Table 3. Stepwise multiple regression analysis between percent changes in lipid parameters and age, %dWC, and %dBMI

	Women					Men				
	β	95%CI		Standardized β	p value	β	95%CI		Standardized β	p value
Model 1										
Dependent variable, %dLDL										
%dBMI	0.72	0.44	0.99	0.15	<0.001	0.86	0.62	1.10	0.16	<0.001
age						−0.08	−0.15	−0.01	−0.05	0.019
Dependent variable, %dHDL										
%dBMI	−0.23	−0.43	−0.03	−0.07	0.026	−0.70	−0.88	−0.53	−0.17	<0.001
Dependent variable, %TG										
%dBMI	2.08	1.42	2.75	0.18	<0.001	4.47	3.78	5.16	0.28	<0.001
Model 2										
Dependent variable, %dLDL										
%dWC	0.16	0.05	0.26	0.08	0.005	0.25	0.09	0.41	0.07	0.002
age						−0.11	−0.18	−0.04	−0.07	0.003
Dependent variable, %dHDL										
%dWC						−0.24	−0.36	−0.12	−0.09	<0.001
Dependent variable, %TG										
%dWC	0.33	0.06	0.60	0.07	0.015	1.12	0.64	1.60	0.10	<0.001

Model 1. Independent variables include age, %dWC, and %dBMI. For model 2, independent variables included age and %dWC. Standardized β values are the estimates resulting from analysis performed on standardized variables.

heart disease or hypertension[12]. They found that changes in WHR were associated with changes in total cholesterol and triglycerides in men; however, statistical significance was lost after controlling for changes in BMI. On the other hand, after controlling for changes in WHR, changes in BMI remained to be associated with changes in total cholesterol and triglycerides in both genders. Of note, even before controlling for changes in BMI, WC change was not found to be associated with either total cholesterol or triglycerides in women. Wing *et al.* concluded that changes in WHR may not be independently related to changes in cardiovascular risk factors. Pascale *et al.* showed that in subjects participating in a year-long weight loss program, weight loss, but not reductions in WHR, was significantly related with improvements in fasting glucose, fasting insulin, and HbA1, although the magnitude of WHR reduction was strongly related to the amount of weight lost especially in men[13].

Similar to Wing *et al.*'s study, the current study indicated certain gender differences in the association between WC change and lipid parameter change, especially in the model not controlled for BMI. As HDL-C and TG are closely related to insulin sensitivity, and thus visceral fat mass, the closer relationship of %dBMI than %dWC with %dHDL and %dTG was rather unexpected. It is possible that WC measurements may be less reliable than weight and height measurements, which reduced the predictive power of %dWC for lipid changes. The correlation between %dWC and %dBMI was relatively weak, especially in women. This finding may indicate that a loss (gain) of BMI did not necessarily result in a loss (gain) in WC over a one-year period, and that men appear to lose (or gain) weight in their abdominal area more readily than women, which was consistent with previous observations[8, 12]. The finding that %dWC did not predict lipid changes independently of %dBMI may suggest that changes in BMI might be a more reliable goal to avoid adverse lipid changes than changes in WC.

It has recently been demonstrated that measuring both general and abdominal adiposity provides a better assessment of the risk of death[14]; therefore, we cannot lessen the importance of reducing WC and thus control visceral adiposity; in this sense, whether percent changes in abdominal fat demonstrated by computed tomography will have a greater impact on serum lipid data than %dWC should be examined in future studies[15].

The current study has several potential limitations. First, individuals who, for unknown reasons, did not visit our institute in the second year were not enrolled in the current study, which may cause some bias. Second, we do not have sufficient information

on the extent to which modifications of lifestyle and dietary habits affect the observed changes in general/abdominal obesity[16]. Third, we excluded subjects who were taking lipid-lowering drugs at either visit, and these individuals may, in general, have higher motivation to obtain information on how to improve serum lipid levels effectively as compared with those not taking such drugs. Finally, a longer follow-up should be performed in future studies.

In summary, during a one-year period, percent changes in BMI (%dBMI) were associated positively with percent changes in LDL-C and TG and negatively with those in HDL-C, especially in both genders. Although percent changes in WC (%dWC) also tended to confer adverse changes in lipid parameters, this relationship did not remain significant after controlling for %dBMI.

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Impacts of Changes in Obesity Parameters for the Prediction of Blood Pressure Change in Japanese Individuals

Nobukazu Ishizaka[a] Yuko Ishizaka[c] Ei-Ichi Toda[c] Kazuhiko Koike[b]
Minoru Yamakado[c] Ryozo Nagai[a]

Departments of [a]Cardiovascular Medicine and [b]Infectious Diseases, University of Tokyo Graduate School of Medicine, and [c]Center for Multiphasic Health Testing and Services, Mitsui Memorial Hospital, Tokyo, Japan

Key Words
Waist circumference · Body mass index · Blood pressure · Health screening

Abstract
Aims and Methods: By analyzing data from 2,861 individuals who underwent general health screening 2 years running, we have investigated the impact of changes in waist circumference (WC) and body mass index (BMI) over a 1-year period on systolic blood pressure (BPs). We termed WC, BMI, and BPs at the first visit as WC1, BMI1, and BPs1, respectively, and those at the second visit as WC2, BMI2, and BPs2, respectively. The %dWC, %dBMI, and %dBPs was defined as (WC2 – WC1)/WC1 × 100, (BMI2 – BMI1)/BMI1 × 100, and (BPs2 – BPs1)/BPs1 × 100, respectively. ***Results:*** In multivariate regression analysis using age, BPs1, WC1, and %dWC as independent variables, %dWC was a significant predictor for %BPs only in men. %dBMI was a significant predictor for %BPs in both genders when age, BPs1, BMI1, and %dBMI were used as independent variables. Compared with individuals with both %dWC <0 and %dBMI <0, age-adjusted %dBPs was significantly greater in those with both %dWC <0 and %dBMI ≥0; however, it did not significantly differ in those with both %dWC ≥0 and %dBMI <0. ***Conclusion:*** Our data suggest that the impact of BMI change might be greater than WC change in terms of BPs change during this short period.

Introduction

Much evidence supports a positive association between obesity parameters and hypertension [1–4], although the strength of such an association may differ according to the parameter used [5]. In addition, a loss or gain in body weight may affect blood pressure levels [6, 7], even in relatively lean or non-obese individuals [8, 9]. Therefore, weight control may be an important target for better blood pressure control, leading to a reduction in mortality from heart and cerebrovascular disease [4]. Compared with weight, or body mass index (BMI), less information seems to be available on whether, or to what extent, a loss (or gain) in waist circumference (WC) would result in a change in blood pressure. We previously reported that a reduction or gain in obesity parameters may affect the status of chronic kidney disease in individuals who underwent general health screening [10]. To this end, here we investigated the mode of association be-

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Dr. Nobukazu Ishizaka, Department of Cardiovascular Medicine
University of Tokyo Graduate School of Medicine
Hongo 7-3-1, Bunkyo-ku, Tokyo 113-8655 (Japan)
Tel. +81 3 3815 5411, ext. 37156, Fax +81 3 5842 5586
E-Mail nobuishizka-tky@umin.ac.jp

tween changes in WC or BMI over a 1-year period and changes in blood pressure levels in Japanese individuals. We analyzed the data separately for each gender, because there may be gender differences in the strength of the association between various obesity parameters and blood pressure [11].

Subjects and Methods

Study Population

The study was approved by the Ethical Committees of University of Tokyo and Mitsui Memorial Hospital. Between October 2005 and October 2006, 3,312 (1,203 women, 2,109 men) individuals underwent general health screening (visit 1), and they visited our institute again in the following year (visit 2). Among these 3,312 individuals, 2,861 (1,114 women, 1,747 men) who reported not taking antihypertensive drugs at both visits were enrolled in the present study. After about 10 min of rest, systolic blood pressure (BPs) and diastolic blood pressure (BPd) were measured in the sitting position by automated sphygmomanometer, BP-203RVIII (Omron Colin, Tokyo, Japan). Blood pressure was measured twice and the mean of these data were taken. With the subject standing, WC was measured at the umbilical level to the nearest 1 cm by trained physicians and technicians [12]. After changing into a robe from our institute, height and weight were measured, and the weight of the robe was subtracted from the value indicated by the scales. Age, WC, BMI, and BPs at visit 1 were designated age1, WC1, BMI1, and BPs1, respectively. Similarly, WC, BMI, and BPs at visit 2 were designated WC2, BMI2, and BPs2, respectively. %dWC, %dBMI, and %dBPs were defined as (WC2 – WC1)/WC1 × 100, (BMI2 – BMI1)/BMI1 × 100, and (BPs2 – BPs1)/BPs1 × 100, respectively.

Laboratory Analysis.

Blood samples were taken from the subjects after an overnight fast. Serum levels of total cholesterol (TC), HDL cholesterol (HDL-C), and triglycerides (TG) were determined enzymatically. Serum uric acid was measured by the uricase-peroxidase method, hemoglobin A_{1C} was determined using the latex agglutination immunoassay. Serum creatinine was measured by TBA-200FR (Toshiba Medical Systems, Tochigi, Japan) using commercially available kits, Accuras Auto CRE (Shino-test, Tokyo, Japan), according to the manufacturer's instructions. Accuracy control was performed every day by constructing X-bar and R charts using commercially available standards. Estimated glomerular filtration rate (eGFR) was calculated by the following equation: $eGFR = 194 \times (\text{serum creatinine})^{-1.094} \times (\text{age})^{-0.287}$ (× 0.739 if female) [13]. Serum insulin was measured by enzyme immunoassay. Homeostasis model assessment insulin resistance (HOMA-IR) was calculated in these individuals according to the following formula: HOMA-IR = [fasting immunoreactive insulin (μU/ml) × fasting plasma glucose (mg/dl)]/405 [14].

