**INTRODUCTION**

Visceral fat accumulation as a result of excessive caloric intake and/or sedentary lifestyle likely contributes to the clustering of metabolic abnormalities and the development of atherosclerotic vascular disease. Metabolic syndrome (MetS) is a well-known highly atherogenic state, and ‘central obesity’ is one of the most important components of MetS. For the MetS diagnosis, waist circumference according to population- and country-specific criteria is used to define central obesity. The Japanese waist circumference cut-off points were determined as 85 cm for men and 90 cm for women based on a visceral fat area (VFA) of 100 cm$^2$ measured by computed tomography (CT), because the mean number of obesity-related disorders exceeded 1.0 when VFA was > 100 cm$^2$.

Several mechanisms linking visceral fat accumulation to endothelial dysfunction and atherosclerosis have been postulated: alterations in adipocytokines, lipids, insulin resistance, liver enzymes, leptin, adiponectin, high sensitivity C-reactive protein (hsCRP) and the degree of atherosclerosis (mean intima-media thickness by carotid ultrasonography).

**ABSTRACT**

**Objective** To investigate the relationship between visceral fat area (VFA) with the anthropometric and biochemical clinical parameters of lifestyle-related diseases, adipocytokines, inflammation and atherosclerosis in Japanese men and women.

**Subjects and methods** The correlation between VFA and the following parameters was evaluated in 94 healthy volunteers (48 men and 46 women): body weight, BMI, waist circumference, body composition, blood pressure, glucose homeostasis, insulin resistance, lipids, uric acid, liver enzymes, leptin, adiponectin, high sensitivity C-reactive protein (hsCRP) and the degree of atherosclerosis (mean intima-media thickness by carotid ultrasonography).

**Results** The mean age was 40.1 ± 9.6 years (42.0 ± 10.2 for men and 38.1 ± 8.6 for women) and the mean VFA was 47.4 ± 40.9 cm$^2$ (70.5 ± 41.4 for men and 23.3 ± 22.3 for women). VFA was positively correlated with blood pressure, fasting glucose, HbA1c, insulin resistance, LDL-C, triglycerides, ALT, $\gamma$GT and uric acid. A negative correlation was observed in HDL-C and adiponectin. Furthermore, hsCRP and carotid intima-media thickness showed a positive association with VFA.

**Conclusion** The present study shows that those with subclinical visceral fat accumulation have potential atherosclerotic risk even though they show no apparent clinical abnormalities.

**Key words** Visceral fat, Lifestyle-related disease, Adiponectin, Inflammation, Atherosclerosis

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**SUBJECTS AND METHODS**

1. Subjects

Ninety four healthy individuals (48 men and 46 women) were
enrolled in this cross-sectional study. The examinations were conducted in February 2013. Written informed consent was obtained from all the subjects to use their health records for analysis. This study was approved by the Ethical Committee of Tokai University, and was conducted in accordance with the Declaration of Helsinki.

2. Measurements

All measurements were performed after overnight fasting. Height and weight were measured in the standing position, and body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Waist circumference was assessed at the end of expiration by measuring the minimum circumference at the level of the umbilicus. Blood pressure (BP) was measured in a sitting position. Visceral and subcutaneous fat area was measured at the level of the umbilicus in the spine position using CT. Bioelectrical impedance analysis (Inbody 720, Biospace Co. Ltd.) was used for evaluating body fat mass, fat-free mass and %body fat.

In addition to the biochemical measurements performed in the routine health check examination, fasting serum immunoreactive insulin (IRI) was measured by chemiluminescent enzyme immunoassay. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as: fasting plasma glucose (in mg/dl) × IRI (in μU/ml)/405^9). The value for HbA1c (%) is expressed as a National Glycohemoglobin Standardization Program (NGSP) equivalent value. Leptin was measured by double antibody radioimmunoassay. Serum level of high molecular adiponectin was obtained using the CLEIA method. High sensitivity C-reactive protein (hsCRP) concentration was measured by turbidimetric immunoassay.

The carotid arteries were examined bilaterally at the levels of the common carotid, the bifurcation, and the internal carotid arteries. The intima-media thickness (IMT) was measured by trained sonographers, and mean IMT was calculated and used for analysis.

3. Statistical analysis

Data are expressed as mean ± standard deviation (SD). SPSS Statistics (version 19.0; SPSS Inc.) was used for the statistical analyses. Pearson’s correlation coefficient was calculated as a measure of association between VFA and the following clinical parameters: anthropometric measurements (body weight, BMI, waist circumference, subcutaneous fat area, body fat mass, fat-free mass and %body fat), systolic and diastolic BP, glucose homeostasis (fasting plasma glucose, HbA1c, insulin and HOMA-IR), lipids (LDL-C, HDL-C and triglycerides), uric acid, liver enzymes (AST, ALT and γGT), adipocytokines (leptin and adiponectin), a marker of inflammation (hsCRP) and the degree of atherosclerosis (mean IMT). p < 0.05 was considered significant.

RESULTS

The clinical characteristics of the subjects are shown in Table 1.

