Table 1b. Baseline Characteristics at the First Visit According to %dBMI

variables	%dBMI-Q1 (range: −21.8−−1.9)	%dBMI-Q2 (range: −1.9−−0.2)	%dBMI-Q3 (range: −0.2−1.4)	%dBMI-Q4 (range: 1.4−15.7)	*p* value
Women					
n	284	268	305	342	
Age, years	53 (52-54)	54 (52-54)	52 (51-53)	49 (49-51)	0.002
Height, cm	156 (156-157)	157 (156-157)	158 (157-158)	157 (157-158)	0.005
Weight, kg	52 (52-54)	51 (52-53)	51 (51-53)	51 (51-53)	0.325
WC, cm	77 (76-78)	76 (76-78)	75 (75-77)	75 (75-77)	0.115
BMI, kg/m^2	21.3 (21.3-22.0)	20.9 (21.1-21.8)	20.5 (20.6-21.2)	20.7 (20.7-21.3)	0.002
Systolic blood pressure, mmHg	117 (118-123)	115 (115-119)	114 (115-119)	113 (115-118)	0.060
Diastolic blood pressure, mmHg	74 (73-76)	73 (72-75)	71 (72-74)	71 (71-74)	0.057
Pulse rate, bpm	63 (63-65)	64 (63-65)	61 (62-64)	63 (63-65)	0.106
LDL-cholesterol, mg/dL	133 (127-135)	132 (129-136)	125 (123-129)	117 (119-125)	<0.001
HDL-cholesterol, mg/dL	67 (66-70)	68 (67-71)	69 (68-71)	67 (67-70)	0.647
Trigryceride, mg/dL	79 (87-102)	76 (80-89)	74 (79-89)	68 (73-81)	0.002
Uric acid, mg/dL	4.5 (4.4-4.7)	4.4 (4.4-4.6)	4.6 (4.5-4.7)	4.4 (4.4-4.6)	0.408
Fasting glucose, mg/dL	88 (88-91)	88 (88-93)	88 (88-91)	88 (88-90)	0.933
Hemoglobin A$_{1C}$, %	5.1 (5.1-5.2)	5.2 (5.1-5.3)	5.1 (5.1-5.2)	5.1 (5.0-5.1)	0.028
Blood urea nitrogen, mg/dL	13.0 (13.2-14.0)	13.0 (13.1-13.9)	13.0 (13.3-14.2)	13.0 (12.8-13.4)	0.174
Serum creatinine, mg/dL	0.60 (0.61-0.63)	0.60 (0.61-0.63)	0.60 (0.62-0.73)	0.60 (0.61-0.63)	0.002
Anti-dyslipidemic medication, *n* (%)	12 (4.2)	10 (3.7)	12 (3.9)	12 (3.5)	0.972
Anti-hypertensive medication, *n* (%)	23 (8.1)	15 (5.6)	16 (5.2)	17 (5.0)	0.352
Current smoker, *n* (%)	21 (7.4)	22 (8.2)	23 (7.5)	41 (12.0)	0.130
Men					
n	504	531	495	484	
Age, years	54 (53-55)	55 (54-55)	54 (53-54)	51 (51-52)	<0.001
Height, cm	169 (169-170)	169 (168-169)	170 (169-170)	170 (169-171)	0.012
Weight, kg	69 (68-70)	67 (67-68)	68 (68-69)	68 (67-69)	0.097
WC, cm	87 (86-87)	85 (85-86)	86 (85-87)	85 (85-86)	0.011
BMI, kg/m^2	24.0 (23.8-24.3)	23.4 (23.4-23.9)	23.7 (23.6-24.1)	23.5 (23.3-23.8)	0.012
Systolic blood pressure, mmHg	126 (127-130)	124 (125-128)	126 (125-129)	123 (123-126)	0.011
Diastolic blood pressure, mmHg	81 (81-83)	79 (79-81)	80 (80-82)	78 (78-80)	0.019
Pulse rate, bpm	62 (62-64)	62 (62-63)	62 (63-64)	62 (61-63)	0.106
LDL-cholesterol, mg/dL	133 (130-135)	129 (128-133)	130 (126-132)	125 (125-130)	0.014
HDL-cholesterol, mg/dL	54 (54-56)	54 (55-57)	54 (55-58)	54 (54-57)	0.437
Trigryceride, mg/dL	111 (126-141)	108 (123-136)	111 (120-135)	107 (118-132)	0.285
Uric acid, mg/dL	6.1 (6.1-6.3)	6.1 (6.0-6.2)	6.0 (6.0-6.2)	6.1 (6.0-6.3)	0.344
Fasting glucose, mg/dL	95 (97-100)	95 (97-100)	95 (95-97)	93 (94-96)	0.002
Hemoglobin A$_{1C}$, %	5.3 (5.3-5.5)	5.3 (5.3-5.4)	5.2 (5.2-5.3)	5.2 (5.2-5.3)	<0.001
Blood urea nitrogen, mg/dL	14.0 (14.3-15.0)	14.0 (14.3-14.8)	14.0 (13.9-14.4)	14.0 (14.3-15.0)	0.130
Serum creatinine, mg/dL	0.80 (0.84-0.92)	0.80 (0.85-0.87)	0.80 (0.83-0.86)	0.90 (0.85-0.87)	0.303
Anti-dyslipidemic medication, *n* (%)	20 (4.0)	18 (3.4)	28 (5.7)	21 (4.3)	0.334
Anti-hypertensive medication, *n* (%)	72 (14.3)	81 (15.3)	47 (9.5)	61 (12.6)	0.035
Current smoker, *n* (%)	155 (30.8)	167 (31.5)	148 (29.9)	157 (32.4)	0.851

underwent a general health screening during this period (first visit) and again the following year (second visit). Among these 3325 individuals, 3213 (2014 men, 1199 women) who reported not taking antidiabetic drugs at either visit were enrolled in the current study. The mean ± standard deviation (SD) of the interval between the two visits of the individuals enrolled was 356 ± 51 days. The percent difference in

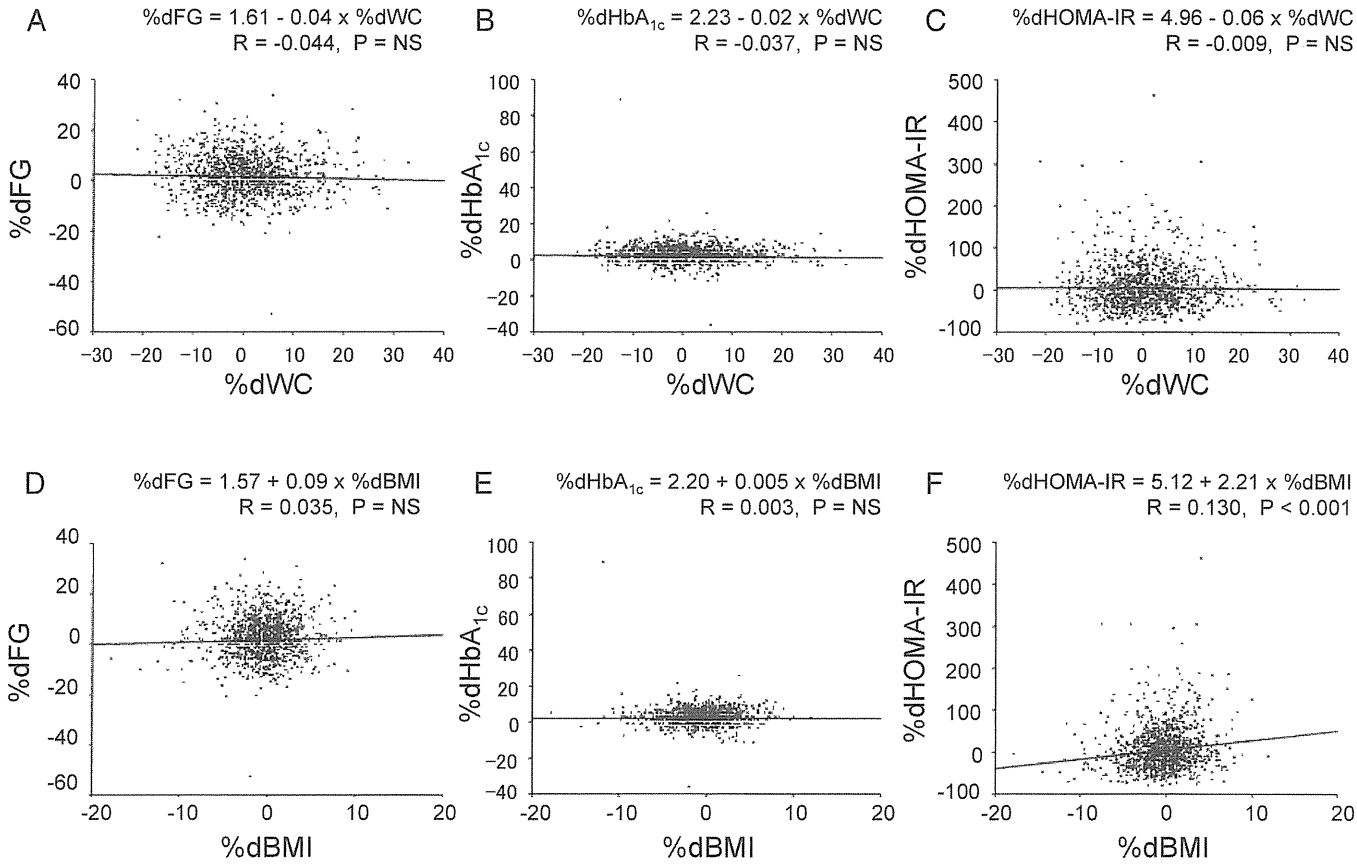

Fig. 1. Scatter plot and linear regression between %dWC and %dFG (A), %dHbA1c (B), and %dHOMA-IR (C) and between %dBMI and %dFG (D), %dHbA1c (E), and %dHOMA-IR (F) in women.

the value of WC, BMI, serum levels of fasting glucose (FG), HbA1c, and HOMA-IR between the first and second visits was designated %dWC, %dBMI, %dFG, %dHbA1c, and %dHOMA-IR, respectively. Blood samples were taken from all subjects after an overnight fast. BMI was expressed as weight (in kilograms) divided by the square of height (in meters). WC was measured at the umbilical level to the nearest 1 cm by trained physicians and technicians[11].

Laboratory Analysis

Serum levels of TC, HDL-C, and TG were determined enzymatically. Serum uric acid was measured by the uricase-peroxidase method; hemoglobin A1c was determined by a latex agglutination immunoassay. Creatinine was measured by TBA-200FR (Toshiba Medical Systems, Tochigi, Japan) using a commercial kit. The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated by the equation: HOMA-IR = (immunoreactive insulin (IRI)) × FBS/405. Blood pressure was measured after about 10 min of rest by an automated sphygmomanometer.

Statistical Analysis

Data are expressed as the median (95% confidence interval (95%CI)) unless stated otherwise. The Kruskal-Wallis test, χ^2 test, logistic regression analysis, and multivariate linear regression analysis were applied as appropriate to assess the statistical significance of differences between groups using computer software, Dr. SPSS II (SPSS Inc., Chicago, IL). A value of $p < 0.05$ was taken to be statistically significant.

