Table 2 | Mean values or frequencies of risk factors for type 2 diabetes according to age groups in 1988 and 2002 by sex

Variable	Age (years)	Men			Women		
		1988	2002	*P*-value	1988	2002	*P*-value
Body mass index (kg/m^2)	40-49	23.5 (2.9)	23.9 (3.3)	0.05	23.2 (3.1)	22.5 (3.7)	0.008
	50-59	23.4 (2.9)	23.9 (3.0)	0.04	23.3 (3.2)	23.0 (3.5)	0.13
	60-69	22.4 (2.9)	23.6 (2.9)	<0.001	23.0 (3.2)	23.4 (3.3)	0.06
	70-79	21.3 (2.6)	22.8 (2.9)	<0.001	22.0 (3.3)	23.3 (3.4)	<0.001
Overall obesity (%)	40-49	29.1	33.1	0.29	24.6	18.7	0.048
	50-59	29.2	31.4	0.53	25.7	24.7	0.73
	60-69	21.8	30.7	0.01	26.2	30.7	0.16
	70-79	7.6	21.9	<0.001	18.5	30.3	0.003
Waist circumference (cm)	40-49	83.1 (8.1)	83.6 (8.4)	0.45	79.3 (9.5)	76.8 (9.7)	<0.001
	50-59	83.3 (7.7)	84.2 (7.9)	0.11	82.1 (9.9)	79.8 (9.4)	<0.001
	60-69	81.5 (8.4)	84.1 (7.8)	<0.001	83.3 (9.8)	83.2 (9.2)	0.87
	70-79	78.8 (7.7)	83.3 (8.5)	<0.001	80.3 (10.6)	84.8 (9.5)	<0.001
Central obesity (%)	40-49	18.5	23.5	0.13	49.4	32.6	<0.001
	50-59	20.6	23.9	0.28	60.0	47.6	<0.001
	60-69	15.5	21.6	0.052	67.5	68.4	0.79
	70-79	10.6	23.5	0.002	50.8	68.8	<0.001
Regular exercise (%)	40-49	10.0	11.0	0.68	6.5	5.1	0.40
	50-59	6.1	8.2	0.28	7.0	9.8	0.13
	60-69	12.7	10.8	0.45	11.8	10.7	0.63
	70-79	26.4	16.2	0.02	14.2	11.4	0.36

All values are given as the mean (standard deviations) or as a percentage. Overall obesity was defined as a body mass index ≥25.0 kg/m^2. Central obesity was defined as a waist circumference ≥90 cm in men and ≥80 cm in women. Regular exercise was defined as engaging in sports at least three times per week during leisure time.

because the present study was carried out in a suburban population, the generalizability of our results to the entire population of Japan is limited. Third, because of issues of overlap and uniformity with the 1988 survey data, we did not use medical history of diabetes in the estimation of prevalence of diabetes. This might have resulted in the underestimation of the prevalence of diabetes. Fourth, there might have been a selection bias resulting from the exclusion of participants who did not have the OGTT. However, the study participants had a similar age distribution and proportion of men (43.3 vs 45.8% in 1988, and 44.1 vs 46.3% in 2002) compared with the original population. Furthermore, the present study had high participation rates in both surveys (approximately 80%). Therefore, we believe that the findings of the present study reflect the actual secular trends in the prevalence of glucose intolerance in our population. Finally, the use of HbA_{1c} has now been recommended for the diagnosis of diabetes by the international expert committee[31], but in the present study, the values of HbA_{1c} were not used for diagnosing diabetes because of the non-standardized HbA_{1c} assay in our 1988 survey. However, HbA_{1c} measurement has been found to be less sensitive for detecting subjects with diabetes compared with the OGTT[32–34]. Furthermore, glucose tolerance status was evaluated in the same way across the two surveys. Thus, this limitation is not likely to distort the prevalence trends in the present study.

In conclusion, the present analysis showed that the prevalence of type 2 diabetes and prediabetes increased significantly in both sexes from the 1980s to the 2000s in a Japanese population. The increasing prevalence of overall and central obesity, and the decline in physical activity seemed to have an influence on this rising trend. More intense efforts for the prevention of type 2 diabetes by modification of lifestyle are required to reduce the burden of type 2 diabetes in Japan.

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CARDIO VASCULAR DIABETOLOGY

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Thresholds of various glycemic measures for diagnosing diabetes based on prevalence of retinopathy in community-dwelling Japanese subjects: the Hisayama Study

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Abstract

Background: There has been controversy over the diagnostic thresholds of hemoglobin A_{1c} (HbA_{1c}) for diabetes. In addition, no study has examined the thresholds of glycated albumin (GA) and 1,5-anhydroglucitol (1,5-AG) for diagnosing diabetes using the presence of diabetic retinopathy (DR). We examined the optimal thresholds of various glycemic measures for diagnosing diabetes based on the prevalence of DR in community-dwelling Japanese subjects.

Methods: A total of 2,681 subjects aged 40-79 years underwent a 75-g oral glucose tolerance test, measurement of HbA_{1c}, GA, and 1,5-AG, and an ophthalmic examination in 2007-2008. The associations of glycemic measures with DR status were examined cross-sectionally. DR was assessed by an examination of the fundus photograph of each eye and graded according to the International Clinical Diabetic Retinopathy Disease Severity Scale. We divided the values of glycemic measures into ten groups on the basis of deciles. The receiver operating characteristic (ROC) curve analysis was performed to determine the optimal threshold of each glycemic measure for detecting the presence of DR.

Results: Of the subjects, 52 had DR. The prevalence of DR increased steeply above the ninth decile for fasting plasma glucose (FPG) (6.2-6.8 mmol/l), for 2-hour postload glucose (PG) (9.2-12.4 mmol/l), for HbA_{1c} (5.9-6.2% [41-44 mmol/mol]), and for GA (16.2-17.5%), and below the second decile for 1,5-AG (9.6-13.5 μg/mL). The ROC curve analysis showed that the optimal thresholds for DR were 6.5 mmol/l for FPG, 11.5 mmol/l for 2-hour PG, 6.1% (43 mmol/mol) for HbA_{1c}, 17.0% for GA, and 12.1 μg/mL for 1,5-AG. The area under the ROC curve (AUC) for 2-hour PG (0.947) was significantly larger than that for FPG (0.908), GA (0.906), and 1,5-AG (0.881), and was marginally significantly higher than that for HbA_{1c} (0.919). The AUCs for FPG, HbA_{1c}, GA, and 1,5-AG were not significantly different.

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Conclusions: Our findings suggest that the FPG and HbA_{1c} thresholds for diagnosing diabetes in the Japanese population are lower than the current diagnostic criterion, while the 2-hour PG threshold is comparable with the diagnostic criterion. 2-hour PG had the highest discriminative ability, whereas FPG, HbA_{1c}, GA, and 1,5-AG were similar in their ability.

Keywords: Diagnostic criteria, Hemoglobin A_{1c}, Glycated albumin, 1,5-anhydroglucitol, Fasting plasma glucose, 2-hour postload glucose, Retinopathy

Background

The International Expert Committee [1,2], the American Diabetes Association [3], and the World Health Organization [4] recently proposed the use of hemoglobin A_{1c} (HbA_{1c}) to diagnose diabetes at a threshold of 6.5% (48 mmol/mol). This threshold was based primarily on the findings of several epidemiological studies in Western populations that investigated HbA_{1c} levels associated with a higher prevalence of diabetic retinopathy (DR), the most specific microvascular complication of diabetes [5-7]. It has been reported that a higher HbA_{1c} level was significantly associated with DR in subjects with diabetes [8], and some clinical trials have demonstrated that lowering HbA_{1c} levels decreased the risk of microvascular complications, such as DR, in diabetes patients [9-11]. These findings suggest that HbA_{1c} levels are intimately related to the risk of DR, and this evidence supports the use of HbA_{1c} as a diagnostic tool for diabetes. However, there has been controversy over the diagnostic threshold of HbA_{1c}. An integrated study of three general populations has shown that the relation between fasting plasma glucose (FPG) levels and the prevalence of retinopathy was continuous, with no clear threshold [12], whereas a prospective study of a French population recently revealed that the optimal threshold of HbA_{1c} for incident retinopathy was 6.0%, which is below the current diagnostic criterion [13]. In addition, several cross-sectional studies of Asian populations, including our previous study, have examined this issue [14-18], but the optimal HbA_{1c} thresholds have differed among these investigations. Thus, a reevaluation of threshold of HbA_{1c} for DR is needed.

Glycated albumin (GA) and 1,5-anhydroglucitol (1,5-AG) levels, which are serum markers of hyperglycemia, have also been found to be significantly associated with microvascular complications [19,20]. There have been a few studies investigating GA [21-23] and 1,5-AG levels [24-26] to detect subjects with glucose intolerance defined by glucose levels, but no study has examined the diagnostic thresholds of these glycemic measures for diabetes based on the presence of DR, and it is uncertain whether GA and 1,5-AG measurements are applicable as a diagnostic tool for diabetes [27,28]. In addition, in the general Asian community, there are limited data assessing FPG and 2-hour postload glucose (PG) levels associated with the prevalence of DR [14,17,29].

