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Dietary glycemic index and risk of type 2 diabetes mellitus in middle-aged Japanese men

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ABSTRACT

This cohort study investigated the association between dietary glycemic index (GI), glycemic load (GL), and the incidence of type 2 diabetes mellitus in middle-aged Japanese men, and the effect of insulin resistance and pancreatic B-cell function on the association. Participants were 1995 male employees of a metal products factory in Japan. Dietary GI and GL were assessed using a self-administered diet history questionnaire. The incidence of diabetes was detected in annual medical examinations over a 6-year period. The association between GI, GL, and the incidence of diabetes was evaluated using Cox proportional hazards models. During the study, 133 participants developed diabetes. Age- and body mass index–adjusted hazard ratios across the GI quintiles were 1.00 (reference), 1.62, 1.50, 1.68, and 1.80; and those of GL were 1.00 (reference), 1.07, 1.48, 0.95, and 0.98. The hazard ratio for the highest GI quintile was significantly greater than that for the lowest quintile. The influence of GI was more pronounced in the lowest insulin resistance subgroups. GI and pancreatic B-cell function were independently associated with the incidence of type 2 diabetes mellitus; participants with low B-cell function and the highest tertile of GI had the highest risk of diabetes. Dietary GI is associated with the incidence of diabetes in middle-aged Japanese men. GI and B-cell function were independently associated with incidence of diabetes.

Author contributions: MS collected the data, performed the analysis, and wrote the manuscript; KN, KM, MI, YM, TK, YN, and HN collected the data, contributed to the "Discussion," and reviewed/edited the manuscript; TT, KY, YS, SK, and SS contributed to the "Discussion" and reviewed/edited the manuscript.

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1. Introduction

The prevalence of type 2 diabetes mellitus is similar in Asian and Western countries even though the prevalence of obesity is lower in Asia [1]. The high incidence of diabetes in the relatively lean Asian population may be explained, in part, by the presence of more abdominal fat in Asians as compared with white people of similar body mass index (BMI) [2,3]. Furthermore, nonobese Asians who have low pancreatic B-cell function are at high risk for diabetes [4-6].

Dietary factors may also play a role in the high incidence of diabetes in the Asian population. An association between dietary glycemic index (GI), glycemic load (GL), and the incidence of type 2 diabetes mellitus has been reported in Western countries [7-9]; however, the association between GI and type 2 diabetes mellitus in the Asian population is not clear because high-GI rice is a significant part of the Asian diet [10-14] and Asian GI values are higher than those in Western countries [15-19]. At present, the only study examining the relationship between GI and type 2 diabetes mellitus in the Asian population was conducted in women [12]; and none have investigated the association in Asian men.

A high-GI diet is associated with insulin resistance and postprandial hyperglycemia and hyperinsulinemia, which may cause pancreatic B-cell failure and diabetes mellitus [20]. However, no studies evaluating the influence of insulin resistance or B-cell function on the association between GI and the incidence of diabetes have been reported.

In this 6-year prospective study of Japanese men, we investigated the relationship between dietary GI, GL, and the risk of developing type 2 diabetes mellitus. The objectives of the study were to investigate whether dietary GI and GL are associated with the risk of diabetes and to examine the effect of insulin resistance and B-cell function on the relationship.

2. Methods

2.1. Participants

The study participants were male employees of a factory that produces zippers and aluminum sashes in Toyama Prefecture, Japan. Detailed information on the study population has been previously reported [6,13]. The Industrial Safety and Health Law in Japan requires that employers conduct annual health examinations for all employees. A test for diabetes mellitus was conducted during annual medical examinations between 2003 and 2009. In 2003, 2275 (89%) of 2543 male employees aged 35 to 55 years received health examinations and responded to the diet survey. Of these 2275 potential participants, 280 (12%) were excluded: 139 were diabetic or had high fasting plasma glucose (≥126 mg/dL) at the time of the baseline examination, 70 did not have fasting plasma insulin levels measured at the baseline examination, 9 men had a total daily calorie intake less than 500 kcal or greater than 5000 kcal, and 62 did not participate in consecutive follow-up annual health examinations. Thus, 1995 participants were included in the present study.

2.2. Data collection

The annual health examination included a medical history, physical examination, anthropometric measurements, and the measurement of fasting plasma glucose, fasting insulin, glycated hemoglobin (HbA_{1c}), and serum lipid levels. Height was measured without shoes to the nearest 0.1 cm using a stadiometer. Weight was measured with participants wearing only light clothing and no shoes to the nearest 0.1 kg using a standard scale. Body mass index was calculated as weight/height^2 (kilograms/square meter). Blood pressure was measured using a mercury sphygmomanometer after the subject rested for 5 minutes in a seated position. All measurements were taken by trained staff.

Plasma glucose levels were measured enzymatically using an Abbott glucose UV test (Abbott Laboratories, Chicago, IL), and plasma insulin levels were determined using radioimmunoassay (Shionogi, Tokyo, Japan). HbA1c was measured by high-velocity liquid chromatography using a fully automated HbA_{1c} analyzer (Kyoto Daiichi Kagaku, Kyoto, Japan). Total cholesterol and triglycerides were measured using an enzyme assay. High-density lipoprotein (HDL) cholesterol was measured using direct methods. Insulin resistance was calculated by the homeostasis model assessment (HOMA) method using the formula: HOMA-IR = fasting insulin (microunits per milliliter) × fasting plasma glucose (milligrams per deciliter)/405 [21]. The HOMA of β-cell function (HOMA-B) was calculated using the following formula: HOMA-B = 360 × fasting insulin (microunits per milliliter)/[fasting plasma glucose (milligrams per deciliter) − 63] [21].

A questionnaire was used to identify voluntary health-related behaviors such as alcohol consumption, smoking, and habitual exercise. A self-administered questionnaire was also used to collect information about a medical history of hypertension, dyslipidemia, diabetes, the use of antidiabetic medication, and a family history of diabetes. High blood pressure and dyslipidemia were defined using the Japanese criteria for metabolic syndrome [22]: *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 HDL cholesterol less than 40 mg/dL.

2.3. Dietary assessment and calculation of dietary GI and GL

Dietary habits during the preceding month were assessed using a self-administered diet history questionnaire (DHQ) [23]. The DHQ was developed to estimate the dietary intakes of macronutrients and micronutrients for epidemiological studies in Japan. A detailed description of the methods used for calculating dietary intakes and the validity of the DHQ have been reported previously [11,24,25]. Estimates of dietary intake for 147 food and beverage items, energy, and nutrients were calculated in 2007 using an ad hoc computer algorithm developed for the DHQ that was based on the Standard Tables of Food Composition in Japan [26].

Of the 147 food and beverage items included in the DHQ, 6 (4.1%) were alcoholic beverages, 8 (5.4%) contained no available carbohydrate, and 63 (42.9%) contained less than 3.5 g of available carbohydrate per serving. The calculation of

dietary GI and GL was thus based on the remaining 70 items. The GI databases used were an international table of GI [27], several publications concerning the GI of Japanese foods [28-30], recent articles on GI values published after the publication of the international GI table [31,32], and an online database provided by the Sydney University Glycemic Index Research Service [33]. Although concerns have been expressed regarding the utility of GI for mixed meals (overall diet) [34,35], many researchers have shown that the GI of a mixed meal can be consistently predicted as the weighted mean of the GI values of each of the component foods [36,37]. We calculated dietary GI by multiplying the percentage contribution of each food to the daily carbohydrate intake by the GI value of the food and then summed these products. GL was calculated by multiplying the dietary GI by the total daily carbohydrate intake and dividing by 100. We used energy-adjusted values by the density method (per 1000 kcal) for dietary GL [11].

2.4. *Diagnosis of diabetes*

Fasting plasma glucose and HbA_{1c} were measured during the annual medical examinations. Participants with HbA_{1c} greater than 6.0% were given a 75-g oral glucose tolerance test (OGTT). According to the definition of the American Diabetes Association [38] and the Japanese Diabetes Society [39], the diagnosis of diabetes was confirmed by at least one of the following observations: (1) a fasting plasma glucose concentration of at least 126 mg/dL, (2) 2-hour glucose level of at least 200 mg/dL in a 75-g OGTT, or (3) treatment with insulin or an oral hypoglycemic agent.

2.5. *Statistical analysis*

We calculated the incidence rates and hazard ratios (HRs) for diabetes according to the quintile of dietary GI, dietary GL, and total energy intake. The Cox proportional hazard model was used to calculate HRs adjusted for multiple variables, including age (<40, 40-44, 45-49, ≥50 years), BMI (<22, 22-25, ≥25 kg/m^2), family history of diabetes (no, yes), alcohol consumption determined by the DHQ (nondrinker, consumed <20 g/d, consumed ≥20 g/d), smoking status (never, ex-smoker, or current smoker), habitual exercise (no, yes), total energy intake (kilocalories per day, quintile), and dietary total fiber intake (grams per 1000 kcal, quintile). The HR for diabetes was calculated separately for BMI (<22, 22-25, ≥25 kg/m^2), the HOMA-IR or HOMA-B tertile in each GI tertile, and the joint effects of GI and BMI, HOMA-IR, or HOMA-B by cross-classifying participants by both variables. The statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS version 12.0J, Tokyo, Japan). A P value < .05 was deemed statistically significant.