Results

Baseline Characteristics

We enrolled 1199 women and 2014 men in this study. The mean age of the individuals enrolled was 51.9 years in women and 53.4 years in men at the first visit. The sex-nonspecific range of the first to fourth %dWC quartiles was −21.3/−3.4, −3.4/−0.1, 0.0/3.3, and 3.3/33.4, respectively, and that of the first to fourth %dBMI quartiles was −21.8/−1.9, −1.9/−0.2, −0.2/1.4, and 1.4/15.7, respectively. Subject characteristics at the first visit are shown according to the

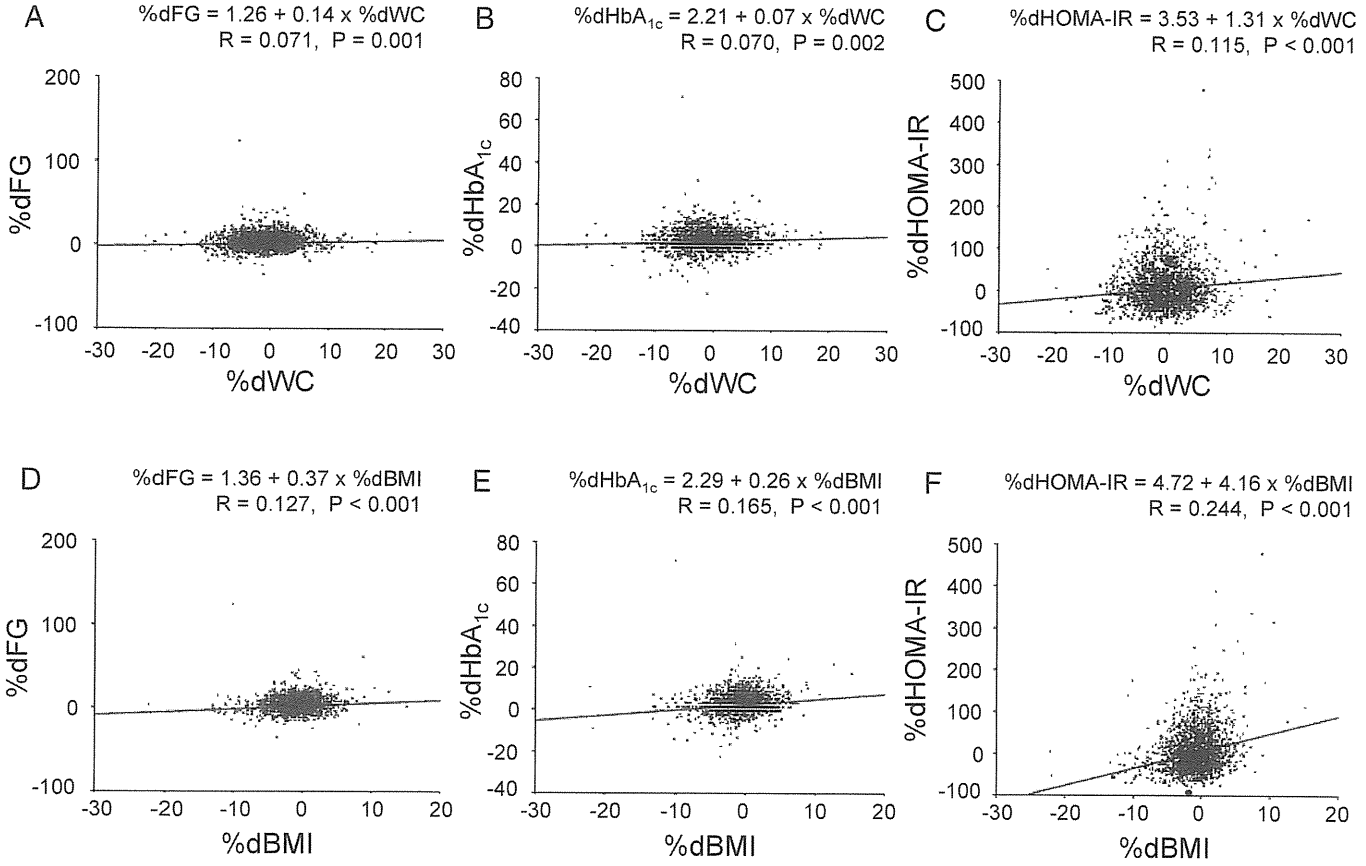

Fig. 2. Scatter plot and linear regression between %dWC and %dFG (A), %dHbA1c (B), and %dHOMA-IR (C) and between %dBMI and %dFG (D), %dHbA1c (E), and %dHOMA-IR (F) in men.

%dWC and %dBMI quartiles in **Table 1**. No statistically significant trends in the rate of anti-dyslipidemic medication or of current smoking were found across the four %dWC or %dBMI quartiles in either gender. The correlation coefficient between %dWC and %dBMI was 0.24 in women and 0.46 in men.

Association between Percent Changes in Obesity Parameters and Percent Changes in Diabetic Parameters

Scatter plots of %dWC and %dBMI versus %dFG, %dHbA1c and %dHOMA-IR, coupled with results of linear regression analyses, are shown in **Fig. 1** and **2**. In women, only the relationship between %dBMI and %dHOMA-IR was significant. In men, by contrast, the relationship was significant between both %dWC and %dBMI and the percent change in each of the diabetic parameters.

Fig. 3 and **4** show the percent changes in diabetic parameters according to the %dWC and %dBMI quartiles. In women, %dHOMA-IR increased with increasing %dBMI. In men, not only %dHOMA-IR but also %dFG and %dHbA1c increased with increasing %dWC and %dBMI.

Logistic Regression Analysis

A multivariate logistic regression analysis, adjusted for age at the first visit, of the second, third, and fourth %dBMI quartiles, showed that the first, second, third, and fourth %dBMI quartiles in men were associated with the highest %dHOMA-IR quartile (%dHOMA-IR >24.3%) with an odds ratio of 1.00 (reference), 1.47 (95%CI 1.08-2.01), 1.51 (95%CI 1.11-2.07), and 2.87 (95%CI 2.13-3.87), respectively. In women, on the other hand, the first, second, third, and fourth %dBMI quartiles were not significantly related to the highest %dHOMA-IR quartile (%dHOMA-IR >24.3%) with an odds ratio of 1.00 (reference), 1.23 (95%CI 0.82-1.85), 1.45 (95%CI 0.98-2.14), and 1.89 (95%CI 1.30-2.74), respectively.

Multivariate Linear Regression Analysis

In a multivariate linear regression analysis with age at the first visit and %dWC as independent vari-

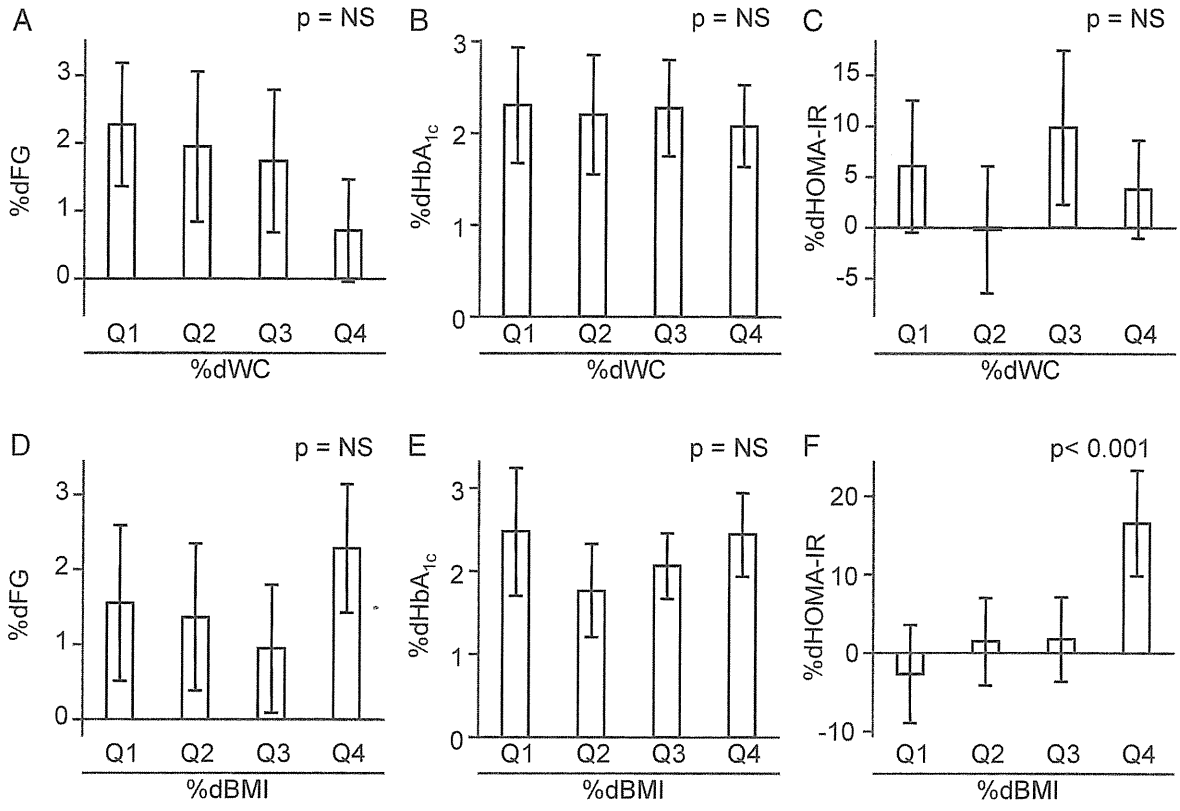

Fig. 3. %dFG (A), %dHbA1c (B), and %dHOMA-IR (C) according to %dWC quartiles, and %dFG (D), %dHbA1c (E), and %dHOMA-IR (F) according to %dBMI quartiles in women. The mean ± 95% confidence interval is shown in each group.

ables (**Table 2**, model 1), %dWC was an independent predictor for %dHOMA-IR in men, but not in women. However, when %dBMI was used as an additional covariate in the statistical model, %dWC did not remain significant (**Table 2**, model 2). In model 2, %dBMI was found to be an independent predictor for %dHOMA-IR, %dFG and %dHbA1c in men, but for only %dHOMA-IR in women.

Discussion

In the current study, we demonstrated that percent changes in obesity parameters (%dWC, %dBMI) were positively correlated with percent changes in glucose metabolism-related parameters (%dFG, %dHbA1c, %dHOMA-IR) in men. In women, by contrast, there was no significant relationship between %dWC and percent changes in diabetic parameters, and %dBMI was not significantly associated with %dFG or %dHbA1c. In the multivariate linear regression analysis, %dWC was a predictor for %dHOMA-IR in men, although it did not remain significant when %dBMI was used as an additional covariate in the statistical model, suggesting that changes in WC are not a predictor for changes in glucose-metabolism-related parameters independent of changes in BMI.

Obesity is associated with a cluster of specific metabolic abnormalities that may be related to cardiovascular risk factors[8, 12]. Wahrenberg *et al.* have reported that WC, which was found to be the strongest regressor among WC, BMI, log-plasma triglycerides, systolic blood pressure, and high-density lipoprotein cholesterol, is a risk factor for insulin resistance[13]. On the other hand, Onat *et al.* prospectively analyzed 1638 men and found that the age-adjusted waist-to-hip ratio (WHR) was significant in predicting diabetes mellitus[14]. Furthermore, Colditz *et al.* analyzed data from 114281 women who did not have diagnosis of diabetes mellitus, coronary heart disease, stroke, or cancer, and showed that BMI was the dominant predictor of risk for diabetes mellitus, although weight gain was also a risk factor for diabetes[15]. It has been shown that even small gains in weight during adulthood lead to a significantly increased risk of many chronic diseases[16]. Several studies showed that weight loss reduced regional depots of adipose tissue and

