

Increased legs-to-total fat percentage ratio in females with a normal body mass index: A change in lifestyle or the adaptation process of the body

Boštjan Jakše¹ , Stanislav Pinter² , and Uroš Godnov³ 

¹Independent Researcher, 1235 Radomlje, Slovenia

²University of Ljubljana, Faculty of Sport, Basics of Movements in Sport, Ljubljana, Slovenia

³University of Primorska, Faculty of Mathematics, Natural Sciences and Information Technologies, Department of Computer Science, Koper, Slovenia

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Abstract

Monitoring the prevalence of obesity is of great importance for assessing lifestyle interventions aimed at preventing or reducing the health and economic burden of obesity. A sedentary Western-type lifestyle results in an increase in the incidence of normal-weight (e.g., thin fat) obesity. In the present cross-sectional study, the regional body composition status (using a multifrequency, medically approved electrical bioimpedance monitor) of 844 Slovene adults was examined. The primary aim was to compare the leg fat percentage to total body fat percentage (LEGFAT%-to-TOTFAT%) and to trunk fat percentage (TRUFAT%) (LEGFAT%-to-TRUFAT%) ratios of participants in the normal and obese body mass index (BMI) and categories according to the BMI and obesity classifications of the World Health Organization. In addition, examined how correlated with, sex, and age, according to obesity classification cut-offs. Results showed, for the whole sample, that increases by an average of 0.13% each year (*ceteris paribus*). However, females in the normal BMI and TOTFAT% categories, but not males, had significantly higher LEGFAT%-to-TOTFAT% and LEGFAT%-to-TRUFAT% ratios than those in the obese category. Furthermore, adjusted R² (linear regression) showed that 82.5% of the variation in LEGFAT% was explained by variations in TOTFAT%, sex, and age. The present results indicated the increasing importance of studying the regional body composition status, especially of TRUFAT% and LEGFAT% compared with TOTFAT% (beyond the known sex differences). The clinical relevance of the increasing disproportionality in the regional body composition status of females with normal BMI and TOTFAT% needs to be further clarified.

Keywords: regional body composition · adults · females · bioimpedance · normal-weight obesity · lifestyle

✉ Correspondence:
Boštjan Jakše
bj7899@student.uni-lj.si

Introduction

In the last few decades, the global prevalence of obesity has nearly tripled (Boutari & Mantzoros, 2022) and the medical and economic costs continue to increase (Vera Anekwe et al., 2020). Today, approximately 650 million adults suffer from obesity (Sørensen, Martinez, & Jørgensen, 2022). The main reasons are the adoption of a progressively more sedentary lifestyle (which includes physical inactivity) and the consumption of an unhealthy diet (Boutari & Mantzoros, 2022). In smaller epidemiological studies evaluating obesity status, to improve the accuracy of obesity screening, body mass index (BMI) is typically combined with the total body fat percentage measurement to define the proportion of overweight and obese people (Hung et al., 2017; Jakše et al., 2021, 2022a, 2022b). Otherwise, the especially female obesity rate could be underestimated (Hung et al., 2017; Jakše, Godnov, & Pinter, 2022) using only the BMI classification of the World Health Organization (WHO) (World Health Organization, 2010). It is well established that adults with a BMI in the overweight or obese category and increased adiposity have a higher risk of mortality (Di Angelantonio et al., 2016; Jayedi et al., 2022). However, this may not necessarily be true in older adult populations (Javed et al., 2020). Therefore, body composition evaluation should be integrated into routine clinical practice for the initial assessment and sequential follow-up of nutritional status (Thibault & Pichard, 2012).

Body composition is known to differ between females and males. Females have more fat mass, while males have more muscle mass. Females also accumulate adipose tissue more often around the hips and thighs, whereas males usually accumulate adipose tissue around the abdominal region (Bredella, 2017). Furthermore, there is an increasingly widespread phenomenon where normal-weight individuals have excess total body fat percentage (TOTFAT%) (Cornier et al., 2011; Kouda et al., 2021). In addition, the prevalence of central obesity in these individuals is rather low (Cornier et al., 2011). Moreover, these individuals are otherwise not classified as having standard BMI and TOTFAT% obesity, according to the BMI and TOTFAT% obesity classification of the WHO (World Health Organization, 1995, 2010), but commonly have metabolic syndrome and increased cardiovascular risk factors (Cornier et al., 2011; Kouda et al., 2021). Due to this phenomenon, the recent global overfat prevalence of 62–76% may

have been significantly underestimated (Maffetone & Laursen, 2020).

In addition, the clinical relevance of disproportionately elevated ratios of these normal-weight individuals (for example, leg fat percentage (LEGFAT%)-to-TOTFAT% or LEGFAT%-to-trunk fat percentage (TRUFAT%) ratios) is still questionable, especially in females. This phenomenon is substantially more common for over-sedentary Western-type lifestyles, especially in high-income countries, which shows the magnitude of the consequences of daily prolonged sitting and general physical inactivity (Dempsey et al., 2020; Katzmarzyk et al., 2022; McLaughlin et al., 2020). Nevertheless, these groups of adults should be analyzed further to better define their adiposity risk (Cornier et al., 2011), especially since BMI better reflects TOTFAT% rather than fat percentage distribution (Kouda et al., 2021).

