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Characteristics of sleep-disordered breathing in Japanese patients with type 2 diabetes mellitus

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Abstract

Sleep-disordered breathing (SDB), especially sleep apnea-hypopnea syndrome, is often observed in patients with type 2 diabetes mellitus; but there are only a few studies on SDB in Japanese diabetic subjects. We investigated the prevalence of SDB in diabetic patients; associations between severity of sleep apnea (SA) and clinical factors, visceral fat, and adiponectin; and associations between type of SA and clinical factors. In the present study, 40 Japanese diabetic patients underwent overnight cardiorespiratory monitoring, and night and morning measurements of serum adiponectin concentrations. Sleep apnea was detected in Japanese diabetic patients at a high prevalence (77.5%). The following variables were associated with SDB: age, body mass index, estimated visceral fat area, and nocturnal reduction in serum adiponectin concentrations. The prevalence of central sleep apnea (CSA, ≥5/h) was 32.3% among diabetic SDB patients. Diabetic SDB patients with CSA had higher hemoglobin, increased intima-media thickness, and higher plasma brain natriuretic peptide levels than those without CSA (<5/h). In conclusion, our study demonstrated a high prevalence of SDB in Japanese diabetic patients, which correlated with visceral fat area and adiponectin. A high frequency of CSA was noted in diabetic SDB patients, together with high hemoglobin, high brain natriuretic peptide, and increased intima-media thickness. The present results of prevalence of SDB may be relevant to the higher incidence of cardiovascular disease in diabetic patients, which need to be clarified in future studies.

1. Introduction

Obstructive sleep apnea-hypopnea syndrome (OSAHS) is the most common form of sleep-disordered breathing (SDB) and is known to be potentially associated with atherosclerosis and cardiovascular disease (CVD) [1-3]. The presence and severity of sleep apnea-hypopnea syndrome (SAS) are defined by the apnea-hypopnea index (AHI), in conjunction with symptoms such as excessive daytime sleepiness [4]. Previous studies demonstrated the possible association between visceral obesity and SAS [5,6], and recent studies reported that obese subjects with SAS also have low serum adiponectin concentrations [7-9].

More recent research demonstrated the likelihood of a relationship between SDB and type 2 diabetes mellitus (T2DM) [10]. In February 2007, the International Diabetes Federation Taskforce on Epidemiology and Prevention stated that the pathophysiologic stress imposed by hypoxemia and sleep fragmentation might be involved in the pathogenesis of insulin resistance and pancreatic β-cell dysfunction through various biological mechanisms, such as direct effects of hypoxemia, sympathetic nervous system activation, systemic inflammation, hypothalamic-pituitary-adrenal dysfunction, dysregulation of adipocytokines, sleep architecture, and other factors [10-14]. Both SAS/SDB and T2DM are strongly associated with CVD [5]. On the other hand, several studies have demonstrated high prevalence of SDB in diabetic subjects [15-20]. The reported prevalence of sleep apnea in American adults with T2DM is 72.4% (AHI levels of ≥5 events per hour) [20]. However, the frequency

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and characteristics of SDB in Japanese patients with T2DM have not been well clarified. We investigated the incidence of SDB in Japanese patients with T2DM to assess the prevalence of SDB and elucidate the clinical variables associated with SDB.

2. Subjects and methods

2.1. Subjects

We enrolled 40 Japanese subjects with T2DM who had been hospitalized at the Department of Endocrinology and Metabolic diseases of Osaka University Hospital because of poor glycemic control and/or the staging of complications during the period from February 2006 to March 2007 (31 men and 9 women; age, 58.7 ± 2.1 years [mean ± SEM]; range, 29-78 years). *Type 2 diabetes mellitus* was defined according to the World Health Organization criteria and/or treatment of diabetes mellitus. The present study was approved by the ethics committee of Osaka University, and a written informed consent was obtained from each participant.

2.2. Cardiorespiratory monitoring

Each participant underwent overnight cardiorespiratory monitoring (Somté; Compumedics, Melbourne, Australia). The recorded signals were analyzed for the number of apneas and hypopneas during sleep. The oxygen desaturation index (ODI), lowest oxygen saturation, baseline oxygen saturation, and time at desaturation less than 90% in minutes of total bedtime for the entire night were measured. *Apnea* was defined if the amplitude of the airflow or respiratory band data decreased to less than 30% of baseline amplitude for at least 10 seconds. *Hypopnea* was defined if airflow or respiratory effort decreased to less than 70% of baseline for at least 10 seconds associated with greater than 4% desaturation but did not meet the criteria for an apnea. *Apnea-hypopnea index* was defined as the total number of apneas/hypopneas per hour of sleep time [4]. Sleep apnea (SA) was categorized into obstructive (OSA), central (CSA), and mixed SA [21-23]. Obstructive sleep apnea represented absence of airflow for at least 10 seconds but presence of thoracoabdominal movement (the amplitude of thoracic or abdominal band data remained at least 15% of baseline amplitude). Central sleep apnea represented lack of airflow for at least 10 seconds without thoracoabdominal movement [4] (the amplitude of thoracic or abdominal band data decreased <15% of baseline amplitude). Mixed apnea represented lack of airflow for at least 10 seconds with an initial central component followed by obstructive component [24]. There is no consensus on the classification of hypopnea into CSA or OSA component; and thus, hypopneas were classified into obstructive apnea. In the present study, we defined subjects who had obstructive sleep apnea-hypopnea index (OSAHI) of at least 5 and central sleep apnea index (CSAI) less than 5 as obstructive dominant, OSAHI less than 5 and CSAI at least 5 as central dominant, and OSAHI at least 5 and CSAI at least 5 as mixed type. All recordings were scored manually by an experienced polysomnographic technologist [25], and the duration of sleep was estimated using the self-reported sleep time and the recording data.

2.3. Anthropometry and laboratory measurements

Height, weight, and waist circumference were measured in standing position. Waist circumference at the umbilical level was measured with a nonstretchable tape in late expiration while standing (in centimeters). Visceral fat area (VFA) was estimated by bioelectrical impedance analysis (BIA) [26]. *Hypertension* was defined as systolic blood pressure of at least 140 mm Hg, diastolic blood pressure of at least 90 mm Hg, or treatment of hypertension. Subjects with a previous diagnosis of dyslipidemia and hypertension who were on medications for any of these conditions were included in this study. *Dyslipidemia* was defined as a low-density lipoprotein cholesterol concentration greater than 140 mg/dL, triglyceride concentration greater than 150 mg/dL, high-density lipoprotein cholesterol concentration less than 40 mg/dL, and/or treatment of dyslipidemia. *Metabolic syndrome* was defined based on the published criteria of the metabolic syndrome for the Japanese population [27]. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: HOMA-IR (microunits per milliliter × milligrams per deciliter) = (fasting immunoreactive insulin × fasting glucose)/405. Diabetic retinopathy was assessed by an ophthalmologist. Subjects with overt diabetic nephropathy represented those with urinary albumin greater than 300 mg/d. Diabetic neuropathy represented the presence of 2 of the following 3 clinical findings: subjective symptoms (pain, numbness, itchiness, coldness, warmness, and weakness), absence of deep tendon reflex, and loss of vibration. Subjects on medications known to increase serum adiponectin levels, such as pioglitazone [28], and subjects with renal dysfunction (creatinine >1.5 mg/dL) were excluded from this study because renal dysfunction is reported to alter serum adiponectin level [29].

Venous blood samples were collected for measurements of hemoglobin, hematocrit, creatinine, hemoglobin A_{1c} (HbA_{1c}), high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, and brain natriuretic peptide (BNP) after awakening while the subject was in the supine position. For the purpose of the present study, serum samples that were obtained from each patient and stored at −20°C were thawed and assayed for adiponectin levels using sandwich enzyme-linked immunosorbent assay (Otsuka, Tokushima, Japan) [7,30]. In 15 (48.4%) of the 31 subjects with both SDB and T2DM, serum adiponectin concentrations were measured before sleep (at 8:00 PM) and after awakening (at 7:00 AM).

Two-dimensional and Doppler echocardiography (SSA-660A; Toshiba, Tokyo, Japan) was performed from standard parasternal views in the resting state, in left lateral position.

This provided left ventricular ejection fraction (LVEF). Maximum carotid intima-media thickness (IMT) and mean IMT of the common carotid artery were also measured in supine position (SSA-660A, Toshiba). Maximum IMT was measured on both the right and left sides in the observation-possible areas of the common carotid artery, bulbus, and internal carotid artery, except the external carotid artery. The mean IMT represented the average of the values of the right and left common carotid arteries, but not the bulbus, determined at 3 points of measurements. The ankle-brachial index (ABI) and the carotid-femoral pulse wave velocity (PWV) were analyzed with a noninvasive autonomic device (model BP-203RPE; Nihon Colin, Tokyo, Japan). To evaluate diabetic autonomic neuropathy, electrocardiogram data were recorded after at least 15 minutes of supine resting during wakefulness; and 100 consecutive R-R intervals were analyzed. The average value (M) of an R-R interval and the standard deviation were determined from this analysis, and coefficient of variation (CVR-R) was computed using the following formula: CVR-R (percentage) = standard deviation/M × 100. However, atrial fibrillation rhythm was not measured.

2.4. Statistical analysis

Data are presented as mean±SEM and compared by 1-way or 2-way analysis of variance with Fisher protected least significant difference test for multiple-group analysis, unpaired Student t test, or Mann-Whitney U test for data with only 2 groups. The frequencies of each group were compared by the χ^2 test. Relationships between 2 continuous variables were analyzed using scatter plots and Pearson correlation coefficients. In all cases, 2-tailed P values were used; and P values < .05 were considered statistically significant. All analyses were performed with the StatView software version 5.0 (HULINKS, Tokyo, Japan).