3. Results

The mean participant age at baseline was 46.0 years, and the mean BMI was 23.4 kg/m^2. The mean dietary GI was 69.2, and the mean dietary GL (/1000 kcal) was 87.9. White rice was the largest contributor to dietary GI (61.2%), followed by noodles (5.4%), bread (5.2%), and confectioneries (4.9%).

The participants' baseline characteristics according to the dietary GI and GL quintile are shown in Table 1 (GI) and Table 2 (GL). No association was observed between dietary GI and age, BMI, serum lipid levels, fasting plasma glucose and insulin, blood pressure, prevalence of high blood pressure, or dyslipidemia. The higher GL quintiles were associated with significantly lower HDL cholesterol, lower fasting plasma glucose, higher fasting insulin, lower systolic/diastolic blood pressure, and a lower prevalence of high blood pressure. Furthermore, high GI and GL were associated with lower dietary energy intake, lower fat intake, lower dietary fiber intake, and higher carbohydrate intake.

During the 6-year follow-up (8988 person-years), we documented 133 cases of diabetes. Among these, 115 diagnoses were based on high fasting plasma glucose levels, 16 were diagnosed according to a 75-g OGTT, and 2 participants had been treated with hypoglycemic medication.

The crude incidence rates (per 1000 person-years) across the GI quintiles from lowest to highest were 10.1, 15.7, 13.6, 16.1, and 18.3, respectively (Table 3). The age- and BMI-adjusted HRs (model 1) across the GI quintiles were 1.00 (reference), 1.62, 1.50, 1.68, and 1.80. The HR of the highest GI quintile was significantly higher than that of the lowest quintile. Further adjustment for family history of diabetes, alcohol intake, smoking, physical activity, the presence of high blood pressure, and dyslipidemia at baseline (model 2) did not affect the HRs. When we used a model adjusted for the variables used in model 2 plus dietary factors (model 3), the HRs across the quintiles were higher than those in models 1 and 2; and the HRs for the fourth and fifth quintiles were significantly higher than that of the first quintile.

The crude incident rates (per 1000 person-years) across the GL quintiles were 13.3, 15.0, 19.5, 12.4, and 14.0 (Table 3). The age- and BMI-adjusted HRs across the BMI quintiles were 1.00 (reference), 1.07, 1.48, 0.95, and 0.98; and no association was found between GL and the incidence of diabetes. The relationships remained nonsignificant even after additional adjustments for potential confounders (models 2 and 3).

Because GI was inversely associated with total energy intake and total fiber intake (Table 1) and positively associated with the incidence of diabetes, we further evaluated the association between total energy intake and total fiber intake and the incidence of diabetes (Table 3). There were no associations between the total energy intake, total fiber intake, and incidence of diabetes.

We analyzed the association between GI and the incidence of diabetes separately in subgroups based on the degree of BMI, insulin resistance, or pancreatic B-cell function at baseline. There were no differences in the associations between GI and baseline characteristics among the different BMI, insulin resistance, and B-cell function subgroups (Supplemental Table 1). High GI was associated with a significantly higher risk of diabetes in participants with a BMI less than 22 kg/m^2, but not in the subgroup with a BMI of 22 to 24.9 kg/m^2 or in participants with a BMI of at least 25 kg/m^2 (Table 4). Similarly, significant positive associations were observed in participants in the lowest HOMA-IR and HOMA-B tertiles, but not in the other tertiles (Table 4). We examined the joint effects of GI and BMI/HOMA-IR/HOMA-B by cross-classifying participants by both variables (Fig. 1). We found a significant

Table 1 – Baseline characteristics of study participants according to dietary GI quintiles

	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	P [b]
GI	<66.2	66.2-68.5	68.6-70.4	70.5-72.6	≥72.7	
Age (y)	45.7 ± 6.0	46.2 ± 6.0	45.7 ± 6.2	46.0 ± 6.1	46.3 ± 5.8	.286
Height (cm)	169.7 ± 6.0	169.7 ± 6.1	170.0 ± 5.9	169.3 ± 5.9	169.1 ± 6.1	.113
Weight (kg)	68.2 ± 9.6	67.5 ± 9.5	67.0 ± 9.0	67.3 ± 9.5	67.3 ± 9.3	.178
BMI (kg/m^2)	23.6 ± 2.9	23.4 ± 2.9	23.1 ± 2.8	23.4 ± 2.8	23.5 ± 2.9	.541
Total cholesterol (mg/dL)	207.5 ± 34.0	208.6 ± 33.5	208.4 ± 35.1	210.8 ± 33.8	201.9 ± 31.5	.101
Triglycerides (mg/dL) [a]	106 (68-157)	103 (69-151)	114 (78-168)	103 (66-156)	97 (67-143)	.073
HDL cholesterol (mg/dL)	57.9 ± 14.9	57.3 ± 13.2	58.7 ± 15.4	57.9 ± 15.1	58.4 ± 14.6	.522
Fasting plasma glucose (mg/dL)	92.5 ± 10.1	92.8 ± 9.4	92.5 ± 9.6	93.4 ± 10.4	93.0 ± 9.6	.300
Fasting insulin (μU/mL) [a]	5.1 (3.0-7.3)	4.9 (3.0-7.0)	4.7 (3.0-7.0)	5.0 (3.0-8.0)	4.7 (3.0-7.0)	.129
HOMA-IR [a]	1.15 (0.73-1.74)	1.10 (0.70-1.67)	1.06 (0.73-1.62)	1.13 (0.69-1.76)	1.07 (0.68-1.53)	.212
HOMA-B [a]	66.2 (43.5-94.1)	60.9 (40.0-92.8)	60.6 (40.0-90.0)	61.4 (41.5-93.9)	59.6 (39.8-90.0)	.026
HbA_{1c} (%)	5.0 ± 0.4	5.0 ± 0.4	5.0 ± 0.4	5.0 ± 0.5	5.0 ± 0.4	.954
Systolic blood pressure (mm Hg)	120.5 ± 18.0	119.8 ± 17.4	120.4 ± 15.1	121.9 ± 18.8	120.2 ± 20.9	.668
Diastolic blood pressure (mm Hg)	77.9 ± 12.9	76.9 ± 12.1	78.0 ± 11.1	78.6 ± 13.4	77.6 ± 14.6	.765
Family history of diabetes (%)	13.9	12.6	14.0	14.7	12.2	.837
Smoking status						.001
Nonsmoker (%)	33.3	32.1	29.7	30.8	28.2	
Ex-smoker (%)	16.2	15.2	14.5	16.4	11.7	
Current smoker (%)	50.5	52.8	55.9	52.7	60.2	
Alcohol intake						.333
Nondrinker (%)	21.4	24.5	24.4	27.1	21.6	
Light drinker (<20 g/d; %)	36.3	34.6	33.7	32.3	30.7	
Moderate/heavy drinker (≥20 g/d; %)	42.3	40.9	41.9	40.5	47.7	
Habitual exercise, yes (%)	33.6	30.8	25.4	25.9	25.1	.021
Prevalence of high blood pressure [c] (%)	8.7	8.8	6.3	10.4	7.9	.302
Prevalence of dyslipidemia [c] (%)	10.2	10.1	9.0	9.0	6.6	.402
GI	63.4 ± 2.8	67.5 ± 0.7	69.5 ± 0.5	71.5 ± 0.6	74.2 ± 1.3	<.001
GL (/1000 kcal)	76.0 ± 16.2	85.1 ± 15.0	87.7 ± 17.0	92.9 ± 16.6	97.7 ± 19.9	<.001
Total energy intake (kcal/d)	2383 ± 695	2270 ± 631	2198 ± 586	2096 ± 518	2044 ± 559	<.001
Total fiber intake (g/1000 kcal)	5.7 ± 1.5	5.3 ± 1.3	4.9 ± 1.3	4.7 ± 1.2	4.0 ± 1.2	<.001
Protein (% energy)	12.5 ± 2.3	12.1 ± 2.2	11.6 ± 2.0	11.6 ± 2.0	10.8 ± 2.1	<.001
Fat (% energy)	24.1 ± 6.7	22.4 ± 6.1	21.6 ± 6.3	20.8 ± 5.9	18.4 ± 6.3	<.001
Carbohydrates (% energy)	54.9 ± 9.1	57.3 ± 8.0	57.3 ± 8.9	58.9 ± 8.2	59.7 ± 9.2	<.001

Values are mean ± standard deviation or percentage.
[a] Values are geometric means (interquartile range).
[b] Linear regression was used for continuous variables based on ordinal variables containing the median value for each quintile, and a χ^2 test was used for categorical variables.
[c] High blood pressure and dyslipidemia were defined using the Japanese criteria for metabolic syndrome.

interaction between GI and HOMA-IR (P = .005), and the influence of GI was more pronounced in the lowest HOMA-IR tertile subgroups. On the other hand, participants in the lowest HOMA-B tertile with the highest GI had the highest risk of diabetes (Fig. 1C). We observed no interaction between GI and BMI or HOMA-B.

