Adiponectin and Visceral Fat Associate with Cardiovascular Risk Factors

Yumi Matsushita[1], Toru Nakagawa[2], Shuichiro Yamamoto[2], Takanori Kato[2], Takanori Ouchi[2], Nobuko Kikuchi[2], Yoshihiko Takahashi[3], Tetsuji Yokoyama[4], Tetsuya Mizoue[5] and Mitsuhiko Noda[6]

Objective: To examine the combined effect of CT-measured visceral fat area (VFA) and adiponectin level against the clustering of metabolic risk factors.

Design and Methods: The subjects were 6,996 Japanese. The subjects were divided according to the combinations of VFA and adiponectin level quartiles and the odds ratio for multiple risk factors of metabolic syndrome were calculated (adjusted for age and lifestyle factors using logistic regression analyses). Group with the lowest VFA and the highest adiponectin level was used as a reference. The correlation between adiponectin level and each metabolic risk factor was evaluated.

Results: The strongest correlation was observed between adiponectin level and high-density lipoprotein cholesterol levels (r = 0.369 and 0.439 for men and women). Both VFA and adiponectin level were independently associated with the clustering of metabolic risk factors (interaction P = 0.58 and 0.11 for men and women). The odds ratio for the clustering of metabolic risk factors in the group with the highest VFA and the lowest adiponectin level, compared with the group with the lowest VFA and the highest adiponectin level, was 12.7 (9.7-16.6) for men and 13.5 (6.0-30.2) for women.

Conclusion: The ability to detect metabolic syndrome could be improved by examining adiponectin level in conjunction with VFA.

Introduction

The prevalence of metabolic syndrome has been growing globally with clusters of obesity, high blood pressure, impaired lipid metabolism, and hyperglycemia. Individuals with metabolic syndrome have a higher risk of cardiovascular disease and a subsequent increase in disease mortality or morbidity (1-3). Several criteria for the diagnosis of metabolic syndrome are used worldwide. The visceral adipose tissue is regarded as an endocrine organ, partly because it secretes adipocytokines and other vasoactive substances that can influence the risk of developing traits of metabolic syndrome (4). We recently demonstrated that measuring the visceral fat area (VFA) is superior in predicting the accumulation of multiple risk factors, compared with the subcutaneous fat area (SFA), BMI, and waist circumference (WC) measurements (5). Regarding the multiple risk factors of metabolic syndrome, the odds ratios for the VFA quintiles were 1.0 (ref.), 2.4, 3.4, 5.0, and 9.7 for men and 1.0 (ref.), 1.5, 2.6, 4.6, and 10.0 for women (P < 0.001 for trends in both sexes) (5).

Adiponectin is predominantly secreted by adipocytes, and the adiponectin level is reduced in individuals with obesity, insulin resistance, and type 2 diabetes (6-10). Low plasma adiponectin levels have recently been shown to predict the risk of developing type 2 diabetes in humans (9,11). The adiponectin level is also inversely associated with other traditional cardiovascular risk factors, such as blood pressure, total and low-density lipoprotein (LDL) cholesterol, and triglyceride (TG) levels (12,13), and is positively related to high-density lipoprotein (HDL) cholesterol levels (12,14).

Some previous studies reported the impact of adiponectin levels on metabolic syndrome and its components (15); however, the sample sizes were insufficient. In addition, the combined effect of the VFA and adiponectin level has not been examined in an epidemiological study. Thus, we have examined the combined effect of the VFA and adiponectin level on the clustering of metabolic risk factors.

Methods

Survey

Among the 17,606 employees of the same company and their spouses who underwent a health examination in Japan between 2008

[1] Department of Clinical Research, National Center for Global Health and Medicine, Tokyo, Japan, Correspondence: Yumi Matsushita (ymatsushita@ri.ncgm.go.jp) [2] Hitachi, Ltd., Hitachi Health Care Center, Ibaraki, Japan [3] Division of Diabetes and Metabolism, Iwate Medical University, Iwate, Japan [4] Department of Health Promotion, National Institute of Public Health, Saitama, Japan [5] Department of Epidemiology and Prevention, National Center for Global Health and Medicine, Tokyo, Japan [6] Department of Diabetes and Metabolic Medicine, National Center for Global Health and Medicine, Tokyo, Japan

18

TABLE 1 Characteristics of the subjects

	Men		Women	
	Mean	(SD)	Mean	(SD)
N	6,221		775	
Age, years	52.8	(10.2)	57.4	(9.7)
Body mass index, kg/m²	24.1	(3.0)	23.0	(3.4)
Visceral fat area, cm²	121.7	(53.4)	81.6	(46.3)
Subcutaneous fat area, cm²	133.6	(56.4)	182.8	(76.9)
Adiponectin, log μg/mL	0.83	(0.20)	1.05	(0.21)
High blood pressure, %	38.9		34.1	
High triglyceride, %	35.4		23.7	
Low HDL cholesterol, %	11.0		17.8	
Hyperglycemia, %	57.8		40.8	
Multiple risk factors of metabolic syndrome, %	45.5		34.2	

and 2009, we analyzed 6,996 subjects ranging in age from 25 to 75 years (6,221 men and 775 women) who had undergone a computed tomography (CT) examination and answered a questionnaire on lifestyle factors and current treatments for metabolic conditions (hyperlipidemia, hypertension, or diabetes). The VFA was measured using a CT scanner and was calculated using a software application (fatPointer; Hitachi Medico, Tokyo, Japan) according to a protocol described elsewhere (11). Briefly, single slice imaging at the umbilical level was performed using a CT machine (Redix turbo; Hitachi Medico) while the subject was in a supine position. The imaging conditions were 120 kV, 50 mA, using a 5 mm thick slice. Height, weight, and body fat were measured using an automated scale (BF-220; Tanita, Tokyo, Japan) with the patient wearing a light gown. The BMI was defined as the weight (kg) divided by the square of the height (m²). A blood sample was collected from each subject after more than 12 hours of fasting. The glucose level was measured using the glucose oxidase enzyme-electrode method (A&T, Tokyo, Japan). TG and HDL cholesterol levels were measured using an enzymatic colorimetric method (Cholestest TG; Sekisui Medical, Tokyo, Japan) and a nonsettling enzymatic method (Cholestest NHDL; Sekisui Medical), respectively. Adiponectin levels were measured using an immunoturbidimetric method (Adiponectin Latex Kit for humans; Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). Blood pressure was measured using an automated sphygmomanometer (Kentaro ADVANCE BP-203RV III A/B; Colin, Tokyo, Japan). This study was approved by the ethics review committee of the National Center for Global Health and Medicine. Written informed consent was obtained from all the subjects.

Definition of the state of risk factor clustering

In this study, subjects were defined using the criteria of the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) guidelines (6) published in 2005: [1] high TG (TG ≥ 150 mg/dL), [2] low HDL cholesterol (HDL cholesterol <40 mg/dL in men and <50 mg/dL in women), [3] high blood pressure (systolic blood pressure ≥130 mm Hg or diastolic blood pressure ≥85 mm Hg), [4] hyperglycemia (fasting glucose ≥100 mg/dL), and [5] multiple risk factors (having two or more of components [1-4] listed

above). Subjects currently receiving treatment for hyperlipidemia, hypertension, or diabetes were deemed as having the respective risk factor, regardless of the biochemical value.