3. Results

3.1. Characteristics of T2DM subjects enrolled in the present study

Table 1 summarizes the characteristics of the subjects enrolled in this study. Of the 40 patients with T2DM studied, 9 were found to have no SA. The prevalence of SA in subjects with T2DM was 77.5% (Fig. 1). The values of AHI, 4% ODI, and the percentage of time spent with arterial O_2 saturation recorded by a pulse oximeter (SpO_2) at less than 90% were significantly higher and the lowest SpO_2 were significantly lower in T2DM patients with SDB than those with DM but without SDB. The T2DM patients with SDB were older and had higher body mass index (BMI), larger waist circumference for men, larger cardiothoracic ratio (CTR), higher HOMA-IR, and lower baseline SpO_2 than SAS patients without T2DM. There was a significant correlation between AHI and VFA measured by BIA in all subjects (n = 21), in men (n = 13), and in women (n = 8) (r = 0.80, P < .001; r = 0.79, P < .001; and r = 0.85, P < .01, respectively; Fig. 2). There was no significant difference in the use of each antidiabetic therapy between diabetic patients with and without SDB.

Table 1
Characteristics of T2DM subjects without or with SDB

	DM without SDB (n = 9)	DM with SDB (n = 31)	P value
Age, y	49.8 ± 4.2	61.2 ± 2.2	<.05
Sex (male/female)	6/3	21/10	NS
BMI, kg/m^2	23.9 ± 1.6	29.6 ± 1.4	<.05
Waist, cm (male)	83.8 ± 4.4	100.2 ± 3.7	<.05
Waist, cm (female)	93.7 ± 9.4	101.9 ± 5.7	NS
Current smoking	4	8	NS
CTR, %	44.0 ± 1.8 (n = 8)	51.3 ± 1.0 (n = 28)	<.01
Hb, g/dL	14.5 ± 0.6	14.0 ± 0.4	NS
Ht, %	42.1 ± 1.8	41.6 ± 1.7	NS
Creatinine, mg/dL	0.76 ± 0.08	1.26 ± 0.25	NS
Duration of DM, y	11 ± 3	15 ± 2	NS
HbA_{1c}, %	8.4 ± 0.7	8.1 ± 0.3	NS
HOMA-IR, U	1.5 ± 0.4 (n = 4)	3.6 ± 0.5 (n = 15)	<.05
DM neuropathy	3 (33%)	18 (62%)	NS
DM retinopathy	1 (11%)	9 (33%)	NS
DM nephropathy	2 (22%)	14 (43%)	NS
Mets	4	22	NS
Hypertension	4	19	NS
Dyslipidemia	4	12	NS
History			
Coronary artery diseases	1	5	NS
Cerebrovascular diseases	0	2	NS
LVEF, %	68.2 ± 3.0 (n = 7)	65.5 ± 1.5 (n = 27)	NS
Mean IMT, mm	0.95 ± 0.12 (n = 6)	0.98 ± 0.05 (n = 25)	NS
Max IMT, mm	1.48 ± 0.12 (n = 6)	1.82 ± 0.17 (n = 23)	NS
ABI	1.16 ± 0.03 (n = 8)	1.14 ± 0.02 (n = 30)	NS
PWV, cm/s	1337 ± 78 (n = 8)	1690 ± 77 (n = 30)	<.05
CVR-R, %	3.7 ± 0.6 (n = 7)	2.6 ± 0.4 (n = 24)	NS
AHI, events/h	2.4 ± 0.49	30.3 ± 3.9	<.01
Baseline SpO_2, %	97.2 ± 0.3	95.0 ± 0.4	<.05
Lowest SpO_2, %	90.3 ± 1.5	78.6 ± 2.1	<.05
4% ODI, events/h	1.6 ± 0.7	21.4 ± 4.9	<.01
% <90% time	0.05 ± 0.04	9.97 ± 3.21	<.01
Current medication (SU/BG/αGI/insulin)	1/1/2/8	4/3/2/20	NS

Mean ± SEM. Numbers of available data are shown in each parenthesis. Ht indicates hematocrit; Mets, metabolic syndrome; 90% <time, time at desaturation less than 90 % in minutes of total bedtime; SU, sulfonylurea; BG, biguanide; αGi; α-glucosidase inhibitor; NS, not significant.

3.2. Nocturnal changes in adiponectin concentrations in diabetic patients with SDB and without SDB

Next, we focused on the nocturnal changes in serum adiponectin concentrations in diabetic patients, as reported previously [7], by measuring night and early morning levels in 24 such patients. Individual data are shown in Fig. 3A. The percentage change in serum adiponectin level (Δadiponectin = [serum adiponectin concentrations after awakening − before sleep]/before sleep [percentage]) in diabetic SDB

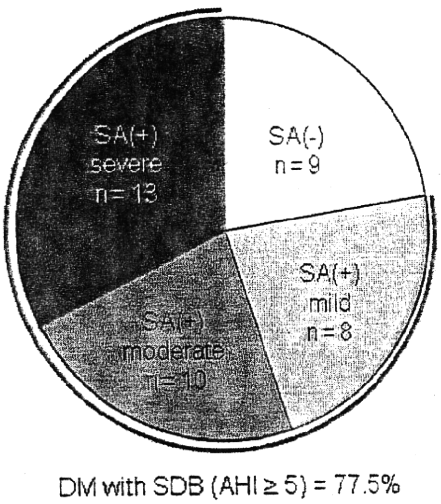

Fig. 1. Prevalence of SA in patients with T2DM. The diagnosis of SA was based on AHI of at least 5 and classified as mild AHI (≥5 to <15), moderate AHI (≥15 to <30), or severe AHI (≥30) according to the guidelines of the American Academy of Sleep Medicine Task Force [4].

patients (n = 9) was −3.4% ± 1.8%, whereas in diabetic non-SDB patients (n = 15), it was 4.7% ± 2.6% ($P < .05$); the difference between the 2 groups was significant (Fig. 3B). In diabetic SDB patients, the aforementioned reduction in serum adiponectin concentration was noted even in patients with mild SA (Fig. 3C).

3.3. Distribution of SA types and characteristics of diabetic patients with SDB

Next, we investigated the frequency of various types of SA in diabetic SDB patients. The incidence of CSA (ie, central dominant [n = 7] and mixed [n = 3]) was 32.3% in diabetic patients. The clinical characteristics of diabetic CSA less than 5 and CSA at least 5 patients are shown in

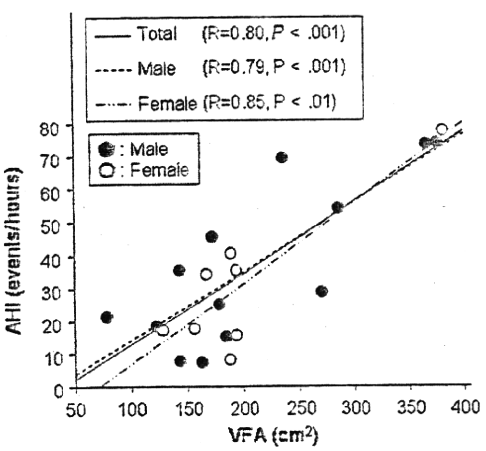

Fig. 2. Relationship between VFA and AHI in 21 patients. Visceral fat area was estimated by BIA [26].

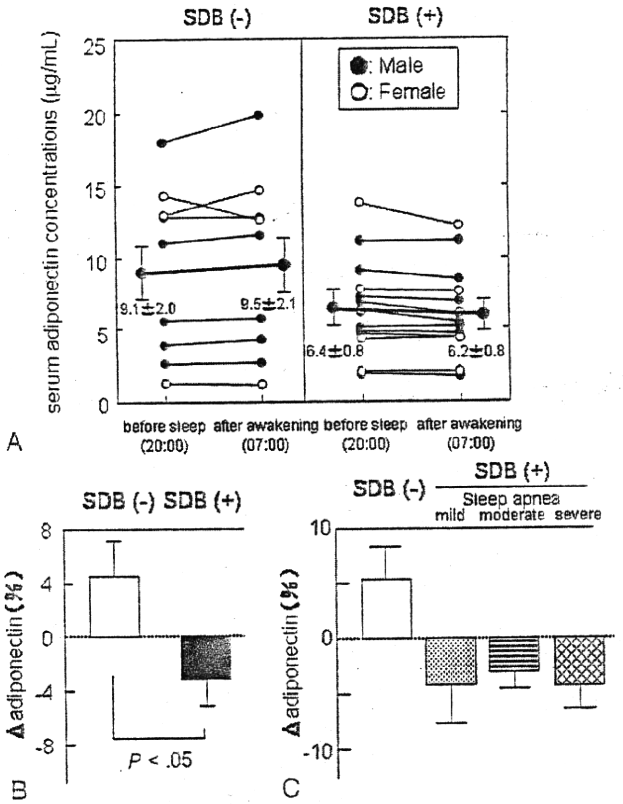

Fig. 3. Serum adiponectin concentrations before sleep and after awakening in T2DM patients without SDB (n = 9) and with SDB (n = 15). A, Serum adiponectin concentrations quantified by enzyme-linked immunosorbent assay. B, ΔAdiponectin in diabetic patients without SDB and with SDB, and in 3 classes of SA severity (C). Data are mean ± SEM.

Table 2. The CSA at least 5 group had higher hemoglobin, hematocrit, and mean IMT compared with the CSA less than 5 group. No difference was found in other variables between the 2 groups. There was no significant difference in Δadiponectin between CSA at least 5 group (n = 9) and CSA less than 5 group (n = 4) (Table 2), although the CSA at least 5 group tended to show a decrease in the morning level, although it was not statistically significant. Plasma BNP concentrations were significantly higher in the CSA at least 5 group than in the CSA less than 5 group (Table 2, $P < .05$).

