metabolic syndrome [23]: high blood pressure was defined as a systolic blood pressure ≥ 130 mmHg or a diastolic blood pressure ≥ 85 mmHg; dyslipidaemia was defined as serum triglycerides ≥ 1.7 mmol/l or HDL cholesterol < 1.03 mmol/l.

We calculated the incidence rates and hazard ratios (HRs) of diabetes according to the sex-specific quintile of waist circumference and BMI. The Cox proportional hazard model was used to calculate age- and sex-adjusted HRs and multivariate-adjusted HRs. In the multivariate-adjusted model, HRs were adjusted simultaneously for potential confounders including age, sex, family history of diabetes, smoking habits, alcohol use and exercise frequency. In each quintile of waist circumference or BMI, the geometric means of HOMA-IR and HOMA-B were calculated and were compared between those who developed Type 2 diabetes and those who did not using a Student *t*-test. Statistical analysis was conducted with the Statistical Package for the Social Sciences (SPSS version 12.0J; SPSS, Tokyo, Japan).

Results

The baseline characteristics of the study participants are presented in Table 1. At the baseline examinations, the participants had a mean age of 44.4 years for both men and women, a mean BMI of 23.2 kg/m^2 for men and 22.6 kg/m^2 for women and a mean waist circumference of 80.0 cm for men and 72.4 cm for women. During the 8-year follow-up (27 861 person-years), we documented 218 incident cases of diabetes (175 men and 43 women). Among these, 172 were diagnosed with diabetes based on high fasting plasma glucose levels, 40 were diagnosed according to a 75-g OGTT and six had been treated with glucose-lowering medications.

Table 2 shows the baseline characteristics and incidence of diabetes according to the sex-specific quintiles of waist circumference. Participants with higher waist circumference tended to be older and to have higher values of fasting plasma glucose, HbA_{1c}, HOMA-IR and HOMA-B and higher

Table 1 Baseline characteristics of study participants

Characteristic	Total	Men	Women
Participants (*n*)	3992	2533	1459
Age (years)	44.4 ± 5.8	44.4 ± 5.9	44.4 ± 5.7
Waist circumference (cm)	77.2 ± 8.8	80.0 ± 7.6	72.4 ± 8.8
Body mass index (kg/m^2)	23.0 ± 2.9	23.2 ± 2.7	22.6 ± 3.2
Fasting plasma glucose (mmol/l)	5.0 ± 0.49	5.0 ± 0.50	4.9 ± 0.45
Fasting insulin (μU/ml)	5.6 ± 4.3	5.7 ± 4.8	5.4 ± 3.3
HbA_{1c} (%)	5.0 ± 0.4	5.0 ± 0.4	4.9 ± 0.4
HOMA-IR*	1.04 (0.69–1.50)	1.04 (0.67–1.55)	1.03 (0.76–1.43)
HOMA-B*	67.2 (47.0–95.3)	64.5 (43.2–93.3)	72.0 (51.4–98.2)
Systolic blood pressure (mmHg)	119 ± 14	122 ± 14	115 ± 13
Diastolic blood pressure (mmHg)	75 ± 11	77 ± 11	72 ± 10
Total cholesterol (mmol/l)	5.3 ± 0.87	5.3 ± 0.86	5.3 ± 0.89
HDL cholesterol (mmol/l)	1.5 ± 0.40	1.4 ± 0.39	1.7 ± 0.38
Triglycerides (mmol/l)*	1.0 (0.69–1.42)	1.2 (0.80–1.67)	0.8 (0.56–0.99)
Family history of diabetes (%)	11.9	12.8	10.5
Smoking (%)			
Never	53.7	28.7	96.9
Ex-smoker	7.4	11.5	0.5
Current smoker	38.9	59.8	2.6
Alcohol drinking (%)			
Non-drinker	43.8	21.5	82.6
Occasional drinker	2.5	2.2	3.0
Light drinker	41.0	56.9	13.2
Moderate/heavy drinker	12.7	19.3	1.2
Regular exercise (%)			
None	70.9	66.5	78.3
Weak	17.1	19.5	13.2
Moderate	8.8	9.9	6.9
Strong	3.2	4.1	1.6
Prevalence of high blood pressure† (%)	29.5	36.0	18.2
Prevalence of dyslipidaemia† (%)	21.3	29.7	6.8

Values are means ± standard deviation or %.
HbA_{1c}, glycated haemoglobin; HDL, high-density lipoprotein; HOMA-B, homeostasis model assessment of pancreatic B-cell function; HOMA-IR, homeostasis model assessment of insulin resistance.
*Values are geometric means (interquartile range).
†High blood pressure and dyslipidaemia were defined by Japanese criteria of metabolic syndrome.

Table 2 Age- and sex-adjusted and multivariate-adjusted hazard ratios for the incidence of Type 2 diabetes according to sex-specific quintile of waist circumference

Parameter	Waist circumference quintile				
	Q1	Q2	Q3	Q4	Q5
Range of waist circumference, men (cm)	51.0–73.0	73.5–78.0	78.5–82.0	82.5–86.0	86.5–110.0
Range of waist circumference, women (cm)	54.0–65.0	65.5–69.0	69.5–73.5	74.0–80.0	80.5–120.0
Participants (*n*)	852	803	820	765	752
Age (years)	43.7 ± 5.7	44.3 ± 5.7	44.4 ± 5.9	44.7 ± 5.8	45.0 ± 5.9
Fasting plasma glucose (mmol/l)	4.9 ± 0.49	4.9 ± 0.46	5.0 ± 0.46	5.1 ± 0.52	5.1 ± 0.49
HbA_{1c} (%)	5.0 ± 0.4	5.0 ± 0.4	5.0 ± 0.4	5.0 ± 0.4	5.1 ± 0.4
Fasting insulin (μU/ml)	4.1 ± 3.9	4.7 ± 3.4	5.3 ± 3.3	6.3 ± 4.0	7.7 ± 5.7
HOMA-IR*	0.75 (0.54–1.05)	0.88 (0.61–1.24)	1.04 (0.72–1.46)	1.22 (0.86–1.67)	1.48 (1.07–2.06)
HOMA-B*	53.4 (37.9–75.0)	60.2 (41.5–83.5)	66.8 (48.0–90.0)	75.0 (53.3–106.7)	87.9 (63.5–120.0)
Family history of diabetes (%)	10.7	11.7	13.3	10.2	13.8
Prevalence of high blood pressure† (%)	21.5	24.8	28.4	35.6	38.7
Prevalence of dyslipidaemia† (%)	7.9	14.8	21.5	26.0	38.7
Total person-years	6143	5787	5689	5242	5000
Incident cases (*n*)	39	23	34	58	64
Rate per 1000 person-years	6.3	4.0	6.0	11.1	12.8
Adjusted hazard ratio (95% CI) (Model 1)	1.78 (1.06–2.98)	1.00 (reference)	1.59 (0.94–2.71)	3.11 (1.92–5.04)	3.30 (2.05–5.31)
Adjusted hazard ratio (95% CI) (Model 2)	1.81 (1.08–3.04)	1.00 (reference)	1.62 (0.95–2.76)	3.27 (2.01–5.31)	3.37 (2.09–5.43)
Adjusted hazard ratio (95% CI) (Model 3)	1.90 (1.13–3.19)	1.00 (reference)	1.50 (0.88–2.56)	2.82 (1.73–4.61)	2.72 (1.67–4.42)
Adjusted hazard ratio (95% CI) (Model 4)	1.62 (0.96–2.72)	1.00 (reference)	1.18 (0.69–2.01)	2.10 (1.28–3.46)	2.03 (1.24–3.33)

Model 1, adjusted for age and sex; Model 2, adjusted for age, sex, family history of diabetes, smoking, alcohol drinking and habitual exercise; Model 3, adjusted for variables used in Model 2 and presence of hypertension and hyperlipidaemia at baseline; Model 4, adjusted for variables used in Model 3 and fasting plasma glucose level.
CI, confidence interval; HbA_{1c}, glycated haemoglobin; HOMA-B, homeostasis model assessment of pancreatic B-cell function; HOMA-IR, homeostasis model assessment of insulin resistance.
*Values are geometric means (interquartile range).
†High blood pressure and dyslipidaemia were defined by Japanese criteria of metabolic syndrome.

prevalence of high blood pressure and dyslipidaemia (P for trend < 0.001 for all). There was no significant difference in prevalence of family history of diabetes among the quintiles of waist circumference.