Table 1  Background characteristics of the study subjects

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>94</td>
<td>48</td>
<td>46</td>
</tr>
<tr>
<td>Age (years)</td>
<td>40.1 ± 9.6</td>
<td>42.0 ± 10.2</td>
<td>38.1 ± 8.6</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>63.2 ± 14.5</td>
<td>72.5 ± 12.4</td>
<td>53.4 ± 9.3</td>
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<tr>
<td>BMI (kg/m^2)</td>
<td>22.7 ± 3.9</td>
<td>24.1 ± 3.8</td>
<td>21.1 ± 3.5</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>80.7 ± 10.8</td>
<td>85.5 ± 10.1</td>
<td>75.8 ± 9.2</td>
</tr>
<tr>
<td>Visceral fat (cm^2)</td>
<td>47.4 ± 40.9</td>
<td>70.5 ± 41.4</td>
<td>23.3 ± 22.3</td>
</tr>
<tr>
<td>Subcutaneous fat (cm^2)</td>
<td>136.3 ± 80.8</td>
<td>139.4 ± 90.2</td>
<td>130.0 ± 70.4</td>
</tr>
<tr>
<td>Body fat mass (kg)</td>
<td>15.6 ± 7.4</td>
<td>16.4 ± 8.3</td>
<td>14.8 ± 6.4</td>
</tr>
<tr>
<td>Fat free mass (kg)</td>
<td>47.5 ± 10.2</td>
<td>56.1 ± 5.9</td>
<td>38.6 ± 4.4</td>
</tr>
<tr>
<td>%Body fat (%)</td>
<td>24.3 ± 7.5</td>
<td>21.8 ± 7.0</td>
<td>26.9 ± 7.1</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>119.6 ± 15.2</td>
<td>124.8 ± 12.2</td>
<td>114.1 ± 16.1</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>77.6 ± 13.2</td>
<td>82.5 ± 11.7</td>
<td>72.4 ± 12.8</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>89.8 ± 8.7</td>
<td>93.2 ± 9.2</td>
<td>86.3 ± 6.7</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.9 ± 0.3</td>
<td>4.9 ± 0.3</td>
<td>4.9 ± 0.3</td>
</tr>
<tr>
<td>Fasting insulin (μU/ml)</td>
<td>4.97 ± 2.92</td>
<td>5.69 ± 3.41</td>
<td>4.21 ± 2.06</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.1 ± 0.7</td>
<td>1.3 ± 0.9</td>
<td>0.9 ± 0.5</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>113.5 ± 32.2</td>
<td>120.2 ± 31.4</td>
<td>106.5 ± 31.9</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>65.1 ± 16.8</td>
<td>57.3 ± 13.2</td>
<td>73.2 ± 16.4</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>107.7 ± 84.0</td>
<td>134.7 ± 99.6</td>
<td>79.5 ± 51.4</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>20.6 ± 5.6</td>
<td>22.9 ± 5.7</td>
<td>18.3 ± 4.5</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>19.9 ± 12.4</td>
<td>25.6 ± 14.0</td>
<td>14.0 ± 6.5</td>
</tr>
<tr>
<td>γGT (U/l)</td>
<td>33.5 ± 30.7</td>
<td>45.9 ± 37.4</td>
<td>20.5 ± 12.0</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>5.3 ± 1.3</td>
<td>6.1 ± 1.1</td>
<td>4.5 ± 0.9</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>7.92 ± 6.24</td>
<td>5.46 ± 3.78</td>
<td>10.49 ± 7.23</td>
</tr>
<tr>
<td>Adiponectin (μg/ml)</td>
<td>3.65 ± 2.46</td>
<td>2.28 ± 1.30</td>
<td>5.08 ± 2.58</td>
</tr>
<tr>
<td>hsCRP (ng/ml)</td>
<td>1055.3 ± 4929.1</td>
<td>1564.3 ± 6792.4</td>
<td>524.2 ± 1212.2</td>
</tr>
<tr>
<td>Right mean IMT (mm)</td>
<td>0.50 ± 0.11</td>
<td>0.52 ± 0.12</td>
<td>0.48 ± 0.10</td>
</tr>
<tr>
<td>Left mean IMT (mm)</td>
<td>0.53 ± 0.15</td>
<td>0.56 ± 0.17</td>
<td>0.51 ± 0.11</td>
</tr>
</tbody>
</table>

Data are mean ± SD. BMI, body mass index; BP, blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γGT, γ-glutamyl transpeptidase; hsCRP, high sensitivity C-reactive protein; IMT, intima-media thickness.
The mean age was 40.1 ± 9.6 years (42.0 ± 10.2 for men and 38.1 ± 8.6 for women). The subjects were not obese on average (BMI 22.7 ± 3.9). Mean waist circumference for men was close to the diagnostic criterion for metabolic syndrome in Japan (85 cm), whereas that for women was lower compared with the criterion (90 cm). The mean VFA was 47.4 ± 40.9 cm² (70.5 ± 41.4 for men and 23.3 ± 22.3 for women). 35 men (75.0%) and 46 women (100%) were VFA < 100 cm². The mean body fat mass was 1.6 kg higher but %body fat was 5.1% lower in men than in women, because of higher fat-free mass in men. The subjects were not hypertensive or diabetic. Lipids, uric acid and liver function tests on average were within the normal range.