4. Discussion

The major findings of the present study were as follows: (1) there was a high prevalence of SDB in Japanese T2DM patients (77.5%), consistent with previous studies [20]; (2) diabetic SDB patients were significantly older and more obese than non-SDB diabetic patients; (3) visceral fat accumulation correlated with the severity of SA in diabetic patients; (4) there were nocturnal falls in serum adiponectin concentrations in diabetic SDB patients, similar to our previous report in patients with severe OSAHS [7]; and

Table 2
Characteristic of diabetic SDB subjects without or with CSA

	CSA (<5) (n =21)	CSA (≥5) (n =10)	Univariate *P* value
Age, y	60.0 ± 2.9	63.8 ± 3.1	NS
Sex (male/female)	12/9	9/1	NS
BMI, kg/m^2	28.9 ± 1.6	31.0 ± 2.6	NS
Waist, cm (male)	94.0 ± 4.0	105.9 ± 6.4	NS
Waist, cm (female)	100.3 ± 2.3	77 (n = 1)	–
Current smoking	3	5	NS
CTR, %	51.5 ± 1.2 (n = 21)	49.4 ± 1.7 (n = 8)	NS
Hb, g/dL	13.1 ± 0.4	15.8 ± 0.4	<.01
Ht, %	39.3 ± 1.3	47.0 ± 1.3	<.01
Creatinine, mg/dL	1.35 ± 0.36	1.02 ± 0.11	NS
Duration of DM, y	16 ± 3	15 ± 3	NS
HbA_{1c}, %	8.1 ± 0.5	8.2 ± 0.5	NS
HOMA-IR, U	2.8 ± 0.5 (n = 10)	4.8 ± 1.3 (n = 5)	NS
DM neuropathy	13 (62%)	8 (80%)	NS
DM retinopathy	7 (33%)	3 (30%)	NS
DM nephropathy	9 (43%)	7 (70%)	NS
Mets	14	7	NS
Hypertension	12	7	NS
Dyslipidemia	10	2	NS
History			
CAD	3	2	NS
Cerebrovascular disease	1	1	NS
LVEF, %	65 ± 2 (n = 18)	65 ± 3 (n = 9)	NS
Mean IMT, mm	0.92 ± 0.05 (n = 17)	1.11 ± 0.06 (n = 8)	<.05
Max IMT, mm	1.62 ± 0.21 (n = 15)	2.19 ± 0.23 (n = 8)	NS
ABI	1.15 ± 0.02	1.13 ± 0.03	NS
PWV, cm/s	1654 ± 107	1787 ± 109	NS
CVR-R, %	2.7 ± 0.4 (n = 17)	2.3 ± 0.3 (n = 7)	NS
AHI, events/h	25.3 ± 4.6	40.8 ± 6.3	NS
Baseline SpO_2, %	95 ± 1	95 ± 1	NS
Lowest SpO_2, %	79 ± 3	78 ± 3	NS
4% ODI, events/h	19 ± 6	24 ± 7	NS
% <90% time	10.3 ± 3.9	8.3 ± 5.3	NS
ΔAdiponectin	8.8 ± 1.9 (n = 9)	5.0 ± 1.1 (n = 4)	NS
BNP	22.2 ± 8.1 (n = 5)	77.7 ± 0.7 (n = 7)	<.05

Mean ± SEM. Numbers of available data are shown in each parenthesis. CAD indicates coronary artery disease.

(5) the CSA phenotype was associated with higher hemoglobin, thickened arterial wall, and elevated BNP in diabetic subjects.

Katsumata et al [15] reported high incidence of SAS in male diabetic Japanese subjects. To our knowledge, the present study is the first to assess the prevalence of SDB in male and female Japanese patients with T2DM. As reported previously in American subjects [20], such patients were also older and more obese, although the BMI of Japanese diabetic SDB patients was lower than that of the American subjects. Previous studies demonstrated the possible association between visceral fat accumulation and severity of SA [5,6]. The present study also suggested a link between visceral fat accumulation and severity of SA in Japanese diabetic patients (Fig. 2). Taken together, visceral fat accumulation, SDB, and T2DM may form a pathologic complex associated with high risk for CVD [31-33]. Measurement of not only BMI but also VFA, using bioelectrical impedance or computed tomography, may be clinically useful in the assessment of diabetic SDB subjects.

Both SDB and T2DM patients exhibit dysregulated production of adipocytokines, such as monocyte chemoattractant protein–1, leptin, and other proteins [18,34-37]. The biological functions of adiponectin, which was identified as an adipocytokine in the human adipose complementary DNA library [38], include improvement of glucose metabolism [39] and prevention of atherosclerosis [40]. Serum adiponectin concentrations are low in visceral obesity [29], insulin resistance [41], T2DM [42], and OSAHS [7]. Furthermore, our recent report showed a nocturnal fall in circulating adiponectin concentrations in severe OSAHS subjects and that such reduction was probably due to nocturnal hypoxic stress [7]. The present study also found a significant reduction in circulating adiponectin concentrations during sleep in diabetic SDB patients (Fig. 3B). Although our previous study in nondiabetic patients found nocturnal reduction in serum adiponectin concentrations only in severe OSAHS [7], the present study demonstrated such reduction to occur also in diabetic patients with mild SA (Fig. 3C). Patients with both DM and SDB who sustain chronic hypoxemia and elevated oxidative stress may be more susceptible to hypoxic stress during sleep, although a larger study is required to confirm these results. Nocturnal reduction in plasma adiponectin concentrations may be important when designing strategies to prevent CVD in diabetic patients with SDB.

The incidence of CSA in the present study was 32.3% in diabetic patients with SDB (n = 31). It has been suggested that an exaggerated ventilatory response to changes in $PaCO_2$ is crucial for the development of CSA [43-45]. To address the underlying cause of the high incidence of CSA in DM, we should analyze arterial blood gases, including systemic arterial $PaCO_2$ and PaO_2. The present study demonstrated that CSA correlates with elevated hemoglobin concentration [45], high BNP [46,47], and increased carotid vessel thickness [48,49]. These data emphasize the need to check for CSA in T2DM patients with atherosclerosis or impaired cardiac function.

We described here the prevalence and characteristics of SDB in Japanese patients with T2DM. The results showed a high prevalence of SDB in T2DM patients, and it is therefore necessary to diagnose and treat SDB from the standpoint of prevention of CVD in diabetic patients. Large-scale interventional trials, such as weight reduction, intensive antidiabetes therapy, treatment using nasal continuous positive airway pressure, or combinations of these therapies, should be provided to assess the effects of appropriate treatment on the outcome of diabetic patients with SDB.

4.1. Study limitations

There are several limitations to this study. First, we enrolled in the present study 40 hospitalized Japanese patients during the period between February 2006 and March

2007, who represent consecutive patients. In the present study, there might be a selection bias admittedly for severely affected patients because they were the ones who required hospital admission. Although inadequate data were collected from those patients who opted not to participate in the study, there appeared to be no selection bias regarding study participation. Second, we could not find a sufficient number of control subjects matched for age and/or weight to diabetic patients with SDB in the present study. Third, stepwise multiple regression analysis was conducted to identify parameters that significantly contribute to SDB. Multivariable analysis could not be conducted because of the sample size. Further large-scale prospective study is required for confirmation. Finally, we performed screening of SA using type 3 cardiopulmonary monitoring in the current study. This device without the electroencephalogram cannot record the sleep stage and is therefore not accurate in patients with certain comorbidities. Accurate evaluation of SA warrants the measurement of esophageal pressure to distinguish CSA event from OSA event, although all recordings in the present study were subsequently scored manually by an experienced registered polysomnographer using a simplified cardiorespiratory monitoring device [25].

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Original Article

Absolute Value of Bioelectrical Impedance Analysis-Measured Visceral Fat Area with Obesity-Related Cardiovascular Risk Factors in Japanese Workers

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Aim: The accumulation of Visceral fat is known to precede metabolic disorders and atherosclerosis. This study aimed to determine the relationships between body mass index (BMI), waist circumference (WC), estimated visceral fat area (eVFA) measured by bioelectrical impedance analysis (BIA), and obesity-related cardiovascular risk factors.
Methods: The study population was 2,870 middle-aged Japanese employees (males/females = 2,322/548), who had undergone a health check-up.
Results: In the receiver operating characteristic (ROC) curve, the cutoff levels yielding maximal sensitivity plus specificity for predicting the prevalence of ≥2 risks were, 24.5 kg/m^2 for BMI, 84.6 cm for WC, and 111 cm^2 for eVFA in males, and 23.6 kg/m^2, 81.5 cm, and 67 cm^2 in females. The average number of risk factors was over 1.0 in those with a BMI ≥25 kg/m^2 and with a WC ≥85 cm for males, ≥28 kg/m^2 and ≥95 cm respectively for females, and those with an eVFA ≥100 cm^2 for both males and females. In males, it was around 1.0 with cutoff levels of BMI, WC, and eVFA from the ROC curve. However, in females, it was around 0.6, because the prevalence of subjects with obesity and multiple risks was very low.
Conclusions: These results suggested that the cutoff level for visceral fat reduction should be set based on an absolute value of risk factors, rather than a calculated value. In regular health check-up, it may be useful to set an absolute cutoff value for eVFA at 100 cm^2 as criteria to screen for multiple obesity-related cardiovascular risk factors.

