suggesting that intervention therapy such as diet and exercise may reduce total and abdominal fat and that these changes in body composition mediate improvements in insulin sensitivity and may improve endothelial vasodilator function. Prospective randomized controlled trials are needed to confirm this issue. Second, as data were exclusively collected from asymptomatic Japanese subjects, it is uncertain whether our findings can be generalized to other ethnic groups or patients with CVD or diabetes. Third, although adiponectin is not the only cytokine that is being secreted by the fat tissue, we could not evaluate the relationship between CAVI and other adiposity-related factors such as leptin, monocyte chemoattractant protein 1, and biochemical markers of endothelial dysfunction, which may not allow us to exclude the potential effects of these influences on arterial stiffness. Fourth, in contrast to CAVI, neither the VAT area nor HMW adiponectin shows a significant relationship with the ABI in the present study (data not shown). This is probably due to only a small proportion (0.5%) of subjects with a low ABI (<0.9). Further studies in the general population are warranted to confirm an association among the 3 variables. Finally, we could not validate the use of VAT and adiponectin as screening tool for other indexes of CVD, such as IMT, flow-mediated vasodilation, or clinical atherosclerosis. However, it was recently reported that CAVI was more associated with the severity of atherosclerosis determined by coronary angiography than IMT and plaque score [ ]. Thus, CAVI may provide a more sensitive predictor of CVD risk.

5. Conclusions

Cardio-ankle vascular index as a marker of arterial stiffness is significantly associated with both amounts of VAT measured by CT and serum HMW adiponectin levels in asymptomatic Japanese subjects. On ROC analysis, the VAT area demonstrated superior discrimination for the extent of CAVI compared with total and HMW adiponectin levels in both sexes.

References

[1] Fox CS, Massaro JM, Hoffmann U, et al. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. Circulation 2007;116: 39-48.
[2] Ohashi N, Yamamoto H, Horiguchi J, et al. Visceral fat accumulation as a predictor of coronary artery calcium as assessed by multislice computed tomography in Japanese patients. Atherosclerosis 2009;202: 192-9.
[3] Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. Nature 2006;444:875-80.
[4] Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. Endocr Rev 2005;26:439-51.
[5] Lara-Castro C, Luo N, Wallace P, Klein RL, Garvey WT. Adiponectin multimeric complexes and the metabolic syndrome trait cluster. Diabetes 2006;55:249-59.
[6] Kobayashi H, Ouchi N, Kihara S, et al. Selective suppression of endothelial cell apoptosis by the high molecular weight form of adiponectin. Circ Res 2004;94:0e27-e31.
[7] Hara K, Horikoshi M, Yamauchi T, et al. Measurement of the high–molecular weight form of adiponectin in plasma is useful for the prediction of insulin resistance and metabolic syndrome. Diabetes Care 2006;29:1357-62.
[8] von Eynatten M, Humpert PM, Bluemm A, et al. High-molecular weight adiponectin is independently associated with the extent of coronary artery disease in men. Atherosclerosis 2008;199:123-8.
[9] Shokawa T, Imazu M, Yamamoto H, et al. Pulse wave velocity predicts cardiovascular mortality: findings from the Hawaii-Los Angeles-Hiroshima study. Circ J 2005;69:259-64.
[10] Shirai K, Utino J, Otsuka K, Takata M. A novel blood pressure–independent arterial wall stiffness parameter; cardio-ankle vascular index (CAVI). J Atheroscler Thromb 2006;13:101-7.
[11] Kadota K, Takamura N, Aoyagi K, et al. Availability of cardio-ankle vascular index (CAVI) as a screening tool for atherosclerosis. Circ J 2008;72:304-8.
[12] Izuhara M, Shioji K, Kadota S, et al. Relationship of cardio-ankle vascular index (CAVI) to carotid and coronary arteriosclerosis. Circ J 2008;72:1762-7.
[13] Anan F, Masaki T, Umeno Y, et al. Correlations of visceral fat accumulation and atherosclerosis in Japanese patients with type 2 diabetes mellitus. Metabolism 2008;57:280-4.
[14] Tsioufis C, Dimitriadis K, Selima M, et al. Low-grade inflammation and hypoadiponectinaemia have an additive detrimental effect on aortic stiffness in essential hypertensive patients. Eur Heart J 2007;28:1162-9.
[15] Ebinuma H, Miyazaki O, Yago H, et al. A novel ELISA system for selective measurement of human adiponectin multimers by using proteases. Clin Chim Acta 2006;372:47-53.
[16] Matsuzawa Y. Metabolic syndrome—definition and diagnostic criteria in Japan. Journal of Atheroscler Thromb 2005;12:301.
[17] Sipilä K, Koivistoinen T, Moilanen L, et al. Metabolic syndrome and arterial stiffness: the Health 2000 Survey. Metabolism 2007;56:320-6.
[18] Sutton-Tyrrell K, Newman A, Simonsick EM, et al. Aortic stiffness is associated with visceral adiposity in older adults enrolled in the study of health, aging, and body composition. Hypertension 2001;38:429-33.
[19] Lee JW, Lee HR, Shim JY, et al. Viscerally obese women with normal body weight have greater brachial-ankle pulse wave velocity than nonviscerally obese women with excessive body weight. Clin Endocrinol (Oxf) 2007;66:572-8.
[20] Araki T, Emoto M, Yokoyama H, et al. The association of plasma adiponectin level with carotid arterial stiffness. Metabolism 2006;55: 587-92.
[21] Arner P. Insulin resistance in type 2 diabetes: role of fatty acids. Diabetes Metab Res Rev 2002;18:S5-S9.
[22] Yamauchi T, Kamon J, Minokoshi Y, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002;8:1288-95.
[23] Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. J Clin Invest 1996;97:2601-10.
[24] Sengstock DM, Vaitkevicius PV, Supiano MA. Arterial stiffness is related to insulin resistance in nondiabetic hypertensive older adults. J Clin Endocrinol Metab 2005;90:2823-7.
[25] Okamoto Y, Arita Y, Nishida M, et al. An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. Horm Metab Res 2000;32:47-50.
[26] Kato A, Odamaki M, Ishida J, Hishida A. Association of high–molecular-weight to total adiponectin ratio with pulse wave velocity in hemodialysis patients. Nephron Clin Pract 2008;109: 0c18-c24.
[27] Ibata J, Sasaki H, Kakimoto T, et al. Cardio-ankle vascular index measures arterial wall stiffness independent of blood pressure. Diabetes Res Clin Pract 2008;80:265-70.

[28] Takahashi K, Miura S, Mori-Abe A, et al. Impact of menopause on the augmentation of arterial stiffness with aging. Gynecol Obstet Invest 2005;60:162-6.

[29] Araki T, Emoto M, Teramura M, et al. Effect of adiponectin on carotid arterial stiffness in type 2 diabetic patients treated with pioglitazone and metformin. Metabolism 2006;55:996-1001.

[30] Yokoyama H, Emoto M, Fujiwara S, et al. Short-term aerobic exercise improves arterial stiffness in type 2 diabetes. Diabetes Res Clin Pract 2004;65:85-93.

[31] Nakamura K, Tomaru T, Yamamura S, Miyashita Y, Shirai K, Noike H. Cardio-ankle vascular index is a candidate predictor of coronary atherosclerosis. Circ J 2008;72:598-604.

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The impact of visceral adipose tissue and high–molecular weight adiponectin on cardio-ankle vascular index in asymptomatic Japanese subjects

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Abstract

Few studies addressed the relation of visceral adiposity and high–molecular weight (HMW) adiponectin to arterial stiffness. We investigated the impact of visceral adipose tissue (VAT) and HMW adiponectin on cardio-ankle vascular index (CAVI) in asymptomatic Japanese subjects. We studied 487 consecutive subjects (271 men and 216 women) who underwent general health examination between October 2005 and May 2008. The abdominal, visceral, and subcutaneous adipose tissue areas were determined by low-dose x-ray computed tomography. Serum levels of total and HMW adiponectin were measured using the enzyme-linked immunosorbent assay system based on a monoclonal antibody to humans. Cardio-ankle vascular index was positively correlated with VAT area and negatively correlated with HMW adiponectin levels. We also found the positive association of the number of metabolic syndrome components with CAVI in both sexes. A stepwise multiple regression analysis revealed that age, VAT area, serum HMW adiponectin levels, and homeostasis model assessment of insulin resistance were independent determinants of CAVI. Receiver operating characteristic analyses demonstrated that the predictive value of the VAT area for the extent of CAVI (mild: <25th percentile vs severe: >75th percentile) exceeded that of total or HMW adiponectin levels in both sexes. In conclusion, increased CAVI is associated with both amounts of VAT measured by computed tomography and serum HMW adiponectin levels in asymptomatic Japanese subjects. Receiver operating characteristic analysis indicates that the VAT area is a lot better predictor of arterial stiffness than adiponectin levels.