The purposes of this study were to determine the thresholds of FPG, 2-hour PG, HbA_{1c}, GA, and 1,5-AG for the diagnosis of diabetes based on the prevalence of DR in a community-dwelling Japanese population, and to compare the ability of these five glycemic measures to differentiate subjects with and without DR.

Methods

Study population

A population-based prospective study of cardiovascular disease and its risk factors has been underway since 1961 in the town of Hisayama, a suburb of the Fukuoka metropolitan area on Japan's Kyushu Island. The population of the town has been stable for 50 years and was approximately 8,400 in 2010. The age and occupational distributions, and nutritional intake of the population were similar to those of Japan as a whole based on data from the national census and nutrition survey [30,31]. In 2007 and 2008, a cross-sectional survey for the present study was performed in the town. A detailed description of this survey was published previously [30]. There were a total of 3,835 residents aged 40-79 years based on the town registry, and 2,957 (participation rate, 77.1%) took part in a comprehensive assessment, including a 75-g oral glucose tolerance test (OGTT), the measurement of HbA_{1c}, and an ophthalmic examination. We excluded the eight subjects who did not consent to participate in the study, 46 who had already had breakfast, 35 who were on insulin therapy, and 156 who refused the OGTT, leaving a total of 2,712 subjects who completed both the 75-g OGTT and HbA_{1c} measurement. Among these, 21 subjects who lacked ophthalmic examination information and 10 for whom there was no measurement of GA or 1,5-AG were excluded, and the remaining 2,681 subjects (1,192 men, 1,489 women) were enrolled in the present study.

Clinical evaluation and laboratory measurements

The study subjects underwent the OGTT between 8:00 and 10:30 A.M. after an overnight fast of at least 12 hours. Blood for the glucose assay was obtained by venipuncture into tubes containing sodium fluoride at fasting and at 2-hour postload, and was separated into plasma and blood cells within 20 min. Plasma glucose

concentrations were determined by the hexokinase method. According to the 1998 World Health Organization criteria [32], diabetes was defined as FPG ≥7.0 mmol/l or 2-hour PG ≥11.1 mmol/l or both, or the use of antidiabetic medications. Those who were diagnosed with diabetes with or without treatment before the examination were considered to be cases of known diabetes. Blood samples were also collected for the determination of HbA_{1c} levels, hemoglobin (Hb) and serum creatinine concentrations. HbA_{1c} levels were measured by latex aggregation immunoassay (Determiner HbA1C; Kyowa Medex, Tokyo, Japan). The values for HbA_{1c} were estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value calculated with the formula: HbA_{1c} (%) = 1.02 × HbA_{1c} (Japan Diabetes Society [JDS]) (%) + 0.25% [33]. A portion of each serum specimen was stored at -80°C for 5 years until it was used for measurement of GA and 1,5-AG in 2012. Serum GA levels were determined by an enzymatic method using an albumin-specific proteinase, ketoamine oxidase, and an albumin assay reagent (Lucica GA-L; Asahi Kasei Pharma, Tokyo, Japan). Serum 1,5-AG concentrations were measured enzymatically (Lana 1,5AG Auto Liquid; Nippon Kayaku, Tokyo, Japan). Hb concentrations were measured by sodium lauryl sulfate-hemoglobin method. Anemia was defined as Hb <13.0 g/dL for men and <12.0 g/dL for women [34]. Serum creatinine concentrations were measured enzymatically, and estimated glomerular filtration rate (eGFR) was calculated using the following new Japanese equation: eGFR (mL/min/1.73 m^2) = 194 × (serum creatinine [mg/dL])$^{-1.094}$ × (age [years])$^{-0.287}$ × (0.739 if female) [35]. Renal failure was defined as an eGFR <30 mL/min/1.73 m^2 (chronic kidney disease stage 4 or 5) [36].

The height and weight were measured with the subject in light clothes without shoes, and the body mass index (BMI) (kg/m^2) was calculated. Each participant completed a self-administered questionnaire covering medical history and antidiabetic treatment.

Ophthalmic examination and definition of diabetic retinopathy

The methods used for the ophthalmic examination have been described in detail previously [14]. Briefly, each participant underwent fundus photographs for the assessment of DR. After pupil dilatation with 1.0% tropicamide and 10% phenylephrine, fundus photographs (45°) were taken from both eyes of each participant using a Topcon digital TRC NW-6SF fundus camera (Topcon Corporation, Tokyo, Japan). The photographs were taken in 1-field per eye, centered on the macula. The photographs were assessed by photographic graders who were masked to the clinical information. The severity of DR was classified into 5 categories according to the International Clinical Diabetic Retinopathy Disease Severity Scale: no retinopathy (equivalent to the Early Treatment of Diabetic Retinopathy Study [ETDRS] scale level 10), mild nonproliferative DR (equivalent to ETDRS level 20), moderate nonproliferative DR (equivalent to ETDRS levels 35, 43, and 47), severe nonproliferative DR (equivalent to ETDRS levels 53A-53E), and proliferative DR (equivalent to ETDRS levels 61 or higher) [37]. The degree of DR was determined according to the grading in the worse eye. The presence of DR was defined as the presence of mild or moderate or severe nonproliferative DR, or proliferative DR in either eye.

Statistical analysis

The SAS software package version 9.3 (SAS Institute, Cary, NC) was used to perform all statistical analyses. We assessed the statistical significance of differences in the prevalence or mean of each factor among the DR status groups by using a logistic or linear regression model, respectively. To analyze FPG, 2-hour PG, HbA_{1c}, GA, and 1,5-AG levels as categorical variables, these values were divided into ten groups on the basis of deciles. The receiver operating characteristic (ROC) curve analysis was performed to determine the optimal threshold of each glycemic measure for detecting the presence of DR. The optimal threshold was obtained from the point on the ROC curve closest to the ideal of 100% sensitivity and 100% specificity. The discrimination of each measure of glycemia for DR was assessed by the area under the ROC curve (AUC). The difference in the AUC was estimated using the method of DeLong et al. [38]. A value of $p < 0.05$ was considered statistically significant in all analyses.

Ethical considerations

This study was conducted with the approval of the Kyushu University Institutional Review Board for Clinical Research, and written informed consent was obtained from all the participants.

Results

Of the study participants, 52 (1.9%) had DR. Mild nonproliferative DR, moderate nonproliferative DR, severe nonproliferative DR, and proliferative DR were found in 33 (1.2%), 6 (0.2%), 13 (0.5%), and 0 (0%) subjects, respectively. The clinical characteristics of subjects are shown in Table 1. The mean age of participants was 60 years, and men comprised 44.5% of the group. The prevalence of diabetes, known diabetes, anemia, and renal failure was 15.2%, 10.0%, 13.2%, and 0.3%, respectively. The mean values of age, FPG, 2-hour PG, HbA_{1c}, GA, diabetes duration and BMI, and the frequencies of men, diabetes, and known diabetes were significantly higher in the subjects with DR than in those without DR, and the subjects with DR had significantly lower

Table 1 Clinical characteristics of subjects, 2007-2008

Variable	Total (n = 2,681)	No retinopathy (n = 2,629)	Diabetic retinopathy (n = 52)	p value
Age (years)	60 (10)	60 (10)	67 (9)	<0.001
Men (%)	44.5	43.9	75.0	<0.001
Fasting plasma glucose (mmol/l)	5.8 (1.1)	5.7 (1.0)	8.7 (2.5)	<0.001
2-hour postload glucose (mmol/l)	7.9 (3.7)	7.7 (3.4)	18.0 (5.3)	<0.001
Hemoglobin A_{1c} (%)	5.5 (0.7)	5.5 (0.7)	7.4 (1.4)	<0.001
(mmol/mol)	37 (8)	36 (7)	57 (15)	<0.001
Glycated albumin (%)	15.2 (2.8)	15.1 (2.4)	22.7 (6.1)	<0.001
1,5-anhydroglucitol (μg/mL)	20.2 (8.3)	20.5 (8.1)	7.7 (7.1)	<0.001
Diabetic retinopathy (%)	1.9	0	100	>0.99
Diabetes (%)	15.2	13.6	96.2	<0.001
Known diabetes (%)	10.0	8.3	94.2	<0.001
Diabetes duration (years)	9.5 (7.9)	8.3 (7.2)	14.8 (9.0)	<0.001
Body mass index (kg/m^2)	23.2 (3.4)	23.2 (3.4)	24.7 (3.6)	0.002
Hemoglobin (g/dL)	13.6 (1.4)	13.6 (1.4)	13.9 (1.5)	0.09
Anemia (%)	13.2	13.1	17.3	0.37
eGFR ($mL/min/1.73\ m^2$)	72.9 (13.9)	72.9 (13.8)	71.4 (18.6)	0.44
Renal failure (%)	0.3	0.3	0	0.99

eGFR: estimated glomerular filtration rate. All values are given as the mean (standard deviations) or as a percentage.
Diabetes was defined as fasting plasma glucose of ≥7.0 mmol/l, and/or 2-hour postload glucose of ≥11.1 mmol/l, and/or the use of antidiabetic medication.
Known diabetes was defined as those who were diagnosed with diabetes with or without treatment before the examination.
Anemia was defined as hemoglobin <13.0 g/dL for men and <12.0 g/dL for women.
Renal failure was defined as an eGFR <30 $mL/min/1.73\ m^2$.

