers were stained using the same protocols as in histochemical or immunohistochemical methods ($n = 3$). Good agreement between the two methods for assessing type I fiber proportion has been demonstrated (32).

No study detailed the subjects' ethnic origins [which may influence the muscle histomorphology (10, 41, 72)]. In addition, smoking status was mentioned in only six studies. Because smoking may have a deleterious effect on muscle function (43, 75) and structure (51, 53), this factor may explain some of the variability in the muscle histology of these healthy subjects. Last but not least, exercise capacity was assessed in 7 of the 19 studies, and physical activity level was assessed by a

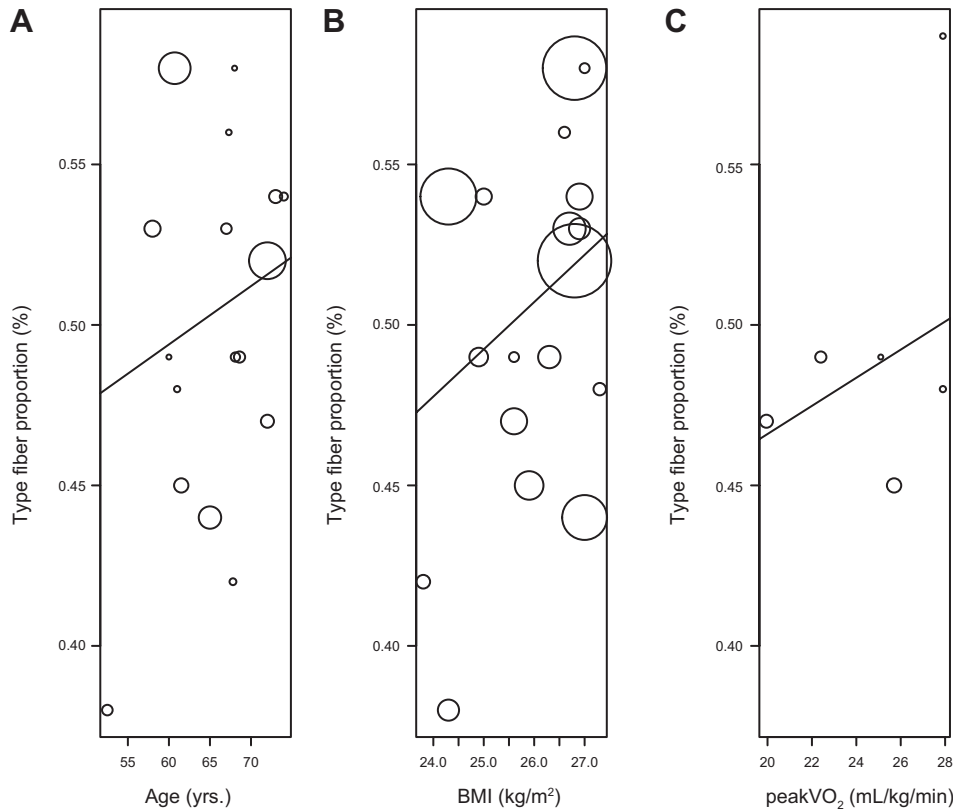


Fig. 4. Relationships between vastus lateralis fiber type I proportion and age (in years) (A); body mass index (in kg/m²) (B), and $\dot{V}O_{2\text{peak}}$ (in ml·kg⁻¹·min⁻¹) (C). Circle sizes represent group sizes. Solid lines represent the weighted linear regression lines (if statistically significant only). Bold circles appear in cases of two overlying identical data sets.

simple question in 9 of the 19 studies. Given the impact of $\dot{V}O_{2\text{peak}}$ and physical activity level on muscle fiber CSA and proportion, the lack of these latter data may be considered as a methodological flaw in these studies and would thus also explain an important part of the variability in the results of the present systematic review.

Conversely, healthy subjects have been defined on the basis of the same criteria, which was a direct clinical examination performed by a medical doctor, allowing the screening of clinical comorbidities and treatments. In addition, a cardiopulmonary exercise testing with measurement of maximal oxygen uptake was available in 8 of the 19 studies. Obese patients were excluded on the basis of body mass index, and at least one specific test for the screening of potential comorbidities had been performed in all studies [electrocardiogram ($n = 10$), body composition analysis ($n = 7$), spirometry ($n = 4$), muscle function test ($n = 12$), glucose tolerance test ($n = 2$), other biological assay ($n = 3$)]. If each infraclinical comorbidity had not been eliminated by the multiplication of systematic investigations, the probability of including subjects with an infraclinical comorbidity and then increasing the variability of our results was reduced to its minimal. Then, the included studies would match the current highest standard for the definition of a “healthy subject” in scientific studies. This definition has constituted the basis of the definition of muscle fiber impairments observed in the context of a chronic disease. Altogether, the results are consistent with the aim of our systematic review and metaanalysis, which was to provide reference values for the vastus lateralis fiber CSA in healthy subjects, to define muscle fiber atrophy in research studies on chronic diseases.