Statistical Analysis

Data are expressed as the mean ± SD unless stated otherwise. Analyses of variance with trend analysis, Tukey's post-hoc analysis and multiple regression analysis were conducted as appropriate to assess the statistical significance of differences between groups using computer software Dr. SPSS II (SPSS, Inc., Chicago, Ill., USA). A value of $p < 0.05$ was taken to be statistically significant.

Results

Baseline Characteristics

As described in the Methods section, among the 3,312 individuals who underwent general health screening visited our institute again in the following year; 2,861 (1,114 women, 1,747 men) who reported not taking antihypertensive drugs at both visits were enrolled in the current study (table 1). The mean ± SD of the interval between the two visits of the individuals enrolled was 355 ± 52 days. The mean ± SD age of the enrolled women (51.3 ± 9.9 years) and men (52.5 ± 10.1 years) was significantly smaller than that of the women (60.7 ± 8.3 years) and men (59.0 ± 8.5 years), respectively ($p < 0.001$), who were excluded because of the antihypertensive medication at either or both visits. Similarly, the mean BMI values of enrolled women (21.2 ± 2.9) and men (23.5 ± 2.7) were significantly smaller than those of the excluded women (22.5 ± 3.2) and men (25.0 ± 2.8), respectively ($p < 0.001$).

WC1 ranged between 51.8 and 118.5 cm, and a WC1 ≥90 cm was found in 71/1,114 women (6.4%), and a WC1 ≥85 cm was found in 183/1,114 men (16.4%). BMI1 ranged between 13.1 and 39.4. A BMI1 ≥25 was found in 110/1,114 women (9.9%) and 453/1,747 men (25.9%), and BMI1 ≥30 was found only in 12/1,114 (1.1%) women and 33/1,747 (1.9%) men. The correlation coefficients between %dWC, %dBMI, %dBPs, WC1, BMI1, and BPs1 are described in table 2. The correlation between %dWC and %dBMI was found to be moderate in men (r = 0.476), whereas it was weak in women (r = 0.241). The relationship between %dBMI and %dBPs was found to be statistically significant in the both genders. On the other hand, the relationship between %dWC and %dBPs was statistically significant only in men. Among the study subjects, it was reported that 60 subjects experienced a WC change of –10 cm or less, and 94 subjects experienced a WC change of +10 cm or more. After excluding these 154 individuals from the study population, the results obtained were not essentially changed (data not shown). It was calculated that a 10% weight gain (loss) over a 1-year period was associated with a 3.88 mm Hg BPs gain (loss) in women and a 9.86 mm Hg BPs gain (loss) in men.

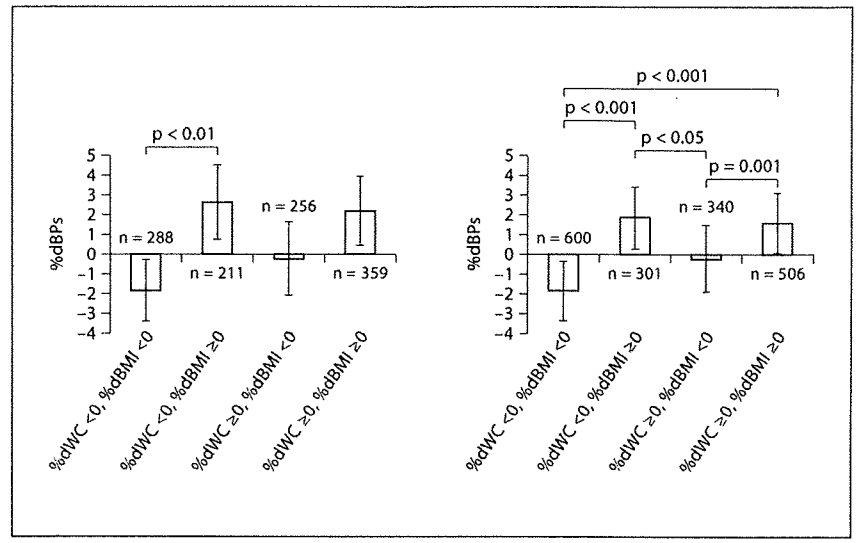

Fig. 1. Comparison of the age-adjusted %dBPs in four subgroups categorized according to the gain or loss of %dWC and %dBMI values. p values were from the result of the Tukey's post-hoc analysis following analyses of variance. Mean ± 95% confidence interval is shown in each group.

Table 1. Clinical characteristics and laboratory data at the first visit

Variables	Whole	%dBPs				p value
		first (range: −40 ~ −7)	second (range: −7 ~ 0)	third (range: +1 ~ +6)	fourth (range: +6 ~ +52)	
Number	2,861	714	809	639	699	
Women/men	1,114/1,747	288/426	314/495	251/388	261/438	0.712
Age, years	52.0 ± 10.1	52.8 ± 10.1	51.4 ± 9.9	51.8 ± 10.0	52.2 ± 10.2	0.047
Height, cm	164.8 ± 8.4	164.5 ± 8.3	165.2 ± 8.5	164.7 ± 8.5	164.7 ± 8.6	0.379
Weight, kg	61.8 ± 11.5	61.8 ± 11.4	62.0 ± 11.6	61.5 ± 11.3	61.8 ± 11.7	0.883
BMI, kg/m^2	22.6 ± 3.0	22.7 ± 3.0	22.6 ± 3.1	22.5 ± 3.0	22.6 ± 3.1	0.781
WC, cm	81.8 ± 9.1	82.0 ± 9.1	81.8 ± 9.3	81.5 ± 9.0	81.9 ± 9.0	0.851
Systolic BP, mm Hg	120.9 ± 18.0	128.7 ± 18.3	121.8 ± 17.0	118.5 ± 16.7	114.2 ± 16.8	<0.001
Diastolic BP, mm Hg	76.4 ± 11.4	79.3 ± 11.3	76.8 ± 10.9	75.5 ± 11.0	73.7 ± 11.5	<0.001
LDL cholesterol, mg/dl	129.2 ± 31.1	131.4 ± 31.5	128.3 ± 29.5	127.1 ± 30.9	130.1 ± 32.4	0.051
HDL cholesterol, mg/dl	61.2 ± 15.3	60.8 ± 15.0	61.8 ± 15.7	61.4 ± 15.6	60.7 ± 15.0	0.465
Triglyceride, mg/dl	109.9 ± 71.4	115.7 ± 69.9	104.7 ± 61.8	109.8 ± 81.0	110.1 ± 73.4	0.030
Uric acid, mg/dl	5.4 ± 1.3	5.4 ± 1.3	5.5 ± 1.3	5.4 ± 1.4	5.5 ± 1.4	0.688
Fasting glucose, mg/dl	95.2 ± 20.0	96.8 ± 20.4	95.1 ± 21.1	94.2 ± 18.0	94.7 ± 20.0	0.072
Hemoglobin A1C, %	5.3 ± 0.7	5.3 ± 0.7	5.3 ± 0.7	5.3 ± 0.7	5.3 ± 0.7	0.506
HOMA-IR	1.5 ± 1.1	1.6 ± 1.1	1.5 ± 1.1	1.4 ± 1.0	1.5 ± 1.0	0.066
Blood urea nitrogen, mg/dl	14.0 ± 3.4	13.8 ± 3.7	14.0 ± 3.2	14.2 ± 3.4	14.1 ± 3.5	0.245
Serum creatinine, mg/dl	0.8 ± 0.3	0.8 ± 0.4	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.764
Estimated glomerular filtration rate	68.6 ± 11.8	68.3 ± 11.4	69.3 ± 12.0	68.4 ± 11.8	68.1 ± 11.8	0.177
Antidiabetic medication, n (%)	51 (1.8)	12 (1.7)	20 (2.5)	10 (1.6)	9 (1.3)	0.335
Current smoker, n (%)	680 (23.8)	179 (25.0)	184 (22.7)	139 (21.8)	178 (25.5)	0.298

Data are means ± SD, unless stated otherwise. BMI = Body mass index; WC = waist circumference; HOMA-IR = homeostasis model assessment of insulin resistance. %dBPs was calculated by the following equation: (BPs at the second visit − BP1 at the second visit)/(BP1 at the second visit) × 100 (%). p value is for trend.