The crude incident rates (per 1000 person-years) across the sex-specific quintiles of waist circumference at baseline were 6.3, 4.0, 6.0, 11.1 and 12.8, respectively [Table 2]. The association between waist circumference and the incidence of diabetes was J-shaped. The age- and sex-adjusted HRs (Model 1) across the quintiles of waist circumference were 1.78, 1.00 (reference), 1.59, 3.11 and 3.30, respectively, and the HRs of the lowest, the fourth and the highest quintile of waist circumference were significantly higher than that of the second quintile. Further adjustment for family history of diabetes, alcohol intake, smoking and physical activity (Model 2) and the presence of high blood pressure and dyslipidaemia at baseline (Model 3) did not change the HRs. The association became slightly weaker after an additional adjustment for fasting plasma glucose at the baseline examination (Model 4). The results were similar for the association between baseline BMI and the incidence of diabetes [Table 3]. The age- and sex-adjusted HRs across the quintiles of BMI were 1.40, 1.00 (reference), 1.21, 1.97 and 3.06, but the association was somewhat weaker than that for waist circumference. The HR for the lowest quintile was not significantly higher than that for the second quintile. Additional adjustments for potential confounders did not substantially change the HRs (Models 2–4). The results were similar when we excluded the 21 participants who developed diabetes within 1 year of follow-up.

We compared the differences in baseline insulin resistance and pancreatic B-cell function between the participants who developed diabetes and those who did not and examined their association with obesity [Table 4]. Among participants in the lowest and the second waist circumference quintile, HOMA-B was significantly lower in those who developed diabetes than in those who did not; however, there were no differences in HOMA-IR between these two groups. In contrast, among participants in the fourth and the highest quintile of waist circumference, HOMA-IR was significantly higher in those who developed diabetes than in those who did not and no significant difference was observed in HOMA-B between these groups. These relationships were somewhat weaker for BMI.

Discussion

In this prospective cohort study of Japanese men and women, there was a J-shaped association between abdominal obesity and the incidence of Type 2 diabetes. The risk of the lowest quintile

Table 3 Age- and sex-adjusted and multivariate-adjusted hazard ratios for incidence of Type 2 diabetes according to sex-specific quintile of body mass index

Parameter	Body mass index quintile				
	Q1	Q2	Q3	Q4	Q5
Range of body mass index, men (kg/m^2)	15.8–20.9	21.0–22.4	22.5–23.8	23.9–25.4	25.5–33.9
Range of body mass index, women (kg/m^2)	15.2–19.9	20.0–21.4	21.5–22.8	22.9–24.9	25.0–41.3
Participants (*n*)	807	813	790	799	783
Age (years)	43.5 ± 5.6	44.1 ± 5.9	44.7 ± 5.8	44.8 ± 5.8	44.9 ± 5.9
Fasting plasma glucose (mmol/l)	4.9 ± 0.50	4.9 ± 0.47	5.0 ± 0.49	5.0 ± 0.48	5.1 ± 0.49
HbA_{1c} (%)	5.0 ± 0.4	5.0 ± 0.4	5.0 ± 0.4	5.0 ± 0.4	5.1 ± 0.4
Fasting insulin (μU/ml)	4.0 ± 3.0	4.7 ± 3.7	5.5 ± 4.0	6.1 ± 4.9	7.7 ± 4.7
HOMA-IR*	0.75 (0.54–1.06)	0.88 (0.63–1.20)	1.04 (0.74–1.48)	1.16 (0.82–1.61)	1.51 (1.08–2.11)
HOMA-B*	53.1 (37.2–72.0)	59.3 (41.5–83.1)	68.1 (49.1–94.7)	72.2 (51.4–98.5)	89.2 (65.5–120.0)
Family history of diabetes (%)	11.6	12.1	10.6	10.5	14.8
Prevalence of high blood pressure† (%)	22.4	23.5	28.4	32.7	41.0
Prevalence of dyslipidaemia† (%)	9.4	15.1	19.0	26.3	37.4
Total person-years	5781	5836	5492	5518	5234
Incident cases (*n*)	36	27	31	50	74
Rate per 1000 person-years	6.2	4.6	5.6	9.1	14.1
Adjusted hazard ratio (95% CI) (Model 1)	1.40 (0.85–2.30)	1.00 (reference)	1.21 (0.72–2.03)	1.97 (1.23–3.14)	3.06 (1.97–4.75)
Adjusted hazard ratio (95% CI) (Model 2)	1.36 (0.82–2.24)	1.00 (reference)	1.23 (0.74–2.07)	2.02 (1.26–3.23)	3.00 (1.93–4.67)
Adjusted hazard ratio (95% CI) (Model 3)	1.42 (0.86–2.35)	1.00 (reference)	1.18 (0.70–1.98)	1.78 (1.11–2.85)	2.46 (1.57–3.86)
Adjusted hazard ratio (95% CI) (Model 4)	1.27 (0.77–2.10)	1.00 (reference)	1.03 (0.61–1.73)	1.59 (0.99–2.56)	2.06 (1.31–3.24)

Model 1, adjusted for age and sex; Model 2, adjusted for age, sex, family history of diabetes, smoking, alcohol drinking and habitual exercise; Model 3, adjusted for variables used in Model 2 and presence of hypertension and dyslipidaemia at baseline; Model 4, adjusted for variables used in Model 3 and fasting plasma glucose level.
CI, confidence interval; HbA_{1c}, glycated haemoglobin; HOMA-B, homeostasis model assessment of pancreatic B-cell function; HOMA-IR, homeostasis model assessment of insulin resistance.
*Values are geometric means (interquartile range).
†High blood pressure and dyslipidaemia were defined by Japanese criteria of metabolic syndrome.

Table 4 Difference in baseline HOMA-IR and HOMA-B between subjects who developed Type 2 diabetes and those who did not, across the sex-specific quintile of baseline waist circumference or body mass index

	HOMA-IR			HOMA-B		
	No diabetes	Incident diabetes	*P**	No diabetes	Incident diabetes	*P**
Waist circumference quintile						
Q1 (lowest)	0.75 (0.54–1.05)	0.79 (0.48–1.08)	0.561	54.8 (38.6–75.8)	31.7 (23.2–40.0)	< 0.001
Q2	0.88 (0.62–1.24)	1.03 (0.76–1.35)	0.158	61.1 (42.4–85.0)	36.2 (25.7–51.4)	< 0.001
Q3	1.03 (0.71–1.45)	1.23 (0.82–1.78)	0.041	67.4 (48.2–90.0)	54.0 (34.1–84.0)	0.053
Q4	1.20 (0.86–1.64)	1.59 (1.01–2.28)	0.003	76.4 (55.4–108.0)	60.1 (32.7–99.4)	0.025
Q5 (highest)	1.45 (1.30–2.02)	1.89 (1.34–2.57)	< 0.001	88.9 (65.5–120.0)	78.1 (50.1–106.5)	0.070
Body mass index quintile						
Q1 (lowest)	0.75 (0.54–1.04)	0.83 (0.50–1.15)	0.268	54.3 (37.9–74.6)	33.1 (24.1–45.0)	< 0.001
Q2	0.88 (0.63–1.20)	0.99 (0.71–1.33)	0.226	60.4 (42.4–83.1)	34.8 (23.2–51.4)	< 0.001
Q3	1.04 (0.73–1.48)	1.15 (0.78–1.54)	0.322	68.9 (49.7–94.7)	50.0 (30.0–74.5)	0.001
Q4	1.15 (0.81–1.61)	1.31 (0.85–1.80)	0.111	73.3 (53.3–99.8)	56.9 (35.0–85.5)	0.038
Q5 (highest)	1.46 (1.04–2.04)	2.12 (1.54–2.95)	< 0.001	90.2 (65.7–120.0)	80.0 (53.7–111.7)	0.087

Values are geometric means (interquartile range).
HOMA-B, homeostasis model assessment of pancreatic B-cell function; HOMA-IR, homeostasis model assessment of insulin resistance.
*Student's *t*-test was used to compare geometric means.

of waist circumference was approximately 80% higher than that for the second quintile, indicating that very lean Japanese individuals are also at high risk for developing diabetes. Among the lean participants, HOMA-B was lower in those who developed diabetes than in those who did not, but there was no difference in HOMA-IR between these two groups. These results suggest that lower B-cell function increases the future risk for developing Type 2 diabetes in lean Japanese people with a very low waist circumference, whereas insulin resistance increases the risk in abdominally obese Japanese individuals.

Previous studies have shown that waist circumference is associated with increased risk for diabetes, independently of BMI, and that waist circumference is a better predictor for diabetes than BMI [1,2,6]. Waist circumference is regarded as a more useful marker for insulin resistance and metabolic abnormalities, because it is more closely associated with visceral adiposity, compared with BMI [24]. Our results show that, in obese people, waist circumference was more strongly associated with the future risk for Type 2 diabetes, compared with BMI, and that waist circumference could effectively predict the higher diabetic risk of obese people.

In contrast, previous studies using populations from Western countries have shown that the association between waist circumference and the incidence of Type 2 diabetes was linear, not J-shaped [1–5]. This discrepancy might have resulted from a difference in the degree of obesity between Western and Asian populations. In our study, the upper limit of waist circumference in the lowest quintile was 73 cm for men and 65 cm for women, which was lower than that in previous studies [1–5]. These previous studies might have been unable to detect a higher risk for developing diabetes in people with very low waist circumference.

