Abstract

Objective To clarify the contributions of visceral fat tissue (VAT) mass deposition to the accumulation of risk factors for atherosclerosis in normal weight subjects.

Subjects and Methods We investigated the relationship between VAT accumulation and both risk factors and waist circumference in non-obese subjects. In the 955 normal weight Japanese subjects (782 men and 173 women), visceral fat area (VFA), subcutaneous fat area (SFA) and waist circumference were measured through computed tomography. Total cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, uric acid, fasting blood glucose, and 2-hour postprandial glucose in the oral glucose tolerance test (OGTT) were measured to assess the risk factors for atherosclerosis.

Results In both men and women with normal body weight, VFA was correlated with total cholesterol (r=0.78 and r=0.71, respectively), HDL cholesterol (r=0.89 and r=0.82, respectively), triglyceride (r=0.72 and r=0.91, respectively), uric acid (r=0.71 and r=0.69, respectively), fasting blood glucose (r=0.84 and r=0.82, respectively), OGTT 2-hour postprandial glucose (r=0.75 and r=0.50, respectively), and systolic (r=0.93 and r=0.73, respectively) and diastolic (r=0.91 and r=0.73, respectively) blood pressure. The average number of risk factors was also correlated with VFA both in men (r=0.88, p=0.005) and women (r=0.91, p=0.003) with normal body weight. Based on the results of the multiple regression analyses, among VFA, SFA, body mass index (BMI) and age, VFA was considered to be the most important predictor for explaining the average number of risk factors. We also demonstrated that VFA was correlated with the waist circumference in normal weight men (r=0.74) and women (r=0.59). In normal weight subjects, the waist circumference equivalent to more than 1.0 of the average number of risk factors was 81 cm in men and 89 cm in women.

Conclusion VAT accumulation itself plays a role as a risk factor for atherosclerosis irrespective of BMI, and measurement of VFA may be more important than that of BMI for predicting the presence of risk factors in normal weight subjects. Measurement of waist circumference also might be important for assessing the possibility of VAT accumulation leading to atherosclerosis.

Key words: visceral fat, risk factor for atherosclerosis, non-obese Japanese

Introduction

Several studies have shown that obesity is a risk factor for type 2 diabetes, hypertension, dyslipidemia and hyperuricemia, which lead to atherosclerotic disorders such as cardiovascular disease (1). Particularly, visceral fat obesity, known to be caused by excess accumulation of visceral adipose tissue (VAT), has been found to be related to the appearance of atherosclerotic-related disorders including dyslipidemia, hypertension, impaired glucose tolerance and hyperuricemia (2). The disproportionate accumulation of VAT can cause metabolic and cardiovascular diseases more frequently than the excess accumulation of subcutaneous adipose tissue (3). For the analysis of VAT, visceral fat area (VFA) measured through computed tomography (CT) is most popularly used (4).
ported to be a risk factor for atherosclerosis, irrespective of body weight (4–6). However, in these studies, the subjects consisted of a small number of men and women, or only men or women. There have been few reports in which the relationship between VAT accumulation in normal weight subjects and risk factors for atherosclerosis were precisely investigated in both genders. Furthermore, few reports are available which determine the cut-off points of VFA and waist circumference for atherosclerosis in non-obese subjects.

In the present cross-sectional study, we investigated the relationship between VAT accumulation and the risk factors for atherosclerosis such as dyslipidemia, hypertension, impaired glucose tolerance and hyperuricemia in normal weight Japanese subjects. We also investigated the relationship between VAT accumulation and the waist circumference to explore the cut-off points of waist circumference for atherosclerosis in non-obese Japanese subjects.

Subjects and Methods

We reviewed data of normal body mass index (BMI) [BMI of less than 25 (kg/m²)] in 955 middle-aged Japanese subjects [782 men and 173 women at the age of 49.8±6.7 and 48.9±5.8 years, respectively (mean±SD)]. All subjects were from a general population who were undergoing a yearly health screening or participating in a precise health check-up program performed in our hospital, and also desired a VFA measurement by CT. Subjects who had any history of endocrinological or metabolic disease or who were regularly taking any drugs were excluded in advance. The health screening included a physical check-up, routine collection of biochemical data, a 75-g oral glucose tolerance test (OGTT), and a CT evaluation of body-fat distribution. All subjects showed normal body mass index (BMI) [BMI of less than 25 (kg/m²)] according to the criteria of the Japan Society for the Study of Obesity (7), had no history of endocrinological or metabolic disease, and were not regularly taking any drugs. Body weight in light clothes was measured by 0.1 kg and height by 0.1 cm. BMI was calculated as weight (kg) divided by the square of height (m²). Morning blood pressure was recorded using a mercury sphygmomanometer in the sitting position.

