Distribution of adipose tissue: Quantification and relationship with hepatic steatosis and vascular profiles of type 2 diabetic patients with metabolic syndrome

F. Illouza,∗ V. Roulier, A. Rod, Y. Gallois, C.-P. Pellé, C. Aubé, V. Rohmer, P. Ritz, P.H. Ducluzeau

Département d’endocrinologie-diabète-nutrition, centre hospitalier universitaire d’Angers, 49933 Angers cedex 01, France
Département de radiologie, centre hospitalier universitaire d’Angers, 49933 Angers cedex 01, France
Laboratoire Lisa UPRES-EA 4014, université d’Angers, 49000 Angers, France
Service de biochimie, centre hospitalier universitaire d’Angers, 49933 Angers cedex 01, France

Received 13 June 2007; received in revised form 1 October 2007; accepted 8 October 2007

Abstract

Aim. – As the distribution of fat is increasingly related to cardiovascular events, we examined whether or not abdominal-fat quantification using magnetic resonance imaging (MRI) software is reliable, and whether or not it is related to clinical markers of fat distribution as well as to metabolic and vascular status.

Methods. – We recorded the anthropometric measurements of 34 obese type 2 diabetic patients with metabolic syndrome. The patients were enrolled to evaluate their abdominal (visceral and subcutaneous) adipose tissue by single-slice L3–L4 MRI. Manual and automated analyses were compared. The relationships between anthropometric measurements, biological markers and intima-media thickness of the common carotid artery were also assessed.

Results. – We validated the automated software to quantify abdominal-fat deposition with MRI compared with manual measurements ($r^2 = 0.95$). The waist-to-hip-circumference ratio (WHR) was the only clinical parameter that correlated with the proportion and quantity of visceral and subcutaneous abdominal-adipose tissue evaluated by MRI ($r = 0.60$). In addition, fat repartition as evaluated by WHR was related to hepatic steatosis parameters (ferritin and ALAT) and to intima-media thickness, whereas simple waist circumference was not a determinant in these obese patients. We also showed that the adiponectin-to-leptin ratio was related to adipose tissue distribution.

Conclusion. – Distribution of abdominal fat, as evaluated by MRI, can be reflected by clinical determination of the WHR. Differences in regional accumulations of abdominal fat may be specifically related to variations in the risks of steatosis and vascular rigidity among obese type 2 diabetic patients.

© 2007 Elsevier Masson SAS. All rights reserved.
SAT in type 2 diabetic patients who displayed the metabolic syndrome, compared with manual segmentation, to measure VAT and long and tedious manual segmentation. Unfortunately, MRI requires from tomodensitometry in that it may be coupled with analysis of liver or muscle fat deposition. Also, MRI is different from tomodensitometry [11,12]. Also, MRI is different

1. Introduction

Individuals with the metabolic syndrome have an increased risk of premature death from cardiovascular disease or all-cause mortality [1]. The distribution of adipose tissue is involved in the obesity-induced metabolic and vascular risk. The accumulation of intra-abdominal-adipose tissue is, at least, partly responsible for the development of the metabolic syndrome. Excess abdominal fat is involved in the pathophysiology of insulin resistance and its clinical evaluation on the basis of waist measurement is among the criteria that define the metabolic syndrome [2,3]. In fact, abdominal obesity has been associated with abnormal carbohydrate [4] and lipid metabolism [5] as well as vascular disorders [6] that carry an increased risk of cardiovascular events [7]. Waist circumference (WC) reflects both visceral-adipose tissue (VAT) and abdominal subcutaneous-adipose tissue (SAT). Waist circumference measurement, alone or associated with the body mass index (BMI), is unable to determine the relative contribution of each type of adipose tissue deposition in the umbilical region. Indeed, the INTERHEART study clearly showed that, more so than WC, an increased waist-to-hip circumference ratio (WHR) is associated with an elevated risk of infarction [8].

Subcutaneous-adipose tissue accounts for the majority of adipose tissue, as about 85% of adipose tissue is located under the skin, while nearly 15% lies within the abdomen [9]. Reduction of subcutaneous-adipose tissue secondary to antiprotease treatment leads to a severe state of insulin resistance and metabolic abnormalities [10]. On the other hand, an increase in hip circumference (HC) has been shown to be associated with a reduction in the risk of myocardial infarction [8]. Thus, the relative proportion of visceral and subcutaneous fat appears to be a good predictor of metabolic and vascular risk associated with obesity.