1,5-AG values. The mean values of Hb and eGFR and the frequencies of anemia and renal failure did not differ between the groups.

Figure 1A shows the prevalence of DR by deciles of the distribution of FPG, 2-hour PG, HbA_{1c}, and GA levels. The prevalence of DR was very low in the first through eighth deciles for each glycemic measure, but started to increase steeply from the ninth decile for FPG (6.2-6.8 mmol/l), 2-hour PG (9.2-12.4 mmol/l), HbA_{1c} (5.9-6.2% [41-44 mmol/mol]), and GA (16.2-17.5%). Figure 1B demonstrates the prevalence of DR by deciles of 1,5-AG levels. The prevalence of DR increased markedly below the second decile for 1,5-AG (9.6-13.5 μg/mL), while there was no apparent increase in the prevalence of DR between the third and the tenth deciles of 1,5-AG.

The optimal thresholds of each glycemic measure for detecting prevalent DR using ROC curve analyses are shown in Table 2. The optimal threshold was 6.5 mmol/l for FPG, 11.5 mmol/l for 2-hour PG, 6.1% (43 mmol/mol) for HbA_{1c}, 17.0% for GA, and 12.1 μg/mL for 1,5-AG. The sensitivity, specificity, positive predictive value, and negative predictive value of these thresholds were 82.7%, 86.6%, 10.9%, and 99.6% for FPG, 90.4%, 89.3%, 14.3%, and 99.8% for 2-hour PG, 86.5%, 88.8%, 13.3%, and 99.7% for HbA_{1c}, 86.5%, 89.0%, 13.5%, and 99.7% for GA, and 78.8%, 85.8%, 9.9%, and 99.5% for 1,5-AG, respectively. Among the five glycemic measures, 2-hour PG threshold of 11.5 mmol/l had the highest sensitivity, while 1,5-AG threshold of 12.1 μg/mL showed the lowest sensitivity. In addition, with the exception of the thresholds for 2-hour PG and 1,5-AG, the thresholds of the glycemic measures were not substantially changed when DR was defined as a moderate or higher level of retinopathy (6.5 mmol/l for FPG, 13.1 mmol/l for 2-hour PG, 6.3% [45 mmol/mol] for HbA_{1c}, 17.2% for GA, and 10.8 μg/mL for 1,5-AG).

To evaluate the ability of each glycemic measure to identify the presence of DR, we compared the AUC among glycemic measures (Figure 2). The AUC for 2-hour PG was 0.947 (95% confidence interval [CI] 0.922-0.971), which was significantly larger than that for FPG (0.908 [95% CI 0.866-0.949]; p = 0.01), GA (0.906 [95% CI 0.853-0.960]; p = 0.03), and 1,5-AG (0.881 [95% CI 0.824-0.937]; p = 0.01), and was marginally significantly higher than that for HbA_{1c} (0.919 [95% CI 0.878-0.959]; p = 0.07). The AUC for 1,5-AG was lower than that for other measures of glycemia, but there was no significant difference in the AUC among FPG, HbA_{1c}, GA, and 1,5-AG.

Discussion

Using data from a cross-sectional survey in a Japanese community, we demonstrated that the optimal thresholds

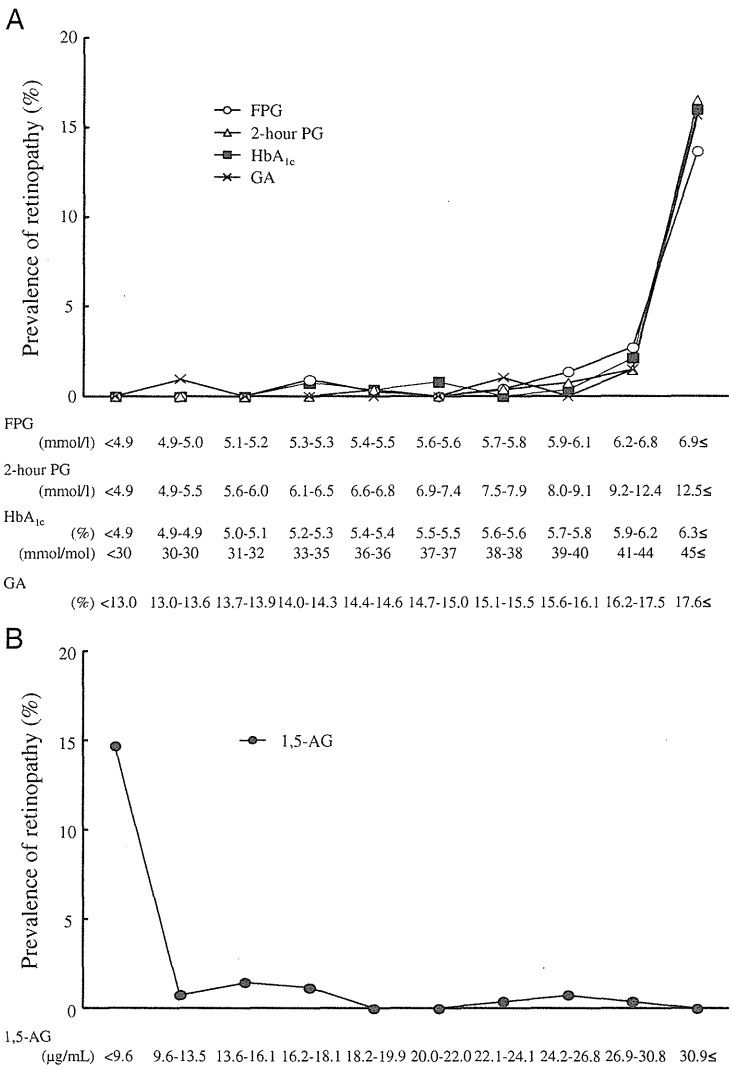


Figure 1 Prevalence of diabetic retinopathy by deciles of the distribution of fasting plasma glucose, 2-hour postload glucose, hemoglobin A_{1c}, glycated albumin (A), and 1,5-anhydroglucitol levels (B). FPG: fasting plasma glucose; 2-hour PG: 2-hour postload glucose; HbA_{1c}: hemoglobin A_{1c}; GA: glycated albumin; 1,5-AG: 1,5-anhydroglucitol.

for detecting prevalent DR from ROC analyses were 6.5 mmol/l for FPG, 11.5 mmol/l for 2-hour PG, 6.1% (43 mmol/mol) for HbA_{1c}, 17.0% for GA, and 12.1 μg/mL for 1,5-AG. These results were in accordance with those from the prevalence analysis of DR by decile levels of these measures of glycemia. These findings suggest that the FPG and HbA_{1c} thresholds for diagnosing diabetes in the Japanese population are lower than the current diagnostic criterion, while the 2-hour PG threshold is approximately 11.1 mmol/l, which is comparable to the diagnostic criterion. To our knowledge, the present study is the first report to determine the GA and 1,5-AG thresholds for the diagnosis of diabetes using the prevalence of DR. Furthermore, 2-hour PG had higher sensitivity and larger AUC than other glycemic measures, whereas the AUCs for FPG, HbA_{1c}, GA, and 1,5-AG were not significantly different. These findings indicate that 2-hour PG has the highest discriminative ability, and measurements of FPG, HbA_{1c}, GA, and 1,5-AG are similar in their ability.