This study is part of a larger study of the nutritional status of Slovene adults in the post-COVID-19 pandemic period (Jakše, Godnov, & Pinter, 2022). In the present study, performed a regional body composition analysis of participants. In addition, our main hypothesis (primary aim) was that females with normal BMI and TOTFAT% would have, on average, higher LEGFAT%-to-TOTFAT% and LEGFAT%-to-TRUFAT% ratios than females with BMI and TOTFAT% obesity, according to the BMI and TOTFAT% obesity classifications of the WHO. In other words, although seemingly unlikely, females in the normal BMI and TOTFAT% categories accumulate more LEGFAT% than those in the obese category. Furthermore, we examined how LEGFAT% correlated with TOTFAT% cut-offs (with normal and obese categories, according to the TOTFAT% obesity classification), sex, and age.

Method

Study Design and Eligibility

Participants from various locations in Slovenia (2.1 million European Union citizens) to cover all regions (Statistical Office of the Republic of Slovenia, 2020) were included. This cross-sectional study protocol was approved on 26 June 2022 by the Ethical Committee in the field of sports in Slovenia (approval document no. 033–41/2022–5). The trial was registered on 30 June 2022 at <https://clinicaltrials.gov> with the number NCT05438966. Furthermore, the study was conducted from 1 July to 31 August 2022. The subject selection and the methods have been

described in detail in previously (Jakše, Godnov, & Pinter, 2022).

Subjects

In the final analysis, we randomly enrolled 844 Slovene adults (females: 64% and males: 36%, $p = 0.304$) who voluntarily underwent body composition measurements. The generally healthy study participants were recruited to participate, without BMI limitations. Furthermore, in the study, we also did not include competitive athletes (the recruitment process was not intentionally or specifically related to healthy and active lifestyle adult), pregnant or lactating females and adults with already diagnosed common chronic disease (the criterion was prescription use of medication).

This study did not examine the physical activity levels of the participants or their effect on body composition and diet. It was part of a larger project

that analyzed food intake only, without supplements, and compared it to recommended levels (Jakše, Godnov, & Pinter, 2022). The results showed that Slovenian adults had an imbalanced diet, including a low intake of complex carbohydrates and high consumption of processed foods. Energy and nutrient intake showed a low intake of carbohydrates, fiber, and a higher intake of total fat, saturated fatty acids (for men), and cholesterol. The micronutrient intake showed a low intake of vitamins C, D, and E (for men) and calcium, but a high intake of sodium and chloride (for men) compared to recommended levels (Jakše, Godnov, & Pinter, 2022).

All participants in this study signed the informed consent form. Furthermore, the participants were not financially remunerated. A detailed flow chart of the study recruitment and the current process studied are presented in Figure 1.