VFA showed a significantly positive correlation with values of anthropometric measurements except for fat-free mass in men (Table 2). Body weight, BMI, waist circumference and body fat mass showed relatively higher correlation coefficients with VFA in men and women. VFA was also significantly positively associated with systolic and diastolic blood pressure in both sexes.

Next, the relationship of VFA with glucose homeostasis was evaluated. Fasting glucose and HbA1c increased along with VFA accumulation, indicating glucose dysregulation (Table 3). VFA was positively associated with insulin resistance in both men and women with relatively higher correlation coefficients (r > 0.6) for HOMA-IR observed in the whole population and in men. VFA was significantly positively correlated with LDL-C and triglycerides, and negatively correlated with HDL-C, which is a characteristic feature of obesity-related dyslipidemia. VFA was also significantly positively correlated with ALT and γGT in both men and women, and with AST and uric acid in the whole population.

Table 3, Fig. 1 and Fig. 2 show the association of VFA with adipocytokines, inflammation and atherosclerosis. Leptin was significantly positively correlated with VFA when men and women were analyzed separately. Adiponectin showed a correlation coefficient of −0.464 (p < 0.001) with VFA in the whole population, and tended to decrease as VFA increased in men and women, although its level did not reach statistical significance. HsCRP, a marker for inflammation, was positively correlated with VFA in the whole population and in women. Furthermore,
the degree of atherosclerosis, evaluated by IMT, showed a positive association with VFA for the whole population. The relationship between VFA and IMT of both carotid arteries appeared similar, and therefore that of VFA and right IMT is shown in Fig. 2.

**DISCUSSION**

In the present study, we demonstrated in healthy volunteers that the levels of parameters of several lifestyle-related diseases, such as hypertension, diabetes, dyslipidemia, hyperuricemia and fatty liver, show significantly positive correlations with VFA. In addition, we found that subclinical visceral fat accumulation is related to an increase in insulin resistance, alterations in adipocytokines, upregulation of inflammation and progression of atherosclerosis.

Visceral fat accumulation has been shown to cause overt hypertension\(^{10}\), diabetes\(^{11-13}\) and dyslipidemia\(^{14,15}\), which are well known per se as cardiovascular risk factors. It has also been known that the clustering of borderline metabolic abnormalities often occurs and is also associated with potential cardiovascular morbidity and mortality. The concept of MetS has been proposed since around 1990 and is called by various names such as “Syndrome X”\(^{16}\) and “Insulin Resistance Syndrome”\(^{17}\). MetS was first defined in 1999 by the World Health Organization (WHO) and it required evidence of insulin resistance for the diagnosis of MetS\(^{18}\). When MetS is in the pre-atherogenic stage with the clustering of mild metabolic abnormalities, insulin resistance may well play a key role in unifying multiple pathological processes\(^{19,20}\). Among the parameters of glucose homeostasis, we found that HOMA-IR exhibited a higher correlation with VFA than fasting glucose or HbA1c, suggesting that visceral fat accumulation appears to be a deteriorative factor related to insulin resistance. Insulin resistance has complex interactions with oxidative stress and hyperglycemia/diabetes leading to endothelial dysfunction (Fig. 3\(^4,21,22\)).

Another important factor linking visceral adiposity to atherosclerosis is adiponectin\(^{23}\). Unlike most other adipokines, adiponectin levels are reduced in individuals with visceral fat accumulation\(^{24}\), although the mechanism is not yet fully clarified. Our result is basically compatible with this fact. The reason for marginal insignificant \(p\)-values when men and women were analyzed separately may be because the sample size was too small to reach a firm conclusion. The negative association between visceral fat accumulation and adiponectin may be partly mediated by tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), which is secreted by adipose tissue.
after macrophage recruitment and is reported to be a strong inhibitor of adiponectin\(^{25}\). TNF-\(\alpha\) and interleukin-6 (IL-6) are inflammatory cytokines secreted from adipocytes, which subsequently induce CRP production in the liver (Fig. 3\(^{4}\)). Although we did not directly measure the levels of TNF-\(\alpha\) or IL-6, the positive correlation between VFA and hsCRP supports the essential role of inflammation in the pathophysiology of visceral adiposity.

Furthermore, quantitative and qualitative changes in serum lipids synergistically promote the atherosclerotic process.

In conclusion, the present study shows that subclinical visceral fat may contribute to progression of atherosclerosis. (Cited and modified from Ref. 4) Accumulation of visceral fat induces insulin resistance, which causes endothelial dysfunction by increasing oxidative stress and hyperglycemia/diabetes. Visceral fat directly deteriorates endothelial function mainly via adiponectin and tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)). TNF-\(\alpha\) and interleukin-6 (IL-6) are inflammatory cytokines and the main inducers of C-reactive protein (CRP) in the liver. In addition, quantitative and qualitative changes in serum lipids synergistically promote the atherosclerotic process.

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The authors state that they have no Conflict of Interest (COI).

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