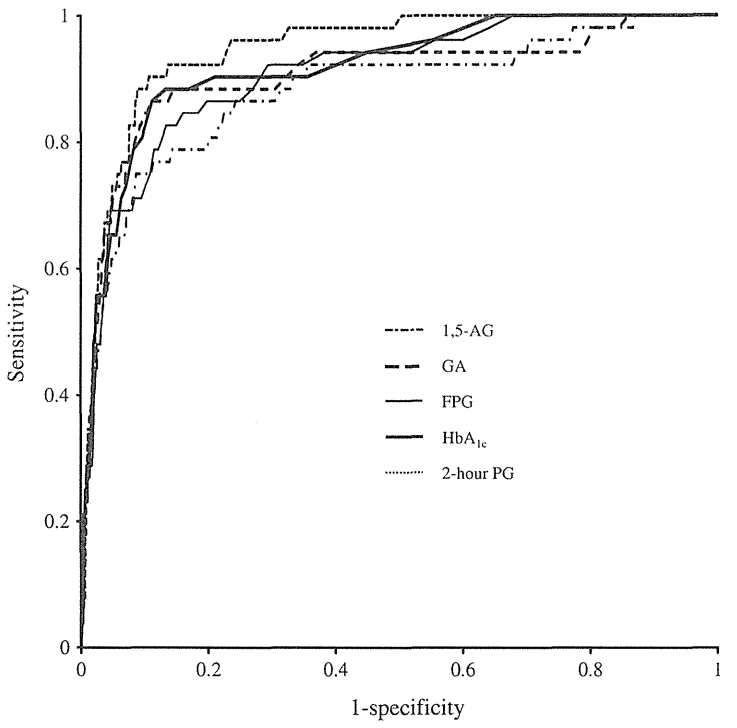
The HbA_{1c} thresholds for identifying presence of DR have varied among prior epidemiological studies of Asian populations, ranging from 6.1% (43 mmol/mol) to 7.0% (53 mmol/mol). In a study in a Singapore population, the optimal HbA_{1c} threshold for DR was 6.6-7.0% (49-53 mmol/mol) [15]. A subanalysis of the DETECT-2, which included three Asian studies in India, Singapore, and Japan, showed that an HbA_{1c} of 6.4% (46 mmol/mol) was the optimal threshold [16]. Similar findings

Table 2 Thresholds of FPG, 2-hour PG, HbA_{1c}, GA, and 1,5-AG levels for detecting diabetic retinopathy

	Threshold	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
FPG	6.5 mmol/l	82.7	86.6	10.9	99.6
2-hour PG	11.5 mmol/l	90.4	89.3	14.3	99.8
HbA_{1c}	6.1% (43 mmol/mol)	86.5	88.8	13.3	99.7
GA	17.0%	86.5	89.0	13.5	99.7
1,5-AG	12.1 μg/mL	78.8	85.8	9.9	99.5

FPG: fasting plasma glucose; 2-hour PG: 2-hour postload glucose; HbA_{1c}: hemoglobin A_{1c}; GA: glycated albumin; 1,5-AG: 1,5-anhydroglucitol; PPV: positive predictive value; NPV: negative predictive value.
The optimal threshold was defined as the point on the receiver operating characteristic curve closest to the ideal of 100% sensitivity and 100% specificity.

were observed in a Chinese population study (6.4% [46 mmol/mol]) [17]. On the other hand, in the present study, the prevalence of DR increased precipitously when HbA_{1c} levels exceeded 5.9-6.2% (41-44 mmol/mol), and the optimal threshold of HbA_{1c} using the ROC analysis was 6.1% (43 mmol/mol). Importantly, this threshold was identical with that from our previous study in 1998 (NGSP: 6.1% [43 mmol/mol]; JDS: 5.7%) [14]. Furthermore, in another epidemiological study in a Japanese population, the ROC analysis indicated that the highest precision for DR was observed at an HbA_{1c} value of 6.2% (44 mmol/mol) [18]. These findings suggest that the HbA_{1c} threshold in the Japanese population was lower than that of other Asians and also lower than the diagnostic criterion of 6.5% (48 mmol/mol). Although the reason for this difference is unclear, the influence of race and ethnicity on HbA_{1c} levels may contribute to this phenomenon. Some epidemiological



Glycemic measures	Area under curve (95% CI)	p value	p value	p value	p value
1,5-AG	0.881 (0.824-0.937)	reference			
GA	0.906 (0.853-0.960)	0.39	reference		
FPG	0.908 (0.866-0.949)	0.24	0.95	reference	
HbA_{1c}	0.919 (0.878-0.959)	0.22	0.56	0.54	reference
2-hour PG	0.947 (0.922-0.971)	0.01	0.03	0.01	0.07

Figure 2 Comparison of the discriminative ability of fasting plasma glucose, 2-hour postload glucose, hemoglobin A_{1c}, glycated albumin, and 1,5-anhydroglucitol for detecting the presence of diabetic retinopathy. FPG: fasting plasma glucose; 2-hour PG: 2-hour postload glucose; HbA_{1c}: hemoglobin A_{1c}; GA: glycated albumin; 1,5-AG: 1,5-anhydroglucitol; CI: confidence interval.

studies have shown that South Asians, Hispanics, and Blacks had higher HbA_{1c} levels than non-Hispanic whites, independent of glucose [39,40]. In our subjects, the mean of HbA_{1c} levels (5.5% [37 mmol/mol]) was lower than those in other Asian population studies (6.0-6.5% [42-48 mmol/mol]) [15-17]. Thus, it is speculated that there are racial and ethnic differences in HbA_{1c} levels even among Asians, and this may be the reason for the lower threshold in our subjects. In addition, the aldehyde dehydrogenase 2*2 (ALDH2*2) allele, which is more common in East Asians than in other ethnic groups, has been identified as a genetic risk factor for incident DR in Japanese subjects with diabetes [41], and thus, such a genetic difference in susceptibility to DR might also affect the HbA_{1c} levels associated with incident DR.

The use of HbA_{1c} measurement to diagnose diabetes remains somewhat controversial [42]. Recent epidemiological studies have revealed that HbA_{1c} measurement alone was less sensitive for detecting subjects with diabetes compared to the OGTT [43,44]. However, in our study, the AUCs for HbA_{1c} and FPG were not significantly different. This finding indicates that the discriminative ability of HbA_{1c} for diagnosing diabetes was comparable to that of FPG. Furthermore, HbA_{1c} measurement can be done without fasting or timed samples, and thus it would be suitable for mass screening in general practice. This advantage has implications for the early identification and treatment of undiagnosed diabetes. For these reasons, HbA_{1c} measurement may be an appropriate tool for detecting undiagnosed diabetes. In addition, some clinical and population-based studies, including our previous study, have shown that elevated HbA_{1c} levels were independently associated with cardiovascular disease [45,46], suggesting that HbA_{1c} is also useful as a predictor of macrovascular complications. Therefore, the use of HbA_{1c} to diagnose diabetes will help to prevent both micro- and macrovascular complications of diabetes, which are increasingly recognized as a global health priority.

In the present analysis, although the prevalence of DR was quite small for GA below 16.2-17.5%, it began to rise sharply above these levels, and the optimal threshold of GA using the ROC analysis was 17.0%. Several studies have examined the use of GA levels for detecting diabetes or glucose intolerance, as defined by glucose levels. In a Japanese population study, the ROC analysis for detecting diabetes identified the GA threshold as 15.5% [23], while another study of Japanese subjects reported a GA level of 17.0% as the lower limit of glucose intolerance [21]. A similar threshold of GA was obtained in a Chinese population study (17.1%) [22]. The thresholds in these studies were in good agreement with our findings. Together with those of other studies, our findings suggest that the optimal GA threshold for diagnosing diabetes is likely to be 17.0%.

There have been a few studies evaluating the optimal threshold of 1,5-AG for identifying individuals with diabetes, as defined by a OGTT. A Japanese population study showed that 14.0 μg/mL was the best value for detecting subjects with diabetes [24]. Similar findings were observed among Japanese male workers (14.2 μg/mL) [26]. In a Chinese study, the mean of 1,5-AG levels was 15.0 μg/mL in subjects with newly diagnosed diabetes and 11.8 μg/mL in subjects with known diabetes [25]. However, no study showed an optimal threshold of 1,5-AG using the presence of DR. The present study revealed that the steepest increment in the prevalence of DR occurred when the 1,5-AG levels fell below 9.6-13.5 μg/mL, and that 12.1 μg/mL was the optimal 1,5-AG threshold in the ROC analysis. Further epidemiological studies are needed to verify our findings.

The FPG of 7.0 mmol/l and the 2-hour PG of 11.1 mmol/l for diagnosing diabetes with the current criterion were also derived mainly from studies in Western populations [7]. In our study, the optimal threshold for detecting prevalent DR was 6.5 mmol/l for FPG, and 11.5 mmol/l for 2-hour PG. The 2-hour PG threshold was compatible with that from our previous report (11.1 mmol/l) [14] and another study of a Japanese population (11.0 mmol/l) [29]. Meanwhile, other Asian population studies have reported that the optimal FPG threshold for DR was 7.0 mmol/l in a Japanese population [29], and 7.2 mmol/l in a Chinese population [17]. These findings are inconsistent with ours. However, a recent meta-analysis in Asian and Western populations evaluated the relationship of glucose levels with DR and concluded that the FPG threshold for diagnosing diabetes was 6.5 mmol/l [16]. Furthermore, our prior studies showed that the threshold of FPG for DR was 6.4 mmol/l [14] and that the FPG threshold corresponding to a 2-h PG of 11.1 mmol/l was 6.2 mmol/l [47]. These results were very similar to those of the present study. Taken together, these findings imply that, in a Japanese population, the threshold of FPG for diabetes is lower than the diagnostic criterion of 7.0 mmol/l, while the threshold of 2-hour PG is 11.1 mmol/l, which is in accord with the diagnostic criterion.