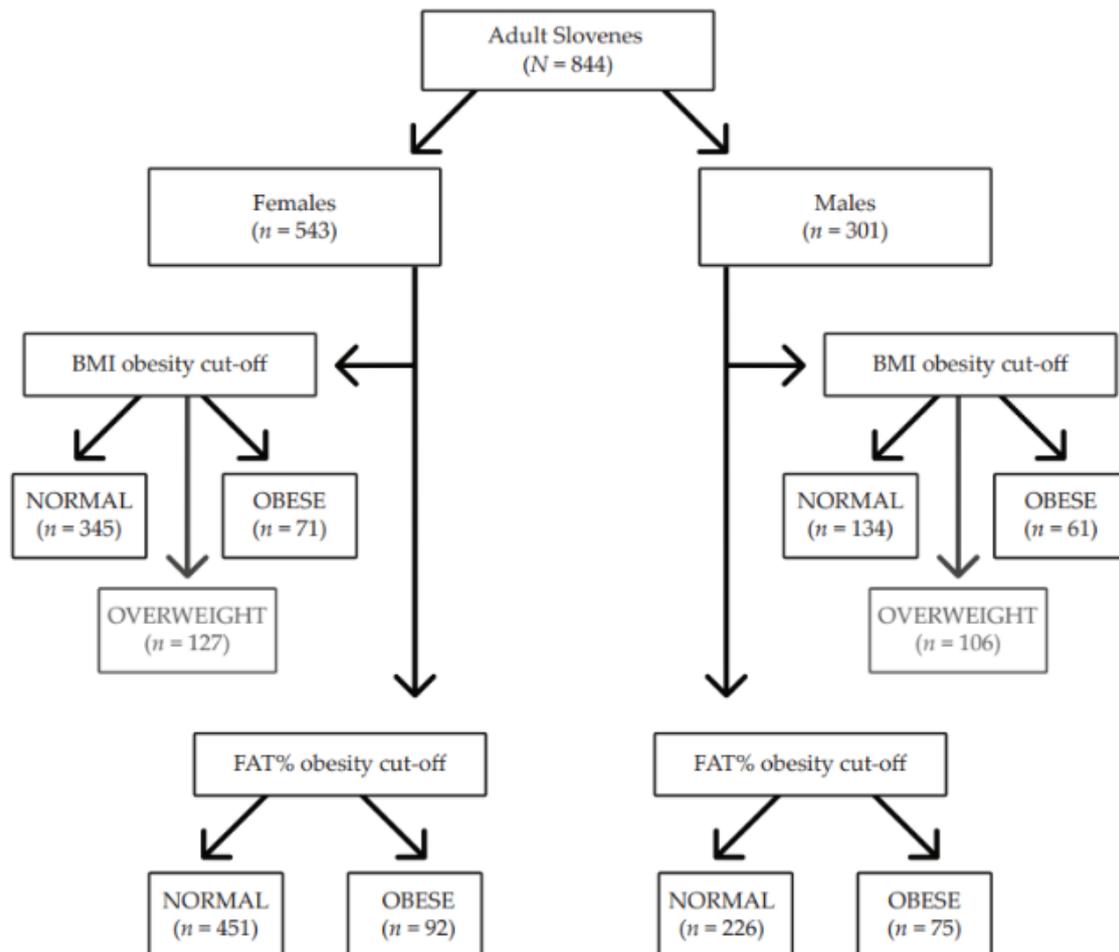


Figure 1. Recruitment process for participation in the study

Outcomes

Regional Body Composition Measures

The regional body composition measures were obtained with a multifrequency segmental, medically approved, and calibrated electrical bioimpedance monitor (BIA: Tanita 780 S MA, Tokyo, Japan). In addition, body height was measured with a standardized, medically approved professional personal floor scale with a stand (Kern, MPE 250K100HM, Kern & Sohn, Balingen, Germany). Furthermore, BMI was calculated as body mass in kilograms, measured by the body composition monitor, and was divided by the square of body height in meters. Of note, the results from the same model of an 8-electrode body composition analyzer that we used were compared with dual-energy X-ray absorptiometry (DXA) and provided accurate measurements of total body fat in healthy young adults females and males, regardless of their level of habitual physical activity (Miller et al., 2016; Verney et al., 2015) and adults with severe obesity (Ballesteros-Pomar et al., 2022). However, a validity study of our multifrequency BIA model compared with DXA on generally healthy normal weight, overweight, and obese class 1 and 2 adults of the age range between 30 and 65 years is still lacking. Nevertheless, currently, BIA analysis is inexpensive, easy to use, portable, and without radiation exposure (Yamada et al., 2017), thus, it is useful on a large sample of the same ethnic group and used throughout the country.

The regional body composition results included the following variables: body height, body mass, BMI, TOTFAT%, average TOTFAT% of both arms (ARMFAT%), TRUFAT%, average total FAT% of both legs (LEGFAT%), and the LEGFAT%-to-TOTFAT% and LEGFAT%-to-TRUFAT% ratios. In addition, we compared the LEGFAT%-to-TOTFAT% and TRUFAT%-to-LEGFAT% ratios within females and males in the normal and obese BMI and TOTFAT% categories according to the BMI for obesity (i.e., ≥ 30 kg/m² for females and males) (World Health Organization, 2010) and TOTFAT% obesity classifications (i.e., $>35\%$ for females and $>25\%$ for males) (World Health Organization, 1995). Finally, we examined how LEGFAT% correlated with TOTFAT% cut-offs (in normal and obese TOTFAT% categories), sex, and age.

Statistical Analysis

Statistical analysis was performed using R 4.1.1. with the tidyverse (Wickham et al., 2019), ggstatsplot (Patil, 2021), and arsenal (Heinzen et al., 2021)

packages. The tidyverse package was used for data transformation, ggstatsplot2 was used for data visualization, and arsenal was used for statistical calculation (e.g., for independent two-sample t-tests and ANOVA with a Tukey posthoc test). In addition, for numerical variables, we used independent sample t-test and linear regression to estimate the relationship between the TOTFAT%-to-LEGFAT% and LEGFAT%-to-TRUFAT% ratios. Variance distribution was tested with a Welch two-sample t-test. Age and sex from these adjustments were statistically significant ($p < 0.001$). Furthermore, an independent two-sample t test was used for the LEGFAT%-to-TOTFAT% and TRUFAT%-to-LEGFAT% ratios for participants in the normal and obese categories, according to the BMI and TOTFAT% obesity classifications. The adjusted R² model was used to assess the contribution of LEGFAT% in explaining variations in TOTFAT%, sex, and age. The threshold for statistical significance was $p < 0.05$. No missing data were present. No sensitivity analysis was performed. Of note, because we have a quite large dataset, we completely rely on the central limit theorem, which is a statistical concept that describes the behavior of the mean of a random sample drawn from any distribution. In this case, checking the normal distribution within a test becomes almost obsolete. Data are presented as the means (standard deviations), and the differences and the p values were further adjusted with the Bonferroni method.

