

## Does Impedance Measure a Functional State of the Body Fat?

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### Summary

The aim was to compare methods of body fat measurement in different BMI groups. An additional aim was to discuss differences reflecting the structural and functional changes of fat tissue. The study group included 130 adult Caucasian women stratified by body mass index (BMI): 18-24.99 (n=30), 25-29.99 (n=26), 30-34.99 (n=33), 35-39.99 (n=30), and BMI≥40 (n=11). Bioelectrical impedance was performed using Tanita TBF 410 GS, Bodystat 1500, and Omron BF 300. A caliper type Best was also applied. Correspondence of four methods with DEXA was assessed using the Bland-Altman and ANOVA analyses. Measurements by BIA were not significantly different from DEXA up to BMI of 30, but DEXA significantly overestimated in the higher BMI subgroup by all three methods. Caliper measurement significantly underestimated DEXA in all BMI subgroups. BIA methods overestimated DEXA for the obese subjects. Tanita did statistically the best. The Caliper test appeared less preferable than the BIA methods, especially in the higher BMI subgroup. DEXA and Caliper measurements seem to be the best estimate of structural (anatomical) fat quantity. We hypothesize that BIA methods could also measure some other physiopathological conditions like inflammation, hydration or cell infiltration of fat.

### Key words

Body composition • DEXA • Fat mass • Obesity • Anthropometric methods • Fat tissue functional properties

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### Introduction

In recent years, there has been a rapid development in methods for assessing body composition and determining the amount of body fat (Ayvaz 2011). The speed of progress in the development of new methods for determining body fat relates well to the increased incidence of excess weight and obesity as well as the need for accurate diagnosis of this worldwide problem. In the last few years, the prevalence of overweight and obese persons has reached epidemic proportions (Berghöfer *et al.* 2008). In this regard, there has been an increased occurrence of diseases associated with obesity – the amount of body fat is one of the risk factors for cardiovascular diseases (Mahabadi *et al.* 2009). Studies have shown a link between high levels of visceral fat and impaired glucose tolerance (Hayashi *et al.* 2003), dyslipidemia, hypertension, insulin resistance and complete metabolic syndrome (Pascot *et al.* 1999, Wagenknecht *et al.* 2003). Thus, quantification of obesity and determination of body fat amount is very important in assessing future health risks (Ayvaz 2011). Besides evaluating the seriousness of obesity, body fat measurement has great importance for monitoring the

effectiveness of its treatment and motivating patients to continue in fat reduction (Zavadilová *et al.* 2011). In sports practice, the analysis of body composition is widely used to optimize the nutrition and training process.

Methods for measuring body fat and the amount of lean mass has stopped being the domain of just specialized centers and can be used for example in obesity and physical education outpatient clinics, in sports clubs, in nutritional counseling offices and even in the commercial fitness centers.

Besides simple anthropometric indicators such as body mass index (BMI) or waist circumference, body composition can be measured with commonly available methods (i.e. methods using bioimpedance or caliper) widely used in clinical practice and in various epidemiological studies (Lemieux *et al.* 1996, Lee *et al.* 2008). The question of accuracy remains with these field methods, and especially with their appropriateness for various groups of the population.

The DEXA method, one of the fat measurement methods evaluated in the present study, belongs among the present reference methods in the field (Heyward and Stolarszyk 1996). This is a morphology method (anthropometrical paging) measuring fat quantitatively. The disadvantage of the majority of the reference methods lies in the high technical and financial operational demands. Therefore, it is necessary to look for more accessible methods that could be used in routine practice as satisfactorily accurate substitutes. Thus, some methods may not only measure anatomical changes in fat but also some functional properties of fat tissue.

The objective of the present study was to compare the most commonly used methods for assessing the amount of body fat, namely three bioelectrical impedance analysis (BIA) methods and anthropometric method caliper test, in terms of their correspondence with DEXA, which is considered the reference method. An additional aim of this study was to show differences in relation to structural and functional changes of fat tissue.

## Methods

The study group included women with a normal weight to the third degree of obesity. This stratification has enabled analysis of whether the differences in accuracy among all methods were similar along the whole scale of BMI or simply BMI-dependent.

### *Fat measurement methods*

#### *Dual energy X-ray absorptiometry (DEXA)*

DEXA uses a whole body scanner with two low dose x-rays at different sources that read bone and soft tissue mass simultaneously. DEXA measurements were performed using QDR 4500A, fan-beam densitometer (Hologic, Waltham, USA), with software version 8.21. A standardized patient positioning procedure was used. The measurement was limited by the instrument to maximum body weight 120 kg.

#### *Bioelectrical impedance analysis (BIA methods)*

Bioelectrical impedance analyses represent electrophysiology methods, used in clinical practice as morphology methods. Three different devices of multi-frequency bio-electrical impedance analysis (BIA) were used: (1) The tetrapolar hand – to foot technique (Bodystat 1500), (2) bipolar hand held technique (Omron BF 300), and (3) bipolar foot to foot technique (Tanita TBF 410 GS). All measurements were taken in accordance with the recommended guidelines. It included (i) no meals and drinks 3 hours prior measurements; (ii) no exhausting exercise 12 hours prior measurements; (iii) no alcohol or caffeine consumption 24 hours prior measurements; (iv) absence of menstruation at the time of measurements.