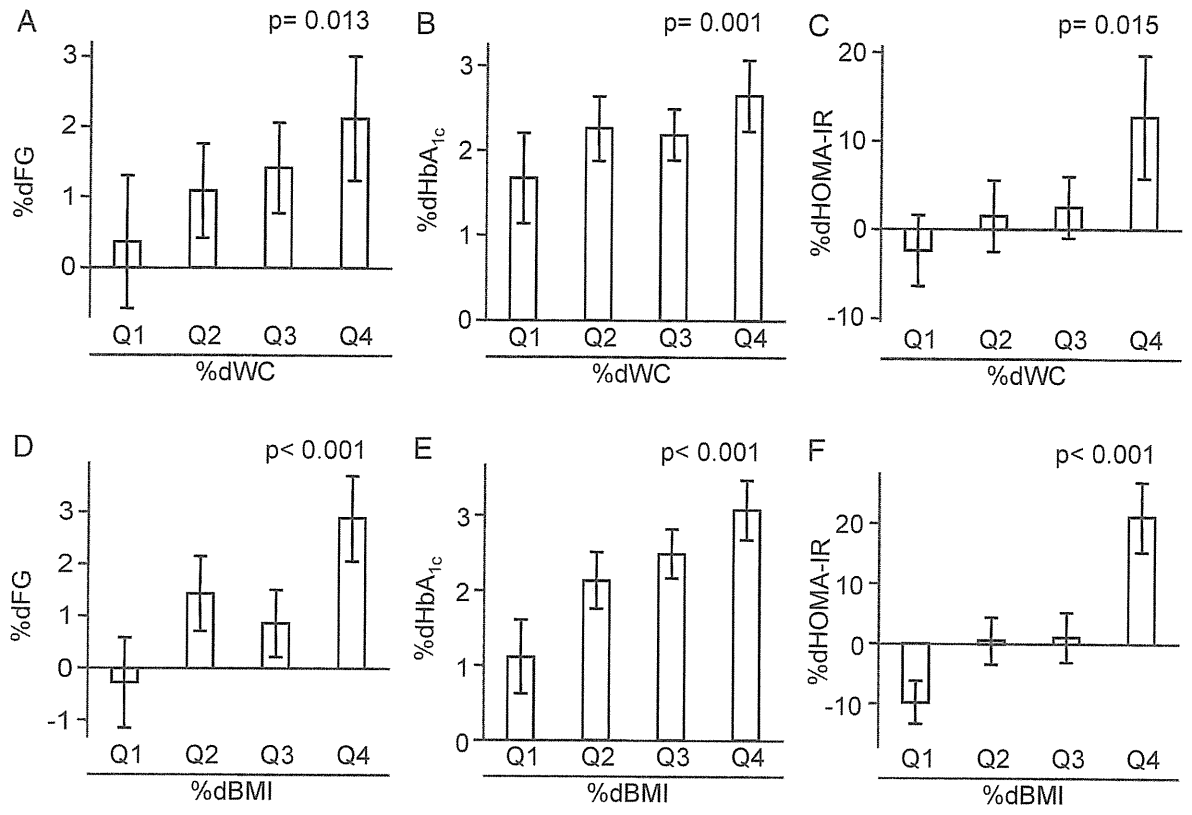

Fig. 4. %dFG (A), %dHbA$_{1c}$ (B), and %dHOMA-IR (C) according to %dWC quartiles, and %dFG (D), %dHbA$_{1c}$ (E), and %dHOMA-IR (F) according to %dBMI quartiles in men. The mean ± 95% confidence interval is shown in each group.

improved insulin sensitivity and cardiovascular risk factors[17, 18]. Pascale *et al.* analyzed 60 women and 33 men participating in a year-long weight loss program and concluded that improvements in FG, fasting insulin, and HbA$_{1c}$ were significantly related to weight loss[19].

Besides body weight, visceral fat has also been reported to be associated with β-cell function in individuals with impaired fasting glycemia and impaired glucose tolerance[9]. In general, BMI is strongly associated with subcutaneous fat area. As parameters of obesity, BMI and WC may have different meanings but similar associations. BMI may have a weaker association with visceral fat; by contrast, WC has a stronger correlation with visceral fat area in both genders[10]. It has been suggested that WC better reflects the accumulation of visceral fat than WHR[20, 21]. Therefore, it is possible that changes in WC have a stronger impact on changes in glucose metabolism as compared with changes in BMI.

In the current study, however, %dBMI was an independent factor predicting %dFG, %dHbA$_{1c}$, and %dHOMA-IR in men, and %dHOMA-IR in women. %dWC was an independent factor predicting %dHOMA-IR in men, only without adjustment for %dBMI. Why %dBMI had a stronger association with %dFG, %dHbA$_{1c}$ and %dHOMA-IR is not clear.

Because Asian women are relatively lean, subcutaneous fat may have a relatively greater influence on WC[22]. For example, Sakurai *et al.* analyzed 2935 men and 1622 women between 35 and 59 years of age: in a multiple logistic regression analysis, WC was associated with FG in both genders. However, the risk ratio of having two or more metabolic disorders was higher for BMI than for WC in women, suggesting WC to be a relatively poor discriminator of visceral fat, and BMI to be a more appropriate index of total and abdominal fat, especially in women[22, 23].

It has recently been demonstrated that the association between WC and cardiovascular risk markers, such as insulin resistance, weakens with age[24]. Janssen *et al.* reported that, although individuals with a moderate and high WC were likely to have elevated cardiometabolic risk markers irrespective of age, there seemed to be a significant correlation between age and WC, indicating that the relation between WC and insulin

Table 2. Multivariate linear regression analysis between percent changes in diabetic parameters and age, %dWC, and %dBMI

		β	95% CI		Standardized β	p value
Women	Model 1					
	Dependent variable, %dFG					
	age	−0.02	−0.06	0.03	−0.02	0.494
	%dWC	−0.05	−0.10	0.01	−0.05	0.118
	Dependent variable, %dHbA1c					
	age	−0.01	−0.04	0.01	−0.03	0.353
	%dWC	−0.02	−0.06	0.01	−0.04	0.181
	Dependent variable, %dHOMA-IR					
	age	0.00	−0.30	0.31	0.00	0.993
	%dWC	−0.06	−0.44	0.32	−0.01	0.753
	Model 2					
	Dependent variable, %dFG					
	age	−0.01	−0.06	0.03	−0.02	0.605
	%dWC	−0.06	−0.12	0.00	−0.06	0.059
	%dBMI	0.12	−0.03	0.27	0.05	0.119
	Dependent variable, %dHbA1c					
	age	−0.01	−0.04	0.02	−0.03	0.374
	%dWC	−0.03	−0.06	0.01	−0.04	0.168
	%dBMI	0.02	−0.08	0.11	0.01	0.741
	Dependent variable, %dHOMA-IR					
	age	0.08	−0.22	0.38	0.01	0.610
	%dWC	−0.28	−0.67	0.10	−0.04	0.152
	%dBMI	2.41	1.42	3.40	0.14	<0.001
Men	Model 1					
	Dependent variable, %dFG					
	age	−0.02	−0.06	0.01	−0.03	0.223
	%dWC	0.14	0.05	0.22	0.07	0.002
	Dependent variable, %dHbA1c					
	age	−0.01	−0.03	0.01	−0.03	0.250
	%dWC	0.07	0.03	0.12	0.07	0.002
	Dependent variable, %dHOMA-IR					
	age	−0.08	−0.29	0.14	−0.02	0.479
	%dWC	1.30	0.80	1.80	0.11	<0.001
	Model 2					
	Dependent variable, %dFG					
	age	−0.01	−0.05	0.02	−0.02	0.434
	%dWC	0.03	−0.07	0.13	0.02	0.544
	%dBMI	0.35	0.20	0.49	0.12	<0.001
	Dependent variable, %dHbA1c					
	age	−0.01	−0.03	0.01	−0.01	0.592
	%dWC	−0.01	−0.06	0.04	−0.01	0.740
	%dBMI	0.26	0.18	0.34	0.17	<0.001
	Dependent variable, %dHOMA-IR					
	age	0.02	−0.19	0.23	0.00	0.840
	%dWC	0.02	−0.52	0.57	0.00	0.932
	%dBMI	4.15	3.33	4.97	0.24	<0.001

For model 1, independent variables include age at the first visit and %dWC. For model 2, independent variables include age at the first visit, %dWC, and %dBMI.

resistance was attenuated in the elderly[24]. With regard to our study, the mean age of the individuals enrolled was 51.9 years in women and 53.4 years in men at the first visit. We may have to analyze the relationship between %dWC or %dBMI and changes in glucose metabolism in a younger population in future studies. In addition, WC measurements may be less reliable or reproducible than weight and height measurements, which might relate to the finding that although %dWC is a predictor for the change in diabetic parameters, the correlation between %dWC and %dBMI was weaker in women, the latter of which is a predictor for the changes in diabetic parameters also in women.

In the current study, interestingly, there was a gender difference in the relationship between %dWC and changes in diabetic parameters. Wing *et al.* reported that the relationship between changes in WHR and changes in lipid parameters differed between women and men: they showed that changes in WHR were associated with changes in total cholesterol and triglycerides levels in men, but not in women[18].

Although we did not look into the mechanisms that may explain the differences in the association of changes in obesity indexes and those in glucose metabolism-related markers between men and women, several explanations may exist. Adipose tissue has been recognized as a significant endocrine organ that releases biologically important cytokines, such as adiponectin, leptin, and vaspin[25, 26]. In several clinical studies, certain gender differences have existed in the serum levels of such adipokines (adiponectin[27, 28], leptin[29], and vaspin[30]), which may account, in part, for the difference in the association between changes in obesity indexes and those in glucose metabolism-related parameters in the current study. Such sexual dimorphism in adipocytokines may be related to the difference in the levels of sex hormones, such as dehydro-epiandrosterone-sulphate (DHEAS), oestradiol, and testosterone[27, 31, 32].

We previously analyzed the relationship between percent changes in obesity parameters and percent changes in serum lipid parameters, uric acid, and systolic blood pressure[33-35]. We found that, as in the current study, the impact of %dBMI was greater than that of %dWC from the viewpoint of changes in serum uric acid and blood pressure.

Our study has several potential limitations. First, we enrolled only individuals who underwent a general health screening at our institute for 2 consecutive years. Second, we analyzed data from participants without considering alcohol consumption or the number of cigarettes smoked. Third, we excluded individuals who were taking antidiabetic drugs at either visit. It has been suggested that these individuals are generally more motivated to improve their own health than those who are not taking such drugs. In addition, a longer follow-up would be required to draw more convincing conclusions in future studies.

In summary, over a one-year period, %dBMI was found to be an independent predictor for %dHOMA-IR in both genders and for %dFG and %dHbA$_{1c}$ only in men. Although %dWC was also associated with percent changes in these diabetic parameters, this relationship did not remain significant after controlling for %dBMI. Conversely, the relationship between %dBMI and percent changes in glucose-related metabolism parameters, especially in men, was independent of %dWC. These findings collectively suggest that controlling body weight, rather than WC, may be the primary target for improving glucose metabolism at least over a one-year period.

