Measurements of body fat is associated with markers of inflammation, insulin resistance and lipid levels in both overweight and in lean, healthy subjects

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ARTICLE INFO

Article history:
Received 16 July 2012
Accepted 12 October 2012

Keywords:
Fat mass
Body composition
Anthropometry
Bioelectric impedance
Inflammation

SUMMARY

Background & aims: Low-grade inflammation is associated with fat mass in overweight. Whether this association exists in lean persons is unknown.

Aims were to investigate associations between anthropometric measures of fat distribution and fat mass (% and kg) assessed by bioelectrical impedance analysis (BIA). Furthermore we wanted to investigate the relationship between fat mass and markers of insulin resistance, inflammation, and lipids in healthy subjects in different BMI categories.

Methods: We compared 47 healthy overweight adults (BMI 26–40 kg/m²) and 40 lean (BMI 17–25 kg/m²) matched for age and sex. Waist and hip circumferences, waist-to-hip ratio, waist-to-height ratio and triceps skinfold were used to evaluate fat distribution. BIA was used to estimate fat mass (% and kg). Markers of insulin resistance, lipids, inflammation and adipokines were measured.

Results: Hip circumference was associated (P < 0.01) with BIA-assessed fat mass (%) in both groups (lean: regression coefficient \( B = 0.4; \) overweight: \( B = 0.5 \)). An increase in hip circumference in all tertiles was associated with higher plasma levels of leptin, CRP and C-peptide in both groups.

Conclusions: Fat mass may play a role in low-grade inflammation also in subjects within the normal range of BMI. Hip circumference may be a surrogate measure for fat mass in subjects in different BMI categories, and may be useful for identification of people with risk of developing overweight-related chronic diseases.

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1. Introduction

The prevalence of overweight and obesity has increased dramatically worldwide. Frequently associated health risks are insulin resistance, elevated blood pressure and hypercholesterolemia, which may lead to type 2 diabetes and cardiovascular disease. The most important determinant of these problems is not the increased body mass per se, but rather the total amount of fat, its distribution in the body and metabolic factors that are related to fat tissue mass. Fat tissue is an active endocrine organ releasing adipokines (leptin, adiponectin, resistin) and inflammatory factors, e.g., interleukin (IL)-6. These mediators modify carbohydrate- and lipid-metabolism and contribute to insulin resistance, hyperlipidemia and inflammatory processes. It is well known that inflammatory markers are associated with fat mass in overweight and obese subjects, but this relation between fat mass and inflammatory markers in lean subjects is not well documented.

Several methods are used to measure the amount of fat in adults. One of the most accurate methods is Dual-energy X-ray absorptiometry (DXA), but measuring fat this way is costly and not readily available in clinical practice. Bioelectrical impedance analysis (BIA) is more available and widely used outside hospitals, and an objective, quick and non-invasive method for assessment of fat and fat free mass. Validation studies of BIA against DXA showed...
that BIA is an adequate tool for prediction of fat (%) in healthy populations.\textsuperscript{11} The most common population-level measure is probably estimation of body mass index (BMI).\textsuperscript{12} Whether BMI is a good marker to define obesity and health status is debated.\textsuperscript{13} Studies have shown that BMI fails to differentiate between elevated body fat and increased lean mass, especially in subjects with a BMI < 30 kg/m\textsuperscript{2}, a frequent cut-off for obesity.\textsuperscript{12} Other anthropometric measures, such as waist circumference, hip circumference, waist-to-hip ratio, waist-to-height ratio and triceps skinfold, are often used to determine fat distribution.\textsuperscript{13,14} Like BMI all these measures are just proxies of fat mass, but may predict adverse outcomes.\textsuperscript{14} The INTERHEART Study showed that all these measures are just proxies of fat mass, but may predict adverse outcomes.\textsuperscript{14} In further studies of the role of adipokines and inflammation in the development of metabolic disorders it will be of interest to investigate if fat mass estimated by anthropometric measures can predict levels of inflammatory markers not only in overweight, but also in lean subjects. Our primary study aim was therefore to determine if any of the frequently used anthropometric measures of fat mass (BMI, waist circumference/hip circumference, waist-to-hip ratio, waist-to-height ratio and triceps skinfold) were associated with BIA-measured fat mass. Furthermore we wanted to investigate the relationship between the anthropometric measure with the strongest correlation with BIA, and adipokines, inflammatory markers, markers of insulin resistance and lipids among healthy subjects in different BMI categories.

