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Association among measurements obtained using portable ultrasonography with sex, body mass index, and age in a large sample of adult population

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Abstract

Background The assessment of the site-specific distribution of subcutaneous tissues (ST) represents an important addition to the body composition (BC) estimation. In this context, ultrasound stands out as a valuable method. However, the absence of reference values complicates both the interpretation of the obtained parameters and the monitoring of their changes during nutritional interventions or training programs.

Methods A total of 6,270 ultrasound images obtained with a technique using a non-diagnostic B-mode 2.5 MHz ultrasound probe (BodyMetrixTM, IntelaMetrix, Inc., Livermore, CA, USA) were obtained from adult men and women and analysed by grouping them according to sex, body mass index (BMI), and age. The thicknesses of total (tSAT), superficial (sSAT), and deep (dSAT) subcutaneous adipose tissue and muscle (MT), their ratios (sSAT/dSAT and MT/SAT), and muscle echogenicity (EG) were measured at the abdomen, thigh, chest, triceps, and hip sites.

Results Women exhibited greater tSAT, sSAT, dSAT, and EG ($p < 0.001$) and lower MT ($p < 0.001$), sSAT/dSAT ($p = 0.008$ for the abdomen and $p < 0.001$ for the thigh), and MT/SAT ($p < 0.001$) compared to men at both the abdomen and thigh sites. SAT-related parameters showed significant correlations with BMI, with thicknesses correlating positively and ratios negatively ($p < 0.001$). Muscle-related parameters correlated significantly with age, with MT and EG showing positive correlations and MT/SAT showing negative correlations ($p < 0.001$) in both sexes. Among SAT parameters, dSAT showed the strongest association with BMI in both men and women.

Conclusions This study is the first to report the mean values of SAT-related quantitative and qualitative measurements (thickness and EG) in various body sites assessed through ultrasound and their associations with

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sex, BMI, and age in a large cohort. These findings could prove valuable in clinical practice for precise monitoring of nutritional and/or training interventions, as well as for a more comprehensive evaluation of nutritional status.

Keywords Ultrasound, Subcutaneous adipose tissue, Muscle, Echogenicity, Tissue quality, Obesity

Background

The use of ultrasound for the assessment of subcutaneous tissues (ST) dates back many decades [1–3]. It has been consolidated over time due to the accuracy of the measurement, as well as the possibility of overcoming the limitation associated with a total body assessment, which becomes regional [2]. Although it is a safe, quick, and relatively inexpensive method, it requires performance by highly skilled medical personnel [2].

Ultrasounds are mechanical, elastic, longitudinal waves with small lengths and high frequencies, not carrying matter and penetrating through body tissue [4, 5]. Ultrasounds are generated from specific materials (quartz crystals or ceramics) that, when subjected to an electric field, undergo mechanical deformation and generate high-frequency vibrations. This property, called piezoelectricity, is characteristic of the crystals contained within the ultrasound probe [5]. When ultrasound passes through a tissue, it generates a reflected beam that is called an echo. The moment it 'hits an obstacle', the echo returns back to the probe, exciting the crystal. In this way, the ultrasound is converted into an electrical signal, which is then processed by specific software [5]. Generally, ultrasound measurements can be performed in two main modes: (i) amplitude mode (A-mode) - in which a graph is produced with spikes referring to and highlighting the transition point between two different tissues [6] and (ii) brightness mode (B-mode) - in which a two-dimensional image is generated [5]. In B-mode, therefore, the ultrasound image is constructed by converting the reflected beam waves into light signals. In this conversion, the brightness (expressed in shades of grey) is directly proportional to the intensity of the echo and, based on the spatial relationships between the echoes, images of the analysed sections are processed, providing a two-dimensional view. In B-mode analysis, image quality is improved by the introduction of the grey scale: in this scale, the amplitude of the echoes, is represented by different shades of grey (referring to the image brightness), ranging from white (hyperechogenic) to black (anaechogenic) [2, 7, 8]. With this encoding, structures such as the skin-subcutaneous adipose tissue (SAT), SAT-muscle, or muscle-bone interfaces will generate stronger reflections and present as a continuous white band in the two-dimensional ultrasound image, allowing the identification of individual ST [2].

By exploiting the basic physical principles, technological advances have simplified the use of traditional ultrasound for evaluation of ST, with portable ultrasound probes operating in A-mode or B-mode, that, by using specific coupled software for the ultrasound scan processing, allow the ST to be measured objectively and non-diagnostically at an entire body site [2], and can therefore be used by non-medical healthcare personnel such as nutritionists or dietitians, for example.

The measurements of ST thus obtained have been previously validated in studies on both dissected cadavers [9] and living humans [10], reporting a high precision comparable with traditional ultrasound methods. Similarly, a recent study emphasised its reliability, repeatability, and absence of intra- and inter- operator variability of the measurement [11].

However, a number of other studies in the past focused on this ultrasound measurement of SAT in sites for the skinfolds method and used these data to estimate body composition (BC). Comparisons of the results obtained with other recognised methods such as skinfolds [12, 13], bioelectrical impedance analysis [14], and air displacement plethysmography [15], demonstrated a high level of agreement. Nevertheless, it should be noted that the usefulness of an ultrasound quantitative evaluation of ST in various settings (including clinical and sport) is beyond the mere estimation of BC.

Being a non-diagnostic method and providing an accurate measurement of ST thicknesses, these ultrasound measurements can be particularly useful in the nutritionist/dietitian's clinical practice in order to highlight and monitor the distribution of SAT and muscle at the site level. Since ultrasound measurement is not affected by intrinsic factors that represent a limitation for common techniques for the estimation of BC, it makes it possible to better overcome the limits linked to the use of classic nutritional indices (including the simple body mass index, BMI) [16], again bypassing the mere concept of total body BC, with a view to a multifactorial evaluation of the nutritional status, as previously emphasised by other authors referring to the use of ultrasound in the nutritional field [17–22].

It must be stressed, however, that despite the discrete number of publications on ultrasound ST measurements, there are currently no population-specific reference values available that would allow the ultrasound measurements to be considered normal or, at the least, acceptable. Also, studies reporting the ultrasound-derived parameters in large cohorts stratified by sex, BMI, and age have not been conducted. These data are of relevance for their use in clinical practice due to the reported relationship of SAT and muscle-related parameters assessed by traditional ultrasound with sex, BMI, and age [23–25]. To overcome this gap, the present observational retrospective study aims to identify for the first time the average values of ST thicknesses (SAT and its components, superficial SAT [sSAT], deep SAT [dSAT], and muscle [MT]) and muscle echogenicity (EG) revealed at various body sites (abdomen, thigh, chest, hip, and triceps) in a large cohort of subjects stratified for sex, BMI, and quartiles of age, evaluating the relative associations.

Materials and methods

Design and setting

This is a retrospective observational study conducted on ultrasound scans (stratigraphies) from different classes of subjects. Specifically, demographic, anthropometric, and ultrasound data were independently collected and provided by Hosand Technologies s.r.l. (Verbania, Italy) for blinded retrospective statistical analysis. All participants signed the informed consent according to current legislation. The protocol of this retrospective study was approved by the Local Ethics Committee of the Università Telematica Pegaso by resolution no. PROT./E 005082 of 19/07/2024.

A total of 7,190 stratigraphies from only adult subjects were analysed. Stratigraphies from subjects with age < 18 years were not collected. Among the stratigraphies collected, those without BMI data or taken on subjects with a BMI < 18.50 kg/m^2 (underweight, $n = 25$ men and 66 women) were excluded from statistical analyses. The flowchart of all collected stratigraphies, those excluded and those analysed, for single body site, in men and women, is shown in Fig. 1.

Demographic and anthropometric parameters

Demographic and anthropometric data were collected during the nutritional assessment where ultrasound evaluations were performed. Given the design of the present study, the only demographic data collected and provided were sex and age of the participants. In addition, no details about physical activity levels or training programs were collected. However, ultrasound scans were performed at least 24 h after the last training session, as *per* the manufacturer's instructions. Weight and height were measured using professional instruments in the possession of the professional who carried out the nutritional assessment. BMI was calculated as the ratio of weight (expressed in kg) over the square of height (expressed in m^2). In accordance with the World Health Organisation classification [26], on the basis of the BMI value, the subjects were classified into the following categories: normal weight (BMI 18.50–24.99 kg/m^2), overweight (BMI 25.0–29.99 kg/m^2), and obesity (≥ 30.0 kg/m^2).

Ultrasound assessment

As previously described [2], ultrasound measurements of SAT and muscle thicknesses (MT) were performed using

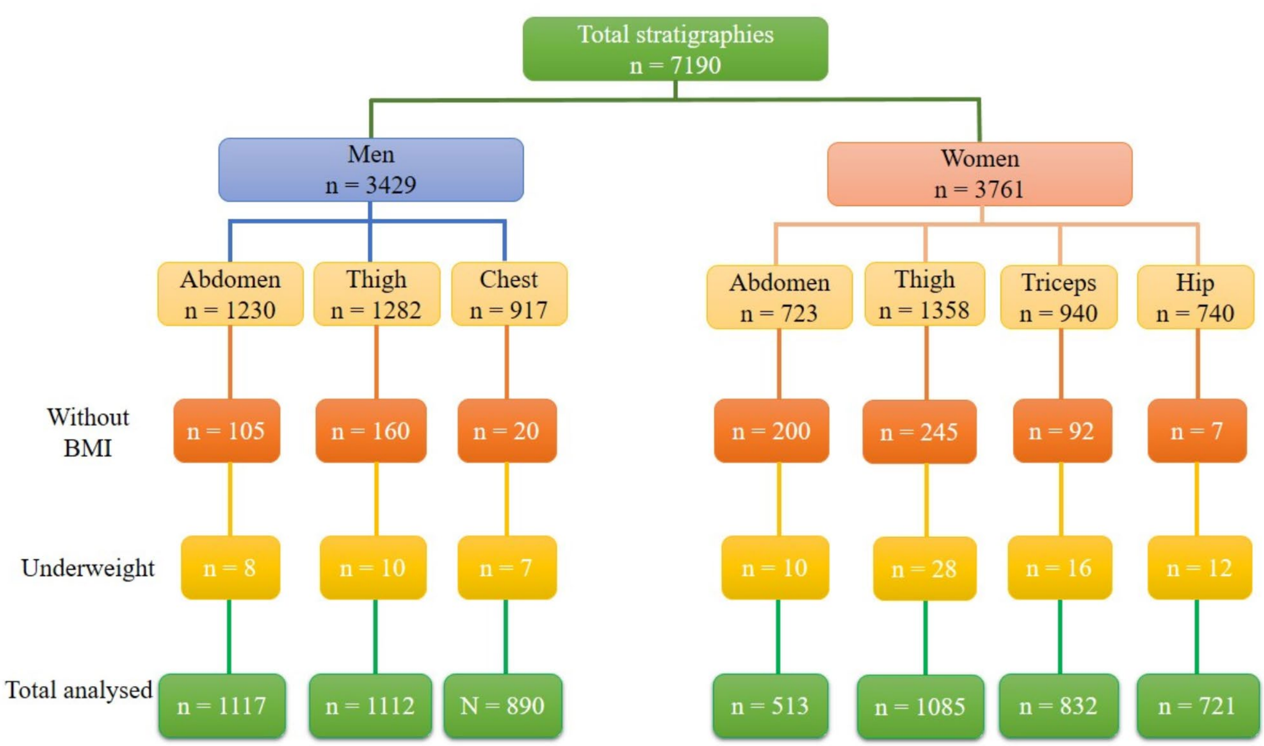

Fig. 1 Flow chart of study participants. BMI, body mass index

the BodyMetrixTM device (IntelaMetrix, Inc., Livermore, CA, USA), a portable ultrasound probe (13 cm × 5 cm), weighing approximately 200 g, with a frequency of 2.5 MHz, non-diagnostic, designed for the measurement of adipose and muscle tissue thicknesses. The probe is connected to a computer or tablet *via* a USB cable and operates through a special application owned by Hosand Technologies s.r.l. (Verbania, Italy). The ultrasound images acquired are subsequently analysed using specific software (Adipometria v 1.8.8, Hosand Technologies s.r.l., Verbania, Italy).

The ultrasound measurements, carried out following the manufacturer's instructions, were obtained at the following body sites (Supplementary Figs. 1–6): abdomen and thigh in men and women; hip and triceps in women only; chest in men only. The acquired ultrasound images (stratigraphies) were processed using dedicated software (Adipometria v 1.8.8, Hosand Technologies s.r.l., Verbania, Italy). The complete ultrasound protocol performed at each body site is detailed in Supplementary Materials.

The thickness measurements obtained were sSAT, dSAT, total SAT (tSAT, given by the sum of sSAT and dSAT), and MT. Starting from these measurements, the following ratios were mathematically calculated directly by the software (Adipometria v 1.8.8., Hosand Technologies s.r.l., Verbania, Italy):

- sSAT/dSAT: ratio of sSAT to dSAT, providing an understanding of the type of SAT most prevalent in a specific body area.
- MT/SAT: ratio between muscle and tSAT, providing how much adipose tissue is present in relation to muscle.

