adiponectin and leptin are also independent factors associated with insulin resistance [11,12]. Moreover, we showed that both triglyceride and adiponectin are associated with visceral fat areas, whereas leptin is associated with subcutaneous fat areas in these patients [11-13]. Thus, abdominal fat areas are likely to be associated with insulin resistance in Japanese type 2 diabetic patients. Not only triglyceride but also leptin and adiponectin are recognized to be associated with atherosclerosis in diabetic patients [14-16].

The metabolic syndrome is reported to be one of the conditions associated with insulin resistance and/or atherosclerosis in humans [17]. The major criteria for the metabolic syndrome, however, are emphasized on the waist circumference. Waist circumference provides a crude but effective measure of visceral fat [18]. Along with increased waist circumference, the minor criteria for the metabolic syndrome such as raised triglyceride/low high-density lipoprotein (HDL) cholesterol, high blood pressure, or high concentration of glucose are suggested to be associated with atherosclerosis in Japanese type 2 diabetic patients. Thus, it may be questioned whether the use of metabolic syndrome to assess atherosclerosis is superior to other risk factors such as hyperglycemia especially in Japanese type 2 diabetic patients. It has been established that hyperglycemia per se is associated with the development of atherosclerosis in diabetic patients. To clarify this, we recruited Japanese type 2 diabetic patients who had no major evidence of atherosclerosis to compare the degree of atherosclerosis between the diabetic patients with and without metabolic syndrome syndrome, taking into account BMI and hemoglobin A_{1c} (HbA_{1c}).

2. Subjects and methods

Fifty-seven Japanese type 2 diabetic patients with BMI of less than 27 kg/m^2 who were well controlled in terms of glycosylated hemoglobin (HbA_{1c}) (7.1% ± 0.1%, mean ± SEM) were enrolled. Type 2 diabetes mellitus was diagnosed based on the World Health Organization criteria [19]. They had no evidence of current acute illness including clinically significant infectious disease. The duration of diabetes was 10.9 ± 1.0 years (range, 1-35 years). Of 57 diabetic patients, 52 were taking sulfonylureas and the rest were treated with diet alone. They had not been treated with insulin or any medications known to alter insulin sensitivity. All subjects had ingested at least 150 g of carbohydrate for the 3 days preceding the study. None of the subjects had significant renal, hepatic, or cardiovascular disease (CVD). Patients did not consume alcohol or perform heavy exercise for at least 1 week before the study.

Metabolic syndrome was diagnosed by the criteria raised by the Japan Internal Medicine Society. Although the use of waist circumference to assess abdominal adiposity is superior to BMI, the cutoff value for waist circumference is likely to be population-specific as there are clear differences across ethnic populations in the relationship between overall adiposity, abdominal adiposity, and visceral fat accumulation. The major criterion in Japanese population is waist circumference of greater than 85 cm in men and greater than 90 cm in women. The minor criteria is as follows: serum triglyceride of ≧150 mg/dL or HDL cholesterol of <40 mg/dL, blood pressure of ≧130/85 mm Hg, and fasting glucose concentration of ≧110 mg/dL. The patients who had both 1 major criteria and 2 or 3 minor criteria were diagnosed as having metabolic syndrome.

Blood was drawn in the morning after a 12-hour fast. Plasma glucose was measured with a glucose oxidase method. The triglycerides, total cholesterol, and HDL cholesterol were also measured. Serum insulin was measured using a 2-site immunoradiometric assay (Insulin Riabead II, Dainabot, Japan). Coefficients of variation were 4% for insulin of greater than 25 μU/mL and 7% for insulin of less than 25 μU/mL, respectively. Serum adiponectin and leptin were measured with a radioimmunoassay kit (Linco Research, St Charles, MO). The intra- and interassay coefficients of variation (CVs) were less than 5% for adiponectin and leptin, respectively. Serum tumor necrosis factor α (TNF-α) concentrations were measured with an enzyme immunoassay kit (Quantikine HS Human TNF-α Immunoassay Kit, R&D Systems, Minneapolis, MN), and serum concentrations of soluble TNF receptor 1 (sTNF-R1) and soluble TNF receptor 2 (sTNF-R2) were measured with an enzyme-linked immunosorbent assay (BIOTRAK, Amersham Life Sciences, Uppsala, Sweden), as described previously [20]. The limits of sensitivity for TNF-α, sTNF-R1, and sTNF-R2 were 0.5, 25, and 50 pg/mL, respectively. The intra-assay CVs for TNF-α, sTNF-R1, and sTNF-R2 were 5.9%, 4.7%, and 3.2%, respectively. The interassay CVs for TNF-α, sTNF-R1, and sTNF-R2 were 10.8%, 5.8%, and 3.6%, respectively. Samples for insulin, adiponectin, leptin, and TNF were prepared, frozen, and stored at −70°C until the assay.

The estimate of insulin resistance index of homeostasis model assessment (HOMA-IR) was calculated with the formula: fasting serum insulin (μU/mL) × fasting plasma glucose (mmol/L)/22.5 [21]. The insulin resistance index of homeostasis model assessment was validated in diabetic patients treated with diet therapy alone and in those treated with sulfonylureas [22,23]. Therefore, we estimated HOMA-IR in diet-treated and sulfonylurea-treated diabetic patients.

Along with ultrasonographically measured carotid atherosclerosis, brachial-ankle pulse wave velocity (ba-PWV) and ankle brachial index (ABI) were used to assess the degree of atherosclerosis.

A carotid sonography was performed with high-resolution B-mode scanning equipment (Logic 500 GE Yokogawa, Milwaukee, WI) with a 7.5-MHz sector scanner probe [24]. The common carotid arteries of both sides were examined with longitudinal and transverse scans because we could not analyze the internal and external carotid arteries fully in all

patients. The CV for interobserver variability was found to be 8.5% and the CV for intraobserver variability was 6.0%. The intimal-medial thickness (IMT) of the common carotid artery was measured in plaque-free segments as the distance from the leading edge of the first echogenic line to that of the second echogenic line. The mean of IMT in plaque-free segments of bilateral common carotid arteries was used for the analysis. The degree of stenosis was also measured in the plaque segments of bilateral common carotid arteries. It was calculated as a percentage ratio between the area of the plaque and that of the lumen using the formula: (lumen area − residual lumen area)/lumen area × 100. Both the areas were automatically measured by the system on a frozen transverse scanning plane at the site of maximal narrowing. When 2 or more plaques were present in the vessel, only that causing the greatest degree of stenosis was considered for analysis.

Brachial-ankle PWV and ABI were measured using a volume-plethysmographic apparatus (from PWV/ABI version-112, Colin, Komaki, Japan). Briefly, after an overnight fast, the subjects were examined in the supine position, with electrocardiogram electrodes placed on both wrists, a microphone for detecting heart sounds placed on the left edge of the sternum, and cuffs wrapped on both the brachia and ankles. The characteristic points of waveforms were determined automatically, and the results were printed out. All procedures took about 5 minutes. The interobserver and intraobserver variation coefficients were 8.4% and 10.0%, respectively. Measurements on different days revealed that slight changes in blood pressure did not correlate with changes in ba-PWV. The mean ba-PWV and ABI values measured on either side of each patient were used for the analysis.

2.1. Data analysis

Data were presented as means ± SEM. Statistical analysis was conducted using the StatView 5 system (Statview, Berkeley, CA). The means of 2 groups were compared using Student t test. $P < .05$ was considered as significant.

3. Results

The subjects studied were all Japanese type 2 diabetic patients (40 men and 18 women) with an age range of 43 to 79 years (62.7 ± 1.1 years) and a BMI of 17.1 to 26.7 kg/m^2 (23.0 ± 0.3 kg/m^2). The fasting plasma glucose was 143 ± 3 mg/dL and HbA_{1c} was 7.1% ± 0.1%. Fasting insulin level was 6.8 ± 0.4 μU/mL. Serum triglycerides and total and HDL cholesterol levels were 119 ± 7, 208 ± 5, and 61 ± 2 mg/dL, respectively. Serum adiponectin and leptin concentrations were 13.6 ± 1.2 μg/mL and 5.8 ± 0.5 ng/mL, respectively. The concentrations of TNF-α, sTNF-R1, and sTNF-R2 were 3.1 ± 0.2, 1132 ± 36, and 2009 ± 54 pg/mL, respectively. On the other hand, there was a wide variation in insulin resistance calculated from HOMA-IR in our diabetic patients (range, 0.71-6.10; 2.40 ± 0.16). Of 57 patients, 24 (41%) patients had HOMA-IR of greater than 2.5, indicating that they are insulin resistant [9,10]. Intimal-medial thickness in plaque-free segments of carotid artery, carotid stenosis in plaque segments, ba-PWV, and ABI were 0.72 ± 0.02 mm (range, 0.40-1.10 mm), 6.6% ± 1.6% (range, 0%-54.5%), 1664 ± 35 cm/s (range, 1139-2294 cm/s), and 1.15 ± 0.01 (range, 1.02-1.26), respectively.

Table 1 shows the clinical profile between the patients with and without metabolic syndrome. Of the 57 patients, 25 were diagnosed as having metabolic syndrome. These patients had significantly higher levels of waist circumference, HOMA-IR, systolic and diastolic blood pressures, and serum triglycerides, but significantly lower concentrations of adiponectin as compared with those without metabolic syndrome. No significant difference was observed in age, sex, fasting glucose, leptin, and HbA_{1c} between the two. The concentrations of TNF-α, sTNF-R1, and sTNF-R2 were not significantly different between the 2 groups. There was no significant difference in the degree of carotid atherosclerosis (IMT in plaque-free segments: 0.72 ± 0.03 vs 0.72 ± 0.02 mm, $P = .435$; carotid stenosis in plaque segments: 6.6% ± 3.0% vs 6.6% ± 1.7%, $P = .497$), ba-PWV (1676 ± 56 vs 1654 ± 44, $P = .380$), and ABI (1.16 ± 0.01 vs 1.15 ± 0.01, $P = .245$) between the 2 groups.