2. Materials and methods

2.1. Subjects

The study population included 47 overweight and 40 lean healthy adult volunteers (M:59/F:28). The overweight group consisted of subjects available for baseline analysis in a contemporary intervention trial performed in 2009. They were approached through mass media and selected in accordance with the following inclusion criteria: waist circumference >94 cm (men), >80 cm (women), and BMI 26–40 kg/m\textsuperscript{2}. Exclusion criteria were type 2 diabetes, kidney, liver, gall bladder, coronary, endocrine or rheumatoid disease, any malignancy the last 5 years, hypertension (\textgt;=160/100 mmHg), pregnancy and lactation. Regular use of anti-inflammatory, lipid lowering and antihypertensive medication was not permitted. In 2010, a reference group of lean subjects was recruited in the same way as the overweight subjects. Inclusion criteria were: waist circumference \textlt;=94 cm (men), \textlt;=80 cm (women), BMI 17–25 kg/m\textsuperscript{2} and age 18–70 years. Exclusion criteria were the same as for the overweight group. The study groups were matched on age and sex. All subjects were instructed to refrain from vigorous physical activity and alcohol the day before the study visit. The study protocol complied with the principles laid down in the Declaration of Helsinki, and approved by the Regional Committee for Medical and Health Research Ethics. Written informed consent was obtained from all participants.

2.2. Laboratory methods

Venous blood samples were collected after an overnight fast (\textgt;=12 h), between 8.00 and 10.00 a.m. Serum was obtained from silica gel tubes (Becton Dickinson vacuumtainer, Plymouth, Great Britain) and kept on ice, centrifuged (1500 g for 12 min), aliquoted and stored at –80 °C until further analyses (inflammatory markers), or kept in room temperature (for standard clinical chemistry) for at least 30 min, until centrifugation at 1500 g for 12 min and immediately prepared for subsequent analysis. Plasma was obtained from EDTA tubes (Becton Dickinson), kept on ice and centrifuged (2000 g, 4 °C, 10 min) within 15 min. Plasma samples were aliquoted and stored at –80 °C until further analysis.

Serum leptin, serum adiponectin, serum resistin, plasma IL-6, and plasma insulin-like growth factor-1 (IGF-1) levels were measured by enzyme immunoassays from R&D Systems (Minneapolis, USA) according the manufacturer’s instructions. All analyses were performed in duplicates. The coefficients of variation for intra-assay and inter-assay variability were <5% and <10%, respectively, for all analyses. Standard blood chemistry and lipid parameters were measured in serum or in EDTA plasma at First Medical Laboratory using routine methods (Oslo, Norway).

2.3. Assessment of fat mass

Subjects wore light clothing and no shoes. Two trained persons performed all measurements, which were performed once, except for triceps skinfold (TSF), which was measured three times. Height was measured by a wall-mounted stadiometer to the nearest 0.1 cm. Weight was measured by a Tanita scale (BC-418 MA, Tanita Corp., Tokyo, Japan) to the nearest 0.1 kg. A correction factor of –1 kg was used to adjust for the weight of light clothing before BMI was calculated. Waist- and hip circumferences were measured by a standard, non-stretch tape to the nearest 0.1 cm while standing in a relaxed position with normal respiration. Waist circumference was measured at a point midway between the iliac crest and the lower rib margin. Hip circumference was measured as the maximum circumference of the posterior buttocks and the anterior symphysis. The waist-hip ratio was calculated as waist circumference/hip circumference and the waist-height ratio was calculated as waist circumference/height. TSF was measured by using a Harpenden Caliper and a standard, non-stretch tape on the non-dominant arm. The midpoint of the arm was measured, with the measuring tape between the shoulder (acromion) and the elbow (olecranon) while the person was bending the arm 90°. TSF and the mid-upper-arm circumference (MUAC) were measured at this midpoint. The mid-upper-arm muscle circumference (MUAMC) was calculated with the equation MUAC−(π×(TSF/10))=MUAMC (cm).\textsuperscript{2}