In contrast, although the authors of the studies in our review confirmed that they did not include healthy subjects with any

condition or treatment susceptible to impact muscle morphology or function, we observed low quality of reporting of the management of potential medication and pathologies. Yet the quality of reporting does not necessarily reflect the quality of the underlying data, methods, or inclusion criteria of a population, and failure to report data or a method does not necessarily mean it has not been used (15, 37, 64). Thus, if this incomplete reporting does not constitute a bias in our systematic review, it is an indication of the required level of reporting the definition of a healthy subject > 40 yr in the included publications.

Reference values. Although some research groups provided reference values for fiber CSA, these data can be disputed because of the low number of subjects included [$n = 74$ (26), $n = 59$ (68)]. In our study, we pooled a larger number of data items ($n = 423$) from 19 studies showing relatively little heterogeneity [and nonsignificant among subgroups (36)] using a validated method to assess the mean and LLN of fiber CSA per fiber type and gender. If the variability in CSA and the number of observations lead to low LLNs per fiber type (type II in particular), the LLNs for fiber CSA according to fiber type proportion provide a relevant LLN for the usual type I fiber proportions of the vastus lateralis in patients with chronic disease.

We used a similar and appropriate systematic screening process to retrieve nine additional studies providing fiber type proportions only, but none met the inclusion criteria of our systematic review. However, because the means and SDs provided for type I fiber proportions resulted from a systematic review process, our calculation of a type I fiber proportion of 32.9% constitutes a valid LLN. In contrast, the 27% in healthy subjects provided by Gosker et al. did not result from a

systematic review process (26). Therefore, the means and LLNs for fiber CSA per fiber type and type I fiber proportion that we present here are currently the most valid reference values for assessing the histomorphological parameters of muscle in chronic diseases.

Consistently with the observations in young subjects (62), the CVs obtained with the simulated SDs for type I and type II fibers were 38% and 51% in men and 46% and 53% in women, respectively, and 20% for the type I fiber proportion, meaning that the variability in fiber CSA and type I proportion remained high (47). Thus, given the impact of anthropometric parameters, tobacco smoking and exercise capacity/physical activity level on fiber CSA and type I proportion, these reference values could probably be improved by further inclusion of studies with well-characterized healthy subjects over 40 yr old.

Research and clinical implications. The critical muscle event in chronic disease is the onset of atrophy because it is a prognostic factor in many chronic conditions such as COPD, CHF (25) and aging (13). However, there is currently no validated tool to define muscle atrophy (71). In research, patients/elderly subjects with muscle atrophy have been isolated according to their body mass index (2, 60) or physical functioning (39). This poor definition of atrophy increases the risk of false-negative results in studies aiming to isolate its underlying biological mechanisms. Cellular atrophy may be a more precise marker of muscle atrophy because it directly reflects an abnormal process in the contractile compartment of the whole organ, in contrast to muscle strength, which depends on several factors. It is therefore widely used as a marker of catabolic/anabolic imbalance. Disuse (14, 22), denervation (57), and cachexia (56, 71) models have demonstrated the direct and early effect on fiber CSA.

In addition, there are currently no technical limitations in assessing fiber CSA. A mini-invasive method has been validated (33), and a rapid automated image analysis system has shown promising accuracy (59). Conversely, body imaging techniques such as computed tomography (6) are limited by cost, accessibility, and concerns about radiation exposure (13). Therefore, the definition of the normal range for muscle fiber CSA will advance the research on muscle atrophy by offering the possibility of selection or stratification of subjects and patients according to occurrence of cellular atrophy. Last, the definition of subjects/patients with cellular atrophy would constitute a gold standard for validating noninvasive diagnostic tools of muscle mass assessment.

In conclusion, our study is the first to provide the lower limits of normal for fiber CSA according to fiber type. According to fiber type and gender, the LLNs for men and women were (%type I-60) – 718 μm^2 and (%type I-70) – 1,485 μm^2 , respectively. There was no significant heterogeneity among subgroups. In addition, the LLN for type I fiber proportion was 32.9%. These reference values will help to better define muscle atrophy in research studies. Ongoing work with inclusions of new studies of well-characterized populations of healthy subjects will further optimize these reference values by reducing the present variability in fiber CSA and proportions.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: F.G., N.M., C.-G.P., and M.H. conception and design of research; F.G. performed experiments; F.G., J. Maury, N.M., P.P., and M.H. analyzed data; F.G., J. Maury, N.M., P.P., J. Mercier, C.-G.P., and M.H. interpreted results of experiments; F.G. and N.M. prepared figures; F.G. drafted manuscript; F.G. edited and revised manuscript; F.G., J. Maury, N.M., P.P., J. Mercier, C.-G.P., and M.H. approved final version of manuscript.

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