Racial differences in the association between obesity and the risk for Type 2 diabetes might also have influenced the results. Although the prevalence of obesity is much lower in Asia than in Western countries, the prevalence of Type 2 diabetes is similar between the two regions [8] and Type 2 diabetes occurs in Asians who are less obese [25,26]. In this study, the range of waist circumference in the fourth quintile was 82.5–86.0 cm for men and 74.0–80.0 cm for women; these values were somewhat lower than the cut-off points of waist circumference proposed by the Japan Medical Association (85 cm for men and 90 cm for women) [23] and also by the International Diabetes Federation (90 cm for men and 80 cm for women) [27]. However, multivariate-adjusted hazard ratios for diabetes in the fourth quintile were significantly higher than those in the second quintile. Thus, participants with high–normal waist circumference would also be at high risk for diabetes even although they were not classified as having abdominal obesity. The definition of abdominal obesity in Asians should be carefully considered from the standpoint not only of identifying the people with cardiovascular disease risk factors as proposed by several previous reports [28–32], but also to detect people at higher risk of future diabetes. It has also been reported that the incidence of Type 2 diabetes was significantly higher in Asian women than in non-Hispanic white women after adjustment for BMI [33]. Some factors beyond obesity, including genetics, may also cause the higher risk for Type 2 diabetes seen in Asian populations.

More Asians have prominent abdominal obesity, compared with those in Western countries with a similar BMI [9,10], indicating that Asians may have a higher predisposition to insulin resistance and thus may be at higher risk for developing diabetes at a lower BMI, compared with people of European descent. Furthermore, not only insulin resistance but also impaired pancreatic B-cell function has been reported to play an important role in the development of Type 2 diabetes in Asians [14,34]. We have shown that lower B-cell function may increase the risk for developing diabetes in lean Japanese individuals. Lower B-cell function may cause hyperglycaemia or marked insulin resistance in the absence of abdominal obesity in these very lean participants.

A study conducted in a relatively lean Taiwanese population found that waist circumference for men and BMI for women were strongly associated with the incidence of diabetes [11]; however, the shape of the relationship could not be determined because the data were analysed using linear logistic regression analysis. Recently, a J-curve association between BMI and the incidence of diabetes was reported in Japanese men and women, aged 60–79 years [16]. It was concluded that aging was a high risk factor for developing diabetes, because it is associated with a decline in B-cell function [35,36]; however, our results suggest that younger and leaner individuals with decreased B-cell function may also be at increased risk for developing diabetes mellitus.

The strength of our study lies in its relatively large sample size compared with those of other Asian studies. Many previous cohort studies used information collected from self-administered questionnaires, whereas our conclusions are based on more reliable data obtained from medical examinations and from determinations of fasting blood glucose and insulin levels, HOMA-IR and HOMA-B. However, our study sample included only people who were employed. As poor health may exclude some individuals from working, the prevalence of obesity and the incidence of diabetes may be lower in our sample population than in the general Japanese population. Another limitation of this study was that the classification of diabetes was not precisely determined in all participants with diabetes. Some lean people with diabetes may not have Type 2 diabetes, but Type 1 diabetes or secondary diabetes. However, the participants with incident diabetes in this study were diagnosed in annual medical check-ups with relatively mild hyperglycaemia (mean HbA_{1c} at diagnosis was 5.9% and there was no difference between the quintiles of waist circumference). Furthermore, the results were similar when we determined the risk of diabetes in participants, excluding those who developed diabetes within 1 year of follow-up, who could have other diseases which may influence anthropometric variables and glucose tolerance. Therefore, most of participants who developed diabetes in this study would have Type 2 diabetes.

In conclusion, although the absolute incident risk of diabetes is higher in obese people, leaner Japanese individuals with a smaller waist circumference would also be at high risk for developing Type 2 diabetes. Moreover, lower B-cell function, but not insulin resistance, may increase the future risk of Type 2 diabetes. Greater attention should be given to very lean Asians, in addition to obese Asians, for the primary prevention of Type 2 diabetes.

Competing interests

Nothing to declare.

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INTERNAL MEDICINE

□ EDITORIAL □

Abnormal Liver Function Tests and Metabolic Syndrome—Is Fatty Liver Related to Risks for Atherosclerosis beyond Obesity?

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Key words: fatty liver, metabolic syndrome

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Oda and colleagues (1) reported that elevated liver enzymes, such as alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT), are related to metabolic syndrome (MetS) in Japanese men and women. One of the main causes of abnormal liver function is fatty liver disease. Recently, it was reported that non-alcoholic fatty liver disease (NAFLD) is related to cardiovascular disease (CVD) (2-5) and to most cardiovascular risk factors, including diabetes, hypertension, hyperlipidemia, and MetS (2, 5-7). Some of the suggested biological mechanisms linking NAFLD and accelerated atherosclerosis are insulin resistance, oxidative stress, inflammation, adiponectin and other adipocytokines, and abnormal lipoprotein metabolism (8, 9).

Insulin resistance and visceral adipose tissue are the two major risk factors underlying MetS, and play a pivotal role in the development of NAFLD and atherosclerosis. These confounding factors should be considered when evaluating the relationships among NAFLD, MetS, and atherosclerosis. Some prospective studies showed that increases in liver function parameters, such as GGT (2, 3) and ALT (4), are associated with the incidence of CVD events, even after adjusting for body mass index (BMI) and other components of MetS. In contrast, in an 11-year follow-up of Australians, Adams et al (6) showed that the presence of NAFLD did not increase the risk of MetS after adjusting for baseline waist circumference and other components of MetS. McKimmie et al (10) evaluated the association between hepatic steatosis and carotid atherosclerosis in the Diabetic Heart Study, and suggested that hepatic steatosis is unlikely a direct mediator of CVD. In other epidemiological studies that evaluated the association between NAFLD and MetS or CVD, the role of obesity was not evaluated fully, although most of those with NAFLD or elevated liver enzymes tended to have higher BMIs or waist circumferences (1, 5, 7). There is insufficient epidemiological evidence in clinical practice to determine whether NAFLD is related to MetS and CVD directly, beyond obesity and insulin resistance.

In Western countries, the prevalence of NAFLD is between 24 and 42% (11, 12), and NAFLD is widely reported to be the most common chronic liver condition. In Asian countries, NAFLD is assumed to be less common. However, the reported prevalence of NAFLD in Asian populations ranges from 5-40% and the increase in NAFLD is also an important problem in Asia (13). Recently, a prospective study in China showed that NAFLD was closely associated with the onset of metabolic disorders, even among non-obese subjects (14). Subsequently, data from lean NAFLD patients in Asia would provide insight into whether NAFLD increases the risk of MetS or CVD beyond obesity.

Patients with NAFLD are at higher risk for MetS and CVD, and thus should be monitored intensively to reduce the risk factors for atherosclerosis. However, further epidemiological studies are needed to evaluate whether the presence of NAFLD or elevated liver enzymes should be dealt with as a component of MetS, as is the case with other classical CVD risk factors.

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Middle-aged Japanese women are resistant to obesity-related metabolic abnormalities

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Abstract

We attempted to determine sex differences in obesity-related metabolic abnormalities in a relatively large middle-aged Japanese population. The study population consisted of 2935 men and 1622 women who were 35 to 59 years old. Metabolic abnormalities were determined using the Japanese criteria for metabolic syndrome, and we evaluated the number of metabolic abnormalities discriminated by waist circumference. In men, the mean number of metabolic abnormalities increased as the waist circumference increased. In women, although the mean number of metabolic abnormalities increased as the waist circumference increased, the mean number was less than 1 even in those with a waist circumference of at least 95 cm. According to the receiver operating characteristic curve, the cutoff levels yielding the maximal sensitivity plus specificity for predicting the prevalence of one or more obesity-related metabolic abnormalities were 80 cm in men and 73 cm in women. However, the positive predictive value was as low as 28.8% in men and 7.1% in women, which may not be suitable for a screening test, especially in women. Middle-aged Japanese women seem to be resistant to obesity-induced metabolic abnormalities, and waist circumference would not effectively predict the existence of metabolic syndrome. In setting the cutoff points in guidelines, a greater emphasis should be placed on the absolute risk of having abnormalities or diseases.