Body composition was estimated using the single frequency bioimpedance analyzer Tanita scale, operating at 50 kHz, with eight-point contact electrodes.\textsuperscript{16} The electrode arrangement in the system allows separate measurements for each arm and leg, the trunk, and whole body. Fat mass (\% and kg) were calculated from the measured resistance values, height, body weight, sex, age, and standard body type (defined in the producer’s manual as less than ten hour of exercise per week). Measurements were performed with the subjects standing barefoot on the platform with arms slightly apart from the body.

2.4. Statistical analysis

Normality distribution was assessed by looking at the QQ-plots and the distribution of the histograms of the variables. Descriptive statistics were used. Independent samples t-test was used for comparison between groups. Univariate linear regression analyses were applied to quantify the relationship between BIA- and anthropometric measurements of body fat. Variables with P-values \textlt;0.2 were included in the multivariate model. A stepwise model reduction procedure was applied, where the F-ratio test was used. In this test we step-by-step eliminated the non-significant variables from the multivariate model. This was done to compare the results with and without the non-significant variables. The reduction (elimination of non-significant variables) was done until it was not possible to reduce the model any further. Although the groups were
3. Results

3.1. Subjects

Forty-seven (33 men and 14 women) overweight (BMI 25–40 kg/m$^2$) and 40 lean (BMI 20–25 kg/m$^2$) subjects (26 men and 14 women) were included. The overweight group had an age range from 37 to 68 years, and the lean from 36 to 65 years (Table 1). The data was normally distributed.

3.2. Insulin resistance markers and lipids

Overweight subjects had higher ($P < 0.05$) levels of all insulin resistance markers (insulin, Homeostasis Model Assessment (HOMA), C-peptide, HbA1c) than their lean counterparts. Glucose was elevated ($P = 0.03$) in overweight men relative to the lean ones, but this was not found among the women (Table 1).

No significant differences in the plasma concentration of total cholesterol were found between the overweight and lean subjects, but the LDL-cholesterol and triglyceride levels were higher ($P < 0.05$) whereas the HDL-cholesterol level was lower ($P < 0.05$) in the overweight relative to the lean subjects (Table 1).

3.3. Inflammatory markers and adipokines

The overweight subjects had higher ($P < 0.05$) levels of CRP and IL-6 than their lean counterparts. Overweight subjects also had elevated ($P < 0.05$) levels of the leptin and resistin, compared to the lean subjects, while the level of adiponectin was lower ($P < 0.05$). Overweight women had higher levels of CRP than overweight men ($P < 0.05$) (Table 2) and women in both groups had higher ($P < 0.05$) levels of leptin and adiponectin than men (Table 2).

3.4. Body composition in overweight and lean subjects

All body composition measures were significantly elevated in overweight compared with lean subjects. Both overweight and lean women had higher TSF ($P < 0.001$), whole body fat (%) ($P < 0.01$),

Table 2

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Concentrations of inflammatory markers and adipokines in healthy overweight and lean subjects.*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
</tr>
<tr>
<td></td>
<td>Overweight, $n = 33$</td>
</tr>
<tr>
<td>IL-6 (ng/ml)</td>
<td>1.4 (0.6)</td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>2.7 (1.2)</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>11.7 (9.6)</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>10.2 (2.8)</td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>126.1 (32.8)</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>1.8 (1.1)</td>
</tr>
</tbody>
</table>

*a Compared to lean subjects, overweight subjects had higher ($P < 0.05$) levels of all variables except IGF-1.

