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## Sex and body mass index implications on gluteofemoral subcutaneous tissue morphology visualized by ultrasonography – preliminary study

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### Keywords

subcutaneous adipose tissue, gluteofemoral, sex, BMI, ultrasonography

### Abstract

**Introduction:** Since the protective value of gluteofemoral subcutaneous adipose tissue against cardiovascular risk factors has already been described in scientific reports, it is important to pay more attention to its evaluation. **Aim of the study:** The purpose of this study was to evaluate sex and body mass index implications on gluteofemoral subcutaneous tissue morphology visualized by ultrasonography. **Material and method:** A population of 40 participants between 20–50 years of age was examined. All individuals underwent the ultrasound examination of subcutaneous adipose tissue in three locations: anterior, posterior and lateral side of a thigh in the 1/3 distal part. All examinations were collected, and the following parameters were evaluated: thickness of subcutaneous adipose tissue in general, thickness of superficial and deep subcutaneous adipose tissue. **Results:** The study revealed significant differences in the architecture of subcutaneous adipose tissue between male and female subgroups. In the group of males, a significantly thinner layer of not only subcutaneous adipose tissue in general (0.65 vs. 1.67 cm,  $p < 0.0001$ ), but also in its main compartments was observed. Moreover, we observed strong positive correlation between body mass index and all subcutaneous adipose tissue layers in the female subgroup. Interestingly, there was no relation between the thickness of the subcutaneous adipose tissue layers between subgroups with a decreased and normal body mass index and an increased body mass index. **Conclusions:** The presented data indicates that sex is an important factor in the determination of subcutaneous adipose tissue architecture of a thigh. The ultrasound examination of this structure can be a useful prognostic tool in the assessment of cardiovascular risk.

## Introduction

Over the last decades, subcutaneous adipose tissue (SAT) has been a subject of interest for many specialists in various fields of medicine, such as cardiology, endocrinology and metabolic diseases. There are numerous reports on the

endocrine function of SAT<sup>(1,2)</sup> and the impact of sex hormones on its distribution<sup>(3,4)</sup>.

SAT is described anatomically as a continuous structure<sup>(5)</sup>. However, recent findings indicate that it is divided into many compartments separately supplied

with blood<sup>(6)</sup>. Its basis is formed of superficial fascia<sup>(7)</sup>, which divides it into two well-defined layers: the superficial subcutaneous adipose tissue (sSAT) and the deep subcutaneous adipose tissue (dSAT)<sup>(8)</sup>. Currently, a growing interest in the relation between the structure and the function of each SAT layer is observed. Distribution of SAT could be examined with anthropometric methods or by using modern diagnostic methods such as ultrasound (US), dual X-ray absorptiometry (DXA) or magnetic resonance imaging (MRI)<sup>(9-11)</sup>. Due to the fact that different types of obesity have various fat distributions, further investigation of SAT morphology in different body locations seems to be important. Negative influence of abdominal subcutaneous adipose tissue (ASAT) on cardiovascular risk (CVR) is already well-established<sup>(12-14)</sup>.

However, the role of adipose tissue in other body localizations has not been fully investigated. Over the last years, numerous reports have been published about the role of adipose tissue in the lower parts of the body<sup>(13,15,16)</sup>. It has been well-documented that adipose tissue of the leg in women, in opposition to intraabdominal and trunk adipose tissue measured by DXA, was consistently negatively related to CVR<sup>(17,18)</sup>. Aasen G. *et al.* stated that a high leg/trunk fat mass ratio assessed in DXA may give protection against diabetes mellitus and a cardiovascular disease in obese postmenopausal women by attenuating the risk of dyslipidemia and insulin resistance measured with Homeostasis Model Assessment of Insulin Resistance<sup>(9)</sup>. The described positive metabolic activity of SAT located in lower body parts in women may be associated with higher lipoprotein lipase activity. Interestingly, this relationship was not observed in the male population<sup>(19)</sup>.