1. Introduction

Cutoff points for waist circumference have been proposed for each racial/ethnic group in diagnosing abdominal obesity and metabolic syndrome [1]. However, the Japanese cutoff points for waist circumference are still controversial. The Japanese cutoff points proposed by the Japan Society of Study for Obesity were derived from a 100-cm^2 area for visceral fat at the umbilical level on computed tomography imaging and are unique in that the value for women (90 cm) is larger than that for men (85 cm) [2,3]. On the other hand, the International Diabetes Federation proposes to use the cutoff point of waist circumference of 90 cm for men and 80 cm for women [1]; and some reports proposed lowering the cutoff point of waist circumference for Japanese women [4,5]. However, previous reports did not pay attention to the absolute risk of the accumulation of metabolic abnormalities in Japanese women. We attempted to determine sex differences in the accumulation of metabolic abnormalities in relation to obesity in a relatively large-scale middle-aged Japanese population.

2. Research design and methods

The study population consisted of 4557 Japanese employees (2935 men and 1622 women) of a metal products factory who were aged 35 to 59 years. Detailed information on this study population has been provided elsewhere [6,7]. The physical examinations for this analysis were held in 1996. Anthropometric markers including waist circumference and blood pressure were measured, and venous blood samples after an overnight fast were withdrawn from each subject during a routine annual medical checkup. Three obesity-related abnormalities—high blood

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pressure, dyslipidemia, and high fasting plasma glucose—were defined by the Japanese guidelines of metabolic syndrome [3]. *High blood pressure* was defined as a systolic blood pressure of at least 130 mm Hg or a diastolic blood pressure of at least 85 mm Hg. *Dyslipidemia* was defined as serum triglycerides of at least 150 mg/dL or high-density lipoprotein cholesterol not exceeding 40 mg/dL, and *high fasting plasma glucose* was defined as a fasting plasma glucose of at least 110 mg/dL. We evaluated the number of obesity-related abnormalities discriminated by waist circumference and body mass index (BMI). We plotted receiver operating characteristic (ROC) curves for waist circumference to predict one or more obesity-related metabolic abnormalities and calculated sensitivity, specificity, and positive predictive value.

3. Results

The participants had a mean age of 45.5 years for men and 45.3 years for women, a mean BMI of 23.3 kg/m^2 for men and 22.6 kg/m^2 for women, and a mean waist circumference of 80.1 cm for men and 72.7 cm for women.

We evaluated the association between waist circumference or BMI and the mean number of obesity-related abnormalities (Fig. 1). In men, the mean number of abnormalities increased as waist circumference or BMI increased. When the waist circumference was 85 cm, which is the cutoff point for the diagnosis of metabolic syndrome in Japanese men [3], the mean number of complicated metabolic abnormalities was approximately 1. In women, although the mean number of abnormalities increased as the waist circumference increased, it was less than 1 even in women with a waist circumference of at least 95 cm or BMI of at least 30 kg/m^2.

The prevalence of one or more obesity-related abnormalities was 50% in men and 21% in women. According to the ROC curve, the area under the curve was higher for men (0.675; 95% confidence interval, 0.655-0.694) than for women (0.627; 95% confidence interval, 0.596-0.657) (Fig. 2). The cutoff levels yielding the maximal sensitivity plus specificity for predicting the prevalence of one or more obesity-related abnormalities were 80 cm in men (sensitivity, 0.59; specificity, 0.69) and 73 cm in women (sensitivity, 0.55; specificity, 0.64). However, the positive predictive value was as low as 28.8% in men and 7.1% in women at these cutoff points.

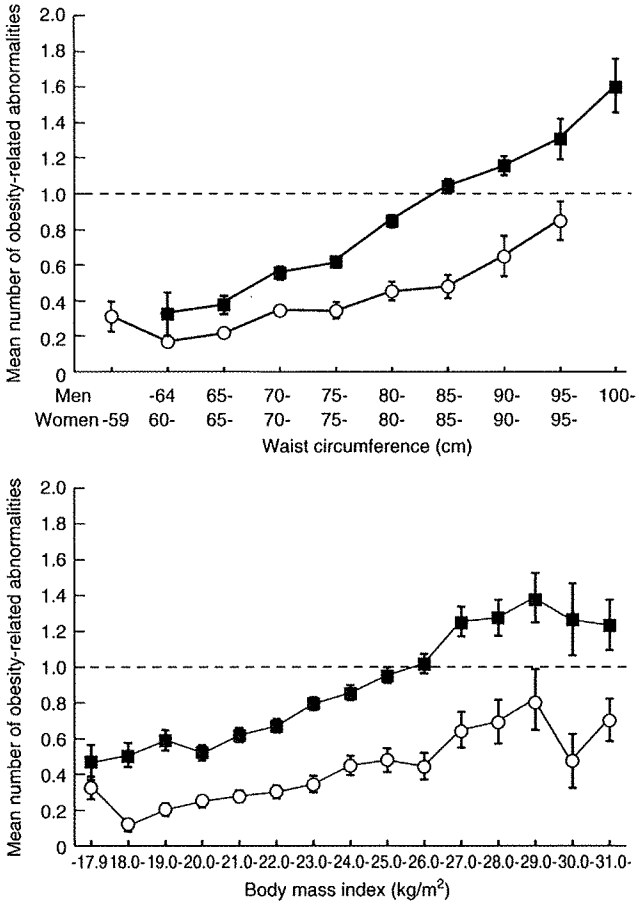
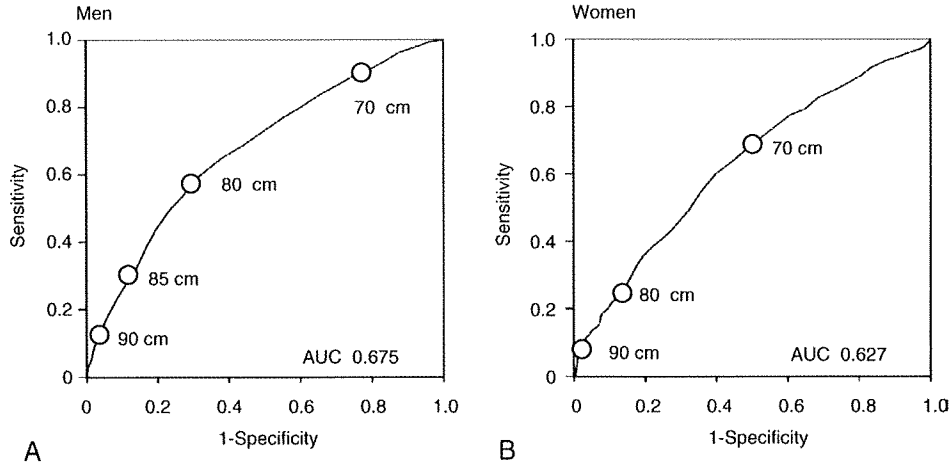


Fig. 1. The mean number of obesity-related abnormalities discriminated by waist circumference or BMI in men (■) and women (○). Obesity-related abnormalities included hypertension, dyslipidemia, and glucose intolerance. The horizontal dotted line shows a mean number of obesity-related abnormalities of 1.0. Data are presented as means ± standard error.

4. Discussion

Hara et al [4] and Ohkubo et al [5] proposed a cutoff point regarding waist circumference for detecting metabolic syndrome or insulin resistance in Japanese based on relatively small, older, and somewhat higher-risk populations. Both reports proposed lowering the cutoff point of waist circumference for Japanese women to 76 cm. We found that a similar cutoff point of 73 cm for women provided the highest sensitivity and specificity for detecting metabolic syndrome as was recently observed [4,5]. Theoretically, sensitivity and specificity (therefore, the ROC curves) are not affected by the prevalence of the detected disease in various populations. However, the positive predictive value was as low as 7.1% in our population of middle-aged Japanese women, which means that only 7.1% of women with waist circumference of 73 cm or higher had the accumulation of metabolic abnormalities. Therefore, this cutoff point may not be suitable for a screening test in women. Two previous reports from Japan did not give the positive predictive values for their cutoff points. The low positive predictive value was caused by the low prevalence of metabolic abnormalities in middle-aged Japanese women and, possibly, the relatively higher resistance of Japanese women than men to obesity-induced metabolic abnormalities. As proposed in previous reports [4,5], lower cutoff points for



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Cutoff point for waist circumference

	Sensitivity	Specificity
Men		
70 cm	0.94	0.16
80 cm	0.59	0.69
85 cm	0.32	0.86
90 cm	0.13	0.96
Women		
70 cm	0.67	0.52
73 cm	0.55	0.64
80 cm	0.27	0.85
90 cm	0.09	0.98

Fig. 2. The predictive performance of waist circumference for one or more obesity-related metabolic abnormalities. The ROC curves for one or more obesity-related metabolic abnormalities in men (A) and in women (B) are shown. The sensitivity and specificity of each cutoff point of waist circumference are given (C).

waist circumference might detect more people with metabolic abnormalities with high sensitivity. However, in a population with a low prevalence of metabolic abnormalities, lower cutoff points would also result in a greater proportion of false positives, with more healthy women in particular being screened as abnormal. Furthermore, because the mean number of complicated metabolic abnormalities was lower than 1 even in those with a waist circumference of at least 95 cm and the area under the ROC curve was lower in women than in men, waist circumference would not effectively predict the existence of metabolic syndrome in Japanese women.