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Key words; Visceral fat, Obesity-related cardiovascular multiple risk factors, Health check-up, Fat distribution, Bioelectrical impedance analysis

Introduction

Obesity is a major risk factor for hyperglycemia, diabetes, and hypertension. However, not all obese subjects have such disorders and, in Japan, mildly obese subjects sometimes have multiple obesity-related cardiovascular risk factors[1-3]. We also demonstrated that a long period of exposure to multiple risk factors, including mild obesity, is involved in the onset of atherosclerosis in Japanese employees[4], and studies of Japanese-Americans in Hawaii and Seattle also suggest that the Japanese as a race can not handle glucose

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Table 1. Clinical characteristics of all subjects

	Males (n=2,322)	Females (n=548)
Age (year)	47.9±10.5 (22-68)	46.8±9.3 (21-63)
Body Weight (kg)	69.1±9.2 (42.6-110.2)	56.3±7.7 (39.7-100.3)
Body mass index (kg/m^2)	24.2±2.9 (16.4-36.7)	23.1±3.1 (17.2-38.3)
Waist circumference (cm)	84.4±7.8 (65.0-114.6)	79.2±8.0 (63.5-114.4)
Estimated visceral fat area (cm^2)	99.6±38.9 (14-254)	62.4±31.1 (22-220)
Systolic blood pressure (mmHg)	130.4±18.6 (84-218)	120.1±18.7 (83-205)
Diastolic blood pressure (mmHg)	80.3±13.0 (46-140)	73.4±13.2 (42-130)
Total cholesterol (mmol/L)	5.24±0.89 (2.51-9.69)	5.37±0.91 (2.98-9.40)
HDL cholesterol (mmol/L)	1.46±0.42 (0.47-3.34)	1.69±0.44 (0.52-3.99)
LDL cholesterol (mmol/L)	2.90±0.76 (0.44-7.04)	2.93±0.79 (0.70-6.58)
Triglyceride (mmol/L)	1.96±1.47 (0.29-19.91)	1.25±0.94 (0.28-14.9)
Glucose (mmol/L)	5.96±1.80 (3.16-24.9)	5.56±1.43 (3.39-25.8)
HbA1c (%)	5.27±0.85 (3.0-12.6)	5.06±0.61 (2.8-9.3)

Data are mean±SD (range).
HDL; high density lipoprotein, LDL; low density lipoprotein, HbA1c; hemoglobin A1c

metabolism as well as Caucasians when over-nourished and are liable to develop intolerance and complications even with a mild degree of adiposity[5-7]. In fact, many obese Japanese have mild adiposity, compared with Europeans and Americans. Clinical research has demonstrated the distribution of body fat rather than the total amount of fat to be associated with obesity-related disorders[1-3]. Recently, there is recognition that the distribution of body fat, especially visceral fat, accompanied by various metabolic disorders, should be distinguished from simple obesity. We and others have demonstrated that the accumulation of intra-abdominal visceral fat precedes obesity-related metabolic disorders, including insulin resistance, glucose intolerance, dyslipidemia, and elevated blood pressure, leading to atherosclerosis based on a clustering of multiple risks[1-3, 8, 9]. The Japanese Visceral Fat Syndrome Study Committee of the Ministry of Health and Welfare was organized to establish diagnostic criteria for obesity-related disease and visceral fat accumulation with obesity-related cardiovascular multiple risk factors[10]. They have also set waist circumference (WC) as an embodiment of visceral fat accumulation[10]. There, a WC over 85 cm in males and over 90 cm in females was decided according to the value of visceral fat area by computed tomography at the umbilical level, which was the cut-off for both male and females in terms of more than one cardiovascular risk factor[10]. In this study[10], the relationship between visceral fat accumulation and the number of obesity-related cardiovascular risk factors was analyzed in 775 men (age, 55±11 years, mean±SD) and 418 women (55±12 years). However, there were some limitations as follows, 1) the cutoff values for VFA were analyzed men and women combined, 2) the study sample w relatively small for females, and 3) the study samp was not a general population but composed of outp tients receiving treatment for metabolic disorders. P viously, we also reported marked gender differen in the proportions of adiposity and marked accele tion of visceral fat accumulation after menopause women[11]. We established a new technique to noni vasively evaluate VFA using an abdominal bioelectri impedance analysis (BIA)[12]. Accordingly, we assu that it is important to establish a correlation betwe BIA-measured visceral fat area and presence of ol sity-related cardiovascular risk factors for middle-ag Japanese men and women, separately.

Aim

The aim of the present study is to establish g der-specific cutoff values for BIA-measured visceral area with obesity-related cardiovascular risk factors urban Japanese workers.

Subjects and Methods

Study Populations

The study group comprised 2,870 Japanese v were employees of Amagasaki city office, Hyogo; urban area, and had underwent an annual he check-up in 2004. **Table 1** summarizes the profiles of male and female subjects (males; n=2,322, fema n=548). The study was approved by the human et committee of Osaka University and written infor

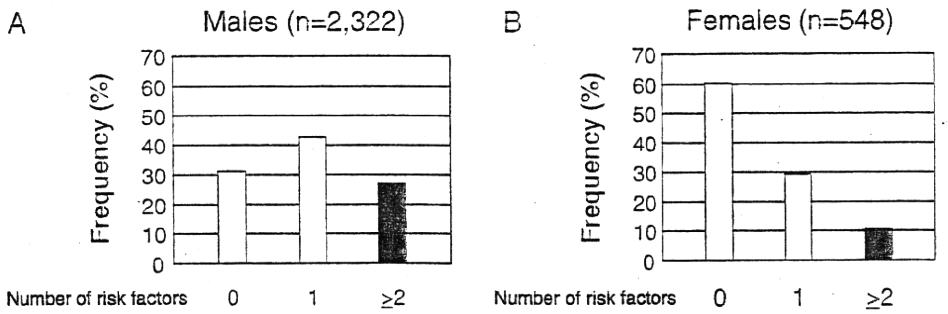

Fig. 1. The frequency of each number of obesity-related cardiovascular risk factors (elevated blood pressure, dyslipidemia, and hyperglycemia) in men (A) and women (B). Solid bar: subjects with two or more risk factors. We investigated the presence of three obesity-related cardiovascular risk factors: 1) elevated blood pressure (systolic blood pressure (SBP) ≥130 mmHg and/or diastolic blood pressure (DBP) (≥85 mmHg), or regular treatment with anti-hypertensive agents), 2) dyslipidemia (hypertriglyceridemia (fasting or postprandial triglyceride of ≥1.69 or 2.27 mmol/L, respectively)[16], and/or low HDL cholesterol (HDL cholesterol <1.04 mmol/L), or regular treatment with hypolipidemic agents), and 3) dysglycemia/impaired glucose tolerance (hyperglycemia (fasting or postprandial serum glucose concentration of ≥6.10 or 7.77 mmol/L, respectively)[17], or regular treatment with anti-diabetic agents).

consent was obtained from each participant. This trial (called The Amagasaki Visceral Fat Study) is registered with the University hospital Medical Information Network, number UMIN 000002391. We already reported the relationship between visceral fat accumulation and metabolic factors in the Amagasaki Visceral Fat Study[13-15], a study of middle-aged Japanese in whom visceral fat accumulation was estimated non-invasively by BIA, using a method described previously[12].

Anthropometry and Laboratory Tests

Height, weight and waist circumference were measured in a standing position. Waist circumference at the umbilical level was measured with a non-stretchable tape in late expiration while standing (in cm). Visceral fat area (VFA) was estimated by BIA, as reported previously[12]. Briefly, the voltage recorded at the flank to the flow of current between the umbilicus and the back correlates significantly with VFA and is not influenced by subcutaneous fat. We reported previously that VFA estimated by BIA (eVFA) correlates significantly with that determined by computed tomography (CT)[12]. The coefficient of variation of BIA with the value of CT was 0.89% in the standing position and with late exhalation.

Blood pressure was measured with a standard mercury sphygmomanometer on the right arm after the subject had rested in a sitting position for at least 10 minutes. Venous blood samples were collected for measurements of blood glucose, high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) levels while the subject was in the supine position.

We investigated the presence of three obesity-related cardiovascular risk factors (**Fig. 1**): 1) elevated blood pressure, 2) dyslipidemia, and 3) dysglycemia/impaired glucose tolerance. Elevated blood pressure was represented by a systolic blood pressure (SBP) of ≥130 mmHg and/or diastolic blood pressure (DBP) ≥85 mmHg, or regular treatment with anti-hypertensive agents. Dyslipidemia was represented by hypertriglyceridemia (fasting or postprandial triglyceride of ≥1.69 or 2.27 mmol/L, respectively)[16], and/or a low HDL cholesterol (HDL cholesterol <1.04 mmol/L), concentration or regular treatment with hypolipidemic agents. Dysglycemia/impaired glucose tolerance was represented by hyperglycemia (fasting or postprandial serum glucose concentration of ≥6.10 or 7.77 mmol/L, respectively)[17], or regular treatment with anti-diabetic agents.

Statistical Analysis

A receiver operating characteristic (ROC) curve was used to determine the appropriate cutoff value for BMI, WC, and eVFA in identifying subjects with two or more of the above risk factors (**Fig. 2**). Differences in the mean number of obesity-related cardiovascular risk factors between males and females were analyzed with the Kruskal-Wallis test (**Fig. 3**). Linear regression and a quadratic curve were used to analyze the relationship between eVFA and WC (**Fig. 4**). The level of significance was set at $p<0.05$. Continuous variables are presented as the mean ± SD (**Table 1**) or mean ±

SEM (**Fig. 3**), as indicated. All statistical analyses were performed with StatView-J 5.0 (Statistical Analysis System Inc, Cary, NC) or the SPSS statistical software package (version 11.0.1J; SPSS, Chicago, IL).

Results

Gender-Specific Receiver Operating Characteristic (ROC) Curve Analysis

Table 1 shows the characteristics of the study population. The participants had a mean age 47.9 years for men and 46.8 years for women, a mean BMI of 24.2 kg/m^2 for men and 23.1 kg/m^2 for women, a mean WC of 84.4 cm for men and 79.2 cm for women, and a mean eVFA of 99.6 cm^2 for men and 62.4 cm^2 for women.

We analyzed the proportion of subjects with each number of obesity-related cardiovascular risk factors (**Table 1**). In males, 42.8% (n=993/2,322) had one risk factor, and 26.5% (n=616/2,322) had two or more (**Fig. 1A**). In females, 29.2% (n=160/548) had one risk factor, and 10.6% (n=58/548) had two or more (**Fig. 1B**). Furthermore, 60.2% of females (n=330/548) were free from any obesity-related cardiovascular risk (**Fig. 1B**), compared with only 30.7% (n=713/2,322) of males (**Fig. 1A**).