Most of the above-cited data were obtained with advanced diagnostic tools such as DXA or MRI, which, due to relatively high costs and limited access, cannot

be easily used in large populations. In our research, we compared the morphological construction of the thigh's SAT between genders using US. It is an easily accessible and cost-effective method that can be widely used in SAT assessment.

The aim of our study was to define the differences in the morphology of thigh's SAT in relation to the gender and body mass index (BMI) with ultrasound imaging.

## Material and method

The research was conducted from September to December 2014. Written consent has been obtained from all participants. According to the medical report, all participants were healthy, without any hormonal or metabolic disorders and did not take any chronic medications. In all individuals, all necessary anthropometric measurements have been carried out. Next, the US examinations were conducted in the Department of Imaging Diagnostics at the Medical University of Warsaw. All individuals underwent the US examination of skin and SAT layers in three locations: anterior, posterior and lateral side of a thigh in the 1/3 distal part. Body characteristic points that helped to determine the proper location were anterior superior iliac spine and the middle of ipsilateral patella basis in the upright position. Measurements of a single patient were performed in a supine position, manually by the same examiner, without using the pressure during the examinations. The measurement was repeated three times in the same position to increase the reproducibility of images. In total, 360 Bmode ultrasound images of thigh's skin and SAT were acquired with the same examination protocol. Imaging depth depended on the BMI level of an individual in the study and the focal distance was always adjusted proportionally to this depth.

**Tab. 1.** Baseline characteristics of the study population

	Male & Female N = 40		Male N = 14 (35%)		Female N = 26 (65%)	
Age [years]	26*	24–36.5**	30.5*	26–37**	26*	24–36**
Weight [kg]	71.74+	15.31++	82.21+	11.69++	66.1+	14.15++
Height [m]	1.71+	0.08++	1.78+	0.07++	1.67+	0.05++
BMI [kg/m <sup>2</sup> ]	23.21*	20.95–27.02**	25.56*	23.25–27.73**	22.09*	20.7–25.95**
Dermis [D; cm]	0.17*	0.15–0.20**	0.17*	0.15–0.18**	0.16*	0.14–0.20**
Subcutaneous adipose tissue [SAT; cm]	1.24*	0.77–1.89**	0.65*	0.47–0.81**	1.67*	1.24–2.02**
Superficial subcutaneous adipose tissue [sSAT; cm]	0.59*	0.34–0.73**	0.30*	0.21–0.37**	0.67*	0.59–0.90**
Deep subcutaneous adipose tissue [dSAT; cm]	0.60*	0.29–1.02**	0.20*	0.15–0.36**	0.86*	0.57–1.40**
Superficial fascia [SF; cm]	0.05*	0.04–0.05**	0.05*	0.04–0.05**	0.05*	0.04–0.05**

\* median; \*\* IQR; + mean; ++ standard deviation

## Population and anthropometric measurements

The examined population included 40 participants (26 women, 14 men) between 20–50 years of age. In those qualified for the study, height and weight were measured. The participants were wearing underwear and no shoes. BMI was calculated by dividing body weight [kg] by height squared [m<sup>2</sup>].

$$\left( BMI = \frac{mass [kg]}{height^2 [m^2]} \right)$$

Then, all individuals were divided into 2 groups: people with a proper weight or underweight (BMI <25) and overweight or obese people (BMI ≥25).

## US examination

The US examinations were carried out using Philips EPIQ 5 Bmode ultrasound machine with a 5–18 MHz linear probe. All measurements were done in an axial view. The following parameters were evaluated: thickness of the dermis (D), SAT, sSAT, dSAT and superficial fascia (SF). SAT thickness was measured from the border of the dermis and SAT – to the upper edge of the deep fascia. sSAT thickness was measured from the border of the dermis and SAT – to the upper edge of the superficial fascia. dSAT thickness was measured from the bottom edge of the superficial fascia to the upper edge of the deep fascia. A ratio of dSAT/sSAT was calculated and compared between genders and BMI values.