Abdominal fat distribution was examined at the umbilicus level in the spine position using CT (Toshiba X Vision scanner, Tokyo, Japan), according to the previously described procedure (8). VFA, subcutaneous fat area (SFA), total fat area (TFA) and waist circumference were measured using a commercial software package (Fat Scan, N2 System, Osaka, Japan). VFA, SFA and TFA were calculated as follows. A region of interest of the subcutaneous fat was defined by tracing its contour on each scan, and the attenuation range of CT numbers for the fat tissue was calculated and expressed as the mean±2SD. Total and intra-peritoneal tissues with attenuation within the mean±2SD were considered to be TFA and VFA, respectively. SFA was calculated as TFA minus VFA.

To measure serum total cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, uric acid and glucose levels, blood samples were obtained at 9 : 00 AM after an overnight fast. Subsequently, all subjects underwent the standard oral glucose tolerance test (OGTT) proposed by WHO (9) between 9 : 00 AM and 11 : 00 AM. Serum total cholesterol and triglyceride levels were measured by enzymatic methods. HDL cholesterol, uric acid, and glucose levels in serum were measured by the direct method using a proprietary detergent, the uricase method, and the hexokinase method, respectively. This study was approved by the ethical committee of NTT West Kyoto Hospital.

The average number of risk factors for atherosclerosis

We regarded glucose impairment, dyslipidemia, hyperuricemia and hypertension as the risk factors for atherosclerosis (7). Dyslipidemia was defined by a total cholesterol level of not less than 220 mg/dl, a triglyceride level of not less than 150 mg/dl, and/or an HDL cholesterol level of less than 40 mg/dl. Glucose impairment was defined by a fasting glucose level of not less than 126 mg/dl and/or a 2-h postprandial glucose level in OGTT of not less than 200 mg/dl, and hyperuricemia by a uric acid level of not less than 7 mg/dl. Hypertension was defined by systolic blood pressure of not less than 140 mmHg and/or diastolic blood pressure of not less than 90 mmHg. In consideration of inter-observer variation, the levels of VFA were grouped by every 10 cm² and the average number of risk factors in each group was calculated (8).

Statistical analysis

Data were expressed as means±SEM. Correlations were assessed using Spearman’s rank correlation test. Multiple regression analyses were performed using a model in which the dependent variable was the average number of risk factors for atherosclerosis, with the following explanatory variables: VFA, SFA, BMI and age. All variables were entered into the analysis simultaneously. Analyses of covariance (ANCOVA) were performed to examine the differences between the ratio of waist circumference to VFA in men and that in women. P values of less than 0.05 were considered to be significant. The statistical software SPSS for Windows (Version 8.0) was used for the analyses.

Results

The baseline characteristics for all subjects are shown in Table 1. In both men and women, BMI showed almost normal distribution with a mode value in the range of 22.5 kg/m² in men, and 21.3 kg/m² in women.

The VFA values in men showed almost a normal distribution with a mode value in the range of 60–100 cm², while those in women showed a peak at a lower portion of the distribution with a mode value in the range of 20–40 cm².

Figure 1 shows the relationship between VFA and meta-
In both men and women with normal weight, VFA was positively correlated with the average levels of total cholesterol (r=0.78, p=0.005; r=0.71, p=0.026, respectively), triglyceride (r=0.72, p=0.005; r=0.91, p=0.003, respectively), uric acid (r=0.71, p=0.001; r=0.69, p=0.022, respectively), fasting glucose (r=0.84, p=0.0005; r=0.82, p=0.007, respectively) and OGTT 2-h postprandial glucose (r=0.75, p=0.001; r=0.50, p=0.04, respectively), and was inversely correlated with HDL cholesterol (r=–0.89, p=0.001; r=–0.82, p=0.006, respectively). VFA in men and women was also positively correlated with the average levels of systolic (r=0.93, p=0.001; r=0.73, p=0.01, respectively) and diastolic (r=0.91, p=0.0002; r=0.67, p=0.001, respectively) blood pressure. Total cholesterol, triglyceride, and fasting glucose levels tended to exceed the upper limit of normal ranges (220 mg/dl, 150 mg/dl, and 110 mg/dl, respectively) as VFA increased.