Statistical analyses

We calculated the Pearson's correlation coefficients between the adiponectin level and each metabolic risk factor. We divided the subjects according to quartiles of the adiponectin level and calculated the odds ratio for multiple risk factors of metabolic syndrome. We adjusted for age, smoking habits (never, past, current), alcohol consumption (nondrinker, drinker consuming less than 2 go per day [the go is a conventional unit of alcohol intake in Japan and contains approximately 23 g of ethanol], or drinker consuming more than 2 go per day), and regular fitness habit (based on a single yes/no question in the questionnaire) using a logistic regression analysis, with the highest adiponectin level group used as a reference. We calculated the odds ratio for the multiple risk factors of metabolic syndrome for a +1 SD increment in the quintile categories of VFA and a +1 SD increment in the quintile categories of adiponectin levels. Furthermore, we divided the subjects according to combinations of VFA and adiponectin level quartiles and calculated the odds ratio for multiple risk factors of metabolic syndrome adjusted for the above-mentioned variables, using the category with the lowest VFA and the highest adiponectin level as the reference. VFA, adiponectin levels, and their interaction term (VFA × adiponectin levels) were included as independent variables in the logistic regression model to examine the interaction effect between VFA and adiponectin levels on the risk of clustering of metabolic risk factors. The stepwise procedure was used to select variables in the multiple logistic regression model with $P < 0.1$ for entry and $P < 0.05$ for removal. All the analyses were performed using SPSS for Windows, Version 15.0 (SPSS Inc., Chicago. IL, USA).

Results

The characteristics of the subjects are shown in Table 1. The mean (SD) age of the subjects was 52.8 ± 10.2 years for men and 57.4 ± 9.7 years for women. The mean VFA was 121.7 ± 53.4 cm² in men and 81.6 ± 46.3 cm² in women. The mean BMI was 24.1 ± 3.0 kg/m² in men and 23.0 ± 3.4 kg/m² in women. The mean log adiponectin level was 0.83 ± 0.20 μg/mL in men and 1.05 ± 0.21 μg/mL in women. The prevalence of multiple risk factors of metabolic syndrome was 45.5% in men and 34.2% in women.

Table 2 shows the partial correlations between adiponectin level and each metabolic risk factor. The HDL cholesterol level positively correlated with adiponectin level ($P < 0.001$). Other metabolic risk factors negatively correlated with the adiponectin level ($P < 0.001$).

The odds ratios for each component of metabolic syndrome according to the adiponectin level are shown in Figure 1. The odds ratios for a high TG level, a low HDL cholesterol level, high blood pressure, and hyperglycemia decreased with increasing quartile categories of adiponectin levels. For the multiple risk factors of metabolic syndrome, the odds ratios (95% confidence intervals [CI]) of the Q1, Q2, Q3, and Q4 adiponectin level categories were 3.4 (3.0-4.0), 2.1 (1.8-2.5), 1.5 (1.3-1.7), and 1.0 (ref.) for men and 4.3 (2.7-6.9), 2.5 (1.6-4.1), 1.5 (0.9-2.4), and 1.0 (ref.) for women. The odds ratio (95% CI) of the lowest (Q1) adiponectin level category

19

TABLE 2 Partial correlation between adiponectin level and each metabolic risk factor

	Men	Women
VFA	−0.364	−0.428
SFA	−0.220	−0.234
BMI	−0.281	−0.237
log TG	−0.340	−0.342
HT	−0.104	−0.153
HDL cholesterol	0.369	0.439
FG	−0.103	−0.177

VFA, visceral fat area; SFA, subcutaneous fat area; BMI, body mass index; TG, triglyceride; HT, hypertension; FG, fasting glucose.
P-values are all less than 0.001.
Values are partial correlation coefficients adjusted for age, smoking habits (never, current, past), alcohol consumption (nondrinker, drinker consuming 2 go or less per day [a go is a conventional unit of alcohol intake in Japan and contains ~23 g of ethanol], or consuming more than 2 go per day), and regular fitness habit (yes/no).

for a high TG level was 3.9 (3.4-4.6) in men and that for a low HDL cholesterol level was 5.8 (4.4-7.8) in men and 9.9 (4.8-20.1) in women.

In Table 3, the odds ratios of adiponectin and VFA levels for the clustering of multiple risk factors of metabolic syndrome are shown according to the VFA and adiponectin quartiles, respectively. For men, the increased adiponectin levels were significantly related to reduced clustering of metabolic risk factors, regardless of the VFA category ($P = 0.58$ for interaction VFA × adiponectin). For women, however, the odds ratio for a +1 SD in the VFA slightly weakened according to the increment of adiponectin level category ($P = 0.11$ for interaction of VFA × adiponectin). The odds ratio for multiple risk factors of metabolic syndrome according to combined groups of VFA and adiponectin are depicted in Figure 2. The odds ratio for the multiple risk factors of metabolic syndrome in the category with the highest VFA and the lowest adiponectin levels were 12.7 (9.7-16.6) for men and 13.5 (6.0-30.2) for women. We conducted stepwise logistic regression analyses among the largest quartile group of VFA, with hypoadiponectinemia (lowest vs. highest quartile group) as the independent variable. The result revealed that smoking, high TG, low HDL cholesterol, and older age were associated with hypoadiponectinemia in men. Low HDL cholesterol and age were associated with hypoadiponectinemia in women (data not shown).

Discussion

We observed a strong association between the combined effect of an increasing VFA and a decreasing adiponectin level and the prevalence of multiple risk factors of metabolic syndrome. Both the VFA and the adiponectin level were independently associated with the clustering of metabolic risk factors. Among the components of metabolic syndrome, the adiponectin level had a particularly strong impact on a high TG level in men and a low HDL cholesterol level in both men and women.

Only one previous report, studying 68 obese Korean subjects, demonstrated an association between the adiponectin level and VFA. The adiponectin level was inversely correlated with the VFA ($r =$

−0.691, $P = 0.009$ in men, $r = -0.319$, $P = 0.002$ in women). Levels of a high molecular weight adiponectin also negatively correlated with the VFA ($r = -0.650$, $P = 0.016$ in men, $r = -0.370$, $P = 0.005$ in women) but not with the BMI or SFA, suggesting hypoadiponectinemia may represent a dysfunction of adipose tissue during obesity (16).

It was unknown whether adiponectin levels were correlated with disease, even when the VFAs were the same. Therefore, we compared the prevalence of multiple risk factors of metabolic syndrome with combinations of the VFA and adiponectin level. We found a markedly increased risk of clustering of metabolic syndrome among individuals who had a low adiponectin level and a high VFA. It was revealed that even when VFAs were the same, hypoadiponectinemia was associated with older age, smoking, and lipid metabolism (high TG and low HDL cholesterol) in men. In women, hypoadiponectinemia was associated with older age and low HDL cholesterol. Thus, it was confirmed that adiponectin correlated with lipid metabolism independent of VFA; from this, we concluded that adiponectin correlated with the clustering of metabolic risk factors. However, even
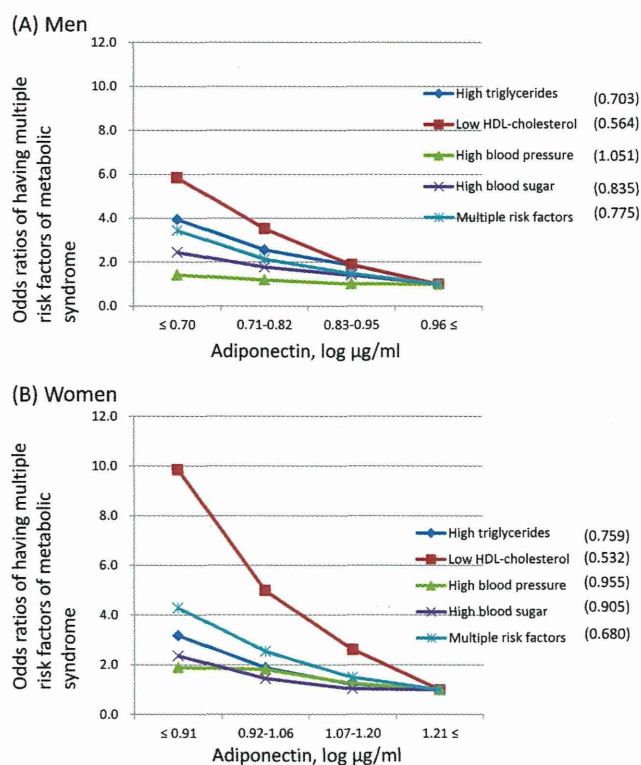
FIGURE 1 Odds ratios for clustering of metabolic risk factors according to the quartiles of adiponectin. The definition of metabolic risk factors is based on the criteria of the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) guidelines published in 2005. Subjects with two or more of the metabolic risk factors (except for waist circumference) were defined as having multiple risk factors. Odds ratios for clustering of metabolic risk factors according to the quartiles (Q1-Q4) of adiponectin adjusted for age, smoking habits (never, current, past), alcohol consumption (nondrinker, drinker consuming 2 go or less per day [a go is a conventional unit of alcohol intake in Japan and contains ~23 g of ethanol], or consuming more than 2 go per day), and regular fitness habit (yes/no). Values in the case arcs are odds ratios for +1 SD increments of adiponectin. (Multiple risk factors; multiple risk factors of metabolic syndrome.)