Acknowledgements

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ORIGINAL ARTICLE

Effects of the AT_1 receptor blocker losartan and the calcium channel blocker benidipine on the accumulation of lipids in the kidney of a rat model of metabolic syndrome

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Unfavorable lipid accumulation may occur in the kidneys in the presence of metabolic syndrome and diabetes. The aim of this study was to investigate whether excess lipids would accumulate in the kidneys of Otsuka Long-Evans Tokushima Fatty (OLETF) rats, an animal model of metabolic syndrome. From 34 weeks of age, OLETF rats were treated orally with a calcium channel blocker, benidipine (3 mg kg^{-1} per day), or an AT1 receptor blocker, losartan (25 mg kg^{-1} per day), for 8 weeks. Blood pressure was slightly but significantly higher in the untreated OLETF rats (149 ± 4 mm Hg) than in Long-Evans Tokushima Otsuka (LETO) rats (136 ± 2 mm Hg), and both losartan (135 ± 3 mm Hg) and benidipine (138 ± 3 mm Hg) reduced blood pressure in OLETF rats to a level comparable to that in LETO rats. Tissue content of triglycerides (TG) was greater in OLETF rats than in LETO rats (6.24 ± 3.77 and 2.85 ± 1.32 μg mg^{-1} · tissue, respectively), and both losartan and benidipine reduced these values. Histological analysis showed lipid droplets in tubular cells in which increased dihydroethidium fluorescence was present. Expression of peroxisome proliferator-activated receptor-α, PGC-1α and uncoupling protein-2 was found to be higher in OLETF rats than in LETO rats; however, the expression of these genes was not altered by treatment with either antihypertensive drug. In contrast, both losartan and benidipine increased the amount of total and phosphorylated forms of AMP kinase and the expression of carnitine palmitoyltransferase-1 (CPT-1). In conclusion, treatment of OLETF rats with losartan and benidipine reduced the tissue content of TG, decreased the production of superoxide and regulated the expression of genes related to fatty acid oxidation such as AMP-activated protein kinase and CPT-1 in the kidneys.
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Keywords: calcium channel blocker; diabetes; kidney; lipotoxicity; renin angiotensin system

INTRODUCTION

Unfavorable lipid accumulation in the kidneys may occur in animal models of diabetes,[1–3] aging,[4] diet-induced obesity[5,6] and nephrectomy.[7] Although the precise mechanism by which lipid content is increased in the kidneys is still not fully elucidated, it may include upregulation of lipogenic gene expression in the kidneys[1,7,8] and uptake of filtered albumin-bound fatty acids by renal tubular cells when increased urinary excretion of albumin is present.[9] Transfer of lipogenic genes induced deposition of lipids and upregulation of fibrosis-related gene expression in the kidneys of diabetic animals, whereas lipogenic gene knockdown had the opposite effect,[1,8] suggesting that the accumulation of excessive lipid in the kidneys is one factor in the pathophysiological process of diabetic nephropathy.[6]

We reported earlier that administration of angiotensin II upregulates the expression of lipogenic genes and increases lipid content in the kidneys,[10] suggesting that activation of the renin angiotensin system may have a role in lipid accumulation in the kidney. It has been reported that lipid content is increased in the liver[11] and pancreas[12] of OLETF rats. In this study, therefore, we investigated whether excessive lipid accumulation occurs in the kidneys of Otsuka Long-Evans Tokushima Fatty (OLETF) rats, which exhibit features of metabolic syndrome,[13] and if present, whether angiotensin II receptor blockers and calcium channel blockers could exert similar effects on renal lipid content in OLETF rats. We used benidipine as the calcium channel blocker because this drug has been reported to reduce the extent of proteinuria in OLETF rats.[14]

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METHODS

Animals

The experiments were performed in accordance with the guidelines for animal experimentation approved by the Animal Center for Biomedical Research, Faculty of Medicine, University of Tokyo. Male OLETF and age-matched Long-Evans Tokushima Otsuka (LETO) rats, a genetic control, were obtained from the Tokushima Research Institute (Otsuka Pharmaceutical, Tokushima, Japan) and maintained under constant temperature and lighting conditions with free access to food and water. At 34 weeks of age, the OLETF rats were given 3 mg kg^{-1} benidipine or 25 mg kg^{-1} losartan per day orally, which was continued for 8 weeks. One day before sacrifice, the rats were kept in a metabolic cage, and urine was collected for 24 h under fasting conditions. Systolic blood pressure and heart rate were measured in conscious rats by tail-cuff plethysmography (BP-98A, Softron, Tokyo, Japan).

Measurement of lipid content in the serum and kidney

Serum levels of total cholesterol (TC), triglycerides (TG) and non-esterified fatty acid were measured by enzymatic methods (SRL, Tokyo, Japan). Contents of TG and TC in the kidney were measured from homogenate extracts by enzymatic colorimetric determination using the Triglyceride-E Test, the Cholesterol-E Test and the Free cholesterol-E Test, respectively (Wako Pure Chemicals, Osaka, Japan).

Histological analysis

Oil red O staining was performed on sections of unfixed, freshly frozen kidney samples (3 μm in thickness). For semi-quantification of lipid deposition, images of each specimen stained with oil red O were taken with an Olympus BX51 microscope and a DP12 digital camera system (Olympus, Tokyo, Japan). Five images taken in the cortical region of each sample were analyzed. The ratio of the areas of lipid deposition to the total tissue region area was calculated using Adobe Photoshop image analysis software (Adobe Systems, San Jose, CA, USA). *In situ* superoxide production was estimated using the oxidative fluorescent dye dihydroethidium (DHE) in unfixed frozen kidney specimens as described earlier.[10] Images were obtained from at least five fields in each section, and signal intensity was presented as a percentage of that in OLETO rats.

Western blot analysis

Western blot analysis was performed as described earlier.[15] Antibodies against total and phosphorylated forms of AMP-activated protein kinase (AMPK) (Cell Signaling Technology, Danvers, MA, USA), sterol regulatory element-binding protein (SREBP)-1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and total and phosphorylated forms of acetyl-CoA carboxylase (ACC) (Cell Signaling Technology) were used at a dilution of 1/1000.

Real time RT-PCR

The mRNA expression of lipid metabolism-related genes was analyzed by real-time quantitative PCR performed using a LightCycler together with Hybri-Probe technology (Roche Diagnostics, Basel, Switzerland). The expression of target genes was normalized to the mRNA expression of the endogenous control, glyceraldehyde-3-phosphate dehydrogenase. The target genes were SREBP-1c, fatty acid synthase (FAS), 3-hydroxy-3-methylglutaryl coenzyme A reductase, peroxisome proliferator-activated receptor (PPAR)-γ, PPAR-α, PPAR-γ coactivator (PGC)-1α, CD36, carnitine palmitoyltransferase (CPT)-1 and uncoupling protein (UCP)-2. The forward and reverse primers used were described earlier.[16]

Statistical analysis

Data are expressed as mean ± s.e.m. ANOVA and Kruskal–Wallis analyses followed by a *post hoc* multiple comparison test were performed using the statistical analysis software Dr. SPSS II (SPSS Inc., Chicago, IL, USA). A value of $P<0.05$ was taken to be statistically significant.

RESULTS

Characteristics of the experimental animals

Body weight, blood pressure, heart rate and blood levels of lipids and glucose in each group have been described elsewhere.[17] Blood pressure was slightly but significantly higher in the untreated OLETF rats (149 ± 4 mm Hg, n=11, P=0.012) than in LETO rats (136 ± 2 mm Hg, n=11), and both losartan (135 ± 3 mm Hg, n=6) and benidipine (138 ± 3 mm Hg, n=11) reduced blood pressure in OLETF rats to a level comparable to that in LETO rats. Treatment of OLETF rats with either antihypertensive drug had no significant effect on circulating levels of triglyceride and glucose, which were higher in OLETF than in LETO rats. Compared with LETO rats, kidney weight was greater in the untreated OLETF rats and the OLETF rats treated with either losartan or benidipine, but no significant difference was observed in creatinine clearance among the groups examined (Figure 1). Urinary protein excretion was greater in OLETF rats than in LETO rats, and both losartan and benidipine reduced proteinuria to a similar extent.

Accumulation of lipids in the kidney

The content of TG in the kidney was significantly greater in untreated OLETF rats than in LETO rats, and both losartan and benidipine treatment reduced renal TG content in the OLETF rats (Figure 2a). The content of TC in the kidney was not significantly different between LETO and untreated OLETF rats; however, both antihyper-

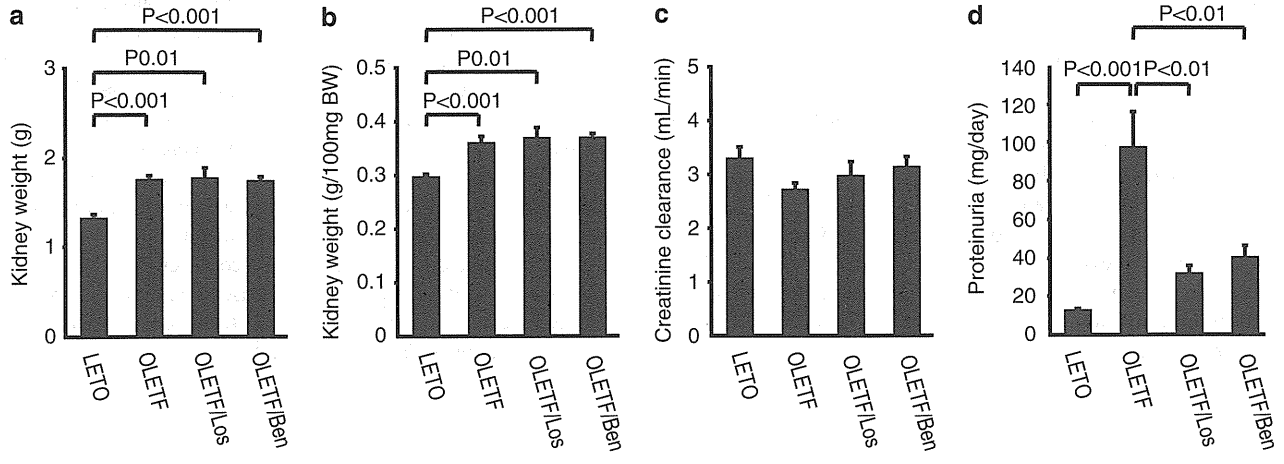

Figure 1 Kidney weight, creatinine clearance and proteinuria in LETO rats and untreated and antihypertensive drug-treated OLETF rats. Absolute values of kidney weight (**a**) and kidney weight expressed per 100 g body weight (**b**). Creatinine clearance (**c**) and daily excretion of urinary protein (**d**). Summary of data from four to six rats in each group.

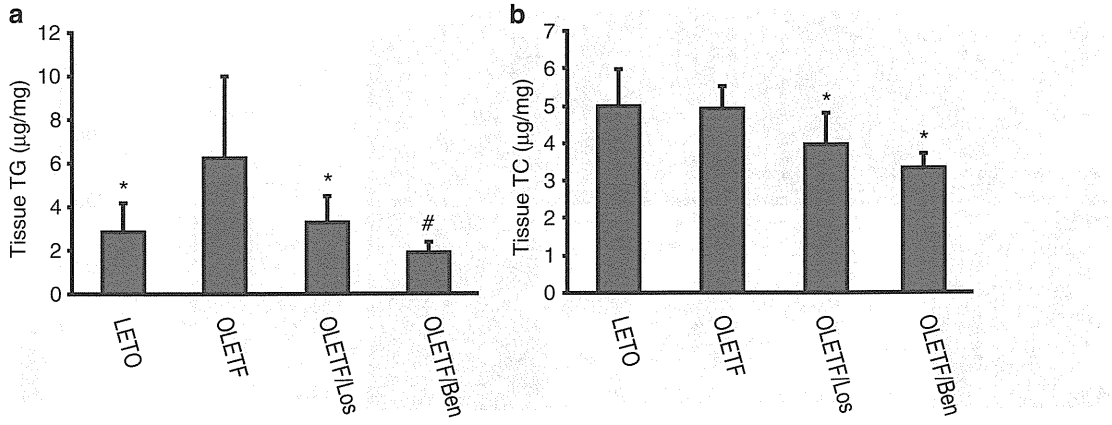

Figure 2 Tissue lipid content in the kidneys of LETO rats and untreated and antihypertensive drug-treated OLETF rats. Content of triglycerides (TG) (**a**) and total cholesterol (TC) (**b**) in the kidneys of LETO (n=4), OLETF (n=8), OLETF/Los (n=8) and OLETF/Ben (n=8) groups is shown.

