assay[10]. The measurements of VFA and APN complied with the Guidelines of the Ethical Committees of Osaka University. Written informed consent was obtained from all subjects.

Statistical Analysis

Comparison of variables between groups was carried out using an unpaired Student's *t*-test. Comparisons of variables between 2004 and 2005 in the NGT group and in the IFG and/or IGT group were carried out using a paired Student's *t*-test. APN, I.I. and homeostasis model of insulin resistance (HOMA-IR) variables were log transformed and analyzed. The area under the receiver operating characteristic (ROC) curve was used to evaluate the predictive power of various parameters. All analyses except ROC analysis were carried out using StatView, version 5 (SAS Institute, Cary, NC, USA). ROC analysis was carried out using Dr. SPSS II, standard version (SPSS, Chicago, IL, USA). Data are expressed as mean ± SD. A *P*-value of <0.05 was considered statistically significant.

RESULTS

The clinical characteristics of the subjects with NGT and IFG and/or IGT at baseline and in the next year are presented in Table 1. In 2004, there were significant differences between the NGT group and the IFG and/or IGT group in sex, age, BMI, WC, VFA, HbA_{1c}, FPG, fasting insulin (F-IRI) and sBP. In contrast, among the 251 NGT subjects in 2004, 26 participants converted to IFG and/or IGT and one participant developed diabetes mellitus (DM) in 2005. Furthermore, among the 107 IFG and/or IGT subjects diagnosed in 2004, 15 participants developed DM and 36 improved to NGT in 2005. In individuals with NGT in 2004, their BMI, WC, VFA, F-IRI and dBP decreased, and HbA_{1c}, TC and HDLC increased significantly in 2005. In individuals with IFG and/or IGT in 2004, their BMI, WC, VFA, F-IRI, LDLC and UA decreased, and HbA_{1c} and HDLC increased significantly in 2005.

Table 2 compares the 2004 clinical variables of subjects of the NGT group who showed deterioration in glucose tolerance in 2005 (worsening group) and of those who retained NGT (retaining group). WC, VFA, sBP, dBP, HbA_{1c}, PG at 0, 30, 60 and 120 min, and AUC ($glucose_{0-120}$) in OGTT were significantly higher and log (I.I.) was significantly lower in the worsening group than in the retaining group. However, BMI, TC, TG, HDLC, LDLC, UA, log (APN), IRI at 0, 30, 60 and 120 min, AUC ($insulin_{0-120}$) in OGTT, and log (HOMA-IR) were not significantly different between the two groups. The area under the ROC curve was used to evaluate the predictive power of the parameters that were significantly different between the two groups. The areas under the ROC curve of all these parameters were significantly higher than 0.5 (Table 3). Among them, the areas under the ROC curve of PG at 0 min, 60 min and AUC ($glucose_{0-120}$) in OGTT were higher than approximately 0.7, showing that these parameters are significant predictors of deterioration of glucose tolerance. ROC curves of these predictors are presented in Figure 1. Next, we determined the optimal cut-off points for these parameters to predict deterioration in glucose tolerance according to the Youden index. The optimal cut-off points for PG at 0 min, 60 min and AUC ($glucose_{0-120}$) in OGTT were 95 mg/dL, 158 mg/dL and 271 mg h/dL, respectively. The sensitivity and specificity of these cut-off points were 0.67 and 0.64 for PG at 0 min, 0.67 and 0.75 for PG at 60 min, and 0.78 and 0.71 for AUC ($glucose_{0-120}$), respectively.

Table 4 compares several of the 2004 clinical variables of individuals diagnosed with IFG and/or IGT who developed DM in

Table 2 | Comparison of baseline variables in normal glucose tolerance subjects who developed glucose intolerance and subjects who retained normal glucose tolerance in 2005

	Worsening group	Retaining group	*P*-value
n (male/female)	27 (27/0)	224 (213/11)	
Age (years)	51.3 ± 6.3	47.9 ± 8.8	0.0563
WC (cm)	93.5 ± 5.7	91.4 ± 4.9	0.0391
BMI (kg/m^2)	27.1 ± 2.2	26.5 ± 2.4	0.2350
VFA (cm^2)	140.6 ± 27.9	130.0 ± 23.0	0.0307
sBP (mmHg)	140.2 ± 13.6	131.3 ± 14.6	0.0029
dBP (mmHg)	88.1 ± 9.3	83.4 ± 9.9	0.0197
TC (mg/dL)	212.5 ± 32.9	214.0 ± 32.7	0.8211
TG (mg/dL)	218.8 ± 198.5	175.6 ± 101.6	0.0714
HDLC (mg/dL)	54.3 ± 18.1	52.7 ± 14.8	0.5959
LDLC (mg/dL)	117.3 ± 34.7	124.4 ± 28.6	0.2373
UA (mg/dL)	6.72 ± 0.93	6.36 ± 1.35	0.1891
HbA_{1c} (%)	5.0 ± 0.4	4.7 ± 0.4	0.0014
Log (APN)	1.8 ± 0.4 (n = 25)	1.8 ± 0.4 (n = 205)	0.9964
OGTT: PG (mg/dL)			
0 min	98.4 ± 7.0	93.2 ± 6.9	0.0003
30 min	167.1 ± 26.2	149.3 ± 27.7	0.0017
60 min	172.4 ± 33.0	138.1 ± 33.9	<0.0001
120 min	114.0 ± 19.7	104.2 ± 19.1	0.0122
OGTT: IRI (μU/mL)			
0 min	7.5 ± 4.2	7.8 ± 4.7	0.7334
30 min	44.1 ± 39.2	44.3 ± 26.6	0.9818
60 min	50.6 ± 34.1	49.5 ± 33.2	0.8636
120 min	35.3 ± 18.7	33.0 ± 21.2	0.5778
log (I.I.)	−0.947 ± 0.907	−0.569 ± 0.832 (n = 216)	0.0284
AUC ($glucose_{0-120}$)	294.5 ± 37.9	253.6 ± 41.0	<0.0001
AUC ($insulin_{0-120}$)	79.6 ± 46.2	77.7 ± 42.4	0.8261
log (HOMA-IR)	0.478 ± 0.509	0.434 ± 0.578	0.7036

Date are mean ± SD. Worsening group consisted of those who showed deterioration of glucose tolerance in 2005.
Retaining group consisted of individuals who retained normal glucose tolerance (NGT) in 2005. APN, adiponectin; AUC, area under the curve; BMI, body mass index; dBP, diastolic blood pressure; HbA_{1c}, hemoglobin A_{1c}; HDLC, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model of insulin resistance; I.I., insulinogenic index; IRI, insulin; LDLC, low-density lipoprotein cholesterol; NS, not significant; OGTT, oral glucose tolerance test; PG, plasma glucose; sBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; UA, uric acid; VFA, visceral fat area; WC, waist circumference.

Table 3 | Areas under the receiver operating characteristic curve of various parameters in relation to worsening from normal glucose tolerance to impaired fasting glucose and/or impaired glucose tolerance and worsening from impaired fasting glucose and/or impaired glucose tolerance to diabetes mellitus

	NGT	*P*	IFG and/or IGT	*P*
WC	0.631	0.027	0.479	NS
VFA	0.627	0.031	0.526	NS
sBP	0.688	0.001	0.536	NS
dBP	0.647	0.013	0.576	NS
HbA_{1c}	0.681	0.002	0.676	0.03
OGTT: PG (mg/dL)				
0 min	0.698	0.001	0.732	0.004
30 min	0.665	0.005	0.701	0.013
60 min	0.773	<0.001	0.618	NS
120 min	0.65	0.011	0.604	NS
AUC ($glucose_{0-120}$)	0.773	<0.001	0.666	0.04
Log (I.I.)	0.368	0.025	0.411	NS
Age	0.584	NS	0.687	0.02

AUC, area under the curve; dBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA_{1c}, hemoglobin A_{1c}; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; I.I., insulinogenic index; NGT, normal glucose tolerance; NS, not significant; OGTT, oral glucose tolerance test; PG, plasma glucose; sBP, systolic blood pressure; VFA, visceral fat area; WC, waist circumference.

2005 (worsening group 2), and those who retained IFG and/or IGT or improved to NGT (retaining or improving group 2) in 2005. Age, HbA_{1c}, PG at 0, 30 min, AUC ($glucose_{0-120}$) in OGTT were significantly higher in the worsening group 2 than in the retaining or improving group 2. The areas under the ROC curve of all these parameters were significantly higher than 0.5 (Table 3). Among them, the areas under the ROC curves of PG at 0, 30 min in OGTT were higher than 0.7, and their optimal cut-off levels were 111 and 182 mg/dL, respectively. The sensitivity and specificity of these cut-off points were 0.73 and 0.72 for PG at 0 min, and 0.87 and 0.49 for PG at 30 min, respectively.

Finally, we investigated whether changes in VFA over the 1-year period were associated with changes in glucose tolerance in subjects with NGT. For this purpose, we divided the subjects into three groups according to the mean ± 1 SD of changes in VFA and calculated the incidence rates of worsening of glucose tolerance. As shown in Figure 2, the rate of worsening of glucose tolerance in NGT subjects did not decrease with decreases in VFA over 1 year. In contrast, the rate of development of DM in IFG and/or IGT subjects tended to decrease with decreases in VFA over 1 year (Figure 2), although not significantly (Table 5). A similar analysis was carried out for data of subjects of the NGT with PG at 0 min of >95 mg/dL or PG at 60 min of >158 mg/dL or AUC ($glucose_{0-120}$) of >271 mg h/dL, who were at high risk of deterioration of glucose tolerance according to the results obtained in the present study. The rate of worsening of glucose tolerance tended to decrease with decrease in VFA in these subjects (Figure 3), although not significantly (Table 5).

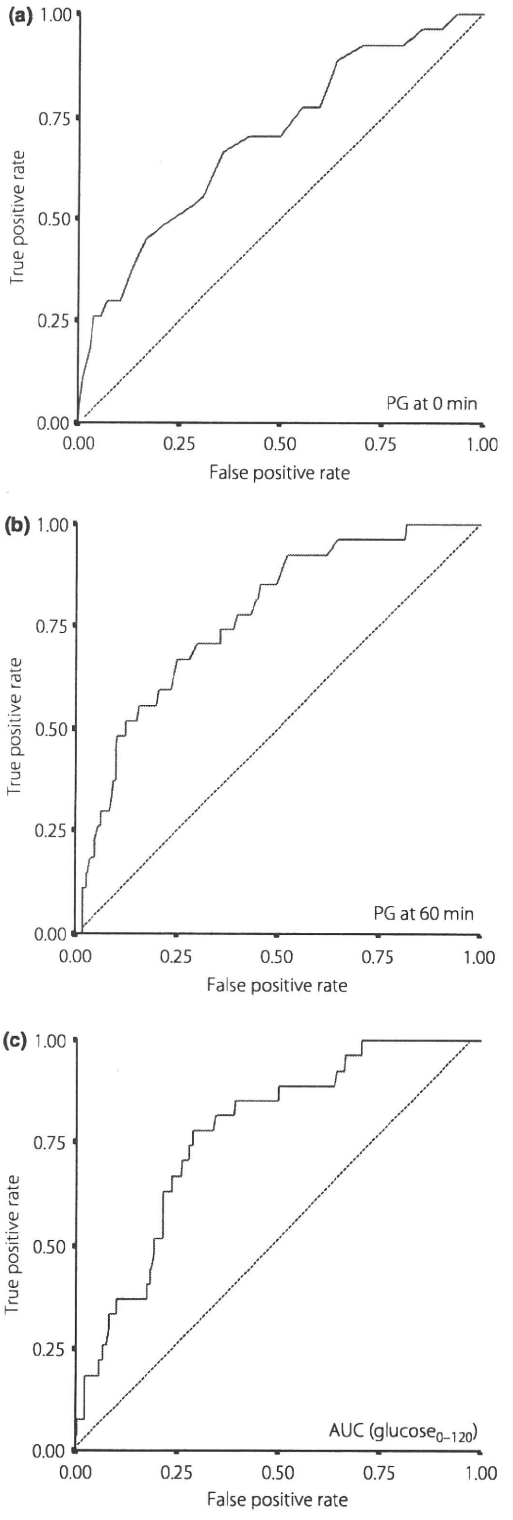

Figure 1 | Receiver operating characteristic curves of (a) plasma glucose (PG) at 0 min, (b) 60 min and (c) area under the curve (AUC; $glucose_{0-120}$) in an oral glucose tolerance test.