1. Introduction

Visceral adipose tissue (VAT) is now generally considered to play an important role in the metabolic syndrome (MetS) and the development of atherosclerosis [1,2]. The adipose tissue is a remarkable endocrine organ that is a source of several adipokines [3]. Although most adipokines appear to promote cardiovascular disease (CVD), adiponectin is thought to possess antiatherogenic and anti-inflammatory effects and may be protective against CVD development [4]. Recent studies have demonstrated that high–molecular weight (HMW) adiponectin is the major active form of this protein associated with insulin sensitivity and protective activities on the vasculature [5,6]. Clinical studies have also reported that HMW adiponectin is more associated with MetS and coronary heart disease than total adiponectin [7,8], suggesting that HMW adiponectin rather than total adiponectin may exert antiatherosclerotic properties that would prevent the development of atherosclerosis.

It has been demonstrated that aortic pulse wave velocity (PWV), which reflects arterial stiffness, is a marker of CVD risk [9]. Recently, the cardio-ankle vascular index (CAVI) has been developed for the quantitative evaluation of vascular wall stiffness in the aorta, femoral arteries, and tibial artery by measuring PWV and blood pressure (BP) [10]. Indeed, CAVI has been reported to be correlated with

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other CVD risk markers, such as intima-media thickness (IMT) and coronary atherosclerosis, thus reflecting the degree of atherosclerotic change in general populations and patients with high CVD risk [11,12].

Previous studies have reported that visceral adiposity is associated with PWV in patients with type 2 diabetes mellitus [13] or that total adiponectin is inversely related to PWV in patients with essential hypertension [14]. However, there are little data assessing the relationship of visceral adiposity, total adiponectin levels, and HMW adiponectin levels to arterial stiffness in apparently healthy subjects without any medication. Furthermore, it is not known which of these adiposity-related measurements is more closely related to arterial stiffness. The purpose of this study was to evaluate the impact of VAT measured by computed tomography (CT) and total and HMW adiponectin levels on CAVI in asymptomatic Japanese subjects.

2. Methods

2.1. Subjects

The study population consisted of 487 Japanese individuals (271 men, 216 women) who underwent general health examination between October 2005 and May 2008 at Grand Tower Medical Court Life Care Clinic. Subjects who were diagnosed as having CVD and received any medication were excluded. We measured abdominal VAT areas by low-dose x-ray CT, the serum total and HMW adiponectin levels, and the CAVI in all subjects. This study was approved by the Medical Ethics Committee of the Grand Tower Medical Court Life Care Clinic. All subjects provided written informed consent before their inclusions in the study.

2.2. Anthropometric measurement and laboratory methods

After an overnight fast, blood samples were obtained; and BP was measured in the sitting position with the right arm. Height (in meters) and body weight (in kilograms) were used to calculate the body mass index (BMI). The waist circumference (WC) was measured at a level of umbilicus in the late exhalation phase while standing. Measurements of the abdominal VAT and subcutaneous adipose tissue (SAT) areas were undertaken using low-dose x-ray CT with a Hitachi Robusto (Hitachi Medical, Tokyo, Japan). Serum lipid profile was determined by an enzymatic method (total cholesterol and triglycerides) or a direct method (low-density lipoprotein [LDL] cholesterol and high-density lipoprotein [HDL] cholesterol) with a Hitachi 7080 analyzer. The fasting plasma glucose (FPG) levels were measured using the hexokinase method. Serum insulin levels were measured using a chemiluminescent enzyme immunoassay. Insulin resistance was evaluated by the homeostasis model assessment (HOMA-IR), calculated as fasting insulin (in microunits per milliliter) × FPG (in millimoles per liter)/22.5. Hemoglobin A_{1c} (HbA_{1c}) was determined by turbidimetric immunoassay. Serum concentrations of high-sensitivity C-reactive protein (hsCRP) were determined by latex turbidimetric immunoassay. Serum levels of total and HMW adiponectin were measured using the enzyme-linked immunosorbent assay system (Sekisui Medical, Tokyo, Japan) based on a monoclonal antibody to humans [15]. For total adiponectin, the intraassay coefficient of variation (CV) was 4.5% and the interassay CV was 3.0%. For HMW adiponectin, the intraassay CV was 7.7% and the interassay CV was 9.1%. *Metabolic syndrome* was defined by the Japanese criteria as a WC level of at least 85 cm in men and at least 90 cm in women and 2 or more of the following 3 risk factors: hypertension (a BP level ≥130/85 mm Hg), dyslipidemia (an HDL cholesterol level ≤40 mg/dL or a triglycerides level ≥150 mg/dL), and glucose intolerance (an FPG level ≥110 mg/dL) [16]. Metabolic syndrome score (MS) means the number of components of the MetS.

2.3. Measurement of CAVI

Cardio-ankle vascular index was recorded using a VaseraVS-1000 vascular screening system (Fukuda Denshi, Tokyo, Japan) by the methods as previously described [10]. Briefly, cuffs were applied to bilateral upper arms and ankles, electrocardiogram leads were attached to both wrists, and a phonocardiogram was placed at the right sternum border in the second intercostal space. The subjects were placed in the supine position for at least 10 minutes, and then measurements were performed automatically. Cardio-ankle vascular index was calculated by the following formula: $CAVI = a[(2\rho/\Delta P) \times \ln (Ps/Pd)PWV^2] + b$, where Ps is systolic BP, Pd is diastolic BP, ΔP is Ps − Pd, ρ is blood density, and a and b are constants to match aortic PWV. The equation was derived from Bramwell-Hill's equation and the stiffness parameter β. The obtained data were analyzed using VSS-10 software (Fukuda Denshi), and the values of right and left CAVI were calculated. Averages of the right and left CAVI were used in the analysis. The average CV of CAVI has been reported to be 3.8% [10]. The ankle brachial index (ABI) was also calculated as the highest ankle systolic pressure divided by the highest brachial systolic pressure on both sides, and the lower ABI from right and left measurements was used in subsequent statistical analysis.

2.4. Statistical analysis

Categorical variables were presented as number of patients (percentage), and continuous variables were expressed as means ± SD or medians (interquartile range). Differences between men and women in the clinical variables were evaluated using Student t test or the Mann-Whitney U test. The mean CAVI values were stratified by the number of components of MetS using analysis of variance. The correlation coefficient was estimated by

Pearson correlation. As triglycerides, fasting insulin, HOMA-IR, total and HMW adiponectin levels, and hsCRP were not normally distributed, logarithmic transformation was performed for the analysis. We performed stepwise multiple regression analysis to evaluate the independent determinants of CAVI in all subjects using age, sex, BMI, WC, systolic and diastolic BP, triglycerides, HDL cholesterol, LDL cholesterol, FPG, fasting insulin, HOMA-IR, HbA_{1c}, prevalence of MetS, VAT area, SAT area, total adiponectin levels, HMW adiponectin levels, and hsCRP as covariates. A receiver operating characteristics (ROC) analysis was used to compare the predictive power of the VAT area, total adiponectin levels, and HMW adiponectin levels in the prediction of the extent of CAVI. Prediction of CAVI extent was based on discrimination between subjects with mild (CAVI <25th percentile) and severe (CAVI >75th percentile) values of CAVI. Statistical comparisons of the area under ROC curves were performed using a computer program (ROCKIT; Charles E Metz, University of Chicago). Statistical analyses were performed using the JMP 5.0.1 statistical software (SAS Institute, Cary, NC). A P value less than .05 was considered statistically significant.