*b Overweight males versus lean males.

*c Overweight females versus lean females.

*d Overweight males versus overweight females.

*e Lean males versus lean females.
and fat mass (P < 0.01) than their male counterparts (Table 3). Males had higher levels for all other measurements than females except for hip circumference, mid-upper-arm circumference and trunk fat mass.

### 3.5. Prediction of fat mass

To quantify the relationship between anthropometric estimates of fat mass and body fat measured with BIA we performed multiple linear regression analyses (Table 4). Hip circumference had the highest standardized coefficient and explained most of the variation in whole body and trunk fat mass (% and kg) in both overweight and lean subjects. Waist-to-hip ratio demonstrated the second highest standardized coefficient for whole body fat mass (% and kg) and trunk fat (%) in overweight subjects. In lean subjects, TSF had the second highest standardized coefficient for all BIA measures of fat mass. In summary, the results showed that measurements of hip circumference were highly associated with whole body and trunk fat mass expressed in kg and percentage, in both lean and overweight subjects. The results also indicated that an increase in hip circumference with one cm in both overweight and lean subjects corresponded to an increase in the trunk body fat mass with 360 g.

### 3.6. Relationship between insulin resistance markers, lipids and inflammatory markers, and body fat

Because measurements of hip circumference were closely related to BIA-derived fat mass in both lean and overweight subjects, tertiles of hip circumference and whole body fat (%) were used to analyze the relation between fat mass and markers of insulin resistance, lipids and inflammatory markers (Tables 5 and 6). We also divided BMI into tertiles and performed the same analysis (Table 7). In overweight subjects, IL-6 was reduced across tertiles of hip circumference (Table 5). Levels of adiponectin and leptin increased, while resistin decreased. There was also an elevation of IGF-1 and CRP concentrations. Levels of HOMA (P < 0.05) and C-peptide (P < 0.05) increased and an elevation of triglycerides was seen, while HDL-cholesterol remained stable (Table 5). The same trends were found regarding tertiles of BMI in the overweight subjects, except for a significant decrease of resistin (P < 0.05) and elevated C-peptide (P < 0.01) levels (Table 7). Across tertiles of whole body fat (%) (Table 6), there were also increasing trends in adiponectin (P < 0.01) and leptin (P < 0.01), IGF-1, CRP, HOMA, and C-peptide. Concentrations of IL-6 and resistin (P < 0.05) increased across tertiles, and triglyceride concentrations decreased.

Regarding the relation to tertiles of hip circumference in lean subjects (Table 5), IL-6 and adiponectin were reduced, and leptin (P < 0.01) and resistin values were increased. Levels of IGF-1, CRP, HOMA, C-peptide and triglycerides were increased, while HDL-cholesterol was reduced. Similar trends were found for tertiles of BMI in the lean subjects, except for resistin which was decreased across tertiles, and CRP (P < 0.05), which was significantly increased (Table 7). Like for the tertiles of hip circumference and BMI, leptin (P < 0.01), IGF-1, CRP, HOMA and C-peptide, increased across tertiles of fat (%) (Table 6). IL-6 values however were stable.