Table 4 | Comparison of baseline variables in subjects with impaired fasting glucose and/or impaired glucose tolerance who developed diabetes mellitus and those who retained impaired fasting glucose and/or impaired glucose tolerance or improved to normal glucose tolerance in 2005

	Worsening group 2	Retaining or improving group 2	*P*
n (male/female)	15 (13/2)	92 (83/9)	
Age (years)	55.3 ± 3.0	51.4 ± 6.9	0.0367
WC (cm)	93.1 ± 5.7	93.7 ± 5.6	0.7192
BMI (kg/m^2)	27.4 ± 2.7	27.3 ± 2.8	0.9016
VFA (cm^2)	143.7 ± 27.4	142.6 ± 26.0	0.8790
sBP (mmHg)	138.1 ± 14.3	136.1 ± 13.3	0.5863
dBP (mmHg)	87.9 ± 9.8	85.2 ± 8.5	0.2644
TC (mg/dL)	211.0 ± 31.5	217.1 ± 35.4	0.5308
TG (mg/dL)	194.5 ± 118.7	171.0 ± 131.5	0.5184
HDLC (mg/dL)	56.1 ± 24.4	53.7 ± 13.2	0.5722
LDLC (mg/dL)	114.0 ± 39.4	129.2 ± 31.7	0.0986
UA (mg/dL)	6.50 ± 1.60	6.33 ± 1.30	0.6465
HbA_{1c} (%)	5.3 ± 0.4	5.0 ± 0.4	0.0461
log(APN)	1.7 ± 0.4	1.8 ± 0.4 (*n* = 79)	0.5546
OGTT: PG (mg/dL)			
0 min	113.3 ± 8.9	105.2 ± 10.3	0.0054
30 min	205.6 ± 30.6	184.8 ± 29.3	0.0126
60 min	221.3 ± 36.2	202.1 ± 41.2	0.0917
120 min	150.7 ± 24.9	150.6 ± 24.5	0.9880
OGTT: IRI (μU/mL)			
0 min	9.9 ± 3.9	10.0 ± 5.5	0.9654
30 min	29.4 ± 16.4	39.7 ± 28.5	0.1752
60 min	47.0 ± 22.0	60.9 ± 39.2	0.1854
120 min	46.6 ± 19.8	62.0 ± 45.0	0.1968
Log (I.I.)	−1.800 ± 1.395 (*n* = 13)	−1.260 ± 0.903 (*n* = 91)	0.0648
AUC ($glucose_{0-120}$)	372.5 ± 44.0	345.6 ± 46.3	0.0384
AUC ($insulin_{0-120}$)	75.8 ± 28.9	99.0 ± 60.6	0.1483
log (HOMA-IR)	0.952 ± 0.409	0.797 ± 0.609	0.3741

Date are mean ± SD. Worsening group consisted of subjects who developed DM in 2005.

Retaining or improving group consisted of subjects who retained IFG and/or IGT or improved to NGT in 2005. APN, adiponectin; AUC, area under the curve; BMI, body mass index; dBP, diastolic blood pressure; HbA1c, hemoglobin A1c; HDLC, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model of insulin resistance; I.I., insulinogenic index; IRI, insulin; LDLC, low-density lipoprotein cholesterol; NS, not significant; OGTT, oral glucose tolerance test; PG, plasma glucose; sBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; UA, uric acid; VFA, visceral fat area; WC, waist circumference.

Because all subjects of the worsening group were male, we focused on the data in male subjects and re-calculated the rate of worsening of glucose tolerance, and we obtained a similar results (data not shown).

DISCUSSION

In the present study, we found that WC, VFA, sBP, dBP, HbA_{1c}, PG at 0, 30, 60 and 120 min, AUC ($glucose_{0-120}$) of

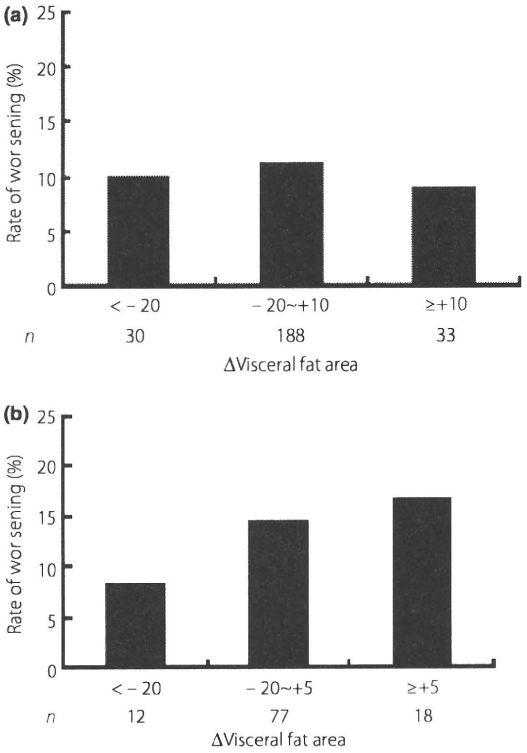

Figure 2 | Rates of worsening of glucose tolerance in the three study groups divided according to the mean ± 1 SD of changes in visceral fat area over the 1-year period of the study. (a) Normal glucose tolerance subjects, (b) impaired fasting glucose and/or impaired glucose tolerance subjects in 2004. ΔVisceral fat area indicates the increment in visceral fat area from 2004 to 2005 in each subject. *n*, number of subjects.

OGTT and log (I.I.) can predict deterioration of glucose tolerance over 1 year in NGT subjects with abdominal obesity, and that the power of PG at 0, 60 min and AUC ($glucose_{0-120}$) was relatively the strongest among these variables. Furthermore, we calculated the optimal cut-off values of these parameters: 95 mg/dL for PG at 0 min, 158 mg/dL for PG at 60 min, and 271 mg h/dL for AUC ($glucose_{0-120}$). It has already been reported that the PG at 60 min during OGTT is a strong predictor of future risk of type 2 diabetes with a cut-off value of 155 mg/dL[11]. Although the report was based on data about the risk of type 2 diabetes during a 7–8-year follow-up period, the cut-off value of PG at 60 min described in the aforementioned study was almost similar to that computed in the present study. In addition, the same study also showed that NGT subjects with PG at 60 min of >155 mg/dL, who also fulfilled the criteria for the metabolic syndrome, were at greater risk of developing diabetes. Together, with the aforementioned study, the present results suggest that NGT subjects with abdominal obesity with PG at 60 min of >155–158 mg/dL are at high risk of deterioration of glucose tolerance over both a short and long period.

The present results also showed that the rate of worsening of glucose tolerance in NGT subjects did not decrease with

Table 5 | Odds ratio and 95% confidence intervals of worsening of glucose tolerance in relation to ΔVisceral fat area

	n	OR	95% CI	*P*-value
NGT				
Mean − SD > ΔVFA	30	1	Reference	
Mean + SD > ΔVFA ≥ mean − SD	188	1.132	0.316–4.058	0.8493
ΔVFA ≥ mean + SD	33	0.900	0.167–4.843	0.9023
IFG and/or IGT				
Mean − SD > ΔVFA	12	1	Reference	
Mean + SD > ΔVFA ≥ mean − SD	77	1.833	0.215–15.653	0.5796
ΔVFA ≥ mean + SD	18	2.200	0.201–24.092	0.5185
NGT PG at 0 min ≥95				
Mean − SD > ΔVFA	12	1	Reference	
Mean + SD > ΔVFA ≥ mean − SD	91	2.347	0.282–19.497	0.4297
ΔVFA ≥ mean + SD	11	2.444	0.189–31.534	0.4933
NGT PG at 60 min ≥158				
Mean − SD > ΔVFA	11	1	Reference	
Mean + SD > ΔVFA ≥ mean − SD	59	3.111	0.365–26.484	0.2989
ΔVFA ≥ mean + SD	8	6.000	0.490–73.470	0.1609
NGT AUC ($glucose_{0-120}$) ≥ 271				
Mean − SD > ΔVFA	11	1	Reference	
Mean + SD > ΔVFA ≥ mean − SD	63	2.857	0.336–24.282	0.3362
ΔVFA ≥ mean + SD	13	8.571	0.836–87.847	0.0704

AUC, area under the curve; IFG, impaired fasting glucose, IGT, impaired glucose tolerance;NGT, normal glucose tolerance; VFA, visceral fat area.

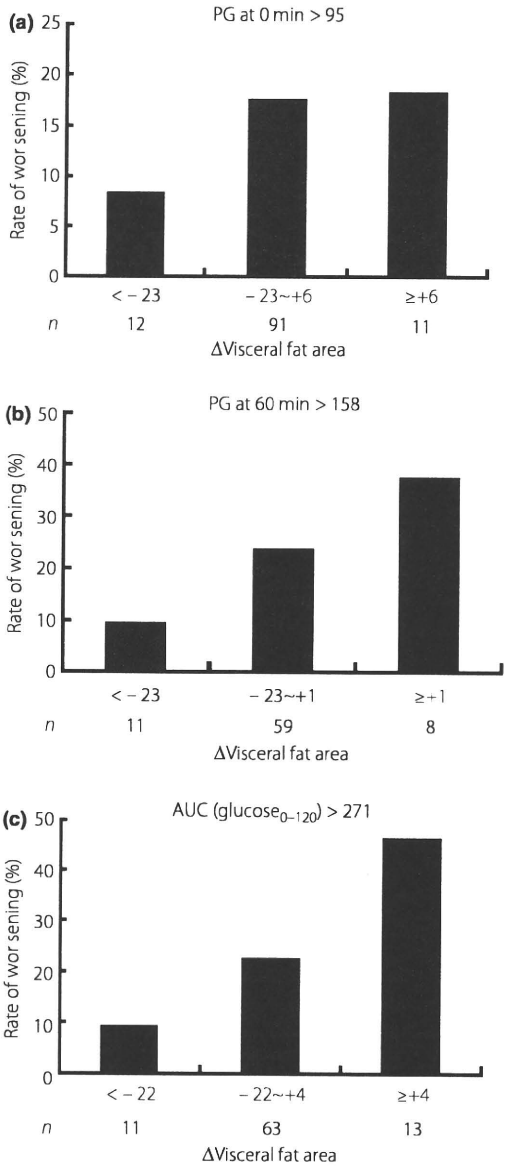

Figure 3 | Rates of worsening of glucose tolerance in subjects with (a) normal glucose tolerance and plasma glucose (PG) at 0 min of >95 mg/dL, (b) PG at 60 min of >158 mg/dL, (c) and area under the curve (AUC; $glucose_{0-120}$) of >271 mg h/dL in an oral glucose tolerance test. ΔVisceral fat area indicates the increment in VFA from 2004 to 2005 in each subject. *n*, number of subjects.

decreases in VFA over the 1-year period, suggesting that reducing visceral fat over 1 year had no beneficial effect on glucose tolerance in NGT. In contrast, the rate of developing DM in IFG and/or IGT subjects tended to decrease with decreases in VFA over a 1-year period. These results are in agreement with those of Schäfer *et al.*[12], who showed that moderate weight loss under a lifestyle intervention program with reduction in visceral fat improved glucose tolerance in individuals with IGT, but not with NGT. Their follow-up period was 7–11 months, and was as short as ours. It is possible that the beneficial effects of reduction of visceral fat in NGT might not become apparent over a short period of time and that such intervention in NGT might prevent future deterioration of glucose tolerance over a longer period of time. In fact, the F-IRI decreased significantly, similar to VFA, over the 1-year period, even in NGT subjects in the present study (Table 1), which should lead to conservation of future insulin secretion capacity. We also addressed the question of whether reductions in visceral fat have beneficial effects in NGT subjects with PG at 0 min of >95, PG at 60 min of >158, or AUC ($glucose_{0-120}$) of >271, who are at high risk of worsening of glucose tolerance based on the results of the present study. The results showed that these subjects benefit from such reduction, similar to persons with IFG and/or IGT. We propose that the individuals with these parameters over the cut-off values should receive a lifestyle intervention program aimed at decreasing visceral fat, even in NGT.