Table 2
Correlation between CAVI and clinical variables in men and women

Clinical variables	Men (n = 271)		Women (n = 216)	
	r	P	r	P
Age (y)	0.474	<.0001	0.568	<.0001
BMI (kg/m^2)	0.048	.42	−0.08	.23
WC (cm)	0.046	.44	0.071	.29
SBP (mm Hg)	0.183	.0025	0.185	.0064
DBP (mm Hg)	0.121	.046	0.184	.0067
Total cholesterol (mg/dL)	−0.038	.53	0.164	.015
Triglycerides (mg/dL)[a]	0.0756	.21	0.25	.0002
HDL cholesterol (mg/dL)	−0.034	.57	−0.115	.091
LDL cholesterol (mg/dL)	−0.049	.41	0.186	.0061
FPG (mg/dL)	0.232	.0001	0.236	.0005
Fasting insulin (μU/mL)[a]	0.211	.0007	0.238	.0005
HOMA-IR[a]	0.260	<.0001	0.284	<.0001
HbA_{1c} (%)	0.232	.0001	0.282	<.0001
VAT area (cm^2)	0.366	<.0001	0.383	<.0001
SAT area (cm^2)	0.15	.013	0.154	.023
Total adiponectin (μg/mL)[a]	−0.113	.061	−0.09	.18
HMW adiponectin (μg/mL)[a]	−0.254	.0001	−0.214	.0021
hsCRP (mg/L)[a]	0.173	.0041	0.112	.1
ABI	0.122	.06	0.091	.2

Abbreviations are the same as in Table 1.
[a] Log-transformed values.

3. Results

3.1. Patient characteristics

The study subjects consisted of 271 men (mean age, 46 years; range, 23-73 years) and 216 women (mean age, 47 years; range, 25-70 years). The clinical and biochemical characteristics of the study subjects are shown in Table 1. Body mass index, WC, systolic and diastolic BP, triglycerides, LDL cholesterol, FPG, fasting insulin, HOMA-IR, HbA_{1c}, prevalence of MetS, hsCRP, and CAVI were significantly higher in men than in women. The VAT area was also significantly larger in men than in women. High-density lipoprotein cholesterol and the serum total and HMW adiponectin levels were significantly lower in men than in women.

Table 1
Clinical and biochemical characteristics of the study subjects

	Men (n = 271)	Women (n = 216)	P
Age (y)	46.3 ± 10.5	46.6 ± 10.8	NS
BMI (kg/m^2)	24.6 ± 3.9	21.0 ± 3.1	<.01
WC (cm)	87.0 ± 10.6	76.7 ± 8.9	<.01
SBP (mm Hg)	125.9 ± 15.5	116.3 ± 14.1	<.01
DBP (mm Hg)	79.0 ± 10.5	72.0 ± 9.2	<.01
Total cholesterol (mg/dL)	208.4 ± 34.2	207.7 ± 38.7	NS
Triglycerides (mg/dL)	120.0 (80.0-189.0)	66.0 (48.0-93.0)	<.01
HDL cholesterol (mg/dL)	55.6 ± 13.9	70.2 ± 16.7	<.01
LDL cholesterol (mg/dL)	124.7 ± 32.4	118.2 ± 32.8	<.05
FPG (mg/dL)	109.3 ± 23.7	94.6 ± 17.3	<.01
Fasting insulin (μU/mL)	6.8 (4.6-10.8)	4.7 (3.5-6.8)	<.01
HOMA-IR	1.75 (1.11-2.90)	1.07 (0.79-1.57)	<.01
HbA_{1c} (%)	5.38 ± 0.81	5.12 ± 0.60	<.01
MetS (n, %)	81 (29.9)	15 (6.9)	<.01
VAT area (cm^2)	99.3 ± 54.0	38.7 ± 31.2	<.01
SAT area (cm^2)	139.6 ± 73.8	143.6 ± 69.6	NS
Total adiponectin (μg/mL)	4.2 (3.1-5.4)	7.6 (5.8-10.0)	<.01
HMW adiponectin (μg/mL)	1.5 (0.8-2.2)	3.8 (2.4-5.3)	<.01
hsCRP (mg/L)	0.68 (0.37-1.36)	0.33 (0.19-0.68)	<.01
CAVI	7.49 ± 1.03	7.21 ± 0.95	<.01
ABI	1.15 ± 0.1	1.10 ± 0.1	NS

Data are expressed as number of subjects (percentage), means ± SD, or medians (interquartile range). SBP indicates systolic blood pressure; DBP, diastolic blood pressure; NS, not significant.

3.2. Correlation between CAVI and clinical variables

Table 2 shows the result of simple linear regression analysis between CAVI and clinical variables. Cardio-ankle vascular index was positively correlated with age, systolic and diastolic BP, FPG levels, fasting insulin, HOMA-IR, HbA_{1c} levels, VAT area, and SAT area and negatively correlated with HMW adiponectin levels in both sexes. Cardio-ankle vascular index was positively correlated with hsCRP in men and with total cholesterol, triglycerides, and LDL cholesterol in women. Fig. 1 shows the comparison of CAVI values according to the MS in men and women. In both sexes, CAVI increased linearly as the MS increased; average CAVI values for those with MS of 0, 1, 2, or at least 3 were 7.13, 7.27, 7.53, and 7.92 in men (P for trend < .0001) and 7.05, 7.32, 7.62, and 8.18 in women (P for trend < .0001), respectively. In addition, subjects with MetS had a significantly higher CAVI than those without in men ([mean ± SD] 7.91 ± 1.0 vs 7.31 ± 0.98, P < .001) and women (8.18 ± 1.54 vs 7.15 ± 0.82, P < .001). Table 3 describes the result of stepwise multiple regression analysis in all subjects. Age, VAT area, HMW adiponectin levels,

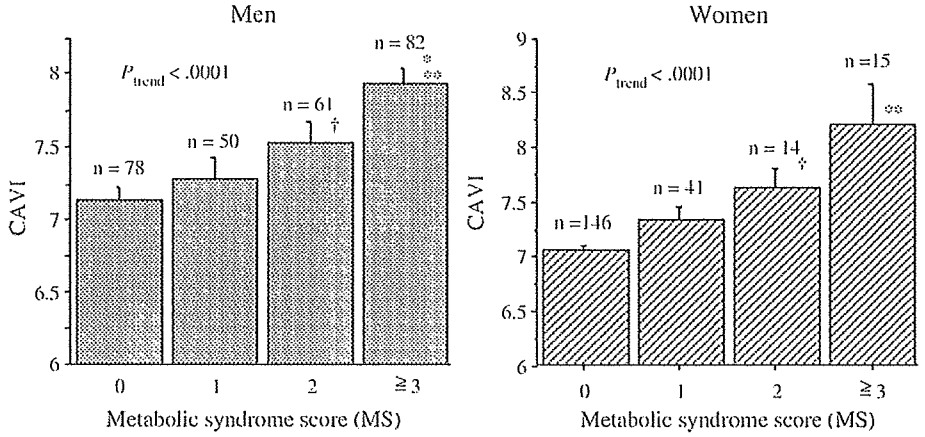

Fig. 1. Comparison of CAVI values according to the MS in men and women. MS means the number of components of the MetS including WC, BP, triglycerides or HDL cholesterol, and FPG. †P less than .05 vs MS = 0 group, *P less than .05 vs MS = 2 group, **P less than .01 vs MS = 0 or MS = 1 group in men, †P less than .05 vs MS = 0 group, **P less than .01 vs MS = 0 or MS = 1 group in women.

and HOMA-IR were found to be independent determinants of CAVI (adjusted R^2 = 0.38, P < .0001).

3.3. Predictive values of the VAT area, total adiponectin levels, and HMW adiponectin levels for the extent of CAVI

Receiver operating characteristic analyses were performed to quantify the power of the VAT area, total adiponectin levels, and HMW adiponectin levels for the prediction of CAVI extent (Fig. 2). Analyses were performed in subjects representing the upper (n = 66, CAVI >8.05 in men; n = 52, CAVI >7.70 in women) and lower quartile (n = 67, CAVI <6.80 in men; n = 50, CAVI <6.60 in women) of CAVI. The area under the curve for the VAT area was significantly larger than that for HMW adiponectin levels in men (VAT area: 0.763 [95% confidence interval {CI}, 0.676-0.836] vs HMW adiponectin levels: 0.653 [95% CI, 0.556-0.741], P = .05) and women (VAT area: 0.762 [95% CI, 0.670-0.837] vs HMW adiponectin levels: 0.627 [95% CI, 0.524-0.720], P = .02). The area under the curve for HMW adiponectin levels was also significantly larger than that for total adiponectin levels in men (HMW adiponectin levels: 0.653 [95% CI, 0.556-0.741] vs total adiponectin levels: 0.583 [95% CI, 0.485-0.677], P = .03) and women (HMW adiponectin levels: 0.627 [95%, CI 0.524-0.720] vs total adiponectin levels: 0.538 [95% CI, 0.436-0.638], P = .01).