## Statistical analysis

SAS 9.2 package was utilized to carry out statistical analyses. All variables were tested for a normal distribution with the Shapiro-Wilk test. Normally distributed continuous variables are represented as a mean ± SD (SD-standard deviation), and nonnormally distributed continuous variables are represented as a median (25<sup>th</sup>–75<sup>th</sup> percentile (IQR)). One-sided Student's t-test and the Wilcoxon test were used to evaluate the significance of changes in quantitative variables. In order to evaluate correlations between changes in quantitative variables, the Pearson or Spearman correlation coefficients were calculated.

**Tab. 2.** dSAT/sSAT ratio analysis (N – number of people)

dSAT/sSAT	Male & Female (N = 40)			
	1.20*; 0.83–1.54**			
	Female (N = 26)		Male (N = 14)	
	1.30* 1.04–1.56**		0.82* 0.54–1.12**	
	BMI ≤25 (N = 7)	BMI >25 (N = 19)	BMI ≤25 (N = 6)	BMI >25 (N = 8)
1.26* 0.96–1.45**	1.75* 1.41–2.22**	0.48* 0.41–0.56**	1.03* 0.86–1.40**	

\* mediana; \*\* IQR; N – number of people)

## Results

### General analysis

Baseline characteristics of the study population are shown in Tab. 1.

The study revealed significant difference in the architecture of SAT between male and female subgroups (Fig. 1). In the group of males, a significantly thinner layer of SAT (0.65 vs. 1.67,  $p < 0.0001$ ), as well as its main compartments – sSAT and dSAT, was observed. The difference in thickness of the SAT layers was more distinctive in the inner adipose layer (dSAT; 0.20 cm vs. 0.86 cm;  $p < 0.0001$ ) than in the superficial one (sSAT; 0.30 cm vs. 0.67 cm;  $p < 0.0001$ ) (Fig. 1 A). Generally, dSAT layer was thicker than sSAT (dSAT/sSAT ratio = 1.20, IQR 0.83–1.54) (Tab. 2).

A detailed analysis of the data received from particular localizations of a thigh (anterior, lateral and posterior surface) revealed analogical relations. The greatest difference in SAT layers thickness between genders was observed on the lateral surface of a thigh (Fig. 2). Fig. 3 shows ultrasound images of SAT layers visualized in different localization on the thigh in both sexes.

Interestingly, there was no relation between the thickness of the SAT layers between subgroups with normal and increased BMI values in general (Fig.1B). Furthermore, there was no significant correlation between BMI and the thickness of the SAT and its compartments (Tab. 3).

### Analysis in gender subgroups

The study revealed that males with normal BMI are characterized by greater thickness of the sSAT layer (0.37 cm vs. 0.23 cm;  $p = 0.0027$ ; dSAT/sSAT = 0.48, IQR 0.41–0.56) (Fig. 4 A, Tab. 2). Moreover, we found a significant positive correlation between BMI and the thickness of dSAT layer in the male subgroup ( $r = 0.830$ ;  $p = 0.0002$ ), which was confirmed by the dSAT/sSAT ratio (Tab. 2). Table 4 shows the results of a correlation analysis between BMI and the studied variables.

Significant differences in SAT anatomy between females with an increased BMI and those within normal ranges of BMI were found (Fig 4B). Overweight and obese women

**Tab. 3.** Correlations between BMI and thickness of SAT compounds

BMI	SAT		dSAT		sSAT	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
	0.082	0.6200	0.180	0.2500	0.056	0.7300

SAT – subcutaneous adipose tissue; sSAT – superficial subcutaneous adipose tissue; dSAT – deep subcutaneous adipose tissue; *p* – *p*-value; *r* – *r* value