The present study has limitations. The number of subjects included in the present analysis was approximately two-thirds of those identified as NGT in 2004, and we could not follow the rest of the subjects by OGTT in 2005. When we compared the

clinical data of the rest of the subjects with those of the present study, VFA in both 2004 and 2005 were significantly different between the two groups (120.3 ± 21.8 *vs* 131.1 ± 23.7 in 2004, 106.2 ± 26.3 *vs* 124.4 ± 25.7 in 2005). It might be that we analyzed subjects with relatively severe abdominal obesity.

Although we found various factors, including WC, VFA and log (I.I.), were involved in significant predictors of deterioration of glucose tolerance from NGT to IFG/IGT, only HbA_{1c}, PG at 0 and 30 min, and AUC ($glucose_{0-120}$) in OGTT were significantly higher in the worsening groups from IFG/IGT to DM. We speculate that WC and VFA dropped out from such factors in IFG/IGT, probably because visceral fat is likely to decrease by intervention in larger WC and VFA individuals, leading to an improvement in glucose tolerance. Thus, we might not be able to predict deterioration of glucose tolerance by only using these parameters in the IFG/IGT group. Regarding log (I.I.), the *P*-value was 0.0648, suggesting that it might be defined as a significant predictor if evaluated in a larger sample size.

In conclusion, the present study identified certain predictors in NGT subjects with abdominal obesity for deterioration of glucose tolerance over a 1-year period, these include PG at 0 and 60 min, and AUC ($glucose_{0-120}$) in OGTT. The results also showed that lifestyle intervention that results in reduction of visceral fat does not prevent deterioration of glucose tolerance in NGT, although such a program seems beneficial in subjects with the aforementioned predictors at levels higher than the cut-off points. We propose that individuals with PG at 0 and 60 min, and AUC ($glucose_{0-120}$) higher than the cut-off values, including NGT, should receive a lifestyle intervention aimed at reducing visceral fat. Further studies of larger population samples and longer follow-up periods are warranted.

ACKNOWLEDGEMENTS

The authors thank Dr. Tetsuya Ohira for his helpful comments. The authors declare no financial support or relationship that may pose conflict of interest.

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Impact of concomitant diabetes and chronic kidney disease on preload-induced changes in left ventricular diastolic filling in hypertensive patients

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Objectives **Concomitant diabetes and/or chronic kidney disease (CKD) in hypertensive patients may portend additive deleterious effects on active left ventricular relaxation. We investigated the effect of a passive leg lifting (PLL) maneuver, a means of increasing preload, on left ventricular filling to assess the relationship of concomitant diabetes mellitus (DM) and/or CKD with diastolic function in hypertensive patients.**

Methods **A total of 155 asymptomatic essential hypertensive patients underwent Doppler echocardiography to compare the echocardiographic indices at baseline and during PLL. In 51 patients, the effect of physiological saline infusion was also examined.**

Results **The changes in echocardiographic indices, including deceleration time of early diastolic filling (EDT) and the ratio of transmitral early left ventricular filling velocity to early diastolic Doppler tissue imaging of the mitral annulus (E/E′) by saline infusion showed a good correlation with those induced by PLL (Bland–Altman plot and linear regression). We next divided the total participants into four groups according to the presence/absence of diabetes and/or CKD [DM(−)/CKD(−); $n = 48$, DM(+)/CKD(−); $n = 25$, DM(−)/CKD(+); $n = 43$, and DM(+)/CKD(+); $n = 39$)] and found that the changes in EDT ($F = 15.92$, $P < 0.01$) as well as those in E/E′ ($F = 8.87$, $P < 0.01$) were significantly different among the subgroups. Multiple logistic regression analysis revealed that these complications were independent predictors of EDT less than 150 ms [DM, odds ratio (OR): 2.82; CKD, OR: 2.18, $P < 0.05$, respectively] as well as E/E′ ratio at least 15.0 during PLL (DM, OR: 4.78; CKD, OR: 3.32, $P < 0.05$, respectively).**

Conclusion **This simple preloading test unmasks latent progression of left ventricular dysfunction in essential hypertension; that is, these complications potentially cause deterioration of left ventricular compliance and preload reserve even in the early stages of diastolic dysfunction.**
J Hypertens **29:144–153 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

Journal of Hypertension 2011, 29:144–153

Keywords: chronic kidney disease, complication, diabetes, diastolic function, hypertension

Abbreviations: A′-velocity, tissue Doppler late diastolic velocity; ARdur–Ad, the time difference between the duration of the atrial filling wave and the duration of flow at atrial contraction; A-velocity, the transmitral late filling velocity; CKD, chronic kidney disease; CVD, cardiovascular disease; DM, diabetes mellitus; E/A, the ratio of peak early to late diastolic filling velocity; E/E′ ratio, the ratio of early diastolic transmitral velocity to early diastolic tissue velocity; E′-velocity, tissue Doppler early diastolic velocity; EDT, the deceleration time of early diastolic left ventricular filling; eGFR, estimated glomerular filtration rate; E-velocity, the transmitral early filling velocity; HOMA index, homeostatic model assessment index; PLL, passive leg lifting; PVa, pulmonary vein atrial reversal; PVd, peak diastolic forward flow velocity; PVs, peak systolic forward flow velocity; S/D ratio, the ratio of the pulmonary venous systolic velocity to diastolic velocity; S′-velocity, tissue Doppler systolic velocity

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Received 16 November 2009 **Revised** 1 August 2010
Accepted 9 August 2010

Introduction

It is increasingly recognized that hypertensive patients with concomitant risk factors, such as diabetes mellitus (DM) and chronic kidney disease (CKD), are at increased risk of subsequent cardiovascular disease (CVD) [1–5]. Although the mechanism of the association of concomitant DM and/or CKD in hypertension with high CVD risk remains to be elucidated, it is possible that latent left ventricular diastolic dysfunction may also be present in these patients before the development of more severe stages of diastolic dysfunction, and, thus, contribute to CVD risk.

Doppler echocardiography permits noninvasive assessment of left ventricular diastolic function in addition to measurement of systolic function. Mitral inflow velocity recorded by Doppler echocardiography has been widely used to evaluate left ventricular diastolic function [6,7]. Previous reports have shown that the baseline mitral flow pattern and its variations after loading manipulations, recorded by means of echocardiography, are a powerful prognostic marker in patients with chronic heart failure [8,9]. The passive leg lifting (PLL) maneuver is a noninvasive and cost-effective method to increase preload through a transient increase in venous return. This simple

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DOI:10.1097/HJH.0b013e32833f387c

maneuver has several advantages over volume loading for the purpose of assessing directional changes in diastolic function, because it does not significantly affect blood pressure, heart rate, or afterload [9,10], and the duration of the effect is short [9,11]. In previous studies, this maneuver was performed only in chronic heart failure patients [8,9,11], healthy individuals [10], or open chest coronary surgery patients [12], and the prognostic value of this maneuver was explored by categorizing diastolic dysfunction according to the severity; that is, 'in heart failure patients, a restrictive filling pattern in response to PLL maneuver has a worse prognosis'. However, even in essential hypertension with left ventricular hypertrophy (LVH), a restrictive left ventricular filling pattern is very uncommon [13]. On the other hand, it is increasingly recognized that left ventricular diastolic dysfunction is more severe in patients with DM and hypertension [14], renal dysfunction such as microalbuminuria [15], and end-stage renal disease [16]. These results suggest that concomitant DM and/or renal dysfunction in hypertension portend additive deleterious effects on active left ventricular relaxation. Accordingly, the present study examined the effect of PLL maneuver on Doppler echocardiographic indices to assess the relationship of concomitant DM and/or CKD to diastolic function in hypertensive patients without previous CVD.

Methods

Study population

We studied 155 consecutive patients referred to Osaka University Hospital, Japan for evaluation of asymptomatic hypertension. Eligible patients aged 32–87 years who had good-quality echocardiographic recordings were enrolled, and all patients included in this study were in stable sinus rhythm. Hypertension was defined as SBP at least 140 mmHg and/or DBP at least 90 mmHg on repeated measurements, or receiving antihypertensive treatment. DM was defined according to the American Diabetes Association criteria [17]. CKD was defined according to the guidelines of the National Kidney Foundation classification of CKD, as estimated glomerular filtration rate (eGFR) less than 60 ml/min per 1.73 m^2 or dipstick proteinuria ($\geq$1+) [18]. Smoking status was determined by interview and defined as follows: never smoker, past smoker (history of habitual smoking but had quit), and current smoker. Ischemic heart disease was defined as a 75% or greater organic stenosis of at least one major coronary artery as confirmed by coronary angiography, or a history of myocardial infarction or percutaneous transluminal coronary angioplasty. Exclusion criteria included ischemic heart disease, acute coronary syndrome, chronic heart failure [New York Heart Association (NYHA) class II or greater], old cerebral infarction, history of transient ischemic attack, and secondary hypertension. Participants with moderate or severe aortic or mitral regurgitation, heart rate more than 100 bpm, abnormal heart rhythm, diabetic retinopathy or neuropathy, receiving hemodialysis or erythropoietin therapy, or undetectable pulmonary venous flow throughout the cardiac cycle or absent reversal were also excluded. The study protocol was approved by the Ethics Committee of Osaka University, and all procedures followed were in accordance with the institutional guidelines of Osaka University. All participants enrolled in this study were Japanese, and all gave written informed consent to participate in the study.

Baseline clinical characteristics

After fasting overnight, venous blood and urine sampling was performed in all participants. Height and body weight were measured, and BMI was calculated. The following parameters were also determined: total cholesterol, triglycerides, high-density lipoprotein cholesterol, hemoglobin A1c, and homeostatic model assessment (HOMA) index; that is, plasma glucose level × (plasma insulin level/22.5). eGFR was calculated using the abbreviated Modification of Diet in Renal Disease (MDRD) formula in ml/min per 1.73 m^2.

Echocardiographic methods and calculation of derived variables

Imaging and Doppler echocardiography were performed in all participants in this study. Studies were performed using phased-array echocardiography with M-mode, two-dimensional, pulsed, and color-flow Doppler capabilities, as previously described [19,20]. Left ventricular internal dimension and septal and posterior wall thickness were measured at end-diastole and end-systole according to the American Society of Echocardiography recommendations [21]. Color-flow Doppler recordings were used to check for aortic and mitral regurgitation, as previously described [22]. End-diastolic dimensions were used to calculate left ventricular mass by a previously reported formula [23]. Left ventricular mass was considered an unadjusted variable and was normalized by body surface area and expressed as left ventricular mass index (LVMI). LVH was considered to be present when LVMI was more than 116 g/m^2 for men and more than 104 g/m^2 for women [24,25].

The left ventricular diastolic filling pattern was recorded from the apical transducer position with the participant in the left lateral decubitus position, with the sample volume situated between the mitral leaflet tips. The leading edge of the transmitral Doppler flow pattern was traced to derive the peak of early diastolic and atrial phase left ventricular filling (E-velocity and A-velocity, respectively), E/A ratio, the deceleration time of early diastolic left ventricular filling (EDT), and the duration of the atrial filling wave (Ad).

Pulmonary venous flow velocity was recorded by placing a sample volume about 1 cm into the right superior pulmonary vein [26]. Pulmonary vein systolic (PVs),

diastolic (PVd), S/D ratio, and atrial reversal (PVa), as well as the duration of flow at atrial contraction (ARdur), were recorded. When a biphasic PVs was detected, the highest peak velocity was used [26]. Ad and ARdur were measured as close to the zero baseline as possible from the start to termination of flow at atrial contraction after the P wave on the simultaneously recorded electrocardiogram, and the difference between Ad and ARdur was calculated (ARdur−Ad).