Table 3
Stepwise multiple regression analysis between CAVI and clinical variables

Independent variables	Standardized β	Standard error	t	P
Age	1.47	0.12	12.4	<.0001
VAT area	0.46	0.18	2.62	.0092
HMW adiponectin[a]	−0.54	0.26	−2.10	.036
HOMA-IR[a]	1.52	0.76	2.00	.046
R^2	0.40			
Adjusted R^2	0.38			

Abbreviations are the same as in Table 1.
[a] Log-transformed values.

4. Discussion

In the present study, we first evaluated the association of the VAT area, total adiponectin levels, and HMW adiponectin levels with CAVI as a marker of arterial stiffness in a group of subjects without previous manifestation of CVD. We found a positive association of MetS components and VAT area with CAVI. We also found an inverse relation between HMW adiponectin levels and CAVI. A stepwise multiple regression analysis revealed that age, VAT area, HMW adiponectin levels, and HOMA-IR were independent predictors for increase of CAVI. On ROC analysis, VAT area demonstrated superior discrimination for the extent of CAVI compared with total and HMW adiponectin levels in both sexes.

We showed that CAVI is higher in subjects with MetS than in those without and increased linearly as the components of MetS increased, which is consistent with prior result that MetS is independently associated with arterial stiffness [17]. Previous studies also reported that the VAT area is significantly associated with arterial stiffness in older adults [18], middle-aged women [19], and patients with type 2 diabetes mellitus [13]. With respect to total adiponectin and arterial stiffness, there is a significant relationship in hypertensive patients [14] and nondiabetic subjects [20]. However, there has been no information regarding the effect of VAT and HMW adiponectin levels on arterial stiffness in asymptomatic subjects. We found that both the VAT area and HMW adiponectin levels remained as independent determinants of CAVI in multivariable analysis even after adjustment for MetS or other indices of adiposity measurement such as BMI, SAT, and WC. These results indicate that the measurement of the VAT area and HMW

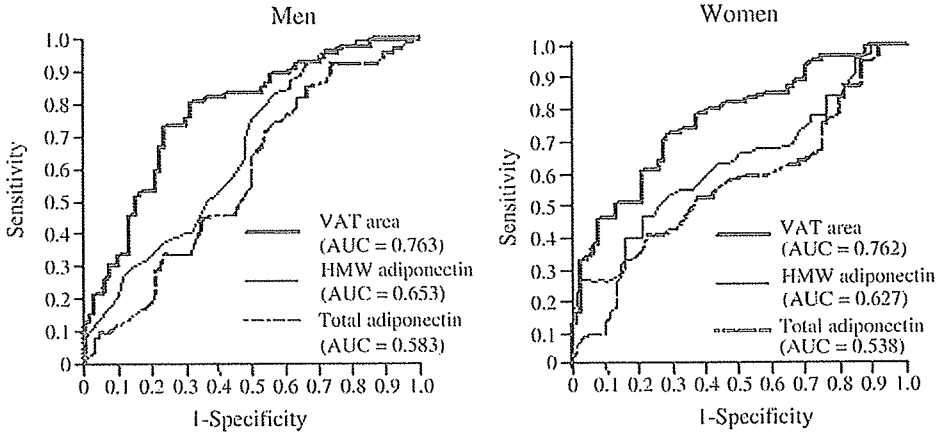

Fig. 2. Receiver operating characteristic curves of the VAT area, total adiponectin levels, and HMW adiponectin levels and predictive values for the extent of CAVI in men and women.

adiponectin levels in conjugation with CAVI may help identify subjects needing more aggressive risk modification in clinical practice.

The mechanisms of linking VAT and adiponectin to arterial stiffness are not entirely understood. In the present study, we demonstrated that HOMA-IR as a marker of insulin resistance was an independent predictor of CAVI, which implies one of the plausible mechanisms of increased arterial stiffness. Arner [21] has suggested that the flux of lipid from the visceral fat to the liver via portal circulation might account for hepatic insulin resistance. Adiponectin was also reported to modulate insulin sensitivity by stimulating glucose utilization and fatty acid oxidation via the phosphorylation and activation of adenosine monophosphate–activated protein kinase in both muscle and liver cells [22]. Clinical study also suggests that HMW adiponectin is a more useful marker to evaluate insulin resistance and MetS than total adiponectin [7]. Insulin resistance is reported to be associated with decreased endothelium-dependent vasodilation [23] and increased arterial stiffness [24]. These results indicated that decreased HMW adiponectin levels concomitant with the accumulation of visceral fat could potentially be involved with the acceleration of the increased arterial stiffness via insulin resistance.

From ROC analysis, we can clearly see that the VAT area was a lot better predictor of arterial stiffness than adiponectin levels. The accumulation of VAT is considered to be causally linked to atherosclerosis as a result of the dysregulation of various kinds of adipokines production and chronic intravascular inflammation [3]. Our findings suggested that other factors than adiponectin, such as inflammatory factors, other proteins secreted by adipose tissue, or insulin or glucose levels may also be important in the pathophysiologic mechanisms by which abdominal adiposity leads to arterial stiffness. In addition, HMW adiponectin was more associated with arterial stiffness than total adiponectin. One possible explanation of this result may be the higher affinity of HMW adiponectin to collagen in the vascular wall compared with low–molecular weight or middle–molecular weight forms, thus possibly exerting better repair on injured vessels [25]. In addition, a recent study revealed that only HMW adiponectin selectively suppressed endothelial cell apoptosis, whereas neither the low–molecular weight nor the middle–molecular weight form had this effect [6]. Clinical data also confirmed that HMW to total adiponectin ratio was an independent determinant for PWV and CAVI in hemodialysis patients [26].

In the present study that represents arterial stiffness by CAVI, neither BP nor sex remained as independent predictors of CAVI in stepwise multiple regression analysis. Whereas BP is a major determinant of PWV, CAVI is designed to be adjusted for BP based on the stiffness parameter β and is hypothesized to measure arterial stiffness independent of BP. It has been reported that CAVI has a lower correlation with BP than PWV [10] or that the significant risk factors of high CAVI were age and HbA_{1c}, whereas systolic BP was not relevant [27], which is consistent with our results in that BP did not contribute to CAVI. In addition, there was no significant sex interaction in our analyses. For instance, menopause is reported to augment the age-related increase in arterial stiffness [28]. However, our study subjects are relatively young and the proportion of postmenopausal women is low, which may be one of the reasons that CAVI was not affected by sex in a multivariable model.

This study had some limitations. First, because this is a cross-sectional study, causality cannot be established. However, it bas been already reported that arterial stiffness defined by stiffness parameter β is partly improved by interventions to insulin resistance such as the insulin sensitizer pioglitazone and aerobic exercise in type 2 diabetes mellitus [29,30]. Our findings in the present study are almost in keeping with these previous observations,

suggesting that intervention therapy such as diet and exercise may reduce total and abdominal fat and that these changes in body composition mediate improvements in insulin sensitivity and may improve endothelial vasodilator function. Prospective randomized controlled trials are needed to confirm this issue. Second, as data were exclusively collected from asymptomatic Japanese subjects, it is uncertain whether our findings can be generalized to other ethnic groups or patients with CVD or diabetes. Third, although adiponectin is not the only cytokine that is being secreted by the fat tissue, we could not evaluate the relationship between CAVI and other adiposity-related factors such as leptin, monocyte chemoattractant protein 1, and biochemical markers of endothelial dysfunction, which may not allow us to exclude the potential effects of these influences on arterial stiffness. Fourth, in contrast to CAVI, neither the VAT area nor HMW adiponectin shows a significant relationship with the ABI in the present study (data not shown). This is probably due to only a small proportion (0.5%) of subjects with a low ABI (<0.9). Further studies in the general population are warranted to confirm an association among the 3 variables. Finally, we could not validate the use of VAT and adiponectin as screening tool for other indexes of CVD, such as IMT, flow-mediated vasodilation, or clinical atherosclerosis. However, it was recently reported that CAVI was more associated with the severity of atherosclerosis determined by coronary angiography than IMT and plaque score [31]. Thus, CAVI may provide a more sensitive predictor of CVD risk.

