known about the regulation of iron homeostasis in hypertensive patients. Piperno *et al.* have reported that increased serum ferritin was more frequent in subjects with essential hypertension than in normotensive subjects (*31*). In their study, increased resistance appeared to be among the possible mechanisms underlying iron overload in an animal model of hypertension (*32*). Urinary transferrin excretion has been reported to be increased when albuminuria is present, not only in diabetic hypertensive cases (*33*), but also in non-diabetic hypertensive cases (*34*); this increase might also in part account for the link between altered iron homeostasis in the kidney and hypertension. It has been reported that treatment of diabetic patients with low-dose candesartan slightly decreased blood pressure, and this in turn reversed the increase in the urinary excretion of transferrin over time (*35*).

In the current study, we targeted the regulation of the expression of several newly discovered iron metabolism–related genes in the kidney. It is well known that the whole-body iron balance is maintained by the regulation of iron absorption by the intestine, as essentially no pathway for iron excretion is present in humans (*20*). Considering that anemia is among the possible side effects of all commercially available AT_1 receptor blockers in Japan, regulation of the expression of iron metabolism–related genes by angiotensin II or by the renin angiotensin system in intestinal cells should also be closely investigated in future studies.

In conclusion, we have characterized the expression patterns of several iron metabolism–related genes, including TfR, DMT1, FPN, and hepc, and their regulation by angiotensin II in the rat kidney. Further studies are necessary for analyzing the relative contribution of these genes to renal iron homeostasis and, presumably, to tubular iron reabsorption in terms of the activity and involvement of the renin angiotensin system.

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Association between serum albumin, carotid atherosclerosis, and metabolic syndrome in Japanese individuals

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Abstract

Serum albumin is a maker of nutritional status and possesses antioxidative properties. Here, we have sought to investigate the mode of association between serum albumin levels, metabolic syndrome, and carotid atherosclerosis by analyzing the data of the cross-sectional data from 8143 individuals who underwent general health screening test. After adjusting for age, total cholesterol, and smoking status, the highest quartile of serum albumin (≥4.7 g/dL) was associated with increased prevalence of metabolic syndrome with an odds ratio of 1.80 (95% CI 1.41–2.23, $P<0.0001$) in women, and 1.60 (95% CI 1.44–1.78, $P<0.0001$) in men, when compared to the lowest serum albumin quartile (<4.3 g/dL). By contrast, when compared with the lowest quartile, the highest quartile of serum albumin was associated with reduced prevalence of carotid plaque with an odds ratio of 0.62 (95% CI 0.42–0.91, $P<0.001$) in women, and 0.76 (95% CI 0.62–0.93, $P<0.01$) in men, and for carotid intima-media thickening with an odds ratio of 0.57 (95% CI 0.35–0.94, $P<0.05$) in women, and 0.71 (95% CI 0.55–0.92, $P<0.01$) in men. Our data showed that higher serum albumin was inversely associated with the prevalence of early carotid atherosclerosis, although it was positively associated with the prevalence of metabolic syndrome. Whether these observations are in part explained by the antioxidative properties of albumin requires further investigation.

Keywords: Metabolic syndrome; Carotid atherosclerosis; Ultrasonography; Albumin; Antioxidants

1. Introduction

Serum albumin level is known to be reduced in various diseased conditions, such as malnutrition, inflammatory states, and liver diseases [1]. It has been shown that lower levels of serum albumin are associated with increased risk of cardiovascular mortality [2,3] and carotid atherosclerosis [4,5]. On the other hand, serum albumin levels show positive association with some of the coronary risk factors, such as body mass index (BMI), blood pressure, and lipid profiles [6–9], although these associations cannot distort the association between low serum albumin levels and cardiovascular disease.

It has recently been suggested that there may be an inverse association between circulating antioxidants, such as Vitamin C and carotenoids, and metabolic syndrome [10]. In the serum, albumin as well as bilirubin and uric acid represent major plasma antioxidant components. In the previous study, we reported negative association between serum bilirubin and metabolic syndrome and positive association between serum uric acid and metabolic syndrome [11]. Serum albumin may be associated positively with several atherogenic risk factors [6]; however, little has been known about the mode of association between serum albumin and metabolic syndrome. Thus, in the present study, we have investigated the mode of association between serum albumin, carotid

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atherosclerosis, and metabolic syndrome by analyzing the cross-sectional data from individuals undergoing general heath screening.

2. Methods

2.1. Study subjects

The study was approved by The Ethical Committee of Mitsui Memorial Hospital. Between September 1994 and December 2003, 49,331 (16,868 women, 32,463 men) subjects aged 20 years old or older underwent a general health screen. Of the 49,331 subjects, 8143 subjects (2671 women, 5472 men) underwent health screening course including carotid ultrasonography, and were enrolled in the present study. In Japan, regular health check-ups for employees are legally mandated, and all or most of the costs of the screening are usually paid by the company to which they belong or by each subject. There are several courses, with different costs, in our health screening. Some courses include carotid ultrasonography, but some do not. Therefore, the study subjects were not considered to be a random selection from the whole subjects who underwent general health screening during the study period; however, which course to be chosen was not recommended by the physicians or health care participants. The interquartile cut off points of serum albumin, 4.3, 4.5, and 4.7 g/dL, were used in both genders. Cigarette smoking outcome data were collected in a structured questionnaire.

2.2. Laboratory data

Blood samples were taken from our subjects after an overnight fast. Serum albumin was measured by Bromcresol Green (BCG) dye-binding method and inter-assay coefficient of variation was 0.6%. Serum levels of total cholesterol (TC), HDL-cholesterol (HDL-C), and triglycerides were determined enzymatically. Serum uric acid was measured by the uricase-peroxidase method, haemoglobin A_{1C} was determined using the latex agglutination immunoassay, and bilirubin was determined by the vanadium oxide method. Plasma glucose was measured by hexokinase method and serum insulin was measured by enzyme immunoassay.

The data of basal insulin levels were available in 6338 subjects (2026 women, 4312 men), and homeostasis model assessment insulin resistance (HOMA-IR) was calculated in these individuals according to the following formula: HOMA-IR = [fasting immunoreactive insulin (IRI; μU/mL) × FPG (mg/dL)]/405.

When converting from mg/dL to mmol/L or mg/dL to μmol/L, following conversion factors would be used: uric acid, 59.48 (mg/dL to μmol/L); bilirubin, 17.10 (mg/dL to μmol/L); TC, 0.0259 (mg/dL to mmol/L); HDL-C, 0.0259 (mg/dL to mmol/L); triglycerides, 0.0113 (mg/dL to mmol/L); and glucose, 0.0555 (mg/dL to mmol/L).

2.3. Carotid ultrasonography

Carotid artery status was studied and analyzed as described previously [11]. In brief, this was examined by high resolution B-mode ultrasonography, using a machine (Sonolayer SSA270A, Toshiba, Japan) equipped with a 7.5 MHz transducer (PLF-703ST, Toshiba). The carotid arteries were examined bilaterally at the levels of the common carotid, the bifurcation, and the internal carotid arteries from transverse and longitudinal orientations by trained sonographers. The intima-media thickness was measured using a computer-assisted method by experienced sonographers who were unaware of the subjects' clinical and laboratory findings. Plaque was defined as a clearly isolated focal thickening of the intima-media layer with thickness of $\geq$1.3 mm at the common or internal carotid artery or the carotid bulb. Carotid intima-media wall thickening was said to occur when the intima-media thickness which was measured at the far wall of the distal 10 mm of the common carotid artery was $\geq$1.0mm.

2.4. Criteria for metabolic syndrome

Diagnosis of metabolic syndrome was made according to the criteria of the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP-III) [12] with BMI used as a surrogate for waist circumference as has been done in previous other studies, because waist circumference was not available in this study sample. The five thresholds used were: (1) triglyceride levels $\geq$150 mg/dL (1.69 mmol/L), (2) HDL-C levels <40 mg/dL (1.04 mmol/L) in men or <50 mg/dL (1.29 mmol/L) in women, (3) fasting plasma glucose levels $\geq$110 mg/dL (6.1 mmol/L), or taking an antidiabetic medication, (4) systolic blood pressure $\geq$130 mmHg or diastolic blood pressure $\geq$85 mmHg or taking an antihypertensive medication, and (5) body mass index (BMI) $\geq$25 kg/m^2. These five parameters were designated 'metabolic syndrome risk factor components' in the current study. Metabolic syndrome was diagnosed when three or more of these components were present.

2.5. Statistical analysis

The data in this study were analyzed by the χ^2-test, ANOVA with a Bonferroni post hoc test, and multivariate logistic regression analysis using computer software, StatView ver. 5.0 (SAS Institute, NC, USA). A value of $P < 0.05$ was taken to be statistically significant. Results are expressed as the mean ± S.D. unless stated otherwise.