Results

The sample consisted of 777 adults (18–64 years) and 67 older adults (≥ 65 years). In addition, the average age of adults and older adults was 38.3 ± 10.9 and 68.4 ± 3.6 years, respectively ($p < 0.001$). The average BMI status for the whole sample was in the overweight BMI category (25.1 ± 5.0 kg/m²), with an average BMI and TOTFAT% of 24.3 ± 4.8 kg/m² and 32.7% and 26.6 ± 5.0 kg/m² and 23% for adult females and males, respectively. In terms of sex differences, females had significantly lower BMI values (24.3 kg/m² vs. 26.6 kg/m², $p < 0.001$) and higher TOTFAT% (27.3% vs. 19.8% , $p < 0.001$). Of note, the complete nonsegmental body composition status by sex and age and the characteristics of the participants, including their dietary intake, dietary pattern identification, and smoking status, are reported elsewhere (Jakše, Godnov, et al., 2022).

Table 1. Regional body composition status by sex and according to the BMI and TOTFAT% obesity classification.

Parameters	According to BMI classification								According to TOTFAT% obesity classification					
	Males				Females				Males			Females		
	NO	OV	OB	p-value	NO	OV	OB	p-value	NO	OB	p-value	N	OB	p-value
N (%)	138 (46)	107 (35)	56 (19)	< 0.001	365 (67)	120 (22)	58 (11)	< 0.001	230 (76)	71 (24)	< 0.001	452 (83)	91 (17)	< 0.001
Age (y)	35.3 ± 12.4	42.4 ± 13.4	46.7 ± 12.2	< 0.001	38.7 ± 12.7	44.1 ± 12.9	47.8 ± 12.8	< 0.001	38.3 ± 13.5	45.3 ± 13.4	0.002	39.6 ± 12.6	47.9 ± 13.0	< 0.001
Body height (cm)	179.7 ± 6.5	179.2 ± 6.1	177.0 ± 7.5	0.999	166.7 ± 6.2	166.2 ± 6.3	164.6 ± 6.0	0.999	179.7 ± 7.0	177.5 ± 6.8	0.781	166.5 ± 6.2	165.6 ± 6.1	0.999
Body mass (kg)	74.1 ± 7.4	86.6 ± 6.5	107.5 ± 19.6	< 0.001	60.0 ± 6.9	75.2 ± 7.0	92.5 ± 15.1	< 0.001	79.3 ± 10.1	102.2 ± 20.0	< 0.001	63.0 ± 9.3	86.1 ± 15.2	< 0.001
BMI (kg/m ²)	22.9 ± 1.5	26.9 ± 1.3	34.2 ± 5.3	< 0.001	21.7 ± 1.7	27.2 ± 1.4	34.1 ± 4.7	< 0.001	24.6 ± 2.6	32.6 ± 5.5	< 0.001	22.7 ± 2.9	31.4 ± 5.2	< 0.001
TOTFAT (%)	14.7 ± 4.5	20.0 ± 4.1	29.8 ± 6.0	< 0.001	23.4 ± 5.6	32.9 ± 4.0	39.3 ± 3.9	< 0.001	16.3 ± 4.5	29.3 ± 5.1	< 0.001	24.8 ± 6.0	38.9 ± 3.1	< 0.001
ARMFAT (%)	14.2 ± 3.9	18.1 ± 4.0	27.9 ± 8.1	< 0.001	23.5 ± 6.3	36.2 ± 3.9	46.4 ± 5.6	< 0.001	15.3 ± 3.9	27.4 ± 6.9	< 0.001	25.7 ± 7.4	43.9 ± 5.9	< 0.001
TRUFAT (%)	15.8 ± 5.5	21.9 ± 4.8	33.1 ± 6.6	< 0.001	18.7 ± 6.8	28.5 ± 5.1	33.7 ± 4.7	< 0.001	17.8 ± 5.6	32.3 ± 6.2	< 0.001	20.1 ± 6.9	34.6 ± 3.3	< 0.001
LEGFAT (%)	12.9 ± 4.2	17.4 ± 4.3	24.8 ± 5.1	< 0.001	30.3 ± 4.6	38.6 ± 3.3	45.1 ± 3.7	< 0.001	14.1 ± 3.6	25.2 ± 5.0	< 0.001	31.7 ± 5.2	43.7 ± 3.7	< 0.001
LEGFAT%-to-TOTFAT%	0.90 ± 0.19	0.88 ± 0.15	0.83 ± 0.08	0.550	1.33 ± 0.20	1.18 ± 0.08	1.11 ± 0.06	< 0.001	0.89 ± 0.17	0.86 ± 0.12	0.999	1.31 ± 0.19	1.12 ± 0.04	< 0.001
LEGFAT%-to-TRUFAT%	0.89 ± 0.34	0.82 ± 0.21	0.75 ± 0.10	0.072	1.85 ± 0.90	1.39 ± 0.23	1.35 ± 0.17	< 0.001	0.85 ± 0.29	0.80 ± 0.19	0.999	1.78 ± 0.83	1.27 ± 0.13	< 0.001

Data are the means ± standard deviations (SDs). Statistically significant values are shown in bold. ANOVA with a Tukey post-hoc test and t tests were used for numerical variables and for the LEGFAT%-to-TOTFAT% and LEGFAT%-to-TRUFAT% ratios, according to the BMI and TOTFAT% obesity classifications. N: the number of participants in the group, BMI: body mass index, TOTFAT: total body fat, ARMFAT: arm fat, TRUFAT: trunk fat, LEGFAT: leg fat, NO: Normal BMI category, OV: overweight BMI category, OB: obese BMI category.