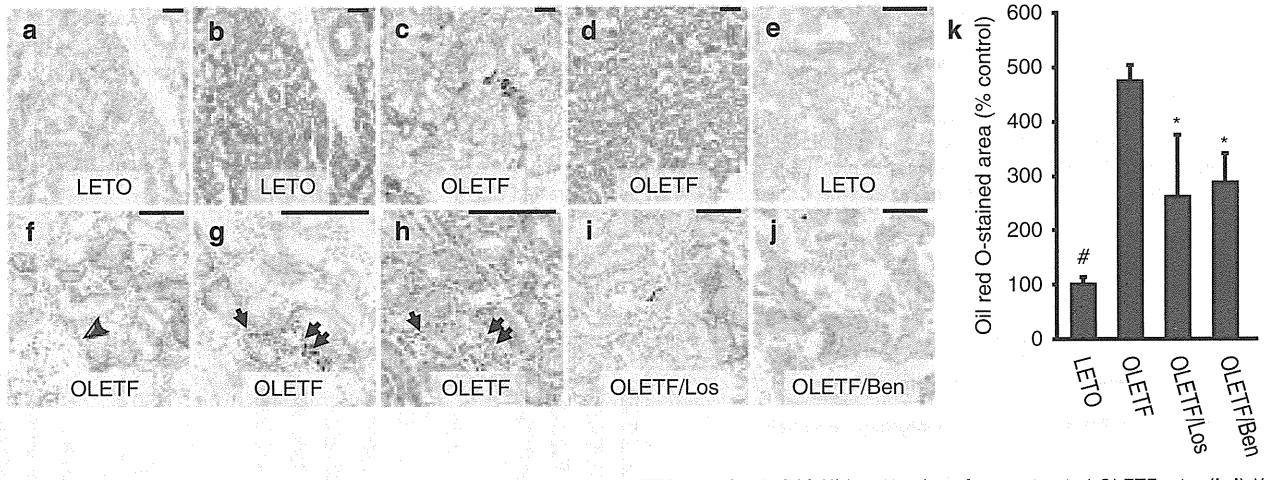

Figure 3 Accumulation of lipids in the kidney. (**a**, **b**, **e**) Kidney sections from LETO rats. (**c**, **d**, **f**–**h**) Kidney sections from untreated OLETF rats. (**i**, **j**) Kidney sections from OLETF rats treated with losartan (**i**) and benidipine (**j**). (**a**, **c**, **e**–**g**, **i**, **j**) Oil red O staining. (**b**, **d**, **h**) Hematoxylin and eosin staining; (**a**, **b**), (**c**, **d**) and (**g**, **h**) are serial sections. Lipid droplets were observed in the tubular (**c**, **g**), but not glomerular (**f**, arrowhead), regions of kidney from untreated OLETF rats. In some hematoxylin and eosin-stained specimens, lipid droplets could be identified by unstained small vesicles (**g**, **h**, arrows). The extent of lipid accumulation in the tubular cells was diminished by treatment of OLETF rats with either losartan (**i**) or benidipine (**j**). Scale bars indicate 100 μm. (**k**) Semi-quantification of the oil red O-stained area. Summary of data from five to seven experiments in each group. Kruskal–Wallis analyses followed by a *post hoc* multiple comparison test were performed. $^{*}P<0.05$ and $^{\#}P<0.01$ versus untreated OLETF rats.

tensive drugs reduced renal TC content in OLETF rats (Figure 2b). Histological analysis showed that only a trace amount of oil red O-positive lipid droplets was present in the kidneys of LETO rats (Figure 3). By contrast, increased lipid droplets were observed in the tubular and interstitial regions of untreated OLETF rats. Some lipid droplets appeared as small cavities on hematoxylin and eosin-stained frozen specimens (Figures 3g and h, arrows). Areas of oil red O-positive deposits were significantly reduced by the treatment of OLEFT rats with losartan or benidipine (Figures 3i–k). The correlation coefficients between tissue TG content and the extent of proteinuria and between the oil red O stained area and the extent of proteinuria were 0.40 ($P<0.05$, n=33) and 0.65 ($P<0.001$, n=36), respectively.

Localization of superoxide

Fluorescent signals on DHE staining were greater in untreated OLETF rats than in LETO rats and were reduced by treatment with either losartan or benidipine (Figure 4). In untreated OLETF rat kidney, DHE signals were found to be increased in tubular epithelial (Figures 4e–j, arrowheads) and vascular wall cells (Figures 4e–g, arrows), the former of which contained lipid droplets.

Regulation of genes related to lipid metabolism

The expression of mature SREBP-1 protein did not significantly differ among the four groups examined (Figure 5). Compared with LETO rats, expression of both total and phosphorylated forms of AMPK α was increased in OLETF rats treated with either losartan or benidipine, although it was not increased in untreated OLETF rats. The expression of total ACC protein was unaffected by losartan or benidipine in OLETF rats; however, treatment with either antihypertensive drug significantly increased the amount of the phosphorylated form of ACC.

Among the genes tested, mRNA expression of SREBP-1c, FAS, 3-hydroxy-3-methylglutaryl coenzyme A reductase, PPAR-γ, LDL-r and CD36 did not significantly differ between untreated OLETF and

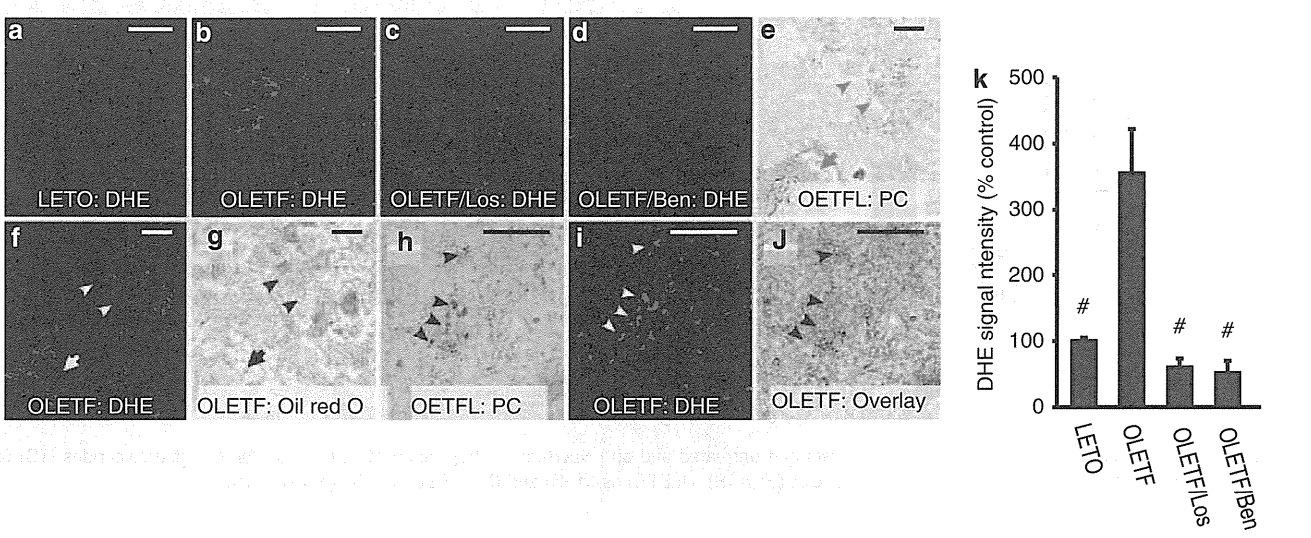

Figure 4 Localization of lipid droplets and superoxide in kidney sections. (**a**) Kidney sections from LETO rats. (**b**, **e–j**) Kidney sections from untreated OLETF rats. (**c**, **d**) Kidney sections from OLETF rats treated with losartan (**c**) and benidipine (**d**). (**a–d**, **f**, **i**) Dihydroethidium (DHE) staining. (**e**, **h**) Phase contrast (PC) microscopic images. (**j**) PC microscopic image overlaid with DHE stained images. (**g**) Oil red O staining; (**e**, **f**) and (**h–j**) are the same section; (**e–g**) are serial sections. DHE signals were more intense in OLETF kidneys (**b**) than in LETO kidneys (**a**), and both losartan (**c**) and benidipine (**d**) reduced DHE signal intensity. Granular droplets could be observed in the tubular regions, presumably lipid droplets, by PC imaging (**e**, arrowheads) of the unstained specimens, and DHE-stained superoxide was increased in these regions and in vascular wall cells (arrow, **e**, **f**). Oil red O staining (**h**) of the serial specimen confirmed that granular materials were lipid droplets. Higher magnification imaging demonstrated that DHE signals were increased in cells with granular droplets. Scale bars indicate 100 μm. (**k**) Semi-quantification of the DHE signals. Summary of data from five to seven experiments in each group. Kruskal–Wallis analyses followed by a *post hoc* multiple comparison test were performed. $^{\#}P<0.01$ versus untreated OLETF rats.

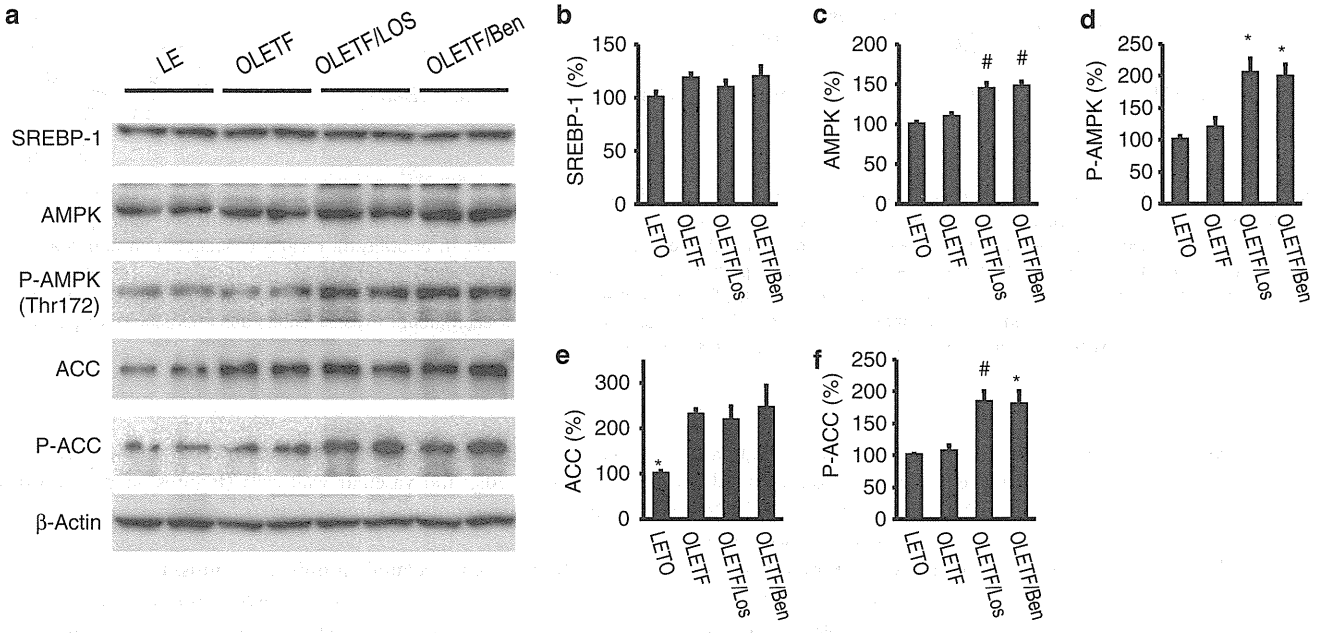

Figure 5 Western blot analysis. Western blot of sterol regulatory element-binding protein-1 (SREPB-1), AMP-activated protein kinase α (AMPK), the phosphorylated (activated) form of AMPK α (P-AMPK), acetyl-CoA carboxylase (ACC) and phosphorylated ACC. (**a–f**) Summary of data from four to six experiments in each group. $^{*}P<0.01$ and $^{\#}P<0.05$ versus untreated OLETF rats by Dunnett's *post hoc* analysis.

LETO rats (Figure 6). By contrast, mRNA expression of PPAR-α and UCP-2 was greater, whereas that of PGC-1α was lower, in untreated, losartan-treated and benidipine-treated OLETF rats than in LETO rats. Although the mRNA expression of CPT-1 did not differ between untreated OLETF and LETO rats, treatment of OLETF rats with either losartan or benidipine significantly increased CPT-1 expression.