#### *Anthropometric method (Caliper test)*

Fat measurement methodology proposed by Pařízková (1977), based on skin fold thicknesses at 10 sites, was applied and the formula transforming measurements into a body fat percentage estimate was used. This is a morphology method measuring fat quantity and partially tissue elasticity. The measurements were performed using the BEST caliper. All subjects were measured by an experienced individual.

Measurements by each of the methods were always provided by the same person in order to minimize the risk of errors while measuring. Measurements of the same subject by different methods were carried out in a short time sequence in order to avoid any possible changes in body hydration, which would influence the results of the BIA methods (Deurenberg 1996).

### *Subjects*

The study group included 130 women with different values of BMI ranging from normality up to the third degree of obesity (Table 1). The subjects were clients of the Center of Physical Activities, which

cooperates with the First Faculty of Medicine, Charles University and General University Hospital in Prague, Czech Republic. The mean age of subjects was 46.8 years, with a standard deviation (SD) of 14.7 years. The mean BMI was 31.1 kg/m<sup>2</sup>; SD=6.7 kg/m<sup>2</sup>. Subjects exceeding the maximum required body weight of 120 kg were excluded because of technical limitations with the DEXA instrument. Pregnant women were also

excluded.

All measurements were taken during one visit at laboratories of the Center of Physical Activities and at the Institute of Sports Medicine, Charles University. DEXA measurements were carried out at laboratories of the Third Department of Medicine, General University Hospital and the First Faculty of Medicine, Charles University.

**Table 1.** Study groups, stratification and descriptive statistics.

BMI range (kg/m <sup>2</sup> )	Number	Mean age (years)	Mean height (cm)	Mean weight (kg)	Mean BMI (kg/m <sup>2</sup> )
18.00-24.99	30	34.7±14.7	166.7±5.8	61.9±6.5	22.2±1.7
25.00-29.99	26	44.3±15.8	166.2±6.1	77.4±7.6	27.4±1.4
30.00-34.99	33	51.0±13.0	164.3±6.3	88.2±8.7	32.5±1.4
35.00-39.99	30	53.3±8.9	161.2±4.6	97.1±7.4	37.2±1.3
≥40.00	11	55.1±6.8	159.3±3.5	110.6±7.1	43.4±2.3
<b>Total</b>	130	46.8±14.7	164.1±6.0	83.9±16.8	31.1±6.7

Data are mean ± SD.

### Statistics

Statistical analyses were performed using the following methods:

- The basic statistical description of the sample is given as means ± SD.
- Four pairs of methods, each consisting of DEXA (taken as a gold standard) and one of the methods (Bodystat, Omron, Tanita, Caliper) were subject, both in the whole sample and in the five BMI bands, to Bland-Altman analysis (Bland and Altman 1986). The data are displayed as a scatter plot of the individual differences between the two methods (y axis) and the average of the methods (x axis). The Bland-Altman limits of agreement (displayed as horizontal lines) are calculated as the mean ± two SDs of the individual differences between the pairs of measurements. The tests performed in the framework of the Bland-Altman analysis were (i) the paired t-test for the bias with respect to DEXA; and (ii) the standard t-test of regression line slope for the dependence between the difference and mean of DEXA and the other method. The limits of agreement for the sample stratified by BMI and all method pairs were displayed using the Cleveland's dot plot (Cleveland 1984), with added error bars.
- The biases of the four methods (Bodystat, Omron,

Tanita, Caliper) with respect to DEXA were tested, as well as mutually compared, using two-way ANOVA with one between-subjects factor of BMI band (5 levels), and one within-subject factor of method (5 levels: DEXA, Bodystat, Omron, Tanita, Caliper). In case of a significant interaction between the two factors, a separate one way ANOVA with method as the only factor was applied, in each of the five BMI subgroups. In those BMI subgroups where the one-way ANOVA yielded significant results, pairwise method comparisons were performed, as a *post-hoc* analysis, with Bonferroni-adjusted paired Student t-tests. (Note that the difference between methods A and B is, at the same time, the difference between the biases of A with respect to DEXA, and B with respect to DEXA.)

- Absolute differences |Bodystat – DEXA|, |Omron – DEXA|, |Tanita – DEXA|, and |Caliper – DEXA| were analyzed in an analogous manner – using first two-way ANOVA with BMI band and a method as factors; in case of significant interaction one-way ANOVA in separate BMI subgroups and, in case of significant one-way ANOVA, Bonferroni-adjusted paired t-tests. The only difference is that the factor of method had four (not five) levels.

The statistical test results were considered to be

significant when  $p < 0.05$ . The following statistical software was used: Standard package Statistica (descriptive analyses, ANOVA, t-tests), R (R Development Core Team 2010), and R extension packages MethComp (Carstensen and Gurrin 2011).