Pulsed wave Doppler tissue imaging was also performed by activating the Doppler tissue imaging function in the same machine. Sample volume was located at the septal side of the mitral annulus. Peak mitral annular systolic (S′) and diastolic early (E′) and late (A′) tissue velocities were measured. All measurements were performed by one trained investigator who was blinded to the clinical data of the participants.

Loading manipulations

All patients underwent simultaneous measurement of blood pressure and echocardiographic parameters. Blood pressure was measured noninvasively using a calibrated semiautomatic cuff connected to a Marquette monitor. After performing Doppler echocardiographic recording as well as blood pressure measurement at baseline, loading manipulations were performed. Specifically, in all patients, measurements were repeated during a PLL maneuver (legs elevated to 45° from the horizontal position) maintained for at least 1 min. The timing of the maximum changes occurring in the transmitral and pulmonary venous flow patterns as well as left ventricular tissue velocity was determined, and blood pressure was measured simultaneously. In 51 patients, after repeat baseline measurements to confirm a return to a hemodynamic steady state (usually 10–20 min), warm (37°C) physiological saline was infused rapidly at a rate of 33 ml/min. Simultaneous blood pressure and echocardiographic measurements were repeated after 15 min (500 ml infusion).

Statistical analysis

Summary statistics are presented as mean (±SD) for continuous variables, or percentages for categorical variables unless otherwise specified. First, the significance of differences in parameters before and after loading manipulations was evaluated using paired *t*-test. To compare the difference in changes in parameters induced by the two loading manipulations between groups, two-sample *t*-test was used. Linear regression analysis was used to assess the correlation of changes in Doppler echocardiographic indices induced by the two loading manipulations. Bland–Altman analysis was used to evaluate the agreement between the two methods, and limits of agreement (mean ± 1.96 times the SD of the differences) were determined. Second, we divided the participants into four categories according to the presence/absence of DM and/or CKD. Differences in characteristics between groups were tested using χ^2 test for dichotomous variables, and one-way analysis of variance (ANOVA) with Sheffe's posttest for continuous variables, as appropriate. Third, paired *t*-test of the differences was used to compare paired measurements within groups before and after the PLL maneuver. Fourth, to determine the significance of the difference in parameters induced by PLL between subgroups, Friedman test was used. Fifth, logistic regression analysis was used to determine the odds ratio (OR) of EDT during PLL less than 150 or E/E′ ratio during PLL at least 15.0. Multivariable logistic regression analysis was used to identify independent determinants of EDT during PLL less than 150 or E/E′ ratio during PLL at least 15.0, after accounting for relevant variables using a *P* value of less than 0.05 as the selection criterion. All *P* values were two sided, and those less than 0.05 were considered statistically significant. All calculations were performed using a standard statistical package (SPSS, version 17.0; SPSS Inc., Chicago, Illinois, USA).

Results

Comparative study

Of the 155 participants, 51 consented to participate in the comparative study (31 men; mean age 67 ± 9 years, 17 without DM and CKD, 26 with DM or CKD, and eight with DM and CKD). The two loading manipulations to evaluate diastolic reserve were well tolerated without adverse events. Blood pressure, heart rate, and echocardiographic indices before and after loading manipulations with physiological saline infusion and PLL maneuver are summarized in Table 1. Blood pressure and heart rate did not change significantly. EDT was significantly decreased, and peak E-velocity, E/A ratio, peak PVs-velocity, peak PVd-velocity, peak E′-velocity, and E/E′ ratio were significantly increased in response to loading manipulations. The changes in blood pressure, heart rate, and echocardiographic indices were not significantly different between the two loading manipulations. The changes in EDT ($y = 11.08 + 0.73x$, $r = 0.85$) and E/E′ ratio ($y = -0.02 + 0.77x$, $r = 0.82$) by physiological saline infusion were strongly correlated with those induced by PLL ($P < 0.01$, respectively). Bland–Altman plot regression showed good agreement of changes in EDT (Fig. 1a) and E/E′ ratio (Fig. 1b) induced by saline infusion with those induced by PLL.

Doppler and hemodynamic characteristics at baseline and in response to passive leg lifting

Baseline clinical and biochemical characteristics of the study participants are shown in Table 2. We divided the total participants into four groups as follows: no DM and no CKD [DM(−)/CKD(−)], DM without CKD [DM(+)/CKD(−)], CKD without DM [DM(−)/CKD(+)], and DM and CKD [DM(+)/CKD(+)]. At baseline, 64 of the total participants had DM and 82 had CKD. Patients with DM(+)/CKD(+) had a worse functional status and

Table 1 Hemodynamics and echocardiographic indices at baseline and during loading manipulations in patients enrolled in comparative study

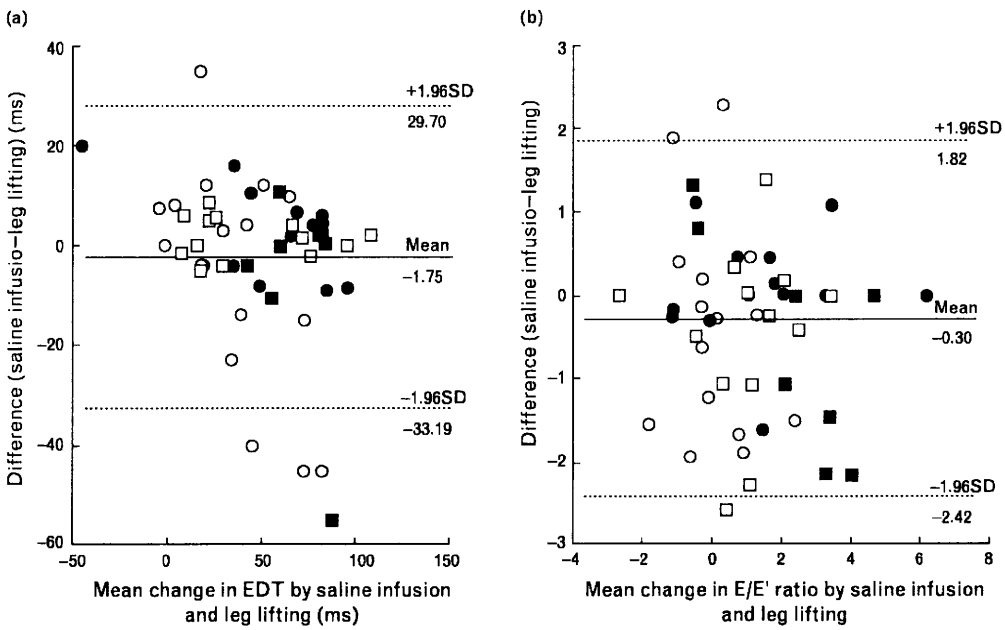
	Physiological saline		PLL		
	Baseline	Saline	Baseline	PLL	*P* value between groups
SBP (mmHg)	136.7 ± 16.3	137.2 ± 17.0	135.4 ± 13.4	137.0 ± 15.7	NS
DBP (mmHg)	76.4 ± 10.5	76.8 ± 11.0	76.0 ± 10.8	76.5 ± 11.9	NS
Heart rate (bpm)	60.1 ± 8.1	61.0 ± 8.6	61.9 ± 7.2	63.2 ± 8.1	NS
E-velocity (m/s)	0.72 ± 0.14	0.88 ± 0.14†	0.72 ± 0.14	0.87 ± 0.15†	NS
A-velocity (m/s)	0.79 ± 0.19	0.84 ± 0.20†	0.78 ± 0.18	0.80 ± 0.20	NS
E/A ratio	0.96 ± 0.26	1.08 ± 0.24†	0.96 ± 0.26	1.14 ± 0.31†	NS
EDT (ms)	235.1 ± 37.7	187.9 ± 38.1†	236.9 ± 40.5	187.4 ± 37.0†	NS
PVs-velocity (m/s)	0.55 ± 0.13	0.64 ± 0.15†	0.55 ± 0.13	0.60 ± 0.11*	NS
PVd-velocity (m/s)	0.34 ± 0.15	0.47 ± 0.15†	0.38 ± 0.13	0.41 ± 0.11†	NS
S/D ratio	1.54 ± 0.45	1.45 ± 0.42	1.58 ± 0.50	1.51 ± 0.35	NS
PVa-velocity (m/s)	0.26 ± 0.09	0.28 ± 0.04†	0.25 ± 0.06	0.27 ± 0.04	NS
ARdur–Ad (ms)	−23.0 ± 14.7	−19.8 ± 12.3	−24.0 ± 13.6	−22.6 ± 15.8*	NS
E′-velocity (cm/s)	6.26 ± 1.18	7.03 ± 1.49†	6.19 ± 1.18	6.91 ± 1.33†	NS
E/E′ ratio	11.80 ± 2.71	12.73 ± 3.29†	11.82 ± 2.62	13.03 ± 3.20†	NS
A′-velocity (cm/s)	9.12 ± 1.42	9.20 ± 1.34	9.13 ± 1.45	9.18 ± 1.70	NS
S′-velocity (cm/s)	6.16 ± 0.93	6.29 ± 0.95	6.18 ± 0.82	6.30 ± 0.94	NS

Values are mean ± SD. The *P* values of two-sample *t*-test are shown. A-velocity, the transmitral late filling velocity; A′-velocity, tissue Doppler late diastolic velocity; ARdur–Ad, the time difference between the duration of the atrial filling wave and the duration of flow at atrial contraction; E-velocity, the transmitral early filling velocity; E′-velocity, tissue Doppler early diastolic velocity; E/E′ ratio, the ratio of early diastolic transmitral velocity to early diastolic tissue velocity; E/A ratio, the ratio of peak early to late diastolic filling velocity; EDT, the deceleration time of early diastolic filling; PLL, passive leg lifting maneuver; PVa, pulmonary vein atrial reversal; PVd, peak diastolic forward flow; PVs, peak systolic forward flow; S′-velocity, tissue Doppler systolic velocity; S/D ratio, the ratio of the pulmonary venous systolic velocity to diastolic velocity. *$P < 0.05$ and †$P < 0.01$ versus baseline.

more compromised hemodynamics, such as lower high-density lipoprotein cholesterol and eGFR, higher HOMA index, hemoglobin A1c, and LVMI. The prevalence of LVH did not significantly differ among the subgroups.

The mean time to the maximum changes occurring in the echocardiographical indices in response to PLL was 18.3 ± 3.9 s. The PLL maneuver produced a significant difference in changes in E-velocity, EDT, peak E′-velocity, and E/E′ ratio between subgroups (Table 3). The change in EDT was significantly different between subgroups (Table 3 and Figure 2a). The DM(+)/CKD(−), DM(−)/CKD(+), and DM(+)/CKD(+) groups showed a significantly greater decrease in EDT than the

Fig. 1



Bland–Altman plots comparing changes in echocardiographic indices by physiological saline infusion and passive leg lifting maneuver. Changes in deceleration time of early diastolic filling (EDT) (a) and the ratio of early diastolic transmitral velocity to early diastolic tissue velocity (E/E′) (b). Open circles indicate participants with diabetes mellitus (DM)(−)/CKD(−). Closed circles indicate participants with DM(+)/CKD(−). Open squares indicate participants with DM(−)/CKD(+). Closed squares indicate participants with DM(+)/CKD(+).