DISCUSSION

In this study, we found that TG and TC contents were increased in the kidneys of untreated OLETF rats compared with LETO rats. In the kidneys of OLETF rats, oil red O-positive lipid droplets were observed mainly in tubular epithelial cells, in which increased superoxide was present. Treatment of OLETF rats with either losartan or benidipine,

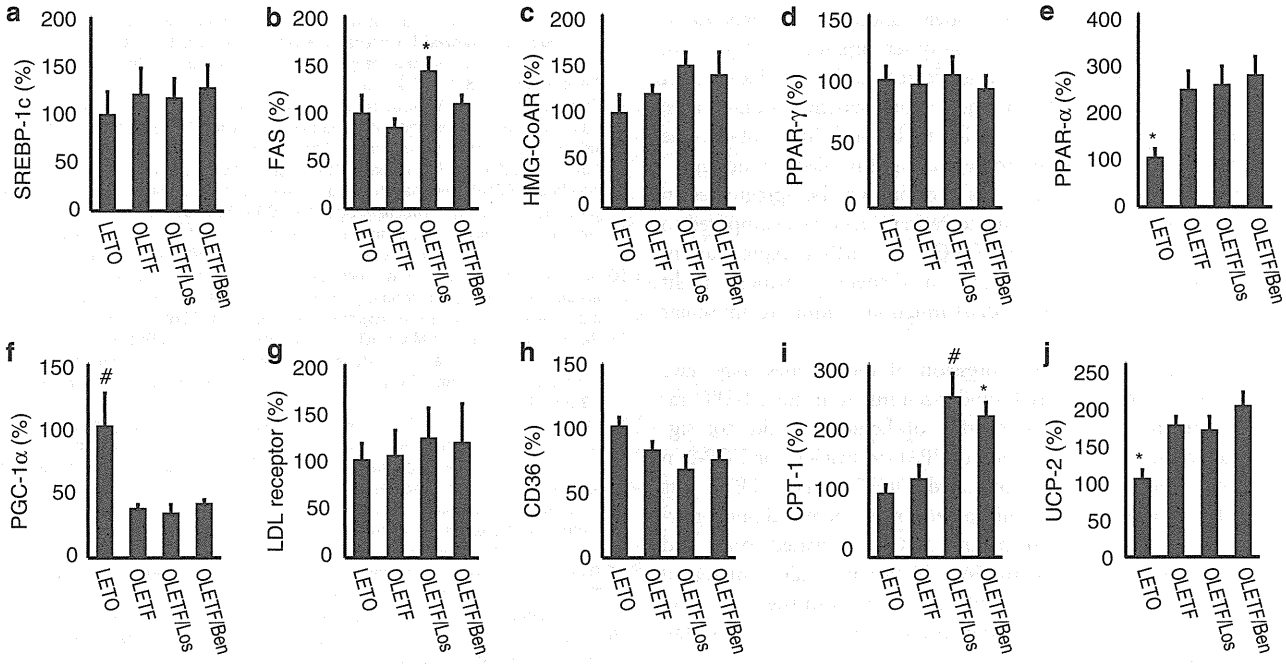

Figure 6 mRNA expression and regulation of lipid metabolism-related genes. (**a**) Sterol regulatory element-binding protein-1c (SREBP-1c). (**b**) Fatty acid synthase (FAS). (**c**) 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoAR). (**d**) Peroxisome proliferator-activated receptor (PPAR)-γ. (**e**) PPAR-α. (**f**) PPAR-γ coactivator (PGC)-1α. (**g**) LDL receptor. (**h**) CD36. (**i**) Carnitine palmitoyltransferase (CPT)-1. (**j**) Uncoupling protein (UCP)-2. Summary of data from six to ten experiments in each group $^{*}P<0.01$ and $^{\#}P<0.05$ versus untreated OLETF rats by Dunnett's *post hoc* analysis.

both of which lowered blood pressure to a similar extent, reduced tissue TG content, oil red O-positive deposits, superoxide signals and urinary protein excretion in the kidneys to a similar extent.

The mechanisms underlying lipid accumulation in the kidney in various animal models and in humans have not been fully elucidated; however, the presumed mechanisms include the regulation of genes related to the uptake, biosynthesis, catabolism and efflux of lipids[1,3,4,10,18] and the uptake of fatty acids carried on filtered albumin by the renal tubules.[7,9] What is the possible mechanism underlying the reduction in the renal lipid content of OLETF rats caused by both losartan and benidipine? We reported earlier that long-term administration of angiotensin II upregulates the expression of SREBP-1 and FAS and increases the renal content of lipids, which was suppressed by losartan. This effect of losartan may, at least in part, be independent of its depressor effect.[19] Therefore, it is possible that benidipine may also reduce renal lipid content through a mechanism independent of its depressor effect. To our knowledge, however, no earlier studies have shown that a calcium channel blocker can regulate the expression of certain lipogenic gene and/or reduce tissue lipid content. Toblli *et al.*[20] reported that renin angiotensin system inhibition, but not calcium channel blockade, suppressed lipid deposition in the heart[20] and liver[21] in obese Zucker rats.

In this study, the expression of SREBP-1 and FAS was not upregulated in the kidneys of OLETF rats compared with LETO rats, and neither losartan nor benidipine reduced the expression of these genes in the kidneys of OLETF rats. Therefore, it can be stated that the mechanism by which lipid content increased in the kidneys of OLETF rats may be, at least in part, different from that in angiotensin II-infused rats. This could be the reason why losartan was not more effective than benidipine in terms of reducing lipid content in the kidney.

As enhanced urinary albumin excretion may lead to the subsequent tubular absorption of lipid-bound albumin,[7,9] another possibility is that both losartan and benidipine reduced renal lipid content by their anti-proteinuric effect. Several studies have reported that benidipine may have a greater anti-proteinuric effect than other CCBs such as amlodipine,[22] and the renoprotective effect of benidipine may be, in part, mediated by the preservation of an essential cofactor of nitric oxide synthase, (6R)-5,6,7,8–tetrahydrobiopterin.[14] As both losartan and benidipine reduced the extent of proteinuria, both drugs might reduce the transport of the albumin-bound fatty acid to tubular cells. The close relationship between the extent of lipid deposition and proteinuria observed in this study may support this notion. Furthermore, the reduction of blood pressure *per se* might have a role in the modulation of lipid content in the kidney.

In this study, tubular cells that contained lipid droplets were positive for DHE signals (Figure 4). A spatial relationship between lipid droplets and superoxide was also observed in the kidneys and heart of angiotensin II-induced hypertensive animals,[10,16] indicating that these two phenomena have a relationship[23] under conditions of hypertension and metabolic syndrome, although a causal and resultant relationship has not yet been determined. Taking these observations into account, future studies should examine whether other antihypertensive drugs such as calcium channel blockers of other subclasses, anti-oxidative agents[24] and other drugs that may have an anti-proteinuric effect are effective in reducing lipid content in the kidneys of OLETF rats.

In this study, we also found several differences between OLETF and LETO rats in terms of the expression of lipid regulatory genes. The expression of PPAR-α and UCP-2 (mRNA) and ACC (protein) was higher, whereas that of PGC-1α (mRNA) was lower, in untreated OLETF rats than in LETO rats. Several earlier studies have shown

altered expression of these genes under conditions of diabetes or metabolic syndrome in the kidney or other organs. Proctor *et al.* reported that PPAR-α expression is decreased in the kidneys of diabetic animals compared to their non-diabetic counterparts, which results in decreased fatty acid oxidation.[2] In addition, the expression of UCP-2 was found to be increased in diabetic kidneys.[25] The mRNA expression of UCP-2 was also found to be upregulated in the liver, skeletal muscles, heart and aorta in OLETF rats compared to LETO rats.[26,27] Downregulation of PGC-1α mRNA expression in skeletal muscles was also reported in diabetes,[28] which might have a role in reducing mitochondrial function, leading to muscular lipotoxicity.[29]

Although regulation of the expression of these genes may have played a role in the increased lipid accumulation in OLETF rat kidneys, treatment with either losartan or benidipine did not significantly alter the mRNA expression of PPAR-α, PGC-1α or UCP-2 in OLETF rats. Compared with untreated OLETF rats, OLETF rats treated with either losartan or benidipine showed increased phosphorylation of AMPK (activated form) and ACC (inactivated form) and increased expression of CPT-1 mRNA, which may result in increased β-oxidation.[11] Therefore, it is possible that changes in the expression of these genes may have a role in the anti-steatotic effects of losartan and benidipine.

In summary, lipid content in the kidney was increased in untreated OLETF rats compared with LETO rats. Oil red O-stainable lipid droplets were primarily found in tubular epithelial cells, which also showed increased superoxide production. Treatment of OLETF rats with either losartan or benidipine, both of which suppressed proteinuria, reduced tissue TG content and modulated the expression of several lipid regulatory genes such as the total and phosphorylated forms of AMPK and CPT-1. These data collectively suggest that losartan and benidipine are both effective in suppressing proteinuria and normalizing lipid homeostasis in the kidneys of a rat model of metabolic syndrome. The underlying mechanisms by which these antihypertensive agents reduced lipid content in the kidney should be investigated in future studies.

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Original Article

Impact of Changes in Waist Circumference and BMI over One-Year Period on Serum Lipid Data in Japanese Individuals

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Aim: Loss or gain in obesity indexes, such as body mass index (BMI) and waist circumference (WC), may affect serum lipid parameters. We therefore analyzed the impact of changes in WC and BMI over a one-year period on serum levels of LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), and triglycerides (TG).
Methods: We analyzed the data of 3,111 individuals who were not on lipid- lowering medication and who underwent general health screening two years running.
Results: The correlation between percent changes of WC (%dWC) and BMI (%dBMI) were both statistically significantly correlated with percent changes in LDL-C (%dLDL), HDL-C (%dHDL), and TG (%dTG) except that between %dWC and %dHDL in women. In multiple regression analysis, %dBMI, but not %dWC, was found to be an independent predictor of %dLDL, %dHDL, and %dTG. When %dBMI was excluded from the variables, %dWC was identified as an independent factor predicting %dLDL and %dTG; however, in individuals with %dBMI of ≥0, %dWC was not found to be a predictor of percent changes in any lipid parameters tested in this model.
Conclusion: Percent changes in BMI were found to be an independent predictor of adverse changes in lipid parameters in both genders. Although percent changes in WC (%dWC) also tended to confer adverse changes in lipid parameters, this relationship did not remain statistically significant after controlling for %dBMI. It is suggested that changes in obesity parameters are an important goal to avoid adverse lipid changes, although there might be some gender differences.

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Key words; Waist circumference, Body mass index, LDL-C, Health screening

Introduction

It is well known that obesity parameters, such as body weight, body mass index (BMI), and waist circumference (WC), may be related with values of serum lipid parameters, including low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), and triglycerides (TG) as well as other established cardiovascular risk factors[1, 2]. Although there are in general substantial correlations among various obesity parameters, some parameters may provide better prediction of insulin resistance than others[3, 4]. On the other hand, fewer studies have analyzed the effect of changes in obesity parameters on changes in these lipid parameters in the general population[5]. To this end, the aim of the current study was to investigate the relationship between changes in obesity parameters over a one-year period and changes in lipid parameters over the same period in Japanese individuals.