In terms of sex differences (for the total sample), females had significantly higher ARMFAT% ($28.8 \pm 9.9\%$ vs. $18.2 \pm 7.0\%$, $p < 0.001$) and LEGFAT% ($33.7 \pm 6.7\%$ vs. $16.7 \pm 6.2\%$, $p < 0.001$) but not TRUFAT% ($22.5 \pm 8.4\%$ vs. $21.2 \pm 8.4\%$, $p = 0.999$) than males. In addition, females had higher LEGFAT%-to-TOTFAT% (1.28 ± 0.18 vs. 0.88 ± 0.16 , $p < 0.001$) and LEGFAT%-to-TRUFAT% ratios (1.70 ± 0.78 vs. 0.84 ± 0.27 , $p < 0.001$) than males. Furthermore, for the total sample, LEGFAT% increased by an average of 0.13% each year (*ceteris paribus*). Participants in the obese TOTFAT% category had, on average, a 10.7% higher LEGFAT% than participants in the normal TOTFAT% category. Moreover, the TOTFAT% in males compared to that in females was 17.6% lower. In addition, females and males in the normal BMI and TOTFAT% categories were the youngest, while females and males in the obese BMI and TOTFAT% categories were the oldest.

Importantly, the LEGFAT%-to-TOTFAT% and the LEGFAT%-to-TRUFAT% ratios in females did not follow the trend of sex differences in regional body composition analysis. For example, females in the normal BMI and TOTFAT% categories had significantly higher LEGFAT%-to-TOTFAT% ratios (BMI category: 1.33 ± 0.20 vs. 1.11 ± 0.06 , $p < 0.001$ and TOTFAT% category: 1.31 ± 0.19 vs. 1.12 ± 0.04 , $p < 0.001$) and LEGFAT%-to-TRUFAT% ratios (BMI category: 1.85 ± 0.90 vs. 1.35 ± 0.17 , $p < 0.001$ and TOTFAT% category: 1.78 ± 0.83 vs. 1.27 ± 0.13 , $p < 0.001$) than males. In other words, females in the normal BMI and TOTFAT% categories, compared with those in the overweight, obese BMI and TOTFAT% categories, accumulated more fat percentage in their legs (and compared to their

TRUFAT%) (e.g., if thin females gain TOTFAT%, they accumulate LEGFAT% the most).

However, females in the obese BMI and TOTFAT% categories had significantly higher absolute LEGFAT mass than those in the normal BMI and TOTFAT% categories (BMI category: 7.7 ± 2.0 kg vs. 3.3 ± 0.7 kg, $p < 0.001$ and TOTFAT% category: 6.7 ± 1.9 kg vs. 3.6 ± 1.0 kg, $p < 0.001$). A similar trend was observed in absolute TRUFAT mass (BMI category: 16.5 ± 3.8 kg vs. 6.3 ± 2.8 kg, $p < 0.001$ and TOTFAT% category: 15.9 ± 3.1 kg vs. 7.1 ± 3.1 kg, $p < 0.001$).

The complete regional body composition status by sex and according to the BMI and TOTFAT% obesity classifications is shown in Table 1.

Table 1. Regional body composition status by sex and according to the BMI and TOTFAT% obesity classification.

Data are the means \pm standard deviations (SDs). Statistically significant values are shown in bold. ANOVA with a Tukey post-hoc test and t tests were used for numerical variables and for the LEGFAT%-to-TOTFAT% and LEGFAT%-to-TRUFAT% ratios, according to the BMI and TOTFAT% obesity classifications. N: the number of participants in the group, BMI: body mass index, TOTFAT: total body fat, ARMFAT: arm fat, TRUFAT: trunk fat, LEGFAT: leg fat, NO: Normal BMI category, OV: overweight BMI category, OB: obese BMI category.

Furthermore, the adjusted R^2 (linear regression, F test) was 0.825, which means that 82.5% of the variation in LEGFAT% was explained by variation in TOTFAT% (according to the TOTFAT% obesity classification), sex, and age (Figure 2).