## Results

### Bland-Altman analysis

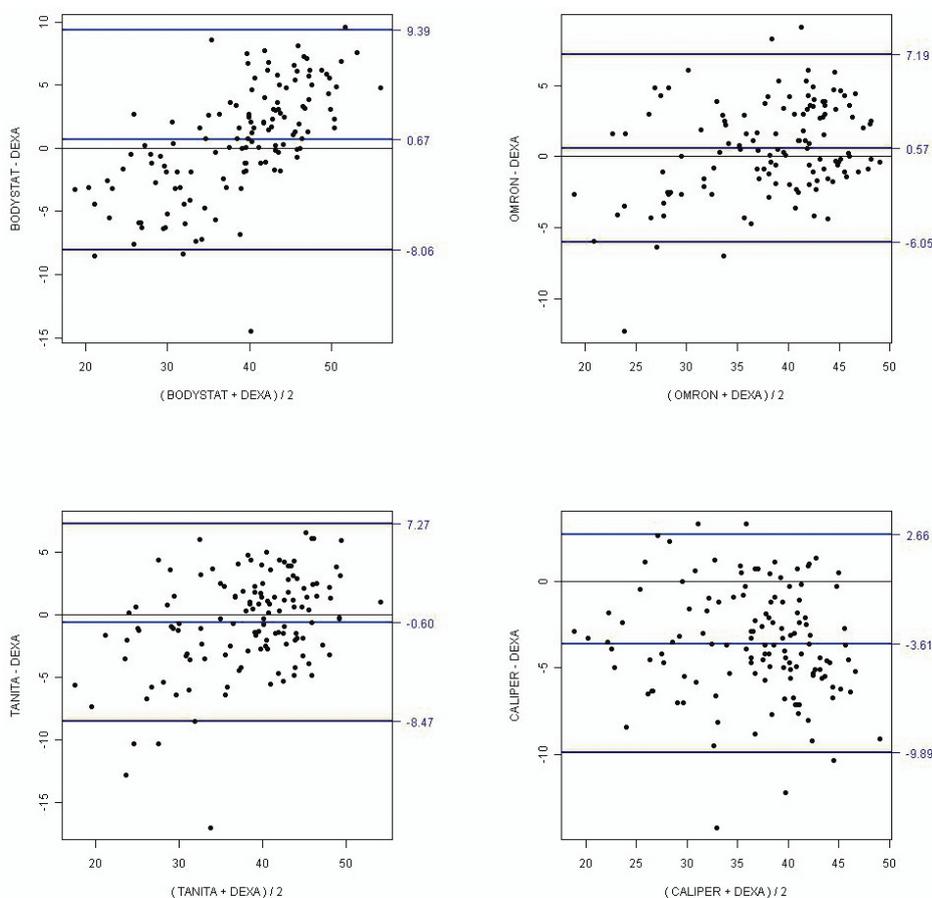
As a first step, a comparison was carried out of each method with the DEXA method for the entire group of women (Fig. 1). The difference between DEXA and Bodystat methods was particularly analyzed based on their mean and the dependence was found to be statistically significant ( $p < 0.0001$ ). Bodystat mostly overestimated DEXA for individuals with lower body fat content and underestimated for individuals with higher body fat content. Considering these results, it would not be appropriate to use a common reference range for the entire group of women with varying degrees of obesity. The solution would be to stratify the group based on BMI

and determine the reference ranges for each subgroup of women individually.

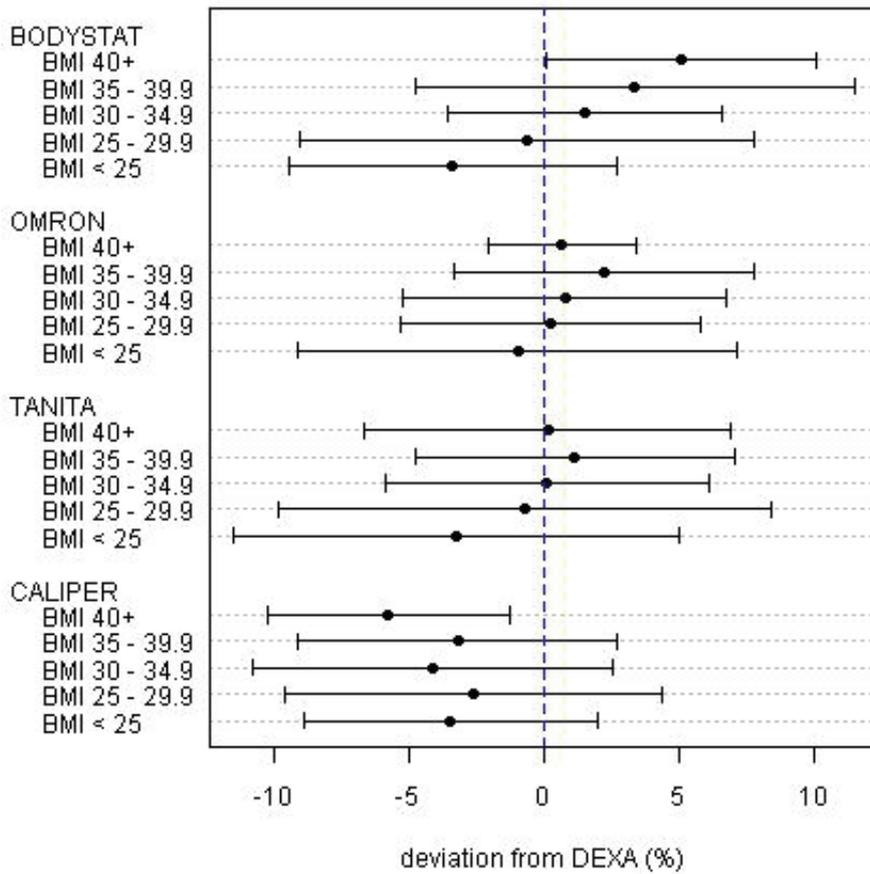
The results of the comparison of DEXA and Omron methods were similar ( $p = 0.0002$ ). Thus, these results once again show that it would be appropriate to stratify the entire group according to BMI into several subgroups.