Table 2. Pearson's correlation coefficient of obesity indices and blood pressure parameters

	%dWC	%dBMI	%dBPs	WC1	BMI1	BPs1
Women						
%dWC						
r	–					
p value	–					
%dBMI						
r	0.241	–				
p value	<0.001	–				
%dBPs						
r	−0.014	0.097	–			
p value	0.635	0.001	–			
WC1						
r	−0.317	−0.053	−0.028	–		
p value	<0.001	0.078	0.350	–		
BMI1						
r	−0.026	−0.087	−0.029	0.787	–	
p value	0.393	0.004	0.331	<0.001	–	
BPs1						
r	−0.025	−0.055	−0.325	0.365	0.409	–
p value	0.396	0.064	<0.001	<0.001	<0.001	–
Men						
%dWC						
r	–					
p value	–					
%dBMI						
r	0.476	–				
p value	<0.001	–				
%dBPs						
r	0.116	0.232	–			
p value	<0.001	<0.001	–			
WC1						
r	−0.268	−0.089	−0.031	–		
p value	<0.001	<0.001	0.189	–		
BMI1						
r	−0.054	−0.071	−0.026	0.830	–	
p value	0.023	0.003	0.286	<0.001	–	
BPs1						
r	−0.090	−0.077	−0.327	0.308	0.322	–
p value	<0.001	0.001	<0.001	<0.001	<0.001	–

BPs = Systolic blood pressure; WC = waist circumference; BMI = body mass index. BPs at visit 1 and visit 2 were designated BPs1 and BPs2, respectively. BMI at visit 1 and visit 2 were designated BMI1 and BMI2, respectively, and WC at visit 1 and visit 2 were designated WC1 and WC2, respectively. %dBMI, %dWC, and %dBPs were calculated by the equation (BMI2 − BMI1)/BMI1 × 100 (%), (WC2 − WC1)/WC1 × 100 (%), and (BPs2 − BPs1)/BPs1 × 100 (%), respectively.

Table 3. Multiple regression analysis between %dBPs and age1, WC1, BMI1, %dWC, and %dBMI

	β	95% CI	Standardized β	p value
Women				
Model 1				
BPs1	−0.23	−0.27 to −0.20	−0.38	<0.001
Age1	0.11	0.05 to 0.18	0.10	0.001
WC1	0.11	0.03 to 0.19	0.09	0.005
%dWC	0.01	−0.06 to 0.09	0.01	0.733
Model 2				
BPs1	−0.24	−0.28 to −0.21	−0.40	<0.001
BMI1	0.47	0.25 to 0.70	0.13	<0.001
Age1	0.13	0.07 to 0.19	0.12	<0.001
%dBMI	0.34	0.15 to 0.53	0.10	0.001
Model 3				
BPs1	−0.24	−0.28 to −0.21	−0.40	<0.001
BMI1	0.65	0.28 to 1.03	0.17	0.001
Age1	0.14	0.07 to 0.20	0.13	<0.001
%dBMI	0.39	0.19 to 0.60	0.11	<0.001
WC1	−0.08	−0.21 to 0.05	−0.06	0.244
%dWC	−0.08	−0.17 to 0.01	−0.06	0.071
Men				
Model 1				
BPs1	−0.22	−0.25 to −0.19	−0.35	<0.001
WC1	0.15	0.08 to 0.22	0.11	<0.001
%dWC	0.28	0.17 to 0.39	0.11	<0.001
Age1	0.02	−0.03 to 0.07	0.02	0.467
Model 2				
BPs1	−0.22	−0.25 to −0.19	−0.35	<0.001
%dBMI	0.80	0.64 to 0.96	0.22	<0.001
BMI1	0.41	0.23 to 0.59	0.10	<0.001
Age1	0.05	0.00 to 0.10	0.05	0.035
Model 3				
BPs1	−0.22	−0.25 to −0.19	−0.35	<0.001
%dBMI	0.82	0.63 to 1.00	0.22	<0.001
BMI1	0.38	0.04 to 0.72	0.10	0.027
Age1	0.05	0.00 to 0.10	0.05	0.046
WC1	0.01	−0.11 to 0.14	0.01	0.845
%dWC	−0.03	−0.16 to 0.11	−0.01	0.705

BPs = Systolic blood pressure; WC = waist circumference; BMI = body mass index. Standardized β values are the estimates resulting from an analysis performed on variables that were standardized. BPs at visit 1 and visit 2 were designated BPs1 and BPs2, respectively. BMI at visit 1 and visit 2 were designated BMI1 and BMI2, respectively, and WC at visit 1 and visit 2 were designated WC1 and WC2, respectively. %dBMI, %dWC, and %dBPs were calculated by the equation of (BMI2 − BMI1)/BMI1 × 100 (%), (WC2 − WC1)/WC1 × 100 (%), and (BPs2 − BPs1)/BPs1 × 100 (%), respectively.

Model 1 = Independent variables include age, BPs1, WC1, and %dWC; model 2 = independent variables include age, BPs1, BMI1, and %dBMI; model 3 = independent variables include model 1 + BMI1, and %dBMI.

Multiple Linear Regression Analysis

In multiple regression analysis, in which age1, WC1, BPs1, and %dWC were used as independent variables (model 1), %dWC was found to be an independent predictive value for %dBPs in men, but not in women (table 3). In a model where age1, BMI1, BPs1, and %dBMI were used as independent variables (model 2), %dBMI was found to be an independent predictive value for %dBPs in the both genders. After including all of the age1, BPs1, WC1, BMI1, %dWC, and %dBMI in a model as independent variables (model 3), %dBMI remained to be a predictor for %dBPs in both genders. In model 3, the variance inflation factor scores of all applied independent variables were <10 (data not shown)

Comparison between Individuals with BMI Gain or Loss together with WC Gain or Loss

We then compared the %BPS values between individuals with both WC loss (%dWC <0) and BMI loss (%dBMI <0), those with both WC loss and BMI gain (%dBMI ≥0), both WC gain and BMI loss, and those with both WC gain and BMI gain during a 1-year period (fig. 1). Age-adjusted %dBPs was significantly greater in individuals with both WC loss and BMI gain compared with those with both WC loss and BMI loss. On the other hand, age-adjusted %dBPs did not significantly differ between individuals with both WC loss and BMI loss and those with WC gain and BMI loss in both genders. When the same analysis was performed after excluding 154 subjects who experienced WC change of −10 cm or less or +10 cm or more, the results obtained were not essentially changed (data not shown).

Discussion

By analyzing data from individuals who underwent general health screening for 2 consecutive years, we showed that a percent difference in BMI (%dBMI) was a statistically significant predictor for a percent difference in BPs (%dBPs) in both genders. A percent difference in WC (%dWC) was also found to be a predictor for %dBPs in men; however, it lost statistical significance after further adjustment for BMI at the first visit and %dBMI, and it was not significant in women before and after such further adjustment.

A body of evidence indicates an association between obesity parameters and blood pressure levels [15, 16]. A reduction in body weight may result in a lowering of blood pressure in overweight or obese subjects [17, 18], although the results may not be always uniform. Moore et al. [19] showed that modest weight loss over a 4-year period substantially lowered the long-term risk of hypertension in overweight adults in Framingham. Haung et al. [20] showed that weight loss occurring after 18 years of age was related to a significantly lower risk, whereas weight gain was related to greater risk of hypertension in middle-aged women. In addition, Yang et al. [21] showed that in men aged between 40 and 74 years, weight gain occurring after 20 years of age was significantly associated with prehypertension. Most of the reports studying the potential association between changes in obesity parameters and changes in blood pressure were carried over a follow-up period longer than that in the current study. Furthermore, Truesdale et al. [22] have more recently shown that weight change over a 3-year period resulted in change in blood pressure levels; men who had experienced a 10% weight gain over the previous 3 years had BPs that was 2.6 mm Hg higher. They found, however, that the impact of weight change was, albeit present, less prominent in women. Women who had experienced a 10% weight gain over the previous 3 years had BPs that was only 0.9 mm Hg higher, suggesting the presence of gender difference in the extent of association between weight change and blood pressure change. We also showed here that the magnitude of the effect of changes in obesity parameters on blood pressure changes may vary by gender (table 3).

As compared to changes in weight, and thus in BMI, fewer analyses have focused on the relationship between changes in WC and blood pressure alterations. Considering that reductions in WC have been recommended more strongly than before for the purpose of prophylaxis and/or resolution of metabolic syndrome by the government in our country [23], the impact of WC reduction (gain) in terms of alterations of atherogenic risk factors, including blood pressure and levels of glucose and lipids, is becoming a more important issue to be investigated. Therefore, we also assessed whether changes in WC were reflected by the BPs change, and whether this relationship, if present, was independent of BMI change. We found that WC change was predictive of BPs change in men but not in women. In addition, the association between %dWC and %dBPs in men lost statistical significance after controlling for BMI1 and %dBMI (table 3). In contrast, %dBMI was a predictor for %dBPs in both genders regardless of the control of %dWC, suggesting that a reduction in BMI may represent a more essential target than WC reduction in terms of blood pressure control. This concept may be further supported by our finding that mean %dBPs did

not differ significantly between individuals with %dWC <0 and those with %dWC ≥0 among individuals with %dBMI <0. In reverse, %dBPs reduction was significantly greater in individuals with %dBMI <0 than in those with %dBMI ≥0 among individuals with %dWC <0 (fig. 1).

It has been reported that, in individuals with a mean BMI of 31, change in BMI was significantly correlated with change in BPs in both genders, even after adjusting for change in waist-hip ratio [24]. In the same study, it was reported that change in waist-hip ratio was not significantly correlated with change in BPs after adjusting for BMI change in men, and that the relationship between change in waist-hip ratio and BPs change was not significant before any adjustment in women. The results of Wing et al. [24] can be said to be similar to our current observation although there is a difference between WC and waist-hip ratio.