3. Results

3.1. Baseline characteristics

The baseline characteristics of the study subjects are given in Table 1. The age of the enrolled subjects ranged from 22

Table 1
Baseline characteristics

Variables	Quartiles of serum albumin				*P*-value
	1	2	3	4	
Women					
Albumin range (g/dL)	3.3–4.2	4.3–4.4	4.5–4.6	4.7–5.4	
No. of subjects	639	826	735	471	
Age (years)	59 ± 11	56 ± 10	56 ± 10	55 ± 10	<0.0001
Body mass index (kg/m^2)	21.9 ± 3.3	21.9 ± 3.1	22.0 ± 3.0	21.7 ± 3.2	0.57
Systolic BP (mmHg)	119 ± 21	120 ± 19	122 ± 21	126 ± 23	<0.0001
Diastolic BP (mmHg)	73 ± 11	73 ± 11	75 ± 12	77 ± 13	<0.0001
Laboratory data					
White blood cells (×10^3/μL)	5.0 ± 1.3	4.9 ± 1.3	5.0 ± 1.3	5.1 ± 1.3	0.045
Hemoglobin (g/dL)	12.8 ± 1.2	13.1 ± 1.0	13.3 ± 1.0	13.6 ± 1.0	<0.0001
Platelet count (×10^4/μL)	23.0 ± 5.7	23.2 ± 5.2	23.7 ± 5.3	23.7 ± 5.6	0.036
Total protein (g/dL)	7.0 ± 0.4	7.2 ± 0.4	7.5 ± 0.3	7.8 ± 0.4	<0.0001
Albumin (g/dL)	4.1 ± 0.1	4.4 ± 0.1	4.5 ± 0.1	4.8 ± 0.1	<0.0001
AST (IU/L)	25.4 ± 36.4	22.4 ± 8.7	22.6 ± 6.6	24.5 ± 11.6	0.0075
Uric acid (mg/dL)	4.6 ± 1.0	4.7 ± 1.0	4.7 ± 1.0	4.8 ± 1.1	0.027
Total bilirubin (mg/dL)	0.7 ± 0.3	0.8 ± 0.3	0.8 ± 0.2	0.9 ± 0.3	<0.0001
γ-GTP (IU/L)	28.5 ± 33.9	28.1 ± 39.0	29.4 ± 24.8	39.1 ± 64.8	<0.0001
CRP > 0.4 mg/dL (%)	7	4	3	3	0.0001
Serum lipid data					
Total cholesterol (mg/dL)	206 ± 33	215 ± 35	221 ± 36	228 ± 36	<0.0001
HDL-cholesterol (mg/dL)	68 ± 15	70 ± 17	70 ± 18	71 ± 18	0.050
Triglycerides (mg/dL)	90 ± 51	94 ± 61	101 ± 70	102 ± 75	0.0028
Glucose metabolism					
Fasting glucose (mg/dL)	91 ± 19	90 ± 13	93 ± 16	95 ± 17	<0.0001
Haemoglobin A_{1C} (%)	5.2 ± 0.6	5.1 ± 0.4	5.2 ± 0.6	5.1 ± 06	0.0013
Smoking status					
Non-smokers (%)	85	84	86	83	0.40
Former smokers (%)	5	5	4	7	
Current smokers (%)	10	11	11	11	
Men					
Albumin range (g/dL)	2.3–4.2	4.3–4.4	4.5–4.6	4.7–5.6	
No. of subjects	1002	1521	1601	1348	
Age (years)	61 ± 10	58 ± 10	56 ± 10	53 ± 10	<0.0001
Body mass index (kg/m^2)	23.7 ± 2.7	24.0 ± 2.9	24.0 ± 2.7	24.0 ± 2.8	0.014
Systolic BP (mmHg)	126 ± 19	127 ± 19	128 ± 19	130 ± 19	<0.0001
Diastolic BP (mmHg)	78 ± 11	79 ± 11	80 ± 12	81 ± 12	<0.0001
Laboratory data					
White blood cells (×10^3/μL)	5.7 ± 1.6	5.6 ± 1.5	5.8 ± 1.6	5.8 ± 1.5	0.0003
Hemoglobin (g/dL)	14.5 ± 1.2	14.9 ± 1.0	15.1 ± 1.0	15.3 ± 1.0	<0.0001
Platelet count (×10^4/μL)	21.5 ± 5.0	22.1 ± 4.9	22.5 ± 4.9	22.6 ± 5.3	<0.0001
Total protein (g/dL)	7.0 ± 0.4	7.2 ± 0.3	7.4 ± 0.3	7.7 ± 0.3	<0.0001
Albumin (g/dL)	4.1 ± 0.1	4.4 ± 0.1	4.5 ± 0.1	4.8 ± 0.1	<0.0001
AST (IU/L)	25.0 ± 13.8	25.9 ± 13.4	25.7 ± 10.2	27.6 ± 12.9	<0.0001
Uric acid (mg/dL)	5.9 ± 1.2	6.1 ± 1.2	6.2 ± 1.2	6.4 ± 1.3	<0.0001
Total bilirubin (mg/dL)	0.8 ± 0.3	0.8 ± 0.3	0.9 ± 0.3	1.0 ± 0.4	<0.0001
γ-GTP (IU/L)	54.3 ± 63.2	61.2 ± 68.0	64.6 ± 64.1	74.2 ± 67.0	<0.0001
CRP > 0.4 mg/dL (%)	10	7	5	5	<0.0001
Serum lipid data					
Total cholesterol (mg/dL)	198 ± 35	201 ± 30	208 ± 31	213 ± 33	<0.0001
HDL-cholesterol (mg/dL)	56 ± 17	56 ± 15	55 ± 14	54 ± 15	0.0003
Triglycerides (mg/dL)	132 ± 131	136 ± 90	151 ± 106	164 ± 149	<0.0001
Glucose metabolism					
Fasting glucose (mg/dL)	100 ± 24	100 ± 21	101 ± 21	102 ± 22	0.021
Haemoglobin A_{1C} (%)	5.5 ± 0.9	5.4 ± 0.7	5.4 ± 0.7	5.3 ± 0.8	<0.0001
Smoking status					
Non-smokers (%)	29	33	28	33	0.0031
Former smokers (%)	32	33	35	33	
Current smokers (%)	39	35	37	33	

BP and CRP indicate blood pressure and C-reactive protein, respectively.

Table 2
Serum albumin quartiles and risk for metabolic syndrome

Quartiles of albumin	Whole odds ratio (95% CI)	Women odds ratio (95% CI)	Men odds ratio (95% CI)
Q1	1 (reference)	1 (reference)	1 (reference)
Q2	1.17 (0.96–1.42)	1.16 (0.72–1.86)	1.17 (0.94–1.45)
Q3	1.30 (1.07–1.58)§	2.19 (1.40–3.42)*	1.14 (0.92–1.42)
Q4	1.54 (1.26–1.89)†	1.74 (1.05–2.91)‡	1.47 (1.17–1.84)*

Age, sex (for whole), total cholesterol, and smoking status were used as covariates. ‡$P<0.05$; §$P<0.01$; *$P<0.001$; †$P<0.0001$ vs. the lowest quartile (reference).

to 88 years (women, 22–87 years; men, 21–88 years) with a mean age of 56.6 ± 10.5 years (women, 56.4 ± 10.4 years; men, 56.7 ± 10.6 years). The mean age either in the second, third, and fourth quartiles was less than that in the first quartile ($P<0.0001$) in both genders. The mean serum albumin level was 4.4 ± 0.3 g/dL in women, and 4.5 ± 0.3 g/dL in men. Only 0.3% (9/2671) of the female and 0.1% (8/5472) of the male subjects had serum albumin levels of less than the lowest normal value, which is 3.7 g/dL. Pearson's correlation coefficients for the relationship between serum albumin and each variable were as follows (women/men): age, −0.13/−0.278; BMI, 0.00/0.05; systolic blood pressure, 0.12/0.08; diastolic blood pressure, 0.14/0.10; total bilirubin 0.20/0.21; uric acid, 0.06/0.11; TC, 0.22/0.17; triglycerides, 0.08/0.11; HDL-C, 0.06/−0.06; plasma glucose, 0.091/0.05; and hemoglobin A1C, −0.05/−0.08. A value of $P<0.001$ was obtained for all of these correlations except for BMI (n.s.), HDL-C ($P<0.01$), and hemoglobin A1C ($P<0.05$) in women. In both genders, albumin level in the former smoker (women, 4.5 ± 0.3 g/dL; men, 4.4 ± 0.2 g/dL) and in current smokers (women, 4.5 ± 0.3 g/dL; men, 4.4 ± 0.2 g/dL) did not significantly differ from that in the never smokers (women, 4.5 ± 0.3 g/dL; men, 4.4 ± 0.2 g/dL).