In addition, muscle EG was measured through the calculation of the ultrasound signal intensity point by point within the entire tissue selected in the stratigraphy by software (Adipometria v 1.8.8., Hosand Technologies s.r.l., Verbania, Italy), returning a percentage value.

Statistical analysis

Results are expressed as mean ± standard deviation (SD). Differences in the single parameters evaluated between women and men were analysed by unpaired Student's *t*-test. The correlation study between the parameters evaluated was performed using the Pearson *r* correlation coefficient. Continuous variables normally distributed were compared by the one-way analysis of variance (ANOVA), followed by the Bonferroni *post-hoc* test. A multiple regression analysis model (stepwise method), expressed as Beta (β), *t*, R^2, with BMI as the dependent variable, was used to estimate the predictive values of age, sSAT, dSAT, tSAT, sSAT/dSAT, MT, and MT/SAT in both women and men for the sites abdomen and thigh. Variables with a variance inflation factor (VIF) > 10 were excluded to avoid multicollinearity. Values ≤ 5% were considered statistically significant. All the statistical analyses were conducted using the IBM SPSS Statistics Software (PASW Version 21.0, SPSS Inc., Chicago, IL, USA).

Results

In statistical analysis, each body site has been considered as a single study population. Therefore, the demographic and anthropometric parameters, as well as the ultrasound parameters were analysed individually. Table 1 reports the parameters for abdomen and thigh sites, while Table 2 reports the parameters for hip, triceps, and chest sites. For all sites analysed, the mean BMI values were in women (kg/m^2): 25.42 ± 5.08, 25.16 ± 4.66, 25.43 ± 4.37, and 25.90 ± 4.74 for abdomen, thigh, hip, and triceps, respectively; in men (kg/m^2): 25.63 ± 4.06, 25.50 ± 3.97, and 25.09 ± 3.42 for abdomen, thigh, and chest, respectively. However, in subsequent analyses, individual populations were stratified by BMI categories. Only for the abdomen and thigh the differences of the averages of individual parameters assessed between men and women were analysed. For both sites, mean BMI values were not statistically different ($p = 0.385$ and 0.066 for abdomen and thigh, respectively), but women were on average older than men (years: 39.27 ± 14.85 vs. 34.55 ± 12.82 and 40.22 ± 14.23 vs. 31.13 ± 12.83, women vs. men in abdomen and thigh, respectively; $p < 0.001$ for both sites). For the abdomen and thigh, women had significantly higher values of tSAT (Δ = + 6.04 mm and + 9.94 mm in abdomen and thigh, respectively; $p < 0.001$ for both sites), sSAT (Δ = + 2.12 mm and + 2.16 mm in abdomen and thigh, respectively; $p < 0.001$ for both sites), dSAT (Δ = + 3.34 mm and + 5.94 mm in abdomen and thigh, respectively; $p < 0.001$ for both sites) and muscle EG (Δ = + 4.79 and + 4.50 in abdomen and thigh, respectively; $p < 0.001$ for both sites) than men and significantly lower values of sSAT/dSAT (Δ = – 0.07 and – 0.33 in abdomen and thigh, $p = 0.008$ and $p < 0.001$, respectively), MT (Δ = – 3.21 mm and – 6.37 mm in abdomen and thigh, respectively; $p < 0.001$ for both sites) and MT/SAT ratios (Δ = – 0.62 and – 4.01 in abdomen and thigh, respectively; $p < 0.001$ for both sites).

Populations were stratified by BMI and age quartiles. The distribution of SAT and its components in the subjects stratified by sex and BMI, and the related statistical differences are reported in Table 3. In general, sSAT, dSAT and tSAT thicknesses increase across BMI categories in all sites in both men and women ($p < 0.001$ for all); although in the triceps site sSAT thicknesses do not differ significantly between women with overweight and obesity. On the other hand, sSAT/dSAT ratio values decrease significantly across BMI categories in all sites in both men and women ($p < 0.001$ for all), although no

Table 1 Descriptive analysis of study populations for abdominal and thigh sites

		Abdomen				Thigh			
Parameters	**Sex**	***N***	**Mean**	**SD**	***p***	***N***	**Mean**	**SD**	***p***
Age (years)	W	513	39.27	14.85	**< 0.001**	1085	40.22	14.23	**< 0.001**
	M	1117	34.55	12.82		1112	31.13	12.83	
Weight (kg)	W	513	68.58	13.40	**< 0.001**	1085	68.15	12.58	**< 0.001**
	M	1117	82.03	14.26		1112	81.87	14.01	
Height (cm)	W	513	164.43	6.61	**< 0.001**	1085	164.68	5.94	**< 0.001**
	M	1117	178.80	7.31		1112	179.06	7.18	
BMI (kg/m^2)	W	513	25.42	5.08	0.385	1085	25.16	4.66	0.066
	M	1117	25.63	4.06		1112	25.50	3.97	
sSAT (mm)	W	513	9.86	4.36	**< 0.001**	1085	6.91	1.82	**< 0.001**
	M	1117	7.74	2.97		1112	4.75	1.36	
dSAT (mm)	W	465	16.88	10.12	**< 0.001**	1077	11.20	6.24	**< 0.001**
	M	939	13.54	10.19		701	5.26	2.97	
tSAT (mm)	W	513	25.16	13.48	**< 0.001**	1085	18.03	7.42	**< 0.001**
	M	1117	19.12	12.67		1112	8.09	4.10	
sSAT/dSAT	W	465	0.78	0.45	**0.008**	1077	0.71	0.32	**< 0.001**
	M	939	0.85	0.46		701	1.04	0.49	
MT (mm)	W	513	13.91	3.21	**< 0.001**	1085	32.51	8.43	**< 0.001**
	M	1117	17.12	3.39		1112	38.88	9.03	
EG (%)	W	513	62.91	12.63	**< 0.001**	1085	48.99	17.18	**< 0.001**
	M	1117	58.12	12.42		1112	44.49	15.29	
MT/SAT	W	513	0.89	0.81	**< 0.001**	1085	2.23	1.17	**< 0.001**
	M	1117	1.51	1.19		1112	6.24	3.17	

Data are expressed as mean and SD. Difference between groups were analysed by paired Student's *t* test. A *p* value in bold type denotes a significant difference ($p < 0.05$)

Abbreviations: body mass index, BMI; superficial subcutaneous adipose tissue, sSAT; deep subcutaneous adipose tissue, dSAT; total subcutaneous adipose tissue, tSAT; muscle thickness, MT; echogenicity, EG; standard deviation, SD

significant differences are observed between women with overweight and obesity in the hip and triceps, as well as between men with normal-weight and overweight for the chest site (Table 3). The values of MT, EG and MT/SAT in the subjects stratified by sex and age, and the related statistical differences are reported in Table 4. MT values decrease significantly across age quartiles in abdomen and thigh in both men and women ($p < 0.001$ for all), although no statistical difference is observed in men in the quartile values between 18 and 45 years. For the other sites analysed, although the values are different, no linear trend is observed in the changes in MT across age quartiles. For EG and MT/SAT ratio, on the other hand, general increasing and decreasing trends are observed across age quartiles, respectively, although these are not linear (Table 4). A possible effect of BMI is therefore also denoted within the individual age quartiles. For this reason, the distributions of (i) SAT and its components and of (ii) muscle-related parameters across BMI categories and age quartiles are reported in Tables 5 and 6.

Correlation studies

SAT- and muscle-related parameters measured at abdomen and thigh levels were correlated with BMI and age (Table 7). This analysis was performed only in these two body sites because (i) they may be considered the most representative sites for SAT accumulation (abdomen) and muscle mass (thigh), and (ii) because only for them were available measurement in both sexes. With regard to SAT, both in women and men, results showed a significant positive correlation between BMI and sSAT, dSAT and tSAT ($p < 0.001$ for all), and a significant negative correlation with sSAT/dSAT ratio ($p < 0.001$ for all), in abdomen (Fig. 2) and thigh (Fig. 3). Regarding to muscle measurements, both in women and men, results showed a significant positive correlation between age and EG ($p < 0.001$ for all), and a significant negative correlation with MT and MT/SAT ratio ($p < 0.001$ for all), in abdomen (Fig. 4) and thigh (Fig. 5).

Finally, to assess the relative prognostic value of age, sSAT, dSAT, tSAT, sSAT/dSAT, MT, and MT/SAT to influence BMI, we performed a multiple linear regression analysis model with these parameters. Results are shown in Table 8. In particular, in the multiple regression model, the variable tSAT was always excluded from the analysis. For the abdominal site, in women the prognostic value of age, sSAT, dSAT and the MT/SAT ratio was significant ($p < 0.001$ for all); in men, sSAT ($p < 0.001$), dSAT ($p < 0.001$) and the sSAT/dSAT ratio ($p = 0.011$) were significant. For the thigh site, the prognostic values of age,

Table 2 Descriptive analysis of study populations for hip and triceps sites

		Hip			Triceps		
Parameters	**Sex**	***N***	**Mean**	**SD**	***N***	**Mean**	**SD**
Age (years)	W	721	43.08	13.31	832	43.86	14.10
Weight (kg)	W	721	68.68	12.27	832	69.64	12.78
Height (cm)	W	721	164.69	5.44	832	164.07	5.78
BMI (kg/m^2)	W	721	25.43	4.37	832	25.90	4.74
sSAT (mm)	W	721	9.11	3.30	832	7.03	1.80
dSAT (mm)	W	457	10.54	5.06	813	10.78	4.31
tSAT (mm)	W	721	15.79	7.74	832	17.57	5.44
sSAT/dSAT	W	457	1.03	0.50	813	0.73	0.36
MT (mm)	W	721	21.05	4.50	832	32.14	6.48
EG (%)	W	721	67.78	12.51	832	45.52	15.15
MT/SAT	W	721	1.80	1.19	832	2.07	0.77
		Chest					
Parameters	**Sex**	**N**	**Mean**	**SD**			
Age (years)	M	890	35.34	12.31			
Weight (kg)	M	890	80.14	12.20			
Height (cm)	M	890	178.61	6.52			
BMI (kg/m^2)	M	890	25.09	3.42			
sSAT (mm)	M	890	5.20	2.04			
dSAT (mm)	M	316	7.44	5.08			
tSAT (mm)	M	890	7.85	6.10			
sSAT/dSAT	M	316	1.14	0.49			
MT (mm)	M	890	27.52	7.34			
EG (%)	M	890	57.27	13.91			
MT/SAT	M	890	5.56	3.64			

Data are expressed as mean and SD

Abbreviations: body mass index, BMI; superficial subcutaneous adipose tissue, sSAT; deep subcutaneous adipose tissue, dSAT; total subcutaneous adipose tissue, tSAT; muscle thickness, MT; echogenicity, EG; standard deviation, SD

sSAT, dSAT, sSAT/dSAT ratio, MT, and MT/SAT ratio were significant ($p < 0.001$ for all) in women; in men, age ($p < 0.001$), dSAT ($p < 0.001$), MT ($p = 0.001$), and MT/SAT ($p = 0.040$) were significant. Interestingly, although the prognostic value of the sSAT/dSAT ratio was significant only in women, in both sexes, as well as for the MT/SAT ratio, the observed relationship was negative (Table 8).

Discussion

The present observational retrospective study reported, for the first time, the average values of SAT and muscle parameters in abdomen, thigh, chest, hip and triceps detected by ultrasound, a promising tool offering accuracy and accessibility for assessing body composition, in a large cohort of adult subjects stratified by sex, BMI, and age.