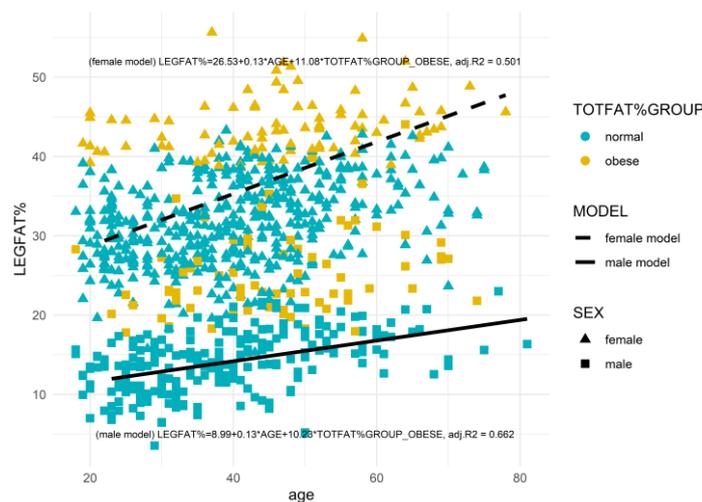


Figure 2. LEGFAT% correlation with TOTFAT% cut-offs, sex, and age

Discussion

This is the first and largest study in Slovenia to examine the (regional) body composition status of Slovene adults published in scientific journals. In addition to this unique point, it reveals a phenomenon observed for the first time, which should be examined and confirmed or refuted by the results of other studies. In confirmed, it is necessary to examine the clinical importance of the phenomenon, the potential cause of its existence/formation, and the possibilities for its health management.

Slovenes are Slavs, which is the most prevalent ethno-linguistic group in Europe (Veldhuis & Underdown, 2017). Furthermore, the study was carried out within two months in the summer.

The predicted results of the study were (a) that males would have a lower TOTFAT% value than females, (b) that the participants in the normal-weight BMI and TOTFAT% categories would be the youngest, those in the overweight BMI category would be slightly older, and those in the obese BMI and TOTFAT% categories would be the oldest, and (c) that participants (females and males) in the obese category would have, on average, higher LEGFAT and TRUFAT mass, and LEGFAT% and TRUFAT% status compared with those in the normal BMI and TOTFAT% categories. However, the LEGFAT%-to-TOTFAT% and LEGFAT%-to-TRUFAT% ratios in females did not follow this trend (our initial hypothesis). Females in the normal BMI and TOTFAT% categories, compared with those in the obese BMI and TOTFAT% categories, accumulated more fat percentage in their legs (and compared to their TRUFAT%). Furthermore, with the linear regression method, we estimated that LEGFAT% would increase by an average of 0.13% points each year (*ceteris paribus*). Finally, 82.5% of the variation in LEGFAT% was explained by variation in TOTFAT% (according to the TOTFAT% obesity classification), sex, and age.

Body Mass Index and (Regional) Body Composition Measurement of Obesity

Although BMI is a measure of overall adiposity, in different proportions, it does not represent fat content (and distribution) because it incorporates both fat and lean mass (Jayedi et al., 2022). If only BMI were used as a central marker of obesity, then the global obesity epidemic and chronic disease rates would be underestimated (Heber, 2010; Okorodudu et al., 2010). However, by combining BMI and TOTFAT% measures the accuracy of obesity screening in adults is markedly improved (Chooi,

Ding, & Magkos, 2019; Chrysant & Chrysant, 2019; Hung et al., 2017). In support of this, in the sample, a comparison of the proportions of obese people between the two cut-off obesity classifications (BMI vs. fat percentage) showed a significantly underestimated proportion of obese female participants based on BMI classification (13% vs. 17%, $p = 0.005$) (Jakše, Godnov, & Pinter, 2022).

Importantly, obesity is a multifactorial problem caused by changes in individuals' lifestyles, including a shift towards frequent consumption of calorie-dense and nutrient-poor foods, physical inactivity, and a sedentary lifestyle (Boutari & Mantzoros, 2022; Sultana et al., 2021). In addition, the sex differences in body composition status have been well established; compared with males, females have higher TOTFAT% and deposit it in a different pattern (e.g., more adipose tissue in the hips and thighs), and males have a higher percentage of muscle mass than females (Karastergiou et al., 2012). Basic sex-specific differences indicate a greater accumulation of adiposity in females than in males without deleterious metabolic consequences, which are not completely understood and are probably related to physiological sex differences (Karastergiou et al., 2012).

Furthermore, a substantial body of evidence demonstrates a harmful effect of obesity and excess adiposity (e.g., of both abdominal and general obesity) on various aspects of health; moreover, obesity is associated with increased overall mortality (Cornier et al., 2011). Generally, studies support the relationship between higher fat content and a higher risk of mortality in a J-shaped manner (Cornier et al., 2011; Jayedi et al., 2022). However, in a recent meta-analysis of prospective cohort studies, researchers found that higher TOTFAT% in older adults was no longer associated with mortality (Jayedi et al., 2022). Nevertheless, in one study, the researchers divided the sample in the normal BMI range using sex-specific TOTFAT% tertiles. The results showed that the males and females that were within the normal BMI range but in the highest TOTFAT% tertile ($> 23.1%$ in males and $> 33.3%$ in females) compared with the low TOTFAT% group were four and seven times more likely to have metabolic syndrome. In addition, these participants also had a higher prevalence of dyslipidemia, hypertension (males), and cardiovascular disease (females) (Romero-Corral et al., 2010). Finally, in addition to the amount of total body fat, the distribution of fat stores in the body (regional body composition) is also important (Cornier et al., 2011).