The present study showed that among the five glycemic measures, 2-hour PG had not only the highest sensitivity but also the largest AUC to identify the presence of DR. These results suggest that the performance and discriminative ability of 2-hour PG for diagnosing diabetes were superior to those of other glycemic measures. Oxidative stress is known to be one of the crucial contributors in the pathogenesis of DR [48]. It has also been reported that acute hyperglycemia had a more specific triggering effect on oxidative stress than chronic

sustained hyperglycemia [49,50]. Thus, 2-hour PG values can be considered a better marker of oxidative stress levels arising from acute hyperglycemia than FPG, HbA_{1c}, GA, and 1,5-AG values. Furthermore, a prospective study demonstrated that postprandial plasma glucose was a stronger predictor of the progression of DR than HbA_{1c} in Japanese subjects with diabetes [51]. Taken together, these findings imply that 2-hour PG levels may be more strongly associated with DR than other glycemic measures. This may explain why 2-hour PG has a high diagnostic accuracy for DR. On the other hand, the AUCs for GA and 1,5-AG did not significantly differ from those for FPG and HbA_{1c}, suggesting that GA and 1,5-AG are acceptable alternatives for the diagnosis of diabetes, and these two measures may be particularly useful for individuals with anemia, renal disease or hemoglobinopathy, for whom interpretation of HbA_{1c} values is problematic. However, the 1,5-AG levels had smaller AUC with lower sensitivity than other glycemic measures. One possible explanation for this phenomenon may be that 1,5-AG levels reflect the degree of glycosuria rather than glucose levels [28], while other glycemic measures directly indicate the degree of hyperglycemia. In addition, it has been reported that 1,5-AG levels were influenced by individual difference in their renal thresholds for glucose [52]. These facts might be the reason for the relatively low discriminative ability of 1,5-AG in our study.

The strengths of our study include the population-based design, high participation rate, and availability of data to evaluate the five glycemic measures. In addition, it is noteworthy that the FPG, 2-hour PG, and HbA_{1c} thresholds in the present study were nearly the same as those from our previous study [14], suggesting the high reproducibility of the results in our population. However, some limitations should also be mentioned. First, our analyses included subjects with antidiabetic medications. Hypoglycemic medications could have affected the levels of glycemia. The optimal thresholds remained substantially unchanged, except for GA, after excluding subjects who received hypoglycemic medications (FPG: 6.3 mmol/l; 2-hour PG: 11.5 mmol/l; HbA_{1c}: 6.2% [44 mmol/mol]; GA: 20.5%; and 1,5-AG: 12.1 μg/mL). However, the precision of this finding may be limited, because of the small number of cases of DR among those not taking hypoglycemic medications. Second, the values of HbA_{1c} were not measured by high-performance liquid chromatography (HPLC) as used in the Diabetes Control and Complications Trial, although the method and reagent used to measure HbA_{1c} in this study have since been NGSP-certified. It would be preferable to measure HbA_{1c} by HPLC to make the results of our study more comparable to those of other studies. Third, the GA and 1,5-AG levels were measured in serum conserved at -80°C for 5 years. However, the stability of GA and 1,5-AG measurements in frozen stored serum sample was preserved [53,54]. Fourth, this study is a cross-sectional design, which might have affected the threshold values of glycemic measures. Diagnostic thresholds would ideally be derived from prospective studies that examine the relationship between measures of glycemia and incident microvascular complications. Lastly, the influence of factors that may affect HbA_{1c} levels, such as anemia, renal failure, and hemoglobinopathy should be considered. We performed sensitivity analyses excluding subjects with anemia or renal failure, and the optimal threshold of HbA_{1c} remained unchanged (6.1% [43 mmol/mol]). Furthermore, the prevalence of hemoglobinopathy in Japan was reported to be very low (0.04%) [55]. Therefore, the influence of this limitation would have been small.

Conclusions

The present analysis showed that, in a Japanese population, the FPG and HbA_{1c} threshold for diagnosing diabetes was lower than the current diagnostic criterion, while the 2-hour PG threshold was consistent with the diagnostic criterion, and the discriminative ability of 2-hour PG was superior to other glycemic measures. These findings suggest that the threshold of 2-hour PG is 11.1 mmol/l, regardless of race, whereas ethnic-specific thresholds of FPG and HbA_{1c} may be necessary. This study also demonstrated the potential applicability of GA and 1,5-AG measurements as a diagnostic tool for diabetes. Further prospective studies are needed to verify these findings, and investigations of HbA_{1c} levels in the intermediate range are also required.

Abbreviations

HbA_{1c}: Hemoglobin A_{1c}; DR: Diabetic retinopathy; FPG: Fasting plasma glucose; GA: Glycated albumin; 1,5-AG: 1,5-anhydroglucitol; PG: Postload glucose; OGTT: Oral glucose tolerance test; Hb: Hemoglobin; NGSP: National Glycohemoglobin Standardization Program; JDS: Japan Diabetes Society; eGFR: Estimated glomerular filtration rate; BMI: Body mass index; ETDRS: Early Treatment of Diabetic Retinopathy Study; ROC: Receiver operating characteristic; AUC: Area under the receiver operating characteristic curve; CI: Confidence interval; HPLC: High-performance liquid chromatography.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

NM contributed to the study concept and design, data collection, data analysis, data interpretation, and drafting of the manuscript. MY contributed to the data collection, data interpretation, and drafting of the manuscript. TN, JH, YH, FI, and MF contributed to the data collection and data interpretation. TH and DK measured the samples and contributed to the data interpretation. MK, UN, and TK contributed to the data interpretation and the critical revision of the manuscript for important intellectual content. YK contributed to the data collection, data interpretation, drafting of the manuscript, obtained funding, and study supervision. All authors provided critical review of the draft and approved the final version.

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CARDIO VASCULAR DIABETOLOGY

ORIGINAL INVESTIGATION Open Access

Elevation of circulating fatty acid-binding protein 4 is independently associated with left ventricular diastolic dysfunction in a general population

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Abstract

Background: Fatty acid-binding protein 4 (FABP4) is expressed in both adipocytes and macrophages. Recent studies have shown secretion of FABP4 from adipocytes and association of elevated serum FABP4 level with obesity, insulin resistance, hypertension, and atherosclerosis. However, little is known about role of FABP4 in cardiac function.

Methods: From the database of the Tanno-Sobetsu Study, data for 190 subjects (male/female: 82/108) who were not treated with any medication and underwent echocardiography in 2011 or 2012 were retrieved for analyses of relationships between serum FABP4 concentration, metabolic markers and parameters of echocardiography.

Results: Serum FABP4 level was positively correlated with age, body mass index (BMI), blood pressure (BP), LDL cholesterol, HOMA-R and mean left ventricular (LV) wall thickness (LVWT, males: $r = 0.315$, females: $r = 0.401$, $p < 0.01$) and was negatively correlated with HDL cholesterol, estimated glomerular filtration rate (eGFR) and peak myocardial velocity during early diastole (e'; males: $r = -0.434$, females: $r = -0.353$, $p < 0.01$), an index of LV diastolic function. However, no significant correlation was found between FABP4 level and LV end-diastolic dimension, LV ejection fraction or LV mass index. There were significant correlations of e' with age, BMI, BP, eGFR, brain natriuretic peptide (BNP), FABP4, metabolic markers and LVWT. Multivariate regression analysis adjusted by HOMA-R, BMI, eGFR, BNP or LVWT in addition to age, gender and BP revealed that serum FABP4 concentration was independently correlated with e'.

Conclusions: Elevation of circulating FABP4 may contribute to LV diastolic dysfunction in a general population.

Keywords: Fatty acid-binding protein 4, Adipokine, Left ventricular diastolic dysfunction

Background

Fatty acid-binding proteins (FABPs) are a group of intracellular lipid chaperones that coordinate lipid responses in cells [1,2]. FABPs are about 14-15-kDa proteins that can reversibly bind hydrophobic ligands, such as saturated and unsaturated long chain fatty acids, with high affinity [1,2]. FABPs have been proposed to facilitate the transport of lipids to specific compartments in the cell. Among FABPs, fatty acid-binding protein 4 (FABP4), known as adipocyte FABP (A-FABP) or aP2, is expressed in adipocytes, macrophages and capillary endothelial cells [1-3]. Emerging evidence indicates that FABP4 acts at the integration between metabolic and inflammatory pathways and plays an important role in the development of insulin resistance and atherosclerosis [4-6]. It has also been demonstrated in experimental models that chemical inhibition of FABP4 could be a therapeutic strategy against insulin resistance, diabetes mellitus, fatty liver disease and atherosclerosis [7].

Adipose tissue is now known to secrete a variety of bioactive molecules called adipokines, such as tumor necrosis factor-α (TNFα), leptin and adiponectin, which are implicated in a wide range of biological phenomena.

