Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 57 (2008) 280–284
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Correlations of visceral fat accumulation and atherosclerosis in Japanese patients with type 2 diabetes mellitus

Futoshi Anan[a,b,*], Takayuki Masaki[b], Yoshikazu Umeno[c], Tetsu Iwao[a], Hidetoshi Yonemochi[d], Nobuoki Eshima[e], Tetsunori Saikawa[d], Hironobu Yoshimatsu[b]

[a]*Department of Cardiology, Oita Red Cross Hospital, Oita 870-0033, Japan*
[b]*First Department of Internal Medicine, School of Medicine, Oita University Oita 870-0033, Japan*
[c]*Department of Endocrinology, Oita Red Cross Hospital, Oita 870-0033, Japan*
[d]*Department of Cardiovascular Science, School of Medicine, Oita University Oita 870-0033, Japan*
[e]*Department of Biostatistics, School of Medicine, Oita University Oita 870-0033, Japan*

Received 1 May 2007; accepted 14 September 2007

Abstract

We tested the hypothesis that increased abdominal visceral fat accumulation (VFA) is associated with insulin resistance and aortic stiffness in patients with type 2 diabetes mellitus not receiving insulin treatment. The study consisted of 22 Japanese patients with type 2 diabetes mellitus and high VFA (≥100 cm^2; age, 61 ± 7 years; high VFA group) and a control group of 18 age-matched patients with normal VFA (<100 cm^2; age, 60 ± 8 years; normal VFA group). Brachial-ankle pulse wave velocity (BaPWV) was measured by automatic oscillometric method. The BaPWV was used as an index of atherosclerosis. The body mass index values ($P < .05$), waist circumferences ($P < .0005$), and waist-to-hip ratios ($P < .05$) were larger in the high VFA group than in the normal VFA group. The BaPWV was higher in the high VFA group than in the normal VFA group ($P < .0001$). Fasting plasma glucose ($P < .05$), insulin concentrations ($P < .0001$), and the homeostasis model assessment (HOMA) index ($P < .001$) were higher in the high VFA group than in the normal VFA group. Multiple regression analysis showed that the VFA level was independently predicted by BaPWV and the HOMA index. Our results indicate that the elevation of VFA in Japanese patients with type 2 diabetes mellitus is characterized by increased aortic stiffness and insulin resistance and that BaPWV and the HOMA index are independent predictors of VFA.
Published by Elsevier Inc.

1. Introduction

A central pattern of body fat distribution, rather than regional or generalized obesity, is now generally considered to play an important role in the metabolic syndrome, which involves insulin resistance, hyperinsulinemia, dyslipidemia, obesity, diabetes mellitus, and hypertension [1-4].

The data confirmed that although the more expensive abdominal computed tomography precisely assesses visceral fat accumulation (VFA), the waist circumference measurement or body mass index (BMI) provides a less expensive means to assess VFA [5]. An increased VFA is a risk factor for cardiovascular disease [6,7] and is associated with insulin resistance in healthy subjects [8] and patients with type 2 diabetes mellitus [9].

Pulse wave velocity (PWV) reflects arterial stiffness, and it has been demonstrated that carotid-femoral PWV relates to the severity of atherosclerosis [10] and predicts future atherosclerotic cardiovascular events [11]. Recently, a simple method of measuring brachial-ankle PWV (BaPWV) has been reported [12-14]. Moreover, BaPWV is a marker of severity of atherosclerosis [13,14]; and increased BaPWV is a risk factor for cardiovascular disease [14] and poor prognosis in patients with acute coronary syndrome [15].

Insulin resistance is linked to established risk factors for atherosclerosis such as hypertension, hyperlipidemia, and obesity, which subsequently accelerate the development and progression of atherosclerosis [16,17]. However, the relationship between VFA levels, insulin resistance, and aortic stiffness has not been adequately investigated.

* Corresponding author. Department of Cardiology, Oita Red Cross Hospital, Oita 870-0033, Japan. Tel.: +81 97 532 6181; fax: +81 97 533 1207.
E-mail address: anan-f@med.oita-u.ac.jp (F. Anan).

0026-0495/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.metabol.2007.09.013

We hypothesized that increased levels of VFA are associated with BaPWV and insulin resistance in patients with type 2 diabetes mellitus. To test our hypothesis, we compared BaPWV and metabolic profiles in Japanese patients with type 2 diabetes mellitus with normal VFA levels to those with high VFA levels; independent predictors of the VFA in these populations were evaluated.