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Table 1a. Baseline Characteristics at the First Visit According to %dWC

Variables	%dWC quartiles				*p* for trend
	First (range: −21.2–−3.4)	Second (range: −3.4–−0.1)	Third (range: 0.0–3.2)	Fourth (range: 3.2–33.3)	
Women					
n	324	193	216	407	
Age, years	52.2±10.2	51.4±10.2	51.4±9.8	50.4±9.3	0.110
Height, cm	156±5	159±6	157±6	158±6	0.021
Weight, kg	51.3±7.5	53.2±8.6	53.7±8.5	52.2±6.9	0.002
WC, cm	78.9±8.5	77.9±9.4	76.8±8.5	73.5±7.9	<0.001
BMI, kg/m^2	21.0±2.9	21.6±3.3	21.7±3.2	21.0±2.7	0.003
Systolic blood pressure, mmHg	117±18	120±20	117±17	116±19	0.242
Diastolic blood pressure, mmHg	73±10	74±12	73±11	72±12	0.225
Pulse rate, bpm	64±8	64±8	64±9	63±9	0.614
LDL-cholesterol, mg/dL	129±32	130±33	129±33	123±32	0.036
HDL-cholesterol, mg/dL	70±14	68±15	68±14	69±15	0.582
riglyceride (interquartile range), mg/dL	75 (55.5–98.5)	75 (55–108)	77 (54–103)	69 (54–90)	0.040
Uric acid, mg/dL	4.6±1.0	4.5±1.0	4.6±0.9	4.4±0.9	0.156
Fasting glucose, mg/dL	89±15	92±18	92±22	90±17	0.188
Haemoglobin A1C, %	5.1±0.5	5.2±0.5	5.2±0.8	5.1±0.6	0.602
Anti-hypertensive medication, n (%)	17 (5.3)	11 (5.7)	6 (2.8)	14 (3.4)	0.306
Anti-diabetic medication, n (%)	1 (0.3)	0	1 (0.5)	4 (1.0)	0.400
Blood urea nitrogen, mg/dL	13.3±3.7	13.5±3.4	13.3±3.1	13.4±3.2	0.928
Serum creatinine, mg/dL	0.66±0.48	0.63±0.09	0.62±0.09	0.63±0.09	0.368
Current smoker, n (%)	35 (10.8)	14 (7.3)	11 (5.1)	44 (10.8)	0.056
Men					
n	453	571	574	373	
Age, years	54.3±10.2	53.2±10.0	53.5±10.5	51.8±10.1	0.008
Height, cm	170±6	169±6	169±6	169±5	0.919
Weight, kg	68.7±10.0	68.3±9.2	68.2±9.1	67.4±8.8	0.246
WC, cm	88.0±7.8	86.7±7.1	85.3±7.2	82.9±7.5	<0.001
BMI, kg/m^2	23.8±3.0	23.8±2.7	23.8±2.8	23.5±2.8	0.278
Systolic blood pressure, mmHg	129±20	128±20	126±18	122±16	<0.001
Diastolic blood pressure, mmHg	82±12	81±12	80±11	78±10	<0.001
Pulse rate, bpm	64±10	63±9	63±9	62±10	0.185
LDL-cholesterol, mg/dL	131±30	130±30	130±30	127±30	0.291
HDL-cholesterol, mg/dL	57±14	55±14	55±13	57±13	0.280
riglyceride (interquartile range), mg/dL	109 (76–154)	109 (79–157)	110 (77–160)	98 (73–143)	0.287
Uric acid, mg/dL	6.1±1.3	6.1±1.2	6.1±1.2	6.1±1.2	0.628
Fasting glucose, mg/dL	102±24	99±20	98±18	99±24	0.013
Haemoglobin A1C, %	5.5±0.8	5.4±0.8	5.3±0.7	5.4±0.9	0.022
Anti-hypertensive medication, n (%)	51 (11.3)	70 (12.6)	74 (12.9)	39 (10.5)	0.676
Anti-diabetic medication, n (%)	17 (3.8)	10 (1.8)	15 (2.6)	15 (4.0)	0.128
Blood urea nitrogen, mg/dL	14.7±4.2	14.6±3.5	14.3±3.2	14.3±3.0	0.144
Serum creatinine, mg/dL	0.87±0.52	0.85±0.13	0.86±0.13	0.84±0.11	0.429
Current smoker, n (%)	140 (30.9)	193 (33.8)	172 (30.0)	127 (34.0)	0.407

Methods

Study Population

The study was approved by the Ethics Committee of Mitsui Memorial Hospital. Between October 2005 and October 2006, 11,558 individuals underwent general health screening at our institute. Of these, 3,312 individuals (1,203 women, 2,109 men)

Table 1b. Baseline Characteristics at the First Visit According to %dBMI

Variables	%dBMI quartiles				*p* for trend
	First (range: −21.8–−1.8)	Second (range: −1.8–−0.2)	Third (range: −0.2–1.4)	Fourth (range: 1.4–15.6)	
Women					
n	267	263	290	320	
Age, years	51.9 ± 10.1	52.7 ± 9.9	51.2 ± 9.2	49.6 ± 9.9	0.001
Height, cm	156 ± 5	156 ± 6	158 ± 6	158 ± 6	0.005
Weight, kg	53.0 ± 7.7	52.7 ± 8.1	51.9 ± 7.4	52.2 ± 7.7	0.290
WC, cm	77.1 ± 8.6	77.1 ± 9.1	75.6 ± 9.0	75.9 ± 8.2	0.076
BMI, kg/m^2	21.6 ± 3.0	21.5 ± 3.1	20.8 ± 2.8	21.1 ± 3.0	0.002
Systolic blood pressure, mmHg	120 ± 20	118 ± 18	116 ± 18	116 ± 18	0.054
Diastolic blood pressure, mmHg	75 ± 12	74 ± 11	73 ± 11	72 ± 11	0.041
Pulse rate, bpm	64 ± 9	64 ± 8	63 ± 9	64 ± 9	0.171
LDL-cholesterol, mg/dL	132 ± 37	132 ± 30	125 ± 29	121 ± 31	<0.001
HDL-cholesterol, mg/dL	68 ± 14	69 ± 15	70 ± 15	69 ± 14	0.293
riglyceride (interquartile range), mg/dL	78 (58–104)	75 (56–105)	72 (53–100)	67 (51.5–91)	<0.001
Uric acid, mg/dL	4.5 ± 1.0	4.5 ± 0.9	4.6 ± 0.9	4.5 ± 1.0	0.470
Fasting glucose, mg/dL	90 ± 17	93 ± 27	89 ± 13	89 ± 12	0.038
Haemoglobin A1C, %	5.1 ± 0.5	5.2 ± 0.8	5.2 ± 0.5	5.1 ± 0.5	0.012
Anti-hypertensive medication, n (%)	11 (4.1)	13 (4.9)	12 (4.1)	12 (3.8)	0.913
Anti-diabetic medication, n (%)	0	3 (1.1)	1 (0.3)	2 (0.7)	0.315
Blood urea nitrogen, mg/dL	13.5 ± 3.0	13.3 ± 3.3	13.7 ± 3.9	13.1 ± 3.1	0.181
Serum creatinine, mg/dL	0.62 ± 0.09	0.62 ± 0.10	0.68 ± 0.51	0.62 ± 0.09	0.022
Current smoker, n (%)	19 (7.1)	22 (8.4)	23 (7.9)	40 (12.5)	0.095
Men					
n	510	515	488	458	
Age, years	54.0 ± 10.2	54.5 ± 10.0	53.4 ± 10.2	51.1 ± 10.3	<0.001
Height, cm	169 ± 6	169 ± 6	170 ± 6	170 ± 6	0.190
Weight, kg	68.7 ± 9.7	67.5 ± 9.0	68.8 ± 9.5	67.7 ± 8.8	0.050
WC, cm	87.0 ± 7.6	85.5 ± 7.3	86.4 ± 7.7	84.9 ± 7.5	0.002
BMI, kg/m^2	23.9 ± 2.8	23.6 ± 2.9	23.9 ± 2.8	23.5 ± 2.7	0.020
Systolic blood pressure, mmHg	128 ± 19	126 ± 19	127 ± 18	124 ± 18	0.004
Diastolic blood pressure, mmHg	81 ± 12	80 ± 12	81 ± 11	79 ± 11	0.010
Pulse rate, bpm	63 ± 9	63 ± 9	64 ± 9	62 ± 9	0.231
LDL-cholesterol, mg/dL	132 ± 31	130 ± 29	129 ± 29	127 ± 31	0.026
HDL-cholesterol, mg/dL	55 ± 13	56 ± 14	56 ± 13	56 ± 14	0.774
riglyceride (interquartile range), mg/dL	111 (79–158)	107 (75–158)	110 (76–153)	102 (75–148)	0.253
Uric acid, mg/dL	6.2 ± 1.3	6.0 ± 1.2	6.0 ± 1.1	6.1 ± 1.2	0.312
Fasting glucose, mg/dL	100 ± 21	101 ± 23	99 ± 18	98 ± 23	0.088
Haemoglobin A1C, %	5.5 ± 0.8	5.4 ± 0.8	5.3 ± 0.7	5.4 ± 0.8	0.049
Anti-hypertensive medication, n (%)	67 (13.1)	70 (13.6)	42 (8.6)	55 (12.0)	0.065
Anti-diabetic medication, n (%)	16 (3.1)	14 (2.7)	11 (2.3)	16 (3.5)	0.692
Blood urea nitrogen, mg/dL	14.6 ± 4.1	14.5 ± 3.2	14.2 ± 3.2	14.5 ± 3.4	0.307
Serum creatinine, mg/dL	0.88 ± 0.50	0.86 ± 0.13	0.84 ± 0.12	0.85 ± 0.13	0.245
Current smoker, n (%)	162 (31.8)	163 (31.7)	151 (30.9)	156 (34.1)	0.758

underwent general health screening during this period (first visit) and again the following year (second visit). Among these 3,312 individuals, 3,111 (1,140 women, 1,971 men) who reported not taking anti-hyperlipidemic drugs at both visits were enrolled in the present study. The mean ± standard deviation (SD) of the interval between the two visits of the individuals enrolled was 355 ± 52 days. The percent difference in

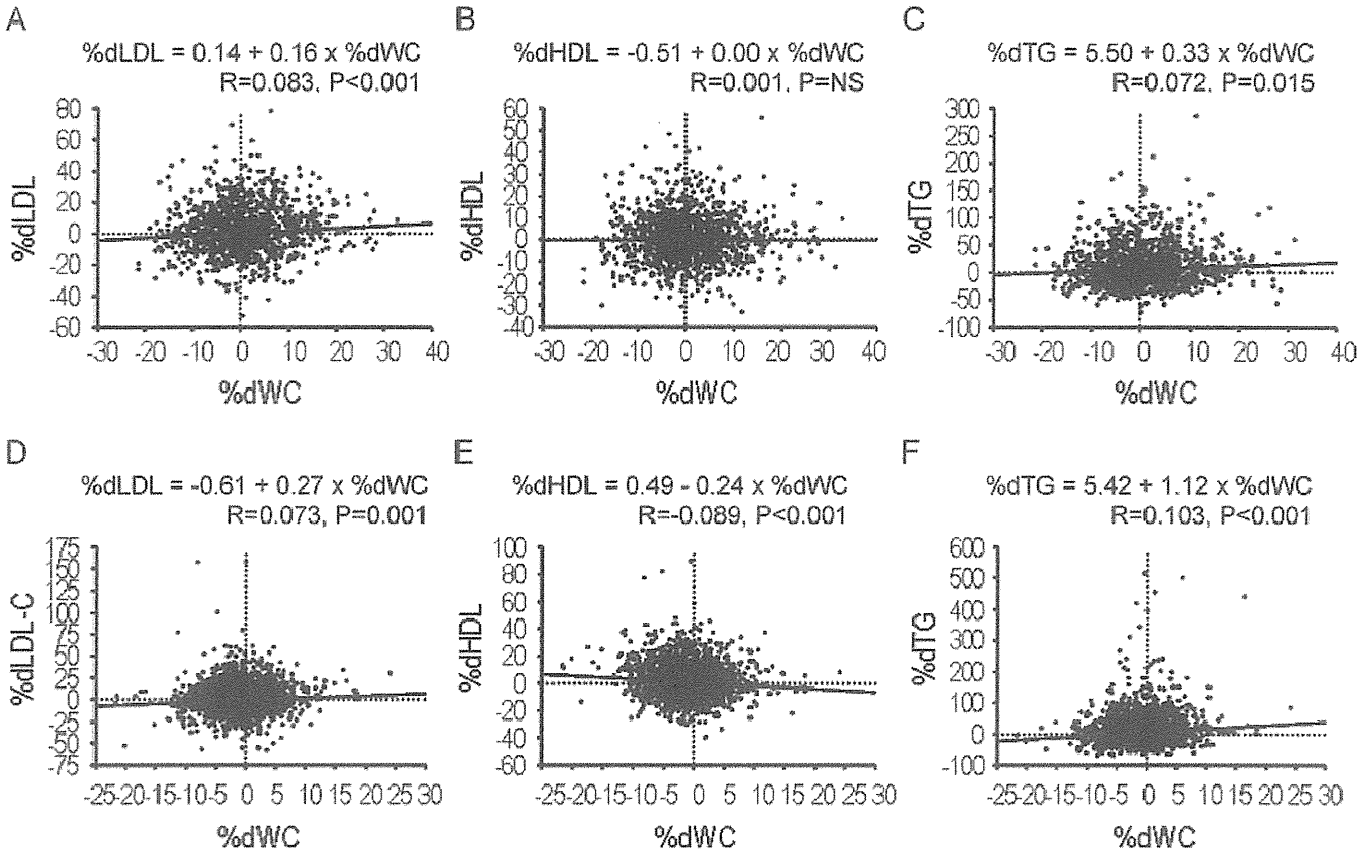

Fig. 1. Linear correlation between %dWC and %dLDL (A), %dHDL (B), and %dTG (C) in women and that between %dWC and %dLDL (D), %dHDL (E), and %dTG (F) in men.

the value of WC, BMI, serum levels of LDL-C, HDL-C, and TG between first and second visits was designated as %dWC, %dBMI, %dLDL, %dHDL, and %dTG respectively. All subjects were seen after an overnight fast. Height and weight were determined, and BMI was expressed as weight (in kilograms) divided by the square of the height (in meters). Waist circumference was measured at the umbilical level to the nearest 1 cm by trained physicians and technicians with the subject standing[6].