The current study has several limitations. First, we retrospectively analyzed data on individuals who underwent general health screening at our institute for 2 consecutive years; as a result, individuals who did not visit our institute the second year for unknown reasons were not enrolled in the current study, which may cause some biases. Second, we could not specify the reasons for weight gain or loss in individuals, however, very few individuals would have been taking antiobesity medications because only one individual in each gender had a BMI of 35 kg/m^2 or more at the first visit. Third, this study population included many non-obese subjects; a BMI1 ≥30 was found only in 1.1% of women and 1.9% of men. Fourth, we excluded those subjects who were taking antihypertensive drugs at either visit. We found that BMI was significantly greater in these excluded subjects than in the study population for both genders. Lastly, although change in BMI may seem to be superior for predicting BPs change than changes in abdominal obesity, abdominal fat volume should be measured by more reliable methods, such as computed tomography, before conclusion. In addition, we have to follow the subjects for a longer period, as a recent study has shown that surrogate measures of abdominal obesity are stronger predictors of all-cause and cardiovascular death than BMI in the general population [25].

In conclusion, in individuals who underwent general health screening for consecutive years, percent change in WC was significantly associated with percent change in BPs in men, but not in women; although this association in men lost statistical significance after controlling for percent change in BMI. By contrast, percent change in BMI was significantly associated with percent change in BPs regardless of controlling for percent change in WC. Our data suggest that controlling BMI, and thus controlling body weight, may represent a more essential goal than a reduction in WC in terms of blood pressure lowering among Japanese individuals who are not taking antihypertensive medication.

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Association between Changes in Obesity Parameters and Incidence of Chronic Kidney Disease in Japanese Individuals

Yuko Ishizaka[a] Nobukazu Ishizaka[b] Mizuki Tani[a] Akiko Toda[a] Ei-Ichi Toda[a]
Kazuhiko Koike[c] Ryozo Nagai[b] Minoru Yamakado[a]

[a]Center for Multiphasic Health Testing and Services, Mitsui Memorial Hospital, and Departments of
[b]Cardiovascular Medicine and [c]Infectious Diseases, University of Tokyo Graduate School of Medicine, Tokyo, Japan

Key Words
Chronic kidney disease · Body mass index · Waist circumference · Health screening

Abstract
Obesity increases the risk for chronic kidney disease (CKD). By analyzing data on individuals who underwent general health screening in two consecutive years, we investigated whether changes in body mass index (BMI) or waist circumference (WC) were associated with the appearance or disappearance of the CKD components; micro-/macroalbuminuria (≥30 mg urinary albumin per gram creatinine) and a low estimated glomerular filtration rate (eGFR; <60 ml/min/1.73 m^2). Logistic regression analysis showed that in men with micro-/macroalbuminuria at the first visit, a BMI reduction of ≥0.42 or a WC reduction of ≥3.0 cm over the 1-year period resulted in a significantly reduced incident of micro-/macroalbuminuria at the second visit. On the other hand, a BMI gain of ≥0.33 over 1 year in men without micro-/macroalbuminuria and a low eGFR at the fist visit significantly increased the incident of micro-/macroalbuminuria and a low eGFR, respectively, at the second visit. These findings indicate that lowering the obesity indexes in men with micro-/macroalbuminuria reduced the incidence of this condition at the 1-year follow-up and that, on the contrary, an increase in BMI in men without micro-/macroalbuminuria and a low eGFR at the first examination increased the risk of these conditions during the 1-year follow-up period.

Introduction

Chronic kidney disease (CKD), now recognized as a potential risk factor for cardiovascular disease as well as for end-stage renal disease [1], is a worldwide public health problem [2]. Several cross-sectional and longitudinal epidemiological studies showed that obesity may increase the prevalence and incidence of CKD [3–8] and end-stage renal disease [9], although there might be differences according to gender and ethnicity [9–11]. However, fewer studies have investigated whether changes in obesity indexes, such as body weight, body mass index (BMI), and waist circumference (WC), are associated with changes in CKD status [12–14]. In the current study, we retrospectively analyzed data on individuals who underwent general health screening at our institute for 2 consecutive years and investigated whether changes in obesity indexes were associated with changes in CKD status in these Japanese individuals.

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Dr. Nobukazu Ishizaka, Department of Cardiovascular Medicine
University of Tokyo Graduate School of Medicine
Hongo 7-3-1 Bunkyo-ku, Tokyo 113-8655 (Japan)
Tel. +81 3 3815 5411, ext. 37156, Fax +81 3 5842 5586
E-Mail nobuishizka-tky@umin.ac.jp

Subjects and Methods

Study Population

The study was approved by The Ethical Committee of Mitsui Memorial Hospital. At our institution, 3,312 (1,203 women, 2,109 men) individuals underwent general health screening including that on urinary albumin excretion between October 2005 and October 2006 (first visit) and also in the subsequent year (second visit). Among the 3,312 individuals, data on 2,861 (1,114 women, 1,747 men) who reported not taking anti-hypertensive drugs at both visits were used for the present study. The mean ± SD of the interval between the two visits of the enrolled individuals by the study subjects was 355 ± 52 days. Individuals who were taking antihypertensive medications were excluded from the analysis because certain depressor drugs may affect renal function and the extent of proteinuria [15, 16] and because the database did not include information on the class of drugs used. At the time of the health examination, recommendations may have given to overweight or obese subjects to reduce body weight. However, in analyzing data for this study, there was no intention to examine which strategies for weight control, if any, would have an impact on the status of CKD during the follow-up.

In Japan, regular health check-ups for employees are legally mandated; thus, the majority of these subjects did not have serious health problems. In addition, all or most of the costs of the screening are usually paid by the company to which they belong or by each subject. In addition, there are several courses in the heath screening program; however, which to choose is up to each individual, but not to physicians or company one belongs to. Therefore, the study population is not considered to be enriched for certain diseased condition.

Laboratory Analysis

Blood samples were taken from the subjects after an overnight fast. Serum levels of total cholesterol (TC), HDL-cholesterol (HDL-C), and triglycerides (TG) were determined enzymatically. Serum uric acid was measured by the uricase-peroxidase method, hemoglobin A_{1C} was determined using the latex agglutination immunoassay, and creatinine was determined by the enzymatic method. Plasma glucose was measured by the hexokinase method and serum insulin was measured by enzyme immunoassay.

Creatinine and urine albumin were measured by TBA-200FR (Toshiba Medical Systems, Tochigi, Japan) and by Accute (Toshiba Medical Systems), respectively, using commercially available kits, Accuras Auto CRE (Shino-test, Tokyo, Japan) and IATRO U-ALB by turbidimetric immunoassay (Mitsubishi Kagaku Iatron, Tokyo, Japan), respectively. Serum creatinine was calibrated using the following formula: serum creatinine (Jaffe method) = 0.2 + serum creatinine (enzyme method). Glomerular filtration rate (GFR) was estimated by equations of the simplified version of Modification of Diet in Renal Disease (MDRD) [17], where 0.881 is a coefficient for eGFR specific to the Japanese population [18]: estimated GFR (eGFR) for Japanese = 186.3 × (serum creatinine)$^{-1.154}$ × (age)$^{-0.203}$ × 0.881 × 0.742 (for females). eGFR values <60 ml/min/1.73 m^2 were classified as low [19]. For the diagnosis of micro-/macroalbuminuria, spot urine samples were collected and analyzed; micro-/macroalbuminuria was defined to be present when the urinary albumin excretion ratio (UAER), expressed as milligrams per gram creatinine, was ≥30 mg/g. Normoalbuminuria, microalbuminuria, and macroalbuminuria were defined as a UAER of <30, 30–299, and ≥300 mg/g, respectively. Micro-/macroalbuminuria and a low eGFR were considered to be the components of CKD [19]. The difference in BMI and WC between the two visits was designated as ΔBMI and ΔWC, respectively.

Statistical Analysis

Skewed variables (TG, UAER) are presented as median values (interquartile range). Other data are expressed as the mean ± SD unless stated otherwise. Analyses of variance, the Mann-Whitney U test, χ^2 tests, and logistic regression analysis were conducted as appropriate to assess the statistical significance of differences between groups using computer software, Dr. SPSS II (Chicago, Ill., USA). A value of $p < 0.05$ was taken to be statistically significant.

Results

Baseline Characteristics

The mean ± SD age of the individuals enrolled was 52.0 ± 10.1 years at the first visit (table 1). Of the 88 females and 149 males with micro-/macroalbuminuria, 83 and 134, respectively, had microalbuminuria and 5 and 15, respectively, had macroalbuminuria.

Changes in BMI and WC Values between the Two Visits

The mean BMI at the second visit was slightly lower than that at the first visit ($p < 0.001$, by paired t test) in men, but did not differ significantly in women. The mean WC at the second visit was slightly larger than that at the first visit ($p < 0.001$, by paired t test) in women and smaller ($p < 0.001$, by paired t test) in men. In this study, we calculated quartiles of WC or BMI by taking into the entire population. Ranges for each quartile for ΔBMI and ΔWC are shown in table 2. About half of the subjects had a decreased WC value at the second visit. The correlation coefficient between the first-visit BMI and ΔBMI was −0.09 ($p = 0.010$) in women and −0.09 ($p = 0.002$) in men, and that between the first-visit WC and ΔWC was −0.31 in women ($p < 0.001$) and −0.28 ($p < 0.001$) in men.