The first interesting observation is the existence of a sex -specific difference in the distribution of SAT (and its components sSAT and dSAT) in both abdomen and thigh, which are greater in women than in men. These expected results are consistent with the observations of Krauze et al. [23] who had measured tSAT, sSAT and dSAT levels using the traditional ultrasound method. Similarly, the authors observed a significant correlation between tSAT, sSAT and dSAT and BMI. Specifically, in men only the dSAT correlated positively and significantly, whereas in women positive and significant correlations were observed with all three variables [23]. In our analyses, sSAT, dSAT and tSAT increased across BMI categories, thus, correlating positively with BMI. These results are in agreement with the observations of Krauze and colleagues, who point out that this relationship between SAT (and its components) and BMI is illustrated by the dSAT/sSAT ratio, which was higher both in women than men, and in subjects with BMI > 25.0 kg/m^2 than in subjects with BMI < 25.0 kg/m^2 [23]. In our study, we calculated the inverse ratio, sSAT/dSAT which was lower in women than in men for abdomen and thigh and correlated negatively and significantly with BMI, thus decreasing across BMI categories. As a mathematical ratio, both a high dSAT/sSAT and a low sSAT/dSAT ratio indicate a greater expansion of the dSAT component compared to the sSAT. Calculation of the sSAT/dSAT ratio is of relevant importance due to the anatomical-functional differences of these two compartments of tSAT, providing objective information regarding the individual predominance of one or the other. In this sense, it is now clearly

Table 3 Subcutaneous adipose tissue distribution in adults stratified for sex and BMI

Abdomen									
Women					**Men**				
BMI	**sSAT (mm)**	**dSAT (mm)**	**tSAT (mm)**	**sSAT/dSAT**	**BMI**	**sSAT (mm)**	**dSAT (mm)**	**tSAT (mm)**	**sSAT/dSAT**
Normal weight	8.18 ± 3.37 (*n* = 300)[a]	11.07 ± 5.56 (*n* = 252)[a]	17.48 ± 8.55 (*n* = 300)[a]	0.92 ± 0.46 (*n* = 252)[a]	Normal weight	6.39 ± 2.16 (*n* = 601)[a]	8.11 ± 5.46 (*n* = 441)[a]	12.34 ± 7.38 (*n* = 601)[a]	1.04 ± 0.47 (*n* = 441)[a]
Overweight	11.22 ± 3.71 (*n* = 132)[b]	19.20 ± 7.69 (*n* = 132)[b]	30.42 ± 9.38 (*n* = 132)[b]	0.69 ± 0.41 (*n* = 132)[b]	Overweight	8.61 ± 2.55 (*n* = 376)[b]	14.26 ± 7.58 (*n* = 358)[b]	22.19 ± 9.50 (*n* = 376)[b]	0.77 ± 0.38 (*n* = 358)[b]
Obesity	11.83 ± 5.22 (*n* = 81)[c]	31.17 ± 8.82 (*n* = 81)[c]	45.00 ± 8.95 (*n* = 81)[c]	0.50 ± 0.30 (*n* = 81)[c]	Obesity	11.25 ± 3.27 (*n* = 140)[c]	28.75 ± 11.44 (*n* = 140)[c]	40.00 ± 12.10 (*n* = 140)[c]	0.46 ± 0.23 (*n* = 140)[c]
p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	***p***	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Thigh									
Women					***Men***				
Normal weight	6.39 ± 1.59 (*n* = 625)[a]	9.24 ± 5.89 (*n* = 618)[a]	15.53 ± 6.89 (*n* = 625)[a]	0.80 ± 0.35 (*n* = 618)[a]	Normal weight	4.45 ± 1.22 (*n* = 617)[a]	4.02 ± 2.08 (*n* = 277)[a]	6.26 ± 2.85 (*n* = 617)[a]	1.19 ± 0.52 (*n* = 277)[a]
Overweight	7.43 ± 1.62 (*n* = 306)[b]	12.86 ± 5.57 (*n* = 305)[b]	20.26 ± 6.43 (*n* = 306)[b]	0.63 ± 0.22 (*n* = 305)[b]	Overweight	4.94 ± 1.42 (*n* = 360)[b]	4.93 ± 2.41 (*n* = 292)[b]	8.94 ± 3.46 (*n* = 360)[b]	1.06 ± 0.44 (*n* = 292)[b]
Obesity	7.95 ± 2.29 (*n* = 154)[c]	15.79 ± 5.52 (*n* = 154)[c]	23.74 ± 6.81 (*n* = 154)[c]	0.53 ± 0.19 (*n* = 154)[c]	Obesity	5.60 ± 1.39 (*n* = 135)[c]	8.59 ± 3.26 (*n* = 132)[c]	14.00 ± 4.15 (*n* = 135)[c]	0.69 ± 0.34 (*n* = 132)[c]
p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	***p***	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Hip					***Chest***				
Normal weight	7.81 ± 2.44 (*n* = 378)[a]	7.44 ± 4.06 (*n* = 151)[a]	10.79 ± 5.33 (*n* = 378)[a]	1.25 ± 0.58 (*n* = 151)[a]	Normal weight	4.27 ± 1.60 (*n* = 511)[a]	4.93 ± 2.90 (*n* = 77)[a]	5.02 ± 3.14 (*n* = 511)[a]	1.32 ± 0.45 (*n* = 77)[a]
Overweight	10.05 ± 3.17 (*n* = 226)[b]	11.18 ± 4.63 (*n* = 198)[b]	19.85 ± 5.71 (*n* = 226)[b]	0.94 ± 0.40 (*n* = 198)[b]	Overweight	6.17 ± 1.87 (*n* = 303)[b]	7.40 ± 5.33 (*n* = 164)[b]	10.18 ± 6.45 (*n* = 303)[b]	1.18 ± 0.51 (*n* = 164)[a]
Obesity	11.48 ± 3.97 (*n* = 117)[c]	13.70 ± 4.73 (*n* = 108)[c]	24.12 ± 5.70 (*n* = 117)[c]	0.88 ± 0.43 (*n* = 108)[b]	Obesity	7.60 ± 1.15 (*n* = 76)[c]	10.12 ± 4.97 (*n* = 75)[c]	17.58 ± 5.85 (*n* = 76)[c]	0.86 ± 0.32 (*n* = 75)[b]
p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	***p***	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Triceps									
Normal weight	6.47 ± 1.53 (*n* = 414)[a]	8.39 ± 3.25 (*n* = 395)[a]	14.47 ± 4.13 (*n* = 414)[a]	0.85 ± 0.40 (*n* = 395)[a]					
Overweight	7.46 ± 1.82 (*n* = 265)[b]	12.34 ± 3.78 (*n* = 265)[b]	19.81 ± 4.60 (*n* = 265)[b]	0.65 ± 0.30 (*n* = 265)[b]					
Obesity	7.80 ± 1.93 (*n* = 153)[b]	14.26 ± 4.00 (*n* = 153)[c]	22.06 ± 4.89 (*n* = 153)[c]	0.57 ± 0.22 (*n* = 153)[b]					
p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**					

Data are expressed as mean ± SD. Difference between groups were compared by one-way ANOVA, followed by Bonferroni test as *post-hoc* test. Letters refer to the differences between groups (a = normal weight vs. overweight and obesity; b = overweight vs. normal weight and obesity; c = obesity vs. normal weight and overweight). A *p* value in bold type denotes a significant difference ($p < 0.05$)

Abbreviations: body mass index, BMI; superficial subcutaneous adipose tissue, sSAT; deep subcutaneous adipose tissue, dSAT; total subcutaneous adipose tissue, tSAT

defined in the literature that these two SAT components do not only differ histologically, but also metabolically. In particular, dSAT is defined as a portion of SAT highly vascularised and innerved, rich in adipocytes (including brown type) and stem cells [27], and it significantly correlates with pro-inflammatory cytokine and adipokines levels [28]. It has been clarified, indeed, that dSAT is involved in neuroendocrine regulation, and it is more metabolically active than sSAT [29]. More specifically, dSAT presents a specific protein expression profile making it biochemically similar to visceral adipose tissue [30]. This explain the relation of dSAT expansion with obesity-related comorbidities, including insulin resistance [31, 32] and liver fat content [33]. Overall, this evidence explains the inverse relationship between sSAT/dSAT ratio and BMI, and suggests the importance to not only measure the thickness of SAT, but also to evaluate and monitor the relative expansion of its components, in particular during both nutritional or training interventions.

MTs were measured for each site, observing, firstly, an expected sex difference for abdomen and thigh. Given the well-known relationship between changes in muscle mass and age, we analysed the MT values in this respect, observing significant negative correlations. In particular, MT values tend to decrease with increasing age, although no statistically significant linear trend is observed for some of the sites analysed across the relative age quartiles. Overall, our observations are in line with recent studies that, although not conducted with the ultrasound method, report not only that the cross-sectional area of

Table 4 Muscle thickness and echogenicity in adults stratified for sex and age

	Abdomen									
	Women					Men				
Quartile	Age quartile	n	MT (mm)	EG (%)	MT/SAT	Age	n	MT (mm)	EG (%)	MT/SAT
I	18–25 years	126	16.06 ± 2.47^{a}	55.15 ± 10.42^{a}	1.48 ± 1.10^{a}	18–23 years	273	17.60 ± 2.99^{a}	54.49 ± 11.91^{a}	2.24 ± 1.51^{a}
II	26–38 years	125	14.75 ± 2.89^{b}	57.44 ± 9.25^{a}	0.99 ± 0.68^{b}	24–31 years	284	18.63 ± 3.48^{b}	55.01 ± 11.00^{a}	1.63 ± 1.00^{b}
III	39–49 years	124	13.33 ± 2.72^{c}	66.90 ± 9.74^{b}	0.65 ± 0.54^{c}	32–45 years	266	16.83 ± 2.70^{c}	59.10 ± 12.04^{b}	1.20 ± 0.79^{c}
IV	50–89 years	138	11.71 ± 2.94^{d}	71.37 ± 12.86^{c}	0.47 ± 0.28^{c}	46–84 years	294	15.47 ± 3.46^{d}	63.59 ± 12.47^{c}	1.00 ± 0.92^{c}
	p		**< 0.001**	**< 0.001**	**< 0.001**	***p***		**< 0.001**	**< 0.001**	**< 0.001**
	Thigh									
	Women					***Men***				
I	18–27 years	270	38.31 ± 7.40^{a}	39.14 ± 14.37^{a}	3.06 ± 1.56^{a}	18–22 years	239	40.95 ± 8.19^{a}	37.94 ± 14.59^{a}	6.76 ± 3.34^{a}
II	28–40 years	257	33.05 ± 8.36^{b}	46.74 ± 15.77^{b}	2.26 ± 0.87^{b}	23–30 years	300	42.00 ± 9.46^{a}	43.08 ± 13.66^{b}	6.68 ± 3.10^{a}
III	41–50 years	282	30.99 ± 7.06^{c}	53.91 ± 15.25^{c}	1.93 ± 0.79^{c}	31–45 years	293	40.27 ± 7.69^{a}	43.77 ± 14.07^{b}	3.63 ± 3.66^{a}
IV	51–89 years	276	27.90 ± 7.33^{d}	55.69 ± 17.94^{c}	1.72 ± 0.80^{c}	46–84 years	280	32.32 ± 7.07^{b}	52.35 ± 15.51^{c}	5.19 ± 2.14^{b}
	p		**< 0.001**	**< 0.001**	**< 0.001**	***p***		**< 0.001**	**< 0.001**	**< 0.001**
Hip						***Chest***				
I	18–33 years	177	22.15 ± 4.75^{a}	61.85 ± 11.84^{a}	2.18 ± 1.36^{a}	18–24 years	202	27.59 ± 6.20^{a}	51.22 ± 11.88^{a}	7.09 ± 3.81^{a}
II	34–43 years	155	20.73 ± 4.26^{b}	64.91 ± 10.90^{a}	2.19 ± 1.46^{a}	25–31 years	202	29.39 ± 7.17^{a}	53.55 ± 12.05^{a}	6.03 ± 3.47^{b}
III	44–51 years	189	$21.28 \pm 4.26^{a, b}$	70.28 ± 12.38^{b}	1.47 ± 0.84^{b}	32–46 years	250	28.57 ± 7.12^{a}	56.87 ± 13.52^{b}	5.06 ± 3.59^{c}
IV	52–74 years	200	20.09 ± 4.33^{b}	72.89 ± 11.65^{b}	1.46 ± 0.86^{b}	47–74 years	236	24.74 ± 7.81^{b}	66.06 ± 13.10^{c}	4.37 ± 3.15^{c}
	p		**< 0.001**	**< 0.001**	**< 0.001**	***p***		**< 0.001**	**< 0.001**	**< 0.001**
Triceps										
I	18–33 years	204	32.17 ± 7.01^{a}	42.55 ± 14.86^{a}	2.40 ± 1.01^{a}					
II	34–44 years	208	32.59 ± 6.93^{a}	$43.03 \pm 14.87^{a, b}$	2.13 ± 0.73^{b}					
III	45–53 years	210	32.65 ± 5.84^{a}	$46.57 \pm 14.11^{b, c}$	1.88 ± 0.56^{c}					
IV	54–89 years	210	31.16 ± 6.03^{a}	49.84 ± 15.68^{c}	1.89 ± 0.59^{c}					
	p		0.069	**< 0.001**	**< 0.001**					

Data are expressed as mean ± SD. Difference between groups were compared by one-way ANOVA, followed by Bonferroni test as *post-hoc* test. Letters refer to the differences between groups (a = quartile I vs. quartile II, quartile III, and quartile IV; b = quartile II vs. quartile I, quartile III, and quartile IV; c = quartile III vs. quartile I, quartile II, and quartile IV; d = quartile IV vs. quartile I, quartile II, and quartile III). A *p* value in bold type denotes a significant difference ($p < 0.05$)

Abbreviations: subcutaneous adipose tissue, SAT; muscle thickness, MT; echogenicity, EG; standard deviation, SD

Table 5 Subcutaneous adipose tissue distribution across BMI categories in adults stratified for sex and age