20

TABLE 3 Odds ratios for clustering of metabolic risk factors

		n		Odds ratios of +1 SD increment of adiponectin	
Men					
VFA (cm²)	≤84.80	1,557	Q1	0.8	(0.7-0.9)
	84.81-120.10	1,559	Q2	0.8	(0.7-0.9)
	120.11-156.90	1,553	Q3	0.8	(0.7-0.9)
	156.91≤	1,552	Q4	0.8	(0.7-0.9)
Women					
VFA (cm²)	≤44.30	194	Q1	0.7	(0.4-1.2)
	44.31-77.90	195	Q2	0.5	(0.3-0.9)
	77.91-113.10	193	Q3	0.8	(0.6-1.2)
	113.11≤	193	Q4	0.6	(0.4-0.9)
		n		Odds ratios of +1 SD increment of VFA	
Men					
Adiponectin (log µg/mL)	≤0.70	1,620	Q1	2.1	
	0.71-0.82	1,556	Q2	2.2	(1.8-2.3)
	0.83-0.95	1,518	Q3	1.9	(1.9-2.5)
	≤0.96	1,527	Q4	2.1	(1.7-2.2)
Women					(1.9-2.4)
Adiponectin (log µg/mL)	≤0.91	198	Q1	2.6	(1.7-3.9)
	0.92-1.06	191	Q2	2.5	(1.6-3.8)
	1.07-1.20	193	Q3	2.1	(1.3-3.3)
	1.21≤	193	Q4	1.7	(1.1-2.5)

VFA, visceral fat area.
Odds ratios of multiple risk factors of metabolic syndrome according to 1 SD increment of VFA or adiponectin adjusted for age, smoking habits (never, current, past), alcohol consumption (nondrinker, drinker consuming 2 go or less per day [a go is a conventional unit of alcohol intake in Japan and contains ~23 g of ethanol], or consuming more than 2 go per day), and regular fitness habit (yes/no).

if the adiponectin levels were the same, weight gain led to a worsening of metabolic risk factors, including lipid. In the largest VFA group, adiponectin itself was related to lipid metabolism and smoking status. Therefore, it was shown that the ability to detect metabolic syndrome would be improved by examining the adiponectin level in conjunction with the VFA, as adiponectin correlated with metabolic risk factors independent of VFA and the correlation was most pronounced between lipid metabolism.

In a case–control study, high plasma adiponectin levels were associated with a lower risk of myocardial infarction (MI) over a follow-up period of 6 years among men without previous cardiovascular disease. After adjustment for matched variables, participants in the highest quintile, compared with the lowest quintile, of adiponectin levels had a significantly decreased risk of MI (relative risk [RR], 0.39; 95% confidence interval [CI], 0.23-0.64; P for trend <0.001). Further adjustment for the hemoglobin A1c or C-reactive protein levels had little impact, but additional adjustment for LDL and HDL cholesterol levels modestly attenuated this association (RR, 0.56; 95% CI, 0.32-0.99; P for trend =0.02) (17). A multiple logistic regression analysis revealed that hypoadiponectinemia was significantly and independently correlated with coronary artery disease (CAD) ($P < 0.0088$) among 450 Japanese men. The multivariate-adjusted odds ratios for CAD in the first, second, third, and fourth quartiles (95% confidence) were 2.051 (1.288-4.951), 1.221 (0.684-2.186), 0.749 (0.392-1.418), and 1.000, respectively (18). Further-

more, another study showed that BMI, serum TG concentration, and the presence of diabetes or CAD remained significantly related to the plasma adiponectin concentration. Weight reduction significantly elevated the plasma adiponectin levels in diabetic obese Japanese subjects (six men and seven women) and nondiabetic obese Japanese subjects (six men and three women). However, the sample size was very small in this study (6).

This study has several strengths and limitations. As one of its strengths, we directly assessed abdominal fat accumulation using CT scanning. This allowed the role of fat deposition in the development of metabolic syndrome and its components to be examined more closely. Secondly, the sample size of our study was sufficiently large (almost 7,000 subjects), and both sexes were included. Thirdly, we adjusted for alcohol consumption and physical activity, which may confound the association between abdominal fat accumulation and metabolic risk factors. However, the study was limited because of its cross-sectional design, and changes in the metabolic risk profile were not monitored.

In this study, we demonstrated a strong association between the combined effect of an increasing VFA and a decreasing adiponectin level with multiple risk factors of metabolic syndrome. Both the VFA and the adiponectin level were independently associated with the clustering of metabolic risk factors in a large Japanese population, which has a relatively low BMI compared with other
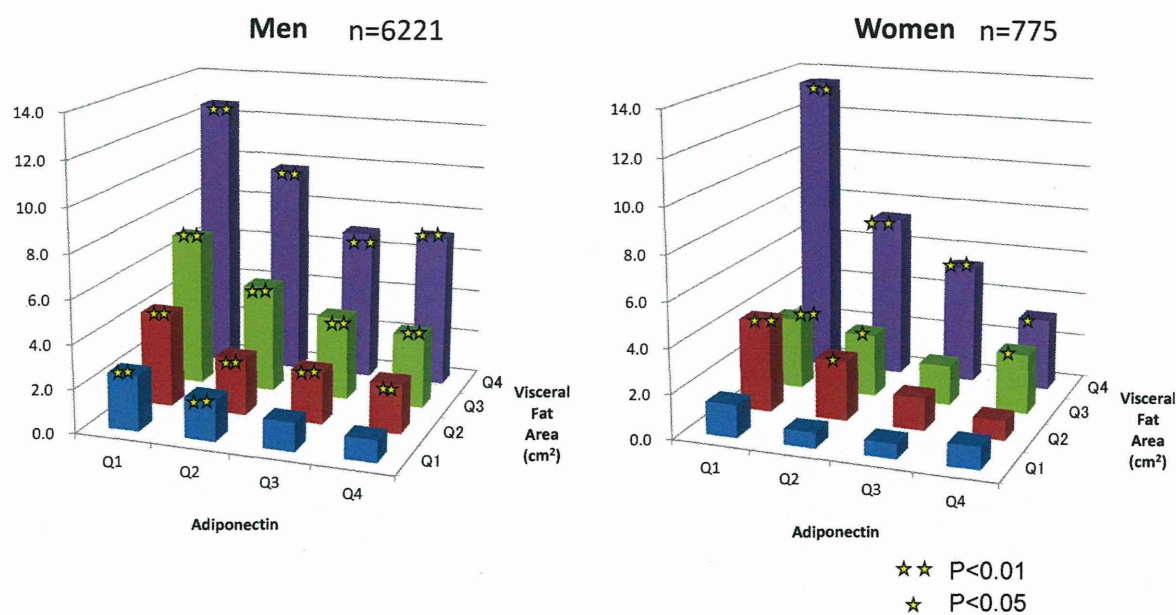
FIGURE 2 Odds ratios for multiple risk factors of metabolic syndrome according to combined groups of VFA and adiponectin. The definition of metabolic risk factors is based on the criteria of the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) guidelines published in 2005. Subjects with two or more of the metabolic risk factors (except for waist circumference) were defined as having multiple risk factors. Odds ratios for multiple risk factors of metabolic syndrome are shown according to combined groups of VFA and adiponectin adjusted for age, smoking habits (never, current, past), alcohol consumption (nondrinker, drinker consuming 2 go or less per day [a go is a conventional unit of alcohol intake in Japan and contains ~23 g of ethanol], or consuming more than 2 go per day), and regular fitness habit (yes/no).