5. Conclusions

Cardio-ankle vascular index as a marker of arterial stiffness is significantly associated with both amounts of VAT measured by CT and serum HMW adiponectin levels in asymptomatic Japanese subjects. On ROC analysis, the VAT area demonstrated superior discrimination for the extent of CAVI compared with total and HMW adiponectin levels in both sexes.

References

[1] Fox CS, Massaro JM, Hoffmann U, et al. Abdominal visceral and subcutaneous adipose tissue compartments: association with metabolic risk factors in the Framingham Heart Study. Circulation 2007;116: 39-48.

[2] Ohashi N, Yamamoto H, Horiguchi J, et al. Visceral fat accumulation as a predictor of coronary artery calcium as assessed by multislice computed tomography in Japanese patients. Atherosclerosis 2009;202: 192-9.

[3] Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. Nature 2006;444:875-80.

[4] Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. Endocr Rev 2005;26:439-51.

[5] Lara-Castro C, Luo N, Wallace P, Klein RL, Garvey WT. Adiponectin multimeric complexes and the metabolic syndrome trait cluster. Diabetes 2006;55:249-59.

[6] Kobayashi H, Ouchi N, Kihara S, et al. Selective suppression of endothelial cell apoptosis by the high molecular weight form of adiponectin. Circ Res 2004;94:0c27-c31.

[7] Hara K, Horikoshi M, Yamauchi T, et al. Measurement of the high-molecular weight form of adiponectin in plasma is useful for the prediction of insulin resistance and metabolic syndrome. Diabetes Care 2006;29:1357-62.

[8] von Eynatten M, Humpert PM, Bluemm A, et al. High-molecular weight adiponectin is independently associated with the extent of coronary artery disease in men. Atherosclerosis 2008;199:123-8.

[9] Shokawa T, Imazu M, Yamamoto H, et al. Pulse wave velocity predicts cardiovascular mortality: findings from the Hawaii-Los Angeles-Hiroshima study. Circ J 2005;69:259-64.

[10] Shirai K, Utino J, Otsuka K, Takata M. A novel blood pressure–independent arterial wall stiffness parameter; cardio-ankle vascular index (CAVI). J Atheroscler Thromb 2006;13:101-7.

[11] Kadota K, Takamura N, Aoyagi K, et al. Availability of cardio-ankle vascular index (CAVI) as a screening tool for atherosclerosis. Circ J 2008;72:304-8.

[12] Izuhara M, Shioji K, Kadota S, et al. Relationship of cardio-ankle vascular index (CAVI) to carotid and coronary arteriosclerosis. Circ J 2008;72:1762-7.

[13] Anan F, Masaki T, Umeno Y, et al. Correlations of visceral fat accumulation and atherosclerosis in Japanese patients with type 2 diabetes mellitus. Metabolism 2008;57:280-4.

[14] Tsioufis C, Dimitriadis K, Selima M, et al. Low-grade inflammation and hypoadiponectinaemia have an additive detrimental effect on aortic stiffness in essential hypertensive patients. Eur Heart J 2007;28:1162-9.

[15] Ebinuma H, Miyazaki O, Yago H, et al. A novel ELISA system for selective measurement of human adiponectin multimers by using proteases. Clin Chim Acta 2006;372:47-53.

[16] Matsuzawa Y. Metabolic syndrome—definition and diagnostic criteria in Japan. Journal of Atheroscler Thromb 2005;12:301.

[17] Sipilä K, Koivistoinen T, Moilanen L, et al. Metabolic syndrome and arterial stiffness: the Health 2000 Survey. Metabolism 2007;56:320-6.

[18] Sutton-Tyrrell K, Newman A, Simonsick EM, et al. Aortic stiffness is associated with visceral adiposity in older adults enrolled in the study of health, aging, and body composition. Hypertension 2001;38:429-33.

[19] Lee JW, Lee HR, Shim JY, et al. Viscerally obese women with normal body weight have greater brachial-ankle pulse wave velocity than nonviscerally obese women with excessive body weight. Clin Endocrinol (Oxf) 2007;66:572-8.

[20] Araki T, Emoto M, Yokoyama H, et al. The association of plasma adiponectin level with carotid arterial stiffness. Metabolism 2006;55: 587-92.

[21] Arner P. Insulin resistance in type 2 diabetes: role of fatty acids. Diabetes Metab Res Rev 2002;18:S5-S9.

[22] Yamauchi T, Kamon J, Minokoshi Y, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002;8:1288-95.

[23] Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. J Clin Invest 1996;97:2601-10.

[24] Sengstock DM, Vaitkevicius PV, Supiano MA. Arterial stiffness is related to insulin resistance in nondiabetic hypertensive older adults. J Clin Endocrinol Metab 2005;90:2823-7.

[25] Okamoto Y, Arita Y, Nishida M, et al. An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. Horm Metab Res 2000;32:47-50.

[26] Kato A, Odamaki M, Ishida J, Hishida A. Association of high–molecular-weight to total adiponectin ratio with pulse wave velocity in hemodialysis patients. Nephron Clin Pract 2008;109: 0c18-c24.

[27] Ibata J, Sasaki H, Kakimoto T, et al. Cardio-ankle vascular index measures arterial wall stiffness independent of blood pressure. Diabetes Res Clin Pract 2008;80:265-70.

[28] Takahashi K, Miura S, Mori-Abe A, et al. Impact of menopause on the augmentation of arterial stiffness with aging. Gynecol Obstet Invest 2005;60:162-6.

[29] Araki T, Emoto M, Teramura M, et al. Effect of adiponectin on carotid arterial stiffness in type 2 diabetic patients treated with pioglitazone and metformin. Metabolism 2006;55:996-1001.

[30] Yokoyama H, Emoto M, Fujiwara S, et al. Short-term aerobic exercise improves arterial stiffness in type 2 diabetes. Diabetes Res Clin Pract 2004;65:85-93.

[31] Nakamura K, Tomaru T, Yamamura S, Miyashita Y, Shirai K, Noike H. Cardio-ankle vascular index is a candidate predictor of coronary atherosclerosis. Circ J 2008;72:598-604.

Value of Estimated Right Ventricular Filling Pressure in Predicting Cardiac Events in Chronic Pulmonary Arterial Hypertension

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Background: Right ventricular (RV) filling pressure can be estimated using tissue Doppler imaging (TDI) from the tricuspid lateral annulus, but few data are available on the usefulness of Doppler-derived RV filling pressure in predicting the prognosis of chronic pulmonary arterial hypertension (PAH).

Methods: In 50 consecutive patients with PAH, TDI was performed within 24 hours of right-sided catheterization to measure early diastolic myocardial velocity at the tricuspid lateral annulus (E_a) and early diastolic tricuspid inflow (E). The tricuspid E/E_a ratio was calculated and compared with the invasive hemodynamic variables. Cardiac events were defined as cardiac death or rehospitalization due to RV failure.

Results: Mean right atrial pressure (RAP) averaged 6 ± 5 mm Hg (range, 1-25 mm Hg). E/E_a correlated positively with mean RAP ($r = 0.80$, $P < .001$), irrespective of RV systolic function. We divided patients into group A with cardiac events (n = 19) and group B without events (n = 31) in a mean follow-up period of 14 months. Plasma brain natriuretic peptide level and E/E_a were significantly higher in group A than in group B (349 ± 310 pg/dL vs 129 ± 136 pg/dL, $P = .001$; 7.0 ± 3.2 vs 4.5 ± 1.9, $P = .004$, respectively), whereas mean pulmonary artery pressure did not differ significantly. In a multivariate model, E/E_a remained predictive for cardiac events (hazard ratio 1.227; 95% confidence interval, 1.042-1.444; $P = .014$). An E/E_a cutoff value of 6.8 discriminated cases with cardiac events with a sensitivity of 42% and specificity of 97% (area under the curve 0.71).

Conclusion: The tricuspid E/E_a ratio provides a reliable estimation of RV filling pressure and predicts cardiac events in patients with PAH. (J Am Soc Echocardiogr 2009;22:1368-74.)