Magnetic resonance imaging (MRI) is now considered the gold-standard method for determining the quantity of SAT and VAT, despite the fact that previous studies of clinical correlation have used tomodensitometry [11,12]. Also, MRI is different from tomodensitometry in that it may be coupled with analysis of liver or muscle fat deposition. Unfortunately, MRI requires long and tedious manual segmentation.

In the present study, we validated an automated method of MRI, compared with manual segmentation, to measure VAT and SAT in type 2 diabetic patients who displayed the metabolic syndrome. The aim of the study was to investigate the relationship between MRI software data and anthropometric measures of obesity (WC, HC, WHR). As the metabolic syndrome and type 2 diabetes are closely associated with vascular abnormalities and liver steatosis, we also searched for a correlation between fat distribution, metabolic and adipokine profiles, and intima-media thickness (IMT).

2. Materials and methods

2.1. Subjects

Thirty-four patients (25 men, 9 women) were recruited by the diabetes department of University Hospital of Angers. The recruitment criteria were:

- age less than 75 years;
- type 2 diabetes with oral or insulin treatment and;
- metabolic syndrome as defined by the NCEP–ATP III classification [1].

All patients received detailed printed and oral information, and gave their informed consent to participate in the study, which was approved by the ethics committee of the hospital.

2.2. Body-fat distribution

The following anthropometric measurements were carefully recorded: weight, height, BMI, WC, HC and WHR. Radiological examinations were performed using a 1.5 T whole-body MRI system (Excite, GE Healthcare, Milwaukee, WI). A T1-weighted, in–out phase, single-slice image at L3–L4 disk level was taken. Manual and automated software calculations of surface area for abdominal VAT and SAT were in square centimetres, corresponding to the real body surface. Body composition was also analyzed by dual-energy X-ray absorptiometry (DEXA; Hologic 4500A) and the system calibrated to differentiate total from truncal fat mass.

2.3. Vascular and metabolic parameters

Blood pressure was determined as the average of five measurements taken in supine position after 5 min of rest.
Ultrasound of the common carotid artery was carried out to measure the IMT. Fasting-metabolic parameters were obtained in the morning: serum glucose; serum total cholesterol, HDL-cholesterol and triglycerides (LDL-cholesterol was estimated according to the Friedwald formula); haemoglobin A1c (HbA1c); serum alanine aminotransferase (ALAT); and serum ferritin. The concentrations of plasma adiponectin and leptin were determined by ELISA (Human Leptin ELISA and Human Adiponectin ELISA kits, Linco Research, St Charles, MO).

2.4. Statistical analysis

Results are given as means ± S.D. Relationships between parameters were calculated by single linear regression analysis. All calculations were performed using StatView 5.0 (Abacus Concepts, Berkeley, CA). Probability (P) values less than 0.05 were considered significant.

3. Results

3.1. Validation of automated measurement of VAT and SAT

Segmentation of visceral and subcutaneous fat requires an image without artefacts (particularly breathhold-related artefacts) (Fig. 1). The reproducibility coefficient of the quantification of VAT and SAT using the software is 0.98. The average areas of VAT were 289 ± 84 cm² and 297 ± 87 cm², and the average areas of SAT were 241 ± 93 cm² and 223 ± 86 cm², by automated and manual analyses, respectively. Comparison of the quantification of fat using purpose-made software and manual segmentation showed an excellent correlation (r² = 0.95, P < 0.05) for VAT as well as for SAT (r² = 0.95, P < 0.05) (Fig. 2). The interoperator reproducibility of the manual quantification of VAT and SAT was done on the 34 MRI images by two different radiologists. Variation coefficients for VAT and SAT were 7.1 and 2.4%, respectively.

Fig. 2. Correlation graphs for automated and manual MRI calculations of abdominal-adipose tissue: A. visceral-adipose tissue (VAT); B. subcutaneous-adipose tissue (SAT).

3.2. Relationships between anthropometric measures and MRI distribution of adipose tissue

Characteristics of the studied population are presented in Table 1. Twenty-five men and nine women with an average age of 59.0 ± 8 years were included in these analyses. All of these patients were obese (BMI = 33.2 ± 4.1 kg/m²), and dis-

Fig. 1. Automated analyses of the distribution of abdominal-adipose tissue from T1-weighted in–out phase, single-slice scans taken at L3–L4 level. Original image (A), subcutaneous (B) and visceral (C) fat segmentation using automated software.
Table 1
Characteristics of the study population

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Means ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>59.0 ± 8.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>93.7 ± 13.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.2 ± 4.1</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>110.3 ± 9.6</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>110.7 ± 10.4</td>
</tr>
<tr>
<td>WHR</td>
<td>1.00 ± 0.08</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>133.1 ± 16.7</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>75.7 ± 8.0</td>
</tr>
</tbody>
</table>