Meanwhile, in both normal weight men and women, BMI and the level of each metabolic or circulatory parameter was slightly or not correlated (Total cholesterol r=0.07, p=0.048; r=0.16, p=0.038, respectively: triglyceride r=0.11 p=0.003; r=0.26, p=0.001, respectively: uric acid r=0.08, p=0.020; r=0.19, p=0.011, respectively: fasting glucose r=0.07, p=0.051; r=0.16, p=0.034, respectively: OGTT 2-h postprandial glucose r=0.11, p=0.048; r=0.08, p=0.059, respectively: HDL cholesterol r=–0.26, p<0.0001; r=–0.18, p=0.018, respectively: systolic blood pressure r=0.19, p<0.0001; r=0.23, p=0.024, respectively: diastolic blood pressure r=0.21, p<0.0001; r=0.20, p=0.009, respectively).

Figure 2 shows that VFA was correlated with the average number of risk factors for atherosclerosis, in both men (r=0.88 p=0.005) and women (r=0.91 p=0.003). The VFA that was equivalent to 1.0 of the average number of risk factors on the regression line was about 68 cm$^2$ in men and 59 cm$^2$ in women.

Multiple regression analyses were performed to determine the variables among VFA, SFA, BMI and age contributing to the average number of risk factors (Table 2). In both men and women with normal weight, the regression procedure indicated VFA as the most important predictive variable (r=0.28, p<0.0001; r=0.35, p<0.0001, respectively) for explaining the average number of risk factors for atherosclerosis, while BMI had a slight correlation to the number of risk factors (r=−0.14, p=0.78; r=−0.03, p=0.79, respectively).

Figure 3 shows the relationship between VFA and waist circumference calculated through CT. In both men and women, VFA was closely correlated with waist circumference (r=0.74, p<0.0001; r=0.59, p<0.0001, respectively). VFA in men was more strongly correlated with waist circumference than that in women, and men had a VFA about 2.2 times larger than that of women with the same waist circumference. ANCOVA was conducted using waist circumference as a covariate, and revealed significant gender differences in VFA (p<0.001). Calculated from the regression line, the waist circumferences which were equivalent to 68 cm$^2$ and 59 cm$^2$ of the VFA in men and women, respectively (equivalent to 1.0 of the average number of risk factors, calculated in Fig. 2), were about 81 cm and 89 cm, respectively.

**Discussion**

In this study, we demonstrated that, in both men and women with normal body weight, the levels of measured parameters of risk factors for atherosclerosis and the average number of risk factors were highly correlated with VFA, compared with BMI. To our knowledge, this is the first study to investigate the precise relationship between VFA and the risk factors for atherosclerosis in both non-obese men and women. Using multiple regression analyses, among VFA, SFA, BMI and age, VFA was the most predictive variable for explaining the number of risk factors. We also demonstrated that VFA was correlated with the waist circumference.
Figure 1. Correlation between VAT accumulation and the average values of risk factors for atherosclerosis in non-obese subjects. Closed circles, men; open circles, women. The broken line represents the upper limits (except lower limits in HDL cholesterol) of the normal range in each parameter.
in both men and women with normal body weight. VFA in men was more strongly correlated with waist circumference than that in women, and men had a VFA about 2.2 times larger than that of women with the same waist circumference. Furthermore, we demonstrated that the waist circumferences in non-obese Japanese subjects, which were equivalent to more than 1.0 of the average number of risk factors, were 81 cm in men and 89 cm in women.

Recent studies have revealed that inappropriate VAT accumulation itself may cause the multiple risk factor syndrome, irrespective of BMI (4, 10, 11). The concept of the multiple risk factor syndrome has been referred to as syndrome X (12), the deadly quartet (13), metabolic syndrome (14) and visceral fat syndrome (10). The mechanisms responsible for these metabolic disorders are not fully understood, but some investigators have reported that insulin resistance due to inappropriate accumulation of VAT may in part cause these metabolic risks in both humans (15–18) and animals (19). One of the mechanisms of insulin resistance by VAT accumulation may be due to an overload of liver free fatty acids (FFA) produced by the high lipolytic activity of VAT. Overexposure of hepatic and extrahepatic tissues to FFA leads to the promotion of aberrations in insulin action and dynamics, and may cause insulin resistance (4, 20).