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Interestingly, recent studies have shown that FABP4 is secreted from adipocytes [8,9], though there are no typical signal peptides for secretion in the sequence of FABP4 [1]. It has also been demonstrated that secretion of FABP4 is via a non-classical secretion pathway and that FABP4 acts as an adipokine for the development of hepatic insulin resistance [9]. Furthermore, elevated serum concentration of FABP4 has been shown to be associated with obesity, insulin resistance, hypertension and atherosclerosis [8-12].

Obesity is a risk factor for several kinds of cardiac insults, such as left ventricular (LV) hypertrophy, LV diastolic dysfunction and heart failure with preserved or reduced ejection fraction. It has been suggested that several adipokines provide a direct pathophysiological link between enlarged adipose tissue and obesity-associated cardiac dysfunction [13]. However, little is known about the relationship between circulating FABP4 and cardiac function, especially in a general population. Therefore, we hypothesized that increase in serum FABP4 reflects LV diastolic dysfunction as an early stage of cardiac insults in a general population. To address this hypothesis, we conducted a study to investigate the cross-sectional associations between serum FABP4 concentration and several echocardiographic parameters in subjects who had not regularly taken any medications.

Methods

Study population

The Tanno-Sobetsu Study is a study with a population-based cohort design recruiting residents of two rural towns, Tanno and Sobetsu, in Hokkaido and includes annual health examination, pathophysiological assessment of metabolic syndrome and cardiovascular disease, and follow-up survey. A total of 357 female subjects (mean age: 66 ± 13 years) in 2011 and 277 male subjects (mean age: 66 ± 13 years) in 2012 received annual examinations in Sobetsu Town. Female and male participants in 2011 and 2012, respectively, were invited to receive echocardiographic examinations. Subjects who were being treated with any regular medications for diseases were excluded. Other exclusion criteria were atrial fibrillation and conductional abnormalities such as left bundle branch block on electrocardiogram or severe valvular disease and left ventricular hypertrophy (wall thickness >12.5 mm) on echocardiogram. A total of 190 subjects who underwent echocardiography (male/female: 82/108, mean age: 63 ± 13 years) contributed to the present analyses. This study conformed to the principles outlined in the Declaration of Helsinki and was performed with the approval of the Ethical Committee of Sapporo Medical University. Written informed consent was received from all of the subjects.

Measurements

Medical check-ups were performed between 06:00 h and 09:00 h after an overnight fast. After measuring anthropometric parameters, blood pressure was measured twice consecutively on the upper arm using an automated sphygmomanometer (HEM-907, Omron Co., Kyoto, Japan) with subjects in a seated resting position, and average blood pressure was used for analysis. Body mass index (BMI) was calculated as body weight (in kilograms) divided by the square of body height (in meters). Peripheral venous blood samples were obtained from study subjects after physical examination for complete blood count and biochemical analyses of the serum. The serum samples were analyzed immediately or stored at −80°C until biochemical analyses.

Serum concentration of FABP4 was measured using a commercially available enzyme-linked immunosorbent assay kit for FABP4 (Biovendor R&D, Modrice, Czech Republic). The accuracy, precision and reproducibility of the kit have been described previously [8]. The intra- and inter-assay coefficient variances in the kit were < 5%. Fasting plasma glucose was determined by the glucose oxidase method. Fasting plasma insulin was measured by a radioimmunoassay method (Insulin RIA bead, Dianabot, Tokyo, Japan). Creatinine (Cr) and lipid profiles, including total cholesterol, high-density lipoprotein (HDL) cholesterol and triglycerides, were determined by enzymatic methods. Low-density lipoprotein (LDL) cholesterol level was calculated by the Friedewald equation. Hemoglobin A1c (HbA1c) was determined by a latex coagulation method and was expressed in national glycohemoglobin standardization program (NGSP) scale. Brain natriuretic peptide (BNP) was measured using an assay kit (Shionogi & Co., Osaka, Japan). High-sensitivity C-reactive protein (hsCRP) was measured by a nephelometry method. As an index of renal function, estimated glomerular filtration rate (eGFR) was calculated by an equation for Japanese: $eGFR(ml/min/1.73m^2) = 194 \times Cr^{(-1.094)} \times age^{(-0.287)} \times 0.739$ (if female). HOMA-R, an indicator of insulin resistance, was calculated by the previously reported formula: insulin(μU/ml) × glucose(mg/dl)/405.

Echocardiography

After medical check-ups and collection of urine and blood samples, echocardiographic examinations were performed by three well-experienced echocardiographers who were blinded to clinical data, using Vivid 9 (GE Health Care, Tokyo, Japan) equipped with a 2.5-MHz frequency transducer. Two-dimensional and color tissue Doppler imaging modes were used to obtain images from standard echocardiographic views, including parasternal long-axis and apical four-, three-, and two chamber views at a left lateral decubitus position. Standard parameters in two-dimensional measurements, including LV end-diastolic

and end-systolic dimensions (mm) and septal and posterior wall thicknesses at end-diastole (mm), were determined. Mean LV wall thickness (mm) was calculated by the average of septal and posterior wall thicknesses at end-diastole. LV ejection fraction (%) was calculated using biplane modified Simpson's method. LV mass was calculated according to the recommendations of the American Society of Echocardiography [14] and normalized for body surface area (LV mass index, g/m^2). Left atrial (LA) dimension (mm) was measured by M-mode echocardiography, and LA volume was measured using biplane Simpson's method and normalized for body surface area (LA volume index, ml/m^2) [14]. Each parameter was evaluated by averaging two to three measurements. Transmitral flow velocities were obtained by pulsed wave Doppler echocardiography, positioning a sample volume at the level of a mitral tip in an apical four-chamber view. Mitral flow parameters, including peak velocities during early (E) and late diastole (A) and E-wave deceleration time, were measured, and the E/A ratio was calculated. Tissue velocity curves were obtained from color tissue Doppler imaging. A sample volume was placed at the lateral annulus in the apical four-chamber view, and peak myocardial velocity during early diastole (e', cm/sec) was measured, and the ratio of mitral to myocardial early diastolic peak velocity (E/e') was calculated.

Statistical analysis

Numeric variables are expressed as means ± SD for normal distributions or medians (interquartile ranges) for skewed variables. The distribution of each parameter was tested for its normality using the Shapiro-Wilk W test, and non-normally distributed parameters were logarithmically transformed for comparison and regression analyses. Comparison between two groups was done with an unpaired *t* test. One-way analysis of variance and Tukey-Kramer *post hoc* test were used for detecting significant differences in data between multiple groups. The correlation between two variables was evaluated using Pearson's correlation coefficient. Multivariate regression analysis was performed to identify independent determinants of e' using the variables with a significant and non-confounding correlation in simple regression analysis as independent predictors, showing the t-ratio calculated as the ratio of regression coefficient and standard error of regression coefficient and the percentage of variance in the object variables that they explained (R^2). A p value of less than 0.05 was considered statistically significant. Holm-Bonferroni sequential correction was also performed in multivariate regression analysis. All data were analyzed by using JMP 9 for Macintosh (SAS Institute, Cary, NC).

Results

Basal characteristics of the study subjects are shown in Table 1. Male subjects were significantly older than the female subjects and they had significantly larger BMI and waist circumference and had higher levels of systolic and diastolic blood pressures, triglycerides, glucose, HbA1c, insulin, HOMA-R and Cr and lower levels of total cholesterol, HDL cholesterol, LDL cholesterol and FABP4 than did the females. No significant difference in eGFR or BNP was found between male and female subjects. In echocardiographic parameters, LA dimension, mean LV wall thickness, LV end-diastolic dimension, LV mass index and E-wave deceleration time were significantly larger in males than in females. On the other hand, LV ejection fraction and E/A ratio were smaller in males than in females. Levels of e' and E/e' were comparable between male and female subjects.

In analyses of data from all study subjects, serum FABP4 level was positively correlated with age, BMI, systolic and diastolic blood pressures, total cholesterol, LDL cholesterol, triglycerides, insulin, HOMA-R, Cr and hsCRP and was negatively correlated with eGFR (Table 2). Similar correlations between the parameters were observed when male and female subjects were separately analyzed.

Regarding echocardiographic parameters, FABP4 concentration was positively correlated with LA dimension, LA volume index and mean LV wall thickness (males: $r = 0.315$, females: $r = 0.401$, $p < 0.01$), though correlation with FABP4 was not significant for LV end-diastolic dimension or LV mass index. FABP4 level was positively correlated with E/e' and negatively correlated with e' (Figure 1; males: $r = -0.434$, females: $r = -0.353$, $p < 0.001$), an index of LV diastolic function, and E/A ratio (Table 2), whereas LV ejection fraction was not correlated with FABP4 level. Among echocardiographic parameters, e' was positively correlated with LV ejection fraction and E/A ratio and was negatively correlated with LA dimension, LA volume index, mean LV wall thickness, LV mass index, E-wave deceleration time and E/e' (Table 3). Of extra-cardiac parameters, age, BMI, waist circumference, systolic and diastolic blood pressures and biochemical markers, including eGFR, BNP, hsCRP and FABP4, were found to be significantly correlated with e' (Table 3).