The correlation between the difference and the average of DEXA and Tanita methods was statistically significant ( $p < 0.0001$ ). Thus, there was again a higher tendency to underestimate the quantity of body fat measured by impedance methods at the lower levels of body fat and, by contrast, at higher levels of body fat to overestimate it. This tendency is somewhat weaker than in the case of Bodystat, but stronger than for Omron.

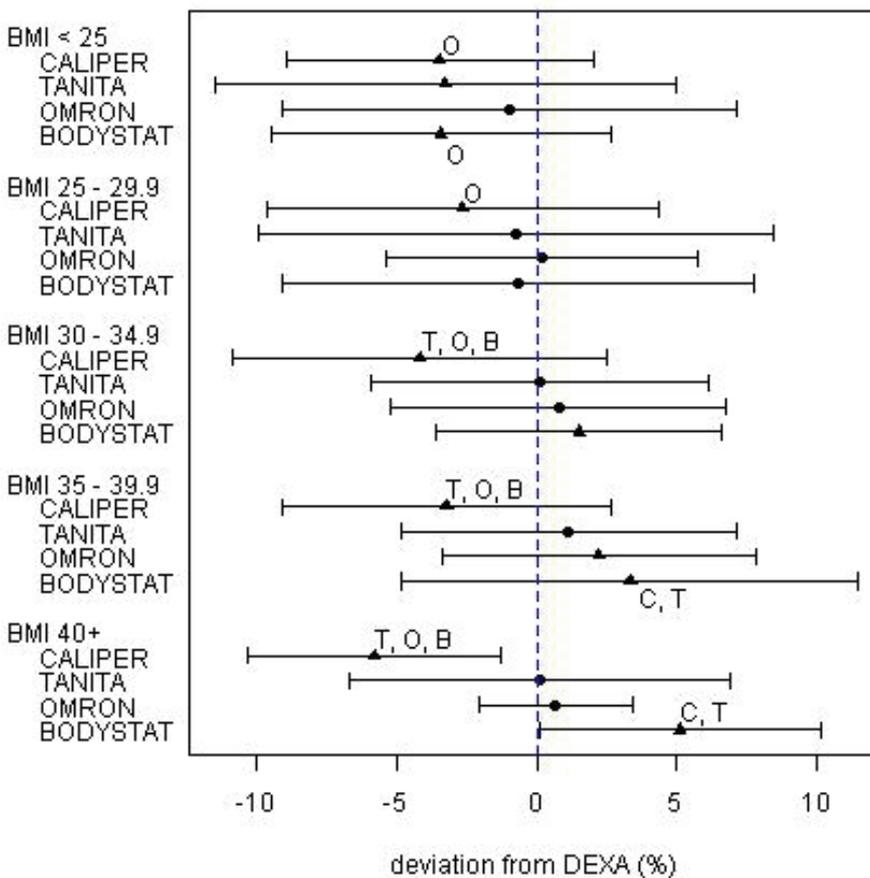
Figure 1 also shows the differences between DEXA and Caliper methods. The DEXA method underestimated body fat values in their entire range. The correlation between the average and the difference of these two methods was not statistically significant ( $p = 0.17$ ).



**Fig. 1.** Comparison of individual methods with DEXA method. Comparison of Bodystat with DEXA method (Bland-Altman graph and limits of agreement) for the entire group of women (**upper left**). Comparison of Omron with DEXA method (Bland-Altman graph and limits of agreement) for the entire group of women (**upper right**). Comparison of Tanita with DEXA method (Bland-Altman graph and limits of agreement) for the entire group of women (**lower left**). Comparison of caliper with DEXA method (Bland-Altman graph and limits of agreement) for the entire group of women (**lower right**).



**Fig. 2.** Mean deviations from DEXA (symbols ●) and the Bland-Altman limits of agreement with DEXA (error bars) for all methods in the study group stratified by BMI.