4. Discussion

This study investigated the association between dietary GI and GL and the incidence of type 2 diabetes mellitus in middle-aged Japanese men. The results indicated that GI, but not GL, had a significant positive association with the incidence of diabetes. The analyses of insulin resistance and dietary GI indicated that the association between high dietary GI and type 2 diabetes mellitus was stronger in the lowest HOMA-IR subgroup. Furthermore, GI and pancreatic B-cell function were independently associated with incidence of type 2 diabetes mellitus; and the participants with low HOMA-B and the highest GI had the highest risk of diabetes.

The results of previous studies that evaluated the association between dietary GI and incidence of diabetes were controversial [8]. Although some reports showed no association between GI and diabetes, other reports and a recent meta-analysis showed positive associations. Differences in these results are probably due to differences in participant characteristics such as age, sex, ethnicity, and lifestyle. All previous studies of the association between GI and GL and the risk of diabetes have been conducted in Western countries [7-9], with the exception of one Chinese study of women [12]. The present study is the first report on an association between GI and GL and the risk of diabetes in Asian men. We found that the HR for the highest GI quintiles was 1.80 (model 1) to 1.96 (model 3); these values are somewhat higher than those reported in previous studies (0.89-1.59 for multivariate-adjusted models) [8].

The GL was not associated with the incidence of diabetes in our study; and our findings agree with those of previous

Table 2 – Baseline characteristics of study participants according to dietary GL quintiles

	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)	P[b]
GL (/1000 kcal)	<72.8	72.8-83.1	83.2-91.5	91.6-103.3	≥103.4	
Age (y)	45.4 ± 6.0	46.5 ± 6.0	45.9 ± 6.2	45.9 ± 5.9	46.2 ± 6.1	.264
Height (cm)	169.7 ± 5.9	169.9 ± 6.0	169.6 ± 5.8	169.4 ± 5.8	169.2 ± 6.4	.102
Weight (kg)	67.9 ± 9.4	67.8 ± 9.3	67.3 ± 9.6	66.8 ± 8.6	67.4 ± 9.9	.178
BMI (kg/m^2)	23.5 ± 2.8	23.4 ± 2.8	23.3 ± 2.8	23.2 ± 2.8	23.5 ± 3.1	.650
Total cholesterol (mg/dL)	206.8 ± 33.4	205.8 ± 34.7	206.4 ± 35.2	208.6 ± 31.6	209.8 ± 33.4	.101
Triglycerides (mg/dL)[a]	108 (69-161)	100 (66-150)	109 (71-160)	99 (67-147)	106 (71-157)	.772
HDL cholesterol (mg/dL)	61.5 ± 15.5	58.8 ± 13.7	57.3 ± 15.3	57.7 ± 14.5	54.9 ± 13.4	<.001
Fasting plasma glucose (mg/dL)	93.6 ± 9.9	93.2 ± 9.6	93.1 ± 10.6	92.3 ± 9.7	92.0 ± 9.3	.010
Fasting insulin (μU/mL)[a]	4.5 (3.0-7.0)	4.8 (3.0-7.0)	5.0 (3.0-7.3)	4.9 (3.0-7.0)	5.1 (3.0-8.0)	.003
HOMA-IR[a]	1.03 (0.66-1.64)	1.09 (0.69-1.66)	1.14 (0.75-1.76)	1.11 (0.72-1.60)	1.15 (0.73-1.76)	.015
HOMA-B[a]	55.3 (37.9-81.3)	59.8 (40.0-83.1)	64.1 (44.7-96.0)	63.7 (41.5-93.9)	66.4 (43.2-102.9)	<.001
HbA_{1c} (%)	5.0 ± 0.4	5.0 ± 0.4	5.0 ± 0.4	5.0 ± 0.4	5.0 ± 0.4	.747
Systolic blood pressure (mm Hg)	123.1 ± 16.7	120.6 ± 18.7	121.1 ± 17.6	119.4 ± 17.1	118.6 ± 20.2	<.001
Diastolic blood pressure (mm Hg)	79.9 ± 12.0	78.4 ± 13.4	78.1 ± 12.2	76.5 ± 12.1	76.1 ± 14.3	<.001
Family history of diabetes (%)	12.0	13.5	16.1	13.8	12.2	.451
Smoking status						.021
Nonsmoker (%)	23.0	29.9	30.9	34.3	36.1	
Ex-smoker (%)	17.8	15.5	14.6	16.5	9.6	
Current smoker (%)	59.3	54.6	54.5	49.3	54.3	
Alcohol intake						<.001
Nondrinker (%)	6.5	12.7	16.3	33.3	50.5	
Light drinker (<20 g/d; %)	17.5	29.9	42.5	40.8	37.1	
Moderate/heavy drinker (≥20 g/d;%)	76.0	57.4	41.2	26.0	12.4	
Habitual exercise, yes (%)	28.8	31.7	29.4	29.5	21.5	.018
Prevalence of high blood pressure[c] (%)	11.8	8.0	8.8	7.0	6.6	.070
Prevalence of dyslipidemia[c] (%)	8.7	7.8	10.1	9.5	8.9	.833
GI	67.1 ± 4.7	68.3 ± 3.7	69.2 ± 3.3	70.0 ± 3.3	71.4 ± 3.0	<.001
GL (/1000 kcal)	62.7 ± 8.8	78.0 ± 3.0	87.2 ± 2.5	97.1 ± 3.3	114.4 ± 9.6	<.001
Total energy intake (kcal/d)	2394 ± 616	2299 ± 581	2183 ± 578	2104 ± 556	2011 ± 653	<.001
Total fiber intake (g/1000 kcal)	4.9 ± 1.6	5.1 ± 1.5	5.0 ± 1.3	4.9 ± 1.4	4.6 ± 1.3	.001
Protein (% energy)	12.7 ± 2.8	12.3 ± 2.1	11.8 ± 1.9	11.5 ± 1.6	10.3 ± 1.6	<.001
Fat (% energy)	25.7 ± 7.7	23.7 ± 5.7	22.1 ± 5.3	20.1 ± 4.2	15.7 ± 4.4	<.001
Carbohydrates (% energy)	46.0 ± 5.6	53.3 ± 3.2	57.5 ± 2.8	62.0 ± 2.9	69.4 ± 4.5	<.001

Values are mean ± standard deviation or percentage.

[a] Values are geometric means (interquartile range).

[b] Linear regression was used for continuous variables based on ordinal variables containing the median value for each quintile, and a χ^2 test was used for categorical variables.

[c] High blood pressure and dyslipidemia were defined using the Japanese criteria for metabolic syndrome.

studies showing that GI, but not GL, was associated with the incidence of diabetes [15,19]. Although some studies have reported that dietary GL was associated with the risk of diabetes [12,16], a meta-analysis comparing the highest and lowest GI and GL quintiles showed that the HR for developing diabetes was more highly associated with GI than GL [8]. Thus, dietary GI is a better predictor of the risk of diabetes than is dietary GL.

High-GI foods are thought to increase insulin resistance, impair pancreatic B-cell function, and eventually lead to type 2 diabetes mellitus [20]. The adverse effects of a high-GI diet have been reported to be more evident in overweight or obese people who, presumably, were insulin resistant at baseline [17,40]. However, evidence of an effect of insulin resistance on the association between GI and diabetes is inconsistent. Some studies have shown that high GI was associated with a higher relative risk of diabetes in people who had a high BMI [12,19], whereas other studies have indicated that high GI was more strongly associated with incidence of diabetes in people with a low BMI [9,15]. These studies used obesity as a marker of insulin resistance; but in our study, insulin resistance was directly measured by HOMA-IR. Thus, we were able to compare the association between GI and the incidence of diabetes according to the degree of insulin resistance. We found a significant interaction between GI and HOMA-IR and also found a significant association between GI and the incidence of diabetes only in participants who were in the lowest tertile of HOMA-IR. Insulin resistance is a strong risk factor for type 2 diabetes mellitus, and it may be difficult to detect the effect of other risk factors in participants with higher insulin resistance.