ethnicities. The present findings have important implications for the prevention of metabolic syndrome. Further prospective studies are needed to assess the impact of the VFA and adiponectin level on the incidence of metabolic syndrome or cardiovascular diseases.**O**

© 2013 The Obesity Society

References

1. Lakka HM, Laaksonen DE, Lakka TA, et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA* 2002;288:2709-2716.
2. Isomaa B, Almgren P, Tuomi T, et al. Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabet Care* 2001;24:683-689.
3. Grundy SM, Brewer HB Jr, Cleeman JI, et al. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* 2004;109:433-438.
4. World Health Organization. Measuring Obesity: *Classification and Description of Anthropometric Data*. Geneva: World Health Org., 1989 (Nutr UD, EUR/ICP/NUT 125).
5. Hara K, Matsushita Y, Horikoshi M, et al. A proposal for the cutoff point of waist circumference for the diagnosis of metabolic syndrome in the Japanese population. *Diabet Care* 2006;29:1123-1124.
6. Hotta K, Funahashi T, Arita Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 2000;20:1595-1599.
7. Halleux CM, Takahashi M, Delporte ML, et al. Secretion of adiponectin and regulation of apM1 gene expression in human visceral adipose tissue. *Biochem Biophys Res Commun* 2001;288:1102-1107.
8. Weyer C, Funahashi T, Tanaka S, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* 2001;86:1930-1935.
9. Lindsay RS, Funahashi T, Hanson RL, et al. Adiponectin and development of type 2 diabetes in the Pima Indian population. *Lancet* 2002;360:57-58.
10. Combs TP, Wagner JA, Berger J, et al. Induction of adipocyte complement-related protein of 30 kilodaltons by PPARγ agonists: a potential mechanism of insulin sensitization. *Endocrinology* 2002;143:998-1007.
11. Spranger J, Kroke A, Möhlig M, et al. Adiponectin and protection against type 2 diabetes mellitus. *Lancet* 2003;361:226-228.
12. Matsubara M, Maruoka S, Katayose S. Decreased plasma adiponectin concentrations in women with dyslipidemia. *J Clin Endocrinol Metab* 2002;87:2764-2769.
13. Kazumi T, Kawaguchi A, Sakai K, Hirano T, Yoshino G. Young men with high-normal blood pressure have lower serum adiponectin, smaller LDL size, and higher elevated heart rate than those with optimal blood pressure. *Diabet Care* 2002;25:971-976.
14. Cnop M, Havel PJ, Utzschneider KM, et al. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia* 2003;46:459.
15. Kajikawa Y, Ikeda M, Takemoto S, Tomoda J, Ohmaru N, Kusachi S. Association of circulating levels of leptin an adiponectin with metabolic syndrome and coronary heart disease in patients with various coronary risk factors. *Int heart J* 2011;52:17-22.
16. Kishida K, Kim KK, Funahashi T, Matsuzawa Y, Kang HC, Shimomura I. Relationships between circulating adiponectin levels and fat distribution in obese subjects. *J Atheroscler Thromb* 2011;18:592-595.
17. Pischon T, Girman CJ, Hotamisligil GS, Rifai N, Hu FB, Rimm EB. Plasma adiponectin levels and risk of myocardial infarction in men. *JAMA* 2004;291:1730-1737.
18. Kumada M, Kihara S, Sumitsuji S, et al. Coronary artery disease. Association of hypoadiponectinemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol* 2003;23:85-89.

DIABETOLOGY &
METABOLIC SYNDROME

RESEARCH   Open Access

How can waist circumference predict the body composition?

Yumi Matsushita[1*], Toru Nakagawa[2], Michihiro Shinohara[2], Shuichiro Yamamoto[2], Yoshihiko Takahashi[3], Tetsuya Mizoue[4], Tetsuji Yokoyama[5] and Mitsuhiko Noda[6]

Abstract

Background: Waist circumference (WC) is used as a risk assessment for metabolic syndrome, diabetes, and cardiovascular disease (CVD). WC consists of visceral fat area (VFA), subcutaneous fat area (SFA), muscle, intramuscular fat, viscera, and bone. Each component of the WC may differ between the sexes and generations, even if they have the same WC. However, this has not been measured in an epidemiological study.

Methods: Between 2004 to 2009, employees and their spouses working at a Japanese company underwent a health examination after more than 12 hours of fasting. We analyzed the data of 11,570 subjects (9,874 men and 1,696 women), aged from 20 to 76 years, who underwent a computed tomography (CT) examination. VFA, SFA, WC, muscle, intramuscular fat, viscera, and bone were measured using a CT scanner. We conducted stratified analyses by generational age, and calculated the Pearson's correlation coefficients between the VFA and WC, BMI, and VFA plus SFA. To establish the equations for converting the WC to the corresponding VFA and VFA plus SFA, linear regression analyses were used to obtain the regression coefficients and intercepts.

Results: As the generations increased in age, the VFA tended to increase. However, the differences in the WC values of each generation did not coincide with the VFA values in men (r = −0.275 and 0.979 for men and women, n = 5 generations), but did correlate with the difference in the sum of the VFA plus SFA for both sexes (r = 0.915 and 0.996 for men and women, n = 5 generations). Older generations had lower WC values when they had the same VFA values as the younger generations.

Conclusions: The WC value corresponding to a certain VFA value differed significantly by generational age. Thus, revised optimal cutoff values for the WC may be needed for each generation.

Background

Metabolic syndrome (MS) is characterized by central obesity, impaired glucose tolerance, high blood pressure, and abnormal lipid metabolism [1]. Individuals with MS have a higher risk of cardiovascular disease, and a subsequent increase in disease mortality and morbidity [2-4]. In 2008, the age-standardized death rates of cardiovascular disease per 100,000 in Japanese, Chinese, Indonesian, and American men were 121.3, 311.2, 186.8, and 366.1, respectively. The rates in Japanese, Chinese, Indonesian, and American women were 76.0, 263.0, 128.4, and 268.7, respectively [5]. The prevalence of MS defined by the modified National Cholesterol Education Program's

Adult Treatment Panel III (NCEP-ATP) guidelines [6] in Japan and China was 20.6% [7] and 27.6% [8], respectively.