Table 1
Clinical characteristics of the diabetic patients included in the study

	Metabolic syndrome (+)	Metabolic syndrome (−)	P
No. of subjects	25	33	–
Waist (cm)	89.6 ± 0.8	77.3 ± 1.2	<.001
Age (y)	62.1 ± 1.8	63.2 ± 1.3	.316
Male/female	20/5	20/12	.071
HOMA-IR	2.71 ± 0.23	2.17 ± 0.20	<.05
Diabetes duration (y)	10.3 ± 1.4	11.1 ± 1.4	.351
Smoking (%)	20	21	.486
SU/diet	22/3	29/3	.343
BMI (kg/m^2)	24.0 ± 0.4	22.2 ± 0.4	<.001
Systolic blood pressure (mm Hg)	143 ± 3	133 ± 3	<.05
Diastolic blood pressure (mm Hg)	88 ± 2	81 ± 2	<.005
Fasting glucose (mg/dL)	141 ± 4	145 ± 4	.237
Fasting insulin (μU/mL)	7.7 ± 0.6	6.1 ± 0.6	<.05
HbA_{1c} (%)	7.0 ± 0.2	7.2 ± 0.2	.227
Triglycerides (mg/dL)	134 ± 12	108 ± 9	<.05
Total cholesterol (mg/dL)	208 ± 7	207 ± 7	.473
HDL cholesterol (mg/dL)	57 ± 3	63 ± 3	.062
LDL cholesterol (mg/dL)	131 ± 6	127 ± 6	.347
adiponectin (μg/mL)	10.7 ± 1.1	15.5 ± 1.9	<.05
Leptin (ng/mL)	6.2 ± 0.8	5.4 ± 0.7	.242
TNF-α (pg/mL)	3.4 ± 0.3	2.9 ± 0.2	.065
sTNF-R1 (pg/mL)	1118 ± 46	1143 ± 52	.366
sTNF-R2 (pg/mL)	1971 ± 68	2036 ± 78	.276
IMT (mm)	0.72 ± 0.03	0.72 ± 0.02	.435
Stenosis (%)	6.6 ± 3.0	6.6 ± 1.7	.497
ba-PWV (cm/s)	1676 ± 56	1654 ± 44	.380
ABI	1.16 ± 0.01	1.15 ± 0.01	.245

SU indicates sulfonylurea; LDL indicates low-density lipoprotein.

4. Discussion

Type 2 diabetes mellitus is a syndrome characterized by insulin resistance and/or defective insulin secretion [1]. There seems to be ethnic difference in insulin resistance in type 2 diabetes mellitus. Haffner et al surveyed the prevalence of white type 2 diabetic patients and found that 92% of type 2 diabetic patients were insulin resistant [7]. Chaiken et al [25] reported that 60% of type 2 diabetic patients with BMI of less than 30 kg/m^2 were insulin resistant in African American populations. We recently demonstrated that 40% of type 2 diabetic patients are insulin resistant in Japanese type 2 diabetic patients [9,10]. Thus, Japanese type 2 diabetic patients are considered to have a unique feature, specifying that they are divided into 2 categories: one with insulin resistance and the other with normal insulin sensitivity [4-6,9,10]. This idea was reconfirmed in the present study.

Another unique feature of Japanese type 2 diabetic patients is that they are not always massively obese. We previously showed that the mean BMI in representative epidemiological studies of Japanese type 2 diabetic patients are 23 to 25 kg/m^2, lower than in the studies of other ethnic populations such as whites [8]. Thus, Japanese type 2 diabetic patients are hypothesized to have another fascinating feature in terms of insulin resistance and atherosclerosis as compared with other ethnic populations.

In the present study, we first found that metabolic syndrome is associated with insulin resistance but not always associated with atherosclerosis in Japanese type 2 diabetic patients. This is a surprising finding because it is a commonly held belief that metabolic syndrome is an important cluster of metabolic abnormalities linked with insulin resistance and CVD [17].

One possible explanation is that the waist circumference, the major criteria for the metabolic syndrome, might not be an accurate measure of intra-abdominal fat areas in Japanese type 2 diabetic patients who are not massively obese. Fujimoto et al [26] previously demonstrated that visceral adiposity, blood pressure, and plasma glucose, but not abdominal circumference, are independent risk factors for incident coronary heart disease in Japanese-American diabetic patients. The BMI of their patients (25.8 kg/m^2) was similar to that of our patients.

The second possible explanation is because of the clinical characteristics or to the degree of atherosclerosis in our patients. The patients studied had no significant CVD and were not accompanied by any major significant abnormalities in the ultrasonographically measured carotid atherosclerosis, PWV, and ABI. The range of IMT, carotid stenosis, PWV, and ABI were 0.4 to 1.1 mm, 0% to 54.5%, 1139 to 2294 cm/s, and 1.02 to 1.26, respectively. Therefore, the association between metabolic syndrome and the degree of atherosclerosis would probably be higher in a population-based study in which the patients with CVD were included in this study.

The third possible explanation is that inflammation including TNF-α and/or hyperglycemia rather than insulin resistance may have unfavorable effects on the atherosclerotic change in Japanese type 2 diabetic patients. It is reported that high glucose can activate monocytes and induce the expression of TNF-α via oxidant stress and nuclear factor–κB transcription factor [27]. Shai et al [28] demonstrated that sTNF-R2 is strongly associated with the risk of coronary heart disease in patients with type 2 diabetes mellitus. Rauchhaus et al [29] demonstrated that elevated sTNF-R1 has shown to be predictive of cardiovascular mortality in patients with chronic heart failure. We recently found that sTNF-R1 was associated with albuminuria in Japanese type 2 diabetic patients [30]. In the present study, we could not find any significant differences in TNF-α system activities (TNF-α, sTNF-R1, sTNF-R2) between the 2 groups. It should be noted that TNF-α system activities are not associated with insulin resistance in Japanese type 2 diabetic patients with BMI of less than 27.0 kg/m^2 [20]. Alternatively, the long-standing diabetic state per se is such a powerful factor on atherosclerosis so that the effect of other risk factors including metabolic syndrome is masked. This idea is supported by the results from the recent 11-year follow-up investigation shown by Bruno et al [31] that diabetic patients with metabolic syndrome had similar all-cause and CVD mortality as compared with those without metabolic syndrome.

Irrespective of this, our present study showed that metabolic syndrome, an insulin-resistant state, is not associated with carotid atherosclerosis, ba-PWV, or ABI in Japanese type 2 diabetic patients. In this respect, Kahn et al [32] very recently warns that clinicians should evaluate and treat all CVD risk factors without regard to whether a patient meets the criteria for diagnosis of the metabolic syndrome.

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Factors responsible for age-related elevation in fasting plasma glucose: a cross-sectional study in Japanese men

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Abstract

To evaluate the factors associated with age-related increase in fasting plasma glucose (FPG) in Japanese men with normal fasting glucose, we measured FPG, fasting immunoreactive insulin, glycated hemoglobin, total cholesterol, triglyceride, and high-density lipoprotein cholesterol levels in health check examinees. Subjects with FPG less than 6.1 mmol/L together with glycated hemoglobin less than 5.6% were enrolled in the study. The homeostasis model assessment of insulin resistance (HOMA-IR) and HOMA-β were used as the indices of insulin sensitivity and insulin secretion, respectively. Fasting plasma glucose increased significantly with age ($r = 0.30$, $P < .0001$), and HOMA-β decreased significantly with age ($r = 0.24$, $P < .0001$). The HOMA-IR had no significant relation with age ($r = 0.06$, not significant), whereas body mass index and serum triglyceride were associated with HOMA-IR ($r = 0.49$, $P < .0001$ and $r = 0.33$, $P < .0001$, respectively). Thus, in Japanese male subjects with normal fasting glucose, it is suggested that the FPG increment with age is associated with decreased β-cell function rather than with insulin resistance. Further analyses were performed by comparing 3 groups: low FPG (FPG <5.0 mmol/L), high FPG (5.0 ≤ FPG < 5.6 mmol/L), and mild impairment of fasting glycemia (mild IFG) (5.6 ≤ FPG < 6.1 mmol/L). The insulin levels in mild IFG and high FPG were significantly higher than in low FPG ($P < .001$), but those in mild IFG were similar to those in high FPG. Analysis of the 3 subgroups revealed that, whereas insulin sensitivity was impaired more in high FPG, there was little compensatory increase in insulin in mild IFG, suggesting that β-cell function is already deteriorated when the FPG level is greater than 5.6 mmol/L.

1. Introduction

Type 2 diabetes mellitus is characterized by both decreasing insulin secretion and insulin sensitivity, partly due to genetic factors [1-3]. Although diabetes is a worldwide health problem [4], it is clear that there are ethnic differences in the pathophysiology of the decreasing glucose tolerance characteristic of its development [5]. Factors responsible for glucose intolerance occur from a prediabetic state: impaired glucose regulation according to the World Health Organization classification. Impaired glucose regulation comprises 2 subgroups: impaired fasting glycemia (IFG) characterized by increasingly impaired fasting plasma glucose (FPG) with 2-hour plasma glucose (2h-PG) within normal limits and impaired glucose tolerance (IGT) characterized by increasingly impaired 2h-PG [6,7]. We previously reported that insulin secretory capacity and insulin sensitivity are both decreased in Japanese subjects with IFG [8-10]. Although β-cell function and insulin sensitivity may well begin to deteriorate earlier, there are few studies of the normal glucose tolerance (NGT) population. Fasting plasma glucose is known to increase with age [11], and both insulin secretory capacity and insulin

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sensitivity are reported to decrease with age [12-14]. We have reported that some subgroups of Japanese NGT subjects show especially decreased β-cell function [15]. However, it is unclear whether deteriorated insulin secretion or insulin sensitivity is the primary factor in the increase in FPG during the period of development from NGT to IFG in Japanese.