The other possible mechanism responsible for insulin resistance related to VAT is the contribution of bioactive substances (adipocytokine) such as tumor necrotizing factor α (TNF-α), adiponectin, and resistin. Previous reports have demonstrated that TNF-α levels are essentially dependent on VAT amount (21) and inversely correlated with insulin sensitivity in obese type 2 diabetic patients (22). Adiponectin, which may increase insulin sensitivity, decreases as VAT increases (23), and hypoadiponectinemia in patients with VAT accumulation may be closely related to insulin resistance and hyperinsulinemia (24). Resistin may also contribute to insulin resistance and its expression in human VAT enhances the expression in abdominal fat (25). The humoral regulators of adipocytokine and neuroendocrine perturbations may play a crucial role in insulin resistance, which leads to atherosclerosis.

We also demonstrated that VFA was an important predictor for explaining the number of risk factors for atherosclerosis in subjects with a normal weight, while BMI had a slight correlation to the number of risk factors. Taken together, these results suggested that, in normal weight sub-

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**Table 2. Multiple Regression Analyses**

<table>
<thead>
<tr>
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<th>Standardized partial regression coefficient</th>
<th>P value</th>
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<tbody>
<tr>
<td><strong>Men</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAT</td>
<td>0.28</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SAT</td>
<td>0.02</td>
<td>0.75</td>
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<tr>
<td>BMI</td>
<td>–0.14</td>
<td>0.78</td>
</tr>
<tr>
<td>Age</td>
<td>0.11</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAT</td>
<td>0.35</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SAT</td>
<td>0.1</td>
<td>0.35</td>
</tr>
<tr>
<td>BMI</td>
<td>–0.03</td>
<td>0.79</td>
</tr>
<tr>
<td>Age</td>
<td>0.23</td>
<td>0.01</td>
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Explanatory variables: VAT, SAT, BMI and age. Dependent variables: The average number of risk factors for atherosclerosis (risk factors: glucose impairment, dyslipidemia, hyperuricemia and hypertension).
objects, measurement of VAT is more important than that of BMI for predicting the presence of risk factors for atherosclerosis. Therefore, lifestyles that may develop VAT, such as taking a high-sucrose diet (26) and alcohol (27), psychological stress (27), decreased exercise (28), and smoking (29), should be avoided even in subjects with normal weight.

Though VFA was the most predictive variable in total for explaining the number of risk factors in the multiple regression analyses, it was less predictive in women than in men. It was speculated that variables that were not investigated in the present analysis such as physical activity and diet might also contribute to the risk factors. Further study is needed to clarify these confounding factors.

In the present study, it was shown that VAT was closely correlated with waist circumference in normal weight Japanese subjects, and that waist circumferences which were equivalent to more than 1.0 of the average number of risk factors for atherosclerosis were over 81 cm in men and over 89 cm in women. Previous studies have indicated a close relationship between a larger waist circumference and deposition of VAT or cardiovascular risks in obesity, and suggested that subjects with a waist circumference which is over approximately 94–102 cm in Western men and 80–100 cm in Western women (not limited to normal weight subjects) have a likelihood of developing atherosclerotic metabolic disturbances (30–32). In addition, the WHO has reported that Asians with a waist circumference of not less than 90 cm in men and not less than 80 cm in women are at high risk (33). The reasons why the cut-off points obtained in this study were different from those described in previous reports might include racial differences in body shape, differences in study population (subjects were limited to those with normal weight in our study), and the measurement method of waist circumference (measurement was made using CT in our study).

In the present study, non-obese Japanese men had a VFA 2.2 times larger than that of non-obese Japanese women with the same waist circumference. It has been reported that the VFA in obese men is 2.6 times larger than that in obese women (34). Based on these results, measurement of the waist circumference in men with normal body weight might be more important than that in women for assessing the accumulation of VAT. Measurement of the waist circumference is a useful and convenient method (35), though the anthropometric measurements comprise both abdominal subcutaneous fat and intra-abdominal visceral fat. When there is insufficient time to measure waist circumference at a medical examination, asking for the size of trousers or skirt of the subjects or patients may be useful for exploring the possibility of VAT accumulation.
In conclusion, in normal weight Japanese subjects, the levels of measured parameters of the risk factors for atherosclerosis and the average number of risk factors were correlated with VAT accumulation. Measurement of VFA was more important than that of BMI for predicting the presence of risk factors in normal weight subjects. VFA was correlated with waist circumference in non-obese Japanese. Therefore, measurement of waist circumference in normal weight subjects, especially in men, might be important for assessing the possibility of VAT accumulation leading to atherosclerosis.

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References