Changes in the Prevalence of Micro-/Macroalbuminuria and a Low eGFR between the Two Visits

Figure 1 shows the number of subjects with micro-/macroalbuminuria and a low eGFR at the first and second visits. Of those with micro-/macroalbuminuria at the first visit, 34% did not have micro-/macroalbuminuria at the second visit, but 4% of subjects who did not have micro-/macroalbuminuria at the first visit had de-

Table 1. Clinical characteristics and laboratory data

Variables	Women (n = 1,114)		Men (n = 1,747)	
	Visit 1	Visit 2	Visit 1	Visit 2
Age, years	51.3 ± 9.9	52.3 ± 9.9	52.5 ± 10.1	53.4 ± 10.1
Height, cm	157.1 ± 5.7	157.1 ± 7.8	169.7 ± 5.9	169.7 ± 5.9
Weight, kg	52.3 ± 7.7	52.3 ± 7.8	67.8 ± 9.2	67.6 ± 9.3
BMI	21.2 ± 2.9	21.2 ± 2.9	23.5 ± 2.7	23.5 ± 2.8
ΔBMI	–	0.0 ± 0.7	–	−0.1 ± 0.7
WC, cm	76.2 ± 8.6	76.9 ± 8.9	85.3 ± 7.5	85.0 ± 7.4
ΔWC, cm	–	0.7 ± 6.0	–	−0.3 ± 3.8
Systolic BP, mm Hg	116 ± 18	115 ± 18	124 ± 17	124 ± 18
Diastolic BP, mm Hg	72 ± 11	72 ± 11	79 ± 11	79 ± 11
Total cholesterol, mg/dl	217 ± 36	215 ± 34	210 ± 32	207 ± 31
LDL-cholesterol, mg/dl	130 ± 30	127 ± 30	128 ± 32	126 ± 31
HDL-cholesterol, mg/dl	69 ± 14	68 ± 14	56 ± 14	56 ± 13
TG, mg/dl	84 ± 46	84 ± 42	127 ± 80	126 ± 101
TG, median (interquartile range)	74 (55–99)	74 (54–101)	107 (77–152)	102 (74–144)
Uric acid, mg/dl	4.5 ± 0.9	4.5 ± 0.9	6.1 ± 1.2	6.0 ± 1.2
Fasting glucose, mg/dl	90 ± 17	91 ± 14	98 ± 21	99 ± 20
Hemoglobin A_{1C}, %	5.2 ± 0.6	5.2 ± 0.5	5.4 ± 0.8	5.5 ± 0.7
Antidiabetic medication, n (%)	5 (0.4)	9 (0.8)	46 (2.6)	58 (3.3)
Blood urea nitrogen, mg/dl	13.4 ± 3.2	13.6 ± 3.3	14.4 ± 3.5	14.5 ± 3.5
Serum creatinine, mg/dl	0.63 ± 0.09	0.62 ± 0.09	0.86 ± 0.28	0.84 ± 0.30
UAER, median (interquartile range)	7.5 (5.1–12.2)	7.9 (5.4–13.1)	5.2 (3.7–10.0)	5.6 (3.9–10.7)
UAER ≥30 mg/g Cr, n (%)	88 (7.9)	91 (8.2)	149 (8.5)	165 (9.4)
eGFR, ml/min/1.73 m^2	69.5 ± 9.3	70.1 ± 9.2	70.9 ± 10.0	71.8 ± 10.2
Low eGFR, n (%)	155 (13.9)	138 (12.4)	212 (13.1)	201 (11.5)
Current smoker	99 (8.9)	93 (8.3)	581 (33.3)	542 (31.0)

Data are means ± SD, median (interquartile range), n, or percentage. BMI = Body mass index; WC = waist circumference; BP = blood pressure; TG = triglycerides; UAER = urinary albumin excretion rate; eGFR = estimated glomerular filtration rate.

Table 2. Range for each quartile of ΔBMI and ΔWC

	Q1	Q2	Q3	Q4
ΔBMI	−5.33/−0.42 (−0.75)	−0.41/−0.04 (−0.21)	−0.04/0.32 (0.13)	0.33/3.67 (0.62)
ΔWC, cm	−21.0/−3.0 (−5.0)	−2.9/−0.1 (−1.5)	0.0/2.7 (1.0)	2.8/23.0 (5.0)

BMI = Body mass index; WC = waist circumference. Medians are given in parentheses.

veloped micro-/macroalbuminuria at the second visit (fig. 1a, b). Of individuals who had a low eGFR at the first visit, 28% did not have a low eGFR at the second visit, but alternatively, 3% of individuals who did not have a low eGFR at the first visit had a low eGFR at the second visit.

Association between Changes in BMI or WC and Albuminuric Status

Next, we investigated whether decreases in BMI and WC values were associated with changes in CKD status (fig. 2a, b). Logistic regression analysis adjusted for age, systolic blood pressure, HDL- and LDL-cholesterol, fast-

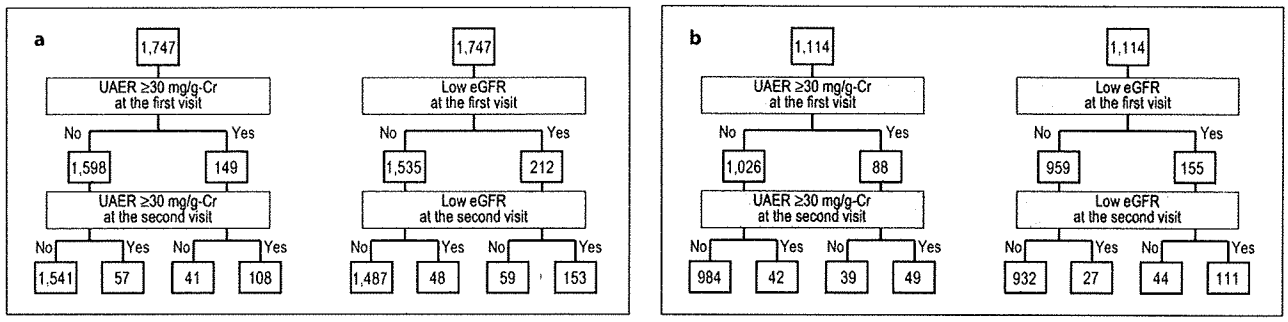

Fig. 1. Flow chart showing the number of men without micro-/macroalbuminuria or a low eGFR at the times of visit 1 and visit 2. **a** Men. **b** Women.

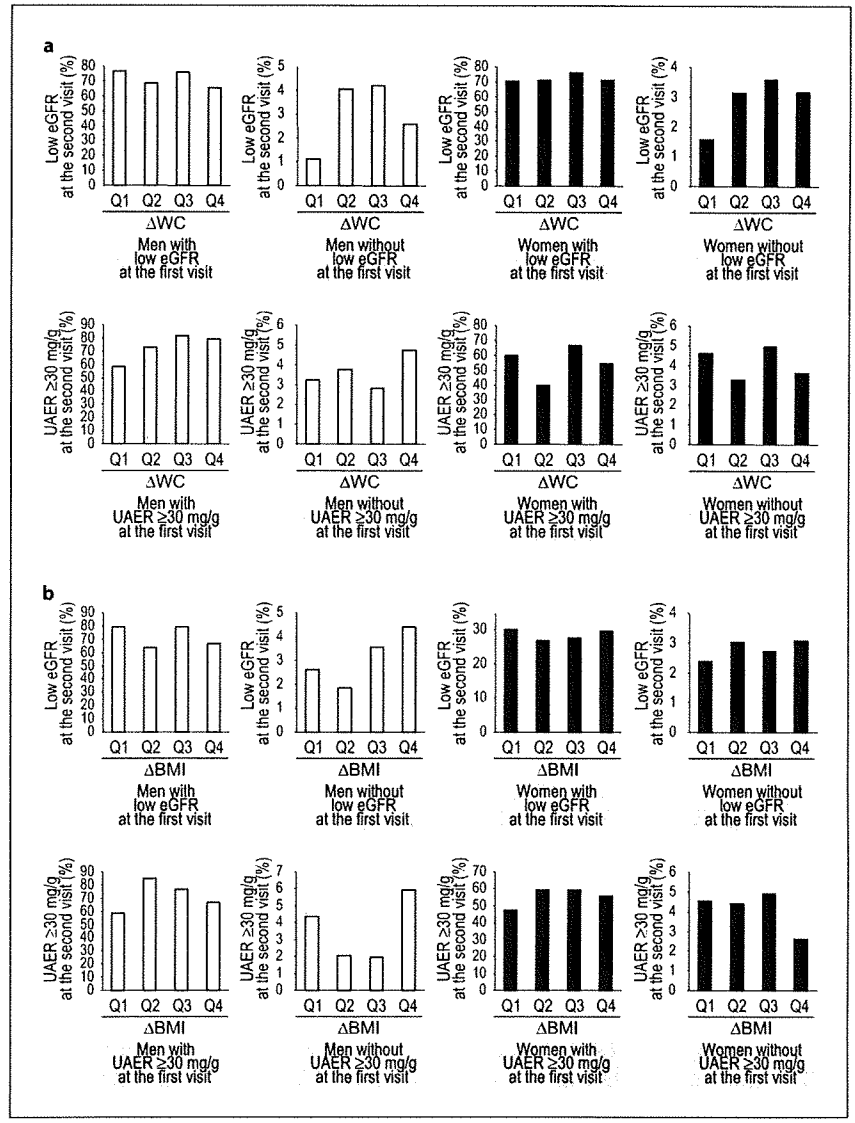

Fig. 2. Prevalence of a low eGFR and elevated levels of albuminuria at visit 2 in subjects with and without a low eGFR or micro-/macroalbuminuria at visit 1 according to quartiles of the difference in waist circumference between visit 1 and visit 2 (ΔWC) (**a**) and the difference in body mass index between the visit 1 and visit 2 (ΔBMI) (**b**).