Fig. 2 shows the gender-specific ROC curves of BMI, WC and eVFA for detecting the clustering of obesity-related cardiovascular risk factors. The area under the curve (AUC) of BMI was 0.661 (95%CI, 0.635-0.686) for males (**Fig. 2A**, left) and 0.705 (95%CI, 0.629-0780) for females (**Fig. 2A**, right). The AUC of WC was 0.702 (95%CI, 0.678-0.72) for males (**Fig. 2B**, left) and 0.687 (0.613-0760) for females (**Fig. 2B**, right), and the AUC of eVFA was 0.711 (0.688-0.734) for males (**Fig. 2C**, left) and 0.710 (0.637-0783) for females (**Fig. 2C**, right). Each AUC value was statistically significant for both males and females. There was no significant difference in the AUC values for BMI, WC and eVFA between males and females.

The cutoff levels yielding maximal sensitivity plus specificity for predicting the prevalence of two or more obesity-related abnormalities were, 24.5 kg/m^2 for BMI (**Fig. 2A**, left), 84.6 cm for WC (**Fig. 2B**, left), and 111 cm^2 for eVFA (**Fig. 2C**, left), in males, and 23.6 kg/m^2 (**Fig. 2A**, right), 81.5 cm (**Fig. 2B**, right), and 67 cm^2 (**Fig. 2C**, right). The cutoff level established from the ROC curve might be influenced by the characteristics of the study participants.

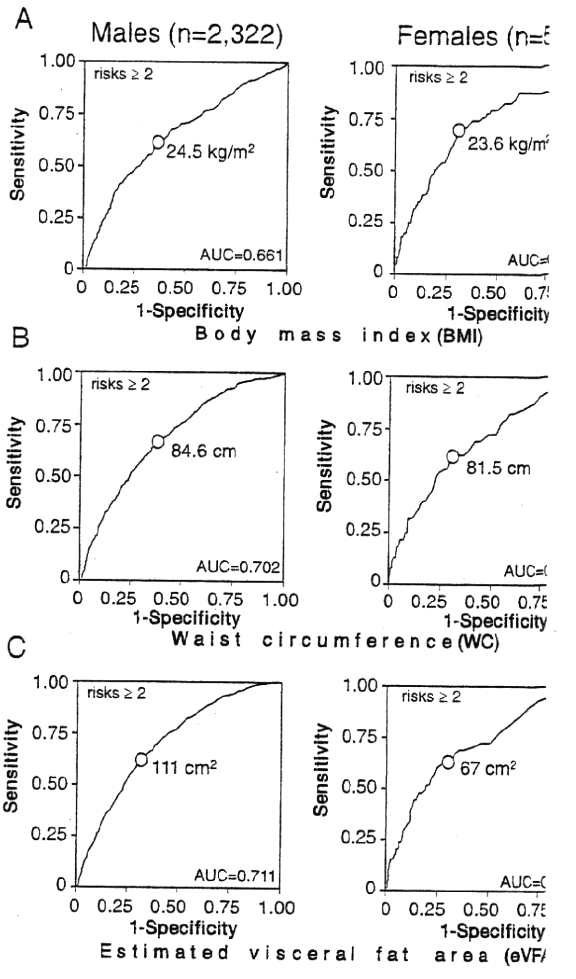

Fig. 2. The predictive performance of BMI (A), WC (B eVFA (C) for two or more obesity-related cardio lar risk factors (elevated blood pressure, dyslipic and hyperglycemia) according to the receiver o ing characteristic (ROC) curve. The ROC curv two or more risk factors in males (left) and fe (right) are shown. Each optimal cut off point for tifying two or more risk factors estimated for the curve is shown by a white circle. AUC; area und specificity-sensitivity curve, 95% CI; 95% confi intervals.

Gender-Specific Number of Obesity-Rel Cardiovascular Risk Factors According to BMI, and eVFA Levels, and Gender-Specific Body Distribution

We evaluated the association between BMI, or eVFA and the number of obesity-related cardi cular risk factors. The male and female groups divided into eight bins of BMI (every 1.5 k **Fig. 3A, 3B**), WC (every 5 cm, **Fig. 3C, 3D**), eVFA (every 20 cm^2, **Fig. 3E, 3F**).

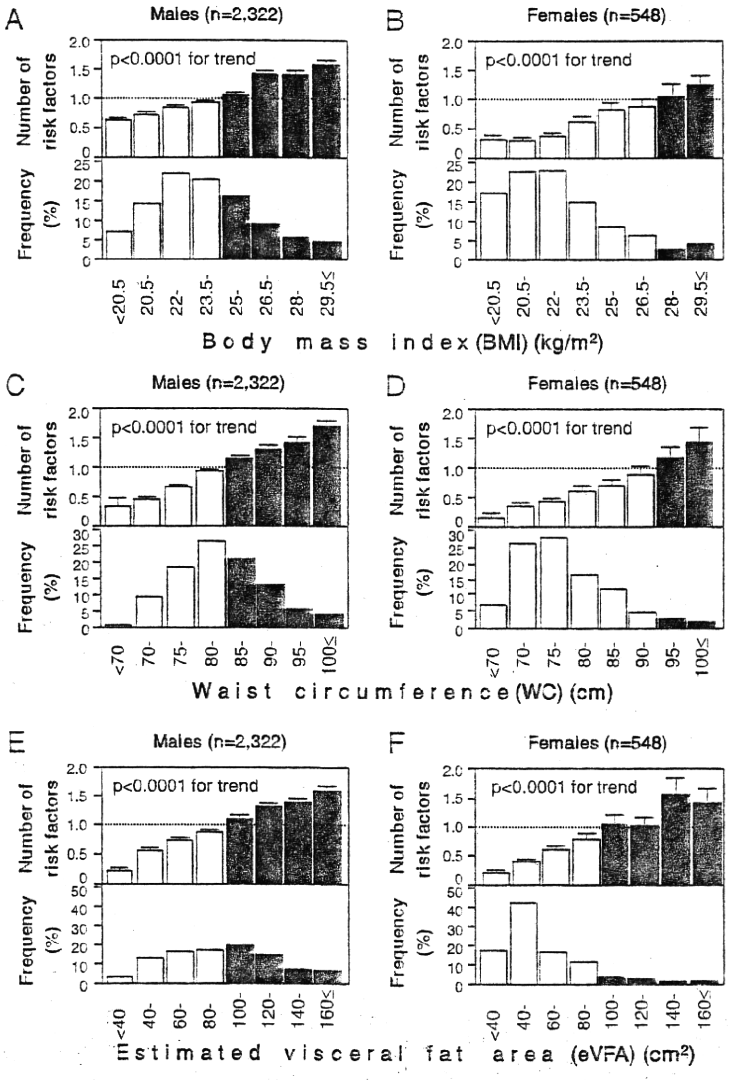

Fig. 3. Correlation between the average number of obesity-related cardiovascular risk factors (elevated blood pressure, dyslipidemia, and hyperglycemia) and body mass index (BMI) (top), and prevalence of BMI in each category divided by (every 1.5 kg/m^2) (bottom) in 2,322 males (A) and 548 females (B), and waist circumference (WC) (top), and prevalence of WC in each category divided by (every 5 cm) (bottom) in males (C) and females (D), and estimated visceral fat area (eVFA) (top), and prevalence of eVFA in each category divided by (every 20 cm^2) (bottom) in males (E) and females (F). The horizontal dotted lines represent an average number of metabolic risk factors of 1.0. Solid bars show each group in which the average number of obesity-related risk factors was over 1.0. Data are the mean ± SEM. Differences in the mean number of obesity-related cardiovascular risk factors were analyzed by the Kruskal-Wallis test.

First, the mean number of obesity-related cardiovascular risk factors increased significantly with a higher BMI, in both males (**Fig. 3A**, upper panel) and females (**Fig. 3B** top) ($p < 0.0001$ for trend, the Kruskal-Wallis test). **Fig. 3A** and **3B** show that, the average number of risk factors was more than 1.0 (black bar)

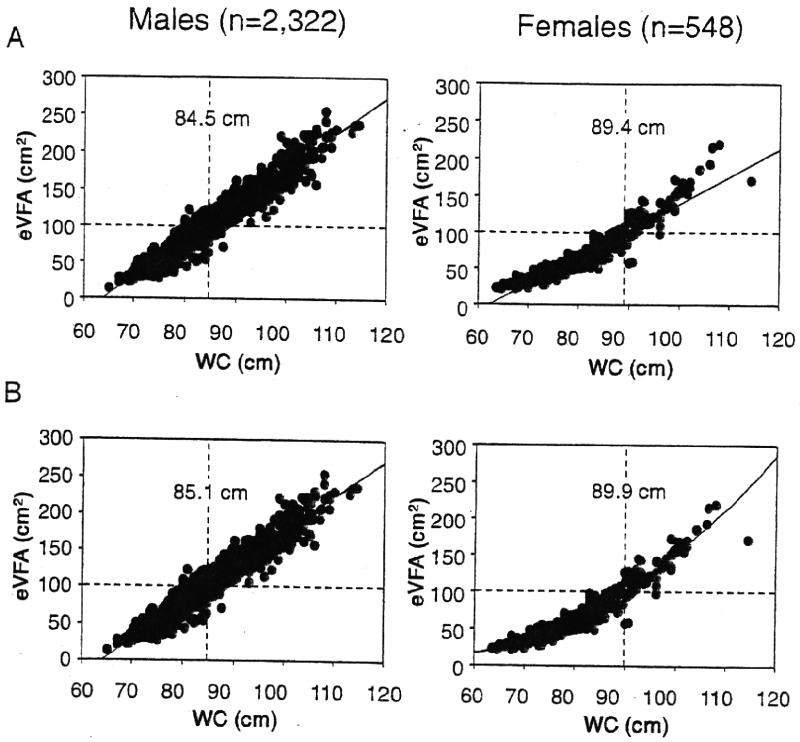

Fig. 4. Regression line and quadratic curve for eVFA and WC in male and female subjects. The horizontal and vertical dotted lines represent an eVFA of 100 cm^2 and WC corresponding to a VFA of 100 cm^2 by a simple regression line (A) and quadratic curve (B).

at over 25 kg/m^2 for males and over 28 kg/m^2 for females. The proportion of subjects with a BMI ≥25 kg/m^2 was 35.8% (n=832/2,322) for males (**Fig. 3A**, bottom, solid bars) and 21.9% for females (n=120/548). That with a BMI ≥28 kg/m^2 was only 6.9% (n=38/548) for females (**Fig. 3B**, bottom, solid bars). When the BMI was 24.5 kg/m^2 for men, which is the cutoff level yielding maximal sensitivity plus specificity for predicting the prevalence of two or more obesity-related abnormalities using the ROC curve, the mean number of metabolic complications was approximately 1.0. In women, although the mean number of abnormalities increased as the BMI increased, it was around 0.6 with a BMI of 23.6 kg/m^2, which is the cutoff level from the ROC curve.