In our study, GI and pancreatic B-cell function were independently associated with the incidence of diabetes; and participants with the lowest pancreatic B-cell function and the highest dietary GI were at the highest risk of diabetes. Dietary GI is higher in Asian populations than in Western populations. For example, the present study showed mean GI values of 69.2, which were similar to

Table 3 – Adjusted HR for type 2 diabetes mellitus according to quintiles of GI, GL, total energy intake, and total fiber intake in 1995 Japanese men

	Q1 (lowest)	Q2	Q3	Q4	Q5 (highest)
GI					
n	402	396	401	402	394
Total person-years	1786	1778	1766	1796	1862
Incident cases (n)	18	28	24	29	34
Rate per 1000 person-years	10.1	15.7	13.6	16.1	18.3
Adjusted HR (95% CI) model 1	1.00 (reference)	1.62 (0.89-2.93)	1.50 (0.81-2.77)	1.68 (0.93-3.03)	1.80 (1.01-3.18)
Adjusted HR (95% CI) model 2	1.00 (reference)	1.68 (0.92-3.04)	1.56 (0.84-2.89)	1.73 (0.96-3.13)	1.88 (1.06-3.35)
Adjusted HR (95% CI) model 3	1.00 (reference)	1.71 (0.94-3.10)	1.66 (0.89-3.10)	1.86 (1.01-3.44)	1.96 (1.04-3.67)
GL					
n	400	401	398	400	396
Total person-years	1733	1735	1739	1856	1924
Incident cases (n)	23	26	34	23	27
Rate per 1000 person-years	13.3	15.0	19.5	12.4	14.0
Adjusted HR (95% CI) model 1	1.00 (reference)	1.07 (0.61-1.88)	1.48 (0.87-2.52)	0.95 (0.53-1.70)	0.98 (0.56-1.72)
Adjusted HR (95% CI) model 2	1.00 (reference)	1.14 (0.65-2.02)	1.54 (0.89-2.65)	1.07 (0.58-1.96)	1.23 (0.67-2.28)
Adjusted HR (95% CI) model 3	1.00 (reference)	1.16 (0.66-2.06)	1.56 (0.89-2.71)	1.07 (0.57-1.99)	1.24 (0.65-2.34)
Total energy intake (range, kcal/d)	(<1703)	(1703-1971)	(1972-2246)	(2247-2641)	(>2641)
n	399	399	399	399	399
Total person-years	1790	1776	1748	1758	1917
Incident cases (n)	24	24	32	24	26
Rate per 1000 person-years	13.4	14.6	18.3	14.2	13.6
Adjusted HR (95% CI) model 1	1.00 (reference)	1.13 (0.65-1.96)	1.49 (0.88-2.54)	1.11 (0.63-1.95)	1.00 (0.57-1.74)
Adjusted HR (95% CI) model 2	1.00 (reference)	1.10 (0.63-1.92)	1.44 (0.84-2.48)	1.06 (0.60-1.87)	0.97 (0.55-1.71)
Adjusted HR (95% CI) model 3	1.00 (reference)	1.12 (0.64-1.97)	1.45 (0.84-2.49)	1.07 (0.60-1.91)	0.97 (0.55-1.72)
Total fiber intake (range, g/1000 kcal)	(<3.7)	(3.8-4.5)	(4.6-5.2)	(5.3-6.0)	(>6.0)
n	400	450	391	370	384
Total person-years	1938	2016	1781	1590	1663
Incident cases (n)	35	26	17	23	32
Rate per 1000 person-years	18.1	12.9	9.5	14.5	19.2
Adjusted HR (95% CI) model 1	1.00 (reference)	0.73 (0.44-1.22)	0.56 (0.31-1.01)	0.80 (0.47-1.35)	0.99 (0.61-1.60)
Adjusted HR (95% CI) model 2	1.00 (reference)	0.73 (0.44-1.23)	0.59 (0.32-1.05)	0.83 (0.48-1.43)	0.98 (0.59-1.64)
Adjusted HR (95% CI) model 3	1.00 (reference)	0.72 (0.43-1.21)	0.59 (0.33-1.06)	0.84 (0.49-1.45)	0.99 (0.59-1.66)

Model 1: adjusted for age and BMI; model 2: adjusted for age, BMI, family history of diabetes, smoking, alcohol intake, habitual exercise, and presence of hypertension and hyperlipidemia at baseline; model 3: adjusted for variables used in model 2 and dietary total energy (for the GI, GL, and total fiber intake) and dietary total fiber intake (for the GI, GL, and total energy intake). CI indicates confidence interval.

those previously reported in Japan [10,14] and higher than the values (range, 48-60) reported in US and European studies [15-19]. Furthermore, both obese and lean Asians who have lower B-cell function are at high risk for developing type 2 diabetes mellitus [4-6]. Our study indicates that the high prevalence of type 2 diabetes mellitus in Asian populations may be explained by high-GI diets in people with lower B-cell function. Thus, an evaluation of the risk of type 2 diabetes mellitus in Asian people must consider lifestyle and food intake as well as genetic background.

Individuals at high risk for diabetes are encouraged to increase their dietary fiber intake and to eat foods containing whole grains [41]. The consumption of such foods is associated with decreased dietary GI. However, the use of GI is recommended as an additional method for management of diabetes in an American Diabetes Association position statement [41] and a recommendation of the American Dietetic Association [42] because the effects of lower-GI diets on glucose metabolism were conflicting [42]. In our study, total fiber intake was not associated with the incidence of diabetes. Furthermore, a higher GI was associated with a higher risk for diabetes, despite a lower total energy intake; and there was no association between total energy intake and the incidence of diabetes. The appropriate energy intake of each person is important for maintaining body weight and preventing obesity and diabetes. However, appropriate energy intake is influenced by many factors, including body composition and physical activity. It is difficult to evaluate the association between total energy intake itself with diabetes; and indices of the quality of food intake such as GI, rather than the quantity of food intake, would be more useful for a population approach.

The strengths of this study include a large sample size, foods contributing to the dietary GI that differed from those in US and European populations, and the fact that it was the first study of the relationship between GI and the incidence of diabetes conducted in Japanese men. Moreover, several previous cohort studies used information collected from self-administered questionnaires, whereas our conclusions are based on more reliable data obtained from medical examinations and fasting blood glucose and insulin levels, HOMA-IR, and HOMA-B. In addition, GI and GL were calculated using responses to a validated questionnaire [11]. A limitation of the present study is that the sample included only people who

Table 4 – Incidence and adjusted HRs[a] for type 2 diabetes mellitus according to GI tertiles of BMI, HOMA-IR, and HOMA-B in 1995 Japanese men

	GI tertiles (range)			P for trend[b]
	T1 (<68.0)	T2 (68.0-71.0)	T3 (≥71.1)	
BMI (kg/m^2)				
<22.0				
Incident cases n/N	3/203	11/227	15/206	
Crude rate per 1000 person-years	3.2	10.4	15.1	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	4.09 (1.13-14.9)	5.78 (1.63-20.5)	.005
22.0-24.9				
Incident cases n/N	14/278	14/257	18/272	
Crude rate per 1000 person-years	11.5	12.4	14.4	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	1.10 (0.52-2.34)	1.20 (0.59-2.44)	.608
≥25.0				
Incident cases n/N	19/196	20/169	19/187	
Crude rate per 1000 person-years	21.9	28.8	22.5	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	1.41 (0.75-2.66)	1.11 (0.58-2.11)	.719
HOMA-IR tertiles				
<0.85				
Incident cases n/N	4/217	8/207	16/219	
Crude rate per 1000 person-years	4.1	8.5	15.4	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	2.07 (0.61-6.95)	3.67 (1.21-11.2)	.015
0.85-1.43				
Incident cases n/N	10/222	9/232	21/240	
Crude rate per 1000 person-years	10.2	8.6	18.6	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	0.78 (0.31-1.94)	1.58 (0.73-3.41)	.221
≥1.44				
Incident cases n/N	22/238	28/214	15/206	
Crude rate per 1000 person-years	20.5	31.4	16.3	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	1.73 (0.98-3.05)	0.83 (0.43-1.62)	.472
HOMA-B tertiles				
<48.4				
Incident cases n/N	16/227	23/230	31/226	
Crude rate per 1000 person-years	16.1	23.0	30.0	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	1.64 (0.86-3.13)	1.86 (1.01-3.44)	.049
48.4-79.3				
Incident cases n/N	10/218	11/205	12/224	
Crude rate per 1000 person-years	10.3	11.8	11.5	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	1.34 (0.56-3.20)	1.26 (0.53-3.00)	.600
≥79.4				
Incident cases n/N	10/232	11/218	9/215	
Crude rate per 1000 person-years	9.4	11.6	8.9	
Multivariate-adjusted HR (95% CI)	1.00 (reference)	1.39 (0.58-3.31)	0.93 (0.37-2.34)	.922

[a] Adjusted for age, BMI, family history of diabetes, smoking, alcohol intake, habitual exercise, and presence of hypertension and hyperlipidemia at baseline.

[b] Linear regression was used for continuous variables based on ordinal variables containing the median value for each GI tertile.

were employed. Poor health may exclude some individuals from working; thus, the prevalence of obesity may be lower in our sample than in the general Japanese population. Another limitation is that we did not measure waist circumference at baseline, which might have provided more information about abdominal fat accumulation and insulin resistance than measuring BMI did. A further limitation of the present study is that we did not determine whether the diabetes mellitus that developed was type 1 or type 2. However, the study participants were middle-aged men; and as the condition was detected in an annual medical checkup, with relatively mild diabetes mellitus being found, it is most likely that the cases were type 2.