Table 3. Logistic regression analysis with the lowest Δwaist circumference or Δbody mass index quartile as an independent variable and micro-/macroalbuminuria at the second visit as a dependent variable in individuals with micro-/macroalbuminuria at the first visit

Variables	Age adjusted		Multivariate adjusted*	
	OR (95% CI)	p value	OR (95% CI)	p value
Male (n = 149)				
ΔWC-Q2, Q3, Q4	1.00	-	1.00	-
ΔWC-Q1	0.36 (0.16–0.80)	0.012	0.31 (0.13–0.73)	0.007
Female (n = 88)				
ΔWC-Q2, Q3, Q4	1.00	-	1.00	-
ΔWC-Q1	1.20 (0.41–3.53)	0.735	1.01 (0.33–3.14)	0.987
Male (n = 149)				
ΔBMI-Q2, Q3, Q4	1.00	-	1.00	-
ΔBMI-Q1	0.37 (0.16–0.84)	0.018	0.36 (0.15–0.84)	0.018
Female (n = 88)				
ΔBMI-Q2, Q3, Q4	1.00	-	1.00	-
ΔBMI-Q1	0.51 (0.17–1.54)	0.232	0.52 (0.16–1.74)	0.289

BMI = Body mass index; WC = waist circumference.
* Multivariate adjusted: Adjusted for age, systolic blood pressure, HDL-cholesterol, LDL-cholesterol, fasting plasma glucose, and smoking status.

ing plasma glucose, and smoking status showed that, compared with the higher three ΔBMI quartiles, the lowest ΔBMI quartile (≥0.42 reduction) was associated with a significantly lower risk for micro-/macroalbuminuria at the second visit in men who had micro-/macroalbuminuria at the first visit (table 3). Similarly, compared with the higher three ΔWC quartiles, the lowest ΔWC quartile (≥3.0 cm reduction) was associated with significantly lower risk for micro-/macroalbuminuria at the second visit in men who had micro-/macroalbuminuria at the first visit. In contrast, in women, who had micro-/macroalbuminuria at the first visit, neither a ≥0.42 reduction in BMI nor a ≥3.0-cm reduction in WC significantly reduced the prevalence of micro-/macroalbuminuria at the second visit. Compared with the lower three ΔBMI quartiles, the highest ΔBMI quartile (≥0.33 gain) was associated with a significantly higher risk for micro-/macroalbuminuria at the second visit in men who did not have micro-/macroalbuminuria at the first visit (table 4).

Association between Changes in BMI or WC and a Low eGFR Status

Compared with the lower three quartiles, the highest ΔBMI quartile (≥0.33 gain) was associated with a significantly higher risk for a low eGFR at the second visit in men who did not have a low eGFR at the first visit (tables 5–6). The lowest quartile of either ΔBMI or ΔWC was not associated with reduced risk for a low eGFR at the second visit in those who had a low eGFR at the first visit in either gender.

Discussion

In the current study, we demonstrated that a WC reduction of ≥2.8 cm or a BMI reduction of ≥0.42 over a period of one year in men with micro-/macroalbuminuria at the first visit significantly reduced the risk for micro-/macroalbuminuria at the second visit (OR 0.31, 95% CI 0.13–0.73 and OR 0.36, 95% CI 0.15–0.84, respectively), after multivariate adjustment. On the other hand, a BMI gain of ≥0.33 over one year in men without micro-/macroalbuminuria or a low eGFR at the first visit significantly increased the risk at these conditions at the second visit (OR 2.50, 95% CI 1.44–4.37 and OR 1.94, 95% CI 1.04–3.61, respectively). Neither of these associations reached statistical significance in women. These data collectively suggest that the albuminuric status may be altered when men with micro-/macroalbuminuria have a substantial decrease in WC or BMI, and, in reverse, the

Table 4. Logistic regression analysis with the highest Δwaist circumference or Δbody mass index quartile as an independent variable and micro-/macroalbuminuria at the second visit as a dependent variable in individuals without micro-/macroalbuminuria at the first visit

Variables	Age adjusted		Multivariate adjusted*	
	OR (95% CI)	p value	OR (95% CI)	p value
Male (n = 1,598)				
ΔWC-Q1, Q2, Q3	1.00	–	1.00	–
ΔWC-Q4	1.52 (0.83–2.78)	0.177	1.62 (0.88–2.99)	0.120
Female (n = 1,026)				
ΔWC-Q1, Q2, Q3	1.00	–	1.00	–
ΔWC-Q4	0.87 (0.44–1.69)	0.674	0.87 (0.44–1.70)	0.677
Male (n = 1,598)				
ΔBMI-Q1, Q2, Q3	1.00	–	1.00	–
ΔBMI-Q4	2.41 (1.39–4.19)	0.002	2.50 (1.44–4.37)	0.001
Female (n = 1,026)				
ΔBMI-Q1, Q2, Q3	1.00	–	1.00	–
ΔBMI-Q4	0.57 (0.25–1.31)	0.185	0.60 (0.26–1.37)	0.221

BMI = Body mass index; WC = waist circumference.
* Multivariate adjusted: Adjusted for age, systolic blood pressure, HDL-cholesterol, LDL-cholesterol, fasting plasma glucose, and smoking status.

Table 5. Logistic regression analysis with the lowest Δwaist circumference or Δbody mass index quartile as an independent variable and a low eGFR at the second visit as a dependent variable in individuals with a low eGFR at the first visit

Variables	Age adjusted		Multivariate adjusted*	
	OR (95% CI)	p value	OR (95% CI)	p value
Male (n = 212)				
ΔWC-Q2, Q3, Q4	1.00	–	1.00	–
ΔWC-Q1	1.39 (0.68–2.88)	0.369	1.33 (0.63–2.80)	0.454
Female (n = 155)				
ΔWC-Q2, Q3, Q4	1.00	–	1.00	–
ΔWC-Q1	0.84 (0.38–1.85)	0.664	0.90 (0.39–2.08)	0.808
Male (n = 212)				
ΔBMI-Q2, Q3, Q4	1.00	–	1.00	–
ΔBMI-Q1	1.60 (0.80–3.23)	0.185	1.49 (0.73–3.04)	0.276
Female (n = 155)				
ΔBMI-Q2, Q3, Q4	1.00	–	1.00	–
ΔBMI-Q1	0.73 (0.30–1.81)	0.500	0.91 (0.34–2.38)	0.833

BMI = Body mass index; WC = waist circumference.
* Multivariate adjusted: Adjusted for age, systolic blood pressure, HDL-cholesterol, LDL-cholesterol, fasting plasma glucose, and smoking status.

Table 6. Logistic regression analysis with the highest Δwaist circumference or Δbody mass index quartile as an independent variable and a low eGFR at the second visit as a dependent variable in individuals without a low eGFR at the first visit

Variables	Age adjusted		Multivariate adjusted*	
	OR (95% CI)	p value	OR (95% CI)	p value
Male (n = 1,535)				
ΔWC-Q1, Q2, Q3	1.00	-	1.00	-
ΔWC-Q4	0.84 (0.39–1.83)	0.667	0.88 (0.40–1.91)	0.737
Female (n = 959)				
ΔWC-Q1, Q2, Q3	1.00	-	1.00	-
ΔWC-Q4	1.30 (0.60–2.86)	0.508	1.37 (0.62–3.03)	0.432
Male (n = 1,535)				
ΔBMI-Q1, Q2, Q3	1.00	-	1.00	-
ΔBMI-Q4	1.98 (1.07–3.66)	0.030	1.94 (1.04–3.61)	0.037
Female (n = 959)				
ΔBMI-Q1, Q2, Q3	1.00	-	1.00	-
ΔBMI-Q4	1.22 (0.53–2.83)	0.644	1.23 (0.53–2.89)	0.631

BMI = Body mass index; WC = waist circumference.
* Multivariate adjusted: Adjusted for age, systolic blood pressure, HDL-cholesterol, LDL-cholesterol, fasting plasma glucose, and smoking status.

status of albuminuria or a low eGFR may be altered when men without micro-/macroalbuminuria or a low eGFR, respectively, gain BMI substantially, although such a relationship was not apparent in female subjects. Future studies should be directed toward elucidating whether these observed gender differences were, in part, due to the greater prevalence of other risk factors, such as increased blood pressure, elevated fasting glucose levels, and reduced insulin sensitivity [20, 21], in men than in women.

Several studies have investigated the possible association between the obesity index and CKD. A high BMI has been reported to be associated with CKD [6, 10, 11]. Chou et al. [22] reported that in elder Taiwanese, the waist-hip ratio, body weight and WC, but not BMI, were predictors of a low eGFR, and that among these predictors, the waist-hip ratio may be the best anthropometric index for predicting a low eGFR. Foster et al. [23] showed that the association between obesity with an increased risk of developing stage 3 CKD was not independent, but was confounded by other cardiovascular disease risk factors. These findings suggest that the mode of association between certain obesity index and CKD might differ according to the study design and population studied.