In addition, the American Diabetes Association (ADA) lowered the cutoff value of IFG from 6.1 to 5.6 mmol/L [16]. Subjects with FPG from 5.6 to 6.1 mmol/L and with normal postprandial glucose level are categorized as having IFG in the ADA criteria, although they are categorized as having NGT in the criteria of the World Health Organization and the Japanese Diabetes Association. Thus, analysis of these subjects with mild IFG (mild impairment of fasting glucose) in view of insulin secretion and insulin sensitivity is crucial to elucidate the characteristic of subjects with borderline glucose dysregulation. To investigate the pathogenesis of prediabetes in Japanese, we compared insulin secretory capacity and insulin sensitivity in health check examinees exhibiting normal fasting glucose (NFG).

2. Subjects and methods

2.1. Subjects

Among health check examinees between 1993 and 2004 at Kyoto University Hospital, Kansai-Denryoku Hospital, and Kyoto Preventive Medical Center, 657 male subjects with FPG <6.1 mmol/L and glycated hemoglobin (HbA_{1c}) <5.6% were enrolled in the study (Table 1). Subjects with known history or signs of diabetes, previous gastrointestinal operation, liver disease, renal failure, endocrine disease, malignancy, hypertension, frequent heavy exercise, or history of medications before the study were excluded.

2.2. Measurements

Physical measurement (body height, body weight) and laboratory measurements (urine test, FPG, fasting immunoreactive insulin [F-IRI], HbA_{1c}, total cholesterol [TC], triglyceride [TG], and high-density lipoprotein cholesterol [HDL-C] level) were taken. The study was designed in compliance with the ethics regulations of the Helsinki Declaration. Blood samples were collected after overnight fasting for 16 hours [8]. Plasma glucose levels were measured by glucose oxidase method using the Hitachi Automatic Clinical Analyzer 7170 (Hitachi, Tokyo, Japan). Serum insulin levels were measured by radioimmunoassay (RIA beads II; Dainabot, Tokyo, Japan), which shows low cross-reaction with C-peptide of less than 0.005% and proinsulin less than 0.5% [8]. Glycated hemoglobin levels were measured by high-performance liquid chromatography methods. Serum TC, TG, and HDL-C levels were measured as reported previously [17]. To evaluate insulin resistance, we used the homeostasis model assessment of insulin resistance index (HOMA-IR) calculated by the formula FPG (in millimoles per liter) × IRI (in microunits per milliliter)/22.5. The HOMA-IR is a reliable measure of insulin resistance, correlating well with values obtained by glucose clamp and minimal model studies [18-20]. To calculate pancreatic β-cell function (HOMA β-cell), we used the formula 20 × IRI (in microunits per milliliter)/[FPG (in millimoles per liter) − 3.5] [18].

Table 1
Clinical characteristics of the subjects with NFG

	Data
n	657
Age (y)	44.9 ± 11.2
BMI (kg/m^2)	23.6 ± 2.8
HbA_{1c} (%)	4.8 ± 0.3
FPG (mmol/L)	5.1 ± 0.4
F-IRI (μU/mL)	5.2 ± 2.9
TC (mmol/L)	5.19 ± 0.88
TG (mmol/L)	1.45 ± 1.01
HDL-C (mmol/L)	1.45 ± 0.35

Data are mean ± SD.

2.3. Statistical analysis

Clinical data are expressed as mean ± SD. Analyses were performed using the STATVIEW 5 system (StatView, Berkeley, CA). Multiple regression analysis was used to compare age and FPG, HOMA-β, HOMA-IR, and body mass index (BMI). The same analysis was performed between HOMA-IR and BMI and TG. The NFG group was divided into low and high FPG and mild IFG, and the metabolic profiles were compared using analysis of variance. The data are expressed as mean ± SE. $P < .05$ is considered significant.

3. Results

3.1. Characteristics of the study population

As shown in Table 1, the mean age of the subjects is 44.9 ± 11.2 years and the mean BMI is 23.6 ± 2.8 kg/m^2. Among them, the number of subjects with BMI more than 30 are 22 (3.4%), concomitant with the representative epidemiologic studies in Japanese [21-23].

3.2. Correlation between age and FPG, HOMA-β, and HOMA-IR

Fig. 1A shows a positive relationship of FPG with age ($r = 0.30$, $P < .0001$; FPG [in millimoles per liter] = 0.011 × age + 4.6). Fig. 1B shows that HOMA-β has a negative correlation with age ($r = 0.24$, $P < .0001$), whereas there is no significant correlation between HOMA-IR and age ($r = 0.06$, not significant).

3.3. Correlation between HOMA-IR and BMI and serum TG levels

Fig. 2A, B shows that BMI and serum TG levels are associated with HOMA-IR ($r = 0.49$, $P < .0001$ and $r = 0.33$,

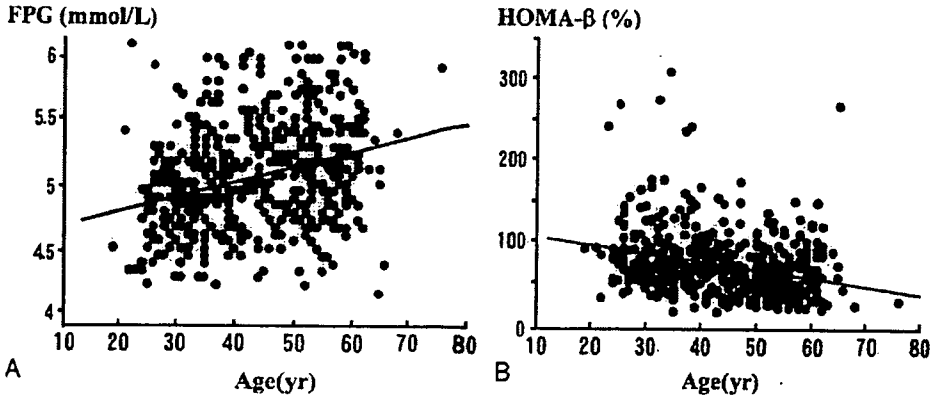

Fig. 1. Distribution of FPG (A) and HOMA-β (B) cell by age. The FPG increases with age ($r = 0.30$, $P < .0001$). The HOMA-β cell is negatively correlated with age ($r = 0.24$, $P < .0001$).

$P < .0001$, respectively). Multiple regression analysis shows that both BMI and TG are independently associated with HOMA-IR (standardized $\beta = 0.41$ and 0.15, respectively). Body mass index was the strongest determinant of HOMA-IR, and BMI did not increase with age significantly in Japanese men ($r = 0.07$, not significant).

3.4. Analysis of 3 subgroups of NFG subjects

To evaluate the factors involved in increasing FPG in Japanese NFG and the ADA recommendation of lowering the threshold of upper limit of normal FPG from 6.1 to 5.6 mmol/L [16], we divided our NFG subjects into 3 subgroups: low FPG (FPG <5.0 mmol/L), high FPG (5.0 ≤ FPG < 5.6 mmol/L), and mild impairment of fasting glucose (mild IFG) (5.6 ≤ FPG < 6.1 mmol/L); and age, BMI, TG, and insulin secretion and sensitivity were compared. As shown in Table 2, high FPG and mild IFG have higher age and BMI than low FPG (both $P < .0001$). Insulin in high FPG and mild IFG is increased compared with that in low FPG ($P < .001$); insulin in mild IFG is similar to that in high FPG. The HOMA-IR in high FPG and mild IFG is increased compared with that in low FPG ($P < .0001$). The HOMA-β in high FPG and mild IFG is decreased compared with that in low FPG ($P < .0001$); the HOMA-β in mild IFG is decreased compared with that in high FPG ($P < .001$).

4. Discussion

In this study, we analyzed the factors responsible for age-related elevation of FPG in Japanese men with NFG. Fasting plasma glucose was found to increase with age primarily because of reduced β-cell function rather than increased insulin resistance. In addition, we have elucidated that there was no compensatory increase in insulin secretion in mild IFG (FPG 5.6-6.1 mmol/L).

Our study subjects were composed only of men because the number of female subjects was 158, which is not comparable with male subjects. Some reports showed a difference between men and women in the elevation of FPG [24-26], and another showed similar results between men and women in the elevation of FPG [27]. We analyzed the results from our 158 female subjects, and we could not find

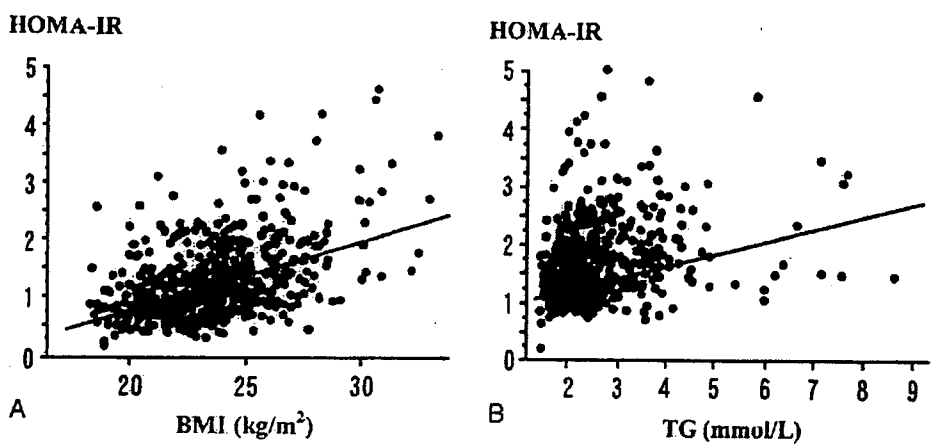

Fig. 2. Distribution of HOMA-IR by BMI (A) and TG (B). Both BMI and TG are associated with HOMA-IR (BMI: $r = 0.49$, $P < .0001$; TG: $r = 0.33$, $P < .0001$).