Keywords: Cardiac events, Pulmonary hypertension, Right atrial pressure, Tissue Doppler imaging

Pulmonary arterial hypertension (PAH) has an accompanying risk of right ventricular (RV) failure and death.[1] Previous studies of long-term outcome of patients with PAH have demonstrated that invasive hemodynamic variables, such as mean right atrial pressure (RAP), cardiac index, and mixed venous oxygen saturation, were independent predictors of adverse outcome.[2,3] Because the clinical course of PAH is variable and the outcome is heterogenous, noninvasive repeatable clinical markers of prognosis may be helpful to clinicians. Doppler echocardiography enables us to estimate pulmonary artery systolic pressure (PASP) noninvasively by the tricuspid regurgitation (TR) pressure gradient calculated from Doppler-derived TR velocity,[4] but this estimated PASP showed no prognostic value in previous studies.[5-7] With the use of tissue Doppler imaging (TDI), recent reports showed that RV filling pressure could be estimated by the ratio of early diastolic tricuspid inflow (E) to early diastolic myocardial velocity at the tricuspid lateral annulus (E_a) in a wide variety of pathologic conditions.[8,9] The methods in those studies, however, were not sufficiently validated in patients with chronic PAH, and the predictive value of Doppler-derived RV filling pressure remains unknown. Thus, this study aimed to validate the correlation between the tricuspid E/E_a ratio and the mean RAP and to evaluate the value of TDI-derived RV filling pressure in predicting cardiac events in patients with PAH with various causes.

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MATERIALS AND METHODS

Study Population

A total of 50 consecutive patients with proven chronic PAH who were admitted to the National Cardiovascular Center were enrolled in this study. All patients were in sinus rhythm. We performed echocardiography including TDI within 24 hours of right-sided catheterization. Medication status remained unchanged between catheterization and TDI measurements. Subjects constituted

a heterogenous group (Table 1). Patients with congenital heart disease and evidence of an intracardiac shunt (eg, atrial septal defect) were excluded. All patients gave informed consent before participation, and the study protocol was approved by the institutional review board. Unencouraged 6-minute walk distance was measured to assess exercise capacity, and plasma brain natriuretic peptide (BNP) level was measured within 1 week before catheterization.

Echocardiographic Examination

A Vivid 7 ultrasound system equipped with an S4 transducer (GE Healthcare, Milwaukee, WI) was used. Measurements were performed with an experienced sonographer blinded to the patients' catheterization data. Left ventricular (LV) end-diastolic diameter and fractional shortening were determined in parasternal long-axis view. Expiratory diameter of the inferior vena cava (IVC) was measured along with its respiratory variation (ie, IVC collapsibility) in a subcostal view within 2 cm of the right atrium.[10] TR was graded on the basis of the vena contracta width (VCW), which is the narrowest portion of the regurgitant jet just distal to the anatomic valve orifice on the atrial side. The maximum VCW measured in multiple views (basal parasternal short-axis, RV inflow, and apical 4-chamber) was used to diagnose severe TR defined as a VCW $\geq$ 6.5 mm.[11] Maximal TR jet velocity was measured from the apical view, and TR pressure gradient was calculated by applying the simplified Bernoulli equation.[4] Pulmonary regurgitation (PR) was examined from a parasternal short-axis view, PR end-diastolic velocity was measured at termination of reverse flow after the "a wave," and PR end-diastolic pressure gradient was calculated by applying the simplified Bernoulli equation.[12] By using the apical 4-chamber view, RV fractional area change was calculated as the RV area difference (end-diastole − end-systole) normalized to RV area in end diastole.[13] RV end-diastolic area, RV end-systolic area, and right atrial end-systolic area were divided by the height to account for differences in body size. The deformity index, a measure of septal displacement, was derived from the ratio of the 2 short-axis diameters measured at early diastole.[14]

Doppler and Tissue Doppler Analysis

Mitral and tricuspid inflows were recorded from an apical 4-chamber view to measure E, peak late velocity (A), deceleration time of E wave (DT), and diastolic filling time. The sum of RV isovolumetric contraction time and isovolumetric relaxation time was obtained by subtracting RV ejection time from the interval between cessation and onset of the tricuspid inflow velocities with Doppler echocardiography. For the analysis of global RV function, RV myocardial performance index was obtained by dividing the sum of both isovolumetric intervals by ejection time.[15] For TDI analysis, pulsed-wave TDI was applied in a modified apical 4-chamber view at a depth of 12 ± 2 cm using a high frame rate by narrowing the sector angle. A 5-mm sample volume was placed at the lateral corner of the tricuspid annulus to measure systolic (S_a), E_a, and late diastolic annular velocities (A_a) (Figure 1).[9] All Doppler measurements were recorded at a sweep speed of 50 to 100 mm/s and represented an average of 5 to 7 consecutive cardiac cycles that have been shown to be equivalent to those obtained at end-expiratory apnea.[8]

Catheterization Data

Right-sided catheterization was performed using a 7F Swan-Ganz catheter to measure pulmonary capillary wedge pressure, mean pulmonary artery pressure (PAP), RV end-diastolic pressure, and mean RAP. Cardiac output (CO) was calculated using the estimated Fick principle. Pulmonary vascular resistance was calculated using the established formula: pulmonary vascular resistance = 80 × (mean PAP − pulmonary capillary wedge pressure)/CO.

Table 1 Clinical characteristics of study patients (n = 50)

Characteristics	Findings
Age (y)	46 ± 13
Gender female	39 (78%)
HR during catheterization (beats/min)	77 ± 14
HR during echocardiography (beats/min)	75 ± 12
SBP during catheterization (mm Hg)	108 ± 14
SBP during echocardiography (mm Hg)	104 ± 10
NYHA functional class, III or IV	21 (42%)
Plasma BNP level (pg/mL)	212 ± 242
6MWD (m)	381 ± 129
Diagnosis	
Idiopathic PAH	23 (46%)
Chronic thromboembolic pulmonary hypertension	14 (28%)
Connective tissue disorder	11 (22%)
Other	2 (4%)
Medication	
Diuretics	15 (30%)
Epoprostenol infusion	22 (44%)

BNP, Brain natriuretic peptide; *HR*, heart rate; *NYHA*, New York Heart Association; *SBP*, systolic blood pressure; *6MWD*, 6-minute walk distance; *PAH*, pulmonary arterial hypertension.
Values are mean ± SD or number (percentage).

Follow-up

All patients were followed up for a minimum of 1 year. The primary end point was defined as adverse outcomes, including cardiac death or rehospitalization due to RV failure, at long-term follow-up (mean duration: 14 ± 1 months).

Statistical Analysis

Continuous data were expressed as means ± standard deviation. Categoric variables were presented as the number and percentages. Student t test or Mann–Whitney *U* test was used to compare continuous variables with or without normal distribution, respectively. Associations between continuous variables were assessed using Pearson correlations. Bland–Altman analysis was used to assess the agreement between Doppler-derived mean RAP and invasively measured mean RAP. Optimal cutoff value of tricuspid E/E_a ratio for prediction of cardiac events was determined using a receiver operating characteristic analysis, and at that point, sensitivity and specificity were determined. The Kaplan–Meier method was used to estimate survival free of adverse outcomes. Univariate Cox proportional hazards analysis was used to examine the relationship between cardiac events and selected demographic and echocardiographic variables. The results are expressed as hazard ratios with 95% confidence intervals. For continuous demographic variables, we chose to separate patients into 2 groups on both sides of the median value. Multivariate analysis based on the Cox proportional hazards regression model was used to examine the independent effect of each variable on cardiac events, controlling for possible confounding variables. The selection of the independent variables was performed using a stepwise forward selection method. Intraobserver and interobserver variability described by the coefficient of variation were assessed in 20 randomly selected subjects. Intraobserver reproducibility was assessed for a single observer on 2 separate occasions, and interobserver variability was