In conclusion, our results indicate that dietary GI is associated with the incidence of diabetes in middle-aged Japanese men. Dietary GI and pancreatic B-cell function were independently associated with the incidence of diabetes. Dietary GI is higher and pancreatic B-cell function is lower in Asian people, as compared with Western people; and these may result in a higher prevalence of diabetes in Asian populations. Our findings suggest that a low-GI diet may be beneficial in preventing type 2 diabetes mellitus in Asian people.

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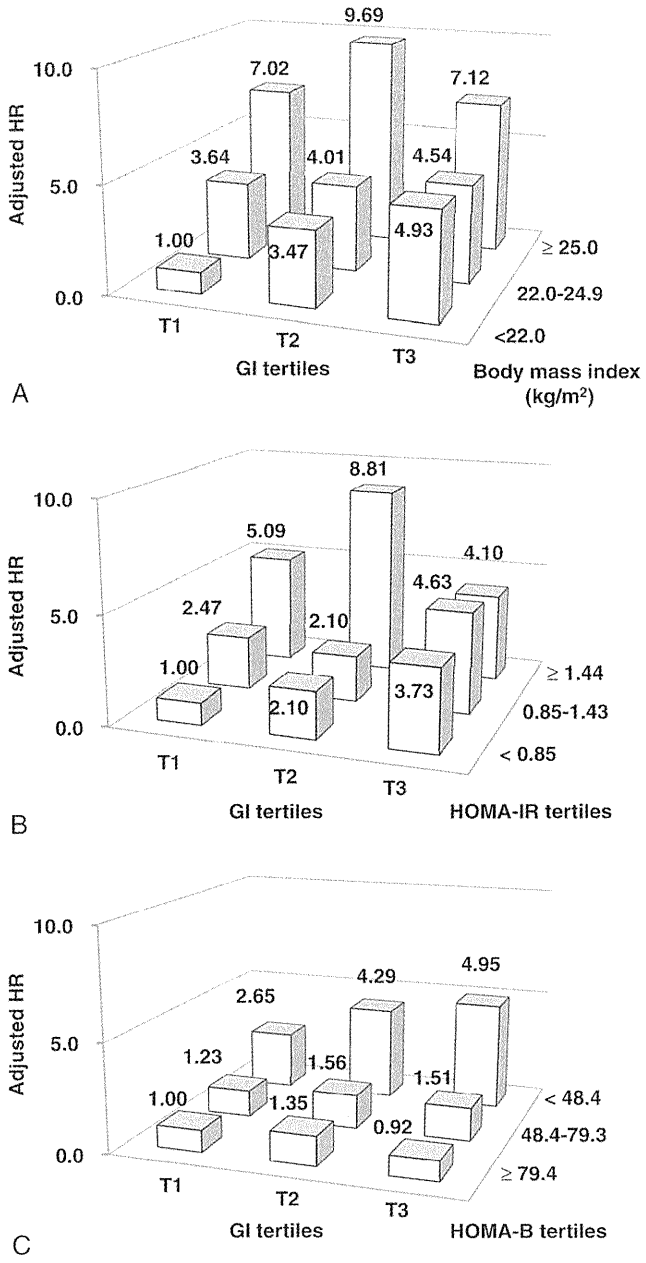

Fig. 1 – Adjusted HRs for type 2 diabetes mellitus by different levels of GI and BMI (A), HOMA-IR (B), and HOMA-B (C) in 1995 Japanese men. The HRs were adjusted for age, BMI, family history of diabetes, smoking, alcohol intake, habitual exercise, and presence of hypertension and hyperlipidemia at baseline.

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Conflict of interest disclosure

None.

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Short Communication

Decreasing Systolic Blood Pressure Is Associated with Improving Estimated Glomerular Filtration Rate (eGFR) with Lifestyle Modification in Japanese Healthy Women

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The link between changes in a subject's metabolic syndrome components and her estimated glomerular filtration rate (eGFR) was evaluated in healthy Japanese women. We used data for 53 Japanese women (46.0 ± 10.9 years) with a 1-year follow up. eGFR was defined by a new equation developed for Japan. There were no significant relationships between eGFR and clinical parameters at baseline. Subjects were given advice for dietary and lifestyle improvement. At the 1-year follow up, eGFR was significantly increased. In addition, changes in eGFR were weakly correlated with systolic blood pressure ($r = -0.306$, $p = 0.0260$). A decrease in systolic blood pressure may be associated with improving eGFR in Japanese women.

Key words: systolic blood pressure, estimated glomerular filtration rate (eGFR), metabolic syndrome, lifestyle modification

Chronic kidney disease (CKD) is a common disorder and has become a public health challenge [1]. For example, about 20% of adults have CKD, which is defined as kidney damage or a glomerular filtration rate (GFR) $< 60\,ml/min/1.73\,m^2$ for at least 3 months regardless of cause, and 4.1% have moderate or severe CKD [2]. We have also previously reported in a cross-sectional study that the estimated glomerular filtration rate (eGFR) [3] in men with abdominal obesity and in women with hypertension was significantly lower than that in subjects without these components of metabolic syndrome [4]. However, whether decreases in metabolic syndrome components are beneficial for improving eGFR, and what effects this has on eGFR remain to be investigated in a longitudinal study.

In this study, we evaluate the link between changes in eGFR and changes in metabolic syndrome components in Japanese women with a 1-year follow up.

Subjects and Methods

Subjects. We used data for 53 Japanese women, aged 46.0 ± 10.9 years, who met the following criteria: (1) received a health check-up including special health guidance and a follow-up check-up 1-year later, (2) received anthropometric measurements, fasting blood examination and blood pressure measurements as part of the annual health check-up, (3) received no medications for diabetes, hypertension, and/or dyslipidemia, and (4) provided written informed consent (Table 1).

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Table 1 Clinical characteristics and changes in parameters with 1-year follow up

	Baseline	Follow up	p
Number of Subjects	53		
Age	46.0 ± 10.9		
Height (cm)	156.1 ± 4.3		
Body weight (kg)	62.4 ± 8.8	60.9 ± 8.5	0.0002
Body mass index (kg/m^2)	25.6 ± 3.3	25.0 ± 3.3	0.0002
Abdominal circumference (cm)	78.7 ± 8.1	76.6 ± 8.3	0.0005
Systolic blood pressre (mmHg)	121.5 ± 14.1	119.8 ± 15.4	0.2772
Diastolic blood pressure (mmHg)	76.2 ± 9.4	74.6 ± 10.8	0.2245
Triglyceride (mg/dl)	98.2 ± 65.8	95.8 ± 62.1	0.7065
HDL cholesterol (mg/dl)	64.5 ± 14.5	64.6 ± 14.9	0.9362
Blood sugar (mg/dl)	95.1 ± 9.4	93.8 ± 9.3	0.2018
Cr (mg/dl)	0.58 ± 0.09	0.56 ± 0.10	0.0148
eGFR (ml/min/1.73m^2)	90.0 ± 17.9	94.2 ± 19.9	0.0215
		Mean ± SD	

At the first health check-up, all subjects were given instructions by well-trained medical staff on how to change their lifestyle as special health guidance. Nutritional instruction was provided with a well-trained nutritionist, who planned a diet for each subject based on their data and provided simple instructions (*i. e.* not to eat too much and to consider balance when they eat). Exercise instruction was also provided by a well-trained physical therapist, who encouraged each subject to increase their daily amount of steps walked.

Ethical approval for the study was obtained from the Ethical Committee of Okayama Health Foundation.

Anthropometric and body composition measurements. Anthropometric and body compositions were evaluated based on the following parameters: height, body weight and abdominal circumference. Body mass index (BMI) was calculated by weight / [height]^2, in kg/m^2. Abdominal circumference was measured at the umbilical level in standing subjects after normal expiration [5].

Blood pressure measurements at rest. Resting systolic and diastolic blood pressures were measured indirectly using a mercury sphygmomanometer placed on the right arm of the seated participant after at least 15 min of rest.

Urine examination. Urine samples were collected from the second- morning urine (before 10 a. m.) and subjected to examination within 1 h. The urine examination was performed using urine test strips (BAYER, Tokyo, Japan). The reagent strip was dipped directly into the urine sample. Just after dipping, the sample was graded as −: negative, ±: trace positive, +: positive (30 mg/dl), 2+: positive (100 mg/dl), 3+: positive (300 mg/dl) or 4 +: positive (1,000 mg/dl) by comparison with a standard color chart found on the container's label.

Blood sampling and assays. We measured overnight fasting serum levels of creatinine (Cr) (enzymatic method), high-density lipoprotein (HDL) cholesterol, triglycerides (L Type Wako Triglyceride · H, Wako Chemical, Osaka, Japan) and plasma glucose. eGFR was calculated using the following equation: eGFR (ml/min/1.73m^2) = $194 \times Cr^{-1.094} \times Age^{-0.287} \times 0.739$ (a constant derived specifically for women) [3]. Reduced eGFR was defined as an eGFR < 60 ml/min/1.73 m^2.