In the diagnosis of MS, the waist circumference (WC) is almost always used as a criteria, and this measurement is typically used as a surrogate measure of the visceral fat area (VFA) [1,6,9,10]. A previous study demonstrated the prevalence of MS under the NCEP-ATP III definitions (the obesity index used BMI at more than 25, instead of WC) in Asian ethnic groups (Japan, Korea, and Mongolia) using the same research design and protocols for all subjects [11]. There were no significant differences in the prevalence of MS among the three Asian ethnic groups, in spite of great differences in BMI and in each metabolic parameter.

Several studies have shown a strong correlation between the VFA and anthropometric values, such as the BMI and WC [12,13], thus, these easily measurable anthropometric

* Correspondence: ymatsushita@ri.ncgm.go.jp
[1]Department of Clinical Research, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan
Full list of author information is available at the end of the article

values have been used as surrogate indices of the VFA, regardless of age. Body composition can differ greatly with age [14,15] and it is unknown whether the relationships between the VFA and BMI or WC are the same throughout the different age groups. Previous studies have shown that visceral adipose tissue positively correlated with age in both men and women [13,16], but there were differences in the mean BMI [14] and the mean WC [14,15] among the different age groups. Differences in body components according to age has not yet been clarified in a large epidemiological study.

In this study, we investigated correlations of the VFA, measured by computed tomography (CT), with WC and BMI, and compared the mean VFA and subcutaneous fat area (SFA) according to gender and age groups. Furthermore, we analyzed the CT image divided into VFA, SFA, muscle, visceral and bones to examine how WC can predict body composition.

Methods
Participants and procedures
Employees and their spouses working at the Hitachi company, Ibaraki Prefecture, between 2004 to 2009, underwent a health examination after more than 12 hours of fasting. Of these participants, we analyzed the data for 11,570 subjects (9,874 men and 1,696 women), aged between 20 to 76 years, who underwent a CT examination. Body height and weight were measured using an automated scale (BF-220; TANITA), and the BMI was defined as the weight (kg) divided by the square of the height (m^2). The VFA, SFA and WC were measured using a CT scanner and protocols described elsewhere [17]. Briefly, single slice imaging was performed at the umbilicus level in a supine position, using a Redix turbo CT machine (Hitachi Medico). The imaging conditions were 120 kV, 50 mA, and a slice thickness of 5 mm. The VFA, SFA and the WC were calculated using fatPointer software (Hitachi Medico). This system also divided and analyzed CT-scanned images into 5 areas (VFA, SFA, muscle, intramuscular fat, and viscera) and others (bone and air). The division process was performed in the following way: first, the body area was extracted from the CT-scanned abdominal image. Then, the specific body area without fat (epidermis, bone, muscle and visceral organs; external fat area) was extracted from the initial extracted body area. Next, the external fat area was segregated into the viscera and subcutaneous fat, based on the location area of the initial extracted body area. And then, areas of muscle, intramuscular fat, and visceral organs were segregated from the located information of the contained visceral and the external fat area. Finally, each separated area was analyzed and displayed in different colors. HbA1c levels were measured using a HPLC method with an ADAMS HA8160 device (Arkrey). Blood glucose levels were measured using the glucose electrode technique with an ADAMS glucose GA1170 device (Arkrey). Blood pressure was measured using an oscillometric method with a Kentaro ADVANCEBP-203RV III A/B device (Colin) while the patient was in a sitting position and after the patient had rested for 3 min. Informed consent was obtained from each examinee regarding the use of his or her data for research purposes. The present study protocol was approved by the ethics review committee of the National Center for Global Health and Medicine.

Statistical analyses
All analyses were performed according to gender. We conducted stratified analyses by generational age (under 39, 40–49, 50–59, 60–69, and over 70 years). To compare the characteristics of the subjects in the five age groups, ANOVA or regression analysis was used for the continuous variables, such as VFA, SFA, WC, BMI, and laboratory values. We calculated the Pearson's correlation coefficients between the VFA and WC, BMI, and VFA plus SFA. We also calculated the Pearson's correlation coefficients between the VFA plus SFA and the anthropometric indices (WC and BMI). To establish the equations for converting the WC to the corresponding VFA and VFA plus SFA, linear regression analyses were used to obtain the regression coefficients and intercepts. All analyses were performed using SPSS software for Windows, Version 15.0 (SPSS Inc., IL, USA).

Results
The characteristics of the subjects are shown in Table 1. The mean age of the study subjects was 52.8 ± 10.0 years for men and 57.2 ± 9.5 years for women. The mean BMI was 24.1 ± 3.0 kg/m^2 in men and 23.1 ± 3.5 kg/m^2 in women. The mean VFA was 123.2 ± 53.5 cm^2 in men and 84.4 ± 47.1 cm^2 in women. The mean WC was 86.6 ± 11.4 cm in men and 83.8 ± 9.5 cm in women. The mean HbA1c was $5.8 \pm 0.7\%$ in men and $5.8 \pm 0.8\%$ in women. The mean fasting plasma glucose was 106.5 ± 20.9 mg/dL in men and 100.9 ± 20.1 mg/dL in women. The mean systolic blood pressure was 121.7 ± 12.3 mmHg in men and 119.9 ± 14.3 mmHg in women. The mean diastolic blood pressure was 77.4 ± 8.3 mmHg in men and 74.1 ± 9.1 mmHg in women. All P for trends across the age groups were significant (P < 0.001). All p-values for homogeneity among the age groups were also significant at p < 0.001 by analysis of variance (data not shown).

Correlations between the VFA and the anthropometric indices are shown in Table 2. The VFA strongly correlated with WC and BMI in both men and women (r > 0.650), but the correlation coefficients were different in each age group.

Correlations between the VFA plus SFA and the anthropometric indices are shown in Table 3. The VFA plus SFA