3.2. Prevalence of metabolic syndrome

When compared with the individuals in the lowest albumin quartile, HOMA-IR was significantly greater in the subjects in the highest quartile ($P<0.01$) in women, and in those in any higher quartiles ($P<0.001$) in men (Fig. 1A). In addition, prevalence of metabolic syndrome in the second to fourth quartiles of serum albumin was greater than that in the lowest quartile (Fig. 1B). After adjustment for age, TC, and smoking status, logistic regression analysis showed that individuals in the highest quartile, in men and those in the highest and the second highest quartiles, in women of serum albumin had a

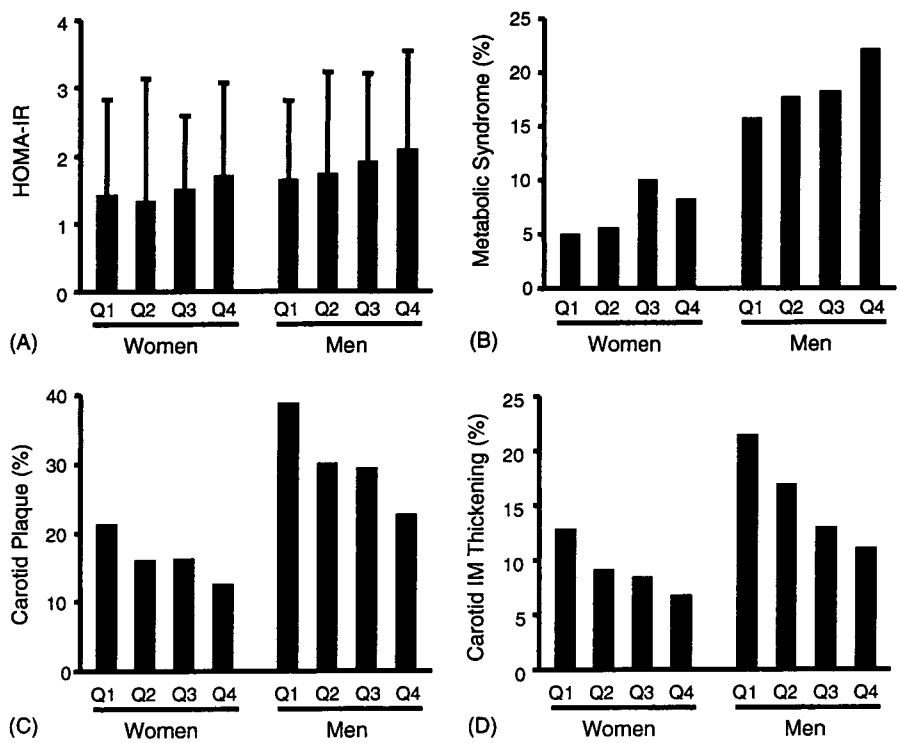

Fig. 1. (A) HOMA-IR of each gender according to the serum albumin quartile and gender. (B) Prevalence of metabolic syndrome according to the serum albumin quartile and gender. (C) Prevalence of carotid plaque according to the serum albumin quartile and gender. (D) Prevalence of intima-media (IM) thickening according to the serum albumin quartile and gender.

Table 3
Serum albumin quartiles and risk for carotid plaque and intima-media thickening

Quartiles of albumin	Whole odds ratio (95% CI)	Women odds ratio (95% CI)	Men odds ratio (95% CI)
For carotid plaque			
Q1	1 (reference)	1 (reference)	1 (reference)
Q2	0.82 (0.70–0.96)‡	0.82 (0.61–1.09)	0.82 (0.68–0.99)*
Q3	0.91 (0.78–1.07)	0.84 (0.62–1.14)	0.94 (0.78–1.14)
Q4	0.72 (0.60–0.89)*	0.62 (0.42–0.91)*	0.76 (0.62–0.93)§
For carotid intima-media thickening			
Q1	1 (reference)	1 (reference)	1 (reference)
Q2	0.88 (0.73–1.01)	0.81 (0.56–1.18)	0.90 (0.72–1.13)
Q3	0.73 (0.60–0.89)§	0.73 (0.49–1.09)	0.73 (0.58–0.92)§
Q4	0.68 (0.54–0.85)*	0.57 (0.35–0.94)‡	0.71 (0.55–0.92)§

Age, sex (for whole), BMI, systolic blood pressure, total cholesterol, HDL-cholesterol, triglycerides, fasting glucose, and smoking status were used as covariates. ‡$P<0.05$; §$P<0.01$; *$P<0.001$ vs. the lowest quartile (reference).

significantly greater prevalence of metabolic syndrome when compared to the individuals in the lowest quartile in each gender (Table 2). Serum albumin levels (g/dL) of women who had zero ($n=1365$), one ($n=791$), two ($n=329$), three ($n=130$), four ($n=46$), and five ($n=10$) risk factor components were 4.41 ± 0.24, 4.42 ± 0.27, 4.46 ± 0.27, 4.54 ± 0.27, and 4.46 ± 0.17, respectively. Serum albumin levels (g/dL) of men who had zero ($n=1405$), one ($n=1790$), two (n = 1271), three ($n=703$), four ($n=270$), and five ($n=33$) risk factor components were 4.44 ± 0.25, 4.47 ± 0.25, 4.50 ± 0.26, 4.53 ± 0.26, and 4.53 ± 0.28, respectively.

3.3. Prevalence of carotid plaque and intima-media thickening

The prevalence of carotid plaque and carotid intima-media thickening showed graded decrease according to the serum albumin quartiles in both genders (Fig. 1C and D). Logistic regression analysis showed that the negative association between serum albumin and carotid plaque or carotid intima-media thickening remained statistically significant after adjusting for age, BMI, systolic blood pressure, TC, HDL-C, triglycerides, fasting glucose, and smoking status (Table 3).

4. Discussion

In the present study, we demonstrated two major findings. First, individuals with higher serum albumin had a significantly reduced odds ratio for early carotid atherosclerosis (carotid plaque and carotid intima-media thickening). Second, individuals with higher serum albumin had a significantly increased odds ratio for metabolic syndrome. Serum albumin levels are considered to be a nutritional marker [13]. Serum albumin levels may be reduced in various diseased conditions, such as malnutrition and inflammatory states [1], and, on the contrary, they may be increased in the individuals with increased total body fat [6]. Although serum albumin levels may be modulated in response to the nutritional status, they may not be much reduced until near terminal starvation in otherwise healthy individuals [14], and thus, they lay within relatively narrow range in general populations [3,15].

It has been shown that lower levels of serum albumin are associated with increased risk of all-cause and cardiovascular mortality [2]. Djoussé et al. have reported that lower serum albumin concentrations are associated with an increased risk of coronary disease in both genders by analyzing the data from participants of the Framingham Offspring Study [3]. It still remains unclear whether the prognostic value of low albumin simply reflects inflammation/nutrition status or if there is an independent effect of albumin. However, there are several possible mechanisms that explain inverse association between serum albumin and cardiovascular disease. First, serum albumin may retard the atherogenesis by its antioxidative properties [16,17], as ROS may play a crucial role in the atherosclerotic process [18]. This possibility may be supported by the observations that oral intake or plasma levels of certain antioxidants, such as Vitamins C and E, and β-carotene, are associated with a reduced risk of coronary artery disease and carotid atherosclerosis [20–23]. Furthermore, we have also shown an inverse association between serum bilirubin, another endogenous antioxidant [19], and carotid atherosclerosis [11,20]. Second, subjects with low serum albumin levels are more likely to be cigarette smokers [15,21]. In the present study, however, this possibility may be rather unlikely, as negative association between serum albumin and carotid atherosclerosis remained statistically significant in both genders even after the adjustment of smoking status. Third, low albumin levels may reflect activation of proinflammatory cytokines and ongoing subclinical inflammation [22], which plays a crucial role in the process of atherogenesis [23]. Consistent with this idea was the finding that positive CRP, defined as CRP > 0.4 mg/dL, was most frequent in the lowest quartile of serum albumin in the current study. Finally, albumin may act as an anti-inflammatory agent towards endothelial cells [24].