Multivariate regression analysis was performed to identify independent determinants of e' using systolic blood pressure, the most strongly correlated factor among anthropometric and biochemical parameters ($r = -0.465$, $p < 0.001$), in addition to age and gender (Model 1) and showed that serum FABP4 concentration was independently correlated with e' (Table 4). Next, the variables with a significant and non-confounding correlation in simple regression analysis were additionally chosen as possible independent predictors in Model 2 ~ 6: a marker of adiposity (BMI, Model 2), glucose and insulin metabolism (HOMA-R, Model 3), renal function (eGFR, Model 4), cardiac damage (BNP, Model 5) or cardiac morphology (LV wall thickness, Model 6). When the each parameter

Table 1 Characteristics of the studied subjects

	Whole	Male	Female
n	190	82	108
Age (years)	63 ± 13	66 ± 13	60 ± 13*
Body mass index (kg/m^2)	23.2 ± 3.7	24.4 ± 3.9	22.3 ± 3.4*
Waist circumference (cm)	84 ± 11	88 ± 11	81 ± 10*
Systolic blood pressure (mmHg)	134 ± 21	138 ± 18	130 ± 22*
Diastolic blood pressure (mmHg)	77 ± 11	79 ± 10	75 ± 12†
Biochemical data			
Total cholesterol (mg/dl)	202 ± 33	188 ± 29	212 ± 32*
HDL cholesterol (mg/dl)	68 ± 18	59 ± 17	75 ± 16*
LDL cholesterol (mg/dl)	119 ± 29	108 ± 28	127 ± 28*
Triglycerides (mg/dl)	83 (64–112)	93 (70–132)	77 (57–100)*
Glucose (mg/dl)	92 (87–99)	97 (91–103)	90 (85–96)*
HbA1c (%)	5.5 ± 0.5	5.6 ± 0.5	5.4 ± 0.4*
Insulin (μU/ml)	4.5 (3.4-6.9)	4.9 (3.6-7.6)	4.2 (3.2-6.1)*
HOMA-R	1.02 (0.74-1.63)	1.21(0.85-1.92)	0.94 (0.68-1.37)*
Creatinine (mg/dl)	0.74 ± 0.17	0.87 ± 0.16	0.65 ± 0.11*
eGFR (ml/min/1.73 m^2)	72 ± 14	71 ± 15	74 ± 14
BNP (pg/ml)	28 (11–31)	14 (9–33)	21 (12–31)
hsCRP (mg/dl)	0.03 (0.02-0.07)	0.04 (0.02-0.08)	0.03 (0.02-0.06)
FABP4 (ng/ml)	12.2 (8.4-16.2)	10.9 (8.1-14.2)	13.3 (9.1-17.0)†
Echocardiographic parameters			
Left atrial dimension (mm)	34 ± 6	36 ± 6	33 ± 6*
Left atrial volume index (ml/m^2)	27 ± 8	26 ± 8	27 ± 8
Mean LV wall thickness (mm)	8.8 ± 1.2	9.6 ± 0.9	8.2 ± 0.9*
LV end-diastolic dimension (mm)	44 ± 5	45 ± 5	43 ± 4*
LV mass index (g/m^2)	93 ± 23	102 ± 22	86 ± 21*
LV ejection fraction (%)	67 ± 5	66 ± 6	67 ± 5†
E/A	0.91 (0.75-1.19)	0.79 (0.68-0.94)	0.98 (0.79-1.31)*
E-wave deceleration time (msec)	214 ± 53	223 ± 59	207 ± 47†
e' (cm/sec)	10.2 ± 3.1	9.7 ± 3.2	10.6 ± 3.0
E/e'	7.0 ± 2.3	6.7 ± 2.3	7.3 ± 2.3

Variables are expressed as number, means ± SD or medians (interquartile ranges). BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; LV, left ventricle. *P < 0.01, †P < 0.05 vs. male.

was additionally incorporated into the adjustment, FABP4 remained as an independent predictor of e' in Model 2 ~ 6 (Table 4), although the independent correlation in Model 2 was cancelled after Holm-Bonferroni sequential correction. Additional multivariate regression analysis using all of the used parameters in Model 1 ~ 6, including age, gender, systolic blood pressure, BMI, HOMA-R, eGFR, BNP, mean LV wall thickness and FABP4, showed that FABP4 level ($t = -2.36$, $p = 0.020$) was independently correlated with e' after adjustment of other variables (overall $R^2 = 0.563$).

In low and middle tertiles of BMI, e' in a group with low levels of FABP4 (FABP4-Low) was significantly higher than that in a group with high levels of FABP4 (FABP4-High) (Figure 2). Furthermore, there was no significant difference in e' between the FABP4-Low and FABP4-High groups in high tertile of BMI, but the FABP4-Low group in high tertile of BMI had significantly lower e' than did that in low tertile of BMI.

Discussion

The salient finding in the present study was that FABP4 was independently and negatively correlated with e', which reflects LV relaxation and is known as one of the most sensitive indexes of LV diastolic function in a healthy population [14]. LV diastolic dysfunction often precedes

Table 2 Simple regression analysis for log FABP4

	Whole (n = 190)		Male (n = 82)		Female (n = 108)	
	r	p	r	p	r	p
Age	0.225	0.002	0.193	0.083	0.314	0.001
Body mass index	0.500	<0.001	0.580	<0.001	0.564	<0.001
Waist circumference	0.518	<0.001	0.642	<0.001	0.518	<0.001
Systolic blood pressure	0.272	<0.001	0.283	0.010	0.329	0.001
Diastolic blood pressure	0.268	<0.001	0.255	0.021	0.329	0.001
Biochemical data						
Total cholesterol	0.213	0.003	0.008	0.943	0.282	0.003
LDL cholesterol	0.268	<0.001	0.058	0.606	0.365	<0.001
HDL cholesterol	−0.107	0.143	−0.125	0.263	−0.238	0.013
log Triglycerides	0.157	0.031	0.097	0.385	0.282	0.003
log Glucose	0.137	0.060	0.081	0.467	0.277	0.004
HbA1c	0.046	0.532	0.044	0.695	0.107	0.272
log Insulin	0.388	<0.001	0.380	0.001	0.480	<0.001
log HOMA-R	0.386	<0.001	0.384	<0.001	0.489	<0.001
Creatinine	0.192	0.008	0.448	<0.001	0.305	0.001
eGFR	−0.350	<0.001	−0.397	<0.001	−0.350	<0.001
log BNP	0.126	0.085	0.076	0.500	0.156	0.108
log hsCRP	0.233	0.001	0.312	0.005	0.218	0.024
Echocardiographic parameters						
Left atrial dimension	0.251	0.001	0.354	0.001	0.290	0.002
Left atrial volume index	0.231	0.002	0.226	0.050	0.225	0.020
Mean LV wall thickness	0.195	0.007	0.315	0.004	0.401	<0.001
LV end-diastolic dimension	−0.042	0.562	0.069	0.541	−0.082	0.399
LV mass index	0.039	0.596	0.085	0.448	0.105	0.281
LV ejection fraction	0.113	0.121	0.219	0.048	−0.023	0.812
log E/A	−0.178	0.016	−0.173	0.131	−0.279	0.004
E-wave deceleration time	0.006	0.934	−0.013	0.906	0.066	0.497
e'	−0.360	<0.001	−0.434	<0.001	−0.353	<0.001
E/e'	0.353	<0.001	0.361	0.001	0.327	0.001

BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; LV, left ventricle.

LV systolic dysfunction in heart diseases, and moderate diastolic dysfunction alone potentially induces heart failure, which is referred to as heart failure with preserved ejection fraction (HFpEF) [15]. A recent study in which data from the Framingham cohort study were analyzed showed that age, diabetes mellitus, BMI, smoking and atrial fibrillation were predictors of HFpEF [16]. It is notable that the correlation of FABP4 level with e' was independent of age, BMI, HOMA-R and LV wall thickness (Table 4). These results suggest that serum FABP4 is a novel marker of LV diastolic dysfunction and potentially a predictor of HFpEF.