**Tab. 4.** Correlations between BMI and thickness of SAT compounds in male

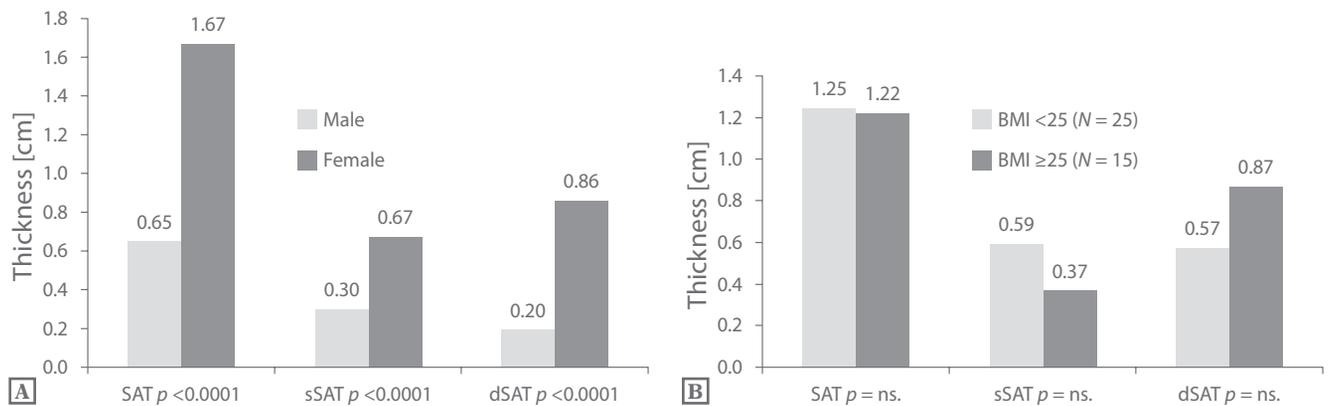
BMI	SAT		dSAT		sSAT	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
	0.480	0.0830	0.830	0.0002	-0.196	0.5000

SAT – subcutaneous adipose tissue; sSAT – superficial subcutaneous adipose tissue; dSAT – deep subcutaneous adipose tissue; *p* – *p*-value; *r* – *r* value

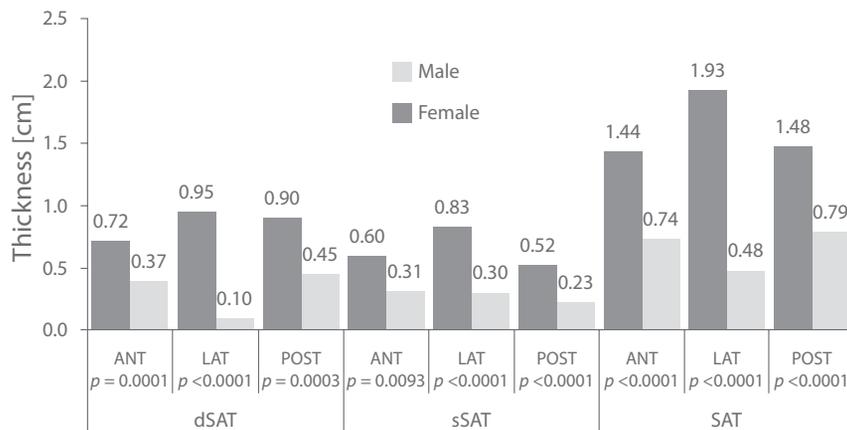
were characterized by the predominant increase in dSAT thickness (dSAT 1.80 cm vs. sSAT 1.00 cm *p* = 0.05), whereas in subjects with a normal BMI, thickness of the discussed parameters remained at the same level (dSAT 0.72 cm vs. sSAT 0.62 cm, *p* = 0.24). This dependence is well illustrated by the dSAT/sSAT ratio (1.75 IQR 1.41–2.22 for BMI > 25 and 1.26 IQR 0.96–1.45 for BMI ≤ 25). The comparison of US images obtained from women with normal and increased BMI values is visualized in Fig. 5. In addition, we observed strong positive correlation between BMI and all SAT layers in the female subgroup. Table 5 presents the analyses of correlations between BMI and the studied variables.