Interestingly, Saito et al. have reported the positive associations of serum albumin with BMI, blood pressure, and lipid profiles after adjusting for gender and age in rural residents

[6]. Similarly, by analyzing the cross-sectional data from individual without a history of coronary heart disease, Danesh et al. have reported that serum albumin levels were positively associated with low-density lipoprotein cholesterol, triglycerides, and blood pressure [7]. Several other studies have also shown that serum albumin was associated with blood pressure and lipid profiles [8,9]. In addition to these observations, we have shown that serum albumin was positively associated with metabolic syndrome and insulin resistance, estimated by HOMA-IR, which are risk factors for carotid atherosclerosis that were independent of conventional atherogenic risk factors [25], in the current study, for the first time to out best knowledge. As serum albumin is considered to be cardioprotective, irrespective of its association with several atherogenic risk factors, several underlying mechanisms have been proposed in previous studies as mentioned above; however, so far, there is no direct evidence.

Although diet restriction may be effective in reducing subcutaneous and visceral fat, and in improving insulin sensitivity, it might also decrease serum albumin levels [26]. It may be questioned whether or not caloric restriction [27], especially when accompanied by the reduction of serum albumin levels, acts favorably in terms of protection of atherosclerotic diseases. Although we cannot answer this question from the current study, this should be investigated in future prospective studies.

Our study does have some limitations. First, as described in Section 2, only individuals who underwent health screening including carotid ultrasonography were selected as the study population; therefore, the study subjects were not considered to be a random selection from the whole subjects who underwent general health screening during the study period. Second, due to the cross-sectional nature of the current study, we are not able to conclude the causal or resultant relationship between serum albumin and metabolic syndrome or carotid atherosclerosis. Third, although serum albumin is postulated to possess antioxidant properties, there are other known circulating antioxidants, such as Vitamins A, C, and E; retinyl esters; and carotenoids. Association between some of these circulating antioxidants and metabolic syndrome were suggested as described above [10]; however, we did not use these factors as covariates. Finally, neither did we adjust the results for physical activity, which may also affect the prevalence of metabolic syndrome [28]. These possible confounding factors should be taken into consideration in future studies.

In conclusion, by analyzing cross-sectional data from individuals undergoing general health screening including carotid ultrasonography, we have demonstrated that higher serum albumin is associated with reduced prevalence of carotid atherosclerosis, whereas it is associated with increased prevalence of metabolic syndrome. Our data may provide evidence that negative association between serum albumin and atherosclerotic disease may not be explained by the hemodynamic and metabolic abnormalities that comprise the components of metabolic syndrome.

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Original Article

Is Metabolic Syndrome a Risk Factor for Carotid Atherosclerosis in Normotensive and Prehypertensive Individuals?

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Aim: We have investigated whether metabolic syndrome is a risk factor for carotid atherosclerosis also in normotensive or prehypertensive individuals.
Methods: We analyzed the data from 851 subjects who had a blood pressure of less than 140/90 mmHg and were not taking antihypertensive medication. Metabolic syndrome was defined according to three different criteria: Japan criteria (Japan-MetS); those of the National Cholesterol Education Program (NCEP)-Adult Treatment Panel III (ATPIII) (NCEP-MetS); and modified NCEP-ATPIII criteria in which body mass index was used as a surrogate for waist circumference (modified NCEP-MetS).
Results: Japan-MetS, NCEP-MetS, and modified NCEP-MetS were found, respectively, in 1%, 4%, and 4%, of women, and in 10%, 5%, and 9%, of men. After the adjustment for gender and age, the association between MetS and carotid atherosclerosis did not reach statistical significance.
Conclusion: Although the number of enrolled subjects was relatively small, these data may further support the importance of controlling blood pressure within the optimal range for the purpose of preventing atherosclerosis in individuals with metabolic syndrome.

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Key words; Metabolic syndrome, Carotid artery, Normotension, Prehypertension

Introduction

Metabolic syndrome (MetS), which is thought to be linked with insulin resistance, is a risk factor for cardiovascular disease[1] and stroke[2]. We have previously shown that MetS is a risk factor for carotid atherosclerosis in those undergoing general health screening[3]. Several other studies have shown an association between atherosclerotic diseases and MetS in individuals of different race and ethnicity, although these studies may have used different, or slightly modified, diagnostic criteria for MetS, such as those proposed by the World Health Organization (WHO)[4], the National Cholesterol Education Program (NCEP)[5], or those used in our country[6]. Although these criteria vary in some components, they all share several conventional atherogenic risk factors as diagnostic criteria[7-9], including impaired glucose metabolism, hypertension, and dyslipidemia.

Individuals with prominent hemodynamic and metabolic abnormalities can readily be recognized and managed by health care providers. Thus, it might be questioned whether, by using the concept of MetS, we can identify individuals at higher risk for atherosclerotic complications among those with only mild hemodynamic and/or metabolic abnormalities. We found that hypertension is the most common component of metabolic syndrome and the greatest contributor to carotid arteriosclerosis in individuals who had undergone general health screening[10]. Recently, we investigated data from subjects with normotension or prehypertension, who underwent general health screening between September 1994 and December 2003, and found that MetS might not be an independent risk factor for carotid atherosclerosis in such a population[11]. As waist circum-

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ference (WC) data were not available in that study, however, body mass index (BMI) was used as a surrogate for waist circumference for the diagnosis of MetS. As WC has recently become a routine measurement during general health screening at our institute, here we re-analyzed whether MetS, as diagnosed by Japan and NCEP criteria, is a risk factor for carotid atherosclerosis in normotensive or prehypertensive individuals.

Methods

Study Subjects

The study was approved by The Ethical Committee of Mitsui Memorial Hospital. Between October 2005 and June 2006, 1323 subjects underwent general health screening including carotid ultrasonography, measurement of other metabolic markers, and waist circumference necessary to assess the presence or absence of MetS at the Center for Multiphasic Health Testing and Services, Mitsui Memorial Hospital. Among them 851 (342 women, 509 men), who were not taking anti-hypertensive medications, were found to have a systolic blood pressure (SBP) of less than 140 mmHg and a diastolic blood pressure (DBP) of less than 90 mmHg, and were enrolled in the current study. Data on basal insulin levels were available for all subjects enrolled, and the homeostasis model assessment of insulin resistance (HOMA-IR) was calculated in these individuals according to the following formula: HOMA-IR = fasting immunoreactive insulin (μU/mL) × fasting plasma glucose (FPG, mg/dL))/405[12]. Normotension or prehypertension was defined according to the criteria of JNC 7[13], unless otherwise stated.

Definition of Metabolic Syndrome

For the diagnosis of MetS, we used three different criteria.

(1) Definition and diagnostic criteria of MetS in Japan[6] (Japan-MetS).

Waist circumference (WC) ≧90 cm in women or ≧85 cm in men plus two or more of the following: HDL cholesterol (HDL-C) <40 mg/dL or triglycerides (TG) ≧150 mg/dL; SBP ≧130 mmHg or DBP ≧85 mmHg; FPG ≧110 mg/dL.

(2) MetS diagnosed by NCEP ATP-Ⅲ criteria[5] (NCEP-MetS).

Subjects with three or more of the following five components were considered to have MetS: TG levels ≧150 mg/dL; HDL-C levels <40 mg/dL in men or <50 mg/dL in women; FPG ≧110 mg/dL; SBP ≧130 mmHg or DBP ≧85 mmHg; waist circumference >102 cm in men and >88 cm in women.

(3) MetS diagnosed by NCEP ATP-Ⅲ criteria using BMI criteria as a surrogate for the WC criterion (modified NCEP-MetS).

In these criteria, BMI ≧25.0 kg/m^2 was used as a surrogate of the WC criterion in NCEP-ATPⅢ criteria.

Of the 851 study subjects, 11 (1%) were taking anti-diabetic medicine, and were considered to fulfill the FPG criterion.

Carotid Ultrasonography

Carotid artery status was assessed using a high-resolution B-mode ultrasonography instrument (Sonolayer SSA270A, Toshiba, Japan) equipped with a 7.5 MHz transducer (PLF-703ST, Toshiba). In the current study, we diagnosed carotid plaque according to the criteria of the Society for the Study of Early Atherosclerosis; these criteria differ from those used in several previous studies[3], where carotid plaque was defined as clearly isolated focal thickening of the intima-media layer with a thickness of ≧1.3 mm. Here, we defined carotid plaque by a portion of the artery with an intima-media complex thickness of ≧1.1 mm[14] with a focal protrusion or point(s) of inflexion. The difference in the prevalence of carotid plaque in health screening participants between the current and previous studies[3] may be due to the difference in criteria used to diagnose carotid plaque. On the other hand, carotid wall intima-media thickening was said to be present when intima-media thickness, measured at the far wall of the distal 10 mm of the common carotid artery, was ≧1.0 mm[3].

Statistical Analysis

The data in this study were analyzed by the χ^2 test, unpaired t test, and multivariate logistic regression analysis using computer software, StatView ver. 5.0. A value of $p<0.05$ was taken as significant. The results are expressed as the mean ± SD.