**Fig. 3.** Results of two-way ANOVA of the deviations from DEXA for all methods and study group stratified by BMI. Symbols ▲/● ... mean deviations from DEXA significant/not significant at the 5 % level. Symbols C/T/O/B ... mean deviation from DEXA of the given method significantly different ( $p < 0.05$ ) from that of Caliper test/Tanita/Omron/Bodystat. Error bars ...  $\pm 2$  SD of the individual deviations from DEXA.

The Bland-Altman analysis was further performed in individual BMI subgroups. The results (reference ranges for different measurement methods and BMI subgroups) are shown in Figure 2.

#### *ANOVA of biases with respect to DEXA*

The individual deviations from DEXA of the measurements by the three BIA methods and the caliper test were analyzed by a two-way ANOVA with repeated measurements. The results are shown in Figure 3 (note that Figure 2 data is displayed here again but it was rearranged and includes additional information). There was a statistically significant relation between the factors of the BMI subgroup and the method of measurement ( $p < 0.0001$ ).

One-way ANOVA for repeated measurements shows the statistically significant differences between individual methods in the first BMI group ( $p < 0.0001$ ). Multiple comparisons (Bonferroni-adjusted t-test) show that DEXA significantly underestimates compared to all the other methods except for Omron ( $p < 0.0001$  in all 3 cases). Moreover, significant differences were found in these pairs of methods: Omron vs. the caliper test ( $p = 0.017$ ) and Omron vs. Bodystat ( $p = 0.029$ ). In the second BMI subgroup, statistically significant differences between the measurement methods were found ( $p = 0.0029$ ), and multiple comparisons show a significant bias with respect to DEXA for the caliper test ( $p = 0.007$ ), and a significant difference between Omron and the caliper test ( $p = 0.001$ ). In the third BMI subgroup, there are statistically significant differences between the measurement methods ( $p < 0.0001$ ) due to the fact that caliper test differs significantly from all the other methods including DEXA ( $p < 0.0001$  in all cases), and, moreover, Bodystat was significantly biased from DEXA ( $p = 0.017$ ). In the fourth subgroup, there are again statistically significant differences between the individual measurement methods ( $p < 0.0001$ ). All the methods except for Tanita are significantly biased from DEXA (Bodystat:  $p = 0.001$ ; Omron:  $p = 0.002$ ; Caliper:  $p < 0.0001$ ). Caliper test is again significantly different from all the other methods ( $p < 0.0001$ ). Furthermore, there is a statistically significant difference between the methods Bodystat and Tanita ( $p = 0.0085$ ). In the fifth BMI subgroup, one-way ANOVA shows a significant difference among the individual methods ( $p = 0.0001$ ), too. Multiple comparisons show a significant bias with respect to DEXA for Bodystat ( $p = 0.0005$ ) and Caliper ( $p = 0.0001$ ), and further significant differences in these

pairs of methods: Bodystat vs. Caliper ( $p < 0.0001$ ), Bodystat vs. Tanita ( $p = 0.0016$ ), Omron vs. Caliper ( $p = 0.0023$ ) and Tanita vs. Caliper ( $p = 0.0031$ ). The difference between Omron and Bodystat methods was close to statistical significance ( $p = 0.0920$ ). Although statistical results were favorable for Omron in the given subgroup, it was necessary to take into account that they were based on only 5 measurements. The other 6 probands were not measurable by Omron.

We summarize the results as follows:

- For BMI less than  $25 \text{ kg/m}^2$ , DEXA underestimates compared to all the other methods.
- The caliper method significantly underestimates the results when compared to the DEXA method in all BMI subgroups.
- For BMI over  $30 \text{ kg/m}^2$  all bioimpedance methods overestimate DEXA.
- In the three highest BMI subgroups, the biases of the Tanita method with respect to DEXA were only non-significant and the smallest of all the considered methods (significantly smaller than the bias of caliper test in all the three subgroups, and of Bodystat in the fourth and fifth subgroups).
- With the exception of the fourth BMI subgroup, the Omron method had relatively small (and statistically non-significant) biases with respect to DEXA. Moreover, Omron had the narrowest Bland-Altman limits of correspondence with DEXA in the highest BMI subgroup. On the other hand, the measurement by Omron failed in 6 of 11 probands in the subgroup of the most obese women (BMI above 40).
- The Bodystat method significantly overestimates DEXA in three highest BMI subgroups (BMI over  $30 \text{ kg/m}^2$ ).