Table 2
Comparison of 3 FPG subgroups of NFG subjects

	Low FPG (FPG <5.0 mmol/L)	High FPG (5.0 ≤ FPG < 5.6 mmol/L)	Mild IFG (5.6 ≤ FPG < 6.1 mmol/L)
n	268	288	101
Age (y)	42.0 ± 0.7	45.7 ± 0.6[a]	49.8 ± 1.0[a,b]
BMI (kg/m^2)	23.0 ± 0.2	23.9 ± 0.1[a]	24.3 ± 0.3[a]
TC (mmol/L)	5.07 ± 0.05	5.23 ± 0.05[c]	5.35 ± 0.08[d]
TG (mmol/L)	1.30 ± 0.05	1.55 ± 0.06[d]	1.56 ± 0.09[c]
HDL-C (mmol/L)	1.45 ± 0.02	1.44 ± 0.02	1.45 ± 0.03
F-IRI (μU/mL)	4.6 ± 0.2	5.7 ± 0.2[a]	5.6 ± 0.3[c]
HOMA-IR	0.96 ± 0.04	1.31 ± 0.04[a]	1.44 ± 0.07[a]
HOMA-β (%)	78.5 ± 3.1	65.2 ± 1.9[a]	49.2 ± 2.4[a,b]

Data are mean ± SE.
[a] $P < .0001$ vs low FPG.
[b] $P < .001$ vs high FPG.
[c] $P < .05$ vs low FPG.
[d] $P < .005$ vs low FPG.
[e] $P < .0005$ vs low FPG.

remarkable differences with male subjects (data not shown). Further studies are necessary to elucidate the sex difference of the factors responsible for elevation of FPG. Although some reports showed an increase in insulin resistance in subjects older than 70 years, our male subjects were younger than 70 years. Insulin resistance in subjects older than 70 years was reported mainly because of the change in abdominal adiposity [28,29]; and in representative epidemiologic studies such as the Funagata study and the Hisayama study, the mean age of developing glucose intolerance is around 50 years in Japanese [21-23]. For these reasons, our subjects being around the age of 50 years was enough for our purpose in this study of elucidating the factors responsible for FPG elevation from normal to borderline glucose dysregulation.

Fasting plasma glucose increased by 0.011 mmol/L per year, in accord with previous reports [30]. The HOMA-β decreased by 0.85% per year, clearly indicating reduced basal insulin secretion. Although previous studies in whites and in other populations have found that insulin resistance is closely associated with age-related FPG elevation [12,31], HOMA-IR did not increase with age significantly in our subjects. To characterize the insulin resistance of our study population, we performed both simple and multiple regression analyses between HOMA-IR and the other measured factors. The BMI and serum TG levels were strongly associated with HOMA-IR ($P < .0001$), in accord with our previous results in Japanese diabetic patients [32]. Although BMI was the strongest determinant of HOMA-IR, it did not increase with age; the mean BMI of 23.6 kg/m^2 is in accord with Japanese statistical data [21-23] and is much lower than in whites [33,34]. The BMI of Asians in other studies is also reported to be lower, suggesting a common metabolic profile [35]. The leaner Japanese subjects in this study might therefore be expected to be less influenced by insulin resistance in comparison with whites.

Impaired fasting glycemia is a prediabetic state characterized by FPG elevation without increased 2h-PG. We previously reported that insulin secretory capacity and insulin sensitivity are both already decreased in IFG [8-10], suggesting the clinical importance of early deterioration of β-cell function and insulin sensitivity in developing prediabetes. In addition, we regarded the PG level of 5.6 mmol/L as an important FPG threshold value according to ADA recommendation [16]. Therefore, we compared insulin secretion and insulin sensitivity in 3 subgroups of NFG subjects: low FPG (FPG <5.0 mmol/L), high FPG (5.0 ≤ FPG < 5.6 mmol/L), and mild IFG (5.6 ≤ FPG < 6.1 mmol/L). Insulin secretion in mild IFG was not increased compared with that in high FPG, indicating impaired compensatory insulin secretion against increasing insulin resistance. Some reports have found that early-phase insulin secretion and insulin sensitivity are both decreased in NGT at a higher range of FPG (FPG >5.1-5.3 mmol/L) [36-38]. Fortunately, we could analyze 56 subjects during the 8-year follow-up period using oral glucose tolerance test results [39]. The subjects who developed from NFG to IFG showed decreasing insulin sensitivity and insulin secretory capacity, and those who developed from NFG to IGT showed decreased early insulin secretory response. These follow-up data were compatible with our previous data of IFG and IGT [5,8,10,39]. Taken together, these data indicate that insulin secretory capacity is already decreased in NGT at the higher range of FPG and that a lack of compensatory insulin secretion appears at greater than 5.6 mmol/L in FPG.

We find in Japanese NFG subjects that age-related FPG elevation is mainly due to decreased β-cell function rather than to increasing insulin resistance as in white subjects. In addition, analysis of 3 degrees of increasing FPG indicates that failure of compensatory insulin secretion is responsible for the elevation in FPG in these subjects. Thus, these data could be helpful in reconsideration of the threshold FPG for prediabetes to be recommended by the ADA [16]. However, decreasing the upper threshold of FPG entails increasing the IFG population, a costly social health problem [40]. Further studies are required to clarify the ethnic differences in the development of diabetes and diabetic complications and the value of clinical interventions in newly diagnosed IFG patients.

Acknowledgment

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Soluble tumor necrosis factor receptor 2 is independently associated with pulse wave velocity in nonobese Japanese patients with type 2 diabetes mellitus

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Abstract

The aim of the present study was to investigate the factors contributing to pulse wave velocity (PWV) in patients with type 2 diabetes mellitus. We focused on tumor necrosis factor (TNF) including soluble TNF receptors (sTNF-R1, sTNF-R2) in this study because TNF seems to be associated with the progression of atherosclerosis and because the relationships between PWV and TNF were not yet examined in type 2 diabetic patients. Univariate regression analyses showed that PWV was positively correlated with age ($r = 0.492$, $P < .001$), diabetes duration ($r = 0.251$, $P = .021$), systolic ($r = .595$, $P < .001$) and diastolic ($r = 0.248$, $P = .022$) blood pressure, antihypertensive medication ($r = 0.268$, $P = .013$), and the concentrations of sTNF-R1 ($r = 0.354$, $P = .001$) and sTNF-R2 ($r = 0.415$, $P < .001$). Although there was a positive correlation between TNF-a and sTNF-R1 ($r = 0.382$, $P < .001$) or sTNF-R2 ($r = 0.394$, $P < .001$), TNF-a was not associated with PWV. Other variables including gender were not associated with PWV. Multiple regression analyses showed that PWV was independently predicted by the level of age ($F = 15.1$), systolic blood pressure ($F = 31.6$), and sTNF-R2 ($F = 5.2$), which explained 49.2% of the variability of PWV. From these results, it can be concluded that serum soluble TNF receptor is an important independent factor associated with aortic PWV in type 2 diabetic patients.

1. Introduction

Type 2 diabetes mellitus is associated with high mortality and morbidity due to atherosclerosis including coronary heart disease (CHD). As regards the risk factors responsible for the evolution of atherosclerosis in diabetic patients, Bierman [1] previously estimated that typical risk factors including blood pressure, cholesterol, and smoking can account for no more than 25% to 30% of excess cardiovascular risk factors in diabetic patients. Thus, other factors seem to play a major role in the progression of atherosclerosis in diabetes.

A number of studies have identified abnormalities of arterial stiffness in subjects with diabetes [2-4]. It has recently been reported that aortic stiffness measured by pulse wave velocity (PWV) is highly predictive of cardiovascular mortality in subjects with type 2 diabetes mellitus [5]. PWV also predicts cardiovascular mortality in nondiabetic subjects [6]. Whereas age and blood pressure are shown to be associated with PWV, age and blood pressure alone do not completely account for the abnormalities of aortic stiffness in subjects with type 2 diabetes mellitus.

Tumor necrosis factor a (TNF-a), the proinflammatory cytokine, seems to be associated with the progression of

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atherosclerosis in type 2 diabetes mellitus. There is, however, a paucity of the literature regarding the relationship between TNF-a and atherosclerosis in type 2 diabetic patients. We recently demonstrated that TNF-a system activity, especially soluble TNF-R1 (sTNF-R1), is strongly and independently associated with albuminuria in type 2 diabetic patients [7]. Klein et al [8] demonstrated that TNF-a influences the metabolism of glycosaminoglycans, which are components of the vascular endothelium and the glomerular basement membrane and are involved in the etiology of microalbuminuria. Shai et al [9] demonstrated that sTNF-R2 is strongly associated with risk of CHD in patients with type 2 diabetes mellitus. It is therefore hypothesized that there is a causal relationship between TNF-a and vascular complications in diabetic patients. To the best of our knowledge, however, the relationships between serum soluble TNF receptor and PWV have not yet been evaluated in type 2 diabetes mellitus.

In this context, a major problem is that atherosclerotic disease such as CHD, renal failure, stroke, and peripheral arterial occlusive disease of the lower extremities might affect PWV. Moreover, it is well recognized that being overweight or hyperglycemic per se might affect serum concentrations of TNF-a and soluble TNF receptor in humans [10,11]. To disclose the mechanisms responsible for the early stage of atherosclerosis, we recruited nonobese, well-controlled, unique Japanese type 2 diabetic patients who had no evidence of vascular complications including CHD, cerebral infarction, renal failure, and peripheral arterial occlusive disease, taking into account body mass index (BMI) and fasting glucose. This is the first description of serum level of soluble TNF receptor being independently associated with PWV in nonobese, well-controlled, unique Japanese type 2 diabetic patients.