Results

Prevalence of MetS Defined by Three Diagnostic Criteria

The age of the subjects ranged from 25 to 91 years (women, 25-91 years; men, 29-89 years) (**Table 1**). Japan-MetS, NCEP-MetS, and modified NCEP-MetS were found, respectively, in 4 (1%), 14 (4%), and 13 (4%) women, and in 49 (10%), 24 (5%), and 47 (9%) men. The prevalence and overlap of MetS defined by these criteria are shown in **Fig. 1**. In the study population, 767 (90.1%) individuals were found to be free from MetS, as defined by any of the three criteria. HOMA-IR in individuals with or without Japan-MetS

Table 1. Baseline characteristics and laboratory data

Variables	Women (n=342)	Men (n=509)
Age, years	55.9±11.0	56.9±10.8
Body mass index, kg/m^2	21.2±2.9	23.6±2.6
Waist circumferrence, cm	77.2±9.2	85.7±7.3
Systolic blood pressure, mmHg	114±13	118±12
Diastolic blood pressure, mmHg	71±9	76±8
Normotension (%)	219 (64)	237 (47)
Prehypertension (%)	123 (36)	272 (53)
Laboratory data		
Total cholesterol, mg/dL	223±34	210±33
HDL-cholesterol, mg/dL	70±16	56±14
Triglycerides, mg/dL	84±43	127±93
Uric acid, mg/dL	4.5±0.9	6.2±1.1
Fasting glucose, mg/dL	90±12	99±18
Hemoglobin A1C, %	5.2±0.5	5.4±0.6
HOMA-IR	1.2±0.8	1.6±1.0
Smoking status		
Never (%)	297 (87)	161 (32)
Former (%)	20 (6)	215 (42)
Current (%)	25 (7)	133 (26)

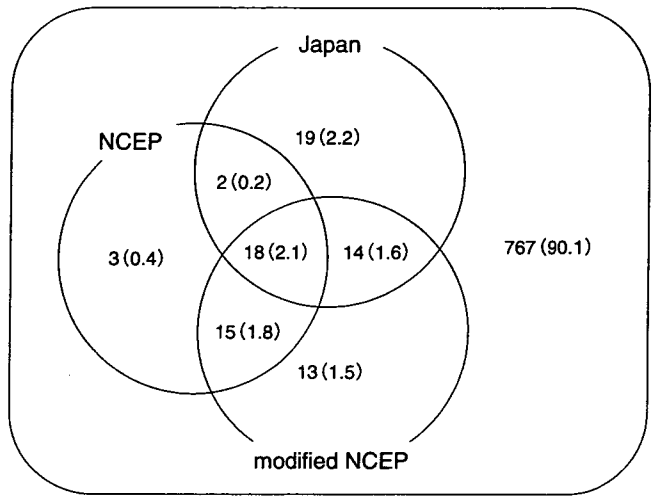

Fig. 1. Venn diagram showing the three target populations. Percentages are shown in parentheses.

was, respectively, 4.0±2.5 or 1.2±0.7 (p<0.0001) in women, and 2.4±1.5 or 1.5±0.9, respectively, (p<0.0001) in men. HOMA-IR in individuals with or without NCEP-MetS was, respectively, 2.7±1.6 or 1.2±0.7 (p<0.0001) in women, and 2.8±1.6 or 1.67±1.06 (p<0.0001) in men. HOMA-IR in individuals with or without modified NCEP-MetS was, respectively, 2.7±1.7 and 1.2±0.7 (p<0.0001) in women, and 2.6±1.6 and 1.5±0.90 (p<0.0001) in men. The characteristics and laboratory data of individuals who had metabolic syndrome defined by either criterion are described in **Table 2**.

In the whole study population, 674 individuals (284 women, 390 men) had a BP of <130/85 mmHg, and in this subpopulation, the prevalence of Japan-MetS, NCEP-MetS, and modified NCEP-MetS was found to be, respectively, 0 (0%), 5 (2%), and 4 (1%) in women, and 9 (2%), 7 (2%), and 14 (4%) in men. In addition, in 456 normotensive individuals (219 women, 237 men) (i.e., individuals with a BP of <120/80 mmHg), the prevalence of Japan-MetS, NCEP-MetS, and modified NCEP-MetS was found to be, respectively, 0 (0%), 5 (2%), and 4 (2%), respectively, in women, and 5 (2%), 3 (1%), and 4 (2%), respectively, in men.

Relation between Carotid Plaque and MetS

Carotid plaque was found in 145 (42%) of the 342 women and 295 (58%) of the 509 men. HOMA-IR in individuals with or without carotid plaque was, respectively, 1.3±0.8 or 1.2±0.8 (p=0.44) in women, and 1.6±1.0 or 1.7±1.1 (p=0.45) in men. When individuals with fasting glucose of ≧140 mg/dL (2 in women and 17 in men) were excluded from this analysis, HOMA-IR in individuals with or without carotid plaque was, respectively, 1.3±0.7 or 1.2±0.7 (p=0.35) in women, and 1.5±0.8 or 1.7±1.1 (p=0.12) in men.

After adjustment for gender and age, logistic regression analysis showed that the association between MetS defined by the three different criteria and carotid plaque did not reach statistical significance. After further adjustment for smoking status and SBP, the odds ratio for carotid plaque was decreased and, again, MetS as defined by any of the three criteria was not found to be associated with carotid plaque (**Table 3**).

Relation between Carotid Intima-Media Thickening and MetS

Carotid intima-media thickening was found in 20/342 (5.8%) women and 61/509 (12.0%) men. After adjustment for gender and age, logistic regression analysis showed that the odds ratio of MetS defined by the three different criteria for carotid intima-media thickening was as follows: Japan-MetS, 1.63 (95% CI 0.69-3.84. p=0.27); NCEP-MetS, 1.25 (95% CI 0.42-3.78, p=0.69); and modified NCEP-MetS, 0.94 (95% CI 0.35-2.58, p=0.92). After further adjustment for smoking status and SBP, the odds ratio for carotid plaque was decreased and, again, MetS as defined by any of the three criteria was not found to be associated

Table 2. Clinical characteristics and laboratory data of individuals who were positive for metabolic syndrome

Variables	Japan-Mets		NCEP-Mets		modified NCEP-Mets	
	Women ($n=4$)	Men ($n=49$)	Women ($n=14$)	Men ($n=24$)	Women ($n=13$)	Men ($n=47$)
Age, years	65.3±13.5	56.2±10.1	59.6±10.1	56.7±8.5	56.0±11.0	55.3±10.1
Bod y mass index, kg/m^2	24.2±1.6	25.2±2.4	24.5±2.3	25.6±3.1	25.8±2.4	26.0±2.4
Waist circumferrence, cm	92.3±1.7	91.0±6.2	89.5±3.9	90.8±9.2	88.5±3.8	91.3±7.4
Systolic blood pressure, mmHg	134±2	130±8	126±11	127±11	128±10	129±9
Diastolic blood pressure, mmHg	84±4	81±5	77±8	79±9	79±7	80±7
Normotension (%)	0 (0)	5 (10)	5 (36)	3 (13)	4 (31)	5 (11)
Prehypertension (%)	4 (100)	44 (90)	9 (64)	21 (88)	9 (69)	42 (89)
Laboratory data						
Total cholesterol, mg/dL	231±16	211±37	226±29	215±34	214±29	211±38
HDL- cholesterol, mg/dL	55±5	47±11	51±9	42±8	50±10	42±8
Triglycerides, mg/dL	129±51	229±149	180±76	252±115	165±66	249±145
Uric acid, mg/dL	4.5±1.2	6.5±1.2	5.0±1.0	6.6±1.5	5.1±0.9	6.6±1.3
Fasting glucose, mg/dL	115±26	117±35	104±16	116±17	104±16	107±17
Hemoglobin A1C, %	5.8±1.1	5.9±1.0	5.5±0.8	6.0±0.7	5.4±0.8	5.6±0.7
HOMA-IR	4.0±2.5	2.4±1.5	2.7±1.6	2.8±1.6	2.7±1.7	2.6±1.6
Smoking status						
Never (%)	4 (100)	11 (22)	11 (79)	8 (33)	11 (85)	15 (32)
Former (%)	0 (0)	16 (33)	1 (7)	9 (38)	1 (8)	18 (38)
Current (%)	0 (0)	22 (45)	2 (14)	7 (29)	1 (8)	14 (30)

Table 3. Odds ratio for carotid plaque

Variables	Odds raio (95% CI)	p value
Japan-MetS		
Adjusted for sex, age	1.62 (0.84-3.11)	0.15
Adjusted for sex, age, SBP, smoking status	1.22 (0.62-2.39)	0.57
NCEP-MetS		
Adjusted for sex, age	1.73 (0.82-3.65)	0.15
Adjusted for sex, age, SBP, smoking status	1.42 (0.67-3.01)	0.36
modified NCEP-MetS		
Adjusted for sex, age	1.52 (0.84-2.74)	0.75
Adjusted for sex, age, SBP, smoking status	1.19 (0.65-2.20)	0.57

with carotid plaque: Japan-MetS, odds ratio 1.27 (95% CI 0.51-3.17. $p=0.60$); NCEP-MetS, 0.97 (95% CI 0.31-3.00, $p=0.96$); and modified NCEP-MetS, 0.68 (95% CI 0.24-1.89, $p=0.45$).