Body Fat Distribution Status

Previous research has documented an increase in body fat percentage and a decrease in muscle mass with aging in females, particularly during menopause, attributed to reduced estrogen levels (Fenton, 2021; Gába & Přidalová, 2014). However, the difference in average age between the compared groups in terms of BMI and TOTFAT% categories (38.7 years versus 47.8 years for BMI categories and 39.6 years versus 47.9 years for TOTFAT% categories), although present, does not entirely account for the potential phenomenon observed in our study in terms of regional fat distribution ratios among females with normal and obese BMI and TOTFAT% categories.

Therefore, regarding regional body composition status (i.e., 'body shape'), our study focused on the existence of potentially unfavorable LEGFAT%-to-TOTFAT% and LEGFAT%-to-TRUFAT% ratios of females in the normal BMI and TOTFAT% categories compared with those in the obese BMI and TOTFAT% categories. We propose several possible reasons for the obtained ratios, namely, (i) sex-specific differences in the pattern (e.g., the proportion, rate, and body region within females) of fat accumulation for a given increase in excess fat, (ii) potentially an overall less healthy lifestyles of (youngest) adult females in the normal BMI and TOTFAT% categories or (iii) combinations of both. Arguments for the first reason may include (a) age differences between females in the obese and normal BMI and TOTFAT% categories, where females in the obese BMI and TOTFAT% categories were significantly older (9.1 and 8.3 years) and (b) a higher absolute TRUFAT mass (2.2–2.6 times) and LEGFAT mass (1.9–2.3 times).

According to data on age differences and absolute LEGFAT mass and TRUFAT mass, the explanation may be that it is probably a protective adaptation of the body, which stores a greater proportion of excess fat in the leg region at first, as long as this is physiologically and anatomically possible (Chang et al., 2018). In other words, this phenomenon probably occurs after the body's capacity to store excess fat exceeds a certain point in an individual's BMI and fat percentage status until the next body's physiological anatomical adaptations occur.

Nevertheless, Japanese researchers determined the specific characterization of stored regional ARMFAT% compared to the LEGFAT% of young healthy adults. With the BIA, the data showed that the ARMFAT% was not different from the LEGFAT% in males, while in females, the

ARMFAT% was significantly higher than the LEGFAT% (Yamauchi et al., 2015). Interestingly, these results are inconsistent with ours, where the males included in our study had higher ARMFAT% than LEGFAT% in all BMI and TOTFAT% categories; furthermore, females also had higher LEGFAT% than ARMFAT%. Of note, we recognized that body composition measures by BIA in a multiethnic sample may demonstrate disparate results between ethnicities (Blue et al., 2021). However, our study sample consisted of one ethnic group (i.e., Slavs). Importantly, viewed from a clinical health impact perspective, although the accumulation of LEGFAT% might represent health benefits (Visaria et al., 2022), excess adiposity is still associated with poor health outcomes (Power & Schulkin, 2008).

Second, in our study, females in the normal BMI and TOTFAT% categories accumulated more LEGFAT% than those in the obese BMI and TOTFAT% categories, and compared to their TRUFAT%, we speculated that this might be due to (a combination) (a) increased physical inactivity in females in the normal BMI and TOTFAT% categories who were also the youngest, (b) an increased sitting time in these groups of females, and (c) more generally unhealthy eating habits of females in the normal BMI and TOTFAT% categories. Although in our study we did evaluate the dietary intake of females and males (Jakše, Godnov, & Pinter, 2022), we did not differentiate between the dietary intake of females in the normal and obese BMI and TOTFAT% categories. However, the dietary intake assessment showed that females diet compared to males was significantly different in terms of carbohydrate (44% E vs. 38% E, recommended \square 50% E) and protein intake (17% E vs. 21% E, recommended 10–15% E). We emphasize that females and males free sugar intake was within the dietary recommendation. However, the females and males did not meet the dietary recommendation for fiber intake (27 g/d vs. 26 g/d, recommended $<$ 30 g/d), while the total fat intake was well above the dietary recommendation (36% vs. 39%, recommended 30% E). In addition, the saturated fatty acids intake in females was at the limit and exceeded in males (10% vs. 11%, recommended \leq 10% E) (Jakše, Godnov, & Pinter, 2022). Importantly, the study was carried out in the summer; the winter period is associated of higher energy intake in adults (Stelmach-Mardas et al., 2016). Consequently, higher energy intake, in addition to greater inactivity during winter, is additionally associated with higher BM and fat percentage.