---

**Table 3**

Body composition of the healthy overweight and lean subjects.\(^a\)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overweight, n = 33</td>
<td>Lean, n = 26</td>
</tr>
<tr>
<td>Mean (s.d.)</td>
<td>Mean (s.d.)</td>
<td>Mean (s.d.)</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>109.0 (7.0)</td>
<td>97.3 (4.7)</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>108.2 (8.4)</td>
<td>85.7 (5.8)</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>1.0 (0.06)</td>
<td>0.9 (0.05)</td>
</tr>
<tr>
<td>Waist-height ratio</td>
<td>0.6 (0.07)</td>
<td>0.4 (0.03)</td>
</tr>
<tr>
<td>Triceps skinfold (mm)</td>
<td>21.1 (9.2)</td>
<td>9.6 (2.8)</td>
</tr>
<tr>
<td>Mid-upper-arm circumference (cm)</td>
<td>34.8 (2.5)</td>
<td>29.2 (2.0)</td>
</tr>
<tr>
<td>Mid-upper-arm muscle circumference (cm)</td>
<td>28.2 (3.3)</td>
<td>26.2 (2.1)</td>
</tr>
<tr>
<td>Whole body fat (%)</td>
<td>29.0 (5.6)</td>
<td>18.3 (3.9)</td>
</tr>
<tr>
<td>Whole body fat mass (kg)</td>
<td>29.4 (8.0)</td>
<td>14.6 (3.8)</td>
</tr>
<tr>
<td>Total fat free mass (kg)</td>
<td>71.1 (7.5)</td>
<td>64.3 (6.3)</td>
</tr>
<tr>
<td>Trunk fat (%)</td>
<td>31.4 (5.6)</td>
<td>19.8 (4.9)</td>
</tr>
<tr>
<td>Trunk fat mass (kg)</td>
<td>18.0 (4.5)</td>
<td>9.1 (2.7)</td>
</tr>
<tr>
<td>Trunk fat free mass (kg)</td>
<td>38.7 (3.8)</td>
<td>36.1 (3.5)</td>
</tr>
</tbody>
</table>

\(^a\) Compared to lean subjects, overweight subjects had higher (P < 0.05) levels of all variables.

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**Table 4**

Predictors of fat mass and fat percentage derived from multiple regression analyses in overweight and lean subjects.\(^a\)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Predictors</th>
<th>Overweight, n = 47</th>
<th>Lean, n = 40</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B</td>
<td>95% CI</td>
<td>B</td>
</tr>
<tr>
<td>Fat %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole body</td>
<td>TSF(^b)</td>
<td>0.32</td>
<td>0.0.06</td>
</tr>
<tr>
<td>HC(^c) (cm)</td>
<td>0.5, 0.3–0.7</td>
<td>0.00</td>
<td>0.4, 0.2–0.7</td>
</tr>
<tr>
<td>WHR(^e)</td>
<td>20.9, 2.3–39.5</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Trunk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC (cm)</td>
<td>0.5, 0.3–0.7</td>
<td>0.5, 0.1–0.9</td>
<td>0.02</td>
</tr>
<tr>
<td>WHR</td>
<td>21.9, 3.1–40.8</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole body</td>
<td>WHR(^f)</td>
<td>0.2, 0.1–0.4</td>
<td>0.04</td>
</tr>
<tr>
<td>HC (cm)</td>
<td>0.4, 0.3–0.5</td>
<td>0.4</td>
<td>0.2–0.5</td>
</tr>
<tr>
<td>Waist(^g) (cm)</td>
<td>0.2, 0.1–0.3</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Adjusted for age and sex.

\(^b\) B: regression coefficient (kg). The predictor with the highest standardized coefficient that explains most of the variation of the dependent variable is ranked 1, the second highest 2.

\(^c\) TSF = triceps skinfold.

\(^d\) HC = hip circumference.

\(^e\) WHR = waist-to-hip ratio.

\(^f\) WHR = waist-to-height ratio.

\(^g\) Waist = waist circumference.
In obesity the fat tissue produces adipokines\(^19\) and cytokines, which may result in chronic inflammation.\(^20\) It has been shown that systemic inflammation is higher in obese than in normal weight persons.\(^21\) Leptin is preferentially secreted by subcutaneous adipose tissue,\(^22\) and the concentration is dependent on adipocyte size\(^23\) as well as energy balance.\(^21\) In our study we found a strong association between hip circumference and whole body fat (\%), and leptin levels. The same association was also found with BMI. Normally, leptin levels are higher in obese individuals as demonstrated here. Interestingly we observed that leptin levels increased with increasing fat (\%) also in the lean group. One could argue that this could be an effect of food intake or macrophage infiltration in adipose tissue due to weight gain, which is known to produce higher leptin levels. However, in both study groups the blood levels were measured during fasting and all subjects reported stable weight for at least two months prior to inclusion. Few studies have shown the same trend with leptin levels in lean people, but a positive association between fat mass accumulation, oxidative stress indices and leptin levels has been observed,\(^7\) suggesting that fat mass-induced oxidative stress may cause a dysregulation of adipokines, also in lean subjects.