Table 2 Baseline clinical and hemodynamic characteristics of study participants

Variables	All	DM(−)/CKD(−)	DM(+)/CKD(−)	DM(−)/CKD(+)	DM(+)/CKD(+)	‖*P* for difference
n	155	48	25	43	39	
Age (years)	67.1 ± 8.6	66.5 ± 6.8	67.6 ± 7.5	66.6 ± 11.4	68.2 ± 7.9	NS
Male/female, *n*	100/55	27/21	12/13	32/11	29/10	<0.05
BMI (kg/m^2)	24.0 ± 3.4	23.2 ± 3.1	25.7 ± 2.8*	23.5 ± 3.5	24.6 ± 3.5	<0.05
Duration of hypertension (years)	17.8 ± 12.5	15.0 ± 11.5	13.1 ± 9.1	21.3 ± 15.0*	20.4 ± 11.0	NS
Smoking status, %						
Never/past/current	40.6/40.0/19.4	54.2/18.7§/27.1	48.0/32.0/20.0	32.6/55.8†/11.6	28.2/53.9†/17.9	<0.01
SBP (mmHg)	140.6 ± 13.8	137.9 ± 10.9	137.0 ± 12.5	143.7 ± 15.5	143.0 ± 14.2	NS
DBP (mmHg)	76.9 ± 9.5	78.9 ± 8.8	76.2 ± 10.3	76.0 ± 10.2	76.0 ± 10.3	NS
Heart rate (bpm)	65.8 ± 8.6	66.8 ± 8.4	67.4 ± 10.5	62.8 ± 7.5	66.9 ± 8.2	NS
Total cholesterol (mmol/l)	4.99 ± 0.98	4.97 ± 0.72	5.19 ± 1.13	5.15 ± 1.16	4.67 ± 0.91	NS
Triglycerides (mmol/l)	1.50 ± 0.75	1.26 ± 0.64†	1.30 ± 0.50	1.65 ± 0.93*	1.80 ± 0.63	<0.05
HDL-chol (mmol/l)	1.21 ± 0.40	1.38 ± 0.37	1.26 ± 0.30	1.19 ± 0.49	1.01 ± 0.31†	<0.01
HOMA index	1.84 ± 2.26	1.36 ± 1.08	2.68 ± 1.87†	1.36 ± 2.16	2.41 ± 3.33†	<0.01
Hemoglobin A1c (%)	5.96 ± 1.10	5.29 ± 0.36	6.74 ± 1.22†§	5.51 ± 0.45	6.74 ± 1.30†§	<0.01
eGFR (ml/min per 1.73 m^2)	51.5 ± 28.1	75.4 ± 12.9§	73.5 ± 10.9§	31.6 ± 21.8†	30.2 ± 20.9†	<0.01
Left ventricular mass index (g/m^2)	136.4 ± 40.8	123.2 ± 30.3	127.8 ± 44.6	142.1 ± 40.5	153.7 ± 45.5†	<0.01
Left ventricular hypertrophy (%)	70.3	58.3	80.9	74.4	75.8	NS
Fractional shortening (%)	39.0 ± 6.5	39.1 ± 6.8	38.5 ± 6.7	39.8 ± 6.3	37.4 ± 6.0	NS
Therapy						
Calcium antagonist, %	71.0	54.2§	60.0	81.4†	87.2†	<0.01
β-Blocker, %	40.0	29.2§	8.0§	58.1†	53.9*	<0.01
ACE inhibitor or ARB, %	61.3	47.9	56.0	67.4	74.4	NS
Diuretic, %	30.3	16.7§	4.0§	46.5†	46.2†	<0.01

Values are mean ± SD for continuous variables, and the percentages of participants for categorical variables. ACE, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; CKD, chronic kidney disease; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HDL-chol, high-density lipoprotein cholesterol; HOMA index, homeostatic model assessment index. ‖The *P* values of one-way analysis of variance (ANOVA) or χ^2 test. *$P < 0.05$ and †$P < 0.01$ versus DM(−)/CKD(−). ‡$P < 0.05$ and §$P < 0.01$ versus DM(−)/CKD(+).

DM(−)/CKD(−) group. Furthermore, when compared with the DM(+)/CKD(−) group or with the DM(−)/CKD(+) group, the DM(+)/CKD(+) group showed a significantly greater decrease in EDT. Similarly, the change in E/E′ ratio was significantly different between subgroups (Table 3 and Figure 2b). E/E′ ratio did not change significantly in the DM(−)/CKD(−) group; however, a significant increase in E/E′ ratio was found in the DM(+)/CKD(−), DM(−)/CKD(+) and DM(+)/CKD(+) groups (Figure 2b). The DM(+)/CKD(−), DM(−)/CKD(+), and DM(+)/CKD(+) groups showed a significantly greater increase in E/E′ ratio than that in the DM(−)/CKD(−) group. In addition, when compared with the DM(+)/CKD(−) group or with the DM(−)/CKD(+) group, the DM(+)/CKD(+) group showed a significantly greater increase in E/E′ ratio.

During PLL, 17 of the total participants had EDT less than 150 ms. Univariate logistic regression analysis found that age, fractional shortening, baseline-EDT, DM, and CKD were significantly associated with the risk of EDT less than 150 ms during PLL (Table 4). Multiple logistic regression analysis including age, fractional shortening, and baseline-EDT was performed and revealed that DM as well as CKD was an independent predictor of EDT less than 150 ms during PLL. The further addition of antihypertensive medication to the model did not meaningfully influence the results [DM, OR: 2.09, 95% confidence interval (CI): 1.52–6.88; CKD, OR: 2.20, 95% CI: 1.21–8.50, $P < 0.05$, respectively].

During PLL, 44 of the total participants had E/E′ ratio at least 15.0. The variables that were significantly associated with the risk of E/E′ ratio at least 15.0 during PLL were age, duration of hypertension, DBP, presence of LVH, baseline-E/E′, DM, and CKD. The independent predictive value of these complications and E/E′ ratio at least 15.0 during PLL was also confirmed by multiple logistic regression analysis including age, duration of hypertension, DBP, presence of LVH, and baseline-E/E′ (Table 5). The further addition of antihypertensive medication to the model did not meaningfully influence the results (DM, OR: 4.49, 95% CI: 1.42–7.94; CKD, OR: 3.69, 95% CI: 1.39–8.45, $P < 0.05$, respectively).

Discussion

The present study demonstrated that, in hypertensive patients, the changes in echocardiographic indices by PLL maneuver showed a good correlation to those induced by physiological saline infusion. The changes in EDT as well as those in E/E′ ratio induced by PLL were significantly different between subgroups, with a joint effect that was greater than the individual effect of either disease separately. The results of multiple logistic regression analysis indicated that concomitant DM and/or CKD in hypertensive patients was an independent predictor of EDT less than 150 ms as well as E/E′ ratio at least 15.0 during PLL.

Our results were partially in accordance with previous reports that PLL resulted in increased peak E-velocity [10], shortened EDT [9,10], and increased E/A ratio [9]. Previous studies have used the PLL maneuver as a means of increasing preload. An increase in preload, produced by PLL or physiological saline infusion,

Table 3 Hemodynamics and Doppler echocardiographic indices at baseline and changes during passive leg lifting maneuver

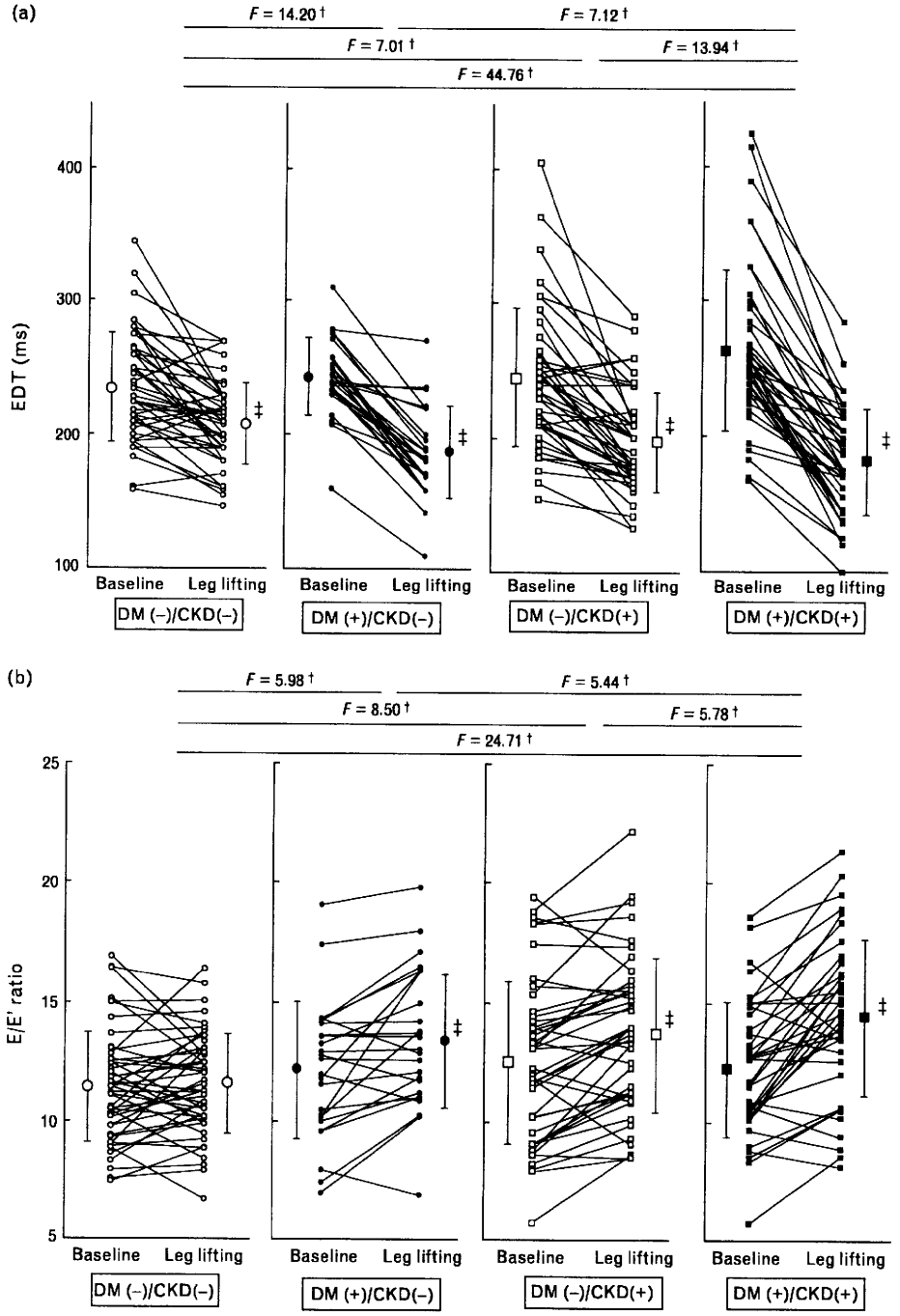
Variables	All	DM(−)/CKD(−)	DM(+)/CKD(−)	DM(−)/CKD(+)	DM(+)/CKD(+)	*P* for difference#
SBP (mmHg)						
Baseline	140.6 ± 13.8	137.9 ± 10.9	137.0 ± 12.5	143.7 ± 15.5	143.0 ± 14.2	NS
Change from baseline	0.7 ± 1.3	0.5 ± 1.0	1.1 ± 2.1	0.3 ± 1.1	1.1 ± 1.5	NS
DBP (mmHg)						
Baseline	76.9 ± 9.5	78.9 ± 8.8	76.2 ± 10.3	76.0 ± 10.2	76.0 ± 10.3	NS
Change from baseline	1.4 ± 1.8	1.1 ± 1.3	1.0 ± 2.2	1.1 ± 2.0	1.8 ± 1.7	NS
Heart rate (bpm)						
Baseline	65.8 ± 8.6	66.8 ± 8.4	67.4 ± 10.5	62.8 ± 7.5	66.9 ± 8.2	NS
Change from baseline	1.1 ± 4.38	0.9 ± 4.1	2.2 ± 4.9	0.4 ± 7.2	1.1 ± 3.3	NS
E-velocity (m/s)						
Baseline	0.74 ± 0.17	0.68 ± 0.15†	0.73 ± 0.17	0.78 ± 0.17*	0.77 ± 0.18*	<0.05
Change from baseline	0.17 ± 0.11	0.17 ± 0.11	0.16 ± 0.11	0.13 ± 0.10	0.20 ± 0.11§	<0.01
A-velocity (m/s)						
Baseline	0.85 ± 0.19	0.78 ± 0.14	0.81 ± 0.18	0.89 ± 0.20	0.93 ± 0.21†	<0.01
Change from baseline	0.00 ± 0.09	0.01 ± 0.10	0.03 ± 0.08	−0.01 ± 0.09	0.00 ± 0.07	NS
E/A ratio						
Baseline	0.89 ± 0.22	0.89 ± 0.21	0.92 ± 0.24	0.90 ± 0.22	0.85 ± 0.24	NS
Change from baseline	0.20 ± 0.17	0.22 ± 0.17	0.18 ± 0.16	0.17 ± 0.20	0.23 ± 0.16	NS
EDT (ms)						
Baseline	246 ± 48	236 ± 41	243 ± 28	245 ± 51	264 ± 59	NS
Change from baseline	−51.1 ± 41.6	−25.2 ± 29.2§	−55.9 ± 29.7*	−48.9 ± 40.5*	−81.7 ± 42.0†§	<0.01
PVs-velocity (m/s)						
Baseline	0.63 ± 0.15	0.63 ± 0.18	0.54 ± 0.14*§	0.67 ± 0.13	0.63 ± 0.13	<0.01
Change from baseline	0.00 ± 0.10	0.02 ± 0.10	0.01 ± 0.11	−0.02 ± 0.09	−0.04 ± 0.11	NS
PVd-velocity (m/s)						
Baseline	0.41 ± 0.12	0.41 ± 0.14	0.37 ± 0.11	0.42 ± 0.10	0.40 ± 0.12	NS
Change from baseline	0.09 ± 0.11	0.07 ± 0.08	0.05 ± 0.11	0.10 ± 0.13	0.12 ± 0.13	NS
S/D ratio						
Baseline	1.62 ± 0.41	1.62 ± 0.48	1.52 ± 0.35	1.67 ± 0.39	1.64 ± 0.39	NS
Change from baseline	−0.27 ± 0.37	−0.15 ± 0.26	−0.17 ± 0.18	−0.30 ± 0.39	−0.41 ± 0.47*	NS
PVa-velocity (m/s)						
Baseline	0.28 ± 0.07	0.29 ± 0.09	0.25 ± 0.04	0.29 ± 0.07	0.28 ± 0.05	NS
Change from baseline	0.00 ± 0.04	−0.01 ± 0.06	0.01 ± 0.02	0.00 ± 0.04	0.01 ± 0.04	NS
ARdur−Ad (ms)						
Baseline	−27.9 ± 23.5	−35.2 ± 20.6	−25.8 ± 19.7	−29.7 ± 25.3	−18.2 ± 20.9	NS
Change from baseline	6.0 ± 14.4	5.7 ± 16.9	7.3 ± 8.7	5.1 ± 15.0	6.6 ± 12.5	NS
E′-velocity (cm/s)						
Baseline	6.24 ± 1.10	6.04 ± 1.04	6.10 ± 1.02	6.43 ± 1.24	6.37 ± 1.05	NS
Change from baseline	0.78 ± 1.09	1.45 ± 0.94§	0.76 ± 0.94*	0.35 ± 1.05†	0.45 ± 1.01†	<0.01
E/E′ ratio						
Baseline	12.03 ± 2.87	11.42 ± 2.30	12.14 ± 2.87	12.47 ± 3.41	12.24 ± 2.82	NS
Change from baseline	1.13 ± 2.03	0.15 ± 1.65†	1.20 ± 1.93*	1.18 ± 1.72*	2.24 ± 2.28†‡	<0.01
A′-velocity (cm/s)						
Baseline	8.85 ± 0.83	8.71 ± 0.80	8.96 ± 1.26	8.85 ± 0.54	8.95 ± 0.79	NS
Change from baseline	0.22 ± 0.53	0.15 ± 0.48	0.22 ± 0.45	0.21 ± 0.48	0.29 ± 0.64	NS
S′-velocity (cm/s)						
Baseline	6.21 ± 0.70	6.17 ± 0.61	6.18 ± 0.79	6.23 ± 0.79	6.25 ± 0.67	NS
Change from baseline	0.05 ± 0.60	0.06 ± 0.58	−0.04 ± 0.59	0.00 ± 0.66	0.16 ± 0.56	NS