**Analysis between genders in BMI subgroups**

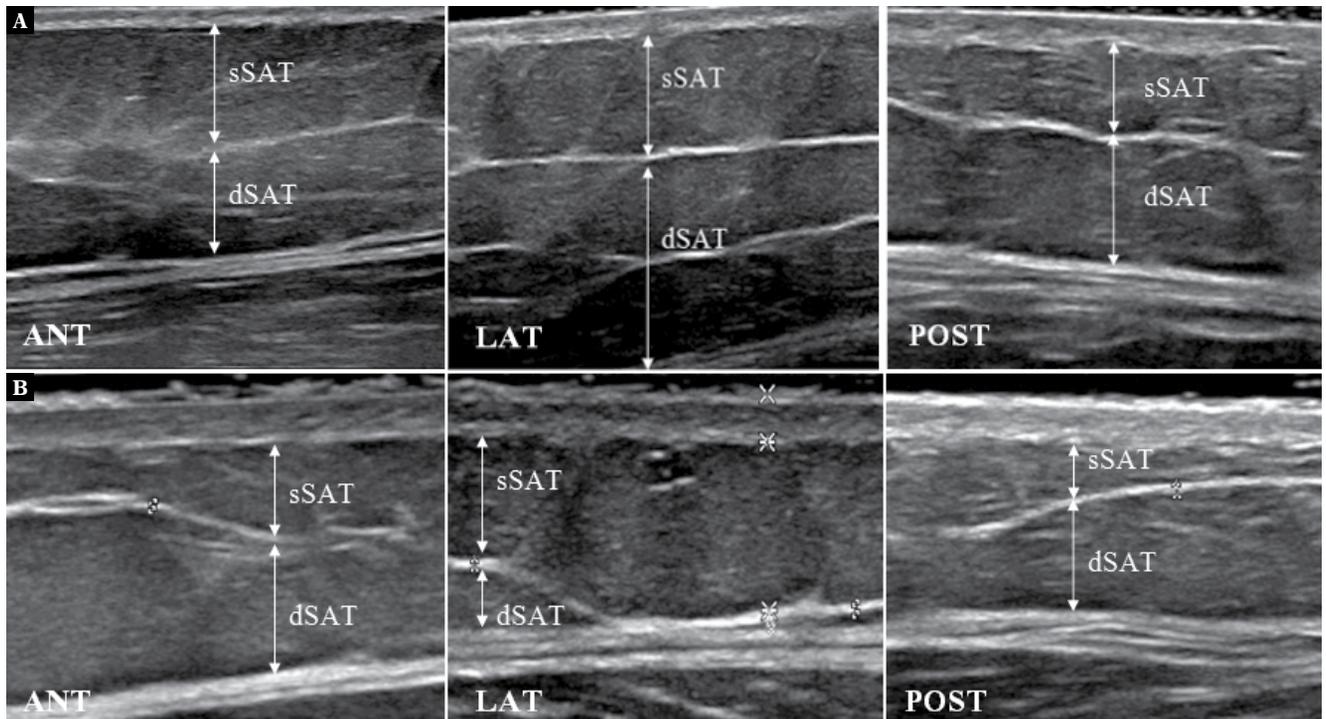
Analysis of SAT morphology between females and males within BMI subgroups (for females: BMI median = 21.8 kg/m<sup>2</sup>; IQR 20.7–22.98 kg/m<sup>2</sup>; for males: BMI median = 25.56 kg/m<sup>2</sup>; IQR 23.25–27.73 kg/m<sup>2</sup>) revealed that SAT layers are generally significantly thinner in the male subgroup (*p* < 0.05) even despite increased BMI values. Table 6 presents a summary comparison of SAT morphology in female and male subgroups. Furthermore, Fig. 5 shows the comparison of ultrasound images of SAT layers visualized in both sexes.