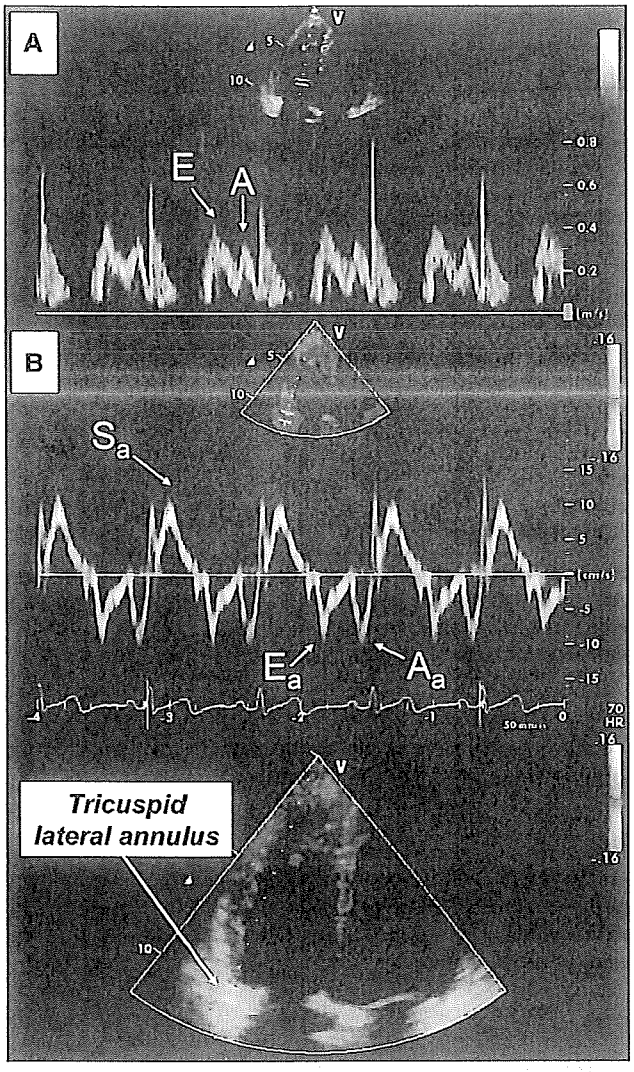

Figure 1 Tricuspid inflow velocities (**A**) and tricuspid annular velocities recorded by TDI at the lateral corner of the tricuspid annulus (**B**) in patients with idiopathic PAH. The tricuspid inflow E is 42 cm/s, and annular E_a is 10.5 cm/s. The tricuspid E/E_a ratio is 4.0, predicting a mean RAP of 4 mm Hg (catheter-derived pressure 3 mm Hg). A_a, Late diastolic velocity; E_a, early diastolic velocity; *RAP*, right atrial pressure; S_a, systolic velocity.

performed by 2 independent experienced investigators who had performed Doppler echocardiography at least for 5 years. A *P* value less than .05 was considered significant. All statistical analyses were performed using SPSS 12.0 (SPSS Inc, Chicago, IL).

RESULTS

Clinical, Hemodynamic, and Echocardiographic Characteristics of the Study Population

Patients' clinical, invasive hemodynamic, and echocardiographic characteristics are presented in Tables 1 and 2. The mean time lag between catheterization and echocardiography was 3 ± 2 hours. Heart rate and systolic blood pressure were similar during cardiac catheterization and echocardiography ($P = .84$ and $P = .81$, respectively). The VCW could not be measured because of mild TR in 15 patients (30%),

Table 2 Invasive hemodynamic and echocardiographic characteristics (n = 50)

Characteristics	Mean ± SD	Range
Catheterization data		
Pulmonary artery systolic pressure (mm Hg)	86 ± 28	45-131
Mean pulmonary artery pressure (mm Hg)	52 ± 17	26-87
Mean right atrial pressure (mm Hg)	6 ± 5	1-25
RV end-diastolic pressure (mm Hg)	10 ± 6	2-25
Pulmonary vascular resistance (dyn/s/cm^{-5})	1195 ± 522	257-2148
Cardiac index (L/min/m^2)	2.2 ± 0.6	1.2-3.1
Pulmonary capillary wedge pressure (mm Hg)	7 ± 3	1-13
Echocardiographic data		
LV end-diastolic diameter (mm)	37 ± 7	21-47
LV fractional shortening (%)	40 ± 8	29-54
IVC expiratory diameter (mm)	20 ± 6	9-32
IVC collapsibility (%)	46 ± 20	4-79
Vena contracta width (mm)	*7.2 ± 3.9	*3.7-22.2
TR jet maximal velocity (m/s)	4.1 ± 0.8	2.8-5.5
TR pressure gradient (mm Hg)	70 ± 25	31-121
PR end-diastolic pressure gradient (mm Hg)	11 ± 9	2-36
Indexed RV end-diastolic area (cm^2/m)	17 ± 5	7-31
Indexed RV end-systolic area (cm^2/m)	12 ± 5	4-23
Indexed RA end-systolic area (cm^2/m)	16 ± 6	7-38
RV fractional area change (%)	30 ± 11	13-53
RV myocardial performance index	0.66 ± 0.20	0.29-1.18
Deformity index	2.3 ± 0.7	1.1-3.8
Tricuspid flow E (cm/s)	49 ± 21	16-134
Tricuspid annular E_a (cm/s)	10.0 ± 3.4	3.7-16.4
Tricuspid E/E_a ratio	5.4 ± 2.7	1.7-13.0
Tricuspid flow A (cm/s)	52 ± 20	23-104
Tricuspid flow DT (ms)	179 ± 58	80-309
Mitral flow E (cm/s)	62 ± 23	27-131
Mitral flow A (cm/s)	61 ± 18	18-104
Mitral flow DT (ms)	211 ± 43	92-315
Diastolic filling time/RR interval (%)	52 ± 6	40-63

DT, Deceleration time; *IVC*, inferior vena cava; *LV*, left ventricular; *PR*, pulmonary regurgitation; *RA*, right atrial; *RV*, right ventricular; *TR*, tricuspid regurgitation; *SD*, standard deviation.
*Thirty-five of 50 subjects.
Data are presented as mean ± SD.

whereas 25 patients (50%) had severe TR. S_a showed a strong correlation with RV fractional area change ($r = 0.80$, $P < .001$). None of the TDI velocities had a significant relation to heart rate and mean PAP.

Estimation of Mean Right Atrial Pressure

Indexed right atrial end-systolic area and RV myocardial performance index were weakly correlated with mean RAP ($r = 0.36$, $P = .011$; $r = 0.31$, $P = .03$, respectively). Tricuspid inflow DT showed a weak negative correlation with mean RAP ($r = -0.32$, $P = .025$). IVC diameter and its collapsibility had a significant relation to mean RAP ($r = 0.60$, $P < .001$; $r = -0.60$, $P < .001$, respectively). Both tricuspid inflow E and annular E_a had a weak but significant relation to mean RAP ($r = 0.41$, $P = .006$; $r = -0.31$, $P = .03$, respectively). When the correlation between the tricuspid E/E_a ratio and the mean RAP was examined, an improved correlation coefficient of 0.80 was revealed (Figure 2A). This correlation was still significant after adjustment for the severity of TR ($r = 0.76$, $P < .001$). In all subgroups of various underlying disorder, this correlation remained significant. The tricuspid E/E_a ratio

correlated well with mean RAP regardless of RV systolic function (decreased RV contraction: fractional area change < 30%, n = 32, $r = 0.81$; preserved RV contraction, n = 18, $r = 0.75$; both $P < .001$). Bland–Altman plot demonstrated a good intermodality agreement between Doppler-derived estimated RAP and measured RAP with a slight overestimation of estimated RAP (Figure 2B). As a whole, the optimal cutoff value of E/E_a ratio for mean RAP ≥ 10 mm Hg was identified as 7.3 (sensitivity 87%, specificity 97%), whereas the expiratory IVC diameter cutoff was 23.5 mm (sensitivity 87%, specificity 83%). The areas under the curve of E/E_a ratio and IVC diameter were 0.92 and 0.85, respectively ($P < .001$).