A positive relationship between BMI, waist circumference and CRP has been documented.\(^24\) This is in accordance with our study as we found that CRP increased with increasing BMI and interestingly, this positive relationship was significant in lean subjects. We also found an association between hip circumference and whole body fat (\%) and CRP, although not significant. These results confirm the findings by Arner et al.\(^25\) of an association between inflammation and fat mass in lean individuals. There is also evidence that IL-6,
BMI> Our study a subgroup of the overweight people, namely those with adiponectin levels as fat mass accumulates and an elevation with weight loss.\(^27\) Earlier studies show a decrease\(^30\) in adiponectin levels as fat mass increases.29 They have high fat mass and high BMI and high HDL, but an inverse reduction of triglyceride levels. Studies have described these markers from the lowest to the highest tertile of whole body fat (%) in both groups. Low level of HDL-cholesterol is an important risk factor for cardiovascular disease.28 One would expect a reduction in HDL-cholesterol as fat mass expands. This was found in our study with increasing hip circumference in lean individuals and with increasing BMI in both groups. In the overweight however, we found stable levels of HDL-cholesterol as hip circumference increased, and elevated levels of HDL-cholesterol from the lowest to highest tertile of whole body fat (%). Elevated HDL-cholesterol levels were followed by an inverse reduction of triglyceride levels. Studies have described a subset of obese individuals, termed metabolically healthy, which appear to be resistant to the development of metabolic disturbances.20 They have high fat mass and high BMI and high HDL, but low triglycerides and visceral fat and normal insulin sensitivity. In our study a subgroup of the overweight people, namely those with BMI > 30 kg/m\(^2\), but no elevated HOMA, triglyceride- or LDL levels, had the highest levels of whole body fat (%). It should be noted that all the overweight women in our study were in the highest tertile of fat (%). This may also explain our findings regarding adiponectin: In the overweight group we found elevated levels of adiponectin in the highest tertiles of hip circumference, whole body fat (%) and BMI. Earlier studies show a decrease\(^26\) in adiponectin levels as fat mass accumulates and an elevation with weight loss.27 The major strength of our study is that we examined a broad range of anthropometric measures. Our study has some limitations since we used indirect measurements as indicators of total and relative contributions of subcutaneous versus visceral fat. The number of subjects was relatively low and the results should be confirmed in a larger population. The age and gender heterogeneity is also a limitation, although the variable was adjusted for. In conclusion, we have showed that measurements of hip circumference to assess total body and trunk fat (%) may represent a valid substitute to BIA measurements in both lean and overweight subjects. Thus this is a highly feasible method outside the hospital setting in order to identify people at risk of increased inflammation and insulin resistance.

Our results may also suggest that fat (%) is associated with elevation of risk factors for lifestyle related disorders among lean persons. Although the choice of fat measure may impact on the magnitude of these associations, adherence to a healthy lifestyle is also important for people within the recommended range of BMI. The relationship between markers of inflammation, insulin resistance and lipids in lean as well as overweight subjects should be studied further in order to understand the role of fat mass in healthy subjects with different BMI. Such knowledge may be of considerable interest for early identification of subjects at risk of type 2 diabetes and cardiovascular disease.

**Conflict of interest**

The authors declare no conflict of interest.

**Funding sources**

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Please cite this article in press as: Wesseltoft-Rao N, et al., Measurements of body fat is associated with markers of inflammation, insulin resistance and lipid levels in both overweight and in lean, healthy subjects, e-SPEN Journal (2012), http://dx.doi.org/10.1016/j.ejme.2012.10.002.