**Fig. 1.** **A.** Differences in subcutaneous adipose tissue's morphology – analysis of genders (SAT – subcutaneous adipose tissue; sSAT – superficial subcutaneous adipose tissue; dSAT – deep subcutaneous adipose tissue). **B.** Comparison of SAT and its compounds in "low-BMI" and "high-BMI" subgroups (SAT – subcutaneous adipose tissue; sSAT – superficial subcutaneous adipose tissue; dSAT – deep subcutaneous adipose tissue; nss – not statistically significant)



**Fig. 2.** Subcutaneous adipose tissue's morphology in different localizations (SAT – subcutaneous adipose tissue; sSAT – superficial subcutaneous adipose tissue; dSAT – deep subcutaneous adipose tissue; ANT – anterior surface; LAT – lateral surface; POST – posterior surface)



**Fig. 3.** Ultrasound images of SAT visualized in different localisation on the thigh of a 26-year-old woman (BMI = 22,8 kg/m<sup>2</sup>) (A) and a 31-year-old man (BMI = 23,3 kg/m<sup>2</sup>) (B). The thinnest layer of the SAT was localised on the lateral surface of thigh in female and in posterior surface in male.

## Discussion

In the study, two morphologically independent layers of thighs' SAT sSAT and dSAT were identified and compared. Similar layers had been described previously mainly at the ASAT<sup>(20,21)</sup>. It was observed that sex is the strongest factor determining the construction of thigh's SAT. Interestingly, there was no significant correlation between BMI and the thickness of the SAT, and its compartments in general population. On that conclusion, the results shown in this paper are consistent with the tests carried out by Störchle *et al.*<sup>(22)</sup>, stating that individual BMI measurement cannot be a diagnostic tool for assessing the amount of adipose tissue in general. In fact, for comparable levels of BMI, the thicknesses of individual layers of SAT are significantly higher in women than in men. When subgroups were analysed, a strong correlation was shown between the BMI and the thickness of all SAT layers in women, with the strongest correlation with the dSAT. These results were additionally confirmed by the calculation of the dSAT/sSAT ratio (see Table 2) which showed a higher value for the women group, with the highest value for obese and overweight women (BMI ≥25). These findings are consistent with the conclusions formulated by Querleux B *et al.*<sup>(10)</sup>.

Since adipose tissue is an active endocrine gland, its examination could be a new tool in the diagnosis of numerous diseases e.g. obesity or glucose intolerance, as well as a way to evaluate the cardiovascular risk<sup>(13,16,23)</sup>. Most of the published studies emphasize a positive correlation between the thickness of ASAT, visceral adipose

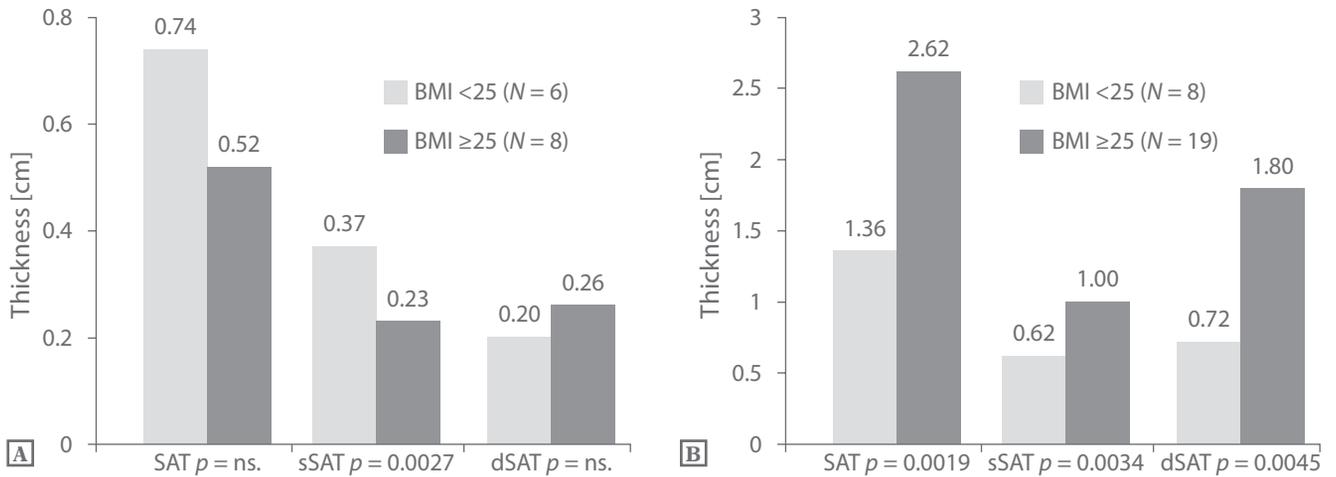
tissue (VAT) and cardiovascular risk. These studies concern less about the structure and function of adipose tissue located elsewhere in the body<sup>(4,24)</sup>. Walker *et al.* conducted a research which showed independent metabolic functions of sSAT and dSAT layers and their association with obesity. Significant correlation was found between the volume of sSAT and obesity, and its metabolic consequences<sup>(21)</sup>. Given the incidence of different types of obesity in both sexes<sup>(25)</sup> and, in consequence, various adipose tissue distribution in the body<sup>(26)</sup>, the extension of the adipose tissue research for other body locations, especially thighs, seems necessary to fully assess the significance of SAT.