Definition of metabolic syndrome. Women with an abdominal circumference in excess of 90 cm were defined as having metabolic syndrome if they also had 2 or more of the following components: 1) Dyslipidemia: triglycerides ≥ 150 mg/dl and/or HDL cholesterol < 40 mg/dl, 2) High blood pressure: blood pressure $\geq 130/85$ mmHg, 3) Impaired glucose tolerance: fasting plasma glucose ≥ 110 mg/dl [5].

Statistical analysis. Data are expressed as means ± standard deviation (SD). A statistical analysis was performed using a paired *t* test: $p < 0.05$ was considered to be statistically significant. Pearson's correlation coefficients were calculated and used to test the significance of the linear relationship among con-

tinuous variables; stepwise multiple regression analysis was also used.

Results

The clinical parameters at the baseline and the 1-year follow up are summarized in Table 1. Anthropometric and body composition parameters such as body weight, BMI and abdominal circumference were significantly reduced with lifestyle modification after 1 year. Cr was significantly decreased and eGFR was significantly increased. No subject was diagnosed as having metabolic syndrome and only one subject was diagnosed with reduced eGFR from baseline to the 1-year follow up. In addition, 2 subjects were identified as positive (+) for proteinuria at baseline and 4 subjects were identified as trace positive at the 1-year follow up.

The relationship between eGFR and clinical parameters at baseline was evaluated. There were no significant relationships between eGFR and other clinical parameters at baseline (Table 2).

Table 2 Simple correlation analysis between eGFR and clinical parameters at baseline

	r	p
Body weight (kg)	0.082	0.5594
Body mass index (kg/m^2)	0.033	0.8165
Abdominal circumference (cm)	−0.154	0.2708
Systolic blood pressre (mmHg)	−0.167	0.2333
Diastolic blood pressure (mmHg)	−0.119	0.3958
Triglyceride (mg/dl)	0.123	0.3785
HDL cholesterol (mg/dl)	−0.063	0.6566
Blood sugar (mg/dl)	−0.193	0.1662

Table 3 Simple correlation analysis between changes in eGFR and changes in clinical parameters with 1-year follow up

	r	p
Body weight (kg)	0.188	0.1775
Body mass index (kg/m^2)	0.181	0.1945
Abdominal circumference (cm)	0.253	0.0672
Systolic blood pressre (mmHg)	−0.306	0.0260
Diastolic blood pressure (mmHg)	−0.112	0.4325
Triglyceride (mg/dl)	0.095	0.5006
HDL cholesterol (mg/dl)	0.227	0.1015
Blood sugar (mg/dl)	−0.214	0.1232

We further evaluated the relationship between changes in eGFR and changes in clinical parameters. Changes in eGFR were weakly correlated with changes in systolic blood pressure ($r = -0.306$, $p = 0.0260$) (Table 3, Fig. 1). However, changes in eGFR were not significantly correlated with changes in other metabolic components. We also used stepwise multiple regression analysis to evaluate the effect of changes in clinical parameters, *i. e.* body weight, BMI, abdominal circumference, systolic blood pressure, diastolic blood pressure, triglyceride, HDL cholesterol and blood sugar, on the change in eGFR, and found that only change in systolic blood pressure was significant [Change in eGFR = 3.632 − 0.349 (change in systolic blood pressure), $r^2 = 0.093$, $p = 0.0260$].

Finally, we further investigated the difference of change in eGFR between subjects who had different levels of systolic blood pressure at baseline [Group L, systolic blood pressure < 140 mmHg; Group H, systolic blood pressure ≥ 140 mmHg]. The changes in systolic blood pressure in Group H subjects (−1.20 ml/min/1.73 m^2) was lower than that in Group L subjects (4.9 ml/min/1.73 m^2) after 1 year, but not at a significant level ($p = 0.2822$).

Discussion

The main objective of this study was to explore the link between changes in eGFR and changes in metabolic syndrome components in Japanese women with a

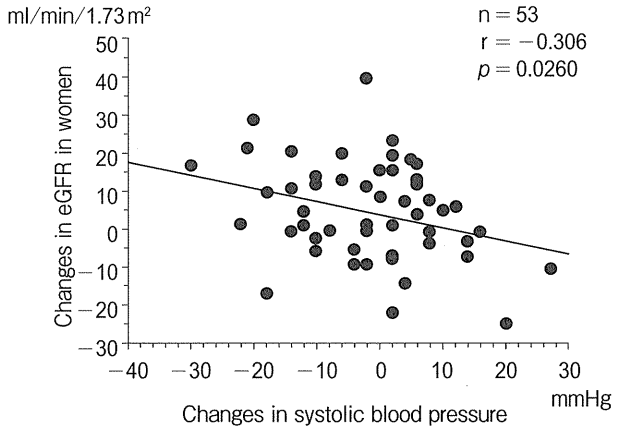

Fig. 1 Simple correlation analysis between changes in eGFR and changes in systolic blood pressure at 1-year follow up.

1-year follow up.

Tanaka *et al.* [6], Ninomiya T *et al.* [7] and Iseki *et al.* [8] reported that metabolic syndrome, using the modified ATP III definition [9], was associated with CKD in the Japanese population. Compared with subjects with 0 or 1 component of metabolic syndrome, subjects with 2, 3 and 4 or more components had odds ratios of 1.13, 1.90 and 2.79 for CKD [7]. In this study, no subject was diagnosed as having metabolic syndrome, using the Japanese criteria, either at baseline or at the 1-year follow up. We have previously reported that the prevalence of metabolic syndrome was 3.6% in Japanese women [10]. However, with lifestyle modification after the initial health check-up, eGFR was significantly increased even in women without metabolic syndrome at the 1-year follow-up.

Hypertension contributes to the development of renal injury and end-stage renal disease [11-15]. Even high-normal blood pressure has been shown to be significantly associated with development of CKD in both sexes. Yamagata *et al.* reported that the baseline-adjusted predictor of developing CKD included age, GFR, hematuria, hypertension, diabetes, serum lipids, obesity, smoking status and consumption of alcohol with 10-year follow up [11]. Tozawa *et al.* also reported a relative risk of 1.34 for end-stage renal failure for every increase of 10 mmHg in systolic blood pressure in 51,878 women investigated [12]. In the present study, there was no significant relationship between eGFR and systolic blood pressure at baseline. However, we revealed that, with lifestyle modification, changes in systolic blood pressure were correlated with changes in eGFR in women without metabolic syndrome. Therefore, the clinical impact of hypertension was noted.

Potential limitations remain in our study. First, the small sample size in our study makes it difficult to infer causality between eGFR and hypertension. Second, we also could not reveal the mechanism of the linkage between eGFR and hypertension. Further prospective studies are needed in Japanese subjects. Third, most of the enrolled subjects were not diagnosed as CKD at baseline. Therefore, the results in this study may not apply for patients with CKD.

In conclusion, a decrease in systolic blood pressure with lifestyle modification was associated with an increase in eGFR. Therefore, lifestyle modification may be a necessary and useful measure for the prevention of CKD.

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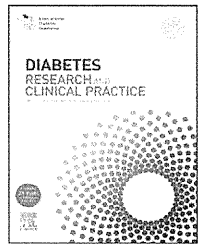

Microinflammation is a common risk factor for progression of nephropathy and atherosclerosis in Japanese patients with type 2 diabetes

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ABSTRACT

Aim: This study aimed to evaluate the change of serum levels of proinflammatory molecules in patients with type 2 diabetes and clarify the involvement of these molecules in diabetic nephropathy and atherosclerosis.

Methods: Sixty-six Japanese type 2 diabetic patients (T2DM) and 39 healthy control subjects were enrolled. We assessed clinical parameters, urinary albumin excretion rate (AER), brachial-ankle pulse wave velocity (baPWV), intima media thickness (IMT) and serum levels of proinflammatory molecules.

Results: Serum levels of IL-6, IP-10 and MCP-1 were significantly higher in T2DM than in control subjects. In T2DM, serum levels of high-sensitivity (hs) CRP, IP-10, hsTNF-α, VCAM-1 and E-selectin were positively correlated with AER. Serum levels of IP-10, hsTNF-α and VCAM-1 were positively correlated with baPWV. Serum levels of hsCRP, IL-6, IP-10 and hsTNF-α were positively correlated with IMT. Multiple linear regression analysis revealed that serum levels of hsTNF-α were independently associated with AER ($\beta = 0.235$, $P = 0.038$) and serum levels of IP-10 were independently associated with baPWV ($\beta = 0.209$, $P = 0.047$) and IMT ($\beta = 0.303$, $P = 0.032$).

Conclusion: Our results suggest that low-grade inflammation, microinflammation, may be a common risk factor for diabetic nephropathy and atherosclerosis in Japanese type 2 diabetic patients.

1. Introduction

Microinflammation, low-grade inflammation occurred on the vascular wall, is involved in the mechanism of atherosclerosis [1]. The elevation of serum C-reactive protein (CRP) level is known to be a risk factor for ischemic heart disease [2,3]. Proinflammatory molecules, such as interferon-γ inducible protein (IP)-10 are reported to be involved in the formation of atherosclerotic lesion [4]. Microinflammation is also occurred in patients with diabetes. Several reports indicated that CRP [5] and proinflammatory cytokines including interleukin (IL)-6 and tumor necrosis factor (TNF)-α [6], are elevated in patients with type 2 diabetes. Elevated levels of CRP and IL-6 predict insulin resistance and development of type 2 diabetes [7,8].