Values are mean ± SD. A-velocity, the transmitral late filling velocity; A′-velocity, tissue Doppler late diastolic velocity; ARdur−Ad, the time difference between the duration of the atrial filling wave and the duration of flow at atrial contraction; CKD, chronic kidney disease; DM, diabetes mellitus; E-velocity, the transmitral early filling velocity; E′-velocity, tissue Doppler early diastolic velocity; E/E′ ratio, the ratio of early diastolic transmitral velocity to early diastolic tissue velocity; E/A ratio, the ratio of peak early to late diastolic filling velocity; EDT, the deceleration time of early diastolic filling; PLL, passive leg lifting maneuver; PVa, pulmonary vein atrial reversal; PVd, peak diastolic forward flow; PVs, peak systolic forward flow; S′-velocity, tissue Doppler systolic velocity; S/D ratio, the ratio of the pulmonary venous systolic velocity to diastolic velocity. #The *P* values of one-way analysis of variance (ANOVA) are shown. *$P < 0.05$ and †$P < 0.01$ versus DM(−)/CKD(−). ‡$P < 0.05$ and §$P < 0.01$ versus DM(−)/CKD(+).

increases the driving pressure at mitral valve opening, resulting in an increase in peak E-velocity because of higher left atrial pressure on mitral valve opening and decreased EDT in parallel with a decline in left ventricular compliance. E′-velocity was determined by left ventricular relaxation, minimal pressure, and preload [27], and E/E′ ratio was modulated by increased preload induced by PLL. In accordance with previous findings [9,10], blood pressure as well as heart rate remained statistically unchanged, and thus, the results of our comparative study confirmed the clinical utility of the PLL maneuver as a means of increasing preload without altering afterload.

EDT is dependent on the rate of increase in left ventricular pressure in early diastole, after it has reached its nadir, and is a measure of the effective operative chamber compliance of the left ventricle [6,28,29]. Because the mitral inflow velocity profile is affected by several factors, including suction, volume status, left atrial pressure, and rate of myocardial relaxation [6,7,30], we also performed tissue Doppler imaging of mitral annular motion. E′-velocity is a less preload-dependent index of early left ventricular relaxation than conventional Doppler parameters, and it has also been validated against the Tau index [31], the time constant of isovolumic relaxation. Nevertheless, E′-velocity was also dependent on

Fig. 2



Changes in deceleration time of early diastolic filling (EDT) (a) ($F = 15.92$, $P_{trend} < 0.01$) and the ratio of early diastolic transmitral velocity to early diastolic tissue velocity (E/E') (b) ($F = 8.87$, $P_{trend} < 0.01$) by passive leg lifting (PLL) maneuver. Data are presented as individual plots and means ± SD. CKD, chronic kidney disease; DM, diabetes mellitus. $^{*}P < 0.05$ and $^{\dagger}P < 0.01$. $^{\ddagger}P < 0.01$ versus baseline.

Table 4 Predictors of EDT less than 150 ms during PLL by crude and multivariate logistic regression analysis

	Crude		Multivariate adjusted	
Variables, unit of increase	OR (95% CI)	*P*	OR (95% CI)	*P*
Age, 1 year	1.08 (1.01–1.16)	<0.05	1.10 (0.98–1.23)	NS
Fractional shortening, 1%	0.89 (0.80–0.98)	<0.01	0.88 (0.76–1.02)	NS
Baseline EDT, 1 ms	0.97 (0.95–0.98)	<0.01	0.94 (0.91–0.97)	<0.01
DM, yes	3.97 (1.32–11.91)	<0.01	2.82 (1.03–7.67)	<0.05
CKD, yes	3.25 (1.01–10.46)	<0.01	2.18 (1.18–8.44)	<0.05

Values are odds ratio [95% confidence interval (CI)]. CKD, chronic kidney disease; DM, diabetes mellitus; EDT, the deceleration time of early diastolic filling; NS, not significant; PLL, passive leg lifting.

preload modulation [27,32], and combining transmitral flow velocity with annular velocity (E/E′ ratio) has been proposed as a tool for assessing left ventricular filling pressures that combines the influence of transmitral driving pressure and myocardial relaxation [33–36]. Previous studies in healthy individuals found that the E/E′ ratio did not change significantly in response to PLL [10] or physiological saline infusion [37]. In contrast, the hemodialysis-related volume reduction produced a significant decrease in E/E′ ratio [38]. Our results found that EDT and E/E′ ratio at baseline were not significantly different among subgroups; however, the changes in EDT as well as those in E/E′ ratio showed a stepwise increase in hypertensive patients with complications such as DM and/or CKD. E/E′ ratio did not change significantly in the subgroup without complications; however, a significant increase in E/E′ ratio was found in the subgroups with complications. In addition, in hypertensive patients, concomitant DM and/or CKD was independently associated with EDT less than 150 ms as well as E/E′ ratio at least 15.0 during PLL, which were proven cut-off values to predict decreased left ventricular stiffness [28,36,39], suggesting that different degrees of latent diastolic dysfunction and preload reserve may account for the divergent response of EDT as well as E/E′ ratio to PLL. Patients with reduced compliance may not have the ability to alter their filling pattern in response to an increase in preload, depending on left ventricular operating chamber compliance, and those with less compliant ventricles are much more sensitive to alterations in filling volume, with a marked increase in filling pressure with a relatively small increase in volume [6,40]. These Doppler changes may result from the development of elevated filling pressures and from accelerated relaxation occurring as a consequence of increased contractility. The baseline Doppler indices and their changes after loading manipulations may represent several steps of left ventricular remodeling, and a decreased response to increased preload may predispose to the development of more severe abnormal left ventricular relaxation. Thus, one novel aspect of this study is the demonstration that hypertensive patients with DM and/or CKD have reduced left ventricular compliance and preload reserve. The results from the Strong Heart Study indicated that concomitant DM further adds to the impairment of left ventricular relaxation associated with hypertension [14]. Left ventricular stiffness, which modulates left ventricular diastolic function, is modulated by changes in the extracellular matrix (collagen accumulation), shifts in titin, and endothelial dysfunction. Previous studies support the view that the diabetic state *per se* affects left ventricular dysfunction, due to decreased vessel wall content of heparin sulfate [41], interstitial accumulation of advanced-glycated end products [42,43], and other factors [44,45]. In CKD, factors in the pathogenesis of myocardial fibrosis include acidosis, slower calcium uptake, angiotensin II, chronically elevated parathyroid hormone, endothelin, aldosterone, and increased plasma catecholamines [46]. In addition, a significantly higher LVMI, which has been implicated in the pathogenesis of left ventricular diastolic dysfunction, was observed in the DM(+)/CKD(+) group, and thus LVH could be one of the possible mechanisms of

Table 5 Predictors of the ratio of E/E′ ratio at least 15.0 during PLL by crude and multivariate logistic regression analysis

	Crude		Multivariate adjusted	
Variables, unit of increase	OR (95% CI)	*P*	OR (95% CI)	*P*
Age, 1 year	1.22 (1.13–1.32)	<0.01	1.29 (1.13–1.48)	<0.01
Duration of hypertension, 1 year	1.05 (1.03–1.08)	<0.01	0.98 (0.92–1.03)	NS
DBP, 1 mmHg	0.92 (0.88–0.96)	<0.01	0.99 (0.91–1.08)	NS
Left ventricular hypertrophy, yes	2.98 (1.22–7.30)	<0.05	0.90 (0.18–4.37)	NS
Baseline-E/E′, 1 unit	1.86 (1.53–2.34)	<0.01	3.00 (1.84–4.90)	<0.01
DM, yes	2.43 (1.19–4.95)	<0.01	4.78 (1.84–8.60)	<0.05
CKD, yes	4.46 (2.01–9.92)	<0.01	3.32 (1.48–8.92)	<0.05

Values are odds ratio (95% CI). CI, confidence interval; CKD, chronic kidney disease; DBP, diastolic blood pressure; DM, diabetes mellitus; E/E′, the ratio of transmitral early left ventricular filling velocity to early diastolic Doppler tissue imaging of the mitral annulus.

our results. Previous reports showed that hypertension with complications such as DM or CKD was associated with increased risk of CVD [1–5], and our results may support investigation of the mechanisms of cardiovascular disorders in hypertensive patients with complications.