24

Table 1 Characteristics of subjects

		Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)	Mean	(SD)	P for trend
	Age (years)	−39		40-49		50-59		60-69		70-		
Men	n	1360		2768		3328		1891		527		
	VFA (cm²)	100.7***	(49.0)	118.7***	(51.8)	125.6***	(53.9)	127.2***	(56.9)	128.6***	(57.4)	<0.001
	SFA (cm²)	146.4	(71.2)	144.3***	(63.7)	129.0***	(50.8)	117.8***	(44.4)	120.6***	(45.0)	<0.001
	Waist circumference (cm)	85.9***	(10.0)	87.0***	(9.2)	86.4***	(8.4)	85.0	(8.2)	85.3	(8.8)	<0.001
	BMI (kg/m²)	24.3***	(3.5)	24.4***	(3.3)	23.9***	(2.8)	23.6*	(2.6)	23.5*	(2.6)	<0.001
	VFA + SFA (cm²)	247.1***	(110.0)	263.0***	(104.6)	254.7*	(94.5)	245.0***	(91.9)	249.2**	(92.7)	0.007
	VFA/SFA (cm²)	0.7***	(0.3)	0.9***	(0.3)	1.0***	(0.4)	1.1***	(0.4)	1.1***	(0.4)	<0.001
	HbA1c (%)	5.6	(0.5)	5.7	(0.7)	5.9**	(0.8)	6.0***	(0.8)	6.0	(0.8)	<0.001
	Fasting plasma glucose (mg/dL)	100***	(13)	104***	(19)	109***	(23)	110***	(22)	109	(22)	<0.001
	Systolic blood pressure (mm Hg)	118***	(11)	120***	(12)	122***	(12)	125*	(13)	127	(12)	<0.001
	Diastolic blood pressure (mm Hg)	74***	(8)	77***	(9)	79***	(8)	79***	(8)	77***	(8)	<0.001
Women	n	133		291		595		587		90		
	VFA (cm²)	45.6	(33.1)	61.6	(41.6)	79.5	(41.6)	93.6	(46.7)	97.5	(48.4)	<0.001
	SFA (cm²)	152.4	(75.3)	169.6	(79.7)	185.2	(75.6)	187.0	(71.0)	186.5	(70.9)	<0.001
	Waist circumference (cm)	78.0	(9.0)	81.1	(10.0)	83.4	(9.3)	84.5	(9.6)	84.5	(8.7)	<0.001
	BMI (kg/m²)	21.6	(3.5)	22.7	(3.6)	23.0	(3.4)	23.2	(3.2)	22.8	(3.1)	<0.001
	VFA + SFA (cm²)	197.9	(103.5)	231.2	(113.8)	264.7	(108.0)	280.6	(106.6)	284.0	(107.0)	<0.001
	VFA/SFA (cm²)	0.3	(0.1)	0.4	(0.2)	0.4	(0.2)	0.5	(0.2)	0.5	(0.2)	<0.001
	HbA1c (%)	5.5	(0.3)	5.7	(0.9)	5.8	(0.9)	5.9	(0.6)	6.0	(0.6)	<0.001
	Fasting plasma glucose (mg/dL)	93	(7)	98	(22)	101	(24)	103	(17)	105	(18)	<0.001
	Systolic blood pressure (mm Hg)	111	(12)	114	(13)	120	(14)	124	(14)	127	(11)	<0.001
	Diastolic blood pressure (mm Hg)	69	(9)	71	(8)	75	(9)	76	(9)	75	(7)	<0.001

VFA; Visceral fat area, SFA; Subcutaneous fat area, BMI; Body mass index.
*P < 0.05, **P < 0.01, ***P < 0.001 for differences in mean values between men and women in each age group by *t*-test.
P for trend across the age groups was calculated by a linear regression analysis where the age group was coded as consecutive integers. All p-values for homogeneity among the age groups were also significant at p < 0.001 by analysis of variance (not shown in table).

strongly correlated with WC in both men and women (r > 0.901), but the correlation coefficients were different in each age group.

Mean values of the VFA, SFA and WC within each age group are shown in Figure 1. In men, the mean WC decreased while the mean VFA paradoxically increased

Table 2 Correlation coefficients between the VFA and the anthropometric indices

	Age	−39	40-49	50-59	60-69	70-	All
Men	n	1360	2768	3328	1891	527	9874
	SFA	0.664	0.637	0.627	0.638	0.634	0.581
	WC	0.780	0.809	0.786	0.794	0.743	0.771
	BMI	0.694	0.681	0.678	0.686	0.685	0.650
Women	n	133	291	595	587	90	1696
	SFA	0.793	0.735	0.671	0.624	0.592	0.662
	WC	0.806	0.798	0.785	0.765	0.800	0.784
	BMI	0.808	0.773	0.761	0.710	0.714	0.727

Values are Pearson's correlation coefficients.
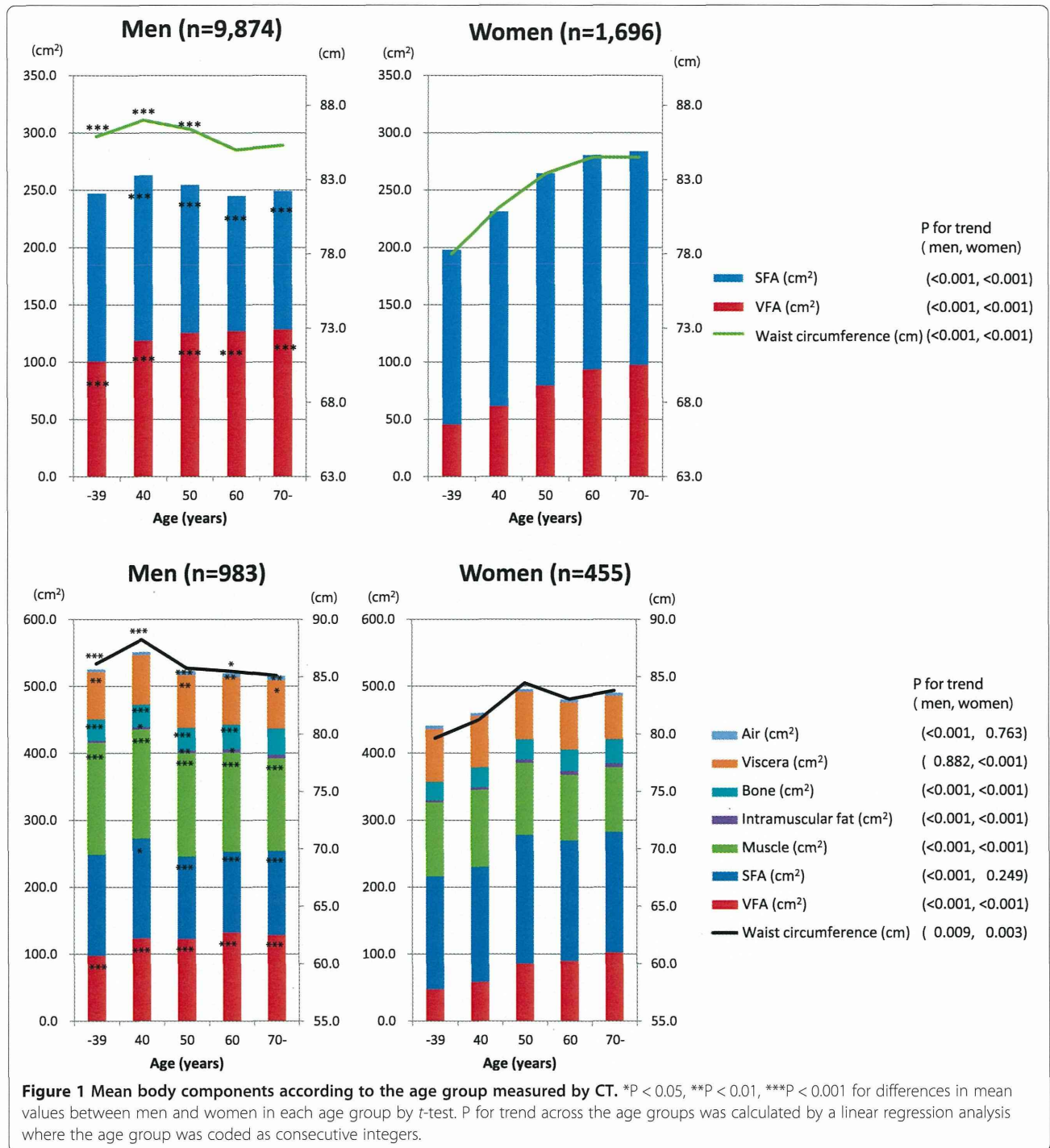All correlation coefficients were statistically significant at p < 0.001.

with age, thus showing almost no correlation between the mean WC and the mean VFA among the 5 age groups (r = −0.275, n = 5). However, a moderate correlation was shown between individuals' WC and VFA (r = 0.771). When the VFA and SFA were summed, the pattern of the change in the mean WC and the mean VFA + SFA among the age groups became very similar (r = 0.915, n = 5) and the correlation between individuals' WC and VFA + SFA

Table 3 Correlation coefficients between the VFA + SFA and the anthropometric indices

	Age	−39	40-49	50-59	60-69	70-	All
Men	n	1360	2768	3328	1891	527	9874
	WC	0.912	0.940	0.885	0.873	0.810	0.901
	BMI	0.869	0.860	0.825	0.803	0.808	0.838
Women	n	133	291	595	587	90	1696
	WC	0.953	0.933	0.954	0.935	0.959	0.945
	BMI	0.869	0.862	0.873	0.848	0.840	0.857

Values are Pearson's correlation coefficients.
All correlation coefficients were statistically significant at p < 0.001.