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The mechanisms for elevation of serum levels of proinflammatory molecules in type 2 diabetes remain unclear, although it might be at least partially caused by oxidative stress [9]. Activation of nuclear factor-κB through oxidative stress-induced by hyperglycemia increases concentrations of circulating proinflammatory cytokines [10].

We previously demonstrated that leukocyte adhesion molecules including E-selectin and P-selectin are up-regulated in the kidney of diabetic patients [11]. We also reported that intercellular adhesion molecules (ICAM)-1 is up-regulated in the kidney of experimental diabetic rats and mediates infiltration of macrophages into diabetic kidney [12]. In another report, serum level of vascular cell adhesion molecules (VCAM)-1 is elevated in diabetic patients [13]. Moreover, macrophage infiltration and renal injuries were prevented in ICAM-1 deficient mice after induction of diabetes [14]. These findings suggest that inflammatory process is also involved in the pathogenesis of diabetic nephropathy.

We have recently reported that serum level of IL-18 is positively correlated with urinary albumin excretion, brachial-ankle pulse wave velocity (baPWV) and intima media thickness of carotid artery (IMT) and both serum and urinary levels of IL-18 are independently correlated with urinary albumin excretion rate (AER) [15]. These findings suggest that IL-18 might be a predictor of progression of diabetic nephropathy as well as atherosclerosis. Elevated level of fibrinogen, an independent risk factor for cardiovascular disease, is also associated with AER [16]. These findings suggest that inflammatory process is involved in both atherosclerosis and diabetic nephropathy. Proinflammatory cytokines, chemokines and adhesion molecules may compose a complex network and contribute to the progression of vascular complications in diabetic patients.

The aim of this study is to evaluate the change of serum profile of proinflammatory molecules including cytokines, chemokines and adhesion molecules in patients with type 2 diabetes and clarify the involvement of these molecules in diabetic nephropathy and atherosclerosis.

2. Subjects, materials and methods

2.1. Study population

A total of 66 Japanese patients (32 females and 34 males) with type 2 diabetes were enrolled in this study. All patients were fulfilled the following inclusion criteria; initially diagnosed diabetes at over 40 of their age, negative about antibody to GAD, no history of ketoacidosis, no renal insufficiency (creatinine clearance >1.00 ml/s), never received any hormone replacement therapy. Cardiovascular disease was defined as an attack of stroke, ischemic heart disease and arteriosclerosis obliterans. Their mean age was 61.0 ± 7.8 (mean ± SD), diabetic duration 10.4 ± 6.4 years, BMI 23.9 ± 3.2 kg/m^2, HbA_{1c} 7.3 ± 1.2%.

A total of 39 age and sex matched healthy adults (24 females and 15 males) were enrolled as control subjects. Inclusion criteria for control subjects was as follows; never diagnosed diabetes, fasting plasma glucose <6.1 mmol/l, HbA_{1c} <5.8%, blood pressure <140/90 mmHg, AER <30 mg/gCr, creatinine clearance >1.00 ml/s, no medication or treatment. Their mean age was 58.1 ± 7.6, BMI 22.1 ± 2.3 kg/m^2, HbA_{1c} 5.1 ± 0.5%.

Informed consent was obtained from all participants and control subjects, and this study was approved by the Ethical Committee of Okayama Saiseikai General Hospital.

2.2. Collection of blood and urine samples

Collection of blood and urine samples was performed in the early morning after overnight fast. Creatinine clearance was determined using Cockcroft-Gault formula. AER was determined with immunoturbidimetric assay Micro Alb (Nitto Boseki Co., Ltd, Tokyo, Japan). Normoalbuminuria was defined as AER 30 mg/gCr, microalubminuria was defined as AER 30–299 mg/gCr and macroalbuminuria was defined as AER >300 mg/gCr. Blood and urine samples were centrifuged immediately after collection and the supernatants were stored at −80 °C and −30 °C until analysis, respectively. All samples were measured at one time after sample collection.

2.3. Measurements of serum proinflammatory molecules

Serum levels of IL-6 were measured using a chemiluminescent enzyme assay (CLEIA kit; Fujirebio, Tokyo, Japan). Serum levels of high-sensitivity (hs) TNF-α, IP-10, monocyte chemotactic protein (MCP)-1, ICAM-1, VCAM-1, E-selectin and L-selectin were measured using a quantitative sandwich enzyme immunoassay (R&D Systems, Minneapolis, USA). Serum P-selectin was measured using an enzyme-linked immunosorbent assay (TaKaRa, Kyoto, Japan).

2.4. Measurements of carotid IMT and baPWV

IMT of the common carotid artery was determined using duplex ultrasonography with a 7.5 MHz linear transducer (SSD-5500; Aloka, Tokyo, Japan). As we previously referred [15], carotid IMT was defined as the distance from the leading edge of the first echogenic line to the second echogenic line on a sonographic image. Measurements of IMT were made at each of the three sites of the greatest thickness on both sides. The mean of these maximal IMT measurements was used for analysis.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice with the patient in a sitting position after 5 min rest. Left and right baPWV was measured automatically using an ABI-form (BP-203RPE II; Nippon Colin, Komaki, Japan). A trained physician at Okayama Saiseikai General Hospital performed all scans. In this study, the highest values of SBP, DBP, IMT and baPWV from the left and the right sides were used for the evaluation of each patient.

2.5. Statistics

All statistical analysis was performed by SPSS for Windows statistical software system. All data are represented as mean ± SD or actual numbers. Comparison of data between type 2 diabetic patients and control subjects was evaluated using unpaired Student's *t*-test and χ^2 for sex. AER, hsCRP, IL-6, hsTNF-α, IP-10, MCP-1, ICAM-1, VCAM-1, P-selectin, E-selectin and L-selectin were not normally distributed (Shapiro–Wilkes test) and natural logarithmic transformation was used to shift

the variables into normal distribution. Correlation was evaluated using univariate and multivariate linear regression analysis. A value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. *Clinical characteristics and serum levels of proinflammatory molecules in diabetic patients and control subjects*

Clinical characteristics and serum levels of proinflammatory molecules between type 2 diabetic patients and control subjects were shown in Table 1. BMI, HDL-cholesterol, fibrinogen, hsCRP in type 2 diabetic patients were significantly higher than in control subjects. As for blood pressure, no significant valiance was observed between type 2 diabetic patients and control subjects. In type 2 diabetic patients, the number of patients with normoalbuminuria, microalbuminuria and macroalbuminuria was 42, 18 and 6, respectively. AER, baPWV and IMT in type 2 diabetic patients were significantly higher than in control subjects (AER 120 ± 480 vs. 10.2 ± 10 mg/gCr, $P < 0.001$; baPWV 17.0 ± 3.5 vs. 14.7 ± 2.5 m/s, $P < 0.001$; IMT 0.86 ± 0.19 vs. 0.73 ± 0.15 mm, $P = 0.001$). There were 11 diabetic patients with episodes of cardiovascular disease. In type 2 diabetic patients, 8 patients received dietary therapy only, 31 patients received oral hypoglycemic agents and 27 patients received insulin replacement therapy. Twenty-four patients were administered angiotensin converting enzyme inhibitors or angiotensin receptor antagonists. Fourteen patients were administered statins.

Serum IL-6 levels were significantly higher in type 2 diabetic patients than in control subjects (1.92 ± 1.24 vs. 1.32 ± 0.78 pg/ml, $P = 0.005$). There was no significant difference in TNF-α levels between type 2 diabetic patients and control subjects. Serum IP-10 levels were significantly higher in type 2 diabetic patients than in control subjects (76.5 ± 37 vs. 55.7 ± 16 pg/ml, $P = 0.004$).

Table 1 – Characteristics of type 2 diabetic patients and control subjects.