Discussion

In the present study, we found that, in individuals with normotension or prehypertension, the prevalence of Japan-MetS, NCEP-MetS, and modified NCEP-MetS was, respectively, 1%, 4%, and 4% in women, and 10%, 5% and 9% in men. MetS defined by Japan-MetS, NCEP-MetS, or modified NCEP-MetS was not found to be statistically significantly associated with carotid plaque after adjustment for age and gender. This statistical non-significance remained after further adjustment for smoking status and systolic blood pressure.

In a previous report, we showed that MetS was associated with carotid plaque, which was independent of other conventional atherogenic risk factors[3]. On the other hand, we found that MetS may not be a risk factor for carotid atherosclerosis in individuals with normotension or prehypertension[11]. In those previous studies, for the diagnosis of MetS, we used NCEP-ATPIII criteria with BMI as a surrogate for WC, designated here as modified NCEP-MetS. In the current study, we extended the concept that there is no association between MetS and carotid atherosclerosis even when using other diagnostic criteria including the WC component. Although the results obtained are in agreement with those of a previous report[11], several points need careful consideration.

First, the number of subjects enrolled was not large in the current study, which may weaken the statistical

power. For example, although not statistically significant, the odds ratio of Japan-MetS for carotid plaque after adjustment for sex and age (1.62) was almost comparable to the odds ratio (1.72) of modified NCEP-MetS for carotid plaque after adjusting for sex, age, total cholesterol levels, and smoking status found previously in health screening participants[15]. Thus, we should re-evaluate these observations in future studies after increasing the number of subjects enrolled. We should also investigate whether the association between MetS, as defined by the Japan criteria or by the NCEP-ATPIII criteria, and carotid atherosclerosis remains statistically significant after further adjustment for other traditional cardiovascular risk factors and/or each component of MetS.

Second, in the current study, we used a different definition of carotid plaque from that used in our previous study, where carotid plaque was defined as clearly isolated focal thickening of the intima-media layer with a thickness of ≧1.3 mm at the common or internal carotid artery or the carotid bulb[11]. In the current study, by contrast, carotid plaque was diagnosed when maximal intima-media thickness was ≧1.1 mm. It may be expected that the prevalence of carotid atherosclerosis would substantially differ according to the criteria used: in individuals with normotension or prehypertension, carotid plaque was found in 42% of women (mean age 56 years) and 58% of men (mean age 57 years) in the current study, and in 13% of women (mean age 55 years) and 24% of men (mean age 55 years) in the previous study[11].

In the current study, the prevalence of Japan-MetS in men was more than 8-fold that in women. This marked difference is in agreement with a recent study reported by another group, in which the prevalence of Japan-MetS in the general Japanese population was found to be 1.7% (mean age 46 ± 1 years) in women and 12.1% (mean age 46 ± 0 years) in men[16]. We have found that in studies using NCEP criteria, the prevalence of MetS (NCEP-MetS) is much closer between genders, which may also be in agreement with studies analyzing the Japanese population[16-19]. When comparing the prevalence of MetS reported in various studies, however, it should be noted that diagnostic criteria with certain minor modifications may have been used in various studies.

We have previously reported that hypertension is the most frequent component of modified NCEP-MetS and the greatest contributor to carotid atherosclerosis among its risk factor components[10]. Mancia *et al.* reported in a population-based study in San Marino that the prevalence of MetS was 24% in hypertensive subjects, whereas it was as little as 4% in subjects with optimal, normal, or high-normal BP[20]. Similarly, during the current study period, we found that the prevalence of Japan-MetS in the whole study population who had undergone carotid ultrasonography was 3% in women and 22% in men (Ishizaka Y, unpublished data); in addition, in the current study, which targeted individuals with optimal, normal, or high normal BP, it was 1% in women and 10% in men. Furthermore, when individuals with a BP ≧130/85 mmHg were removed from our analysis (corresponding to optimal or normal BP according to JNC VI classification[21]), the prevalence of Japan-MetS was 0% in women and 2% in men. Kanauchi *et al.* analyzed the prevalence of MetS, as diagnosed by NCEP-MetS with a modified WC criterion, in individuals who were not taking anti-hypertensive medication, and found it to be 10%, 19%, and 36% in those with normotension, prehypertension, and hypertension, respectively[22]. These data collectively indicate that the prevalence of MetS, regardless of the diagnostic criteria used, is substantially lower in individuals whose BP is in the preferable range.

In summary, we analyzed individuals who underwent general health screening including carotid ultrasonography, and found that Japan-MetS, NCEP-MetS, or modified NCEP-MetS was not a predictor for carotid atherosclerosis in normotensive or prehypertensive individuals after adjusting for sex and age. Our data suggest that maintaining BP within the optimal range is crucial for reducing the risk for both metabolic syndrome and carotid atherosclerosis. As our study involved a relatively small study population, further investigation should be performed after increasing the size of the study population in the future.

Acknowledgement

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ORIGINAL ARTICLE

Ningen Dock 2007 ; **21** : 51－55

Albuminuria in General Health Screening in Japan : Relationship with Insulin Resistance and Atherosclerosis

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Background and Purpose The presence of microalbuminuria is a risk factor for advanced renal failure and atherosclerotic diseases. In the current study, we investigated the association between albuminuria, insulin resistance, and carotid atherosclerosis.

Methods and Results We analyzed data from 3674 individuals (female 1228, male 2446) who underwent general health screening. Microalbuminuria was defined as a urine albumin to urine creatinine ratio, termed the albumin excretion index (AEI), of 30 and 299 mg/g ; macroalbuminuria was defined as an AEI ≥300 mg/g. The prevalence of micro- and macroalbuminuria was 11.7% and 1.5%, respectively. When compared to the lowest AEI quartile (AEI <4.5 mg/g), the highest AEI quartile (AEI ≥150 mg/g) was found to be associated with metabolic syndrome (MetS) with an odds ratio of 5.7 (95% CI 1.7–19.3) in women and 3.9 (95% CI 2.9–5.3) in men after adjusting for total cholesterol (TC) and smoking status. In addition, after adjusting for sex, age, TC, smoking status, systolic BP, and fasting glucose, the highest AEI quartile was associated with carotid plaque with an odds ratio of 1.33 (95% CI 1.06–1.67).

Conclusion Our data show that the presence of albuminuria in individuals undergoing general health screening, even when it is below the cut-off value for "micro-" albuminuria, is a risk factor for MetS and carotid plaque. (*Ningen Dock* 2007 ; **21** : 51－55)

Key Words : albuminuria, ningen dock, metabolic syndrome (MetS), carotid atherosclerosis

The presence of microalbuminuria and macroalbuminuria is an established risk factor for cardiovascular morbidity and mortality as well as for end-stage renal disease in individuals with hypertension or diabetes mellitus[1-3]. Albuminuria is thought to reflect endothelial dysfunction, and this may explain the observed association between albuminuria and cardiovascular disease, although there might be other interlinking factors. Importantly, a link between albuminuria and cardiovascular disease may be present also in low-risk subjects, such as non-diabetic individuals[3] and those that are both non-hypertensive *and* non-diabetic[4]. In the current study, we analyzed the prevalence of microalbuminuria and macroalbuminuria in individuals undergoing general health screening, and assessed whether such albuminuria was a risk factor for insulin resistance, metabolic syndrome (MetS), and carotid plaque in this population.

Methods

Study Subjects

We have analyzed the data from 3674 individuals (female 1228, male 2446) who underwent general health screening at our institute between 2004 and 2006. Blood samples were taken from subject who had been fasting overnight. Serum levels of total cholesterol (TC), HDL cholesterol (HDL-C), and TG were determined enzymatically. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to the following formula : HOMA-IR=[fasting serum insulin (μU/ml) × fasting plasma glucose (mg/dl)]/405.

Diagnosis of Micro- and Macroalbuminuria

For the diagnosis of albuminuria, spot urine samples were collected and analyzed ; albuminuria was expressed the albumin excretion index (AEI). Normoalbuminuria, microalbuminuria, and macroalbuminuria were defined as an AEI of <30 mg/g, 30–299 mg/g, and 300 mg/g respectively[5]. Interquartile cut-off values of AEI were 4.5 mg/g, 7.5 mg/g, and 15.0 mg/g, respectively.