Whether changes in obesity parameters would result in changes in CKD status has also been investigated in several previous studies. Changes in body weight were found to be associated with parallel changes in albuminuria in 6,894 participants of the Prevention of Renal and Vascular End-Stage Disease (PREVEND) study during a 4.2-year follow-up period [12]. In addition, moderate weight loss induced by a hypocaloric and normoproteinic diet in overweight patients with chronic proteinuria resulted in a significant decrease in proteinuria [13]. Furthermore, weight loss induced by an inhibitor of gastrointestinal lipase was associated with the reduction of urinary albumin excretion [14]. Therefore, most, if not all, studies showed that body weight reduction in overweight subjects resulted in a reduction of proteinuria, which was in agreement with the observation in the current study. Compared to the association between changes in obesity parameters and proteinuria, fewer numbers of studies have analyzed the relationship between change in body weight and change in eGFR. In the above-mentioned analysis in the PREVEND study, weight loss or gain did not significantly bring about a change in GFR [12]. Other studies showed that GFR was decreased after weight loss in extremely obese patients, presumably by the mechanisms of amelioration of obesity-associated hyperfiltration [24, 25]. In the current study, BMI gain of ≥0.33 was associated with a significantly higher risk for a low eGFR

at the second visit in men, but not in women, who were free from a low eGFR at the first visit. Taking all these results together, it is suggested that the relationship between weight loss and GFR change may also differ according to the target population. Interestingly, high BMI is known to be associated with better survival in dialysis patients [26] designated as a risk factor paradox [27].

The current study has several limitations. First, we retrospectively analyzed data on individuals who underwent general health screening at our institute in two consecutive years; therefore, individuals who did not visit our institute the following year for unknown reasons were not enrolled in the current study, which may cause some biases. Second, we excluded subjects those who were taking anti-hypertensive agents during either visit. This may have excluded from the study population some hypertensive subjects with proteinuria. Whether or not a body weight change results in a change in CKD status in such hypertensive individuals is nonetheless an important question. However, we do not have data on which class of anti-hypertensive agents had been used, which might affect the development, amelioration or elimination of CKD. Third, we used the MDRD equation for the estimation of GFR, which may result in a certain degree of inaccuracy. In addition, changes in weight will be affected not only by the changes in fat mass, but also those in muscle mass, and eGFR determined by MDRD formula is also highly dependent on muscle mass, as this formula takes only serum creatinine into account. We have to be careful in interpreting the results of the current study, as changes in muscle mass will lead to bias when assessing the association between obesity parameters and eGFR. Fourth, our findings may not be immediately applicable to non-Japanese populations, as the GFR estimated using serum creatinine is again more than slightly affected by muscle mass.

In conclusion, a BMI reduction of ≥ 0.42 or a WC reduction of ≥ 3.0 cm over a 1-year period in men with micro-/macroalbuminuria at the first visit significantly reduced the risk for micro-/macroalbuminuria at the second visit, and a BMI gain of ≥ 0.33 over a period of a year in men without micro-/macroalbuminuria or a low eGFR at the first visit significantly increased the risk for micro-/macroalbuminuria or a low eGFR during the 1-year follow-up. Such associations were not statistically significant in female subjects. Our data indicated that reducing body weight in overweight/obese men with micro-/macroalbuminuria and that maintaining an ideal body weight in non-overweight men without micro-/macroalbuminuria or a low eGFR are both important targets of lifestyle in terms of renoprotection.

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Announcement

The Verband Deutsche Nierenzentren e.V. (Association of German Nephrology Centers) Announces the Bernd Tersteegen Award 2009

Dr. Bernd Tersteegen, the founder of the Verband Deutsche Nierenzentren (DN) e.V., was dedicated to the improvement of outpatient treatment modalities in end-stage renal disease. Specifically, he focused on further technical development of hemodialysis. The Bernd Tersteegen award was established following Dr. Tersteegen's death in 1995. The prize is awarded internationally both for basic and particularly for clinical research related to chronic renal insufficiency and to advances in the treatment of end-stage renal disease.

The annual award of EUR 8,000 is provided by Roche Pharma AG (Grenzach, Germany). The award is usually given to a single applicant but may be shared under certain circumstances. Applicants should be physicians, researchers or engineers who are involved in research in the area of renal failure and renal replacement therapy. Only research papers that have been published in 2008 or 2009 or have not yet been published are suitable for submission. Papers should be written in German or English. Review articles, dissertations, university habilitation works and manuscripts already entered in other competitions may not be submitted.

Five copies of the work must be submitted by July 15, 2009, to the following address:

Verband Deutsche Nierenzentren (DN) e.V.
Priv. Doz. Dr. med. Werner Kleophas, President
Kleine Klotzbahn 23
DE-42105 Wuppertal (Germany)

The members of the prize committee are chosen by the executive board of the DN. The president of the DN serves as chairman of the committee.

In the case in which no work is found suitable for the award, the prize money is carried over to the following year. An appeal is not allowed.

The award will be conferred at the Annual Meeting of the Association of German Nephrology Centers of the DN in Mannheim, Germany, on November 21, 2009. The presence of the award winner at the award ceremony is required. The award winner will be informed in due time.

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Adipose tissue-derived stem cells inhibit neointimal formation in a paracrine fashion in rat femoral artery

Masao Takahashi,[1]* Etsu Suzuki,[4]* Shigeyoshi Oba,[1] Hiroaki Nishimatsu,[2] Kenjiro Kimura,[4] Tetsuo Nagano,[3] Ryozo Nagai,[1] and Yasunobu Hirata[1]

Departments of [1]Internal Medicine and [2]Urology, Faculty of Medicine, and [3]Graduate School of Pharmaceutical Sciences, University of Tokyo, Tokyo; and [4]Saint Marianna University School of Medicine, Kawasaki, Japan

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Takahashi M, Suzuki E, Oba S, Nishimatsu H, Kimura K, Nagano T, Nagai R, Hirata Y. Adipose tissue-derived stem cells inhibit neointimal formation in a paracrine fashion in rat femoral artery. *Am J Physiol Heart Circ Physiol* 298: H415–H423, 2010. First published November 25, 2009; doi:10.1152/ajpheart.00391.2009.— Subcutaneous adipose tissue contains a lot of stem cells [adipose-derived stem cells (ASCs)] that can differentiate into a variety of cell lineages. In this study, we isolated ASCs from Wistar rats and examined whether ASCs would efficiently differentiate into vascular endothelial cells (ECs) in vitro. We also administered ASCs in a wire injury model of rat femoral artery and examined their effects. ASCs expressed CD29 and CD90, but not CD34, suggesting that ASCs resemble bone marrow-derived mesenchymal stem cells. When induced to differentiate into ECs with endothelial growth medium (EGM), ASCs expressed Flt-1, but not Flk-1 or mature EC markers such as CD31 and vascular endothelial cadherin. ASCs produced angiopoietin-1 when they were cultured in EGM. ASCs stimulated the migration of EC, as assessed by chemotaxis assay. When ASCs that were cultured in EGM were injected in the femoral artery, the ASCs potently and significantly inhibited neointimal formation without being integrated in the endothelial layer. EGM-treated ASCs significantly suppressed neointimal formation even when they were administered from the adventitial side. ASC administration significantly promoted endothelial repair. These results suggested that although ASCs appear to have little capacity to differentiate into mature ECs, ASCs have the potential to secrete paracrine factors that stimulate endothelial repair. Our results also suggested that ASCs inhibited neointimal formation via their paracrine effect of stimulation of EC migration in situ rather than the direct integration into the endothelial layer.

vascular endothelial cells; endothelial repair

CELL-BASED THERAPY HAS BEEN recently applied to the field of cardiovascular medicine. Among a variety of stem or progenitor cells that can be used for regeneration of heart and blood vessels, endothelial progenitor cells (EPCs) and bone marrow-derived mesenchymal stem cells (BMMSCs) are the ones that are most popularly used in this field. Although EPCs were originally isolated from human peripheral blood using the hematopoietic stem cell marker CD34 for positive selection, they are believed to reside mainly in the bone marrow (1, 19). EPCs can differentiate into vascular endothelial cells (ECs) in vitro and stimulate angiogenesis in vivo through integration in the endothelial layer of new forming capillaries (1). EPCs that are induced to differentiate into ECs are engrafted in the endothelial layer and inhibit neointimal formation via stimulation of EC regeneration (5). EPCs that are differentiated into ECs also form the endothelial layer on the surface of prosthetic grafts (5). BMMSCs have the potential to differentiate into mesenchymal tissues such as bone, cartilage, fat, and muscle (14). In addition to their capacity to differentiate into mesenchymal tissues, CD34-negative BMMSCs have been shown to differentiate into ECs (13). Furthermore, BMMSCs reportedly secrete paracrine factors that potentially stimulate angiogenesis (10). Bone marrow-derived cells have been used clinically to treat cardiovascular diseases and have turned out to be useful in some reports (22, 24). The problem in the clinical application of EPCs and BMMSCs is that bone marrow aspiration is usually necessary to prepare EPCs and BMMSCs. This procedure is somewhat painful for patients.