Abdomen									
Women					**Men**				
Age/BMI	**sSAT (mm)**	**dSAT (mm)**	**tSAT (mm)**	**sSAT/dSAT**	**Age/BMI**	**sSAT (mm)**	**dSAT (mm)**	**tSAT (mm)**	**sSAT/dSAT**
18–25 years					**18–23 years**				
Normal weight	7.22 ± 2.54 (*n* = 100)	8.84 ± 5.31 (*n* = 72)	13.58 ± 7.71 (*n* = 100)	1.07 ± 0.49 (*n* = 72)	Normal weight	5.86 ± 2.14 (*n* = 176)	7.64 ± 5.73 (*n* = 84)	9.51 ± 7.12 (*n* = 176)	1.10 ± 0.43 (*n* = 84)
Overweight	13.53 ± 4.66 (*n* = 18)	22.82 ± 7.01 (*n* = 18)	36.35 ± 9.54 (*n* = 18)	0.63 ± 0.22 (*n* = 18)	Overweight	8.06 ± 2.75 (*n* = 88)	14.20 ± 10.00 (*n* = 78)	20.64 ± 12.50 (*n* = 88)	0.83 ± 0.53 (*n* = 78)
Obesity	14.15 ± 2.10 (*n* = 8)	42.53 ± 7.36 (*n* = 8)	56.68 ± 6.13 (*n* = 8)	0.35 ± 0.12 (*n* = 8)	Obesity	13.02 ± 3.81 (*n* = 9)	34.52 ± 15.65 (*n* = 9)	47.54 ± 16.30 (*n* = 9)	0.47 ± 0.26 (*n* = 9)
p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	***p***	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
26–38 years					**24–31 years**				
Normal weight	8.95 ± 4.34 (*n* = 91)	10.10 ± 4.88 (*n* = 76)	17.38 ± 8.68 (*n* = 91)	1.02 ± 0.48 (*n* = 76)	Normal weight	6.23 ± 1.87 (*n* = 169)	6.65 ± 4.01 (*n* = 134)	11.50 ± 5.47 (*n* = 169)	1.15 ± 0.52 (*n* = 134)
Overweight	11.53 ± 4.21 (*n* = 22)	18.86 ± 7.51 (*n* = 22)	30.39 ± 10.37 (*n* = 22)	0.69 ± 0.31 (*n* = 22)	Overweight	7.85 ± 1.96 (*n* = 72)	11.59 ± 6.30 (*n* = 68)	18.79 ± 7.89 (*n* = 72)	0.84 ± 0.39 (*n* = 68)
Obesity	16.53 ± 7.17 (*n* = 12)	31.07 ± 6.16 (*n* = 12)	47.61 ± 6.90 (*n* = 12)	0.57 ± 0.32 (*n* = 12)	Obesity	11.27 ± 3.22 (*n* = 43)	21.91 ± 8.10 (*n* = 43)	33.18 ± 8.97 (*n* = 43)	0.59 ± 0.27 (*n* = 43)
p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	***p***	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
39–49 years					**32–45 years**				
Normal weight	7.97 ± 2.41 (*n* = 60)	12.77 ± 5.46 (*n* = 56)	19.90 ± 7.18 (*n* = 60)	0.78 ± 0.43 (*n* = 56)	Normal weight	6.29 ± 1.61 (*n* = 108)	8.00 ± 4.53 (*n* = 83)	12.44 ± 6.09 (*n* = 108)	0.98 ± 0.46 (*n* = 83)
Overweight	11.27 ± 3.60 (*n* = 40)	19.72 ± 8.78 (*n* = 40)	31.00 ± 9.39 (*n* = 40)	0.77 ± 0.63 (*n* = 40)	Overweight	9.17 ± 2.75 (*n* = 120)	13.73 ± 6.05 (*n* = 118)	22.67 ± 7.73 (*n* = 120)	0.75 ± 0.25 (*n* = 118)
Obesity	15.43 ± 5.80 (*n* = 24)	28.23 ± 7.61 (*n* = 24)	43.66 ± 7.88 (*n* = 24)	0.62 ± 0.38 (*n* = 24)	Obesity	11.16 ± 4.11 (*n* = 38)	27.63 ± 9.85 (*n* = 38)	38.79 ± 10.51 (*n* = 38)	0.46 ± 0.22 (*n* = 38)
p	**< 0.001**	**< 0.001**	**< 0.001**	0.383	***p***	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
50–89 years					**46–84 years**				
Normal weight	8.89 ± 3.31 (*n* = 49)	13.97 ± 5.36 (*n* = 48)	22.67 ± 7.83 (*n* = 49)	0.71 ± 0.28 (*n* = 48)	Normal weight	7.26 ± 2.56 (*n* = 148)	9.88 ± 6.48 (*n* = 140)	16.61 ± 8.49 (*n* = 148)	0.95 ± 0.41 (*n* = 140)
Overweight	10.26 ± 2.85 (*n* = 52)	17.68 ± 6.80 (*n* = 52)	27.94 ± 8.02 (*n* = 52)	0.65 ± 0.25 (*n* = 52)	Overweight	8.99 ± 2.27 (*n* = 96)	16.91 ± 7.08 (*n* = 94)	25.55 ± 8.33 (*n* = 96)	0.65 ± 0.33 (*n* = 94)
Obesity	11.84 ± 3.78 (*n* = 37)	30.65 ± 8.89 (*n* = 37)	42.49 ± 8.72 (*n* = 37)	0.44 ± 0.24 (*n* = 37)	Obesity	10.97 ± 2.38 (*n* = 50)	34.44 ± 10.99 (*n* = 50)	45.42 ± 11.72 (*n* = 50)	0.35 ± 0.14 (*n* = 50)
p	**0.001**	**< 0.001**	**< 0.001**	**< 0.001**	***p***	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Thigh									
Women					***Men***				
18–27 years					**18–22 years**				
Normal weight	6.34 ± 1.72 (*n* = 215)	8.91 ± 6.71 (*n* = 210)	15.04 ± 7.92 (*n* = 215)	0.87 ± 0.44 (*n* = 210)	Normal weight	4.43 ± 1.32 (*n* = 166)	4.26 ± 2.30 (*n* = 85)	6.61 ± 3.28 (*n* = 166)	1.12 ± 0.63 (*n* = 85)
Overweight	7.84 ± 1.44 (*n* = 41)	14.37 ± 7.54 (*n* = 41)	22.21 ± 8.33 (*n* = 41)	0.61 ± 0.22 (*n* = 41)	Overweight	5.13 ± 1.18 (*n* = 64)	5.89 ± 3.46 (*n* = 54)	10.10 ± 4.43 (*n* = 64)	1.03 ± 0.47 (*n* = 54)
Obesity	8.78 ± 2.64 (*n* = 14)	17.09 ± 9.67 (*n* = 14)	25.87 ± 11.68 (*n* = 14)	0.59 ± 0.21 (*n* = 14)	Obesity	7.67 ± 1.00 (*n* = 9)	12.25 ± 2.51 (*n* = 9)	19.92 ± 3.16 (*n* = 9)	0.56 ± 1.13 (*n* = 9)
p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	***p***	**< 0.001**	**< 0.001**	**< 0.001**	**0.018**
28–40 years					**23–30 years**				
Normal weight	6.29 ± 1.45 (*n* = 180)	8.60 ± 4.05 (*n* = 180)	14.89 ± 4.91 (*n* = 180)	0.79 ± 0.28 (*n* = 180)	Normal weight	4.44 ± 1.16 (*n* = 192)	3.95 ± 1.73 (*n* = 99)	6.48 ± 2.64 (*n* = 192)	1.17 ± 0.46 (*n* = 99)
Overweight	7.91 ± 1.72 (*n* = 52)	13.95 ± 7.03 (*n* = 52)	21.86 ± 7.79 (*n* = 52)	0.64 ± 0.29 (*n* = 52)	Overweight	5.08 ± 1.64 (*n* = 75)	4.71 ± 1.95 (*n* = 61)	8.91 ± 3.07 (*n* = 75)	1.08 ± 0.37 (*n* = 61)
Obesity	7.85 ± 2.32 (*n* = 25)	16.07 ± 4.20 (*n* = 25)	23.92 ± 3.70 (*n* = 25)	0.54 ± 0.32 (*n* = 25)	Obesity	5.44 ± 1.53 (*n* = 33)	7.97 ± 3.19 (n)33)	13.41 ± 3.93 (*n* = 33)	0.72 ± 0.47 (*n* = 33)
p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	***p***	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
41–50 years					**31–45 years**				
Normal weight	6.49 ± 1.43 (*n* = 127)	9.75 ± 5.93 (*n* = 126)	16.17 ± 6.71 (*n* = 127)	0.75 ± 0.30 (*n* = 126)	Normal weight	4.33 ± 1.29 (*n* = 117)	3.65 ± 1.56 (*n* = 45)	5.73 ± 2.31 (*n* = 117)	1.21 ± 0.36 (*n* = 45)

Table 5 (continued)

Abdomen									
Women					**Men**				
Age/BMI	**sSAT (mm)**	**dSAT (mm)**	**tSAT (mm)**	**sSAT/dSAT**	**Age/BMI**	**sSAT (mm)**	**dSAT (mm)**	**tSAT (mm)**	**sSAT/dSAT**
Overweight	7.10 ± 1.48 (*n* = 107)	12.52 ± 4.69 (*n* = 107)	19.63 ± 5.63 (*n* = 107)	0.60 ± 0.18 (*n* = 107)	Overweight	4.77 ± 1.50 (*n* = 130)	4.62 ± 1.54 (*n* = 113)	8.79 ± 2.73 (*n* = 130)	1.05 ± 0.48 (*n* = 113)
Obesity	8.46 ± 2.77 (*n* = 48)	16.20 ± 5.70 (*n* = 48)	24.66 ± 7.55 (*n* = 48)	0.52 ± 0.17 (*n* = 48)	Obesity	5.52 ± 1.07 (*n* = 46)	9.74 ± 3.19 (*n* = 46)	15.26 ± 3.85 (*n* = 46)	0.60 ± 0.22 (*n* = 46)
p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	***p***	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
51–89 years					**46–84 years**				
Normal weight	6.57 ± 1.72 (*n* = 103)	10.45 ± 6.55 (*n* = 102)	16.92 ± 7.57 (*n* = 103)	0.72 ± 0.28 (*n* = 102)	Normal weight	4.60 ± 1.09 (*n* = 142)	4.08 ± 2.85 (*n* = 48)	5.98 ± 2.94 (*n* = 142)	1.34 ± 0.55 (*n* = 48)
Overweight	7.34 ± 1.70 (*n* = 106)	12.05 ± 4.47 (*n* = 105)	19.35 ± 5.31 (*n* = 106)	0.65 ± 0.21 (*n* = 105)	Overweight	4.93 ± 1.26 (*n* = 91)	4.89 ± 2.80 (*n* = 64)	8.37 ± 3.77 (*n* = 91)	1.08 ± 0.43 (*n* = 64)
Obesity	7.45 ± 1.67 (*n* = 67)	15.11 ± 4.66 (*n* = 67)	22.57 ± 5.65 (*n* = 67)	0.51 ± 0.14 (*n* = 67)	Obesity	5.39 ± 1.33 (*n* = 47)	7.11 ± 2.50 (*n* = 44)	12.05 ± 3.25 (*n* = 47)	0.80 ± 0.33 (*n* = 44)
p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	***p***	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Hip					***Chest***				
18–33 years					**18–24 years**				
Normal weight	7.25 ± 2.34 (*n* = 109)	7.54 ± 4.76 (*n* = 32)	9.46 ± 5.29 (*n* = 109)	1.18 ± 0.52 (*n* = 32)	Normal weight	3.73 ± 1.35 (*n* = 126)	5.30 ± 1.73 (*n* = 2)	3.82 ± 1.65 (*n* = 126)	1.20 ± 0.32 (*n* = 2)
Overweight	10.84 ± 4.03 (*n* = 50)	11.69 ± 4.74 (*n* = 40)	20.19 ± 6.37 (*n* = 50)	0.89 ± 0.32 (*n* = 40)	Overweight	5.48 ± 1.89 (*n* = 73)	5.51 ± 2.22 (*n* = 24)	7.29 ± 4.11 (*n* = 73)	1.34 ± 0.42 (*n* = 24)
Obesity	11.24 ± 5.13 (*n* = 18)	15.75 ± 5.44 (*n* = 17)	26.11 ± 6.90 (*n* = 18)	0.70 ± 0.27 (*n* = 17)	Obesity	9.32 ± 1.58 (*n* = 3)	12.48 ± 1.66 (*n* = 3)	21.80 ± 2.14 (*n* = 3)	0.75 ± 0.17 (*n* = 3)
p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	***p***	**< 0.001**	**< 0.001**	**< 0.001**	0.077
34–43 years					**25–31 years**				
Normal weight	7.56 ± 2.19 (*n* = 118)	7.23 ± 3.40 (*n* = 46)	10.38 ± 4.63 (*n* = 118)	1.20 ± 0.59 (*n* = 46)	Normal weight	4.58 ± 1.42 (*n* = 136)	4.65 ± 1.83 (*n* = 19)	5.23 ± 2.63 (*n* = 136)	1.44 ± 0.60 (*n* = 19)
Overweight	10.62 ± 3.22 (*n* = 21)	9.38 ± 3.33 (*n* = 18)	18.66 ± 4.41 (*n* = 21)	1.22 ± 0.59 (*n* = 18)	Overweight	5.74 ± 1.69 (*n* = 51)	5.25 ± 2.76 (*n* = 13)	7.08 ± 3.58 (*n* = 51)	1.29 ± 0.42 (*n* = 51)
Obesity	13.30 ± 3.82 (*n* = 16)	12.33 ± 4.82 (*n* = 11)	21.78 ± 5.29 (*n* = 16)	1.06 ± 0.52 (*n* = 11)	Obesity	8.06 ± 1.68 (*n* = 15)	9.57 ± 6.41 (*n* = 14)	17.00 ± 7.45 (*n* = 15)	1.04 ± 0.42 (*n* = 14)
p	**< 0.001**	**< 0.001**	**< 0.001**	0.735	***p***	**< 0.001**	**0.003**	**< 0.001**	0.089
44–51 years					**32–46 years**				
Normal weight	8.55 ± 2.45 (*n* = 76)	8.34 ± 4.24 (*n* = 42)	13.16 ± 6.10 (*n* = 76)	1.22 ± 0.60 (*n* = 42)	Normal weight	4.52 ± 1.83 (*n* = 115)	4.63 ± 2.92 (*n* = 22)	5.41 ± 3.53 (*n* = 115)	1.38 ± 0.35 (*n* = 22)
Overweight	9.88 ± 2.99 (*n* = 71)	10.75 ± 4.37 (*n* = 63)	19.42 ± 5.10 (*n* = 71)	0.94 ± 0.36 (*n* = 63)	Overweight	6.33 ± 1.94 (*n* = 104)	5.38 ± 2.58 (*n* = 67)	9.79 ± 4.29 (*n* = 104)	1.35 ± 0.50 (*n* = 67)
Obesity	10.87 ± 2.97 (*n* = 42)	13.91 ± 4.70 (*n* = 42)	24.78 ± 5.88 (*n* = 42)	0.85 ± 0.34 (*n* = 42)	Obesity	7.29 ± 1.44 (*n* = 31)	8.68 ± 3.49 (*n* = 31)	15.97 ± 3.81 (*n* = 31)	0.90 ± 0.29 (*n* = 31)
p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	***p***	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
52–74 years					**47–74 years**				
Normal weight	8.29 ± 2.70 (*n* = 75)	6.45 ± 3.85 (*n* = 31)	10.96 ± 4.88 (*n* = 75)	1.43 ± 0.61 (*n* = 31)	Normal weight	4.26 ± 1.66 (*n* = 134)	5.25 ± 3.43 (*n* = 34)	5.59 ± 3.97 (*n* = 134)	1.23 ± 0.42 (*n* = 34)
Overweight	8.59 ± 2.64 (*n* = 84)	11.68 ± 4.96 (*n* = 77)	20.30 ± 6.08 (*n* = 84)	0.89 ± 0.39 (*n* = 77)	Overweight	6.93 ± 1.57 (*n* = 75)	10.87 ± 6.95 (*n* = 60)	15.63 ± 8.58 (*n* = 75)	0.91 ± 0.47 (*n* = 60)
Obesity	11.49 ± 4.27 (*n* = 41)	12.94 ± 4.23 (*n* = 38)	23.49 ± 4.81 (*n* = 41)	0.93 ± 0.53 (*n* = 38)	Obesity	7.52 ± 1.38 (*n* = 27)	11.79 ± 5.42 (*n* = 27)	19.31 ± 6.57 (*n* = 27)	0.72 ± 0.24 (*n* = 27)
p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	***p***	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Triceps									
18–33 years									
Normal weight	6.40 ± 1.70 (*n* = 125)	7.42 ± 2.73 (*n* = 111)	13.00 ± 3.61 (*n* = 125)	0.93 ± 0.45 (*n* = 111)					
Overweight	7.75 ± 2.62 (*n* = 51)	12.03 ± 3.40 (*n* = 51)	19.78 ± 3.61 (*n* = 51)	0.71 ± 0.48 (*n* = 51)					
Obesity	8.27 ± 2.47 (*n* = 28)	14.79 ± 5.72 (*n* = 28)	23.06 ± 7.60 (*n* = 28)	0.60 ± 0.21 (*n* = 28)					