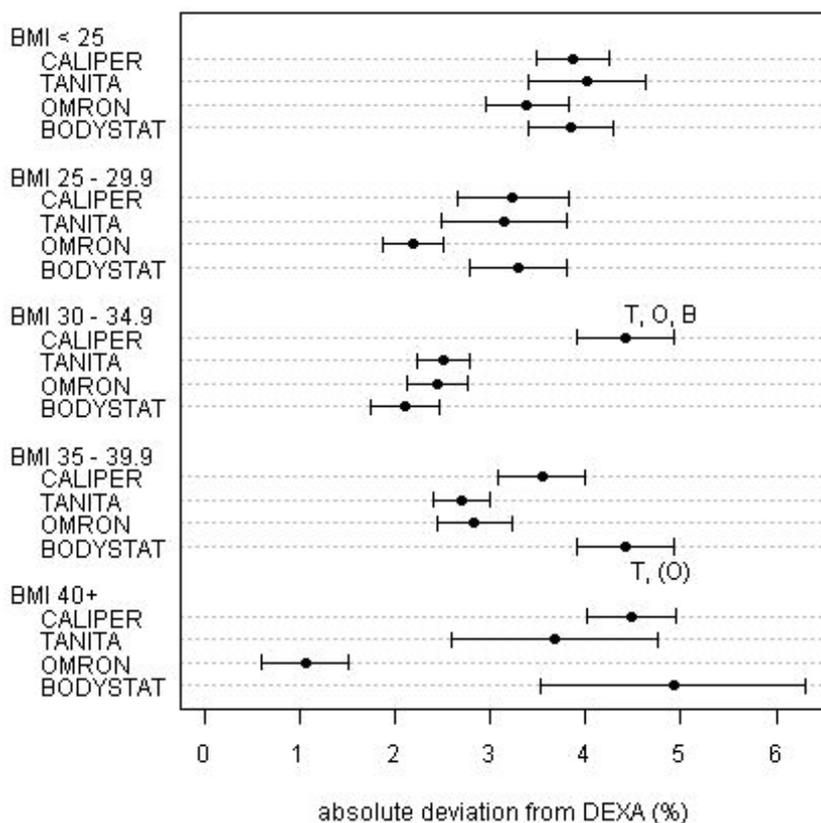
#### *ANOVA of absolute differences from DEXA*

Correspondence with DEXA has its aspects of accuracy and precision. In the Bland-Altman analysis, the former is represented by the mean deviation from DEXA (bias), and the latter by the width of the limits of correspondence. Mean absolute deviation from DEXA is sensitive to both of these parameters, so that it can express the measure of correspondence by the means of a single parameter.

Figure 4 shows the results of the two-way ANOVA of the individual absolute deviations from DEXA of all the BIA and anthropometric methods. The two-way ANOVA yielded a statistically significant interaction between the factors of BMI subgroups and of

the measurement method ( $p=0.0065$ ). In the first and second subgroups of women (BMI below 30), the Omron method had the smallest mean absolute deviations from the DEXA method, however, the differences between the

four methods were not significant (one-way ANOVA:  $p=0.7559$  and  $p=0.3189$  for the first and second subgroup, resp.).



**Fig. 4.** Results of two-way ANOVA of the absolute deviations for all methods and study group stratified by BMI. Symbols • ... mean absolute deviations from DEXA. Symbols T/O/B ... mean absolute deviation from DEXA of the given method significantly different ( $p<0.05$ ) from that of Tanita/Omron/Bodystat. Symbol (O) ... borderline statistical significance ( $0.05<p<0.10$ ) with respect to Omron. Error bars ...  $\pm$  SE of the individual absolute deviations from DEXA.

In the third BMI subgroup, the four methods differed statistically significantly ( $p=0.0001$ ). *Post-hoc* analysis shows that the caliper test is statistically significantly worse than the other methods (vs. Bodystat:  $p=0.012$ , vs. Omron:  $p=0.0162$ , vs. Tanita:  $p=0.0168$ ). In the fourth BMI subgroup, there was also a statistically significant difference between the methods ( $p=0.0103$ ). *Post-hoc* analysis shows that the method Bodystat is less statistically significant than the Tanita method ( $p=0.0084$ ) and than the Omron method, the difference being non-significant but close to the significance limit ( $p=0.0702$ ).

In the fifth BMI subgroup, there are large empirical differences between the methods but, due to small number of subjects, one-way ANOVA yields only a result close to significance ( $p=0.0579$ ). Compared to correspondence with DEXA, Figure 4 suggests that four methods differ very little in the two lowest BMI subgroups, their differences grow with the growing obesity degree when starting with a BMI of 30.

To summarize, Tanita and Omron were the only

methods that showed no inferiority over the whole range of the BMI scale. Omron, however, showed another disadvantage, as it was unable to take measurements in some of the morbidly obese persons (6 of 11 women in the highest BMI subgroup).

## Discussion

Adipose tissue has been historically known to have three main functions (Fantuzzi and Mazzone 2007) – i.e.: energy source, thermoregulation and mechanical protection. Energy is stored in the form of lipids represents the most important one. Lipids, particularly triglycerides, break down into fatty acids and glycerol during starvation. Thermoregulation is the second important function. Adipose tissue prevents body from excess of heat loss during winter by acting as an insulating layer in the body. The third role is mechanical. Particularly visceral adipose tissue helps in providing mechanical protection by forming a thick layer of padding and support around some of the vital organs