Criteria for Metabolic Syndrome

The diagnosis of MetS was made by the criteria of the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP-III)[6] with BMI as a surrogate for waist circumference. MetS was said to be present when three or more of the following conditions were met : (1) TG levels ≥150 mg/dl, (2) HDL-C levels <40 mg/dl in men or <50 mg/dl in women, (3) fasting plasma glucose ≥110 mg/dl or taking an antidiabetic medication, (4) systolic BP ≥130 mmHg or diastolic BP ≥85 mmHg or taking an antihypertensive medication, and (5) BMI >25 kg/m^2.

Carotid Ultrasonography

Carotid artery status was assessed by using a high-resolution B-mode ultrasonography instrument (Sono-

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layer SSA270A, Toshiba Medical Systems Corp., Tochigi, Japan) equipped with a 7.5MHz transducer (PLF-703ST, Toshiba Medical Systems Corp.). Here, we defined carotid plaque by the presence of portion(s) of the artery with an intima-media complex thickness of ≥1.1 mm[7] with a focal protrusion or point(s) of inflexion. This diagnostic criteria was different from the one we had used in several previous studies[8].

Statistical Analysis

The data in this study were analyzed by the χ^2 test, analysis of variance (ANOVA), and a multivariate logistic regression analysis using computer software, StatView ver. 5.0. Results are expressed as the mean±SD.

Results

Prevalence of Micro- and Macroalbuminuria

Mean ages±SD of the enrolled female and male subjects were 56.9±11.2 and 57.3±10.6 years, respectively. Mean±SD (median) value of the AEI was 27±127 (9) in women and 32±211 (6) in men. The overall prevalence of microalbuminuria was 9.8% in women and 10.4% in men, and that of macroalbuminuria was 1.4% and 1.6%, respectively (**Table 1**). Individuals with micro- or macroalbuminuria had significantly higher HOMA-IR values compared with those with normoalbuminuria. The prevalence of microalbuminuria increased with age in both genders (**Fig. 1 A**). The prevalence of micro- or macroalbuminuria according to diabetes and hypertension status is presented in **Fig. 1**

Table 1. Baseline characteristics of the subjects

Variables	Normo-albuminuria	Micro-albuminuria	Macro-albuminuria	*p* value
Women				
Subjects, *n* (%)	1090 (89)	121 (10)	17 (1)	
Age (years)	56 ± 11	62 ± 11	62 ± 11	<0.0001
BMI (kg/m^2)	21.5 ± 3.0	22.9 ± 4.2	23.8 ± 3.5	<0.0001
Systolic BP (mmHg)	120 ± 19	136 ± 23	145 ± 25	<0.0001
Diastolic BP (mmHg)	74 ± 12	82 ± 14	87 ± 12	<0.0001
Laboratory data				
CRP (mg/dl)	0.11 ± 0.39	0.19 ± 0.75	0.22 ± 0.37	0.069
Total cholesterol (mg/dl)	219 ± 35	226 ± 35	221 ± 48	0.13
HDL cholesterol (mg/dl)	68 ± 16	66 ± 17	59 ± 16	0.017
TG (mg/dl)	88 ± 49	96 ± 46	124 ± 55	0.0028
Fasting glucose (mg/dl)	92 ± 12	102 ± 27	111 ± 34	<0.0001
HOMA-IR	1.3 ± 0.9	1.8 ± 1.2	2.9 ± 2.5	<0.0001
Smoking status				
Non-smokers (%)	944 (87)	104 (86)	13 (76)	0.53
Former smokers (%)	61 (6)	9 (7)	1 (6)	
Current smokers (%)	85 (8)	8 (7)	3 (18)	
Men				
Subjects, *n* (%)	2153 (88)	255 (10)	38 (2)	
Age (years)	57 ± 11	61 ± 10	64 ± 12	<0.0001
BMI (kg/m^2)	24.0 ± 2.9	24.9 ± 2.7	24.7 ± 4.3	<0.0001
Systolic BP (mmHg)	127 ± 18	139 ± 20	142 ± 24	<0.0001
Diastolic BP (mmHg)	80 ± 11	87 ± 12	86 ± 14	<0.0001
Laboratory data				
CRP (mg/dl)	0.14 ± 0.43	0.26 ± 1.13	0.15 ± 0.20	0.0041
Total cholesterol (mg/dl)	208 ± 31	212 ± 35	213 ± 38	0.12
HDL cholesterol (mg/dl)	55 ± 13	54 ± 13	57 ± 20	0.45
TG (mg/dl)	136 ± 91	148 ± 112	153 ± 115	0.087
Fasting glucose (mg/dl)	101 ± 19	112 ± 30	123 ± 45	<0.0001
HOMA-IR	1.8 ± 1.3	2.7 ± 5.8	2.5 ± 1.8	<0.0001
Smoking status				
Non-smokers (%)	712 (33)	70 (27)	8 (21)	0.020
Former smokers (%)	878 (41)	128 (50)	16 (42)	
Current smokers (%)	563 (26)	57 (22)	14 (37)	

HOMA-IR : Homeostasis model assessment of insulin resistance.

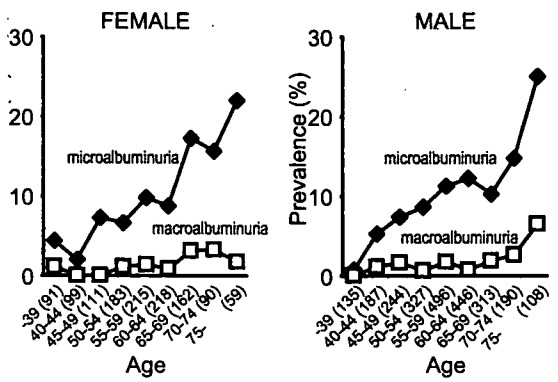
where diabetes was defined as either fasting glucose ≥126 mg/dl *or* taking antidiabetic medication, and hypertension was defined as either systolic BP ≥130 mmHg, diastolic BP ≥85 mg/dl, *or* taking antihypertensive medication. As shown in **Fig. 1B**, individuals with either hypertension or diabetes were more likely to have an AEI ≥30 mg/g (i.e., either micro- or macroalbuminuria) than those with neither of these conditions. In addition, in comparison with individuals who had either of these conditions, an AEI ≥30 mg/g was more than 7- and 8-times more prevalent in women and men, respectively, when both hypertension and diabetes were present (**Fig. 1B**).

Albuminuria and Insulin Resistance

We can see that AEI increased according to HOMA-IR in both genders (**Figs. 2A** and **B**). After adjusting for age and TC, logistic regression analysis showed that AEI was associated with increased insulin resistance, defined here as HOMA-IR ≥2.0, in a value-dependent manner (**Fig. 2B**). Similar observations could be obtained when micro- and macroalbuminuria group was used in the place of AEI quartiles. As expected, albuminuria was also associated with the prevalence of MetS in a value-dependent manner (**Figs. 3A** and **B**).

Albuminuria and Carotid Atherosclerosis

Next, we investigated whether albuminuria was associated with carotid plaque, which is a marker for early atherosclerosis. The prevalence of carotid plaque increased according to the AEI quartiles in both genders (**Fig. 4A**). After adjusting for age and TC, the highest AEI quartile was found to be associated with a significantly higher prevalence of carotid plaque when the lowest AEI quartile was taken as reference. The association between carotid plaque and microalbuminuria was statistically significant in women, but not in men (**Fig. 4B**). Importantly, even after adjustment for sex, age, TC, *systolic BP, and fasting glucose*, the highest AEI quartile was found to be associated with carotid plaque with an odds ratio of 1.33 (95% CI 1.06–1.67, p=0.013) when the lowest AEI quartile was used as reference. When micro- and macroalbuminuria were put combined (i.e., an AEI ≥30 mg/g), age- and TC-adjusted logistic regression analysis showed that an AEI ≥30 mg/g was statistically significantly associated with carotid plaque in women with an odds ratio of 1.93 (95% CI 1.30–2.86, p=0.0011), but not in men (odds ratio 1.15 [95% CI 0.86–1.53, p=0.87]).