Table 5 (continued)

Abdomen									
Women					**Men**				
Age/BMI	**sSAT (mm)**	**dSAT (mm)**	**tSAT (mm)**	**sSAT/dSAT**	**Age/BMI**	**sSAT (mm)**	**dSAT (mm)**	**tSAT (mm)**	**sSAT/dSAT**
p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**					
34–44 years									
Normal weight	6.49 ± 1.62 (*n* = 130)	8.65 ± 3.40 (*n* = 129)	15.07 ± 4.29 (*n* = 130)	0.83 ± 0.37 (*n* = 129)					
Overweight	7.38 ± 1.52 (*n* = 50)	12.12 ± 3.39 (*n* = 50)	19.50 ± 4.38 (*n* = 50)	0.63 ± 0.17 (*n* = 50)					
Obesity	7.64 ± 1.62 (*n* = 28)	13.58 ± 2.66 (*n* = 28)	21.22 ± 3.83 (*n* = 28)	0.54 ± 0.14 (*n* = 28)					
p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**					
45–53 years									
Normal weight	6.53 ± 1.30 (*n* = 79)	8.59 ± 2.51 (*n* = 78)	15.02 ± 3.16 (*n* = 79)	0.79 ± 0.25 (*n* = 78)					
Overweight	7.70 ± 1.44 (*n* = 86)	13.49 ± 3.66 (*n* = 86)	21.19 ± 4.33 (*n* = 86)	0.59 ± 0.19 (*n* = 86)					
Obesity	7.93 ± 1.94 (*n* = 45)	14.40 ± 3.35 (*n* = 45)	22.33 ± 3.69 (*n* = 45)	0.58 ± 0.24 (*n* = 45)					
p	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**					
54–89 years									
Normal weight	6.46 ± 1.35 (*n* = 80)	9.16 ± 3.98 (*n* = 80)	15.27 ± 4.86 (*n* = 80)	0.81 ± 0.47 (*n* = 80)					
Overweight	7.07 ± 1.69 (*n* = 78)	11.42 ± 4.11 (*n* = 78)	18.49 ± 5.05 (*n* = 78)	0.68 ± 0.29 (*n* = 78)					
Obesity	7.53 ± 1.72 (*n* = 52)	14.22 ± 4.06 (*n* = 52)	21.75 ± 4.47 (*n* = 52)	0.57 ± 0.25 (*n* = 52)					
p	**0.001**	**< 0.001**	**< 0.001**	**0.001**					

Data are expressed as mean ± SD. Difference between groups were compared by one-way ANOVA, followed by Bonferroni test as *post-hoc* test. A *p* value in bold type denotes a significant difference ($p < 0.05$)

Abbreviations: body mass index, BMI; superficial subcutaneous adipose tissue, sSAT; deep subcutaneous adipose tissue, dSAT; total subcutaneous adipose tissue, tSAT

the thigh tends to decrease with increasing age [24, 25], but also that this site shows the most profound differences between young and old, and that the quadriceps femoris shows the greatest differences in muscle volume between these two classes of subjects, compared to other muscles of the lower leg [24].

For each stratigraphy analysed, SAT and MT were integrated into the calculation of the MT/SAT ratio which, like the sSAT/dSAT ratio, being mathematically determined does not need to be validated against other methods. We observed that MT/SAT ratios in abdomen and thigh were lower in women than in men, suggesting that, at least in these two sites, women exhibit a predominance of SAT compared to MT. Also, as expected, we noted that this ratio negatively correlated with both age and BMI. The MT/SAT ratio expresses the thickness of adipose tissue in relation to that of muscle, thus providing useful information that may serve for a comprehensive baseline evaluation of the body region-specific fat distribution and MT, as well as to monitor the progress of a nutritional intervention or training plan, having objective indications for their eventual optimisation.

In addition to the thickness measurements, the specific software coupled to the ultrasound probe used for the scans analysed in this study automatically measured the muscle EG through the calculation of the ultrasound signal intensity, point-by-point within the entire tissue selected in the stratigraphy. It was, therefore, provided a direct and objective numeric value referring to muscle EG. In our study, we observed that muscle EG was significantly higher in women than in men for abdomen and thigh, and increased significantly across age quartiles for individual sites, showing a positive and significant correlation with age. This relationship between muscle EG and age is in accordance with previous observations made with the traditional ultrasound [34]. Noteworthy, a directly calculated EG of muscle can be translated in a broader concept of tissue quality. Various studies, indeed, proposed the use of ultrasound for the quality assessment of muscle suggesting the measure of EG, which was found to correlate with typical parameters referring to muscle quality, including handgrip strength and bioelectrical parameters related to body cell mass, such as reactance and phase angle [18–20, 22]. To understand

Table 6 Muscle thickness and echogenicity across age categories in adults stratified for sex and BMI

	Abdomen							
Women					**Men**			
Quartile	**Age**	**MT (mm)**	**EG (%)**	**MT/SAT**	**Age**	**MT (mm)**	**EG (%)**	**MT/SAT**
Normal weight					**Normal weight**			
I	18–25 years	16.09 ± 2.30 (*n* = 100)	53.48 ± 9.53 (*n* = 100)	1.75 ± 1.08 (*n* = 100)	18–23 years	17.30 ± 2.96 (*n* = 176)	53.86 ± 12.26 (*n* = 176)	2.77 ± 1.53 (*n* = 176)
II	26–38 years	14.83 ± 2.91 (*n* = 91)	58.64 ± 8.57 (*n* = 91)	1.17 ± 0.69 (*n* = 91)	24–31 years	18.43 ± 3.30 (*n* = 169)	56.24 ± 10.73 (*n* = 169)	2.01 ± 0.98 (*n* = 169)
III	39–49 years	12.80 ± 2.64 (*n* = 60)	66.61 ± 8.53 (*n* = 60)	0.86 ± 0.68 (*n* = 60)	32–45 years	16.84 ± 2.11 (*n* = 108)	59.08 ± 10.84 (*n* = 108)	1.76 ± 0.84 (*n* = 108)
IV	50–89 years	11.31 ± 2.82 (*n* = 49)	76.10 ± 8.95 (*n* = 49)	0.61 ± 0.40 (*n* = 49)	46–84 years	16.32 ± 3.45 (*n* = 148)	62.48 ± 11.80 (*n* = 148)	1.39 ± 1.04 (*n* = 148)
	p	**< 0.001**	**< 0.001**	**< 0.001**	***p***	**< 0.001**	**< 0.001**	**< 0.001**
Overweight					**Overweight**			
I	18–25 years	15.85 ± 3.22 (*n* = 18)	59.96 ± 9.75 (*n* = 18)	0.48 ± 0.15 (*n* = 18)	18–23 years	18.18 ± 2.94 (*n* = 88)	55.96 ± 10.53 (*n* = 88)	1.35 ± 0.86 (*n* = 88)
II	26–38 years	15.58 ± 2.66 (*n* = 22)	57.12 ± 10.33 (*n* = 22)	0.63 ± 0.31 (*n* = 22)	24–31 years	18.42 ± 3.70 (*n* = 72)	54.79 ± 10.80 (*n* = 72)	1.29 ± 0.82 (*n* = 72)
III	39–49 years	13.51 ± 2.66 (*n* = 40)	69.04 ± 11.06 (*n* = 40)	0.52 ± 0.28 (*n* = 40)	32–45 years	17.09 ± 3.00 (*n* = 120)	59.68 ± 12.91 (*n* = 120)	0.92 ± 0.47 (*n* = 120)
IV	50–89 years	11.65 ± 3.16 (*n* = 52)	73.64 ± 11.79 (*n* = 52)	0.45 ± 0.12 (*n* = 52)	46–84 years	14.89 ± 3.41 (*n* = 96)	66.10 ± 14.81 (*n* = 96)	0.75 ± 0.62 (*n* = 96)
	p	**< 0.001**	**< 0.001**	**0.019**	***p***	**< 0.001**	**< 0.001**	**< 0.001**
Obesity					**Obesity**			
I	18–25 years	16.18 ± 2.90 (*n* = 8)	65.28 ± 14.30 (*n* = 8)	0.30 ± 0.06 (*n* = 8)	18–23 years	17.83 ± 3.47 (*n* = 9)	52.51 ± 17.18 (*n* = 9)	0.47 ± 0.27 (*n* = 9)
II	26–38 years	12.39 ± 1.93 (*n* = 12)	48.85 ± 8.18 (*n* = 12)	0.27 ± 0.10 (*n* = 12)	24–31 years	19.78 ± 3.68 (*n* = 43)	50.54 ± 11.50 (*n* = 43)	0.69 ± 0.24 (*n* = 43)
III	39–49 years	14.36 ± 2.83 (*n* = 24)	64.08 ± 9.85 (*n* = 24)	0.36 ± 0.10 (*n* = 24)	32–45 years	15.97 ± 3.04 (*n* = 38)	57.36 ± 12.62 (*n* = 38)	0.45 ± 0.13 (*n* = 38)
IV	50–89 years	12.35 ± 2.73 (*n* = 37)	61.90 ± 14.00 (*n* = 37)	0.32 ± 0.11 (*n* = 37)	46–84 years	14.04 ± 2.87 (*n* = 50)	62.07 ± 8.26 (*n* = 50)	0.33 ± 0.08 (*n* = 50)
	p	**0.001**	**0.004**	0.137	***p***	**< 0.001**	**< 0.001**	**< 0.001**
	Thigh							
Women					***Men***			
Normal weight					**Normal weight**			
I	18–27 years	38.64 ± 7.48 (*n* = 103)	38.00 ± 14.14 (*n* = 103)	3.36 ± 1.57 (*n* = 215)	18–22 years	41.14 ± 7.63 (*n* = 166)	35.97 ± 13.63 (*n* = 166)	7.78 ± 3.34 (*n* = 166)
II	28–40 years	32.81 ± 8.06 (*n* = 180)	47.90 ± 15.88 (*n* = 180)	2.51 ± 0.85 (*n* = 180)	23–30 years	40.61 ± 8.73 (*n* = 192)	44.63 ± 11.74 (*n* = 192)	7.50 ± 3.26 (*n* = 192)
III	41–50 years	29.93 ± 6.91 (*n* = 127)	54.62 ± 14.90 (*n* = 127)	2.20 ± 0.88 (*n* = 127)	31–45 years	40.25 ± 7.66 (*n* = 117)	41.86 ± 13.01 (*n* = 117)	8.47 ± 3.66 (*n* = 117)
IV	51–89 years	28.42 ± 7.26 (*n* = 103)	56.60 ± 16.65 (*n* = 103)	2.12 ± 1.02 (*n* = 103)	46–84 years	32.25 ± 6.30 (*n* = 142)	48.65 ± 14.02 (*n* = 142)	6.28 ± 1.99 (*n* = 142)
	p	**< 0.001**	**< 0.001**	**< 0.001**	***p***	**< 0.001**	**< 0.001**	**< 0.001**
Overweight					**Overweight**			
I	18–27 years	36.93 ± 7.58 (*n* = 41)	41.55 ± 14.64 (*n* = 41)	1.93 ± 0.68 (*n* = 41)	18–22 years	39.73 ± 9.00 (*n* = 64)	44.11 ± 15.48 (*n* = 64)	4.70 ± 1.85 (*n* = 64)
II	28–40 years	33.09 ± 6.80 (*n* = 52)	46.81 ± 15.71 (*n* = 52)	1.74 ± 0.59 (*n* = 52)	23–30 years	41.50 ± 8.77 (*n* = 75)	44.15 ± 15.79 (*n* = 75)	5.47 ± 2.18 (*n* = 75)
III	41–50 years	32.16 ± 7.05 (*n* = 107)	50.67 ± 14.23 (*n* = 107)	1.84 ± 0.65 (*n* = 107)	31–45 years	40.48 ± 7.42 (*n* = 130)	46.48 ± 13.74 (*n* = 130)	5.67 ± 3.04 (*n* = 130)
IV	51–89 years	27.07 ± 6.71 (*n* = 106)	56.41 ± 16.83 (*n* = 106)	1.55 ± 0.47 (*n* = 106)	46–84 years	32.23 ± 8.28 (*n* = 91)	56.41 ± 15.83 (*n* = 91)	4.54 ± 1.68 (*n* = 91)
	p	**< 0.001**	**< 0.001**	**< 0.001**	***p***	**< 0.001**	**< 0.001**	**0.001**
Obesity					**Obesity**			