2. Subjects and methods

We screened 101 consecutive Japanese patients with type 2 diabetes mellitus who were admitted to our department between January and December 2005.

Among them, 62 patients did not have organic heart disease as determined by physical examination and routine laboratory tests, including serum electrolytes, serum creatinine, blood urea nitrogen, fasting blood glucose, fasting immunoreactive insulin (F-IRI), chest x-ray, 12-lead electrocardiography, echocardiography, treadmill exercise electrocardiography, and thallium 201 cardiac scintigraphy.

All patients underwent clinical examination to exclude the presence of secondary hypertension. *Essential hypertension* was defined as diastolic blood pressure (BP) ≥90 mm Hg, systolic BP ≥140 mm Hg, or self-reported use of antihypertensive medication [18].

2.1. Laboratory methods

Blood was taken at 7:00 AM from the antecubital vein with the patient in the recumbent position after an overnight fast. All patients underwent routine laboratory tests including assays for serum electrolytes, serum total cholesterol, serum triglycerides, serum high-density lipoprotein (HDL), fasting plasma glucose (FPG), and F-IRI. Insulin resistance was evaluated by the homeostasis model assessment (HOMA) index: [fasting plasma insulin (in microunits per milliliter) × FPG (in millimoles per liter)]/22.5 [19]. All subjects underwent computed tomography at the level of the umbilicus level to measure cross-sectional abdominal visceral fat areas and were analyzed with Fat Scan version 3 software (N2 Systems, Osaka, Japan). Details of the procedures have been described previously [20]. This method is validated by other determinations of VFA [21] and widely adopted as a practical method to evaluate regional adiposity.

Twenty-two patients were determined to have high VFA (≥100 cm^2, high VFA group). We also included 18 age-matched patients from the original 62 enrolled patients who had normal VFA (<100 cm^2, normal VFA group), the classification of which has previously been validated [22]. The clinical characteristics of patients in the normal and high VFA groups are summarized in Table 1. Fourteen of the 22 patients in the high VFA group and 11 of the 18 patients in the normal VFA group met the criteria for essential hypertension; and all of these patients were being treated with calcium channel antagonists, angiotensin-converting enzyme (ACE) inhibitors, and/or angiotensin II receptor blockers with diuretics. None of the patients were being treated with insulin. *Dyslipidemia* was defined as fasting triglyceride levels ≥200 mg/dL or an HDL cholesterol (HDL-C) concentration <45 mg/dL for women and <35 mg/dL for men [18]. Ten of the 22 patients in the high VFA group and 7 of the 18 patients in the normal VFA group met the criteria for dyslipidemia. Patients with abnormal plasma creatinine concentrations (≥1.5 mg/dL) were excluded from the study.

Table 1
Clinical characteristics of studied patients

	Normal VFA group	High VFA group	*P*
Age (y)	61 ± 8	60 ± 7	NS
Sex (men/women)	9/9	12/10	NS
VFA (cm^2)	68.9 ± 17.8	183.8 ± 52.8	<.0001
Duration of diabetes (y)	7.1 ± 3.0	7.5 ± 4.1	NS
Hypertension (%)	61	64	NS
Dyslipidemia (%)	39	45	NS
Drug use (%)			
Sulfonylurea	39	41	NS
α-Glucosidase inhibitors	33	36	NS
Pioglitazone	17	14	NS
Statin	33	36	NS
Calcium channel antagonists	44	41	NS
β-blockers	17	18	
ACE inhibitors	22	23	NS
Angiotensin receptor blocker	39	36	NS
BMI (kg/m^2)	25.0 ± 2.9	27.3 ± 3.1	.0203
Waist circumference (cm)	83.0 ± 8.1	92.5 ± 9.6	.0019
Hip circumference (cm)	96.6 ± 6.3	99.5 ± 8.4	NS
Waist-to-hip ratio	0.86 ± 0.10	0.93 ± 0.09	.0309
Systolic BP (mm Hg)	127 ± 11	132 ± 13	NS
Diastolic BP (mm Hg)	77 ± 9	78 ± 8	NS
Heart rate (beats/min)	68 ± 7	69 ± 6	NS
Total cholesterol (mg/dL)	199 ± 30	205 ± 23	NS
Triglyceride (mg/dL)	125 ± 46	156 ± 40	.0397
HDL-C (mg/dL)	47 ± 9	41 ± 8	.0207
FPG (mg/dL)	134 ± 18	163 ± 53	.0413
F-IRI (μU/mL)	5.9 ± 1.9	8.3 ± 2.3	.0009
HOMA index	2.0 ± 0.9	3.3 ± 1.1	<.0001
Hemoglobin A_{1c} (%)	7.7 ± 1.3	7.8 ± 0.9	NS
Uric acid (mg/dL)	5.5 ± 1.6	6.6 ± 1.3	.0178
Creatinine (mg/dL)	0.7 ± 0.2	0.8 ± 0.2	NS