Prevalence of micro- or macroalbuminuria

FEMALE		
	Hypertension	
Diabetes		
	10%	43%
	5%	20%

MALE		
	Hypertension	
Diabetes		
	16%	36%
	5%	15%

1. *Prevalence of micro- and macroalbuminuria according to age (A) and diabetes and smoking status (B)*

Discussion

By analyzing the data of individuals with a mean age of 57 years old, we found that the prevalence of micro- and macroalbuminuria was 9.9% and 1.4%, respectively, in women, and 10.4% and 1.6%, respectively, in men. Albuminuria was value-dependently associated with insulin resistance (defined as a HOMA-IR ≥2.0) and MetS, defined by modified NCEP ATP III criteria. Presence of either hypertension or diabetes increased the prevalence of an AEI ≥30 mg/g (i.e., either micro- or macroalbuminuria), and when compared with those who had neither of these conditions, this prevalence became more than 7-times greater when both hypertension and diabetes were present. Furthermore, we have shown that the highest AEI quartile

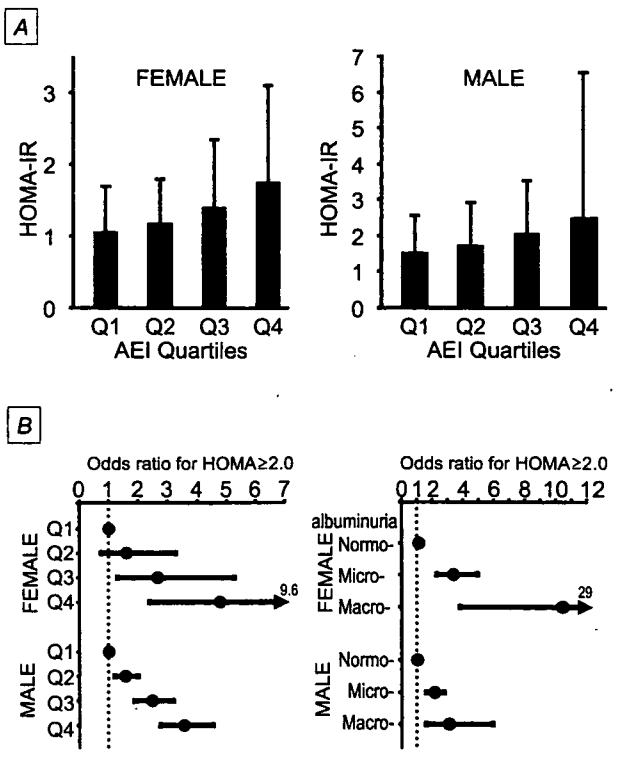


Fig. 2. *A. Homeostasis model assessment of insulin resistance (HOMA-IR) according to albumin excretion index (AEI) quartiles. B. Age-adjusted logistic regression analysis showing the association between urinary albumin excretion and increased insulin resistance, defined as HOMA-IR of ≥2.0.*

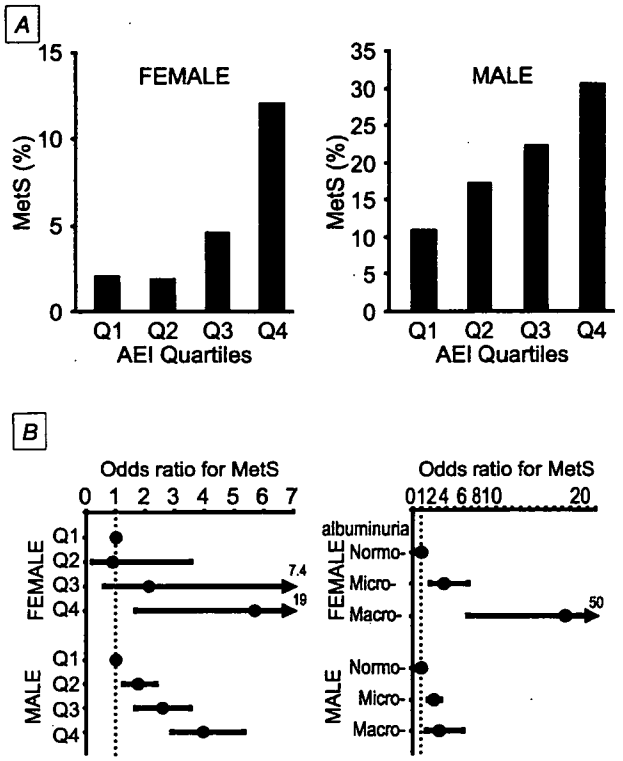


Fig. 3. *A. Prevalence of metabolic syndrome (MetS) according to albumin excretion index (AEI) quartiles. B. Age-adjusted logistic regression analysis showing the association between urinary albumin excretion and MetS.*

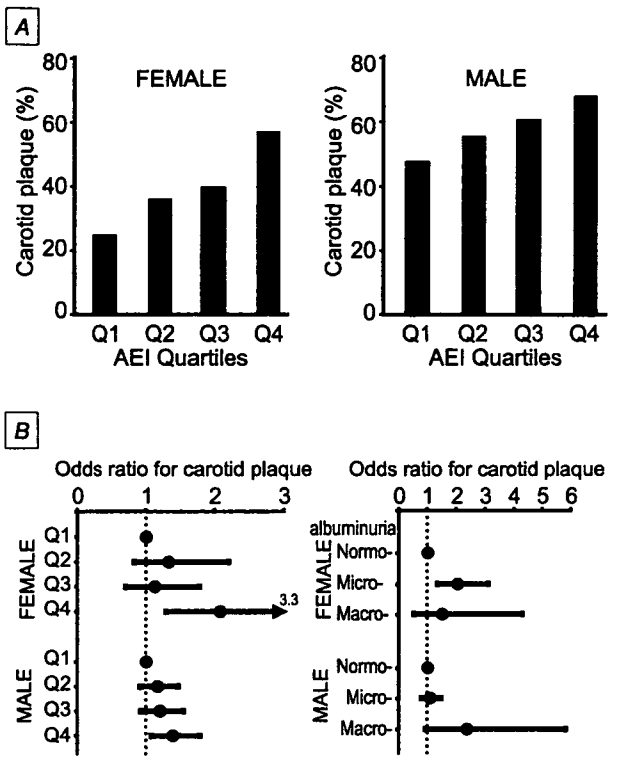


Fig. 4. *A. Prevalence of carotid plaque according to albumin excretion index (AEI) quartiles. B. Age-adjusted logistic regression analysis showing the association between urinary albumin excretion and carotid plaque.*

was significantly associated with carotid plaque after adjustment for age and TC in both genders. We also showed that individuals in the highest AEI quartile (AEI ≥15 mg/g) had a significantly higher prevalence of carotid plaque when compared with those in the lowest AEI quartile (AEI <4.5 mg/g) (**Fig. 4B**).

The prevalence of microalbuminuria has been compared in various races and ethnicities. Metcalf *et al.* discussed how the prevalence of microalbuminuria may be higher in non-Europeans (between 8% and 28%) than in Europeans (between 2% and 10%), although several different criteria may have been used for the diagnosis of microalbuminuria[9]. In Japan, the prevalence of microalbuminuria has been reported to be 13.2%[10] and 13.7%[11] in the general population, which were slightly greater than that found in the current study. The reported prevalence of microalbuminuria in non-diabetic subjects in our country seems to be similar to that in general population[12,13]. In the non-diabetic population, the prevalence of microalbuminuria in Japan was comparable to figures reported in UK[14], USA[15], and Korea[16], whereas Aborigines have exceedingly high rates of albuminuria (36% in men and 39% in women)[17].

Several studies have shown that the presence of albuminuria is a risk factor for atherosclerotic diseases[18-20]. Importantly, microalbuminuria may be associated with atherosclerotic diseases even in the general population[21] and in non-diabetic subjects[22]. In the current study, we also found that the highest AEI quartile was significantly positively associated with carotid plaque in both genders (**Fig. 4B**) after adjusting for age and TC, and, when both genders were analyzed together, this association remained statistically significant with an odds ratio of 1.33, even after further adjustment for systolic BP and fasting glucose. These observations indicate that an association between microalbuminuria and the early stages of atherosclerosis might already be present in relatively low-risk Japanese subjects, such as health-screening participants, and this association might be independent of other atherogenic risk factors in such a population. These observations might provide a clinical basis for deciding on the usefulness and/or necessity of measuring urinary albumin excretion as part of general health screening or Ningen Dock programs, although cost-effectiveness is another issue that needs to be discussed. It should also be stressed that the clinical importance of measuring albuminuria has recently received international attention[23]. Researchers are now focusing on trying to establish the true normal range of urinary albumin excretion[4,24], and whether or not microalbuminuria itself might be a meaningful therapeutic target[25].

In conclusion, in general health-screening participants, albuminuria was found to be value-dependently associated with insulin resistance and MetS. The highest AEI quartile (AEI ≥15 mg/g) was associated with carotid plaque in both genders. Based on these findings, it is obviously of value to keep on evaluating and discussing the importance of measuring albuminuria in the setting of the general population for the purpose of estimating the risk for insulin resistance

and atherosclerosis, with the ultimate goal of establishing guidelines for a healthier lifestyle.

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