In this study, similar to previous reports [2,4,5,8], cutoff points of waist circumference were proposed using ROC curves for predicting the metabolic abnormalities. However, abdominal obesity is important in metabolic syndrome because it has been linked to the development of cardiovascular disease. Further investigations are needed to evaluate the association between waist circumference and future incidence of cardiovascular events to establish an appropriate cutoff point for waist circumference. Another limitation was that the participants of this study did not include older people and might be healthier than general Japanese people because they were identified at a work place. Similar analyses are needed in older Asian populations.

In conclusion, middle-aged Japanese women seem to be resistant to obesity-related metabolic abnormalities; and waist circumference would not effectively predict the existence of metabolic syndrome. In setting cutoff points in guidelines, a greater emphasis should be placed on the absolute risk of having abnormalities or diseases.

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Circulating Matrix Metalloproteinase-2 Is an Independent Correlate of Proteinuria in Patients with Chronic Kidney Disease

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Key Words
Cardiovascular · Chronic kidney disease · Collagen · Proteinuria · Kidney disease · Diabetic nephropathy

Abstract
Background/Aim: Matrix metalloproteinase-2 (MMP-2) has been implicated in chronic kidney disease (CKD) and cardiovascular disease. However, there is no knowledge about the correlations between serum levels of MMP-2, proteinuria and atherosclerosis in patients with CKD. We investigated whether serum MMP-2 levels were associated with proteinuria, intima media thickness (IMT), and the presence of carotid atherosclerotic plaque in CKD patients. ***Methods:*** CKD patients without hemodialysis (n = 99) were enrolled. MMP-2 levels were measured by an ELISA system. IMT and carotid atherosclerotic plaque were evaluated by a high-resolution ultrasonography. ***Results:*** Multivariate analyses revealed that low-density lipoprotein ($p < 0.001$), MMP-2 ($p = 0.001$) and systolic blood pressure ($p = 0.011$) were independent correlates of proteinuria. Age- and serum creatinine-adjusted MMP-2 levels were significantly increased ($p = 0.001$) in proportion to the increasing levels of proteinuria. Further, age ($p < 0.001$), systolic blood pressure ($p = 0.015$) and MMP-2 levels ($p = 0.042$) were independent correlates of IMT. MMP-2 levels were significantly ($p < 0.01$) higher in patients with atherosclerotic plaque than those without it. ***Conclusions:*** The present study demonstrated that serum levels of MMP-2 were one of the independent correlates of proteinuria and IMT in patients with CKD. Our results suggest that serum MMP-2 levels may be one of the risk factors for renal damage and atherosclerosis in CKD patients.

Introduction

Chronic kidney disease (CKD) is recognized by the presence of persistent proteinuria and/or renal dysfunction [1]. Recently, several papers have reported that proteinuria is not just a marker of CKD, but that it could cause renal damage in patients with CKD [2, 3]. Further, there is a growing body of evidence demonstrating that proteinuria is an independent risk factor for cardiovascular disease (CVD) as well [4]. These observations suggest the link of proteinuria to CKD and CVD. However, the underlying mechanisms by which proteinuria could cause renal and vascular damage in patients with CKD are not fully understood.

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Matrix metalloproteinase-2 (MMP-2) is one of the metalloproteinases which can degrade extracellular matrix (ECM) proteins in the kidney and vessels, including fibronectin, laminin and collagens [5]. There is accumulating evidence that MMP-2 is involved in the development and progression of CKD and CVD by eliciting tissue remodeling via structural alterations in the glomerular and tubular areas as well as blood vessels [5–8]. Further, the plasma MMP-2 level is shown to be positively correlated with serum creatinine (Cr) levels in patients with CKD [9, 10]. In addition, Pawlak et al. [11] have demonstrated that plasma MMP-2 but not MMP-9 significantly increased in hemodialysis patients and correlated with intima media thickness (IMT) levels. Since the circulating level of MMP-2 is higher in patients with acute coronary syndromes than in those with stable angina, it could be a biomarker for plaque rupture and/or instability in atherosclerosis [12]. These findings led us to speculate that an elevated circulating level of MMP-2 could link proteinuria to renal and vascular damage in patients with CKD. However, as far as we know, there are no data about the correlations between the serum level of MMP-2, proteinuria and carotid atherosclerosis in patients with CKD. Therefore, we first investigated whether the serum MMP-2 level was associated with proteinuria in patients with CKD. Then, we explored the association between serum MMP-2 levels and surrogate markers of CVD such as IMT and the presence of atherosclerotic plaque in the carotid arteries of our subjects.

Patients and Methods

The study involved CKD patients without hemodialysis in Kurume University Hospital (58.3 ± 17.9 years old, 49 males and 50 females): diabetic nephropathy (n = 20); chronic glomerulonephritis (CGN, n = 43); rapid progressive glomerulonephritis (RPGN, n = 11); lupus nephritis (n = 4); hypertensive nephrosclerosis (n = 3); amyloidosis (n = 1); polycystic kidney disease (PCKD, n = 1); interstitial nephritis (n = 1), and unknown etiology (n = 15). The diagnosis of CKD and its staging were determined according to the National Kidney Foundation Disease Outcome Quality Initiative [13]. Eight patients had angiographically documented CVD and a history of coronary vascular events. The remainder had no history of angina, cardiovascular events or other heart disease. We excluded patients with active inflammatory disease and cancers. Age- and sex-matched healthy volunteers (55.9 ± 5.52 years old, 16 males and 14 females) were also enrolled for the measurement of serum MMP-2 levels as controls.

Data Collection

The medical history was ascertained by a questionnaire. Height and weight were measured, and BMI (kilograms per meter squared) was calculated as an index of the presence or absence of obesity. Blood pressure (BP) was measured in the sitting position (first) and supine position (second) at 3-min intervals using an upright standard sphygmomanometer. Vigorous physical activity and smoking were avoided for at least 30 min before BP measurement.

Fasting blood was drawn from the antecubital vein for determinants of lipids (total cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, and triglycerides), hemoglobin, blood urea nitrogen (BUN), creatinine (Cr) and uric acid. Spot urine was collected for determination of proteinuria (g/g Cr). HDL-cholesterol and LDL-cholesterol were measured using a commercially available laboratory method (Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan). The other chemistries were measured using a commercially available laboratory method (Wako Pure Chemical Industries Ltd., Osaka, Japan). The serum level of MMP-2 was determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (GE Healthcare UK Ltd., Buckinghamshire, UK). Informed consent was obtained from all patients, and the study protocol was also approved by the Institutional Ethics Committee of Kurume University School of Medicine.

Estimated glomerular filtration rate (eGFR) was calculated according to the following formula: eGFR = $0.741 \times 175 \times \text{serum Cr}^{-1.154} \times \text{age}^{-0.203} \times 0.742$ (if female) [14, 15]. IMT and atherosclerotic plaque in the carotid artery were evaluated by a high-resolution ultrasonography with a 7.5-MHz linear probe. Briefly, longitudinal B-mode images at the diastolic phase of the cardiac cycle were recorded by a single trained technician who was blind as to the subject's background. Measurements of IMT were made by the same technician using fine slide calipers at 3 levels of the lateral and medial walls 1–3 cm proximal to the carotid bifurcation as reported previously [16]. The mean of these 6 measurements was taken as the value of the IMT.

Statistical Analysis

Results are presented as mean ± SD. The medications for hypertension (renin-angiotensin system (RAS) inhibitors and calcium channel blockers) and the presence or absence of diabetes were coded as dummy variables. Univariate analysis was performed for determinants of proteinuria. To determine independent determinants of proteinuria or IMT, multiple linear regression analysis was performed. Mean MMP-2 levels, stratified by the severity of proteinuria, were compared using analysis of covariance adjusted for age and Cr. Statistical significance was defined as $p < 0.05$. All statistical analyses were performed with the use of the SPSS system.

Results

Demographic Data

Demographical data of the subjects are presented in table 1. The average serum level of MMP-2 was 1.488 ± 0.504 μg/ml (table 2). Serum MMP-2 levels in CKD patients were significantly ($p < 0.05$) higher than those in age- and sex-matched normal controls (1.276 ± 0.475 μg/ml; table 2). When we compared the values of MMP-2 among different disease entities, MMP-2 levels in pa-

Table 1. Clinical characteristics of the patients

Number of patients	99
Age, years	58.3 ± 17.9
Sex, male/female	49/50
Body mass index	22.9 ± 3.61
Systolic blood pressure, mm Hg	134.1 ± 20.8
Diastolic blood pressure, mm Hg	77.8 ± 12.4
Hemoglobin, g/dl	10.9 ± 2.39
BUN, mg/dl	42.8 ± 28.4
Serum creatinine, mg/dl	3.43 ± 2.82
Uric acid, mg/dl	7.25 ± 2.63
24-Hour creatinine clearance, ml/min	38.5 ± 38.4
eGFR, ml/min	31.9 ± 33.2
Total cholesterol, mg/dl	196.1 ± 54.3
HDL-cholesterol, mg/dl	46.5 ± 17.7
LDL-cholesterol, mg/dl	117.2 ± 47.3
Triglyceride, mg/dl	161.6 ± 92.7
Proteinuria, g/g Cr	4.23 ± 4.93
Diabetes, –/+	69/30
IMT, mm	0.87 ± 0.22
Medication	
RAS inhibitors, –/+	39/60
CCB, –/+	55/44

Values are mean ± SD. eGFR was estimated by a modification of diet in renal disease equation.