Figure 1 Mean body components according to the age group measured by CT. *P < 0.05, **P < 0.01, ***P < 0.001 for differences in mean values between men and women in each age group by *t*-test. P for trend across the age groups was calculated by a linear regression analysis where the age group was coded as consecutive integers.

became very strong (r = 0.901). Thus, it was demonstrated that the difference in the age-specific mean WC did not reflect the VFA alone, but represented the VFA + SFA because of the difference in the SFA among the age groups. In women, the association of age with the mean WC was very similar to the association of age with the mean VFA and mean VFA + SFA: the association being stronger with the latter (r = 0.979 and 0.996, n = 5). At the

individual level, VFA + SFA was more strongly correlated to WC (r = 0.945) than VFA (r = 0.784). With respect to the differences between men and women, the ratios of WC to the VFA + SFA were greater in men than in women (i.e., the line of the mean WC was located at a higher position in men than in women). To clarify the reason for the greater ratios in men, we measured body components (VFA, SFA, muscle, intramuscular fat, bone, viscera, and

air) in randomly selected men (n = 983) and women (n = 455). As shown in Figure 1, the muscle area was larger in men than in women in any age group; bone area was slightly larger in men than in women; and viscera were similar between men and women. It was clearly shown that the difference in WC to the VFA + SFA was due to the larger muscle area in men.
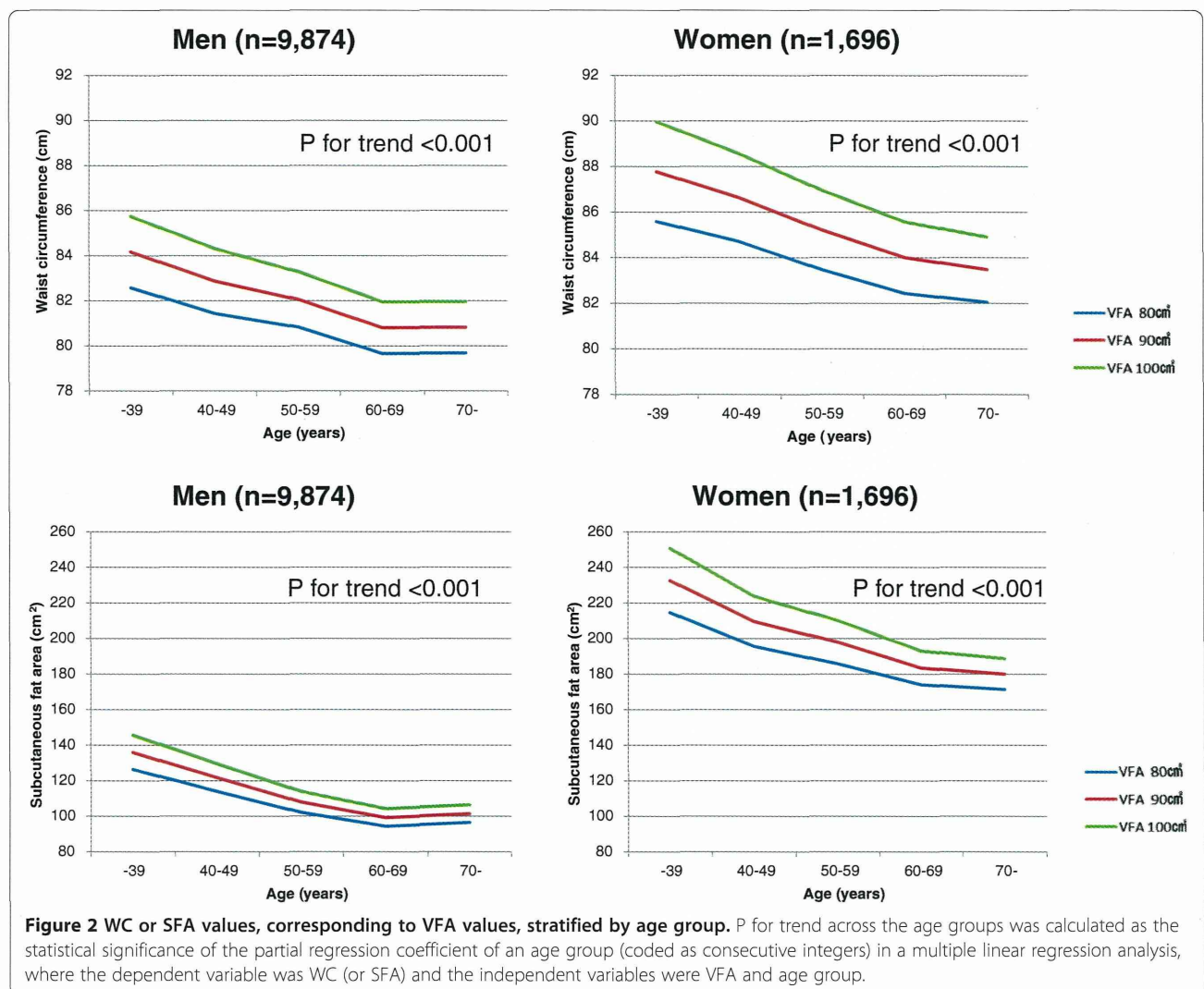
The difference in the WC values in each age group, with corresponding VFA values of 80 cm², 90 cm², and 100 cm², is shown in Figure 2. In both men and women, the older generations had lower WC values compared to the younger generations when the VFA values were the same.

Discussion

The present study showed that, as the generations become older, the VFA tended to increase in both men and women. However, the differences in the WC values

in the age groups did not coincide with the VFA values, but did correlate with the difference in the sum of the VFA plus SFA in both genders. In the current criteria for metabolic syndrome, we used a common cut-off value for the WC for all ages. When using this cut-off threshold, it raises a problem in that the VFA for the younger generations is underestimated in both genders. Therefore, different cut-off values for the WC according to age should be considered.

There is no study which have evaluated the sex- and age-specific differences in body composition in large populations using more 'direct' methods, such as CT. Our study is the first report describing the body composition by age and sex using CT. In a previous study, Ito et al. determined body composition by DXA for the whole body and three anatomical regions: arms, legs, and trunk [15]. They reported curvilinear relationships in men for variables associated with adiposity, i.e. the BMI, WC, total

Figure 2 WC or SFA values, corresponding to VFA values, stratified by age group. P for trend across the age groups was calculated as the statistical significance of the partial regression coefficient of an age group (coded as consecutive integers) in a multiple linear regression analysis, where the dependent variable was WC (or SFA) and the independent variables were VFA and age group.

or regional FM and % FM, with peaks observed in the forties age group. In women, these variables increased linearly in older subjects. The trends of the BMI and WC with age were similar to the results of our study. Although an age-related decline in lean mass was described [18], studies on large populations are scarce [19]. Furthermore, we expanded the calculations of the VFA and SFA separately using CT. The WC has been used as a simple index to measure the VFA in criteria used to diagnose metabolic syndrome [6]. However, this study showed that WC values corresponding to certain VFA values differed in each generation. Thus, a uniform value for the WC should not be used.