Table 6 (continued)

	Abdomen							
Women					**Men**			
Quartile	**Age**	**MT (mm)**	**EG (%)**	**MT/SAT**	**Age**	**MT (mm)**	**EG (%)**	**MT/SAT**
I	18–27 years	37.27 ± 5.12 (*n* = 14)	49.54 ± 12.96 (*n* = 14)	1.78 ± 0.81 (*n* = 14)	18–22 years	46.01 ± 10.65 (*n* = 9)	30.39 ± 12.66 (*n* = 9)	2.60 ± 0.93 (*n* = 9)
II	28–40 years	34.71 ± 12.62 (*n* = 25)	38.28 ± 12.83 (*n* = 25)	1.53 ± 0.64 (*n* = 25)	23–30 years	51.30 ± 10.11 (*n* = 33)	31.65 ± 13.86 (*n* = 33)	4.66 ± 2.03 (*n* = 33)
III	41–50 years	31.19 ± 7.16 (*n* = 48)	59.22 ± 16.87 (*n* = 48)	1.40 ± 0.42 (*n* = 48)	31–45 years	39.73 ± 8.61 (*n* = 46)	41.00 ± 16.38 (*n* = 46)	2.93 ± 1.02 (*n* = 46)
IV	51–89 years	28.40 ± 8.32 (*n* = 67)	53.14 ± 21.29 (*n* = 67)	1.38 ± 0.57 (*n* = 67)	46–84 years	32.68 ± 6.81 (*n* = 47)	55.68 ± 16.70 (*n* = 47)	3.17 ± 1.15 (*n* = 47)
	p	**0.012**	**< 0.001**	0.891	***p***	**< 0.001**	**< 0.001**	**< 0.001**
Hip					***Chest***			
Normal weight					**Normal weight**			
I	18–33 years	20.91 ± 4.85 (*n* = 109)	60.90 ± 10.94 (*n* = 109)	2.77 ± 1.41 (*n* = 109)	18–24 years	27.07 ± 5.98 (*n* = 126)	47.57 ± 10.88 (*n* = 126)	8.23 ± 3.53 (*n* = 126)
II	34–43 years	20.37 ± 4.63 (*n* = 118)	64.55 ± 10.67 (*n* = 118)	2.49 ± 1.51 (*n* = 118)	25–31 years	29.30 ± 7.10 (*n* = 136)	52.14 ± 11.49 (*n* = 136)	6.87 ± 3.58 (*n* = 136)
III	44–51 years	21.05 ± 4.21 (*n* = 76)	71.70 ± 11.19 (*n* = 76)	2.00 ± 1.06 (*n* = 76)	32–46 years	28.91 ± 7.23 (*n* = 115)	52.45 ± 11.97 (*n* = 115)	7.05 ± 3.79 (*n* = 115)
IV	52–74 years	18.60 ± 3.80 (*n* = 75)	75.76 ± 9.64 (*n* = 75)	2.10 ± 1.08 (*n* = 75)	47–74 years	24.96 ± 7.50 (*n* = 134)	64.22 ± 12.02 (*n* = 134)	6.07 ± 2.93 (*n* = 134)
	p	**0.002**	**< 0.001**	**< 0.001**	***p***	**< 0.001**	**< 0.001**	**< 0.001**
Overweight					**Overweight**			
I	18–33 years	23.51 ± 4.03 (*n* = 50)	63.31 ± 13.63 (*n* = 50)	1.29 ± 0.40 (*n* = 50)	18–24 years	28.13 ± 6.39 (*n* = 73)	56.60 ± 10.74 (*n* = 73)	5.34 ± 3.51 (*n* = 73)
II	34–43 years	22.61 ± 3.69 (*n* = 21)	64.33 ± 12.82 (*n* = 21)	1.39 ± 0.74 (*n* = 21)	25–31 years	28.49 ± 7.38 (*n* = 51)	56.58 ± 13.67 (*n* = 51)	4.91 ± 2.52 (*n* = 51)
III	44–51 years	21.07 ± 0.55 (*n* = 71)	68.95 ± 13.91 (*n* = 71)	1.19 ± 0.34 (*n* = 71)	32–46 years	27.57 ± 7.30 (*n* = 104)	62.31 ± 13.95 (*n* = 104)	3.73 ± 2.54 (*n* = 104)
IV	52–74 years	20.32 ± 3.95 (*n* = 84)	72.15 ± 12.98 (*n* = 84)	1.10 ± 0.29 (*n* = 84)	47–74 years	24.36 ± 8.71 (*n* = 75)	68.21 ± 14.01 (*n* = 75)	2.37 ± 1.91 (*n* = 75)
	p	**< 0.001**	**0.002**	**0.005**	***p***	**0.004**	**< 0.001**	**< 0.001**
Obesity					**Obesity**			
I	18–33 years	25.84 ± 2.77 (*n* = 18)	63.56 ± 11.84 (*n* = 18)	1.07 ± 0.20 (*n* = 18)	18–24 years	36.55 ± 3.19 (*n* = 3)	73.38 ± 2.46 (*n* = 3)	1.71 ± 0.30 (*n* = 3)
II	34–43 years	20.85 ± 3.07 (*n* = 16)	68.35 ± 10.02 (*n* = 16)	1.04 ± 0.33 (*n* = 16)	25–31 years	33.27 ± 6.18 (*n* = 15)	55.91 ± 9.29 (*n* = 15)	2.27 ± 0.77 (*n* = 15)
III	44–51 years	22.06 ± 3.80 (*n* = 42)	69.64 ± 11.70 (*n* = 42)	0.98 ± 0.27 (*n* = 42)	32–46 years	30.67 ± 5.54 (*n* = 31)	55.03 ± 11.12 (*n* = 31)	2.14 ± 0.58 (*n* = 31)
IV	52–74 years	22.36 ± 4.97 (*n* = 41)	69.46 ± 11.11 (*n* = 41)	1.03 ± 0.30 (*n* = 41)	47–74 years	24.72 ± 6.92 (*n* = 27)	69.23 ± 13.68 (*n* = 27)	1.53 ± 0.73 (*n* = 27)
	p	**0.002**	0.241	0.657	***p***	**< 0.001**	**< 0.001**	**0.002**
Triceps								
Normal weight								
I	18–33 years	31.59 ± 7.26 (*n* = 125)	41.15 ± 14.72 (*n* = 125)	2.79 ± 1.07 (*n* = 125)				
II	34–44 years	31.57 ± 7.35 (*n* = 130)	43.50 ± 14.99 (*n* = 130)	2.31 ± 0.79 (*n* = 130)				
III	45–53 years	32.80 ± 6.77 (*n* = 79)	48.60 ± 12.81 (*n* = 79)	2.29 ± 0.59 (*n* = 79)				
IV	54–89 years	31.33 ± 6.43 (*n* = 80)	50.37 ± 15.49 (*n* = 80)	2.28 ± 0.67 (*n* = 80)				
	p	0.536	**< 0.001**	**< 0.001**				
Overweight								
I	18–33 years	32.67 ± 7.12 (*n* = 51)	46.45 ± 15.14 (*n* = 51)	1.85 ± 0.46 (*n* = 51)				
II	34–44 years	35.61 ± 5.56 (*n* = 50)	41.59 ± 14.13 (*n* = 50)	1.86 ± 0.54 (*n* = 50)				
III	45–53 years	33.30 ± 4.78 (*n* = 86)	42.87 ± 14.41 (*n* = 86)	1.71 ± 0.35 (*n* = 86)				
IV	54–89 years	31.03 ± 6.23 (*n* = 78)	49.18 ± 13.88 (*n* = 78)	1.75 ± 0.43 (*n* = 78)				

Table 6 (continued)

	Abdomen							
Women					**Men**			
Quartile	**Age**	**MT (mm)**	**EG (%)**	**MT/SAT**	**Age**	**MT (mm)**	**EG (%)**	**MT/SAT**
	p	**< 0.001**	**0.009**	**0.006**				
Obesity								
I	18–33 years	33.81 ± 5.37 (*n* = 28)	41.69 ± 14.18 (*n* = 28)	1.65 ± 0.46 (*n* = 28)				
II	34–44 years	31.93 ± 5.63 (*n* = 28)	43.43 ± 15.94 (*n* = 28)	1.59 ± 0.23 (*n* = 28)				
III	45–53 years	31.12 ± 5.75 (*n* = 45)	50.09 ± 14.36 (*n* = 45)	1.49 ± 0.32 (*n* = 45)				
IV	54–89 years	31.10 ± 5.11 (*n* = 52)	50.02 ± 18.54 (*n* = 52)	1.52 ± 0.27 (*n* = 52)				
	p	0.150	0.055	0.165				

Data are expressed as mean ± SD. Difference between groups were compared by one-way ANOVA, followed by Bonferroni test as *post-hoc* test. A *p* value in bold type denotes a significant difference ($p < 0.05$)

Abbreviations: subcutaneous adipose tissue, SAT; muscle thickness, MT; echogenicity, EG; standard deviation, SD

this relationship, it is necessary to consider that a healthy muscle (therefore, of good quality) is hypoechogenic, with hyperechogenic intra- and peri- muscular lines referring to the perimysium and epimysium [27]. Based on these considerations, the observation of an increase in EG over time can be interpreted as a (negative) change in the quality of that muscle [27]. This can be due to various factors, such as increased intramuscular lipid content, altered physiological muscle architecture, fibrosis or inflammation. All these factors are manifested by an increase in intratissue sound reflexes [27]. Furthermore, as reported in a recent meta-analysis, muscle EG is associated with physical function in older subjects and, thus, it can be referred again to the muscle quality. Also, the authors suggest for a better assessment, (i) the integration of muscle EG with other evaluations, including the muscle size, as well as (ii) to combine the evaluations of multiple muscle in order to improve the correlations [35]. In line with these evidence and suggestions, ultrasound assessments may represent a valid tool, since with it is possible to directly and immediately measure the EG of various muscles in the same sites and integrate it with the single MT and dimension.

Further interesting insights come from the multiple regression analysis carried out which shows (i) that tSAT is always excluded from the model, suggesting that in understanding the influence of subcutaneous fat on BMI it should be not only considered SAT as such, but the distribution of its single components (sSAT and dSAT) and (ii) that the sSAT/dSAT and MT/SAT ratios show a negative relationship, suggesting that although it is the SAT that influences BMI, its component dSAT appears to have a greater influence. Even in men, in fact, although the influence of the sSAT/dSAT ratio on BMI is not significant, that of the dSAT is.