It must be emphasized that no 'cut-off' values were provided based on the results of this study. Despite the significant difference in the change in EDT as well as that in E/E′ ratio during PLL between hypertensive patients with/without DM and/or CKD, overlap was present. Other investigators have used the criterion of a baseline 'E/A ratio less than 1.0 (whatever the value of EDT)', becoming 'E/A ratio more than 1.0 and EDT of 130 or less' during the PLL maneuver [8,9]. However, this criterion could not be applied to our patients because only a few patients had a decrease in EDT to 130 or less during PLL. Mitral flow pattern is usually assessed only once, at baseline, and this may be a limitation because it may change spontaneously in some patients. Accordingly, we hypothesized that changes in Doppler echocardiographic indices in response to loading manipulations such as PLL could provide an estimate of cardiovascular reserve and add information on latent diastolic dysfunction. Previous studies have confirmed the validity of this maneuver in patients with chronic heart failure [8,9]; however, the prognostic value of this maneuver in hypertensive patients remains to be elucidated. Therefore, this study should be viewed as a preliminary investigation to support future studies to determine the ultimate clinical utility of this maneuver.

In hypertensive patients, the PLL maneuver is a useful procedure as a means of increasing preload and could be useful to estimate the effective operative left ventricular compliance. Concomitant DM and/or CKD was independently associated with an exaggerated change in EDT as well as change in E/E′ ratio in response to the PLL maneuver. Our results suggest that this simple preloading test may unmask the latent progression of left ventricular dysfunction; that is, these complications potentially cause deterioration of left ventricular compliance and preload reserve even in the early stages of diastolic dysfunction. The present findings may support investigation of the actual mechanisms of the increased CVD risk in hypertensive patients with DM or CKD. These findings need to be confirmed in larger prospective studies investigating the importance of early detection and treatment of latent diastolic dysfunction in hypertension, which may be an important risk factor for heart failure as well as CVD.

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Hypertension Research (2010) 33, 214–218

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ORIGINAL ARTICLE

Association of gene polymorphism of the fat-mass and obesity-associated gene with insulin resistance in Japanese

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It was reported that gene polymorphisms in the fat-mass and obesity-associated gene (*FTO*) were associated with obesity and diabetes in several genome-wide association studies. A recent report indicated that *FTO*-knockout mice exhibited phenotypes of skinny body shape and normal metabolic profiles. Thus, *FTO* could be important in metabolic disorders. The aim of this study was to clarify the role of single nucleotide polymorphisms (SNPs) in *FTO* in metabolic disorders such as hypertension, obesity, diabetes, dyslipidemia, insulin resistance and metabolic syndrome in the Japanese general population using data from a cohort study in Hokkaido, namely the Tanno–Sobetsu study. Written informed consent for the genetic analysis was obtained from each subject participating in the study. A total of 1514 subjects were genotyped by TaqMan PCR methods for three SNPs, rs9939609, rs1121980 and rs1558902, in *FTO*. Association analyses between the SNPs and metabolic parameters were performed. Although two SNPs, rs9939609 and rs1558902, were not significantly associated with hypertension, obesity, metabolic syndrome or any metabolic parameters, additive and recessive models of rs1121980 were strongly associated with plasma immunoreactive insulin (IRI) level and homeostasis model assessment insulin resistance (HOMA-IR), even after adjusting for confounding factors such as age, gender and body mass index. A haplotype of three SNPs was also significantly associated with IRI and HOMA-IR. One SNP, rs1121980, and a haplotype of three SNPs in *FTO* that contains this SNP, might be important in the progression of insulin resistance in Japanese subjects.
Hypertension Research (2010) **33,** 214–218; doi:10.1038/hr.2009.215; published online 15 January 2010

Keywords: *FTO*; insulin resistance; metabolic syndrome; obesity; SNP

INTRODUCTION

Metabolic syndrome (MS) consisting of central obesity, high blood pressure, abnormal glucose tolerance or abnormal lipid profiles is considered an independent risk factor for cardiovascular diseases such as ischemic heart disease and stroke.[1,2] In Japan, central obesity based on visceral fat accumulation is an essential diagnosis criterion for MS. One of the main pathways to central obesity is considered to be an imbalance in the secretion of adipocytokines from adipose tissues and subsequent following insulin resistance.[3] Genetic background influences metabolic disorders. Recently, several genome-wide association studies revealed that single nucleotide polymorphisms (SNPs) of the fat-mass and obesity-associated gene (*FTO*) might be predisposing factors for obesity, diabetes and MS.[4–6]

Recently, it was reported that *FTO*-knockout mice showed several characteristic phenotypes, which included skinny body shape and normal metabolic profiles.[7] Thus, *FTO* could be important in fat accumulation and the regulation of glucose or lipid metabolism.

In previous genetic analyses, SNPs rs9939609, rs1121980 and rs1558902 in *FTO* were strongly associated with obesity, defined by body mass index (BMI).[8–11] There remain questions concerning whether these SNPs affect obesity and other metabolic disorders in Japanese subjects, who have a quite different body shape and diet from subjects in Western countries. Furthermore, the allele frequencies of *FTO* SNPs in Japanese subjects are quite different from those in Caucasian subjects (http://hapmap.ncbi.nlm.nih.gov/).

It was reported by Japanese investigators that rs1558902, but not rs9939609, in *FTO* was associated with BMI in a case (severe obesity group: BMI $\geqslant 30\,kg\,m^{-2}$)–control (nonobesity group: BMI $\leqslant 23\,kg\,m^{-2}$) study.[9] However, there are no reports on investigations of the relationship between *FTO* SNPs and metabolic disorders including hypertension, obesity, DM and MS as well as other parameters such as insulin resistance in the Japanese general population. In this study, we investigate the role of *FTO* SNPs in metabolic disorders in a cohort study, namely, the Tanno–Sobetsu study in Hokkaido, northern Japan.

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Received 23 October 2009; revised 10 November 2009; accepted 12 November 2009; published online 15 January 2010

METHODS

Study subjects

We recruited 1514 subjects (803 in Tanno town and 711 in Sobetsu town) who had undergone medical checkups in these towns in Hokkaido, Japan, in 2002. The detailed epidemiological findings have already been reported.[12–17] Subjects completed a standard questionnaire regarding their medical history, and smoking and drinking habits. We measured the systolic blood pressure (SBP), diastolic blood pressure (DBP), BMI, abdominal circumference, total cholesterol, triglyceride, high-density lipoprotein cholesterol, plasma glucose and immunoreactive insulin (IRI). Blood samples were collected during fasting in the early morning. Obesity was defined as a BMI $>25\,kg\,m^{-2}$. Dyslipidemia was defined as total cholesterol $\geqslant 220$ mg per 100 ml and/or drug treatment for hypercholesterolemia. Diabetes was defined as fasting blood sugar $\geqslant 126$ mg per 100 ml and/or drug treatment for hyperglycemia. The Japanese definition of MS [18] was used as the diagnosis for MS. Briefly, criterion 1 and two of criteria 2–4 needed to be met.

1. visceral fat: (male) abdominal circumference $\geqslant 85$ cm
   (female) abdominal circumference $\geqslant 90$ cm
2. lipid abnormality: treatment for dyslipidemia or
   triglyceride $\geqslant 150$ mg per 100 ml and/or high-density lipoprotein cholesterol <40 mg per 100 ml
3. blood pressure: treatment for hypertension or
   SBP $\geqslant 130$ and/or DBP $\geqslant 85$ mm Hg
4. hyperglycemia: treatment for diabetes or fasting blood sugar $\geqslant 110$ mg per 100 mL

Homeostasis model assessment insulin resistance (HOMA-IR) was used to determine insulin sensitivity, and was calculated as plasma glucose (mg per 100 ml) × IRI ($\mu U\,ml^{-1}$)/405.[19] Blood pressure was measured twice after 5 min of rest, with the subjects seated. Hypertension was defined as SBP $\geqslant 140$, DBP $\geqslant 90$ mm Hg or the current use of antihypertensive agents. Three hundred and ninety-five subjects were taking antihypertensive agents, and these subjects were included in the study. Individuals undergoing medical treatment and receiving diet therapy or exercise therapy for diabetes mellitus (n=84) were also included. Precise information on the types of antihypertensive agents or the nature of the treatment for diabetes was not obtained. All participants gave written informed consent to participate in the genetic analyses and in all other procedures associated with the study. The ethics committee of Osaka University approved the study protocol. The final number of subjects participating in the genetic study was 1488.

Genotyping

Genomic DNA was extracted from 200 µl of buffy coat using a QIAamp DNA Blood Kit (Qiagen, Hilden, Germany). We selected three SNPs, rs9939609, rs1121980 and rs1558902, in *FTO*, which were identified as being associated with obesity and/or diabetes in previous reports.[6,9,20,21] These SNPs were genotyped using TaqMan PCR methods with the following probes: C__30090620_10 for rs9939609, C__2031261_10 for rs1121980 and C__8917111_10 for rs1558902 (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Hardy–Weinberg equilibrium was calculated by a χ^2-test. Linkage disequilibrium was evaluated by SNP Alyze version 2.1 (DYNACOM Co., Ltd, Mohara, Japan). Associations between the polymorphisms and clinical variables were analyzed using one-way analysis of variance and analysis of covariance adjusted for confounding factors. Differences in genotype or allele distribution were examined by χ^2-analysis. All numerical values are expressed as mean ± s.d. Values of $P<0.05$ were considered to indicate statistical significance. To adjust for multiple testing of the three gene polymorphisms by Bonferroni's correction, we arbitrarily adopted $P<0.017$ (=0.05/3) as the level of statistical significance. Haplotype estimation was performed by the expectation-maximization algorithm. All analyses except analysis of covariance were performed with JMP statistical software (version 5; SAS Institute Inc., Cary, NC, USA), and analysis of covariance was performed with SPSSα statistical software (release 11.0.1; SPSS Inc., Chicago, IL, USA).

RESULTS

The total number of study subjects who were successfully genotyped for all three SNPs, rs9939609, rs1121980 and rs1558902 of *FTO*, was 1488. The characteristics of study subjects are shown in Table 1. In this cohort, the average BMI was much lower than that of study subjects in previous studies.[5,9,22,23] The prevalence of obesity defined as BMI $\geqslant 25\,kg\,m^{-2}$ according to Japanese criteria was 33%. DM, hypertension and MS by the Japanese definitions were exhibited by the study subjects at 7.8, 44.4 and 15.9%, respectively.

From the genotyping, the prevalences of each genotype in the three SNPs were determined to be AA/AT/TT=56/475/957 in rs9939609, AA/AG/GG=81/519/885 in rs1121980 and AA/AT/TT=59/468/959 in rs1558902. These allele frequencies are in accordance with Hardy–Weinberg equilibrium (data not shown).

The three SNPs, rs9939609, rs1121980 and rs1558902 in *FTO*, were tested for associations with hypertension, diabetes, dyslipidemia, obesity and MS in all the subjects using χ^2-tests. As shown in Table 2, there were no significant associations with hypertension or metabolic disorders in additive, dominant or recessive models. Table 3 shows blood pressure level and various metabolic parameters compared among genotypes for the three models of three SNPs in *FTO* using analysis of variance.

Additive and dominant models of rs1121980 showed significant differences in levels of IRI (P=0.022, 0.01, respectively) and HOMA-IR (P=0.029, 0.008, respectively), as shown in Table 3. Table 4 shows detailed data of IRI and HOMA-IR values composed among the genotypes in rs1121980. After adjusting for confounding factors including age, gender, BMI, abdominal circumference and presence of DM, there were still significant differences in IRI and HOMA-IR among genotypes in both additive and dominant models (P=0.005, 0.001, respectively) as determined by analysis of covariance.