BUN = Blood urea nitrogen; eGFR = estimated glomerular filtration rate; HDL = high-density lipoprotein; LDL = low-density lipoprotein; IMT = intima media thickness; RAS = renin-angiotensin system; CCB = calcium channel blockers.

Table 2. Circulating MMP-2 serum levels (mean ± SD)

	MMP-2, μg/ml
CKD patients	
All subjects (n = 99)	1.488 ± 0.504#
CGN (n = 43)	1.403 ± 0.419
Diabetic nephropathy (n = 20)	1.781 ± 0.626*
Others (n = 36)	1.436 ± 0.478
Healthy controls (n = 30)	1.276 ± 0.475

MMP-2 = Matrix metalloproteinase-2; CKD = chronic kidney disease; CGN = chronic glomerulonephritis.

# $p < 0.05$ vs. healthy controls; * $p < 0.001$ vs. CGN.

tients with diabetic nephropathy were significantly higher than those in patients with CGN (1.781 ± 0.626 vs. 1.403 ± 0.419 μg/ml, $p < 0.001$).

Systolic and diastolic BPs were 134.1 ± 20.8 and 77.8 ± 12.4 mm Hg, respectively. Average levels of BUN and serum Cr were 42.8 ± 28.4 and 3.43 ± 2.82 mg/dl, respectively. The mean of eGFR was 31.9 ± 33.2 ml/min. Total, HDL- and LDL-cholesterol were 196.1 ± 54.3, 46.5 ± 17.7 and 117.2 ± 47.3 mg/dl, respectively. Average levels of proteinuria were 4.23 ± 4.93 g/g Cr. Mean IMT was 0.87 ± 0.22 mm. Twenty patients had obvious atherosclerotic plaque in carotid artery. Thirty subjects had already been diagnosed as having diabetes mellitus.

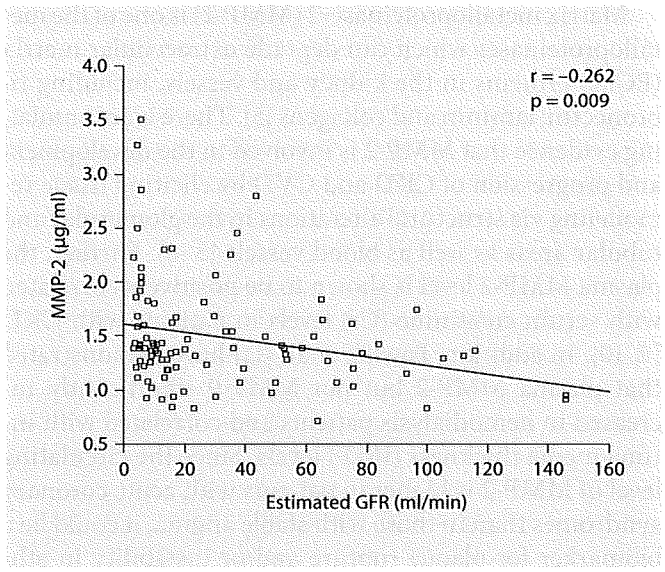

Fig. 1. Linear regression line of serum MMP-2 levels and estimated GFR in patients with CKD (n = 97).

Correlation between Renal Function and Serum MMP-2 Levels

Univariate analysis showed that serum Cr ($p = 0.001$) and eGFR ($p = 0.009$) were significantly correlated with serum MMP-2 levels in CKD patients (fig. 1), whose findings were consistent with previous observations [9].

Correlates of Proteinuria

Univariate analysis revealed that systolic BP ($p = 0.004$), total cholesterol ($p < 0.001$), LDL-cholesterol ($p < 0.001$), MMP-2 ($p < 0.001$; fig. 2) and the presence of diabetes ($p = 0.004$) significantly correlated with proteinuria (table 3). For these significant factors, we performed a stepwise multiple regression analysis. LDL-cholesterol ($p < 0.001$), MMP-2 ($p = 0.009$) and systolic BP ($p = 0.011$) remained significant and were independently related to proteinuria ($r^2 = 0.418$; table 4). Further, mean MMP-2 levels stratified by the severity of proteinuria were compared using analysis of covariance adjusted for age and

Table 3. Univariate regression analysis for the correlates of proteinuria

Variables	β	SE	p
Age	0.064	0.028	0.526
Sex	−0.074	0.993	0.466
Systolic BP	0.284	0.023	0.004
Serum creatinine	0.021	0.177	0.839
eGFR	0.045	0.015	0.659
Total cholesterol	0.470	0.008	<0.001
LDL-cholesterol	0.514	0.009	<0.001
MMP-2	0.468	0.885	<0.001
Diabetes	0.285	1.038	0.004
Plaque	0.129	1.49	0.382

β = Standardized regression coefficients. Sex: male = 0, female = 1. Diabetes: no = 0, yes = 1. Plaque: no = 0, yes = 1.

SE = Standard error; BP = blood pressure; eGFR = estimated glomerular filtration rate; LDL = low-density lipoprotein; MMP-2 = matrix metalloproteinase-2.

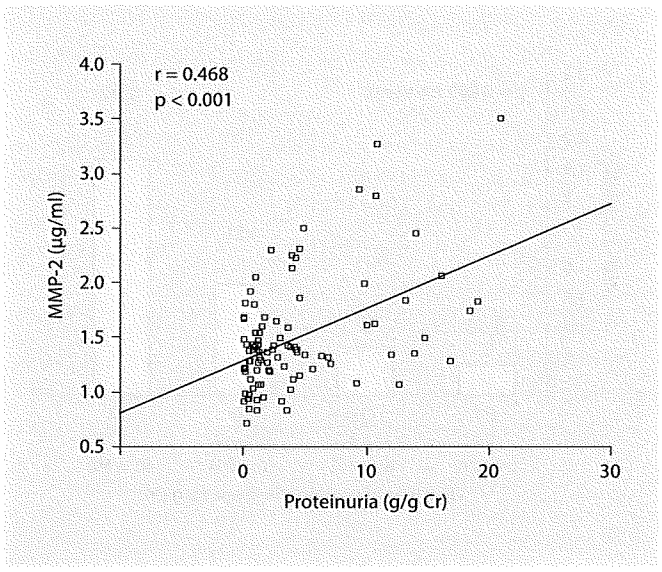

Fig. 2. Linear regression line of serum MMP-2 levels and proteinuria in patients with CKD (n = 97).

Table 4. Multiple stepwise regression analysis for the correlates of proteinuria

Variables	β	SE	p
LDL-cholesterol	0.430	0.009	<0.001
MMP-2	0.296	0.830	0.001
Systolic BP	0.210	0.020	0.011

$r^2 = 0.418$. β = Standardized regression coefficients.

SE = Standard error; LDL = low-density lipoprotein; MMP-2 = matrix metalloproteinase-2; BP = blood pressure.

Cr. A linear and significant trend (p = 0.001) was demonstrated (fig. 3).

Correlates of Atherosclerosis

Univariate analysis revealed that IMT levels were significantly correlated with age (p < 0.001), MMP-2 (p < 0.001; fig. 4) and systolic BP (p = 0.018). Stepwise multiple regression analysis revealed that age (p < 0.001), systolic BP (p = 0.015) and serum MMP-2 (p = 0.042) were independent predictors of atherosclerosis defined by IMT ($r^2 = 0.541$; table 5). Further, serum MMP-2 levels were also significantly higher in patients with atherosclerotic plaque than those without it (fig. 5).

Discussion

The salient findings of the present study are: (1) serum MMP-2 levels are significantly higher in patients with CKD than those in normal healthy subjects; (2) serum MMP-2 levels are one of the independent correlates of proteinuria in CKD patients; (3) mean serum MMP-2 levels are increased in proportion to the severity of proteinuria, and (4) MMP-2 levels are one of the independent predictors of atherosclerosis and are significantly higher in patients with carotid atherosclerotic plaque than in those without it. These observations suggest that MMP-2 is one of the causative risk factors that could link proteinuria to renal and vascular damage in patients with CKD.