2. Subjects and methods

Eighty-six Japanese type 2 diabetic patients who visited Kansai-Denryoku Hospital were enrolled for the present study. They had no abnormal electrocardiogram findings suggestive of ischemic heart disease. They also had normal serum creatinine level (b1.0 mg/dL), ankle brachial index greater than 1.0, and no signs of cerebral stroke. Thus, they were considered to have no major cardiovascular disease at the time of the study. Type 2 diabetes mellitus was diagnosed based on the criteria of the World Health Organization [12]. The subjects had no evidence of current acute illness or infectious process. The duration of diabetes was 11.1 F 0.8 years (mean F SEM). Seventy-five of 86 patients were taking sulfonylureas (gliclazide) and the rest were controlled with diet alone. None had received insulin therapy or any medications known to enhance insulin sensitivity such as biguanide or pioglitazone. Before the study, 2 dietitians confirmed, by checking daily food records, that the subjects had ingested at least 150 g of carbohydrate for the 3 days preceding the study. Blood pressure was measured twice with Colin BP-103ill (Tokyo, Japan) while the patients were in the sitting position after at least 5 minutes rest and the mean value was used for the analysis. Hypertension was defined as systolic blood pressure of 140 nbsp;mm Hg or higher and/or diastolic blood pressure of 90 mm Hg or higher or current use of antihypertensive medication. On the day of the examination, they were told not to take all medications including sulfonylurea, antihypertensive medications, and lipid-lowering agents. Thirty-four (40%) patients were treated with antihypertensive medications. Twenty-nine (34%) of 86 patients were receiving lipid-lowering agents (bezafibrate, 17; HMG-CoA reductase inhibitor, 12). Cigarette smoking was dichotomized into bneverq and beverq (including past and current) by use of a questionnaire. They were told not to smoke at least 1 day before the study. They did not consume alcohol or perform heavy exercise for at least 1 week before the study.

Blood was drawn in the morning after a 12-hour fast. Plasma glucose was measured with the glucose oxidase method. Triglyceride, total cholesterol, and high-density lipoprotein (HDL) cholesterol levels were also measured. The low-density lipoprotein (LDL) cholesterol level was calculated with the Friedewald formula [13]. Serum insulin was measured by a two-site immunoradiometric assay (Insulin Riabead II, Dainabot, Osaka City, Japan). Coefficients of variation were 4% and 7% for insulin greater than and insulin less than 25 l U/mL, respectively.

The estimate of insulin resistance by homeostasis model assessment (HOMA-IR) was calculated with the formula: fasting serum insulin (l U/mL) • fasting plasma glucose (mmol/L)/22.5 [14]. HOMA-IR was validated in diabetic subjects with diet therapy alone and in those treated with sulfonylureas [15,16]. HOMA-IR greater than 2.5 was defined as insulin resistance [17,18]. Serum TNF-a concentrations were measured by an enzyme immunoassay kit (Quantikine HS Human TNF-a immunoassay kit, R&D Systems, Minneapolis, MN), and serum concentrations of sTNF-R1 and sTNF-R2 were measured by enzyme-linked immunosorbent assay (BIOTRAK, Amersham Life Sciences, Uppsala, Sweden) as described previously [7,19]. The limits of sensitivity for TNF-a, sTNF-R1, and sTNF-R2 were 0.5, 25, and 50 pg/mL, respectively. The intra-assay coefficients of variation for TNF-a, sTNF-R1, and sTNF-R2 were 5.9%, 4.7%, and 3.2%, respectively. The inter-assay coefficients of variation for TNF-a, sTNF-R1, and sTNF-R2 were 10.8%, 5.8%, and 3.6%, respectively.

2.1. Measurement of PWV

Pulse wave velocity was measured with a volume plethysmographic apparatus (form PWV/ABI version-112, Colin, Komaki, Japan). Briefly, after an overnight fast, the subjects were examined in the early morning while in the supine position. Electrocardiogram electrodes were placed on both wrists. A microphone for detecting heart sounds was placed on the left edge of the sternum. The cuffs were wrapped on both the brachia and ankles. The characteristic

Table 1
Clinical profiles of patients studied

	All patients	Male patients	Female patients
n	86	61	25
Age (y)	62.8 F 1.0	61.3 F 1.1	66.3 F 1.54
Duration of diabetes (y)	11.1 F 0.8	11.1 F 1.0	11.2 F 1.3
Smoking (%)	26	33	844
BMI (kg/m^2)	22.8 F 0.3	23.1 F 0.2	22.2 F 0.64
Systolic blood pressure (mm Hg)	136 F 2	134 F 2	140 F 4
Diastolic blood pressure (mm Hg)	82 F 1	83 F 1	81 F 2
Sulfonylurea/diet	76/10	55/6	21/4
HMG-CoA reductase inhibitor (%)	15	11	244
Bezafibrate (%)	21	21	20
Antihypertensive agent (%)	42	36	56
Fasting glucose (mg/dL)	142 F 3	144 F 3	133 F 5
HbA_{1c} (%)	7.0 F 0.1	7.0 F 0.1	7.1 F 0.2
Fasting insulin (I U/mL)	6.6 F 0.4	6.7 F 0.4	6.3 F 0.7
HOMA-IR	2.32 F 0.15	2.40 F 0.17	2.13 F 0.24
Triglyceride (mg/dL)	122 F 6	128 F 8	105 F 94
HDL cholesterol (mg/dL)	59 F 2	55 F 2	66 F 344
Total cholesterol (mg/dL)	204 F 4	201 F 4	210 F 9
LDL cholesterol (mg/dL)	126 F 4	126 F 4	128 F 8
Serum creatinine (mg/dL)	0.76 F 0.02	0.82 F 0.02	0.63 F 0.0444
Serum urea nitrogen (mg/dL)	15.3 F 0.4	15.2 F 0.4	15.6 F 0.9
TNF-a (pg/mL)	3.3 F 0.2	3.60 F 0.29	2.65 F 0.194
sTNF-R1 (pg/mL)	1184 F 44	1184 F 41	1185 F 100
sTNF-R2 (pg/mL)	2053 F 57	2070 F 59	2013 F 115
PWV (cm/s)	1661 F 34	1663 F 37	1655 F 59

4 P b .05 vs male patients.
44 P b .01 vs male patients.

points of wave forms were determined automatically and the results were printed out. All procedures took about 5 minutes. The interobserver and intraobserver variation coefficients were 8.4% and 10.0%, respectively. Measurements on different days revealed that slight changes in blood pressure did not correlate with changes in PWV. The mean PWV value measured on either side of each patient was used for the analysis.

2.2. Statistical analysis

Data were presented as mean F SEM. Statistical analyses were conducted with the StatView 5 system (Statview, Berkeley, CA). Simple (Spearman rank) correlation coefficients between PWV and measures of variables were calculated, and a stepwise multiple regression analysis was then used to evaluate the independent association of these variables with PWV. The means of the 2 groups (male vs female patients) were compared with Student t test. P b .05 was considered as significant. In multivariate analysis, an F value of 4 or greater was considered significant.

3. Results

Clinical characteristics of all subjects are summarized in Table 1. They were all Japanese type 2 diabetic patients (61 men and 25 women) with an age range of 43 to 84 years and a BMI of 17.1 to 26.7 kg/m^2. They all were nonobese [20]. The ranges of fasting glucose and glycosylated hemoglobin (HbA_{1c}) were from 92 to 194 mg/dL and from 4.9% to 10.1%, respectively. There was a wide variation in insulin resistance calculated from HOMA-IR (range, 0.51-7.17). Thirty-one (36%) of the 86 subjects had HOMA-IR greater than 2.5, indicating that they were insulin resistant [17,18].

Clinical features of male and female patients are shown in Table 1. Although a significant difference was observed in age, smoking status, BMI, triglycerides, HDL cholesterol, creatinine, and TNF-a between the 2 groups, there was no significant difference in some variables including sTNF-R1, sTNF-R2, and PWV between the 2 groups.

Values of PWV ranged from 1139 to 2728 cm/s (mean, 1661 cm/s; SD, 292 cm/s) (Table 1). Only 17 (20%) of 86 patients had PWV less than 1400 cm/s (range, 1139-1385 cm/s). This finding was far different from the recent report by Kim et al [21] in which 90% of the PWV values were between 525 and 1399 cm/s in 2488 healthy individuals. We therefore considered all patients as a group and investigated the relationships between PWV and some variables including TNF-a with univariate and multiple regression analyses.

Table 2
Correlation of brachial-ankle PWV with measures for variables in all diabetic patients

	Univariate		Multivariate
	r	P	F
Age	0.492	b.001	15.1
Diabetes duration	0.251	.021	2.9
Systolic blood pressure	0.595	b.001	31.6
Diastolic blood pressure	0.248	.022	0.4
TNF-a	0.167	.123	-
sTNF-R1	0.354	.001	0.1
sTNF-R2	0.415	b.001	5.2
Gender	• 0.032	.765	-
Smoking	• 0.047	.663	-
BMI	• 0.113	.296	-
Fasting glucose	0.075	.492	-
HbA_{1c}	0.054	.620	-
Insulin	• 0.007	.950	-
HOMA-IR	0.019	.861	-
Triglycerides	• 0.095	.382	-
Total cholesterol	• 0.035	.747	-
HDL cholesterol	0.036	.743	-
LDL cholesterol	• 0.043	.692	-
Serum creatinine	0.079	.465	-
Therapy for diabetes	• 0.023	.831	-
Therapy for hypertension	0.268	.013	2.1
Therapy for triglyceride	0.055	.610	-
Therapy for cholesterol	0.016	.881	-

Table 2 illustrates the correlation between PWV and the measures of variables including age, sex, and TNF in all diabetic patients. PWV was positively correlated with age (r = 0.492, P b .001), diabetes duration (r = 0.251, P = .021), systolic blood pressure (r = 0.595, P b .001), diastolic blood pressure (r = 0.248, P = .022), sTNF-R1 (r = 0.354, P = .001), and sTNF-R2 (r = 0.415, P b .001). The difference in PWV was also observed between the patients taking antihypertensive medications and those who were not (r = 0.268, P = .013). However, other variables including TNF-a, sex, smoking status, BMI, and therapy for diabetes or hyperlipidemia were not associated with PWV.