Data are means ± SD. NS indicates not significant.

All subjects gave their written informed consent to participate in the study, and the study protocol was approved by the ethics committee of the Oita Red Cross Hospital.

2.2. Measurement of PWV

Brachial-ankle PWV was measured using a volume plethysmogram (Form/ABI; Colin, Komaki, Japan). The details of the measurement, validity, and reproducibility of this method have been reported previously [12-14]. The subject was examined while resting in the supine position, with electrocardiogram electrodes placed on

both wrists, a microphone for detecting heart sounds placed on the left edge of the sternum, and cuffs wrapped on both the brachia and ankles. The cuffs were connected to the plethysmogram sensor that determines the volume pulse form and the oscillometric pressure sensor that measures BP. The volume waveforms for the brachium and ankle were recorded. The stored samples included sufficient waveform data. The characteristic points of waveforms were determined automatically according to the phase velocity theory. The components more than 5 Hz were stored using a pass filter, and the wave front was determined. The time interval between the wave front of the brachial waveform and that of the ankle waveform was defined as the time interval between the brachium and ankle (ΔTba). The distance between sampling points of BaPWV was calculated automatically according to the height of the subject. The path length from the suprasternal notch to the brachium (Lb) was obtained from superficial measurements and was expressed using the following equation: Lb = [0.2195 × height of the subject (in centimeters) − 2.0734]. The path length from the suprasternal notch to the ankle (La) was obtained from superficial measurements and was expressed using the following equation: La = [0.8129 × height of the subject (in centimeters) + 12.328]. Finally, the following equation was used to obtain BaPWV: BaPWV = [(La − Lb)/ΔTba]. In all the studies, BaPWV was measured after at least a 5-minute rest. The interobserver coefficient of variation was 8.4%, and the intraobserver coefficient of variation was 10.0% [13].

2.3. Anthropometric and body composition measurement

The anthropometric and body composition characteristics of the patients were evaluated using the following parameters: height, body weight, BMI, waist circumference, hip circumference, and waist-to-hip ratio. Body mass index was calculated as weight/(height^2) (in kilograms per square meter). The waist circumference was measured midway between the lower rib margin and the iliac crest, and the hip circumference was measured at the widest circumference over the trochanter in standing subjects after normal expiration.

2.4. Statistical analysis

Data are presented as mean ± SD. Differences between the 2 groups were analyzed by the unpaired Student t test, χ^2 test, or Fisher exact probability test (Table 1, Fig. 1). A P value < .05 was considered statistically significant. Simple (Spearman rank) correlation coefficients between VFA and various parameters were calculated (Table 2). Stepwise multiple regression analysis was then used to evaluate the association between the levels of VFA and other factors, such as the BMI, waist circumference, waist-to-hip ratio, triglyceride levels, HDL-C levels, uric acid levels, FPG concentrations and plasma insulin concentrations, HOMA index values, and BaPWV. In our multivariate analysis, F values ≥4 were considered significant (Table 3).