The mean serum MMP-2 level in our CKD subjects was 1.488 ± 0.504 μg/ml, which was one order of magnitude higher than those previously reported in CKD patients [9, 17]. Indeed, Chang et al. [9] have shown that the mean plasma level of MMP-2 was 98.43 ± 3.13 ng/ml in CKD patients. We do not know the exact reasons for the different MMP-2 levels in our study and in theirs. However, the difference in ELISA kits used for the measurement of MMP-2 could account mainly for the discrepancies because (1) there was no significant difference in the subject population (age, sex and CKD patients) and ethnicity (Asian) between our study and theirs [9], and (2) serum MMP-2 levels measured by the

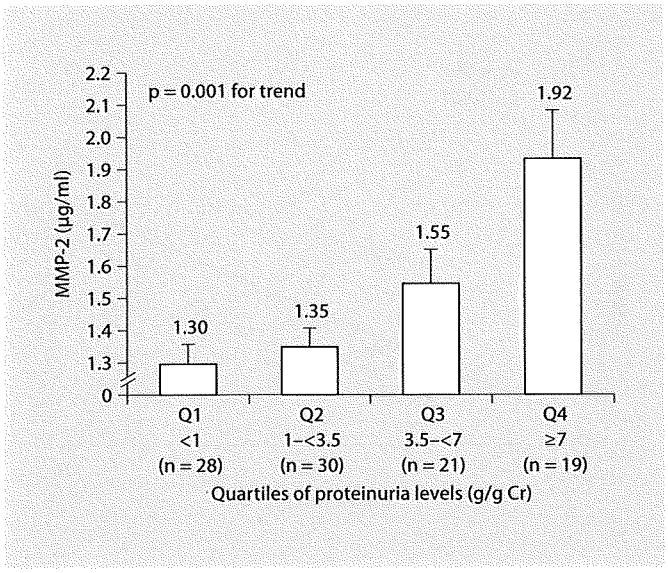

Fig. 3. Age- and serum creatinine-adjusted MMP-2 levels stratified by quartiles of proteinuria.

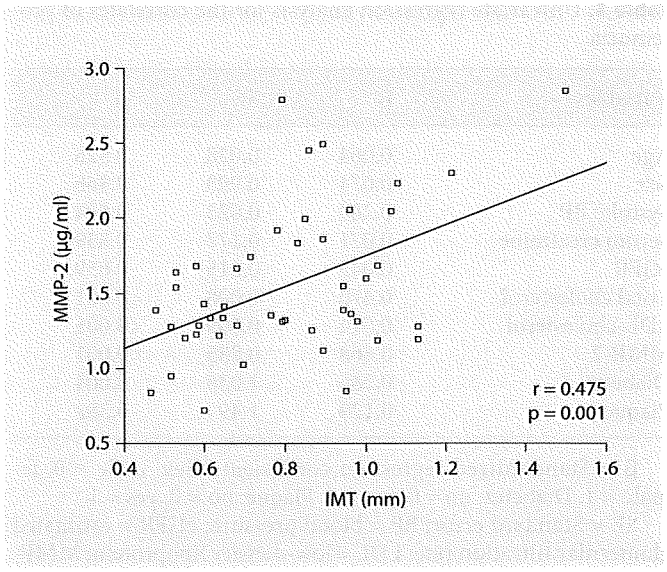

Fig. 4. Linear regression line of serum MMP-2 levels and IMT in patients with CKD (n = 47).

Table 5. Multiple stepwise regression analysis for the correlates of IMT

Variables	β	SE	p
Age	0.526	0.002	<0.001
Systolic BP	0.269	0.001	0.015
MMP-2	0.240	0.052	0.042

$r^2 = 0.541$. β = Standardized regression coefficients.
SE = Standard error; BP = blood pressure; MMP-2 = matrix metalloproteinase-2.

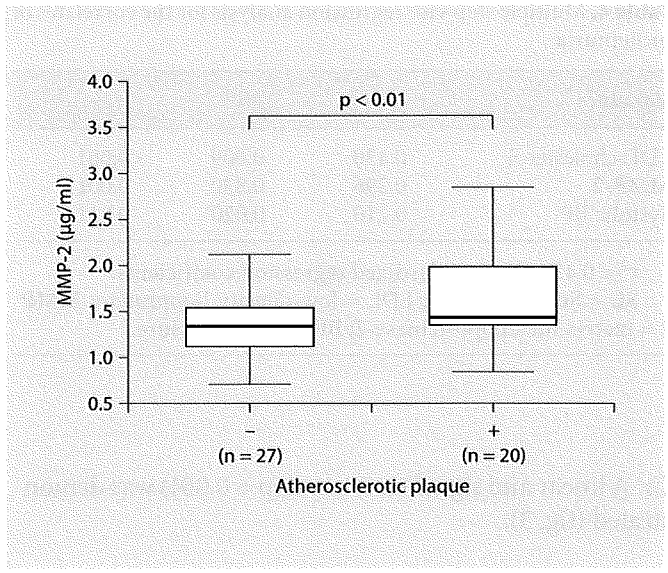

Fig. 5. Serum MMP-2 levels in patients with or without atherosclerotic plaque.

same ELISA kit as ours were 1.141 ± 0.291 and 1.280 ± 0.277 μg/ml in pre- and post-menopausal women, respectively [18].

There is growing evidence that traditional risk factors for CVD, such as hypercholesterolemia and hypertension, could induce renal damage, thus playing a role in the pathogenesis of CKD [19–21]. This is consistent with the observations we made here that LDL-cholesterol and systolic BP are also independent correlates of proteinuria in our CKD patients. Since serum MMP-2 levels were positively associated with proteinuria independent of these traditional risk factors, our present study supports the concept that MMP-2 itself is involved in proteinuria and suggests that the circulating MMP-2 level could be a marker of renal damage in patients with CKD. In support of this, several in vitro and animal studies have reported the implication of MMP-2 in mesangioproliferative changes and/or tubulointerstitial injury, thus being involved in proteinuria [22–25]. Circulating MMP-2 has re-

cently been shown to be correlated with proteinuria in kidney transplant recipients as well [26].

There is also accumulating evidence that proteinuria itself is an independent risk factor for CVD [4]. In this study, we found that serum MMP-2 levels were positively associated with the severity of IMT and the presence of atherosclerotic plaque in the carotid artery, surrogate markers of CVD, in our CKD patients. These observations suggest that MMP-2 is one of the causative molecules that could link proteinuria to CVD. Indeed, MMP-2 has been shown to play a role in both plaque vulnerability and in expansive arterial remodeling [27]. Serum MMP-2 levels were higher in patients with acute coronary syndrome than those in stable angina patients, thus suggesting that the circulating MMP-2 level could be a marker of plaque instability in CVD [28]. Further, since circulating MMP-2 levels were strongly correlated with IMT in chronic renal failure patients on hemodialysis [11, 29], MMP-2 could be one of the factors that could link CKD and CVD as well. In this study, it may be interesting to note that strong well-known risk factors for CVD such as LDL-cholesterol and serum Cr were not related to IMT. Hyper-LDL-cholesterolemia and/or renal dysfunction may promote atherosclerosis, at least in part, via overproduction of MMP-2 in CKD patients.

Although serum levels of MMP-2 may be influenced by agents that blocked the RAS [30, 31], it is unlikely that they could confound the present results because the use of RAS inhibitors was not the independent correlate of proteinuria or IMT in multiple stepwise regression analyses (tables 4, 5). However, we cannot totally exclude the possibility that the use of RAS inhibitors could affect the present findings because this study is probably underpowered.

In conclusion, the present study demonstrates that serum levels of MMP-2 are one of the independent correlates of proteinuria and could be associated with IMT and atherosclerotic plaque in patients with CKD. These findings suggest that serum MMP-2 levels may be one of the causative risk factors for renal damage and atherosclerosis in CKD patients. Since this study is a cross-sectional one, future longitudinal and/or interventional studies are needed to assess the question of whether an elevation in MMP-2 was a cause or a consequence of renal and vascular damage in patients with CKD and if MMP-2 is a biomarker for CVD in patients with CKD.

Limitations

Since the majority of our subjects were late stage renal patients (CKD stages III and IV) and MMP-2 levels were inversely correlated with renal function (fig. 1), we cannot exclude the possibility that the association between increased MMP-2 levels and proteinuria was an epiphenomenon. However, in the present study we found that proteinuria, but not eGFR, was an independent correlate of MMP-2 levels in our patients ($\beta = 0.361$, $p = 0.005$). Further, we found that there was a significant correlation between MMP-2 levels and proteinuria in patients with CKD stages I and II as well ($n = 19$, $\beta = 0.59$, $p = 0.008$). These observations suggest that circulating MMP-2 may be involved in the progression of proteinuria in patients with CKD. A more homogenous group of subjects is required to truly clarify this issue.