In addition, depending on the availability of published data from a recent Slovenian nationally representative dietary survey (SI.Menu 2017/2018) of 780 adults (adults: 191 females and 173 males and older adults: 203 females and 213 males) who were, on average, mostly in the overweight or obese BMI category (59% of adults and 75% of older adults) (Hribar et al., 2021), we suggest an observation based on their results in terms of dietary intake. Researchers have found that older adults have a higher intake of fibre and folate than adults (Pravst et al., 2021; Seljak et al., 2021), which was further reflected in the higher intake of legumes, vegetables, and fruits and the lower intake of meat and meat products, eggs, milk, cereal products, and high sugar foods in older adult females than in adult females, while the representation of low-, moderate- and high-intensity physical activity was comparable (Gregorič et al., 2022). Importantly, a dietary pattern with a lower total fat intake (Hooper et al., 2020; Schick et al., 2020), a more unprocessed plant-based diet (Jakše et al., 2017; Najjar & Feresin, 2019) or even a more consistent implementation of the current nutritional guidelines for an omnivorous diet (Jakše et al., 2021) has been shown to significantly improve or maintain BMI and fat status compared to a typical (unhealthy) Western-type diet (Tran et al., 2020).

Nevertheless, considering the size of the differences in dietary intake between the sexes, we estimate that the aforementioned phenomenon in the female population may not be attributed to differences in dietary intake. This can be further supported, even though we did not measure physical activity (of note, as we emphasized in the inclusion criteria for the study, the recruitment process was not intentionally or specifically related to a healthy and active lifestyle adults) with the observation that females in the normal BMI category significantly accumulated more fat percentage in their legs (and compared to their TRUFAT%) also compared with females in overweight BMI category. Furthermore, this trend was statistically insignificant in males. Based on what has been said, as well as the different results obtained by other researchers (e.g., Japanese) with the same body composition measurement technology (BIA), we conclude that the existence of the phenomenon may be first about ethnic (anatomical) differences and only then about physiological anatomical adaptations of the body (different in females than a males) in the accumulation of body fat/mass. Finally, perhaps the mentioned phenomenon within the female but not male population is a consequence of the cultural, social roles, and processes of economic

development of younger females compared to older (in overweight and obese BMI categories) females. Namely, in a relatively developed European country such as Slovenia, it may be the existence of dietary and lifestyle differences among adult females in a different period of life, which unfortunately we did not evaluate with our study (i.e., hypothetically, younger females of childbearing age, when they are just becoming economically empowered and establishing priorities and balance in life, eat less healthy food, are less physically active, and more sedentary).

Regardless, the results should be taken with caution when drawing conclusions. However, if similar results are confirmed by other researchers (i.e., in the same ethnic group) and the clinical relevance of this phenomenon (potentially unfavourable LEGFAT%-to-TOTFAT% and TRUFAT%-to-LEGFAT% ratios) is assessed, it will be necessary to address possible reasons for this phenomenon (i.e., also in terms of possible differences recently in the sitting duration of younger adult females compared to older adult females) and measures to mitigate them properly.

Strengths and Limitations

This is the first and largest study in Slovenia to examine the (regional) body composition status of Slovene adults and to be published in a scientific journal. The study sample was also large in terms of the number of adult inhabitants in Slovenia and had relatively balanced regional coverage. It was performed in the post-COVID-19 pandemic period within two months; therefore, the study problem represents an actual potential problem in society. In addition, adult participants were randomly recruited and did not include patients diagnosed with common chronic diseases.

In regard to the study aim, we acknowledge a few important limitations. To assess the possible reasons for the obtained results, data on dietary intake and lifestyle in a larger population of females should be obtained according to age and/or BMI status, which has been shown to be potentially valuable when interpreting results. Second, to assess the relevant clinical features of the assessed phenomenon, we would also need to measure common chronic health risk factors, for example, cardiovascular diseases. However, based on unpublished data from one of our previous studies (Jakše et al., 2019, 2020a, 2020b), we are planning to perform a secondary analysis of the data using regional body composition analysis and examine the relationship with cardiovascular disease risk factors while adjusting (in addition to age and sex) for dietary intake, physical

activity status, sitting duration, stress and sleep hygiene status. Last, the validity study of our multifrequency BIA model compared with DXA on generally healthy normal weight, overweight, and obese class 1 and 2 (but not 3) adults of the age range between 30 and 65 years is still lacking.

Our main findings showed that females in the normal BMI or TOTFAT% categories accumulated more LEGFAT% (also compared to their TRUFAT%) than those in the obese BMI or TOTFAT% categories. We estimated that LEGFAT% increases by an average of 0.13% each year. In addition, 82.5% of the variation in LEGFAT% was explained by variation in TOTFAT% (according to the TOTFAT% obesity classification), sex and age.

The reproducibility and clinical relevance of the obtained data must be confirmed by other studies (cross-sectional and interventional). Given the increasing importance of body composition data, regular (regional) body composition monitoring should be integrated into annual medical examinations and clinical practice, together with other measurements, to assess the state of an individual or society status and changes more holistically.

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Informed Consent Statement:

Written informed consent has been obtained from the participants to publish this manuscript.

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