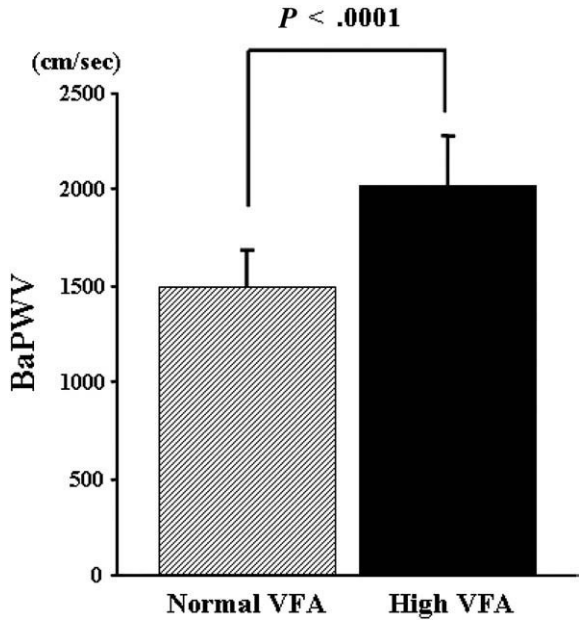

Fig. 1. Comparison of BaPWV between patients with type 2 diabetes mellitus with normal VFA and with high VFA. Data are mean ± SD.

3. Results

As shown in Table 1, the mean ages of the high and normal VFA groups were similar; and there were no significant differences between the groups with respect to sex, duration of diabetes, or administered medications. The BMI values, waist circumferences, and waist-to-hip ratios were larger in the high VFA group than in the normal VFA group (P = .0038, P = .0029, and P = .0042, respectively).

The resting heart rates and systolic and diastolic BPs were not significantly different between the 2 groups. Regarding glucose metabolism, FPG and insulin concentrations and HOMA index values were higher in the VFA group than in the normal VFA group (P = .0080, P = .0034, P = .0001, respectively). However, there was no significant difference in hemoglobin A_{1c} between the 2 groups. With regard to lipid metabolism, the concentration of serum triglyceride was higher and the concentration of serum HDL-C was lower in the high VFA group than in the normal VFA group (P = .0144 and P = .0064, respectively), whereas serum total cholesterol levels were not significantly different between the groups. The concentration of uric acid was higher in the high VFA group than in the normal VFA group (P = .0224). The parameter measuring renal function, serum creatinine concentration, was not significantly different between the groups.

Fig. 1 shows the BaPWV in the normal VFA group and in the high VFA group of patients with type 2 diabetes mellitus. The BaPWV was higher in the high VFA group

Table 2
Correlations between VFA and various parameters

Parameters	Univariate analysis	
	r	*P*
Age	0.051	.7555
Duration of diabetes mellitus	0.086	.5962
BMI	0.448	.0038
Waist circumference	0.459	.0029
Hip circumference	0.024	.8670
Waist-to-hip ratio	0.445	.0042
Systolic BP	0.145	.3717
Diastolic BP	0.049	.7627
Heart rate	0.121	.4588
Total cholesterol	0.259	.1060
Triglyceride	0.384	.0144
HDL-C	−0.424	.0064
Uric acid	0.360	.0224
FPG	0.413	.0080
F-IRI	0.454	.0034
HOMA index	0.566	.0001
Hemoglobin A_{1c}	0.079	.6294
Creatinine	0.286	.0738
BaPWV	0.510	.0008

than in the normal VFA group (2014 ± 269 cm/s vs 1497 ± 175 cm/s, $P < .0001$).

Table 2 depicts the correlation between the VFA level and age, BMI, and other variables in both the high VFA and the normal VFA groups. The VFA levels were positively correlated with the BMI values, waist circumference, waist-to-hip ratio, triglyceride levels, FPG, fasting plasma insulin concentration, uric acid levels, HOMA index values, and BaPWV, and were negatively correlated with HDL-C levels.

Multiple regression analysis was performed using the stepwise procedure. The level of VFA was independently predicted by BaPWV and HOMA index (Table 3).

4. Discussion

In the present study, patients with type 2 diabetes mellitus with VFA manifested increased arterial stiffness evaluated by BaPWV. Among the metabolic parameters, fasting plasma concentrations of glucose and insulin and the HOMA index were higher in patients with high VFA than in those with normal VFA. In addition, multiple regression analysis revealed that the levels of VFA in the patients could be independently predicted by the HOMA index values and BaPWV in Japanese patients with type 2 diabetes mellitus.

There are several reports indicating that an elevated VFA concentration is associated with insulin resistance in healthy subjects [8] and patients with type 2 diabetes mellitus [9]. Raji et al [8] investigated the association between VFA levels and insulin resistance using a hyperinsulinemic-euglycemic clamp in nonobese healthy subjects. They found a significant increase in the VFA levels of healthy subjects with insulin resistance. Gastaldelli et al [9] demonstrated that VFA has a significant negative impact on glycemic control through a decrease in peripheral insulin sensitivity in patients with type 2 diabetes mellitus. In the present study, the level of VFA correlated with the BMI, triglyceride levels, HDL-C levels, fasting plasma insulin concentration, and HOMA index values.