Acknowledgement

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BASIC RESEARCH www.jasn.org

RAGE-Induced Cytosolic ROS Promote Mitochondrial Superoxide Generation in Diabetes

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ABSTRACT

Damaged mitochondria generate an excess of superoxide, which may mediate tissue injury in diabetes. We hypothesized that in diabetic nephropathy, advanced glycation end-products (AGEs) lead to increases in cytosolic reactive oxygen species (ROS), which facilitate the production of mitochondrial superoxide. In normoglycemic conditions, exposure of primary renal cells to AGEs, transient overexpression of the receptor for AGEs (RAGE) with an adenoviral vector, and infusion of AGEs to healthy rodents each induced renal cytosolic oxidative stress, which led to mitochondrial permeability transition and deficiency of mitochondrial complex I. Because of a lack of glucose-derived NADH, which is the substrate for complex I, these changes did not lead to excess production of mitochondrial superoxide; however, when we performed these experiments in hyperglycemic conditions *in vitro* or in diabetic rats, we observed significant generation of mitochondrial superoxide at the level of complex I, fueled by a sustained supply of NADH. Pharmacologic inhibition of AGE-RAGE–induced mitochondrial permeability transition *in vitro* abrogated production of mitochondrial superoxide; we observed a similar effect *in vivo* after inhibiting cytosolic ROS production with apocynin or lowering AGEs with alagebrium. Furthermore, RAGE deficiency prevented diabetes-induced increases in renal mitochondrial superoxide and renal cortical apoptosis in mice. Taken together, these studies suggest that AGE-RAGE–induced cytosolic ROS production facilitates mitochondrial superoxide production in hyperglycemic environments, providing further evidence of a role for the advanced glycation pathway in the development and progression of diabetic nephropathy.

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During oxidative phosphorylation (OXPHOS) under physiologic conditions, there is minimal superoxide leakage, which is immediately scavenged by the antioxidant enzyme manganese superoxide dismutase (MnSOD, SOD2); however, damaged or dysfunctional mitochondria generate excessive superoxide, creating a state of redox imbalance.[1] In diabetic complications, a unifying hypothesis has been postulated: That the excessive generation of mitochondrial superoxide by hyperglycemia is the primary initiating event that activates all other pathways of tissue damage.[2] There remains debate, however, as to whether oxidative stress is an important early link between hyperglycemia and compli-

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cations or is a byproduct of other primary pathogenic mechanisms.[3]

It is thought that excess concentrations of mitochondrial reactive oxygen species (ROS) are mediated by disruption of the activity of OXPHOS enzymes *via* electron leakage at complex I (NADH:ubiquinone oxidoreductase) or complex III (ubiquinol:cytochrome c oxidoreductase).[1,4,5] For example, in Friedreich ataxia, a genetically inherited mitochondrial disease, deficiencies of OXPHOS complexes I, II, and III are associated with excessive steady-state generation of mitochondrial superoxide.[6] Of interest is that progressive renal disease has been reported in patients with mitochondrial respiratory chain abnormalities, with some patients presenting with renal abnormalities as their primary pathology.[7,8] In addition, a decline in the activity of complex III[9] and enhanced susceptibility to mitochondrial permeability transition (mPT)[10] have been shown in diabetic kidneys. In other contexts, mPT decreases the activity of complex I, leading to a specific leakage of superoxide.[11] Furthermore, the hyperglycemia characteristic of diabetes increases the complex I substrate NADH,[12] which is likely to potentiate ROS production by the respiratory chain.[13]

Advanced glycation end products (AGEs), formed by the nonenzymatic irreversible modification of proteins, are known contributors to the pathogenesis of diabetic renal disease,[14] in particular as effector molecules for the receptor for AGEs (RAGE).[15] Ligand engagement of RAGE by AGEs also results in the production of cellular ROS.[16,17]

In this study, we investigated the *in vitro* and *in vivo* effects of the AGE-RAGE interaction on mitochondrial function, under both normal- and high-glucose environments. We show that engagement of RAGE by AGEs induces mPT, which subsequently leads to NADH-dependent excessive generation of mitochondrial superoxide radical *via* complex I deficiency in a high-glucose milieu.

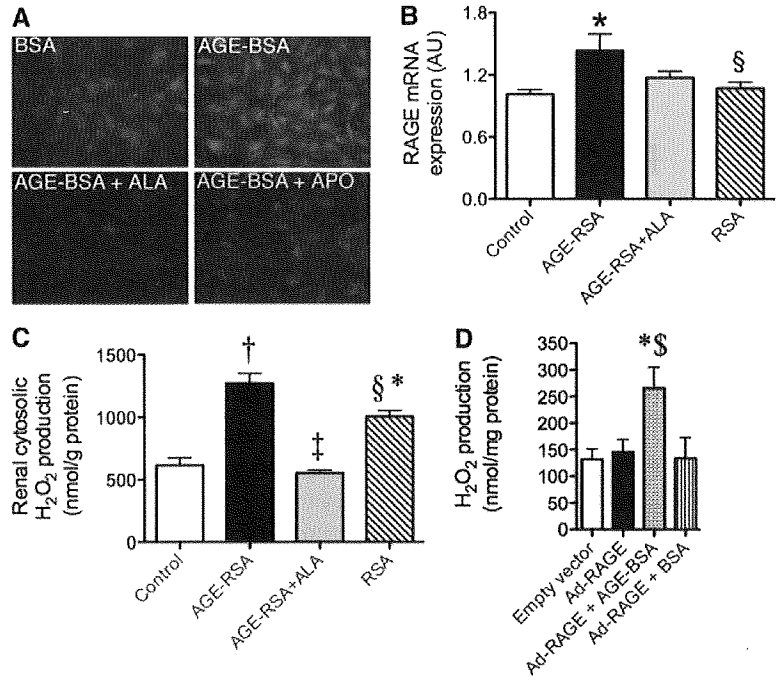

Figure 1. AGEs induce RAGE expression and generate cytosolic H_2O_2 in normal glucose. Primary rat mesangial cells cultured in the presence of 5.5 mM D-glucose were treated with 100 μg/ml glycated BSA (AGE-BSA) or native BSA for 3 d (n = 3 separate experiments). (A) Fluorescence micrographs of intracellular H_2O_2 production (5 μM CM-H_2DCFDA for 10 min at 37°C; ALA 40 μM, APO 1 μM). (B and C) *In vivo*, rats were injected with saline (control), AGE-RSA or native RSA (20 mg/kg per d) for 16 wk (n = 6 to 10 rats per group). One group of rats was co-administered ALA (AGE-RSA+ALA, 10 mg/kg per d). (B) RAGE gene expression in renal cortex. (C) Cytosolic H_2O_2 production as assessed using the Amplex Red reagent. (D) Cytosolic H_2O_2 in primary rat mesangial cells infected with the human full-length (FL) Ad-RAGE or the control empty vector for 72 h. At 24 h after infection, AGE-BSA (100 μg/ml) was added to one group (Ad-RAGE+AGEs). Samples shown are representative of three independent experiments. Bars represent means ± SEM, n = 3. *$P < 0.05$ *versus* control or empty vector; †$P < 0.001$ *versus* control; ‡$P < 0.001$ *versus* AGE-RSA; §$P < 0.05$ *versus* AGE-RSA; \$$P < 0.05$ *versus* Ad-RAGE.

RESULTS

Exogenous AGEs Induce RAGE Expression and Generate Cytosolic Hydrogen Peroxide in Normal-Glucose Environments

Primary rat mesangial cells exposed to 100 μg/ml AGE-modified BSA (AGE-BSA) had increased cellular hydrogen peroxide (H_2O_2) production, as compared with BSA vehicle–treated cells alone (Figure 1A). H_2O_2 production was attenuated by the inhibitor of AGE accumulation alagebrium (ALA) and by the cytosolic NADPH-oxidase inhibitor apocynin (APO; Figure 1A).

Exogenously prepared AGE-BSA and AGE-modified rat serum albumin (AGE-RSA) had approximately 100-fold increases in CML modification (AGE-BSA 67.0 ± 1.2 mmol/mol lysine; AGE-RSA 38.2 ± 3.6 mmol/mol lysine) as compared with the original preparations (BSA 0.5 ± 0.0 mmol/mol lysine and RSA 0.3 ± 0.0 mmol/mol lysine).[18,19] Importantly, no endotoxin was detected in any of the albumin preparations (as measured by the Limulus Amoebocyte Lysate assay). In addition, no anti-CML antibodies in excess of those seen in RSA-injected rats could be detected in the plasma from AGE-RSA–injected rats by ELISA (data not shown).

AGE-RSA injection did not change plasma glucose, glycated hemoglobin, renal function, or systolic BP (Table 1) when compared with RSA-injected rats. Renal cortical RAGE gene (Figure 1B, gene accession no. L33413) and protein expression