Furthermore, Ito et al. calculated the percentage fat mass of the whole body (% FM) as $100 \times (FM)/(FM + lean mass + bone mineral content)$ [15]. They showed that the % FM in every generation was about 1.5-fold higher in women than in men. The results of the present study showed that in the older generations, men and women in their 60s or over, had similar WC values, but the sum of the VFA plus SFA differed between the genders. The sum of the fat content (VFA + SFA) was greater in women than in men, while the physical size of the viscera did not differ largely between the genders. We clarified that the muscle area contributed to the men's greater WC. Our results were consistent with previously reported results. The correspondence of WC to VFA also differed by sex and age group. For example, for the women in the youngest group, the WC corresponding to a VFA of 100 cm^2 was about 90 cm, whereas this was about 85 cm in the most elderly group: a discrepancy of about 5 cm. Similarly, in men, the elderly group had mean WC values about 4 cm smaller than the younger group.

The present study has several strengths. One of its strengths is the direct assessment of the VFA, SFA, WC, muscle, intramuscular fat, viscera, and bone using CT scanning. This allowed for precise determination of WC component. This is the first study to analyze the WC component using CT. In addition, the sample size of our study was extremely large (> 11,000 subjects). Nevertheless, the current study also has a limitation: the study subjects were chosen from a particular place (Hitachi Company) which is not representative of the general population. However, we confirmed similar mean BMI of each age group between our data and the National Nutrition Survey in Japan [20] (data not shown), suggesting our results could be used to be indicative of the general population.

Conclusions

We concluded that differences in the values of WC by generational age did not coincide with the VFA. However, differences in the values of the sum of the VFA plus SFA did correlate with WC in both genders. The WC has been used as a simple index to measure the VFA in the criteria used to diagnose metabolic syndrome, but a WC value corresponding to a certain VFA value differed significantly by generational age. Thus, revised optimal cutoff values for the WC may be needed for each generation.

Authors' contributions
The principal investigator is YM, National Center for Global Health and Medicine. YM takes full responsibility for the work as a whole, including the study design, access to data, and the decision to submit and publish the manuscript. YM, TN, MS, and SY researched the data. YM, TN, YT, TM, TY, and MN contributed to discussions. YM wrote the manuscript. YM, TN, SY, TM, TY, and MN reviewed and edited the manuscript. All authors read and approved the final manuscript.

Author details
[1]Department of Clinical Research, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan. [2]Hitachi, Ltd. Hitachi Health Care Center, 4-3-16, Ose-cho, Hitachi-shi, Ibaraki-ken 317-0076, Japan. [3]Division of Diabetes and Metabolism, Iwate Medical University, 19-1 Uchimaru, Morioka, Iwate 020-8505, Japan. [4]Department of Epidemiology and Prevention, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan. [5]Department of Health Promotion, National Institute of Public Health, 2-3-6 Minami, Wako, Saitama 351-0197, Japan. [6]Department of Diabetes Research, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan.

References
1. Matsuzawa Y: Metabolic syndrome-definition and diagnostic criteria in Japan. *J Jpn Soc Int Med* 2005, **94**:188–203.
2. Lakka HM, Laaksonen DE, Lakka TA, Niskanen LK, Kumpusalo E, Tuomilehto J, Salonen JT: The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA* 2002, **288**:2709–2716.
3. Isomaa B, Almgren P, Tuomi T, Forsén B, Lahti K, Nissén M, Taskinen MR, Groop L: Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care* 2001, **24**:683–689.
4. Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C, American Heart Association, National Heart, Lung, and Blood Institute: Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation* 2004, **109**:433–438.
5. World Health Organization: *Mondiale De La Santé.* Department of Measurement and Health Information; 2011 [article online], April 2011. [http://apps.who.int/gho/data/node.main.1005?lang=en].
6. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F, American Heart Association; National Heart, Lung, and Blood Institute: Diagnosis and management of the metabolic syndrome: an American Heart Association/ National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005, **112**:2735–2752.
7. Takami H, Nakamoto M, Uemura H, Katsuura S, Yamaguchi M, Hiyoshi M, Sawachika F, Juta T, Arisawa K: Inverse correlation between coffee consumption and prevalence of metabolic syndrome: baseline survey of the Japan Multi-Institutional Collaborative Cohort (J-MICC) study in Tokushima, Japan. *J Epidemiol* 2013, **23**:12–20.
8. Xi B, He D, Hu Y, Zhou D: Prevalence of metabolic syndrome and its influencing factors among the Chinese adults: the China Health and Nutrition Survey in 2009. *Prev Med* 2013. in press.

9. World Health Organization: *Definition, Diagnosis and Classification of Diabetes and its Complications: Report of a WHO Consultation.* Geneva: World Health Org; 1999. WHO/NCD/NCS/99.2.
10. International Diabetes Federation: *IDF Worldwide Definition of the Metabolic Syndrome [Article Online].* 2005. [http://www.idf.org/node/1271?unode=1120071E-AACE-41D2-9FA0-BAB6E25BA072] Accessed Feb 3, 2014.
11. Shiwaku K, Nogi A, Kitajima K, Anuurad E, Enkhmaa B, Yamasaki M, Kim JM, Kim IS, Lee SK, Oyunsuren T, Yamane Y: **Prevalence of the metabolic syndrome using the modified ATP III definitions for workers in Japan, Korea and Mongolia.** *J Occup Health* 2005, **47**:126–135.
12. Examination Committee of Criteria for 'Obesity Disease' in Japan; Japan Society for the Study of Obesity: **New criteria for 'obesity disease' in Japan.** *Circ J* 2002, **66**:987–992.
13. Oka R, Miura K, Sakurai M, Nakamura K, Yagi K, Miyamoto S, Moriuchi T, Mabuchi H, Yamagishi M, Takeda Y, Hifumi S, Inazu A, Nohara A, Kawashiri MA, Kobayashi J: **Comparison of waist circumference with body mass index for predicting abdominal adipose tissue.** *Diabetes Res Clin Pract* 2009, **83**:100–105.
14. Yoshiike N, Seino F, Tajima S, Arai Y, Kawano M, Furuhata T, Inoue S: **Twenty-year changes in the prevalence of overweight in Japanese adults: the National Nutrition Survey 1976–95.** *Obes Rev* 2002, **3**:183–190.
15. Ito H, Ohshima A, Ohto N, Ogasawara M, Tsuzuki M, Takao K, Hijii C, Tanaka H, Nishioka K: **Relation between body composition and age in healthy Japanese subjects.** *Eur J Clin Nutr* 2001, **55**:462–470.
16. Fox CS, Massaro JM, Hoffmann U, Pou KM, Maurovich-Horvat P, Liu CY, Vasan RS, Murabito JM, Meigs JB, Cupples LA, D'Agostino RB Sr, O'Donnell CJ: **Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study.** *Circulation* 2007, **116**:39–48.
17. Nakagawa T, Yamamoto S, Irokawa M: **Development of the automated diagnosis CT screening system for visceral obesity.** *Asian Pacific Journal of Disease Management* 2008, **2**:31–38.
18. Forbes GB: *Human Body Composition.* New York: Springer; 1987.
19. Rico H, Revilla M, Villa LF, Ruiz-Contreras D, Hernández ER, Alvarez de Buergo M: **The four-compartment models in body composition: data from a study with dual-energy X-ray absorptiometry and near-infrared interactance on 815 normal subjects.** *Metabolism* 1994, **43**:417–422.
20. Division of Health Promotion and Nutrition, Ministry of Health, Labour and Welfare: *Annual report of the National Nutrition Survey in 2006.* Tokyo: Daiichi Publishing Co; 2008. in Japanese.