Multiple regression analyses were carried out by using the stepwise procedure in all diabetic patients (Table 2). The analysis included PWV as a dependent variable and candidate risk factors (age, diabetes duration, systolic blood pressure, diastolic blood pressure, sTNF-R1, sTNF-R2, therapy for hypertension) as independent variables (Table 2). PWV was independently predicted by age (F = 15.1), systolic blood pressure (F = 31.6), and serum concentration of sTNF-R2 (F = 5.2), which explained 49.2% of the variability of PWV in our diabetic patients. Other variables including diabetes duration, diastolic blood pressure, therapy for hypertension, and sTNF-R1 were not independently associated with PWV in our nonobese Japanese type 2 diabetic patients. Finally, smoking status and BMI were incorporated as candidate risk factors. PWV was independently predicted by age (F = 15.1), systolic blood pressure (F = 31.6), and serum concentration of sTNF-R2 (F = 5.2), which explained 49.2% of the variability of PWV in the patients. Smoking status (F = 3.1) and BMI (F = 3.2) were not independently associated with PWV in our patients.

4. Discussion

The main novel finding in the present study is that sTNF-R2 is strongly and independently associated with brachial-ankle PWV in nonobese Japanese type 2 diabetic patients.

Type 2 diabetes mellitus is a heterogeneous syndrome characterized by insulin resistance and/or defective insulin secretion [22]. As distinct from white populations, Japanese type 2 diabetic patients are unique in that they are not always obese, but some individuals are both insulin sensitive and insulin resistant [23-25]. The present study reconfirmed that 36% of the nonobese Japanese type 2 diabetic patients were insulin resistant.

Atherosclerosis is the leading cause of mortality and morbidity in subjects with type 2 diabetes mellitus. Although the mechanisms by which atherosclerosis occurs are not fully clarified, it has been shown that most clinical events result from mild to moderate arterial lesions that abruptly progress to severe obstructions [26]. Thus, detecting the early stage of atherosclerosis is considered to be the first line to clarify the mechanisms responsible for the evolution of atherosclerosis in type 2 diabetic patients.

Pulse wave velocity, a measure of aortic distensibility, is a noninvasive method to detect the early stage of atherosclerosis in humans. There are some reports suggesting that gender per se might affect the value of PWV [27-29]. We could not, however, find any significant relationship between PWV and gender in our patients.

Pulse wave velocity is shown to predict mortality in patients with hypertension and older healthy individuals, independently of known confounding factors [30]. Several studies have demonstrated that PWV correlates with diabetic complications. Tanokuchi et al [31] reported that PWV is related to serum creatinine level in patients with type 2 diabetes mellitus. Okada et al [32] showed the relationship between PWV and autonomic neuropathy in type 2 diabetic patients. Aso et al [33] demonstrated that PWV was associated with retinopathy and albuminuria in type 2 diabetic patients. PWV is also confirmed to be highly predictive of cardiovascular mortality in subjects with type 2 diabetes mellitus [5]. Thus, it may be hypothesized that micro- and macrovascular complications of type 2 diabetes mellitus share the common pathophysiologic mechanisms. This hypothesis is supported by the report from Neil et al [34] that microalbuminuria, a component of microvascular disease, has strongly and independently been associated with the development of cardiovascular disease and mortality in type 2 diabetic patients. Retinopathy, another microvascular complication, has been shown to be associated with increased cardiovascular and all-cause mortality risk in type 2 diabetic patients [35].

It is well recognized that low-grade inflammation per se seems to have a major role in the pathogenesis of

Table 3
Correlation of brachial-ankle PWV with measures for variables in 52 diabetic patients who have not received antihypertensive medications

	Univariate		Multivariate
	r	P	F
Age	0.291	.038	2.8
Diabetes duration	0.086	.537	-
Systolic blood pressure	0.533	b.001	15.2
Diastolic blood pressure	0.297	.034	1.6
TNF-a	0.198	.157	-
sTNF-R1	0.465	b.001	0.5
sTNF-R2	0.482	b.001	11.2
Gender	0.021	.879	-
Smoking	0.084	.549	-
BMI	• 0.045	.747	-
Fasting glucose	• 0.026	.853	-
HbA_{1c}	0.004	.974	-
Insulin	0.035	.804	-
HOMA-IR	• 0.003	.982	-
Triglycerides	• 0.018	.896	-
Total cholesterol	• 0.144	.303	-
HDL cholesterol	• 0.074	.599	-
LDL cholesterol	• 0.153	.275	-
Serum creatinine	0.180	.198	-
Therapy for diabetes	• 0.031	.827	-
Therapy for triglyceride	0.074	.598	-
Therapy for cholesterol	• 0.021	.884	-

atherosclerosis and diabetes [36]. Ridker et al [37] showed that increased levels of inflammatory markers such as the high-sensitivity C-reactive protein (CRP) and interleukin 6 (IL-6) can predict increased risk of cardiovascular disease in humans. Serum IL-6 is shown to be predictive of the development of type 2 diabetes mellitus in women [38]. Stehouwer et al [39] confirmed that increased urinary albumin excretion, endothelial dysfunction, and chronic inflammation are interrelated processes that are associated with risk of death in type 2 diabetic patients. In the present study, however, we could not find any significant relationships between PWV and serum concentrations of CRP or IL-6 in our diabetic patients (data not shown). It may be argued that antihypertensive medications affect PWV by altering blood pressure in our patients. We therefore investigated 52 patients who have not received antihypertensive medications and found that PWV was independently predicted by the level of systolic blood pressure ($F = 15.2$) and sTNF-R2 ($F = 11.2$), which explained 35.6% of the variability of PWV (Table 3). This finding also supports our idea that TNF system activity per se plays an important role in PWV in Japanese type 2 diabetic patients.

Tumor necrosis factor a, the proinflammatory cytokine, seems to be associated with the progression of atherosclerosis in type 2 diabetes mellitus. There is, however, a paucity of the literature regarding the relationship between TNF-a and atherosclerosis in type 2 diabetic patients. As an index of TNF-a system activities, we measured serum TNF-a, serum sTNF-R1, and serum sTNF-R2 and found that serum sTNF-R2 is strongly and independently associated with PWV in nonobese Japanese type 2 diabetic patients. However, we could not find any independent relationship between PWV and serum TNF-a. It should be noted that TNF receptor levels remain elevated for a longer time than TNF-a itself and TNF receptors might reflect the degree of TNF-a activation more accurately than the measurement of TNF-a itself. Soluble TNF receptor is thus suggested to be a more valuable factor for monitoring the degree of TNF-a system activity in humans.

The mechanisms by which TNF-a system activities are associated with PWV in nonobese Japanese type 2 diabetic patients are not known at present. There is some evidence that TNF-a is associated with the evolution of atherosclerosis. TNF-a has been shown to contribute to the synthesis of inflammatory markers such as CRP and fibrinogen in liver [40], to mediate chemotaxis of monocytes and fibroblasts [41], and to enhance the expression of vascular cell adhesion molecules such as intercellular adhesion molecule 1 [42]. Irrespective of this, our present study showed that sTNF-R2 but not sTNF-R1 was independently associated with PWV. The validity of the present study is supported by the recent longitudinal investigation by Shai et al [9], who showed that sTNF-R2 is an independent predictor of CHD events in patients with type 2 diabetes mellitus.

The reason why TNF-R2 but not TNF-R1 was associated with PWV in our patients remains to be clarified. These 2 receptors seem to differ in signaling and functional properties [43]. Most biological responses such as cytotoxicity and nuclear factor j B activation are mediated by TNF-R1 but not by TNF-R2 [44]. There are some data available regarding the potential role of TNF-R2 in studies in humans. TNF-a has shown to up-regulate TNF-R2 expression in humans [45]. Obese subjects are shown to overexpress TNF-a and TNF-R2 in adipose tissue and have higher levels of TNF-R2 compared with lean subjects [46,47]. In contrast, we previously demonstrated that plasma TNF-R2 but not TNF-R1 was significantly higher in patients with bulimia nervosa [18]. Thus, it might be suggested that the adipose tissue is not the immediate source of TNF-a in our nonobese Japanese patients with type 2 diabetes mellitus. Recent studies have demonstrated that the binding of advanced glycation end products to specific cell-surface receptor molecules expressed on kidney cells can induce local cytokine and initiate local inflammatory reaction [48]. Angiotensin II, a substance that is associated with the development of renal injury in diabetic patients is shown to up-regulate expression of TNF-a [49]. The source of TNF-a in our patients, however, has yet to be determined. It should be noted that macrophages from diabetic patients release more TNF-a than do control macrophages [50]. Furthermore, high glucose can activate monocytes and induce the expression of TNF-a via oxidant stress and nuclear factor j B transcription factor [51].

In summary, although our present study was performed on a limited number of patients without major clinical signs of macrovascular complications, serum sTNF-R2 is likely to be involved in the brachial-ankle PWV in nonobese Japanese type 2 diabetic patients.