Because some antihypertensive drugs might affect insulin sensitivity, we investigated the genotype comparison of HOMA-IR in both additive and dominant models for rs1121980 in subjects without hypertension (n=740). Significant differences were again identified: AA (n=38): 1.82 ± 4.04 *vs.* AG(223): 1.09 ± 0.84 *vs.* GG(418): 1.10 ± 0.94 in the additive model (P=0.004) and AA: 1.82 ± 4.04 *vs.* AG+GG(641): 1.10 ± 0.91 in the dominant model (P=0.0009).

Table 1 Characteristics of study subjects

	N=*1488*
Age (year)	62.7 ± 11.63
Gender (n, male/female)	M/F=582:906 (M: 39.1%)
BMI ($kg\,m^{-2}$)	23.81 ± 3.24
Abdominal circumference (cm)	83.96 ± 10.20
Systolic blood pressure (mm Hg)	137.43 ± 22.79
Diastolic blood pressure (mm Hg)	76.37 ± 11.67
Total cholesterol (mg per 100 ml)	201.18 ± 31.64
HDL-CHO (mg per 100 ml)	50.59 ± 12.28
LDL-CHO (mg per 100 ml)	130.04 ± 29.62
Triglyceride (mg per 100 ml)	102.72 ± 57.86
FBS (mg per 100 ml)	97.8 ± 24.83
IRI ($\mu U\,ml^{-1}$)	5.203 ± 3.71
HOMA-IR	1.32 ± 1.43
	(mean ± s.d.)

Abbreviations: BMI, body mass index; FBS, fasting blood sugar; HDL-CHO, high-density-lipoprotein cholesterol; IRI, immunoreactive insulin; LDL-CHO, low-density-lipoprotein cholesterol; HOMA-IR, homeostasis model assessment insulin resistance.
HOMA-IR=(FBS×fasting IRI)/405.

Table 2 Relationships between three SNPs in *FTO* and metabolic diseases

	rs9939609			*rs1121980*			*rs1558902*		
	Additive model	*Dominant model AA vs. AT+TT*	*Recessive model TT vs. AT+AA*	*Additive model*	*Dominant model AA vs. AG+GG*	*Recessive model GG vs. AG+AA*	*Additive model*	*Dominant model AA vs. AT+TT*	*Recessive model TT vs. AT+AA*
Dyslipidemia	0.5628	0.729	0.2845	0.5386	0.3025	0.4837	0.7339	0.5975	0.4805
Diabetes	0.4729	0.4982	0.4221	0.5166	0.3312	0.7613	0.4923	0.4362	0.514
Hypertension	0.5142	0.3994	0.3291	0.6366	0.9944	0.3623	0.5557	0.6346	0.2859
Obesity	0.9303	0.9001	0.7057	0.8787	0.9718	0.6202	0.8242	0.9073	0.5357
Treatment history									
Dyslipidemia	0.86	0.8151	0.6768	0.6608	0.5948	0.3888	0.8033	0.9036	0.5535
Diabetes	0.4843	0.2792	0.822	0.403	0.4852	0.3648	0.4943	0.3398	0.677
Hypertension	0.4274	0.2045	0.5304	0.6523	0.3601	0.6956	0.4885	0.3156	0.3666
Metabolic syndrome	0.7067	0.9954	0.4213	0.6112	0.9917	0.3415	0.6153	0.8625	0.3767

Values are indicated as *P*-values.

Table 3 Relationships between three SNPs in *FTO* and metabolic parameters

	rs9939609			*rs1121980*			*rs1558902*		
	Additive model	*Dominant model AA vs. AT+TT*	*Recessive model TT vs. AT+AA*	*Additive model*	*Dominant model AA vs. AG+GG*	*Recessive model GG vs. AG+AA*	*Additive model*	*Dominant model AA vs. AT+TT*	*Recessive model TT vs. AT+AA*
T-CHO (mg per 100 ml)	0.1799	0.6608	0.0641	0.3366	0.351	0.1719	0.3976	0.7541	0.1748
HDL-CHO (mg per 100 ml)	0.4853	0.5142	0.4232	0.8663	0.948	0.6243	0.5043	0.4449	0.5204
LDL-CHO (mg per 100 ml)	0.2946	0.5909	0.1191	0.5649	0.4123	0.3708	0.4825	0.7386	0.2273
TG (mg per 100 ml)	0.872	0.6059	0.8236	0.6308	0.6027	0.3557	0.6878	0.4164	0.9499
FBS (mg per 100 ml)	0.9072	0.7465	0.7072	0.9321	0.7166	0.9876	0.9539	0.8355	0.7836
IRI ($\mu U\ ml^{-1}$)	0.3919	0.2543	0.3002	0.022	0.01	0.0876	0.2408	0.311	0.1145
HOMA-IR	0.7414	0.4844	0.6122	0.0294	0.008	0.3577	0.6697	0.5585	0.4156
SBP (mm Hg)	0.7555	0.6449	0.4893	0.3885	0.8893	0.1772	0.6958	0.9078	0.3991
DBP (mm Hg)	0.9621	0.9567	0.7814	0.3831	0.8359	0.2113	0.7915	0.6504	0.7127
BMI	0.6663	0.9483	0.3768	0.4627	0.9628	0.23	0.3762	0.9994	0.1787

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; FBS, fasting blood sugar; HDL-CHO, high-density-lipoprotein cholesterol; HOMA-IR, homeostasis model assessment insulin resistance; IRI, immunoreactive insulin; LDL-CHO, low-density-lipoprotein cholesterol; SBP, systolic blood pressure; T-CHO, total cholesterol; TG, triglyceride.
Values are indicated as *P*-values.

There was strong linkage disequilibrium among the three SNPs in *FTO*. The r^2 values were 0.811 between rs9939609 and rs1121980, 0.956 between rs9939609 and rs1558902 and 0.821 between rs1121980 and rs1558902. Table 5 shows the results of comparison for metabolic parameters for each haplotype of the three SNPs in additive, dominant and recessive models analyzed by analysis of variance. Low-frequency haplotypes, namely, those with frequencies below 1%, were excluded from analysis. Five haplotypes were analyzed. H2 had a strong association with insulin resistance (Table 5), although it was present at a low frequency (n=21).

DISCUSSION

This study is the first reported investigation of the association between genetic variations in *FTO* and detailed metabolic parameters in the Japanese general population. We selected three SNPs, rs9939609, rs1121980 and rs1558902 in *FTO*, that had been found to be strongly associated with obesity, defined by BMI, in genome-wide association

Table 4 Detailed data of IRI and HOMA-IR values compared among genotypes in rs1121980

SNP rs1121980	*Genotype group*	*HOMA-IR*	*P-value*	*IRI ($\mu U\ ml^{-1}$)*	*P-value*
	GG (828)	1.29±1.42		5.06±3.38	
	AG (482)	1.30±1.02	0.006	5.27±3.67	0.020
	AA (76)	1.74±2.99		6.26±6.34	
	GG	1.29±1.42	0.794	5.05±3.38	0.421
	AG+AA	1.36±1.46		5.41±4.15	
	GG+AG	1.29±1.29	0.002	5.14±3.49	0.013
	AA	1.73±2.99		6.26±6.34	

Abbreviations: HOMA-IR, homeostasis model assessment insulin resistance; IRI, immunoreactive insulin; SNP, single nucleotide polymorphism.
Values are shown as mean±s.d.
Subjects with data of HOMA-IR and IRI were analyzed in Table 4.

Table 5 The results of comparison between haplotypes with three SNPs for metabolic parameters

	rs9939609/rs1121980/rs1558902	*T-CHO*	*HDL-CHO*	*LDL-CHO*	*IRI*	*HOMA-IR*
H1	AA/AA/AA (56) *vs.* others	0.6608	0.5142	0.5909	0.2543	0.4844
H2	AT/AA/AT (21) *vs.* others	0.1853	0.2637	0.3121	0.0006[a]	<0.0001[b]
H3	AT/AG/AT (441) *vs.* others	0.2977	0.5497	0.3463	0.9984	0.4927
H4	TT/AG/TT (67) *vs.* others	0.8063	0.4806	0.4044	0.5983	0.7029
H5	TT/GG/TT (878) *vs.* others	0.1305	0.5129	0.3147	0.1002	0.39

Abbreviations: HDL-CHO, high-density-lipoprotein cholesterol; HOMA-IR, homeostasis model assessment insulin resistance; IRI, immunoreactive insulin; LDL-CHO, low-density-lipoprotein cholesterol; T-CHO, total cholesterol.
Values are indicated as *P*-values.
[a]AT/AA/AT 8.11±9.89 (*N*=19). Others 5.16±3.54 (*N*=1370).
[b]AT/AA/AT 2.72±5.59 (*N*=19). Others 1.30±1.28 (*N*=1370).

studies in various ethnicities.[22,24–29] In Japan, Hotta *et al.*[9] reported that rs1558902 in *FTO* was most significantly associated with obesity in a case–control association study using severely obese Japanese subjects (average BMI$\geqslant$30 kg m^{-2}).[9] In this study, we investigated the association between various metabolic parameters including hypertension, DM, obesity and MS in subjects participating in the Tanno–Sobetsu cohort study, a study of a Japanese representative rural cohort in Hokkaido. The average BMI of the study subjects was 23.81 ± 3.24 kg m^{-2}, which is close to the national average in Japan. In this study, none of the three SNPs was associated with obesity, defined by BMI, higher abdominal circumference or prevalence of MS, defined by Japanese criteria. In addition, none of the three SNPs was associated with hypertension, dyslipidemia or prevalence of DM.

Only one SNP, rs1121980, showed a strong correlation with HOMA-IR, which is an index of insulin resistance, in additive and dominant models. Subjects with AA in rs1121980 had a much higher HOMA-IR and a higher insulin resistance than subjects without the AA genotype (P=0.008). This P-value is considered significant (P<0.017) after Bonferroni's correction to adjust for multiple testing of the three SNPs. Subjects with the haplotype H2, which includes AA in rs1121980, had a higher HOMA-IR than other subjects (Table 5). Thus, we conclude that rs1121980 in *FTO* is associated with insulin resistance in the Japanese general population. Because a recent report indicates that a gain of function of *FTO* induces insulin resistance,[7] rs1121980 located in an intron may regulate *FTO* gene function by affecting splicing variation. After adjusting for obesity and the prevalence of DM, rs1121980 is independently associated with insulin resistance. Therefore, rs1121980 may affect insulin resistance, directly and not only indirectly by obesity.

In this study, three SNPs in *FTO* were not associated with obesity. Several reasons for this are considered. One is the difference between Caucasian and Japanese general populations in the severity of obesity. In fact, SNPs in *FTO* were associated with obesity in a study using Japanese subjects with severe obesity (average BMI$\geqslant$30 kg m^{-2}).[9] Another reason is the differences in allele frequency among *FTO* SNPs. In the cases of rs9939609, allele frequency information obtained from HapMap database (http://hapmap.ncbi.nlm.nih.gov/) shows significant differences between Caucasian (AA/AT/TT=0.117/0.667/0.217) and Japanese populations (0.067/0.200/0.733).

In summary, an SNP located in an intron, rs1121980, and a haplotype of three SNPs in *FTO* that includes this SNP, may be important in the progression of insulin resistance in Japanese subjects. This SNP may be an independent risk factor for future MS, hypertension and DM in Japanese subjects. However, this study has limitations because of its cross-sectional design. Prospective studies investigating the relationship between these SNPs and the development of MS, hypertension and DM over a long time scale are necessary.

ACKNOWLEDGEMENTS

We thank Ms Kazuko Iwasa for continuous support of this investigations. This study was supported by research grant from the Japanese Ministry of Health, Labor, and Welfare and Osaka Heart Club.

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