Limitations and strengths

The main strength of this study lies in the fact that, for the first time, population-specific reference values of ST thicknesses (tSAT, sSAT, dSAT and MT), their distribution (sSAT/dSAT and MT/SAT) and quality (EG) obtained by ultrasound were obtained in various body sites in a large cohort stratified for sex, BMI and age. This provides important insights into the overall nutritional assessment of the subject, which goes beyond the estimation of BC. Similarly, however, it has certain limitations. Firstly, the retrospective nature of this study implies that there is not the same number of analyses for the various BMI categories and age quartiles or for the various sites. At the same time, there is no important information available on individual subjects, such as eventual current drug therapies and the presence of any endocrine-metabolic alteration that may have an impact on BC, as well as details regarding physical activity levels or training programs. Secondly, the evidence currently available does not allow definitive conclusions to be drawn about the significance of muscle EG as an indicator of muscle quality, although various authors have taken up this concept. Studies, indeed, have demonstrated the existence of a correlation between muscular EG and certain parameters considered to be indicators of muscle quality, such as phase angle and hand grip strength [18–20, 22]. Overall, therefore, this evidence allows us to speculate on the hypothesis that EG may be considered as an indicator of muscle quality. However, further studies are needed to investigate this relationship and, possibly, validate the value of EG as an indicator of muscle quality, comparing it with golden standard methods. Thirdly, it has been reported in the literature how muscle EG is influenced by muscle size, making it necessary to normalise EG for MT, in order both to better assess muscle quality and to compare it more correctly between different populations [36]. Fourthly, although the ST thickness measurements obtained with the ultrasound probe object of this study were compared in previous studies with the traditional ultrasound [9, 10], no studies are currently available that compare the muscle EG with these two methods. However, two important concepts should be taken into

Table 7 Correlation between SAT-muscle measurements in abdomen and thigh and age-BMI for women and men

Abdomen (women)					Abdomen (men)				
Parameter	**Age**		**BMI**		**Parameter**	**Age**		**BMI**	
	Simple correlation					**Simple correlation**			
	r	***p*-value**	**r**	***p*-value**		**r**	***p*-value**	**r**	***p*-value**
sSAT (mm)	0.103	**0.020**	0.550	**< 0.001**	sSAT (mm)	0.211	**< 0.001**	0.569	**< 0.001**
dSAT (mm)	0.236	**< 0.001**	0.736	**< 0.001**	dSAT (mm)	0.227	**< 0.001**	0.728	**< 0.001**
tSAT (mm)	0.284	**< 0.001**	0.787	**< 0.001**	tSAT (mm)	0.326	**< 0.001**	0.754	**< 0.001**
sSAT/dSAT	-0.292	**< 0.001**	-0.337	**< 0.001**	sSAT/dSAT	-0.232	**< 0.001**	-0.458	**< 0.001**
MT (mm)	-0.526	**< 0.001**	-0.120	**0.006**	MT (mm)	-0.348	**< 0.001**	-0.050	0.097
EG (%)	0.499	**< 0.001**	0.021	0.639	EG (%)	0.331	**< 0.001**	-0.011	0.718
MT/SAT	-0.434	**< 0.001**	-0.502	**< 0.001**	MT/SAT	-0.392	**< 0.001**	-0.483	**< 0.001**
Thigh (women)					**Thigh (men)**				
sSAT (mm)	0.055	0.071	0.375	**< 0.001**	sSAT (mm)	0.023	0.436	0.305	**< 0.001**
dSAT (mm)	0.134	**< 0.001**	0.447	**< 0.001**	dSAT (mm)	0.027	0.480	0.557	**< 0.001**
tSAT (mm)	0.130	**< 0.001**	0.474	**< 0.001**	tSAT (mm)	0.004	0.903	0.623	**< 0.001**
sSAT/dSAT	-0.215	**< 0.001**	-0.393	**< 0.001**	sSAT/dSAT	0.009	0.803	-0.353	**< 0.001**
MT (mm)	-0.446	**< 0.001**	-0.111	**< 0.001**	MT (mm)	-0.392	**< 0.001**	0.059	**0.047**
EG (%)	0.376	**< 0.001**	0.096	**0.020**	EG (%)	0.318	**< 0.001**	0.047	0.117
MT/SAT	-0.402	**< 0.001**	-0.470	**< 0.001**	MT/SAT	-0.207	**< 0.001**	-0.458	**< 0.001**

A *p*-values in bold type denotes a significance difference ($p < 0.05$)

Abbreviations: body mass index, BMI; superficial subcutaneous adipose tissue, sSAT; deep subcutaneous adipose tissue, dSAT; total subcutaneous adipose tissue, tSAT; muscle thickness, MT; echogenicity, EG; standard deviation, SD

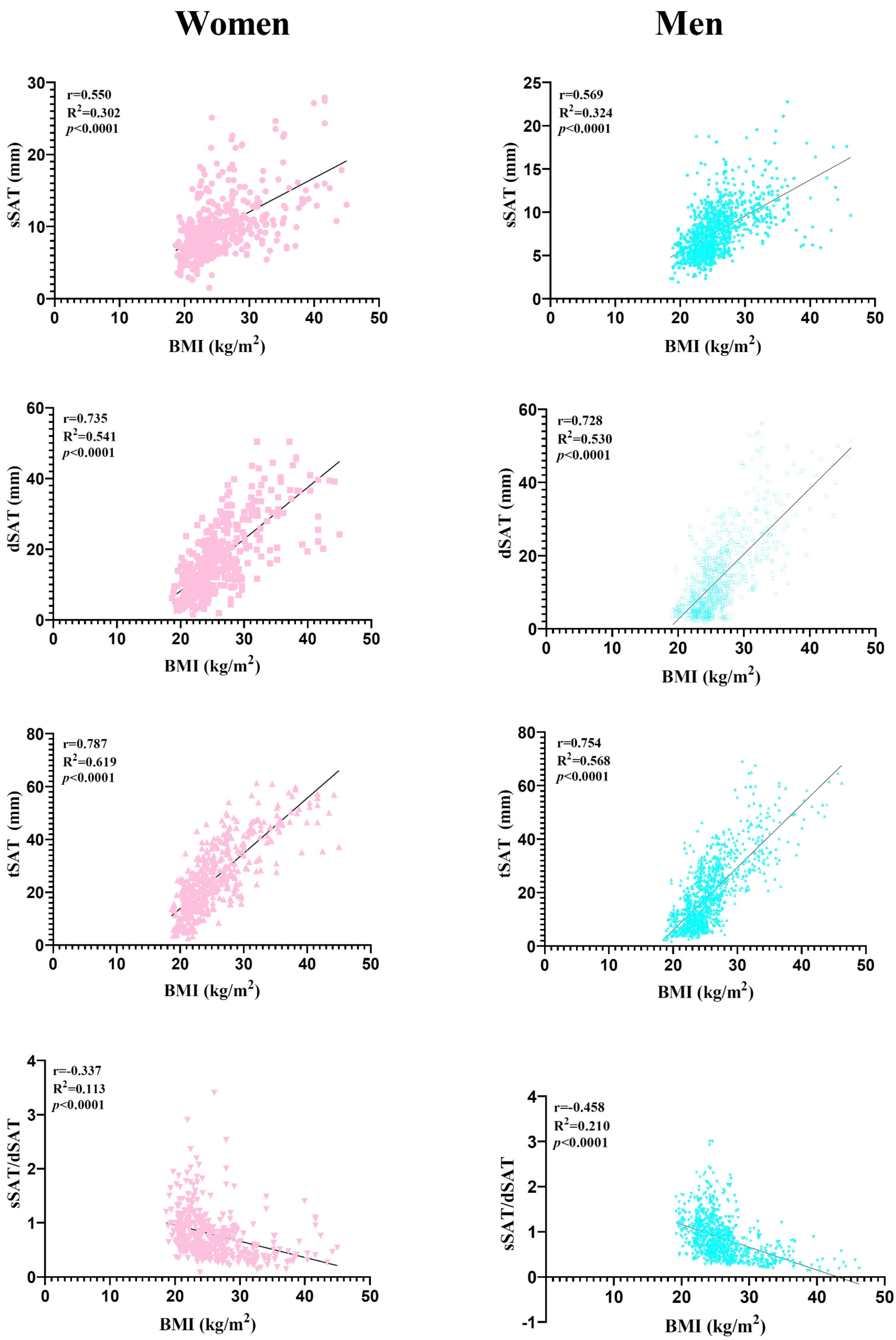

Fig. 2 Correlation between SAT measurements and BMI in abdomen. Correlations between sSAT, dSAT, tSAT, sSAT/dSAT ratio measured in abdomen and BMI in women and men. sSAT, superficial subcutaneous adipose tissue; dSAT, deep subcutaneous adipose tissue; tSAT, total subcutaneous adipose tissue

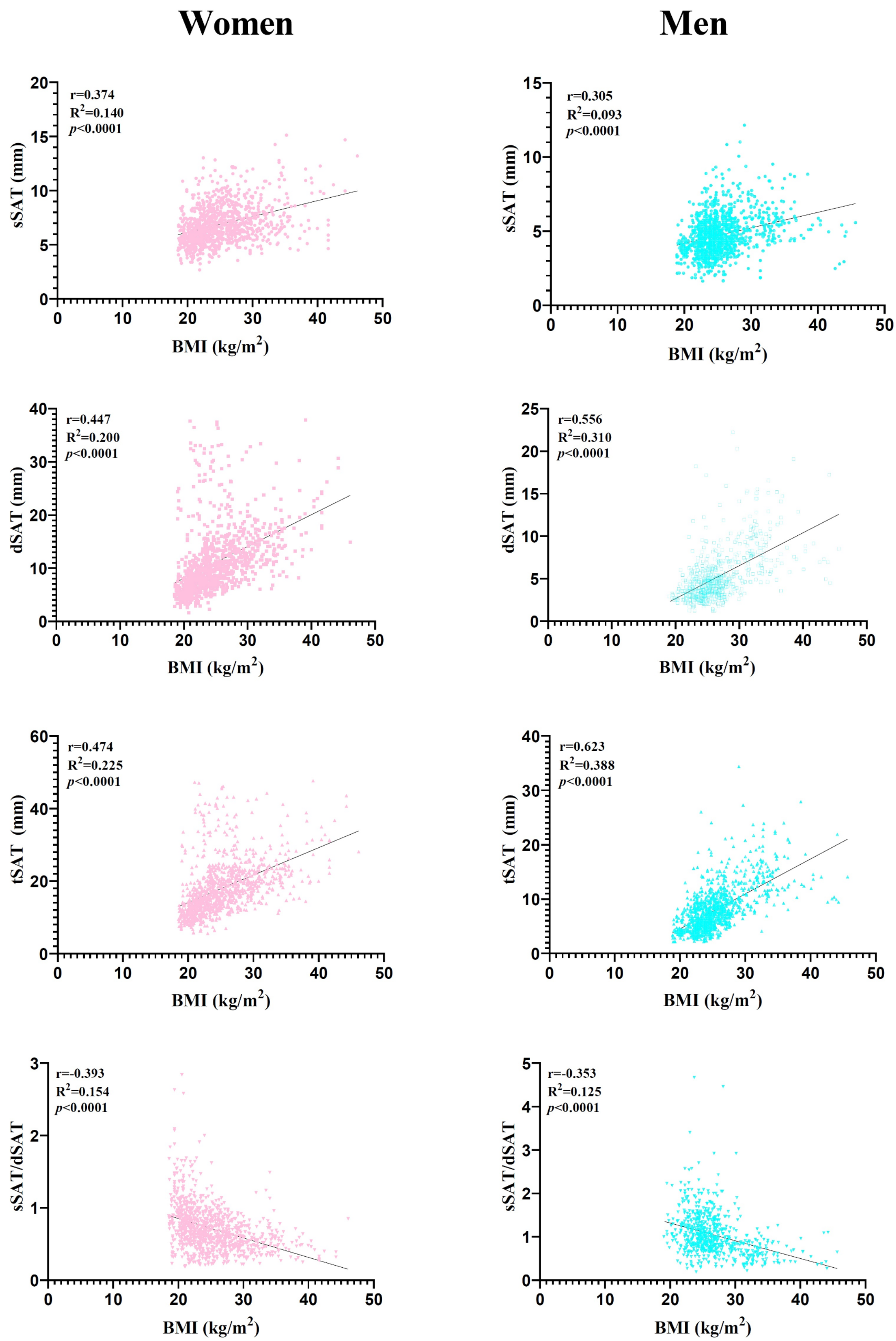

Fig. 3 Correlation between SAT measurements and BMI in thigh. Correlations between sSAT, dSAT, tSAT, sSAT/dSAT ratio measured in thigh and BMI in women and men. sSAT, superficial subcutaneous adipose tissue; dSAT, deep subcutaneous adipose tissue; tSAT, total subcutaneous adipose tissue