Over recent years, there has been an increased interest in methods that are useful in evaluation of both morphology and function of the discussed tissue. So far, most common superficial fascia and SAT layers were visualized by the means of histology<sup>(27,28)</sup>, DXA<sup>(9,17,18)</sup> and MRI<sup>(10,29)</sup>, but few reports present utilization of US in its evaluation<sup>(22)</sup>. US is a credible and repeatable method, readily

**Tab. 5.** Correlations between BMI and thickness of SAT compounds in female

BMI	SAT		dSAT		sSAT	
	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>	<i>r</i>	<i>p</i>
	0.657	0.0003	0.775	<0.0001	0.751	<0.0001

SAT – subcutaneous adipose tissue; sSAT – superficial subcutaneous adipose tissue; dSAT – deep subcutaneous adipose tissue; *p* – *p*-value; *r* – *r* value



**Fig. 4. A.** Relationship between BMI and SAT thickness – analysis of male subgroup (*nss* – not statistically significant). **B.** Relationship between BMI and SAT thickness – analysis of female subgroup (SAT – subcutaneous adipose tissue; sSAT – superficial subcutaneous adipose tissue; dSAT – deep subcutaneous adipose tissue)

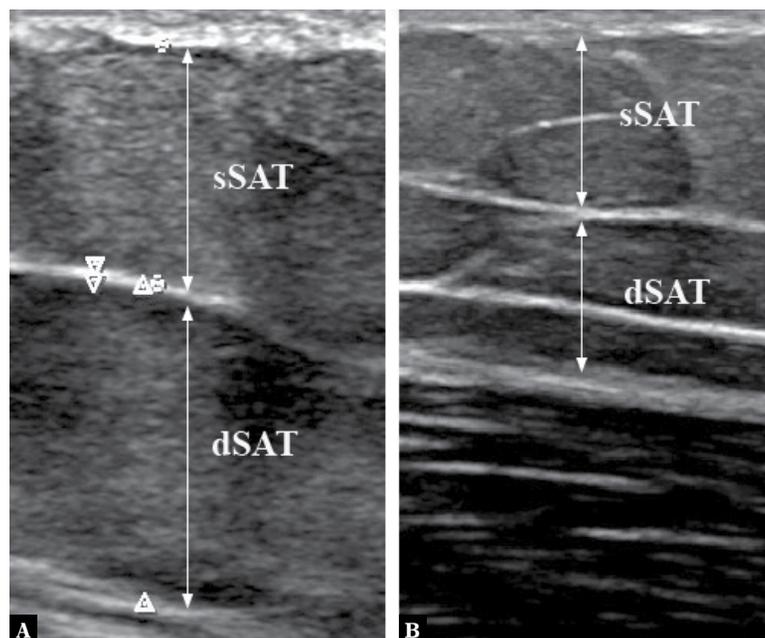
available, inexpensive, non-invasive, and therefore may be used as a common method of studies of subcutaneous tissue<sup>(22,30)</sup>. US examination used in our research made it possible to visualize the superficial fascia with higher resolution, which, in turn, allowed obtaining accurate measurements.