inside the abdominal and thoracic cavity. Many other new functions have been recently described (Table 2). (Ravussin and Smith 2002, Juge-Aubry and Henrichot 2005, Lavie *et al.* 2011, Pedersen 2012, Fujii *et al.* 2014, Proenca *et al.* 2014, Reverchon *et al.* 2014). These new functions are related to the presence of many adipose tissue hormones, significant macrophages presence and their function, and adipose cell capacity of various hormonal regulations. Another function of adipose tissue is neutralization of many toxins and pro-inflammation. Adipose tissue is also responsible for a new phenomenon

called the obesity paradox, which is a situation in which obesity is not harmful but might be beneficial (Lainscak *et al.* 2012). This is particularly true for the older population and in the presence of chronic inflammation, for example in polyarthritis, chronic pulmonary obstructive disease, or psoriasis. Some physiopathological processes may also affect bioimpedance properties of the adipose tissue – fibrosis, vascularisation, blood cell infiltration, apoptosis, hydration changes, loss of elasticity, white fat transformation to brown fat (Table 2).

**Table 2.** Adipose tissue functions, its pathyophysiological changes and their potential relation to BIA/ densitometry diference.

Adipose tissue function	Possible relation to BIA/densitometry diference
Energy source (lipogenesis and lipolysis)	No
Thermoregulation	No
Mechanic protection of the organs and bones from injury	No
Pathological and physiological secretion of adipose hormones into the blood circulation	No
Protection from ectopic fat deposition	No
Immunology function- macrophages activation	Probably yes
Toxic agents storage, e.g. organic pollutants	Probably yes
Protection from endogenous toxins	Probably yes
Paracrine function of adipose cells in organs, e.g. vascular wall, suprarenal glands, etc.	No
Energy output in brown and beige adipose tissue	No
Bone adipose tissue impact on the bone marrow	No
<b>Physiopathological phenomena arising from adipose tissue</b>	
Inflammation	Probably yes
Adipose cells apoptosis	Probably yes
Change of macrophages infiltration from M2 to M1	Probably yes
Insulin sensitivity change of adipose tissue	Probably no
Hydration changes	Yes
Fibrosis	Yes
Vascularisation changes	Yes
Aging	Probably yes
Elasticity loss	Probably yes
Hormonal impact on adipose tissue	Probably yes
White fat conversion into beige and brown fat	Probably yes

In the present study we compared commonly available bioimpedance methods with the DEXA method in subjects with the entire BMI range. The results of the Bland-Altman analyses, as well as a two-way ANOVA (interaction between the factors of BMI band and

measurement method) suggest stratifying the group by BMI and using these methods correctly for each BMI subgroup.

While we succeeded to recruit about 30 subjects from each of the four lower BMI subgroups (BMI<40),

the number of subjects in the group of the morbidly obese women was relatively small ( $n=11$ ). This was due to the limitations of the DEXA instrument which is only capable of measuring individuals up to 120 kg, so tall and very obese women, otherwise eligible for the study, had to be rejected. Moreover, the Omron device was unable to measure 6 of the 11 probands in this group. Thus, Omron by no means should be recommended for the assessment of very obese subjects.

Bodystat was significantly biased in the two highest BMI subgroups and its mean absolute deviations from DEXA were there the highest of all measurement methods. Statistical comparisons with Tanita in the two aforementioned groups clearly favors Tanita, which had significantly smaller mean biases in both subgroups and significantly smaller mean absolute deviation from DEXA in the fourth subgroup. This is rather unexpected finding since four-electrode methods have generally better reputation than the two-electrode ones.

The results yielded by the Tanita method can be summarized as generally favorable with the exception of the lowest BMI subgroup, of which the mean biases with respect to DEXA were small and statistically not significant. In the first BMI subgroup, the measurements by Tanita were significantly negatively biased, but the bias was approximately the same as that of Bodystat and the caliper test, and only insignificantly higher than that of Omron. Regarding both mean bias and mean absolute deviation from DEXA, the Tanita method was not significantly inferior to any method of the triplet Bodystat – Omron – caliper test in any BMI subgroup, and was significantly superior to some of them in some subgroups (mean bias: to Bodystat in subgroups No. 4 and 5, and to the caliper test in the 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> group; mean absolute deviation: to Bodystat in the 4<sup>th</sup> subgroup and to the caliper test in the 3<sup>rd</sup> group).

Measurements by the caliper test are consistently significantly negatively biased, and statistical comparisons with the BIA methods do not appear favorable, especially in the subgroups of obese subjects.

A relatively large number of published studies dealing with the comparison of measuring methods for estimating body composition is available. However, the problem is that observation groups of the individual studies differ substantially in terms of age, gender, BMI, and ethnicity. Moreover, they are using different (and sometimes inappropriate) methods of data analysis. Namely, despite criticism by Bland and Altman (Carstensen and Gurrin 2011), the correlation coefficient

is often accepted as a measure of method correspondence. These differences suggest possible explanation of the fact that the results of previous studies are not entirely consistent and some of them contradict the findings of the present study. Another study (Braulio *et al.* 2010) on a group of obese Brazilian women showed only a small correspondence of bioimpedance methods with DEXA and proposed specific prediction equations in order to reach the results that corresponding to DEXA.