How does VFA relate to BaPWV in the present study? The mechanism of any relationship cannot be established in a cross-sectional analysis. In our opinion, there are some possible explanations for this observation. Arner [23] has suggested that the flux of lipid from the visceral fat depot to the liver might account for hepatic insulin resistance. In a canine model, development of insulin resistance occurred concomitant with visceral adiposity because of a modest fat content in the diet but without increased calories [24]. Steinberg et al [25] reported that insulin-resistant states such as diabetes and obesity are associated with decreased endothelium-dependent vasodilation [25], and arterial compliance may be a partially nitric oxide–dependent process [26]. In addition, insulin has been shown to induce vascular smooth muscle proliferation and migration in cell cultures [27].

Taken together, the findings in this study show for the first time that the VFA levels correlate with BaPWV, which may relate to insulin resistance in patients with type 2 diabetes mellitus.

There are some limitations to this study. Firstly, 67% and 64% of our patients with high VFA and low VFA, respectively, had been diagnosed earlier with associated essential hypertension. All these patients were being treated with one or more antihypertensive drugs, including ACE inhibitors, angiotensin II receptor blockers, and calcium channel antagonists, before enrollment. In this regard, all of these 3 drug classes have been reported to improve insulin resistance [28,29] and aortic stiffness [30,31]. Therefore, these medications might have beneficially affected our results. As to antidiabetic medications, a considerable number of patients were being treated with sulfonylurea and/or α-glucosidase inhibitors, whereas only one patient in each group was treated with pioglitazone, an insulin-sensitizing drug reported to reduce VFA in patients with type 2 diabetes mellitus [32]. Our small cross-sectional study did not allow us to statistically analyze and exclude the potential effects of these influences on the HOMA index and BaPWV.

Secondly, no patients enrolled in the present study underwent coronary angiography and vascular echocardiography. Finally, it has been recognized that there is a sex difference in various aspects of VFA and metabolism. In

Table 3
Stepwise regression analyses between VFA and various parameters

Independent variables	Regression coefficient	Standard error	Standard regression coefficient	F
To VFA intercept	−72.037			
BaPWV	0.074	0.027	0.364	7.794
HOMA index	26.504	7.717	0.447	11.442

the present study, there was no significant difference in these measures between male and female subjects (data not shown). A large-scale study is needed to clarify the sex difference.

In conclusion, the present results indicate that in Japanese patients with type 2 diabetes mellitus, the levels of VFA are associated with aortic stiffness and insulin resistance and that in these patients, BaPWV and HOMA index are independent predictors of VFA.

References

[1] Kaplan NM. The deadly quartet. Upper-body obesity, glucose intolerance, hypertriglyceridemia, and hypertension. Arch Intern Med 1989;149:1514-20.

[2] Despres JP, Moorjani S, Lupien PJ, et al. Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. Arteriosclerosis 1990;10:497-511.

[3] Boyko EJ, Fujimoto WY, Leonetti DL, et al. Visceral adiposity and risk of type 2 diabetes: a prospective study among Japanese Americans. Diabetes Care 2000;23:465-71.

[4] Hayashi T, Boyko EJ, Leonetti DL, et al. Visceral adiposity and the prevalence of hypertension in Japanese-Americans. Circulation 2003; 108:1718-823.

[5] Matsuzawa Y. Pathophysiology and molecular mechanisms of visceral fat syndrome: the Japanese experience. Diabetes Metab Rev 1997;13: 313.

[6] Fujimoto W, Bergstrom R, Boyko E, et al. Visceral adiposity and incident coronary heart disease in Japanese-American men: the 10-year follow-up results of the Seattle Japanese-American Community Diabetes Study. Diabetes Care 1999;22:1808-12.

[7] St-Pierre J, Lemieux I, Vohl MC, et al. Contribution of abdominal obesity and hypertriglyceridemia to impaired fasting glucose and coronary artery disease. Am J Cardiol 2002;90:15-8.

[8] Raji A, Seely EW, Arky RA, et al. Body fat distribution and insulin resistance in healthy Asian Indians and Caucasians. J Clin Endocrinol Metab 2001;86:5366-71.