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Effect of Vietnamese Common Diet on Postprandial Blood Glucose Level in Adult Females

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Summary To elucidate the effect of a typical Vietnamese diet including a high content of white rice on postprandial blood glucose levels, the present study was designed. Thirty healthy female subjects with a similar body mass index, 10 each in their twenties, forties and sixties, were recruited. Four meals with a similar protein energy percentage (13–15%) but different energy ratios of fat and carbohydrate (FC ratio) and vegetable contents were provided by cross-over design. Meal A was designed according to the commonly consumed diet in Vietnam. The FC ratio was 14 : 71 and 84 g of carbohydrate was from rice. Meal B contained carbohydrate in a lower ratio than meal A by fat replacement and its FC ratio was 30 : 57. Meal C was similar to meal A except lacking vegetables. The energy of meal A, B and C was about 2.1 MJ. Meal D was designed to match the amount of carbohydrate and fat within A and B, respectively. The FC ratio of meal D was 26 : 61 and the energy was about 2.4 MJ. Fasting blood glucose was measured before consumption of a test meal. Postprandial blood glucose was measured every 30 min for 2 h. Areas under the curve (AUC) were calculated to compare the glycemic response among the four test meals. There was no significant difference in AUC among the four test meals in the subjects in their twenties. In the subjects in their forties, the AUC of meal A tended to be lower than that of meal C (p=0.07). In the subjects in their sixties, the AUC of meal A was significantly higher than that of meal B (p<0.001). Glycemic responses showed a significant relationship with age (r=0.26, p<0.01); however, there was no association between glycemic responses and BMI (p=0.20). Dietary fat ratios were inversely associated with glycemic responses (r=−0.28, p<0.01). In conclusion, the diet with about 70% energy from carbohydrate which is commonly consumed by Vietnamese may increase glycemic response, especially in elderly people and dietary vegetables may be beneficial to prevent such an increase in glycemic response.

Key Words Vietnamese common diet, glycemic response

Evidence shows an increasing prevalence of diabetes in the Asian population and also indicates the risk of diabetes in Asians may be higher than that of other racial groups at the same body mass index (BMI) (*1–6*). The use of white rice as a staple food, since white rice has been demonstrated and classified as a high glycemic index (GI) food, has been considered as a risk factor for diabetes (*5–7*).

Willett and colleagues (*8*) proposed a "Healthy Eating Pyramid" recently which suggested refined carbohydrates, such as white rice, should be used sparingly, as are sweets. Plant oil was suggested to be near the foundation of the pyramid to meet the fact that Americans get 35% or more of their daily energy from fat. Based on evidence, plant oil contains plenty of polyunsaturated fatty acid, which is considered superior to animal fat (*8*). It has been considered that the prevalence of diabetes increased severely in recent years because Americans consumed too much carbohydrate instead of fat, following the "Food Guide Pyramid" published in 1992 (*9*). The suggestions to use white rice sparingly (*8*) may bring about a great impact on the dietary culture of Asians.

Compared with America, the fat intake ratio was low in Asian countries, especially in Vietnam. According to the National Nutrition Survey in Vietnam, energy from protein, fat, and carbohydrate (PFC ratio) were 13, 12, and 75%, respectively (*10*). Some rural regions in Vietnam were reported to get more than 80% of their energy intake from carbohydrate (*10*). We hypothesized that the use of rice as a staple food is not the only risk factor for diabetes as there might be a synergistic effect with others, among which is a deficient ratio of macro-

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nutrients. Though rice had been classified as high-GI food, most of the time individuals do not eat rice only but combine it in mixed meals. Some researchers also suggested that a single food consumed with other dishes has a lower GI value than a single food consumed by itself (*11, 12*). Glycemic load (GL) was then introduced to represent the combination of quality and quantity of carbohydrate consumed (*13*). However, the utility of GI/GL is still controversial (*12, 13*). A review of the literature indicated the dietary GI or GL was used as a measure of glycemic response in young subjects or diabetes patients (*13–15*). There have been no adequate studies on comparison of postprandial glycemic responses among the young or elderly healthy subjects.

This study was aimed to provide data on postprandial glycemic responses based on the Vietnamese common diet with high carbohydrate intake by involving three different age groups.

MATERIALS AND METHODS

Subjects. This study was conducted in Khanh Van Commune, Ninh Binh Province, Vietnam. Healthy female subjects of three age groups with similar BMI (those in their twenties, forties and sixties) were recruited from a pool of farmers and participated after harvesting season. Ten subjects were enrolled in each group. Informed consent was received from all subjects and approval for the study was given by the Ethical Committee of the Ministry of Health, National Institute of Nutrition, Vietnam. Hypertension, hyperlipidemia, diabetes, impaired fasting glucose, alcohol drinking, and smoking were exclusion criteria. A self-monitored blood glucose device (SMBG device, Precision Xtra, Abbott Laboratories, Abbott Park, Illinois, USA) was used to measure blood glucose for screening. Fasting blood glucose more than 110 mg/dL was excluded according to American Diabetes Association's (ADA) criteria (*16*). Height and weight were measured to 0.1 cm and 0.1 kg, respectively, using a digital weight balance and height scale.

Test meals. Four test meals (A, B, C and D) were designed using white rice as a staple food and pork with or without vegetables as side dishes. Seasonings were almost the same for all the meals. The composition of test meals is shown in Table 1. Since the purpose of this study was to elucidate the effect of dietary FC ratio, the proportion of energy from protein of the four test meals was kept relatively constant (13–15%). Meal A was designed to represent the Vietnam common diet according to the National Nutrition Survey of Vietnam (*10*). Its FC ratio and energy intake were as commonly consumed in Vietnam. The total energy of meal A, B and C was about 2.1 MJ. FC ratios in test meals A and B were 14 : 71 and 30 : 57, respectively. Studies also indicated that dietary vegetables improved glycemic control by reducing or delaying the absorption of carbohydrate (*17–20*). Meal C was designed to be similar to meal A except lacking vegetables and its FC ratio was 15 : 71. To elucidate whether glycemic response was also influenced by a deficient FC ratio, and not only because of the amount of carbohydrate, meal D was designed to match the amount of carbohydrate and fat within A and B, respectively. The total energy of meal D was 2.4 MJ with an FC ratio of 26 : 61. Energy intake and food composition were determined by Nutritive Composition Table of Vietnamese Foods (*21*). All test meals were measured, prepared and divided into portions each morning.

Table 1. Dietary composition and PFC ratios of four test meals.

	Meal A*	Meal B	Meal C	Meal D
White rice (g)	110	86	110	110
Oil (g)	4	13	4	13
Lean pork (g)	40	40	40	40
Vegetable (g; cabbage)	100	100	0	100
Fish sauce (g)	5	5	5	5
Protein (g)	18.4	16.5	16.6	18.4
Fat (g)	7.9	16.7	7.9	16.9
Carbohydrate (g)	89.2	70.9	83.8	89.2
Fiber (g)	2.0	1.9	0.4	2.0
P : F : C ratio	15:14:71	13:30:57	14:15:71	13:26:61
Total energy (MJ)	2.1	2.1	2.0	2.4

*Meal A was designed to represent the Vietnam common diet. 1 kcal=4.186 kJ.

Study design and blood glucose measurement. Each subject took the 4 test meals on separate mornings by cross-over design. Subjects were asked to fast before testing for at least 10 h. Nothing was allowed to be eaten or drunk except water. On the test morning, the subjects arrived at the local health center by the least strenuous means of transportation. After resting in a comfortable position for at least 10 min, body weight was measured. Fasting blood glucose was measured before consumption of the test meal. Test meals were consumed within 15 min. Postprandial blood glucose was measured every 30 min for 2 h from the consumption of the test meals. The incremental area under the curve (AUC) changes in blood glucose was computed by the trapezoidal method (*22*). The AUC for each test meal was expressed as glycemic response for 2 h. AUCs of the four test meals among the three age groups were compared. The blood glucose was measured using a SMBG device. This biosensor glucose test strip is based on an electron-transferred glucose oxidase reaction (*23*). A fill trigger electrode was designed to minimize the possibility of inaccurate results due to low sample volume. The accuracy of the glucose test strip was demonstrated by comparing 393 capillary blood glucose obtained by YSI Glucose analyzer (YSI Inc., Yellow Springs, OH). Agreement between the two methods of measurements was observed with a correlation coefficient (r)>0.98, mean absolute bias of 4.9% and a 3.5% reproducibility of normal glucose concentration. Capillary blood samples were measured with three test strips in three SMBG devices and the average of the three readings was used.

Statistical analysis. Incremental AUCs for each test meal were calculated and the data were expressed as

Table 2. Characteristics of the study subjects.

Group	Age (y)	Height (cm)	Weight (kg)	BMI (kg/m²)	Fasting Blood Glucose (mg/dL)
Twenties (n=10)	23.4±0.5	155.1±1.2	44.0±2.3	18.2±0.8	85.0±2.8
Forties (n=9)	42.3±0.6	153.5±1.6	46.1±2.2	19.5±0.6	88.3±2.3
Sixties (n=10)	61.4±0.4	153.2±1.1	47.6±1.7	20.3±0.8	92.6±1.3

Data are presented as means±SE.