Second, the mean number of obesity-related cardiovascular risk factors increased significantly with a higher WC, in both males (**Fig. 3C** top) and females (**Fig. 3D**, top) (p<0.0001 for trend, the Kruskal-Wallis test). **Fig. 3C** and **3D** show that, the average number of risk factors was more than 1.0 at a WC of over 85 cm for males and over 95 cm for females. In males, 44.5% (n=1,033/2,322) had a large WC (≥85 cm, solid bars) (**Fig. 3C**, bottom), while only 4.9% (n=27/548) of females had a large WC (≥95 cm, solid bars) (**Fig. 3D**, bottom). When WC was 84.6 cm for men, which is the cutoff level obtained from the ROC curve, the mean number of metabolic abnormalities was approximately 1.0. In women, although the mean number of abnormalities increased as WC increased, it was around 0.6 with a WC of 81.5 cm, which is the cutoff obtained from the ROC curve.

Finally, the mean number of obesity-related cardiovascular risk factors increased significantly with a higher eVFA, in both males (**Fig. 3E**, top) and females (**Fig. 3F**, top) (p<0.0001, the Kruskal-Wallis test). The horizontal dotted lines in **Fig. 3E** and **3F** represent eVFA of the 100- cm^2 category and the average number of risk factors of 1.0 for both male and female subjects. In males, 45.2% (n=1,049/2,322) had accumulated visceral fat (eVFA ≥100 cm^2, solid bars) (**Fig. 3E**, bottom), while in females, this figure was 9.6% (n=57/548) (**Fig. 3F**, bottom). When the eVFA was 111 cm^2 for men, the cutoff level from the ROC curve, the mean number of metabolic abnormalities was approximately 1.0. In women, although the mean

number of abnormalities increased as the eVFA increased, it was around 0.6 with an eVFA of 67 kg/m^2, the cutoff level obtained from the ROC curve.

Gender-Specific Correlation between eVFA and WC

WC and eVFA were plotted using a simple regression equation and quadratic curve. Based on the simple regression equation, WC correlated with eVFA in both men ($p<0.0001$, $r=0.971$) and women ($p<0.0001$, $r=0.947$); a WC value of 84.5 cm corresponded to an eVFA of 100 cm^2 in males, while a WC of 89.4 cm corresponded to an eVFA of 100 cm^2 in females (**Fig. 4A**). In addition, calculations using a quadratic curve showed that WC correlated with eVFA in both males ($p<0.0001$, $r=0.971$) and females ($p<0.0001$, $r=0.963$): a WC of 85.1 cm corresponded to an eVFA of 100 cm^2 in men whereas a WC of 89.9 cm corresponded to an eVFA of 100 cm^2 in women (**Fig. 4B**).

Discussion

The present analysis of a large number of middle-aged Japanease demonstrated that the average number of obesity-related cardiovascular risk factors was more than 1.0 in those with a BMI over 25 kg/m^2 for males and over 28 kg/m^2 for females, in those with a WC over 85 cm for males and over 95 cm for females, and in those with an eVFA over 100 cm^2 for both sexes. Several clinical studies have demonstrated that the accumulation of visceral fat is more closely associated with obesity-related metabolic disorders than is the BMI in Japanese[1-3, 13]. Therefore, waist circumference and eVFA may be more useful for detecting obesity-related cardiovascular risk factors than the BMI, although the current study showed the cutoff value for the BMI, over 25 kg/m^2 for males and over 28 kg/m^2 for females. The current study demonstrated a relationship between eVFA measured non-invasively and multiple cardiovascular risk factors.

Over-eating and physical inactivity cause visceral fat to accumulate, leading to the development of obesity-related risk factors and an increased risk of cardiovascular diseases[1-3, 8, 9]. This condition is called "metabolic syndrome"[18, 19] and prevalent in the general Japanese population[20]. Metabolic syndrome is a useful clinical entity to predict cardiovascular risk, beyond the classic risk factors such as age, hypercholesterolemia and smoking[21]. Therefore, a decrease in multiple risk factors by reducing accumulated visceral fat would be important in preventing cardiovascular diseases. Several studies reported that WC and VFA correlated with the number of cardiovascular risk factors in Japanese subjects[10, 22-29]. Some reports proposed using cutoff values for WC and VFA obtained from ROC curves[22-25, 28]. Here, we performed a gender-specific ROC analysis to assess WC and eVFA as an indicator of at least two of the three obesity-related cardiovascular risk factors (**Fig. 2**). Similar to previous reports[22-25, 29], low cutoff points for WC and eVFA for women yielding maximal sensitivity plus specificity might lead to the detection of more subjects with metabolic abnormalities. However, as we showed here, among women with a significantly low prevalence of obesity-related abnormalities, low cutoff points resulted in a high rate of false positives, with women not at risk being identifiel as abnormal. Hence, the relative cutoff points for WC and eVFA for women from the ROC curve may not be effective to screen multiple obesity-related cardiovascular risk factors in middle-aged Japanese women in regular health check-ups. Therefore, a proposed relative cut-off value of VFA estimated from the ROC curve in middle-aged Japanese females should be read with caution. These results suggested that the cutoff value for visceral fat reduction should be based on an absolute value, rather than a relative value calculated from the ROC curve, in middle-aged Japanese. More practically, the average number of risk factors was over 1.0 at an eVFA of 100 cm^2 for both males (**Fig. 3E**, top) and females (**Fig. 3F**, top), indicating that an eVFA of over 100 cm^2 to be suitable to screen multiple obesity-related cardiovascular risk factors in regular health check-ups.

One limitation of this study was that the mean age of the female subjects was 46.8 years (**Table 1**), relatively young compared to the age of retirement (age ≤60). Therefore, the ratio of post-menoposal women was small. We and others have shown that visceral fat starts to accumulate after menopause in women[11, 30, 31]. Hayashi *et al.* recommended an age-specific assessment of VFA when evaluating obesity-related cardiovascular risk factors in females[32]. Other studies have also confirmed increases in cardiovascular events and thr frequency of metabolic risk factors after menopause[33, 34]. Taken together, it is possible that the female subjects in the present study were too young to assess the effect of visceral fat on the number of metabolic risk factors. In other words, adiposity and visceral fat may not matter as much in pre-menoposal women in terms of multiple obesity-related cardiovascular risk factors. Further study is needed to examine this issue in a large number of post-menopausal females with a wide range of body mass values.

Visceral adiposity is usually measured by computed tomography. However, the procedure is expensive, takes time, and involves radiation. The measure-

ment of visceral adiposity by BIA (eVFA) is much more feasible and relatively reliable It may be meaningful that WC equivalent to a visceral fat area of 100 cm^2 as estimated by BIA was ≅85 cm for males and ≅90 cm for females in middle-aged Japanese (**Fig. 4**).

Conclusion

In conclusion, setting an absolute cutoff value for BIA-measured visceral fat area when screening for multiple obesity-related cardiovascular risk factors rather than a relative cutoff value using the ROC curve in a general health check-up, may be effective in preventing cardiovascular diseases.

Limitations of the Study

Our study measured VFA by BIA. Measurements of both visceral and subcutaneous fat areas by computed tomography are more accurate. Subjects who were on medication for diabetes, hyperlipidemia, or hypertension were included (male; n=513, female; n=71). The results were similar to the eVFA value when subjects using medication were excluded (data not shown). The proportion of subjects who received blood tests postprandially anytime between the afternoon and early evening for the annual health check-up was 82.7% in males (n=1921/2322) and 84.9% in females (n=465/548). Therefore, we evaluated each parameter using postprandial criteria reported previously[16, 17].

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Disclosure statement

The authors have no conflict of interest to declare.

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Dysregulation of glucose, insulin, triglyceride, blood pressure, and oxidative stress after an oral glucose tolerance test in men with abdominal obesity

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Abstract

Postprandial metabolic dysregulation plays a role in the development of atherosclerosis. Visceral fat accumulation is an important component of various metabolic disorders including glucose intolerance, dyslipidemia, and hypertension, which correlate with atherosclerotic cardiovascular disease. The aim of the present study was to compare the postprandial response of various metabolic parameters, blood pressure, adiponectin, and oxidative stress to 75-g oral glucose tolerance test (OGTT) in men with (n = 23) and without (n = 7) abdominal obesity based on waist circumference (WC) cutoff value of 85 cm (based on the Japanese criteria for the metabolic syndrome). The cross-sectional prospective study included 30 male subjects who were on no medications and newly diagnosed with mild hypertension and/or dyslipidemia. The percentage change in each parameter ([each parameter at 120 minutes after an OGTT − that before an OGTT]/that before an OGTT × 100) was calculated. The percentage systolic blood pressure, percentage diastolic blood pressure, and percentage triglyceride were −6.3% ± 3.5%, −9.4% ± 3.0%, and −10.2% ± 2.1%, respectively, in the WC less than 85 group (vs baseline: $P = .10$, $P < .01$, and $P < .001$) and 2.0% ± 1.7%, 0.9% ± 2.4%, and 2.8% ± 3.3%, respectively, in the WC at least 85 group (vs WC <85 group: $P < .05$, each). However, there were no significant differences in percentage total cholesterol and percentage high-density lipoprotein cholesterol between the 2 groups. The percentage thiobarbituric acid–reacting substances tended to be lower in the WC less than 85 group (vs baseline: $P = .07$), but not in the WC at least 85 group, albeit statistically insignificant (WC <85 vs ≥85 group: $P = .057$). The maximum carotid intima-media thickness was larger in the WC at least 85 group than the WC less than 85 group ($P < .05$). Evaluation of postprandial changes in obesity-related parameters may be important in preventing atherosclerotic diseases.