Van Loan (1998) compared the measurement of skinfolds and two types of bioimpedance methods with DEXA on 162 Asians (BMI ranging from 16.4 to 34.4 kg/m<sup>2</sup>) came to the conclusion that all the methods show quite a good correlation with DEXA, with the exception of the simplest bioimpedance device, only using a pair of electrodes placed in the hands of the proband (hand-held impedance).

Lintsi *et al.* (2004) compared two types of hand-held bioimpedance devices – the Omron 300 and the Omron 306 – with two types of calculations from anthropometric data according to Deurenberg *et al.* (1991) and Durnin and Womersley (1974), and with the DEXA method. Lintsi *et al.* (2004) concluded that the closest results to the DEXA method were achieved by the Omron 306 device and anthropometric calculations according to Deurenberg. The study group, however, was completely different than that of Van Loan (1998) since it consisted of Estonian soldiers ( $n=32$ ) 17 to 18 years of age (in contrast to the mean age of 45.1±9.0 in the Van Loan's study).

Lloret Linares *et al.* (2011) proved (in agreement to our study) on a large group of obese women ( $n=5740$ ) that the Tanita bioimpedance device overestimated in comparison with the DEXA method (1.1±6.1 kg, i.e. 0.8±5.6 %). This, however, differs from the study of Erselcan *et al.* (2000), where women were divided by BMI into obese ( $n=21$ ) and non-obese ( $n=16$ ) groups. The authors concluded that the bioimpedance method, as well as the method based on measuring skinfolds, underestimated compared to the DEXA results in both groups.

The usefulness of stratifying the study groups was demonstrated by Boneva-Asiova and Boyanov (2008) on 159 women and 124 men divided into three subgroups by BMI, since the mutual agreement of several fat measurement methods decreased with increasing BMI values.

Each of the fat measurement methods used in the present study had its specific limitations:

- In the caliper test, the human factor errors play an important role, since the skinfold measurement requires skills and experience. The measurement is particularly difficult in extremely obese people, as finding a fold of skin may be a problem. The method uses a specific equation to calculate the percentage of body fat from the skinfold sizes. The equation was designed many years ago (Pařízková 1977) and its validity for the current population might be controversial. We expect, however, that despite the fact that there has been a general shift to higher values, higher or lower percentage of total fat still corresponds more or less similarly to the values of skinfolds, as few decades ago. A specific advantage of the caliper method is in evaluation of fat distribution in more places on the surface of the body (which enables assessment of some of the indices that may correlate with various metabolic parameters).
- The bioimpedance methods are much less dependent on the human factor error than the caliper method but they are highly sensitive to the proband's compliance. It is well known that violation of standard conditions (see Methods paragraph) may change hydration of the organism affecting the measurement results.
- In our study, as well as in most of the papers referred above, the DEXA method was accepted as the gold standard. Indeed, according to the literature we consider the DEXA method as a sufficiently accurate technique, and therefore, as the reference method. It should be, however, noted that there are studies questioning the reliability of the DEXA method. Thus, although it is one of the highly recognized methods for body composition evaluation, it is still error-prone when measuring extremely obese people. Inaccuracies may result from the limited size of the scanned field, into which the obese may not fit, weight limitations of equipment and a high degree of photon absorption which increases with the thickness of fatty tissue (R Development Core Team 2010, Všetulová and Bunc 2004).
- The DEXA and caliper methods seem to measure a structural (anatomical) quantity of fat. Caliper method seems to under- or overestimate the percentage of fat. The BIA methods provide with different results in lean and obese and perhaps also in young and older people. A hypothesis could be proposed that BIA methods may also measure

some other physiopathological conditions like vascularisation, inflammation, hydration or cell infiltration of fat tissue as mentioned above.

- The impedance methods are reported to correlate very well with hydration (Goldberg *et al.* 2014). They can be used clinically in geriatric population for hydration measurements. They also are reported to be in relation to inflammatory markers and hypertension (Prujm *et al.* 2013).
- In women BIA methods can correlate with atherosclerosis (Goldberg *et al.* 2014) and it is expected that they can be influenced not only by inflammation but also by vessel wall changes (Sezer *et al.* 2012).

## Conclusion

In the present study, the BIA methods tended to overestimate the DEXA method. Two of the three BIA methods had serious problems, especially in the highest BMI subgroups where Bodystat appeared significantly (positively) biased, and Omron was unable to provide measurements in more than 50 % of the morbidly obese women. The Tanita method did relatively the best from the point of view of measurability problems as there were small biases in obese BMI subgroups and favorable statistical comparisons with other methods in all BMI subgroups. The caliper test was negatively biased in the whole range of BMI scale and appeared less preferable than BIA methods for its worse agreement with DEXA, especially in the higher BMI subgroup.

DEXA and caliper measurement seem to measure the structural (anatomical) quantity of fat. BIA methods may also measure some physiopathological conditions like inflammation, hydration, arterial perfusion, and cell infiltration of fat tissue. In the present study, the validity of these findings is limited to adult Caucasian women and there is a need for some different method for functional testing of fat tissue.

## Conflict of Interest

There is no conflict of interest.

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