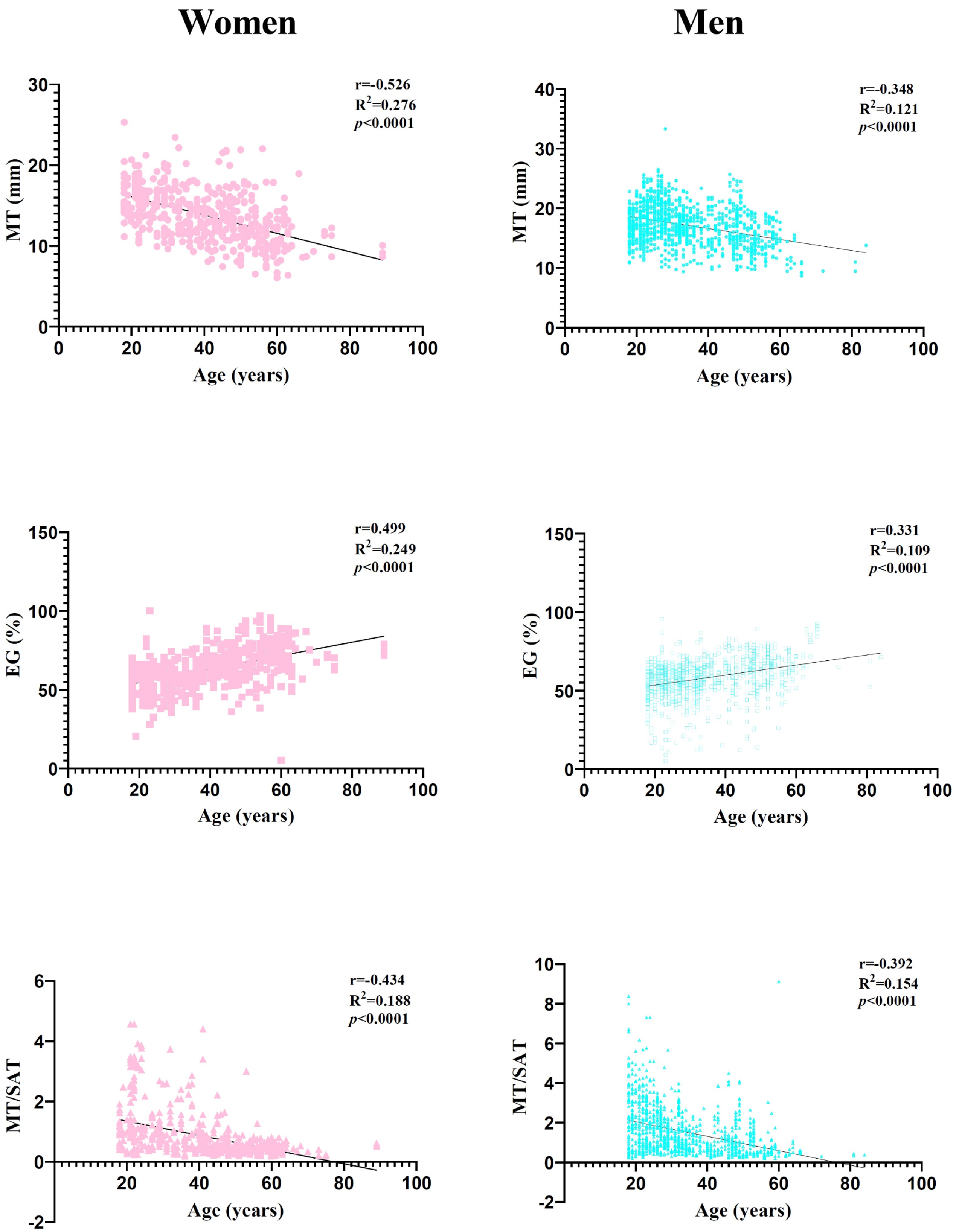

Fig. 4 Correlation between muscle measurements and age in abdomen. Correlations between MT, EG, MT/SAT ratio measured in abdomen and age in women and men. MT, muscle thickness; EG, echogenicity; SAT, subcutaneous adipose tissue

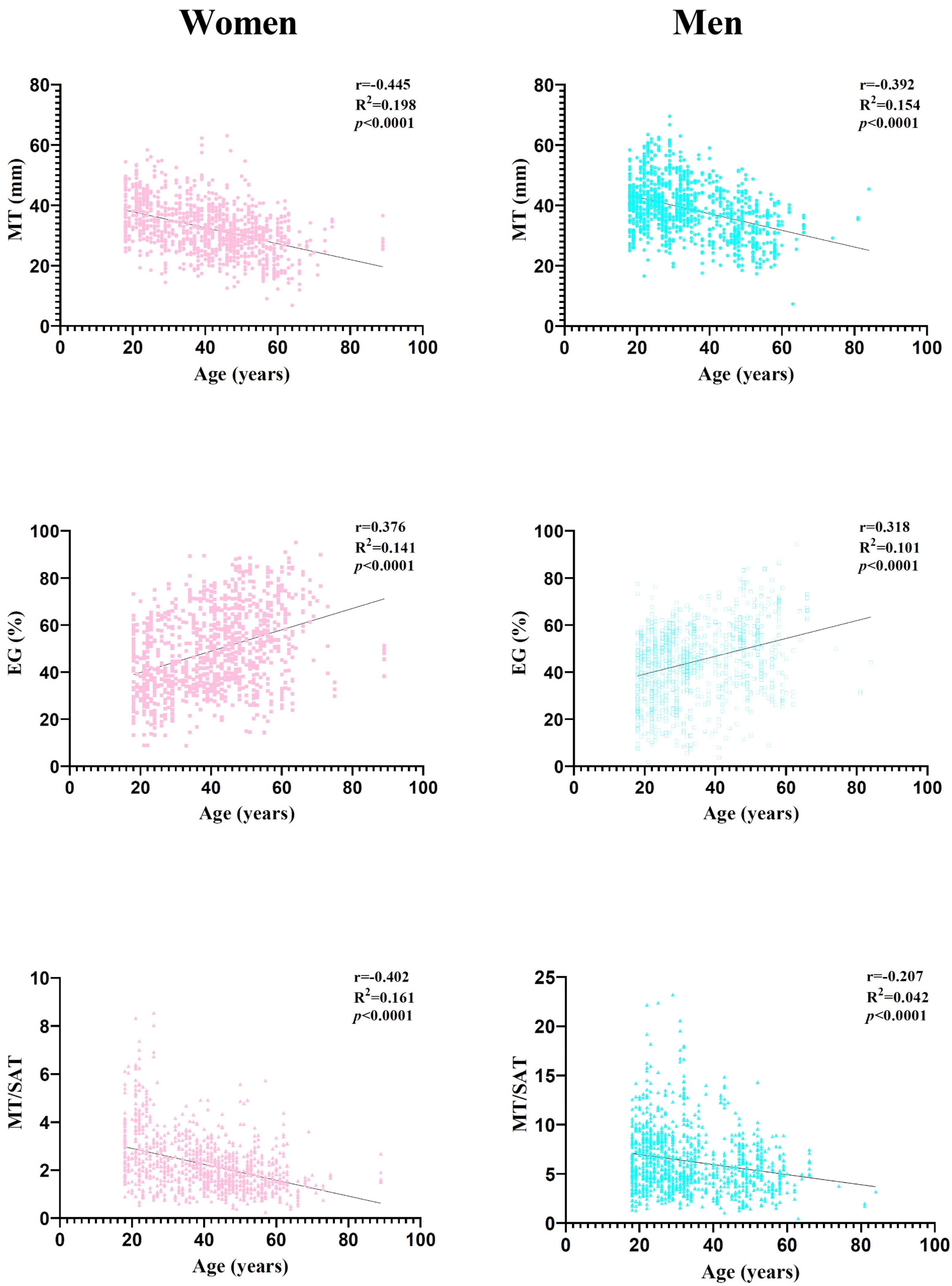

Fig. 5 Correlation between muscle measurements and age in thigh. Correlations between MT, EG, MT/SAT ratio measured in thigh and age in women and men. MT, muscle thickness; EG, echogenicity; SAT, subcutaneous adipose tissue

Table 8 Multiple regression analysis model (stepwise method) with BMI as dependent variable to estimate the prognostic value of age, sSAT, dSAT, tSAT, sSAT/dSAT, MT and MT/SAT in abdomen and thigh, for women and men

Parameters	**Multiple Regression Analysis**								
Abdomen (women)					**Abdomen (men)**				
Model 1	R^2	**β**	**t**	***p* value**	**Model 1**	R^2	**β**	**t**	***p* value**
Age	0.629	0.177	5.087	**<0.001**	Age	0.557	-0.024	-1.017	0.310
sSAT		0.292	6.046	**<0.001**	sSAT		0.152	4.358	**<0.001**
dSAT		0.752	14.868	**<0.001**	dSAT		0.762	19.202	**<0.001**
sSAT/dSAT		0.064	1.213	0.226	sSAT/dSAT		0.100	2.560	**0.011**
MT		-0.065	-1.712	0.088	MT		0.026	0.938	0.348
MT/SAT		0.215	3.737	**<0.001**	MT/SAT		0.065	1.484	0.138
Variable excluded: tSAT					Variable excluded: tSAT				
Thigh (women)					**Thigh (men)**				
Model 1	R^2	**β**	**t**	***p*value**	**Model 1**	R^2	**β**	**t**	***p*value**
Age	0.327	0.231	8.064	**<0.001**	Age	0.398	0.305	9.679	**<0.001**
sSAT		0.181	4.213	**<0.001**	sSAT		-0.011	-0.207	0.836
dSAT		0.04	0.953	0.341	dSAT		0.446	7.940	**<0.001**
sSAT/dSAT		-0.162	-3.539	**<0.001**	sSAT/dSAT		-0.049	-1.080	0.280
MT		0.181	3.999	**<0.001**	MT		0.150	3.336	**0.001**
MT/SAT		-0.265	-3.882	**<0.001**	MT/SAT		-0.121	-2.061	**0.040**
Variable excluded: tSAT					Variable excluded: tSAT				

A *p*-values in bold type denotes a significance difference ($p<0.05$). Abbreviations: body mass index, BMI; superficial subcutaneous adipose tissue, sSAT; deep subcutaneous adipose tissue, dSAT; total subcutaneous adipose tissue, tSAT

account: (i) the measured values of EG (understood as a percentage value) depend on the software used to analyse the ultrasound scan and (ii) it is not possible (or, at least, would not be canonical) to compare the percentage values of EG measured with two different software for analysing ultrasound scans, regardless of the type of probe used. This implies that the data here presented can only refer to ultrasound measurements performed with the probe and analysed with the software used in this study. These limitations also imply the need for further studies aimed at determining the same reference values in more specific classes of subjects and in different clinical settings, as well as at identifying, and possibly validating, additional directly calculated objective indices that integrate EG values with MT values. In order to obtain a more precise and global view of the significance of these indices/parameters, they should, in future studies, be related to other parameters considered as surrogate indicators of muscle quality or, at least, closely related to nutritional status, such as those obtained by means of bioelectrical impedance analysis or handgrip strength, to give examples.

Conclusion

With the advent of ultrasound for the study of ST, a higher and more refined level has been reached in the assessment of the nutritional status, going beyond the mere BC, in terms of estimating %FM. This approach, however, must not be considered as an alternative to other assessment techniques, but must be interpreted in an integrative sense, combining regional ultrasound assessments with measurements and parameters obtained with other methods or instruments, in order to acquire a global and multifactorial evaluation of the subject health status. In this context, the non-diagnostic ultrasound assessments represent a valuable support for nutritionists and dietitians and other non-medical health professionals using the mean values we reported as population-specific reference values. At the same time, due (i) the metabolic implication of SAT components, MT and EG, and (ii) their correlations with BMI and age, their rapid and non-diagnostic assessment and monitoring may be a useful tool for the evaluation of the progress of nutritional interventions and intervention plans, as well as their possible adjustments. However, the observational nature of the present study is intended to emphasise, firstly, the importance of ultrasound monitoring of both thicknesses and EG of STs. In this sense, based on the current available evidence, we believe that the observations we have reported regarding the thicknesses of the STs can be translated into the broad context of ultrasound measurements, regardless of the probe used. As far as muscular EG is concerned, on the other hand, given the absence of evidence that has compared the values obtained with the software for the analysis of ultrasound scans that we used with others that are commonly available, we suggest considering the trend of the differences that we observed by sex, BMI categories and age, rather than the mere numerical value, which could be different if different software is used.

Abbreviations

ST	Subcutaneous tissues
SAT	Subcutaneous adipose tissue
BC	Body composition
BMI	Body mass index
sSAT	Superficial subcutaneous adipose tissue
dSAT	Deep subcutaneous adipose tissue
MT	Muscle thickness
EG	Echogenicity
SD	Standard deviation

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12967-025-06159-1.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

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Author contributions
Conceptualization: GA and LB; Data curation: GA; Formal analysis GA; Methodology GA and GM; Supervision: LB; Roles/Writing - original draft: GA, LV, ARAG, TS; Writing - review & editing. LB, GM and AC. All authors have read and agreed to the published version of the manuscript.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was conducted in accordance with the guidelines outlined in the Declaration of Helsinki, which provides ethical principles for medical research involving human subjects. Additionally, the Ethics Committee of the Università Telematica Pegaso granted a positive opinion on the study protocol (reference no. PROT./E 005082 of 19/07/2024).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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