1. Introduction

The postprandial phenomenon is a key factor in the development of atherosclerosis [1]. Postprandial hyperglycemia, hyperinsulinemia, and dyslipidemia with hypertriglyceridemia (also referred to as *postprandial dysmetabolism*) are individually and collectively recognizable cardiovascular risk factors [2-5]. The cardiovascular toxicity of postprandial dysmetabolism is mediated by oxidative stress, which is directly proportional to the levels of glucose and triglycerides (TGs) after high-caloric meals [2-5]. These transient spikes acutely trigger inflammation, sympathetic hyperactivity, endothelial dysfunction, and a cascade of other atherogenic changes, leading to future cardiovascular events [6-8].

Several studies analyzed the relationship between severity of cardiovascular diseases (CVDs) and postprandial hyperinsulinemia using an oral glucose tolerance test (OGTT) challenge [9-11]. Moreover, there are several reports that high levels of nonfasting serum TGs could predict the risk of CVDs, independent of total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) [12-14]. Postprandial dysregulated metabolism is a major contributor to the pathogenesis of atherosclerosis and

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CVD events [3]. Usually, glucose and lipids are systematically measured in the fasting state. Most studies on the characterization of blood pressure, glucose, and TG levels in obese subjects, especially those with accumulation of abdominal adipose tissue, that is, abdominal obesity, were conducted by measuring visceral fat accumulation or greater waist circumference (WC) during the fasting state, with postprandial changes in these metabolic parameters being barely investigated.

Visceral fat accumulation is an important component of the metabolic syndrome, which encompasses various metabolic disorders such as glucose intolerance, dyslipidemia, and hypertension, and is associated with atherosclerotic CVDs [15]. Adipose tissue is not only a passive reservoir for energy storage but also an important endocrine organ, secreting a variety of bioactive molecules collectively known as *adipocytokines*. We identified adiponectin as an adipocytokine in the human adipose tissue complementary DNA library [16], which exhibits direct antiatherosclerotic and antidiabetic properties in experimental studies [17]. The blood levels of adiponectin are low in obesity [18]. There is increased oxidative stress in subjects with obesity [19,20]. Recent studies confirmed the inverse relationship between oxidative stress and blood levels of adiponectin in human [19,21]. It has been reported that enhanced oxidative stress in the adipose tissue causes dysregulation in the production of adipocytokines, for example, low expression of adiponectin [19].

In the present study, we investigated postprandial metabolic parameters, blood pressure, adiponectin, and oxidative stress using OGTT in Japanese men with abdominal obesity.

2. Subjects and methods

2.1. Study populations

Thirty consecutive Japanese middle-aged men on no medications, who visited the clinic and were newly diagnosed with mild hypertension and/or dyslipidemia between February 2008 and March 2009, were enrolled in the present study (age mean ± SD, 54.7 ± 14.0 years). *Hypertension* was defined as systolic blood pressure (SBP) of at least 130 mm Hg and/or diastolic blood pressure (DBP) of at least 85 mm Hg. Dyslipidemia represented high fasting TG levels of at least 1.69 mmol/L and/or low HDL-C levels of less than 1.04 mmol/L, according to the Japanese criteria of the metabolic syndrome [22,23]. We divided the subjects into those with WC less than 85 cm (WC <85, n = 7) and those with WC at least 85 cm (WC ≥85, n = 23), according to the Japanese criteria of the metabolic syndrome [22,23]. All individuals underwent a standardized 75-g OGTT (Trelan G 75; Shimizu, Shizuoka, Japan) in the clinic after overnight fast. To investigate the OGTT overloading, the subjects with overt diabetes (fasting glucose levels ≥7.8 mmol/L) were excluded. The study was approved by the human ethics committee of Osaka University, and a written informed consent was obtained from each participant. This trial (called *The Hokusetsu Trial*) is registered with University hospital Medical Information Network (no. UMIN 000001454).

2.2. Anthropometry and laboratory measurements

Each subject was asked to complete a questionnaire on family history, medical history, current medication, and smoking history. Height and body weight were measured in standing position. Body mass index was calculated as weight (in kilograms) divided by the square of height in meters (square meters). Waist circumference was measured at the umbilical level using a nonstretchable tape in late expiration while standing (in centimeters). Blood pressure was measured with a standard mercury sphygmomanometer on the right arm after the subjects had rested in the supine position for at least 10 minutes in the clinic. Mean values were determined from 2 independent measurements made at 3-minute intervals. In the fasting state before an OGTT after overnight fasting, venous blood samples were collected for measurements of blood glucose, hemoglobin A_{1c} (HbA_{1c}), immunoreactive insulin (IRI), TC, HDL-C, TG, adiponectin, tumor necrosis factor–α (TNF-α), high-sensitivity C-reactive protein (hCRP), and thiobarbituric acid–reacting substances (TBARS), as an oxidative stress marker, while the subject was in the supine position.

Next, an OGTT was performed after overnight fasting. Venous blood samples were obtained from the forearm while the subject was in the supine position and were collected in the fasting state and at 30, 60, and 120 minutes after an OGTT to evaluate blood glucose and IRI concentrations. Each parameter, such as TC, TG, HDL-C, adiponectin, and TBARS, and blood pressures were measured both in the fasting state and at 120 minutes after the ingestion of 75-g OGTT. The plasma glucose and the IRI area under the concentration-time curve values (AUC glucose and AUC IRI, respectively) during the OGTT were calculated. The summation values of glucose and IRI (Σglucose and ΣIRI) were calculated. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: HOMA-IR (milliunits per liter × milligrams per deciliter) = [(fasting IRI × fasting glucose)/405]. The homeostasis model assessment of pancreatic β-cell function (HOMA-Iβ) was calculated using the following formula: HOMA-Iβ [(milliunits per liter)/(milligrams per deciliter)] = [(fasting IRI × 360)/(fasting glucose − 63)]. The *insulinogenic index* was defined as the ratio of IRI to glucose at 30 minutes after glucose loading. Insulin sensitivity index was calculated using the following formula: 10 000/square root of [(fasting glucose × fasting IRI) × (mean glucose × mean IRI during an OGTT)] [24]. The percentage changes in SBP, DBP, TC, TG, and HDL-C (%SBP, %DBP, %TC, %TG, and %HDL-C, respectively) were calculated using the following

formula: [(each parameter at 120 minutes after an OGTT − that in the fasting state before an OGTT)/that before an OGTT × 100]. Plasma glucose concentrations were determined by the glucose oxidase method. Serum IRI concentrations were determined by an immunochemiluminometric assay (ADVIA Centaur IRI; Siemens Healthcare Diagnostics, Tarrytown, NY). The value of HbA_{1c} was determined by high-performance liquid chromatography. Serum concentrations of TC, TG, and HDL-C were determined by enzymatic methods. The serum concentration of adiponectin was measured by a sandwich enzyme-linked immunosorbent assay system (Adiponectin ELISA Kit; Otsuka Pharmaceutical, Tokushima, Japan). Serum concentration of TNF-α was measured by a quantitative sandwich enzyme immunoassay technique (Quantikine HS Immunoassay kit; R&D Systems, Minneapolis, MN). Serum concentration of hCRP was measured with hCRP assay (N-Latex CRP II; Dade Behring, Marburg, Germany). Serum concentration of TBARS, reflecting serum lipid peroxidation products, was determined by the method of Yagi (Japan Institute for the Control of Aging, Nikken SEIL, Shizuoka, Japan) [25].

The maximum (max) carotid intima-media thickness (IMT) and mean IMT of the common carotid artery were also measured in supine position (LOGIQ S6; GE Healthcare, Waukesha, WI). The max IMT was measured on both the right and left sides in the observation-possible areas of the common carotid artery, bulbus, and internal carotid artery, except the external carotid artery.

2.3. Statistical analysis

Data are presented as mean ± SEM and were compared by unpaired Student *t* test or Mann-Whitney *U* test for experiments with only 2 groups. Differences in frequencies were examined by the χ^2 test. In all cases, *P* values <.05 were considered statistically significant. All analyses were performed with the StatView software version 5.0 (HULINKS, Tokyo, Japan).

Table 1
Characteristics of enrolled subjects

	WC <85 cm (n = 7)	WC ≥85 cm (n = 23)	*P* value
Age (y)	57.1 ± 7.1	53.9 ± 2.6	NS
BMI (kg/m^2)	22.6 ± 0.6	29.1 ± 1.1	<.01
WC (cm)	80.7 ± 2.1	99.7 ± 2.6	<.001
SBP (mm Hg)	131.4 ± 9.7	137.3 ± 3.6	NS
DBP (mm Hg)	71.1 ± 5.1	79.2 ± 2.7	NS
Fasting glucose (mmol/L)	5.24 ± 0.28	5.66 ± 0.14	NS
Fasting IRI (mU/L)	4.3 ± 0.4	16.2 ± 3.2	<.05
HbA_{1c} (%)	4.8 ± 0.1	5.3 ± 0.1	<.05
Fasting TC (mmol/L)	5.45 ± 0.25	6.03 ± 0.15	NS
Fasting TG (mmol/L)	1.43 ± 0.21	2.30 ± 0.26	NS
Fasting HDL-C (mmol/L)	1.47 ± 0.21	1.26 ± 0.07	NS
Adiponectin (μg/mL)	10.3 ± 1.4	5.8 ± .04	<.001
TNF-α (pg/mL)	1.0 ± 0.3	1.3 ± 0.1	NS
hCRP (mg/dL)	0.04 ± 0.01	0.16 ± 0.04	.114
TBARS (nmol/L)	3.17 ± 0.18	3.76 ± 0.18	.101
Mean IMT (mm)	0.8 ± 0.1	0.9 ± 0.1	NS
Max IMT (mm)	0.9 ± 0.1	1.3 ± 0.1	<.05
Smoking (%)	57.1 (n = 4)	78.3 (n = 18)	NS

Data are mean ± SEM. NS indicates not significant.