	Control subjects ($n = 39$)	Diabetic patients ($n = 66$)	P-value
Sex (female) (*n*)	24	32	0.228
Age (years)	58.1 ± 7.6	61.0 ± 7.8	0.062
BMI (kg/m^2)	22.1 ± 2.3	23.9 ± 3.2	0.001
SBP (mmHg)	125 ± 12	130 ± 15	0.064
DBP (mmHg)	77 ± 10	77 ± 9	0.954
Fasting plasma glucose (mmol/l)	5.46 ± 0.5	8.44 ± 3.3	<0.001
HbA_{1c} (%)	5.1 ± 0.5	7.3 ± 1.2	<0.001
Creatinine clearance (ml/s)	1.53 ± 0.3	1.55 ± 0.4	0.811
Total cholesterol (mmol/l)	5.69 ± 1.0	5.37 ± 0.8	0.082
HDL-cholesterol (mmol/l)	1.57 ± 0.5	1.39 ± 0.4	0.044
LDL-cholesterol (mmol/l)	3.47 ± 0.9	3.25 ± 0.8	0.180
Triglyceride (mmol/l)	1.27 ± 0.6	1.59 ± 1.1	0.101
Fibrinogen (g/l)	2.76 ± 0.4	3.01 ± 0.5	0.013
hsCRP (mg/l)	0.054 ± 0.06	0.11 ± 0.1	–
(ln)hsCRP (ln[mg/l])	−3.28 ± 1.2	−2.69 ± 0.9	0.006
Duration of diabetes (years)	–	10.4 ± 6.4	–
History of cardiovascular events (yes) (*n*)	–	11	–
Albumin excretion rate (mg/gCr)	10.2 ± 10	120 ± 480	–
(ln)AER (ln[mg/gCr])	1.92 ± 0.9	2.92 ± 1.6	<0.001
baPWV (m/s)	14.7 ± 2.5	17.0 ± 3.5	<0.001
IMT (mm)	0.73 ± 0.15	0.86 ± 0.19	0.001
Serum IL-6 (pg/ml)	1.32 ± 0.78	1.92 ± 1.24	–
(ln) serum IL-6 (ln[pg/ml])	0.12 ± 0.58	0.47 ± 0.63	0.005
Serum hsTNF-α (pg/ml)	1.18 ± 0.46	1.23 ± 0.48	–
(ln) serum hsTNF-α (ln[pg/ml])	0.06 ± 0.54	0.15 ± 0.37	0.363
Serum IP-10 (pg/ml)	55.7 ± 16	76.5 ± 37	–
(ln) serum IP-10 (ln[pg/ml])	3.98 ± 0.27	4.25 ± 0.40	0.004
Serum MCP-1 (pg/ml)	213 ± 50	249 ± 57	–
(ln) serum MCP-1 (ln[pg/ml])	5.33 ± 0.26	5.49 ± 0.23	0.001
Serum ICAM-1 (ng/ml)	225 ± 66	236 ± 62.1	–
(ln) serum ICAM-1 (ln[ng/ml])	4.39 ± 0.62	5.42 ± 0.28	<0.001
Serum VCAM-1 (ng/ml)	651 ± 181	701 ± 181	–
(ln) serum VCAM-1 (ln[ng/ml])	6.44 ± 0.27	6.52 ± 0.25	0.151
Serum P-selectin (ng/ml)	230 ± 96	243 ± 119	–
(ln) serum P-selectin (ln[ng/ml])	5.33 ± 0.26	5.37 ± 0.51	0.583
Serum E-selectin (ng/ml)	45.2 ± 21	47.2 ± 18	–
(ln) serum E-selectin (ln[ng/ml])	3.72 ± 0.42	3.78 ± 0.38	0.434
Serum L-selectin (ng/ml)	863 ± 222	799 ± 208	–
(ln) serum L-selectin (ln[ng/ml])	6.73 ± 0.26	6.65 ± 0.26	0.140

Data are mean ± SD. *P* for type 2 diabetic subjects versus control subjects.

Table 2 – Simple correlation between logarithmic AER, baPWV or IMT and clinical characteristics and serum levels of proinflammatory molecules in Japanese type 2 diabetic patients.

Variables	(ln)AER		baPWV		IMT	
	r	*P*	*r*	*P*	*r*	*P*
Sex (female) (*n*)	0.023	0.857	0.079	0.533	0.235	0.062
Age (years)	0.222	0.073	0.627	<0.001	0.242	0.054
BMI (kg/m^2)	0.161	0.198	0.191	0.130	0.231	0.066
SBP (mmHg)	0.229	0.064	0.323	0.009	−0.006	0.960
DBP (mmHg)	0.096	0.442	0.135	0.286	0.060	0.637
Fasting plasma glucose (mmol/l)	0.187	0.133	0.032	0.803	0.045	0.721
HbA_{1c} (%)	0.282	0.022	0.199	0.115	0.184	0.145
Creatinine clearance (ml/s)	−0.081	0.519	−0.095	0.458	0.058	0.653
Total cholesterol (mmol/l)	0.046	0.715	−0.028	0.828	−0.053	0.676
HDL-cholesterol (mmol/l)	−0.120	0.343	−0.024	0.855	−0.374	0.003
LDL-cholesterol (mmol/l)	−0.012	0.923	−0.088	0.492	0.163	0.198
Triglyceride (mmol/l)	0.203	0.103	0.057	0.656	0.103	0.420
(ln) hsCRP (ln[mg/l])	0.263	0.037	0.100	0.442	0.296	0.021
(ln) serum IL-6 (ln[pg/ml])	0.020	0.874	0.013	0.920	0.298	0.017
(ln) serum hsTNF-α (ln[pg/ml])	0.329	0.010	0.307	0.018	0.332	0.011
(ln) serum IP-10 (ln[pg/ml])	0.371	0.002	0.509	<0.001	0.265	0.036
(ln) serum MCP-1 (ln[pg/ml])	0.090	0.471	0.152	0.229	0.107	0.401
(ln) serum ICAM-1 (ln[ng/ml])	0.203	0.101	0.040	0.757	−0.016	0.900
(ln) serum VCAM-1 (ln[ng/ml])	0.341	0.005	0.331	0.008	−0.028	0.826
(ln) serum P-selectin (ln[ng/ml])	0.101	0.418	0.020	0.876	0.227	0.071
(ln) serum E-selectin (ln[ng/ml])	0.444	<0.001	0.018	0.889	0.156	0.218
(ln) serum L-selectin (ln[ng/ml])	−0.004	0.973	−0.006	0.960	−0.183	0.148

Data are mean ± SD. *r*, simple correlation coefficient.

Serum MCP-1 levels were significantly higher in type 2 diabetic patients than in control subjects (249 ± 57 vs. 213 ± 50 pg/ml, $P = 0.001$). Among adhesion molecules, significant difference was observed only in ICAM-1 levels between the two groups.

3.2. *Simple correlation between AER, baPWV or IMT and clinical characteristics and serum levels of proinflammatory molecules in patients with type 2 diabetes*

We investigated simple correlation between clinical characteristics or proinflammatory molecules and AER, baPWV or IMT in type 2 diabetic patients. The correlation was shown in Table 2. A significant positive correlation between AER and HbA_{1c} (correlation coefficient [r] = 0.282, $P = 0.022$), serum levels of hsCRP ($r = 0.263$, $P = 0.037$), hsTNF-α ($r = 0.329$, $P = 0.010$), IP-10 ($r = 0.371$, $P = 0.002$), VCAM-1 ($r = 0.341$, $P = 0.005$) and E-selectin ($r = 0.444$, $P < 0.001$) was observed. BaPWV correlated positively with Age ($r = 0.627$, $P < 0.001$), SBP ($r = 0.323$, $P = 0.009$), serum levels of hsTNF-α ($r = 0.307$, $P = 0.018$), IP-10 ($r = 0.509$, $P < 0.001$) and VCAM-1 ($r = 0.331$, $P = 0.008$). IMT correlated positively with HDL-cholesterol ($r = -0.374$, $P = 0.003$), serum levels of hsCRP ($r = 0.296$, $P = 0.021$), IL-6 ($r = 0.298$, $P = 0.017$), hsTNF-α ($r = 0.332$, $P = 0.011$) and IP-10 ($r = 0.265$, $P = 0.036$).

As previously reported [17,18], significant simple correlation between AER, baPWV and IMT was observed both in all participants and in type 2 diabetic patients (data not shown).

3.3. *Multiple linear regression analysis of relationships between AER, baPWV or IMT and clinical characteristics and serum levels of proinflammatory molecules*

Serum levels of hsTNF-α and IP-10 were simply correlated with AER, baPWV and IMT. We next performed multiple linear regression analysis for relationships between AER, baPWV or IMT and hsTNF-α, IP-10 and clinical parameters that were significantly correlated with AER, baPWV or IMT. The correlation was shown in Table 3. Previous history of

Table 3 – Multiple linear regression analysis of relationships between AER, baPWV or IMT and clinical characteristics and serum levels of proinflammatory molecules.

Variables	β	P
Dependent variable: (ln)AER, $R^2 = 0.498$, $P < 0.001$		
Independent variable		
Previous history of cardiovascular events	0.549	<0.001
ln serum hsTNF-α	0.235	0.038
ln hsCRP	0.137	0.265
ln serum IP-10	0.127	0.230
HbA_{1c}	0.062	0.575
Dependent variable: baPWV, $R^2 = 0.538$, $P < 0.001$		
Independent variable		
Age	0.398	<0.001
SBP	0.227	0.035
Duration of diabetes	0.210	0.041
ln serum IP-10	0.209	0.047
ln serum hsTNF-α	0.118	0.260
Dependent variable: IMT, $R^2 = 0.249$, $P = 0.016$		
Independent variable		
ln serum IP-10	0.303	0.032
ln serum hsTNF-α	0.144	0.316
HDL-cholesterol	−0.155	0.328
ln hsCRP	0.052	0.734
ln serum IL-6	0.049	0.774

β, standard correlation coefficients; R^2, multiple coefficients of determination.