Laboratory Analysis

Blood samples were taken from the subjects after an overnight fast. Serum levels of TC, HDL-C, and TG were determined enzymatically. Serum uric acid was measured by the uricase-peroxidase method and hemoglobin A1C was determined using the latex agglutination immunoassay. Blood pressure was measured after about 10 min of rest using an automated sphygmomanometer.

Statistical Analysis

Data are expressed as the mean ± SD unless otherwise stated. Analyses of variance with trend analysis, linear regression analysis and stepwise multiple regression analysis were conducted as appropriate to assess the statistical significance of differences between groups using computer software, StatView ver. 5.0 (SAS Institute, NC) and Dr. SPSS II (SPSS Inc., Chicago, IL). A value of $p < 0.05$ was taken to be significant.

Results

Baseline Characteristics

The mean ± SD age of the individuals enrolled was 51.3 ± 9.8 years in women and 53.3 ± 10.2 years in men at the first visit. The sex-nonspecific ranges (min/max) was −21.2/−3.4, −3.4/−0.1, 0.0/3.2, and 3.2/33.3 for each %dWC quartile, and −21.8/−1.8, −1.8/−0.2, −0.2/1.4, and 1.4/15.6 for each %dBMI quartile. Baseline characteristics of the subjects according to %dWC quartile and %dBMI quartile are described in **Table 1a**, **1b**. There was no statistically

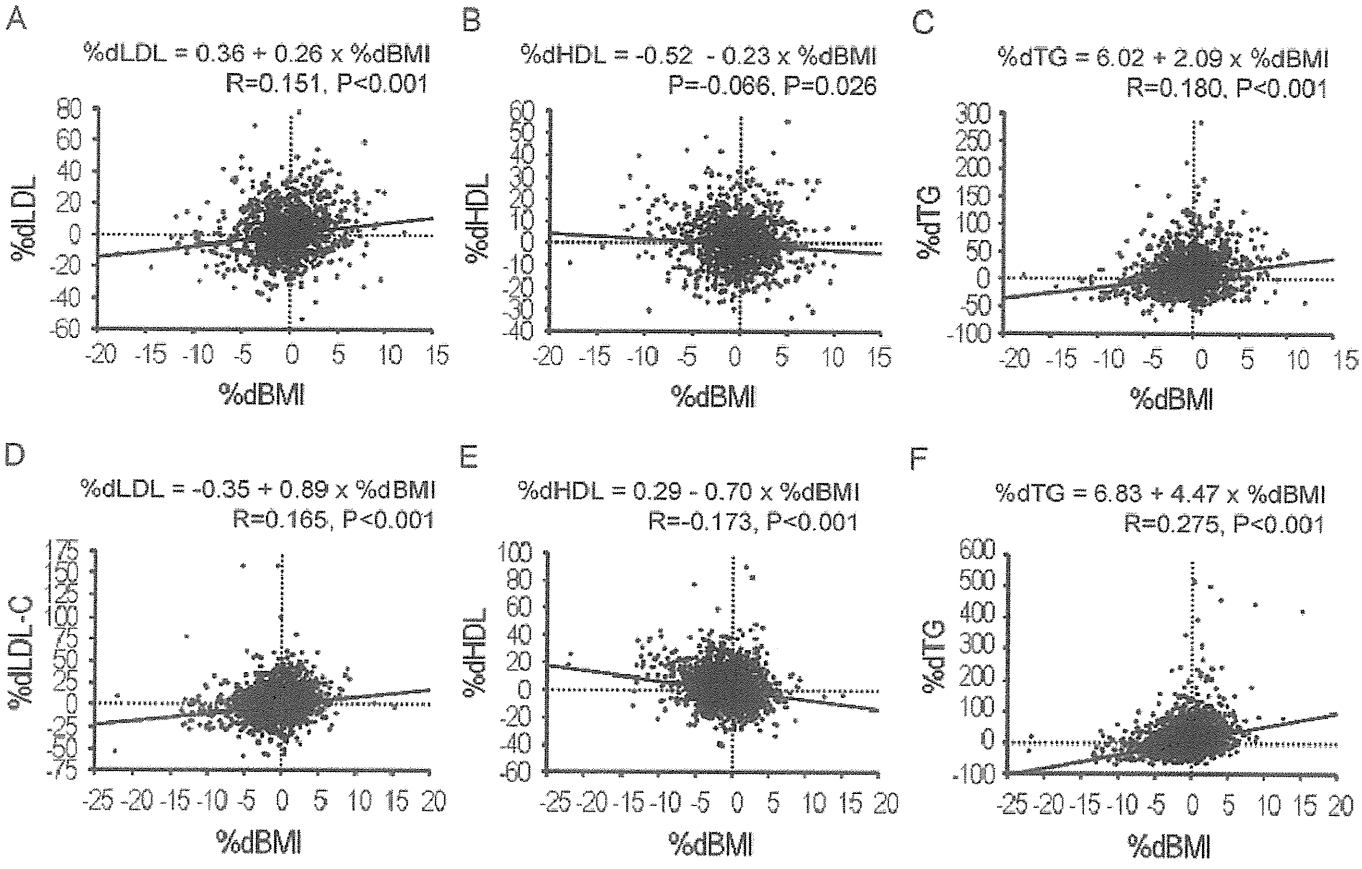

Fig. 2. Linear correlation between %dBMI and %dLDL (A), %dHDL (B), and %dTG (C) in women and that between %dBMI and %dLDL (D), %dHDL (E), and %dTG (F) in men.

significant trend in the rate of anti-hypertensive and anti-diabetic medications across four %dWC or %dBMI quartiles.

Association between Percent Changes in Obesity Parameters and Percent Changes in Lipid Parameters

Scatter plots of %dWC and percent changes in lipid parameters (**Fig. 1**) and %dBMI and percent changes in lipid parameters (**Fig. 2**) are shown. Except between %dWC and %dHDL in women, the correlation was found to be statistically significant between percent changes in obesity parameters and percent changes in lipid parameters; however, the coefficients of correlation were relatively small.

Table 2 describes the percent changes in lipid parameters by %dWC and %dBMI quartiles. In women, %dTG increased with increasing %dWC and with %dBMI. In men, %dLDL and %dTG increased and %dHDL decreased with increasing %dWC (**Table 2a**) and with %dBMI (**Table 2b**). Kappa coefficient between %dWC quartiles and %dBMI quartiles were found to be slight (women, 0.079, $p<0.001$; men, 0.171, $p<0.001$).

Stepwise Multiple Regression Analysis

The correlation coefficient between %dWC and %dBMI was 0.24 in women and 0.47 in men. The regression equation in each gender is as follows: $\%dBMI = -0.181 + 0.096 \times \%dWC$ (women), $\%dBMI = -0.287 + 0.319 \times \%dWC$ (men). We put both %dBMI and %dWC together with age into the statistical model of stepwise multiple regression analysis (**Table 3**, model 1) and it was found that %dBMI, but not %dWC, significantly predicts percent changes in all lipid parameters tested. When %dBMI was excluded from the independent variables, %dWC was identified as an independent factor predicting percent changes in lipid parameters, except for %dHDL in women (**Table 3**, model 2). In women or men with %dBMI of ≥0 (580 women, 890 men), %dWC was not found to be a predictor of percent changes in any lipid parameters tested (data not shown).

Table 2a. Percent changes in lipid parameters according to %dWC quartiles

Variables	%dWC quartiles				*p* for trend
	First (range: −21.2–−3.4)	Second (range: −3.4–−0.1)	Third (range: 0.0–3.2)	Fourth (range: 3.2–33.3)	
Women					
%dLDL	−1.24±14.32	0.44±14.68	0.39±15.80	1.43±15.31	0.127
%dHDL	−0.41±10.97	−0.35±11.93	−0.53±11.02	−0.64±10.90	0.989
%dTG	2.92±35.05	1.49±33.53	9.02±40.49	8.60±37.33	0.034
Men					
%dLDL	−0.26±17.05	−0.36±15.56	−0.31±17.25	0.38±14.93	0.040
%dHDL	2.15±13.23	0.36±11.83	0.08±12.17	−0.21±11.35	0.016
%dTG	−1.25±39.56	5.17±47.12	6.67±53.51	9.66±53.00	0.009

Table 2b. Percent changes in lipid parameters according to %dBMI quartiles

Variables	%dBMI quartiles				*p* for trend
	First (range: −21.8–−1.8)	Second (range: −1.8–−0.2)	Third (range: −0.2–1.4)	Fourth (range: 1.4–15.6)	
Women					
%dLDL	−1.48±16.44	−1.26±12.79	−0.06±14.24	3.42±15.81	<0.001
%dHDL	0.78±12.40	−1.38±10.75	−0.62±9.46	−0.76±11.59	0.147
%dTG	−2.31±33.46	3.58±33.84	6.59±41.32	13.90±35.91	<0.001
Men					
%dLDL	−4.34±16.61	−0.96±15.58	0.01±15.69	2.80±16.68	<0.001
%dHDL	2.94±13.12	0.75±11.37	−0.40±10.50	−1.18±13.29	<0.001
%dTG	−10.21±33.76	1.90±40.05	7.07±48.69	23.13±63.63	<0.001

Discussion

In the current study, both %dWC and %dBMI were positively associated with %dLDL and %dTG in both genders. In addition, %dWC and %dBMI were inversely associated with %dHDL in men, but not in women; however, the association between percent changes in these obesity parameters and percent changes in lipid parameters, when present, was weak. Similar results were obtained when either %dWC or %dBMI was used as a potent predictor of percent changes in lipid data; however the correlation between %dWC and %dBMI was found to be relatively weak, especially in women; the correlation coefficient was 0.47 in men and 0.24 in women. Stepwise multiple regression analysis showed that %dBMI, but not %dWC, was identified as an independent factor predicting % changes in lipid data tested. Notably, even when %dBMI was excluded from the variables, %dWC was not identified as a predictor of %dHDL in women.

Several previous studies showed an association between changes in obesity indexes and lipid parameter changes. For example, in a community-based sample of 3,325 young adults, a 10-year weight gain tended to confer adverse changes in levels of LDL-C, HDL-C, and TG[7]. Bonithon-Kopp *et al.* reported that changes in BMI and the waist to hip ratio (WHR) were positively associated with changes in TG[8]. Williams *et al.* reported that changes in BMI as well as WC had a greater probability of inducing hypercholesterolemia during 7 years of follow-up[9]. In middle-aged subjects free from known cardiovascular diseases and diabetes[10], a gain or loss of WC over 9 years significantly affected serum lipid data and the incidence of metabolic syndrome[11].

On the other hand, only a few studies have investigated whether WC change was associated with changes in lipid parameters independent of BMI. Wing *et al.* analyzed whether changes in WHR led to improvements in serum lipid concentrations independent of weight change in subjects with no history of