[9] Gastaldelli A, Miyazaki Y, Pettiti M, et al. Metabolic effects of visceral fat accumulation in type 2 diabetes mellitus. J Clin Endocrinol Metab 2002;87:5098-103.

[10] Zureik M, Bureau JM, Temmar M, et al. Echogenic carotid plaques are associated with aortic arterial stiffness in subjects with subclinical carotid atherosclerosis. Hypertension 2003;41:519-27.

[11] Laurent S, Boutouyrie P, Asmar R, et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension 2001;37:1236-41.

[12] Tomiyama H, Yamashina A, Arai T, et al. Influences of age and gender on results of noninvasive brachial-ankle pulse wave velocity measurement—a survey of 12517 subjects. Atherosclerosis 2003;166:303-9.

[13] Yamashina A, Tomiyama H, Arai T, et al. Brachial-ankle pulse wave velocity as a marker of atherosclerotic vascular damage and cardiovascular risk. Hypertens Res 2003;26:615-22.

[14] Yamashina A, Tomiyama H, Takeda K, et al. Validity, reproducibility, and clinical significance of noninvasive brachial-ankle pulse wave velocity measurement. Hypertens Res 2002;25:359-64.

[15] Tomiyama H, Koji Y, Yambe M, et al. Brachial-ankle pulse wave velocity is simple and independent predictor of prognosis in patients with acute coronary syndrome. Circ J 2005;69:815-22.

[16] Reaven GM. Role of insulin resistance in human disease. Diabetes 1988;37:1595-607.

[17] DeFronzo RA, Ferrannini E. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 1991; 14:173-94.

[18] Liao D, Sloan RP, Cascio WE, et al. Multiple metabolic syndrome is associated with lower heart rate variability. The Atherosclerosis Risk in Communities Study. Diabetes Care 1998;21:2116-22.

[19] Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28:412-9.

[20] Yoshizumi T, Nakamura T, Yamane M, et al. Abdominal fat: standardized technique for measurement at CT. Radiology 1999;211: 283-6.

[21] Kvist H, Chowdhury B, Sjostrom L, et al. Adipose tissue volume determination in males by computed tomography and ^{40}K. Int J Obes 1988;12:249-66.

[22] The Examination Committee of Criteria for 'Obesity Disease' in Japan, Japan Society for the Study of Obesity. New criteria for 'obesity disease' in Japan. Circ J 2002;66:987-92.

[23] Arner P. Insulin resistance in type 2 diabetes: role of fatty acids. Diabetes Metab Res Rev 2002;18:S5-9.

[24] Mittelman SD, Van Citters GW, Kim SP, et al. Longitudinal compensation for fat-induced insulin resistance includes reduced insulin clearance and enhanced β-cell response. Diabetes 2000;49: 2116-25.

[25] Steinberg HO, Chaker H, Leaming R, et al. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. J Clin Invest 1996;97: 2601-10.

[26] Hu CT, Dinenno FA, Monahan KD, et al. Acute effects of nitric oxide blockade with L-NAME on arterial haemodynamics in the rat. Br J Pharmacol 1997;122:1237-43.

[27] Indolfi C, Torella D, Cavuto L, et al. Effects of balloon injury on neointimal hyperplasia in streptozotocin-induced diabetes and in hyperinsulinemic nondiabetic pancreatic islet-transplanted rats. Circulation 2001;103:2980-6.

[28] Gavras HP. Issues in hypertension: drug tolerability and special populations. Am J Hypertens 2001;14(Pt 2):231S-6S.

[29] Lender D, Arauz-Pacheco C, Breen L, et al. A double blind comparison of the effects of amlodipine and enalapril on insulin sensitivity in hypertensive patients. Am J Hypertens 1999;12:298-303.

[30] Takami T, Shigemasa M. Efficacy of various antihypertensive agents as evaluated by indices of vascular stiffness in elderly hypertensive patients. Hypertens Res 2003;26:609-14.

[31] Anan F, Takahashi N, Ooie T, et al. Effects of valsartan and perindopril combination therapy on left ventricular hypertrophy and aortic stiffness in patients with essential hypertension. Eur J Clin Pharmacol 2005;61: 353-9.

[32] Miyazaki Y, Mahankali A, Matsuda M, et al. Effect of pioglitazone on abdominal fat distribution and insulin sensitivity in type 2 diabetic patients. J Clin Endocrinol Metab 2002;87:1784-91.