Recently, subcutaneous adipose tissue has been drawing much attention, because it contains a lot of mesenchymal stem cells that potentially differentiate into a variety of cell lineages including adipocytes, chondrocytes, osteocytes, and skeletal muscle (4). If these mesenchymal stem cells are useful for the regeneration of heart and blood vessels, adipose tissue will be a promising source of stem cells in the field of cardiovascular medicine, because it is easy to collect by local anesthesia. In fact, it has been reported that adipose-derived stem cells (ASCs) stimulate angiogenesis in the mouse hindlimb ischemia model (9, 11, 15, 16). However, the mechanism by which ASCs stimulate angiogenesis remains to be debated. ASCs promoted angiogenesis by being engrafted in the endothelial layer and stimulating neovascular formation (9, 15) or by producing angiogenesis-stimulating factors without integration into the endothelial layer (11). It also remains controversial whether ASCs can efficiently differentiate into ECs in vitro. Furthermore, it remains unclear whether ASCs can be efficiently integrated in the endothelial layer and inhibit neointimal formation.

In this study, we isolated ASCs from Wistar rats and examined whether ASCs would efficiently differentiate into ECs in vitro. We also examined whether ASCs would be engrafted in the endothelial layer and inhibit neointimal formation using a wire injury model of the rat femoral artery.

MATERIALS AND METHODS

Reagents. Anti-CD34, anti-vascular endothelial (VE)-cadherin, anti-Flk-1, anti-Flt-1, and anti-proliferating cell nuclear antigen (PCNA) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-CD31 antibody was obtained from ABR Affinity BioReagents (Golden, CO). Anti-CD29-FITC antibody was purchased from BioLegend (San Diego, CA). Anti-CD90 antibody was obtained from AbD Serotec (Oxford, UK). Anti-angiopoietin-1 (Ang-1) antibody was purchased from Abcam (Cambridge, MA), and human Ang-1 was obtained from R&D Systems (Minneapolis, MN).

* M. Takahashi and E. Suzuki contributed equally to this work.
Address for reprint requests and other correspondence: E. Suzuki, Inst. of Medical Science, St. Marianna Univ. School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki, 216-8512, Japan (e-mail: esuzuki-tky@umin.ac.jp).

Table 1. *Primers used for real-time PCR analysis*

Rat Flk1 sense	5′-TCTTTATTGTGCACTGCAGATAGAAA-3′
Rat Flk1 antisense	5′-GGACCGATGTTGCCTGTGA-3′
Rat Flt1 sense	5′-TCGCCAGAAGTCGTATGGTTAA-3′
Rat Flt1 antisense	5′-GCACCGAATAGCGAGCAGAT-3′
Rat CD31 sense	5′-GGCGTCCTGTCCGGAATC-3′
Rat CD31 antisense	5′-AGAACTCCTGCACAGTGACGTATT-3′
Rat VE-Cad sense	5′-GGTGAAGAAAGAAGAGGCAGACA-3′
Rat VE-Cad antisense	5′-CGTAGCCGTAGATGTGCAGTGT-3′
Rat VEGF-A sense	5′-GAGGAAAGGGAAAGGGTCAAAA-3′
Rat VEGF-A antisense	5′-CACAGTGAACGCTCCAGGATT-3′
Rat bFGFsense	5′-GTCAAACTACAGCTCCAAGCAGAA-3′
Rat bFGF antisense	5′-AGGTACCGGTTCGCACACA-3′
Rat Ang-1 sense	5′-CAGGAGGTTGGTGGTTTGATG-3′
Rat Ang-1 antisense	5′-TTTGCCCTGCAGTGTAGAACATT-3′
Rat HGF sense	5′-CAATCCAGAGGTACGCTACGAA-3′
Rat HGF antisense	5′-TTTCACCGTTGCAGGTCATG-3′
Rat IGF-1 sense	5′-CCTACAAAGTCAGCTCGTTCCA-3′
Rat IGF-1 antisense	5′-TCCTTCTGAGTCTTGGGCATGT-3′
Rat GAPDH sense	5′-GTATGACTCTACCCACGGCAAGT-3′
Rat GAPDH antisense	5′-TTCCCGTTGATGACCAGCTT-3′

VE-Cad, vascular endothelial cadherin; bFGF, basic fibroblast growth factor; Ang-1, angiopoietin-1; HGF, hepatocyte growth factor.

Cell culture. ASCs were cultured from male Wistar rats as previously reported with slight modification (15). In brief, inguinal subcutaneous adipose tissue was excised and minced in phosphate-buffered saline (PBS) on ice. The minced tissue was then digested at 37°C for 1 h in PBS containing 2% bovine serum albumin and 2 mg/ml collagenase (Sigma, St. Louis, MO). The digested tissue was filtered through a 100-μm nylon mesh and centrifuged at 600 *g* for 10 min. After lysis of red blood cells in 1× lysis buffer containing (in mM) 154 NH_4Cl, 14 $NaHCO_3$, and 0.1 EDTA (pH 7.3), the pellets were plated in 100-mm dishes at a density of 30,000 cells/cm^2 in a 1:1 mixture of Dulbecco's modified Eagle's medium (DMEM) and F12 medium containing 10% fetal bovine serum (FBS). Six hours after the cells were plated, the medium was changed to remove nonadherent cells. The adherent cells were cultured in DMEM-F12-10% FBS and split several times to expand the cells. *Passages 2* to *3* were used for the experiments. To induce differentiation into ECs, ASCs were cultured in endothelial growth medium-2MV (EGM; Lonza Walkersville, Walkersville, MD) on fibronectin-coated dishes. EGM consists of endothelial basal medium-2 (Lonza Walkersville) containing 5% FBS plus growth factors such as epidermal growth factor, hydrocortisone, vascular endothelial growth factor (VEGF)-A, basic fibroblast growth factor (bFGF), and insulin-like growth factor (IGF)-1. ASCs were also cultured on fibronectin-coated dishes in endothelial basal medium-2 containing 5% FBS (EBM) as the negative control. Human umbilical vein ECs (HUVECs) were purchased from Sanko-Junyaku (Tokyo, Japan) and cultured using HuMedia-EG (Kurabo, Osaka, Japan). Rat vascular smooth muscle cells (VSMCs) were cultured from rat thoracic aortas following the explant method, as previously described (17), and maintained in DMEM containing 10% FBS. NRK-52E cells, a cell line derived from rat renal tubular cells, were cultured in DMEM containing 5% FBS.

Flow cytometry. Cultured ASCs were trypsinized and incubated in a blocking buffer (PBS-containing 3% FBS) for 30 min on ice. Approximately 5 × 10^5 cells were incubated with primary antibodies reactive to CD34, VE-cadherin, CD29, CD90, or isotype-matched control IgGs. After being washed, the cells were incubated with secondary antibodies coupled with FITC, when the primary antibodies were unlabeled. Following the wash, samples were analyzed with an EPICS XL flow cytometer (Beckman Coulter, Fullerton, CA).

RNA extraction and real-time PCR. Total RNA was extracted using TRIzol reagent (Gibco, Rockville, MD) according to the instructions provided by the manufacturer. Total RNA was subjected to reverse transcription using an Omniscript RT kit (Qiagen, Tokyo, Japan). The expression of a variety of genes including Flk-1, Flt-1, CD31, VE-cadherin, VEGF-A, bFGF, Ang-1, hepatocyte growth factor (HGF), IGF-1, and glyceraldehyde-3-phosphate dehydrogenase was examined

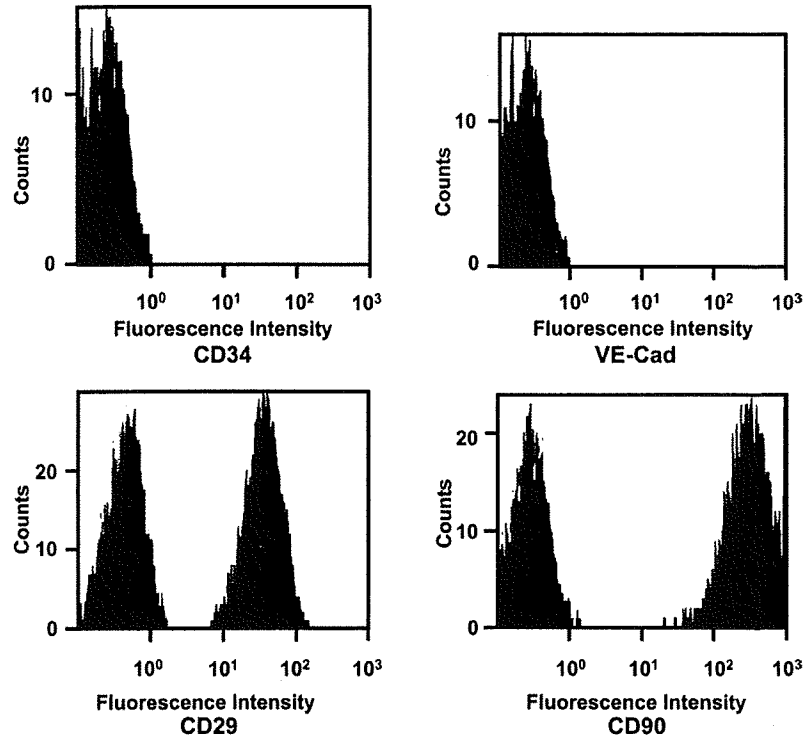

Fig. 1. Flow cytometry analysis of cell surface marker expressions in adipose-derived stem cells (ASCs). Specific fluorescence of CD34, vascular endothelial cadherin (VE-Cad), CD29, and CD90 (black-filled area) and nonspecific fluorescence derived from their isotype matched control IgGs (gray-filled area) are shown. Shown are representative results of 3 independent experiments.