MRI (abdominal)
- VAT (cm²) 289 ± 84
- SAT (cm²) 241 ± 93
- Percentage VAT 54.9 ± 14.0

DEXA
- Total fat mass (kg) 30.6 ± 7.0
- Truncal fat mass (kg) 17.9 ± 4.4
- Truncal fat mass (% of fat mass) 58.6 ± 5.0
- Fat-free mass (kg) 62.4 ± 10.3
- Fat-free mass (% of total weight) 65.5 ± 5.7

Biochemical values (range)
- Serum glucose (mmol/L) (4–5.6) 9.1 ± 2.6
- HbA1c (%) (4.2–5.8) 7.5 ± 1.1
- Ferritin (µg/L) (45–350) 263 ± 249
- ALAT (U/L) (12–45) 39 ± 25
- TC (g/L) (1.6–2.8) 1.93 ± 0.45
- HDL-C (g/L) (0.5–0.8) 0.51 ± 0.12
- LDL-C (g/L) (0.7–2.1) 1.07 ± 0.36
- TG (g/L) (0.5–2.3) 1.84 ± 1.37
- Adiponectin (µg/mL) (2.3–10.5) 6.6 ± 2.1
- Leptin (ng/mL) (2.5–15) 26.7 ± 17.0
- Adiponectin-to-leptin ratio 0.38 ± 0.34

Fig. 3. Graphs of waist-to-hip-circumference ratios correlated to V AT (A); % V AT (B) and total fat-free mass (C). V AT: visceral-adipose tissue; % V AT: proportion of visceral-ladipose tissue; ns: not significant.
coefficients between anthropometric data and MRI calculations. The WC was positively correlated with BMI – even more so than with VAT \( r = 0.50 \) or SAT \( r = 0.45 \) – but there was no correlation with the proportion of VAT. On the other hand, HC was highly correlated with SAT \( r = 0.79 \) and displayed an inverse relationship with the proportion of visceral fat \( r = -0.56 \). Thus, the WHR was the only parameter associated with both VAT \( r = 0.60 \) and the proportion of visceral fat \( r = 0.58 \) (Fig. 3).

The WHR was also associated with truncal-fat distribution as evaluated by DEXA \( r = 0.62 \). Even more striking were the associations between WHR and the quantity \( r = 0.53 \); Fig. 3C) and proportion \( r = 0.56 \) of fat-free mass, evaluated by DEXA, as well as between the WC and quantity of fat-free mass \( r = 0.60 \) (Table 2).

### 3.3. Associations between anthropometric measurements, and metabolic and vascular parameters

Given that the only clinical parameter able to predict the proportion of visceral fat in the abdomen is the WHR, we looked for relationships with clinical and biological parameters. The results are shown in Table 2. In our homogeneous population, WHR was positively correlated to ferritin \( r = 0.41 \), ALAT \( r = 0.39 \) (Fig. 4) and intima-media thickness \( r = 0.34 \). Neither the WC nor HC were associated with metabolic parameters nor was any correlation found with blood pressure, HbA1c or lipid profile.

### 3.4. Relationship with adipokine plasma levels

We also searched for links between the adipose-tissue compartment and plasma-adipokine concentrations. The results are presented in Table 3. As expected, adiponectin levels were inversely associated with WHR \( r = -0.39 \) and VAT \( r = -0.34 \) as well as with the proportion of VAT \( r = -0.33 \). On the other hand, leptin was positively related to HC and SAT, and negatively related to fat-free mass and proportion of VAT. As leptin and adiponectin appear to reflect different fat depositions, we calculated the adiponectin-to-leptin ratio and found that it was positively associated with the proportion of VAT \( r = 0.38 \). The ratio was also inversely related to subcutaneous-fat mass as evaluated by HC \( r = -0.41 \) and SAT \( r = -0.55 \). However, the adiponectin-to-leptin ratio showed a strong correlation to the proportion of fat-free mass \( r = 0.62 \).

### 4. Discussion

Recent studies analyzing the relationships between BMI and total or cardiovascular mortality have revealed yet more contradictory results [13,14]. The importance of the BMI as a
cardiovascular risk factor has even been recently subjected to further discussion [15]. However, an elevated HC could correct some of the excess risk associated with obesity [8].