Table 2
Changes in metabolic parameters measured during 75-g OGTT in subjects with and without *abdominal obesity*, defined based on WC cutoff value of 85 cm

	WC <85 cm (n = 7)	WC ≥85 cm (n = 23)	*P* value
2-h glucose (mmol/L)	5.1 ± 0.9	8.0 ± 0.5	<.05
2-h IRI (mU/L)	25.1 ± 4.4	95.8 ± 14.3	<.05
Peak glucose (mmol/L)	8.2 ± 0.9	10.9 ± 0.5	<.05
Peak IRI (mU/L)	55.3 ± 8.4	120.6 ± 18.3	.064
AUC glucose (mmol/L per 2 h)	13.1 ± 1.5	17.9 ± 0.8	<.01
AUC IRI (mU/L per 2 h)	67.0 ± 7.8	170.0 ± 27.3	<.05
Σglucose (mmol/L)	25.2 ± 2.5	33.5 ± 1.4	<.01
ΣIRI (mU/L)	111.9 ± 11.6	299.2 ± 49.2	<.05
HOMA-IR	1.0 ± 0.2	4.1 ± 0.8	.052
HOMA-Iβ	54.6 ± 8.3	135.0 ± 20.2	<.05
Insulin sensitivity index	9.6 ± 1.0	3.5 ± 0.4	<.001
Insulinogenic index	0.3 ± 0.5	1.3 ± 0.4	NS

3. Results

3.1. Baseline characteristics according to WC

The baseline characteristics of the WC <85 and WC ≥85 groups are summarized in Table 1. The WC ≥85 group had a higher body mass index, IRI, and HbA_{1c} and lower adiponectin than the WC <85 group. The blood levels of hCRP and TBARS tended to be higher in the WC ≥85 group, although the difference was not significant. There were no differences between the 2 groups with regard to SBP, DBP, fasting glucose, TC, TG, and HDL-C concentrations. The max IMT was larger in the WC ≥85 group (1.3 ± 0.1 mm) compared with the WC <85 group (0.9 ± 0.1 mm, $P < .05$).

3.2. Changes in metabolic parameters and blood pressure after an OGTT

First, we evaluated the postprandial glucose, insulin, lipid, and blood pressure using OGTT in both WC groups. The results of glucose and insulin metabolism are shown in Table 2 and in Fig. 1A, B. Six patients had impaired glucose tolerance, 3 had type 2 diabetes mellitus, and 14 had normal glucose tolerance. Both blood glucose and IRI levels after OGTT were higher in the WC ≥85 group than the WC <85 group (Fig. 1A, B). Two-hour glucose, 2-hour IRI, peak glucose, AUC glucose, AUC IRI, Σglucose, and ΣIRI were also significantly higher in the WC ≥85 group than in the WC <85 group. The HOMA-Iβ, a marker of pancreatic β-cell function, was higher in the WC ≥85 group than in the WC <85 group. In addition, HOMA-IR, a marker of insulin

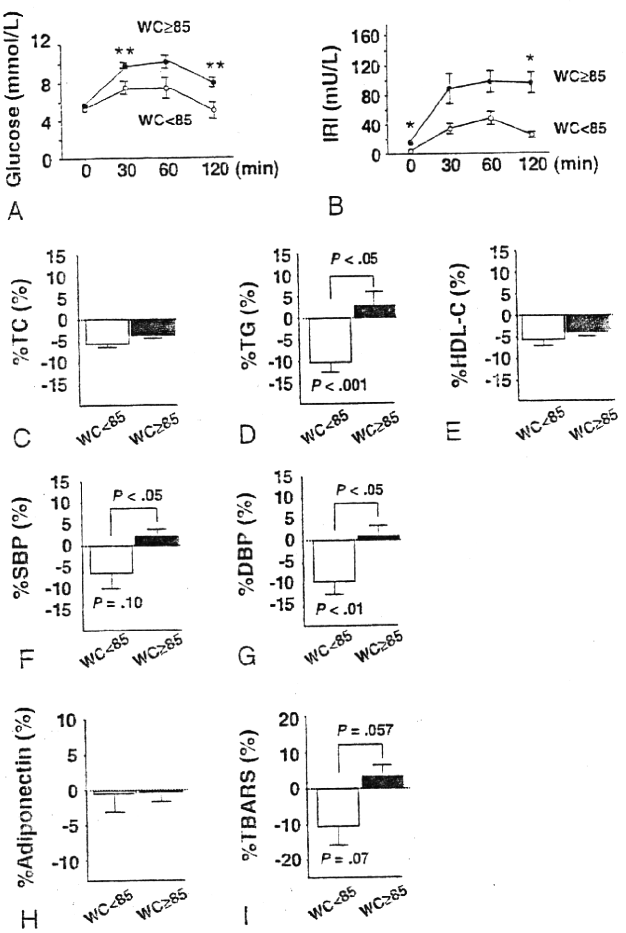

Fig. 1. Differences in plasma glucose (A) and serum IRI (B) levels during 75-g OGTT in subjects with (WC ≥85) and without (WC <85) abdominal obesity. *$P < .05$, **$P < .01$; between the WC ≥85 and WC <85 groups. Percentage changes in TC (C), TG (D), HDL-C (E), SBP (F), DBP (G), serum adiponectin (H), and serum TBARS (I) in response to glucose loading in the WC ≥85 and WC <85 groups. [(TC, TG, HDL-C, SBP, DBP, adiponectin, or TBARS at 120 minutes after an OGTT − that in the fasting state before an OGTT)/that before an OGTT (%)]. The OGTT was performed as described in "Subjects and methods." P values: vs baseline in the WC <85 group; between WC ≥85 and WC <85 groups. Data are mean ± SEM; error bars represent 95% confidence intervals.

resistance, was also higher in the WC ≥85 group, albeit statistically insignificant; and insulin sensitivity index, a marker of insulin sensitivity, was significantly lower in the WC ≥85 group than in the WC <85 group. There was no difference in insulinogenic index, a marker of initial insulin response to glucose, between the 2 groups.

Next, we evaluated the postprandial changes in lipid parameters and blood pressure during OGTT (Fig. 1C-G). The %SBP and %DBP of the WC <85 group were −6.3% ± 3.5% and −9.4% ± 3.0%, respectively (vs baseline: $P = .10$ and $P < .01$; Fig. 1F, G), whereas those of the WC ≥85 group were 2.0% ± 1.7% and 0.9% ± 2.4%, respectively (vs WC <85 group: $P < .05$, each; Fig. 1F, G). The %TG in the WC <85 group was −10.2% ± 2.1% (vs baseline: $P < .001$, Fig. 1D), whereas that in the WC ≥85 group was 2.8% ± 3.3% (vs WC < 85 group: $P < .05$, Fig. 1D). However, there were no differences in %TC and %HDL-C between the 2 groups (Fig. 1C, E).

3.3. Changes in serum adiponectin and oxidative stress level after OGTT

In addition, we investigated the changes in serum adiponectin and oxidative stress levels after OGTT. There was no significant difference in percentage adiponectin (% adiponectin) between the 2 groups (Fig. 1H). However, percentage TBARS (%TBARS) was −10.8% ± 5.3% in the WC <85 group (vs baseline: $P = .07$, Fig. 1I) and 3.3% ± 3.4% in the WC ≥85 group, albeit the difference was statistically insignificant (vs WC < 85 group: $P = .057$, Fig. 1I).

4. Discussion

The main findings of the present study were as follows: (1) postprandial %TG, %SBP, and %DBP were lower significantly in the WC <85 group (vs baseline: $P = .10$, $P < .01$, and $P < .001$, respectively), but not in the WC ≥85 group; and the differences between the 2 groups were significant ($P < .05$, each); (2) %TBARS tended to be lower in the WC <85 group (vs baseline: $P = .07$), but not in the WC ≥ 85 group, albeit statistically insignificant (the WC <85 vs WC ≥85 group, $P = .057$); and (3) the response of serum adiponectin to glucose loading was similar in the 2 WC groups.

Postprandial hyperglycemia, hypertriglyceridemia, and hyperinsulinemia are recognized predictors of cardiovascular pathology [9-14,26,27]. The present study demonstrated that glucose overload resulted in a significant fall in %SBP and %DBP in the WC <85 group vs baseline (Fig. 1). This finding is similar to another study in elderly subjects, which reported a fall in blood pressure after drinking simple sugar solution [28], and adds support to the notion that glucose affects the decrease in systemic postprandial blood pressure after an increase of splanchnic blood flow in healthy subjects [28].

The present study also showed a significant postprandial decrease in %TG in the WC <85 group vs baseline. This finding is also similar to those reported previously in healthy adults [29] and healthy volunteers [30] under similar glucose challenge. What is the mechanism of the decrease in %TG in the WC <85 group? It is possible that TG decreases gradually after insulin-induced activation of lipoprotein lipase, which generally increases in response to a rise in blood glucose level, as reported previously [29,30]. The present study also demonstrated for the first time postprandial dysregulation of blood pressure and TG in the WC ≥85 group (Fig. 1). This observation might be partly due to the fact that insulin resistance affects excess sodium reservoir and dysregulation of lipoprotein lipase activity. Another potential mechanism regarding postprandial