To conclude, we have shown that US is a useful tool in the *in vivo* evaluation of SAT anatomy of thighs. It is one of the first studies evaluating thigh's SAT using this technology on such a large group of people, and it allows to expand the knowledge about the structure of this area. Figures presenting the sSAT and dSAT layers and the

relationship between them can provide additional information for the assessment of the cardiovascular risk. If the results are confirmed on a large group of patients and a comparison between healthy group and group with the elevated risk of the cardiovascular disease is carried out, US will become a perfect tool for the evaluation of the CVR.

## Conclusions

The presented data indicates that sex is an important factor in the determination of SAT architecture of a thigh.



**Fig. 5.** Ultrasound images of SAT localized on the anterior surface of thigh visualised in a 40-year-old woman (BMI = 32 kg/m<sup>2</sup>) (A) and a 23-year-old woman (BMI = 20 kg/m<sup>2</sup>) (B). Abnormal BMI value is accompanied by the thicker layer of the SAT with the asymmetric increase in the dSAT layer

**Tab. 6.** Comparison of SAT morphology between genders in „low-BMI” and „high-BMI” subgroups

sSAT (cm)			
	Male (in general) (N = 14)	Male; BMI ≤25 (N = 6)	Male; BMI >25 (N = 8)
Female (in general) (N = 26)	0.30 vs. 0.67 <i>p</i> < 0.0001	0.37 vs. 0.67 <i>p</i> = 0.0027	0.23 vs. 0.67 <i>p</i> = 0.0002
Female; BMI ≤25 (N = 19)	0.30 vs. 0.62 <i>p</i> < 0.0001	0.37 vs. 0.62 <i>p</i> = 0.0014	0.23 vs. 0.62 <i>p</i> = 0.0006
Female; BMI >25 (N = 7)	0.30 vs. 1.0 <i>p</i> = 0.0017	–*	–*
dSAT (cm)			
	Male (in general) (N = 14)	Male; BMI ≤25 (N = 6)	Male; BMI >25 (N = 8)
Female (in general) (N = 26)	0.20 vs. 0.86 <i>p</i> < 0.0001	0.20 vs. 0.86 <i>p</i> = 0.0008	0.26 vs. 0.86 <i>p</i> = 0.0013
Female; BMI ≤25 (N = 19)	0.20 vs. 0.72 <i>p</i> = 0.0002	0.20 vs. 0.72 <i>p</i> = 0.0017	0.26 vs. 0.72 <i>p</i> = 0.004
Female; BMI >25 (N = 7)	0.20 vs. 1.80 <i>p</i> = 0.0020	–*	–*
SAT (cm)			
	Male (in general) (N = 14)	Male; BMI ≤25 (N = 6)	Male; BMI >25 (N = 8)
Female (in general) (N = 26)	0.65 vs. 1.67 <i>p</i> < 0.0001	0.74 vs. 1.67 <i>p</i> = 0.0014	0.52 vs. 1.67 <i>p</i> = 0.0004
Female; BMI ≤25 (N = 19)	0.65 vs. 1.36 <i>p</i> = 0.0001	0.74 vs. 1.36 <i>p</i> = 0.0032	0.52 vs. 1.36 <i>p</i> = 0.0012
Female; BMI >25 (N = 7)	0.65 vs. 2.62 <i>p</i> = 0.0017	–*	–*

\* Analysis was not valid due to the small number of subjects; N – number of people

Study revealed significantly thickened layers of the SAT, with the predominance of dSAT layer in the female subgroup. Even though in males, the conclusive interpretation of the collected data seems to be stymied by the small number of patients in subgroups, we observed a positive correlation between BMI and thickness of the dSAT layer.

### Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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