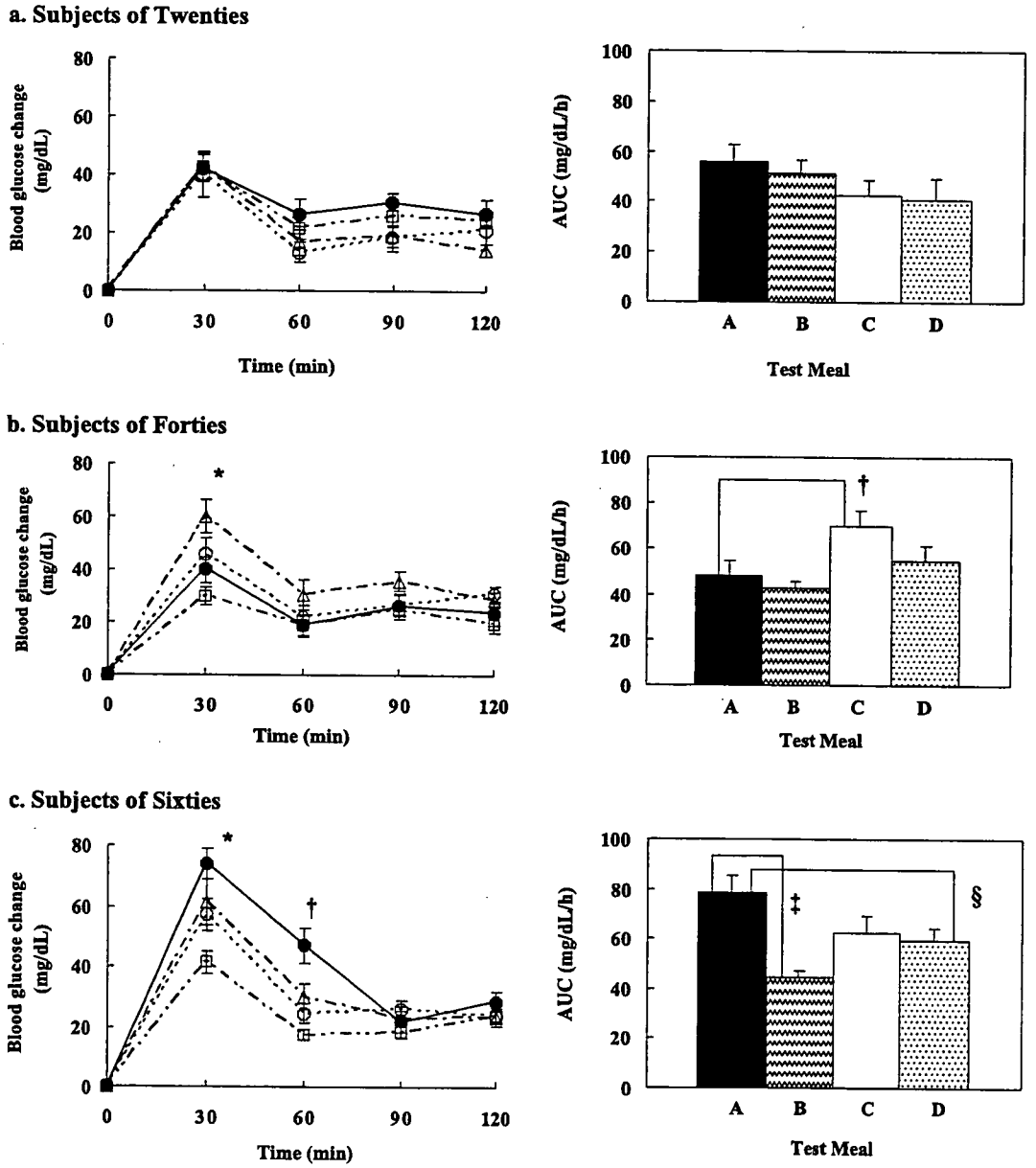

Fig. 1. Postprandial blood glucose level and the AUC after subjects consumed four test meals (●, A; □, B; △, C; ○, D) for 120 min. Meal A was compared with the other three test meals. Two-way ANOVA showed a main effect of age and a significant age-by-test meal interaction (both $p<0.01$). (a) Data for subjects in their twenties. (b) Data for subjects in their forties. $^{*}p<0.05$, and $^{\dagger}p=0.07$ for A vs. C. (c) Data for subjects in their sixties. $^{*}p<0.01$ A vs. B. $^{\dagger}p<0.001$ A vs. B, $p<0.05$ A vs. C, and $p<0.01$ A vs. D. $^{\ddagger}p<0.001$, $^{\S}p=0.07$.

mean±SE. Two-way ANOVA was used to test the main effect and the interaction between age and test meal. Mean contrasts according to modified Bonferroni inequalities were used to analyze significance. Mean values of the four test meals in each age group were compared by analysis of variance (ANOVA). Tukey's multiple comparison test of means was used to compare treatments pairwise. Simple correlations were determined by Pearson's correlation coefficient (r). Partial correlations were measured between postprandial gly-

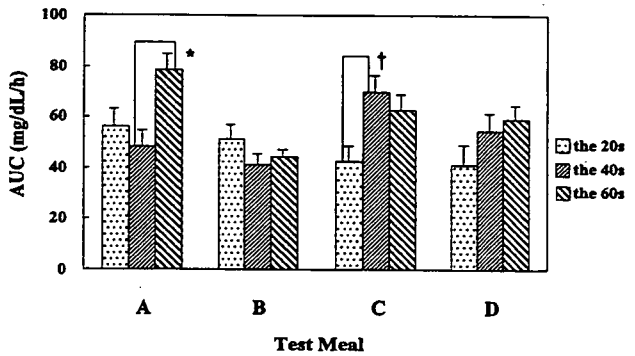

Fig. 2. Postprandial glycemic responses (AUCs) to the same test meals when compared among the three age groups. *$p<0.01$, †$p<0.05$.

cemic response (AUC) and age, and BMI and dietary fat ratios by controlling for the impact of energy intake. Analysis of the data was carried out with SPSS version 11.5 J statistical software. p values less than 0.05 were considered statistically significant.

RESULTS

Originally there were 10 subjects in each age group; however, one subject in her forties was dropped from the study due to a physical problem. Characteristics of the study subjects are shown in Table 2. The heights, weights, BMIs and fasting blood glucose showed no significant difference among the three age groups ($p>0.05$). A main effect of age and age-by-test meal interactions was significant (both $p<0.01$; Fig. 1). In the subjects in their twenties, glycemic response to meal A was higher than that to the others after 60 min, although the difference was not significant (Fig. 1a). The AUCs of meal A, B, C and D were 56±7, 51±6, 43±6 and 40±7 mg/dL/h, respectively, with no significant difference among them.

In the subjects in their forties, meal A, which contained vegetables, had significantly lowered glycemic response at postprandial 30 min when compared with meal C ($p<0.05$, Fig. 1b). The AUCs of meal A, B, C and D were 48±6, 41±4, 70±7 and 55±7 mg/dL/h, respectively. Meals A and C tended to show different glycemic response for the 2 h ($p=0.07$).

In the subjects in their sixties, the greater amount of carbohydrate contained in meal A showed a considerable effect on glycemic response, which was significantly higher than for meal B at postprandial 30 min ($p<0.01$) and 60 min ($p<0.001$) (Fig. 1c). At postprandial 60 min, meal A had a significant converse effect on glycemic response compared with meal C ($p<0.05$). Glycemic response to meal D was significantly lower than that to meal A at postprandial 60 min ($p<0.01$). The AUCs of meals A, B, C and D were 79±6, 45±3, 63±6 and 59±5 mg/dL/h, respectively. The glycemic response for 2 h was significantly different between meals A and B ($p<0.001$), and it tended to show significance between meals A and D ($p=0.07$).

When comparing the glycemic responses to the same test meal among three age groups (Fig. 2), significant differences in meal A was observed for those in their forties versus their sixties ($p<0.01$) and in meal C for the subjects in their twenties compared to their forties ($p<0.05$). Pearson's correlation analyses indicated age was significantly correlated with postprandial glycemic response (AUC) ($r=0.26$, $p<0.01$) (Table 3). A significant negative correlation was observed between AUC and dietary fat ratio ($r=-0.28$, $p<0.01$). There was no association between BMI and AUC ($r=-0.12$, $p=0.20$). After partial correlation analysis, the correlations between AUC vs. age and AUC vs. dietary fat ratio remained significant (beta=0.26, $p<0.01$ and beta=-0.27, $p<0.01$, respectively).

Table 3. Correlation analyses between postprandial glycemic response (AUC) and age, BMI and dietary fat ratio.

	Postprandial glycemic response (AUC)			
	r*	p-value	beta*	p-value
Age	0.26	<0.01†	0.26	<0.01†
BMI	−0.12	0.20	−0.12	0.20
Dietary fat ratio	−0.28	<0.01†	−0.27	<0.01†

*r for Pearson's correlation coefficient, and beta for partial correlation coefficient.
†$p<0.01$ significant difference.

DISCUSSION

The diet with about 70% energy from carbohydrate, which is commonly consumed by Vietnamese, increased glycemic response, especially in the elderly subjects. The increased glycemic response was considered to be mainly due to a low dietary fat ratio and excess carbohydrate since protein intake levels were similar, being 13–15% of total energy intake. The additional amount of fat in meal D reduced glycemic response when compared with meal A, despite the identical amount of carbohydrate. Furthermore, the glycemic response to meal A displayed marked postprandial hyperglycemia compared with meal B. Our results were consistent with other studies which pointed out that fat contained in a mixed meal would delay the absorption of carbohydrate and attenuate the glycemic response (*11*, *12*). Though fat was thought as non-GI (*8*) to attenuate the glycemic response, its high energy density could not be ignored.

Glycemic response to meal A displayed marked postprandial hyperglycemia in the subjects in their sixties compared with meal B, while the same effects couldn't be observed in the subjects in their twenties and forties. It may be speculated that the small difference in the AUC observed in the subjects in their twenties and forties might be due to the leveling off in glycemia as indicated by Brand-Miller et al. (*24*). In their study involving lean healthy volunteers, Brand-Miller et al. (*24*) pointed out that increasing the glycemic load produced a stepwise increase in glucose AUC only observed at the low doses. In that study, five doses (one, two, three, four