Radiologically-based quantification of fat deposits was developed over 20 years ago, and it is now recognized that MRI is a safe and reliable method that correlates with anthropometric measurements [16]. The classical segmentation approach usually uses the threshold method for between-subject standardization. The method is less effective in the presence of intensity variations within the same tissues as is frequently observed with the latest MRI systems. Establishing a threshold would lead to a loss of surface area in some patients and a gain in others. This is why our software was developed as a pixel-by-pixel method of analysis over two algorithm steps. The first classification algorithm allows merging of pixel intensity into fat, background and organs. The second algorithm step corrects any fat classification errors (due to inhomogeneity) and allows discrimination of visceral from subcutaneous-adipose tissue.

Moreover, it has been shown that the use of only one MRI slice is sufficient to reflect the VAT volume, particularly if taken above the L4 vertebral level [17]. As a single-slice MRI scan of abdominal fat makes it possible to quantify the distribution of adipose tissue, we developed a semi-automated method for measuring visceral- and subcutaneous-adipose tissue. Our results show excellent correlations with no dispersion along the data range. Our study shows that the WC is correlated with VAT and also with SAT, but it cannot predict the proportion of visceral and subcutaneous fat in the abdominal compartment. On the other hand, HC is closely related to MRI abdominal subcutaneous-fat mass. The ratio (WHR) of these two clinical variables reflects both the VAT area and proportion (% VAT). Thus, the WHR, but not the WC alone, appears to be a reliable clinical marker of abdominal-adipose distribution, especially in patients who are already obese.

The question of the metabolic and vascular impact of abdominal fat repartition in patients who are morbidly obese was not assessed in our study. In the case of severe obesity, the increase of fat mass is so great that it must be present in both depots. But morbidly obese subjects most likely display a dramatic increase in subcutaneous-fat deposition, including the abdominal region. Unfortunately, such patients rarely benefit from MRI examination and their waist- and hip-circumference measurements are often impressive.

Ferritin appears to be related to nonalcoholic fatty-liver disease. In our study population of obese type 2 diabetic patients with metabolic syndrome, serum ferritin was positively associated with WHR. This result is in accordance with that of Gillum, who found a link between ferritin and WHR regardless of BMI [18]. Iwasaki et al. showed a positive relationship between ferritin and visceral-fat mass [19]. The link between ferritin and WHR, but not WC or HC, leads us to theorize that ferritin might be an early marker of the impact of adipose-tissue distribution on the liver. Among patients displaying increases in ALAT levels, the risk of developing fibrosis and cirrhosis is 20–30 and 1.5–6%, respectively. Moreover, diabetes amplifies these risks [20]. We showed that ALAT was also related to WHR, reflecting the usefulness of the ratio in the prognosis for nonalcoholic fatty-liver disease. So, in obese type 2 diabetic patients, the presence and severity of liver metabolic disturbances appears to be inversely contingent on subcutaneous-fat deposition, although the visceral-fat mass is large in these individuals.

An increase in intima-media thickness is considered a marker of early atherosclerotic disease and predicts the risk of cardiovascular events [21]. Here, we found that the WHR is positively associated with IMT. This is in agreement with a Japanese study that, after adjusting for BMI, demonstrated an association between IMT and WHR, but not WC [22]. It has been shown that IMT is related to mesenteric fat, as determined by ultrasound examination [23]. We could find no direct relationship between IMT and VAT on MRI, although there was a tendency towards a link with the proportion of truncal-fat mass on DEXA ($r = 0.32, P = 0.08$).

It is well-known that the plasma adiponectin level is inversely associated with insulin resistance [24] and visceral-adipose tissue [25], although the data for their interdependence is inconsistent [26,27]. Our study confirms the negative relationship between adiponectin level and VAT as well as % VAT. As leptin is closely related to total adipose tissue and, in particular, subcutaneous-adipose tissue, as evaluated by HC and SAT, it may be that the adiponectin-to-leptin ratio could offer information on adipose distribution. The adiponectin-to-leptin ratio has recently been studied as an index of insulin resistance [28] and atherogenicity [29]. Despite the relatively small number of patients in our study, we found that this ratio was closely and positively correlated to the distribution of abdominal-fat mass (% VAT). Although the association with WHR did not reach statistical significance ($P = 0.12$), the adiponectin-to-leptin ratio may be a useful indicator of adipose distribution.

In conclusion, the WHR was the only clinical parameter that enabled us to estimate the subcutaneous and visceral distribution of abdominal-adipose tissue as evaluated by abdominal MRI. Differences in the regional accumulation of abdominal fat could account specifically for variations in the risk of steatosis and vascular rigidity among obese type 2 diabetic patients who have similar waist circumferences.

References


[23] Liu KH, Chan YL, Chan WB, Chan JC, Chu CW. Mesenteric fat thickness is an independent determinant of metabolic syndrome and identifies subjects with increased carotid intima-media thickness. Diabetes Care 2006;29:379–84.