Previous studies using animal models indicated that FABP4 plays a significant role in several aspects of metabolic syndrome, including insulin resistance, type 2 diabetes and atherosclerosis, through its action at the interface of metabolic and inflammatory pathways in adipocytes and macrophages [1,2,4-6]. Epicardial fat has been reported to directly influence cardiac function because of the absence of a fibrous fascial layer between fat and the underlying myocardium [17,18]. FABP4 mRNA expression in epicardial adipose tissue was recently reported to be profoundly increased compared with its expression in paraaortic adipose tissue in patients with metabolic syndrome [19]. Furthermore, it has recently been reported that exogenous FABP4 acutely suppresses shortening amplitude in cardiomyocytes by attenuating intracellular systolic peak Ca^{2+} level in a dose-dependent manner [20] and impairs the insulin-dependent nitric oxide pathway in vascular endothelial cells [21]. Therefore, it is possible that either FABP4

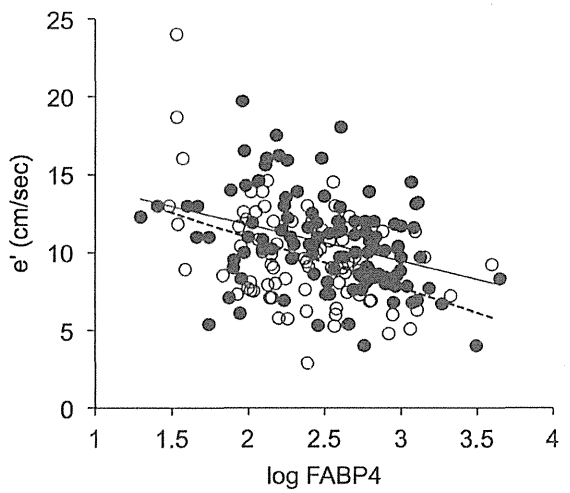

Figure 1 Association of serum FABP4 level with LV diastolic dysfunction. Peak myocardial velocity during early diastole (e'), an index of LV diastolic function, was plotted against log FABP4 for each subject. There was a significant correlation between the two parameters (males: r = −0.434, females: r = −0.353, p < 0.001). Open circles: males (n = 82), closed circles: females (n = 108). Solid regression line: males, broken regression line: females.

Table 3 Simple regression analysis for LV diastolic function

	e'	
	t	p
Age	−0.713	< 0.001
Body mass index	−0.200	0.006
Waist circumference	−0.324	< 0.001
Systolic blood pressure	−0.465	< 0.001
Diastolic blood pressure	−0.233	0.001
Biochemical data		
Total cholesterol	−0.071	0.334
LDL cholesterol	−0.140	0.054
HDL cholesterol	0.175	0.016
log Triglycerides	−0.148	0.042
log Glucose	−0.195	0.007
HbA1c	−0.210	0.004
log Insulin	−0.156	0.032
log HOMA-R	−0.187	0.010
Creatinine	−0.231	0.001
eGFR	0.376	< 0.001
log BNP	−0.394	< 0.001
log hsCRP	−0.201	0.006
log FABP4	−0.360	< 0.001
Echocardiographic parameters		
Left atrial dimension	−0.289	< 0.001
Left atrial volume index	−0.233	0.002
Mean LV wall thickness	−0.370	< 0.001
LV end-diastolic dimension	0.024	0.739
LV mass index	−0.294	< 0.001
LV ejection fraction	0.148	0.043
log E/A	0.644	< 0.001
E-wave deceleration time	−0.205	0.005
E/e'	−0.580	< 0.001

BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitivity C-reactive protein; LV, left ventricle.

secreted from epicardial fat tissue or circulating FABP4 released from subcutaneous and/or visceral adipose tissue or from macrophages may directly modulate cardiac function. In the heart, FABP3 known as heart-type FABP (H-FABP) is abundant and is rapidly released from cells into the circulation after onset of cardiomyocyte damage. Serum concentration of FABP3 has been characterized as an early biochemical marker of acute myocardial infarction and a sensitive marker of ongoing myocardial damage in patients with heart failure [22,23]. Impact of circulating FABP3 is apparently different from that of FABP4.

Inflammation is an important factor in the pathogenesis and progression of heart failure. It has been shown that increased inflammatory cytokines produced by mononuclear cells including macrophages and/or damaged myocardium impaired myocardial function by inducing apoptosis, necrosis and hypertrophic response in cardiomyocytes [24]. In the Framingham Heart Study, increased inflammatory markers, such as CRP, interleukin-6 and TNFα levels, were able to identify asymptomatic older subjects in the community who were at high risk for the future development of heart failure [25]. In the present study, FABP4 was positively correlated with hsCRP, being consistent with the results of several previous studies [10,12]. The macrophage is a critical site of FABP4 action, and macrophage-specific FABP4 deficiency leads to reduced activation of nuclear factor κ B (NF-κB) and c-Jun N-terminal kinase (JNK), resulting in reduced production of a cluster of inflammatory cytokines [5]. Conversely, several inflammatory stimuli have been shown to cause significantly increased expression of FABP4 in macrophages [5]. Local inflammation mediated by FABP4 in macrophages of the heart may participate in mediating cardiac dysfunction.

Up-regulation of FABP4 expression and other adipokines in heart failure has been demonstrated in recent studies [26-28], indicating complex neurohormonal and metabolic abnormalities associated with heart failure. Of note, up-regulation of inflammatory cytokines, catecholamines and natriuretic peptides in heart failure is known to mediate increased lipolysis and insulin resistance [29]. It has been reported that lipolysis is mediated in part through the interaction of FABP4 with hormone-sensitive lipase in adipocytes [30]. A recent study also showed that FABP4 is

Table 4 Multivariate regression analysis for LV diastolic function

	e′			e′	
Model	**t**	**p**	**Model**	**t**	**p**
Model 1			**Model 4**		
Age	−10.64	< 0.001	Age	−10.27	< 0.001
Gender (Male)	−0.31	0.755	Gender (Male)	−0.39	0.695
Systolic blood pressure	−1.83	0.069	Systolic blood pressure	−1.88	0.062
log FABP4	−3.69	< 0.001	eGFR	−1.34	0.181
	R^2 = 0.558		log FABP4	−3.93	< 0.001
				R^2 = 0.562	
Model 2			**Model 5**		
Age	−10.64	< 0.001	Age	−9.11	< 0.001
Gender (Male)	0.27	0.788	Gender (Male)	−0.33	0.744
Systolic blood pressure	−1.57	0.118	Systolic blood pressure	−1.80	0.073
Body mass index	−1.28	0.203	log BNP	−0.33	0.742
log FABP4	−2.29	0.023	log FABP4	−3.70	< 0.001
	R^2 = 0.562			R^2 = 0.556	
Model 3			**Model 6**		
Age	−10.45	< 0.001	Age	−10.34	< 0.001
Gender (Male)	0.09	0.925	Gender (Male)	0.17	0.866
Systolic blood pressure	−1.74	0.083	Systolic blood pressure	−1.62	0.107
log HOMA-R	−0.89	0.376	Mean LV wall thickness	−0.67	0.503
log FABP4	−3.01	0.003	log FABP4	−3.40	< 0.001
	R^2 = 0.560			R^2 = 0.559	

BNP, brain natriuretic peptide; eGFR, estimated glomerular filtration rate; LV, left ventricle.

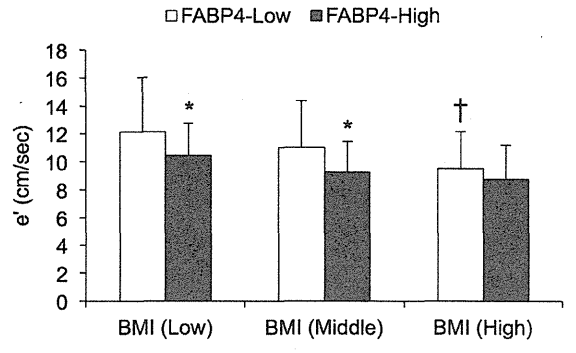

Figure 2 Comparison of LV diastolic function between subjects with low and high levels of FABP4 in tertiles of BMI. Peak myocardial velocity during early diastole (e'), an index of LV diastolic function, was compared between subjects with low and high levels of FABP4 divided by median value of FABP4 levels (FABP4-Low and FABP4-High, respectively) in tertiles of body mass index (BMI). Tertile of BMI consists of low (<21.4 kg/m^2; n = 63), middle (≥21.4 and <24.4 kg/m^2; n = 63), and high (≥24.4 kg/m^2; n = 64) groups. *P < 0.05 vs. FABP4-Low. †P < 0.05 vs. FABP4-Low in BMI (Low).

secreted from adipocytes in a non-classical secretion pathway in relation to lipolysis [9]. Although most of the recruited subjects in the present study were considered to be healthy, relatively high level of lipolytic stimuli, such as inflammatory cytokines, catecholamines and natriuretic peptides, in asymptomatic cardiac dysfunction may increase serum FABP4 concentration.

Circulating FABP4 level was associated with increased LV mass in overweight and obese women [31] and in patients with obstructive sleep apnea syndrome [32]. Recent studies also showed an independent correlation of elevated serum FABP4 with NT-proBNP in heart failure patients [33] or deterioration of LV systolic function in non-obese patients hospitalized for acutely decompensated heart failure [26] and in patients with coronary artery disease [34]. In contrast, there was no significant association between FABP4 level and concurrent [32] or subsequently developed [27] systolic dysfunction in subjects without obvious cardiac disease. In the present study using apparently healthy subjects with no medication, serum FABP4 level was weakly correlated with mean LV wall thickness but with LV mass index or LV ejection fraction. These findings suggest only a marginal