Adverse Cardiac Events in Relation to Echocardiographic Variables

Long-term follow-up was completed in all patients. Patients were followed for a mean period of 14 ± 1 months (range 12-18 months). During the follow-up period, 19 patients were rehospitalized for RV failure, 3 of whom subsequently died in the hospital (pump failure in 2 and sudden cardiac death in 1). We divided patients into group A with cardiac events (n = 19) and group B without events (n = 31). Clinical, invasive hemodynamic, and echocardiographic data for group A and group B are presented in Table 3. There was no significant difference in mean PAP between the groups (57 ± 13 mm Hg vs 50 ± 20 mm Hg, $P = .193$), whereas plasma BNP level and tricuspid E/E_a ratio were significantly higher in group A than in group B (349 ± 310 pg/dL vs 129 ± 136 pg/dL, $P = .001$; 7.0 ± 3.2 vs 4.5 ± 1.9, $P = .004$, respectively). Severe TR was more common in group A than in group B (63% vs 42%, $P = .145$), but not significantly. Diuretics and epoprostenol were administered more frequently in group A than in group B (χ^2 4.6, $P = .032$; χ^2 4.6, $P = .033$, respectively). LV fractional shortening was preserved in both groups A and B, but group A had a tendency for smaller LV end-diastolic diameter and shorter LV diastolic filling time, and had lower mitral inflow E, indicating limited LV preload or impairment of LV relaxation. The results of univariate and multivariate Cox analyses in the final selected model are shown in Table 4. In a multivariate model using a stepwise forward selection method, New York Heart Association functional class, 6-minute walk distance, and tricuspid E/E_a ratio remained predictive of cardiac events. Cardiac events were not associated with patient age or gender, heart rate, and systolic blood pressure. No other variables, including the IVC diameter, added any further useful prognostic information. As shown in Figure 3, the receiver operating characteristic analysis revealed that an E/E_a ratio > 6.8 had a sensitivity of 42% and a specificity of 97% for prediction of cardiac events (area under the curve 0.71; 95% confidence interval, 0.55-0.86; $P = 0.015$). Kaplan–Meier analysis showed that patients with an E/E_a ratio > 6.8 had a significantly worse outcome than those with an E/Ea ratio ≤ 6.8 (Figure 4). The mean coefficients of variations within and across observers were within acceptable range for tricuspid annular E_a (2.2% and 4.3%, respectively) and E/E_a ratio (3.5% and 5.0%, respectively).

DISCUSSION

The current study demonstrated the following: 1) The tricuspid E/E_a ratio provides a reliable estimation of RV filling pressure over a wide variety of disease severity in PAH of various underlying causes without intracardiac shunt diseases. 2) The tricuspid E/E_a ratio is independently predictive in a multivariate analysis and an E/E_a ratio > 6.8 suggests poor prognosis for patients with PAH.

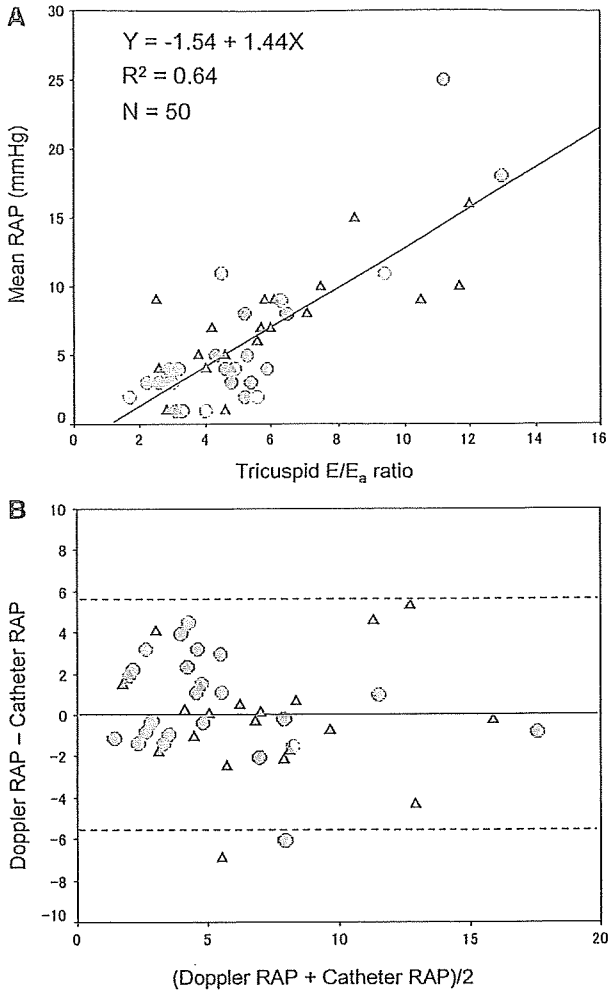

Figure 2 Linear regression analysis between mean RAP and tricuspid E/E_a ratio (A). Patients with chronic thromboembolic pulmonary hypertension (*red circles*), idiopathic PAH (*blue triangles*), connective tissue disorder (*purple circles*), and others (*cyan circles*) were distributed among the study population. Bland-Altman plot (B) shows the mean difference (*solid line*) ± 2 standard deviations (*dotted line*) between Doppler-derived and catheter-derived RAPs. E_a, Early diastolic velocity; *RAP*, right atrial pressure.

Echocardiographic Estimation of Right Ventricular Filling Pressure

Estimation of mean RAP is crucial for a patient's management and determination of Doppler-derived PASP.[4] Until now, various echocardiographic parameters have been used to evaluate mean RAP. In an earlier study, the size and collapsibility of IVC attracted a lot of attention.[10] IVC estimation of RAP is still the "standard" echocardiographic method. However, the optimal subcostal view is not always feasible, and a recent study showed that traditional classification of RAP based on IVC size and collapsibility had a relatively-low diagnostic accuracy.[16] In this study, E/E_a ratio had a larger area under the curve for high RAP than IVC size. In addition, the IVC diameter had no predictive value after a multivariate adjustment. Nagueh et al[17] reported that systolic filling fraction of hepatic vein flow related well to mean RAP, but their study population was not limited to those

Table 3 Clinical, invasive hemodynamic, and echocardiographic data for group A with cardiac events and group B without cardiac events

Characteristics	Group A (n = 19)	Group B (n = 31)	*P* value
Clinical data			
Age (y)	48 ± 12	45 ± 14	.437
Female	14 (74%)	25 (80%)	.564
HR during echocardiography (beats/min)	79 ± 14	72 ± 10	.059
SBP during echocardiography (mm Hg)	107 ± 15	110 ± 13	.470
NYHA functional class, III or IV	14 (74%)	7 (23%)	<.001
6MWD (m)	294 ± 137	434 ± 91	<.001
Catheterization data			
Pulmonary artery systolic pressure (mm Hg)	92 ± 20	83 ± 32	.195
Mean right atrial pressure (mm Hg)	9 ± 6	4 ± 3	.001
RV end-diastolic pressure (mm Hg)	14 ± 6	9 ± 5	.003
Pulmonary vascular resistance (dyn/s/cm^{-5})	1390 ± 377	1076 ± 567	.023
Cardiac index (L/min/m^2)	2.0 ± 0.5	2.3 ± 0.7	.036
Echocardiographic data			
LV end-diastolic diameter (mm)	35 ± 6	38 ± 7	.070
LV fractional shortening (%)	40 ± 8	40 ± 7	.896
IVC expiratory diameter (mm)	23 ± 6	18 ± 6	.006
IVC collapsibility (%)	34 ± 23	54 ± 15	.003
Vena contracta width (mm)	*7.4 ± 3.5	†7.1 ± 4.0	.234
TR jet maximal velocity (m/s)	4.3 ± 0.6	4.0 ± 0.9	.178
PR end-diastolic pressure gradient (mm Hg)	15 ± 8	9 ± 9	.027
Indexed RV end-diastolic area (cm^2/m)	19 ± 4	15 ± 6	.014
Indexed RV end-systolic area (cm^2/m)	15 ± 4	11 ± 5	.002
Indexed RA end-systolic area (cm^2/m)	19 ± 6	14 ± 6	.011
RV fractional area change (%)	24 ± 6	33 ± 11	.002
RV myocardial performance index	0.75 ± 0.17	0.61 ± 0.21	.016
Deformity index	2.6 ± 0.5	2.1 ± 0.8	.012
Tricuspid flow E (cm/s)	52 ± 27	47 ± 17	.412
Tricuspid annular E_a (cm/s)	8.7 ± 3.6	10.9 ± 3.0	.025
Tricuspid E/E_a ratio	7.0 ± 3.2	4.5 ± 1.9	.004
Tricuspid flow DT (ms)	160 ± 59	191 ± 56	.069
Mitral flow E (cm/s)	53 ± 14	68 ± 26	.012
Mitral flow DT (ms)	208 ± 44	212 ± 43	.709
Diastolic filling time/RR interval (%)	49 ± 7	54 ± 6	.078

HR, Heart rate; *NYHA*, New York Heart Association; *SBP*, systolic blood pressure; *6MWD*, 6-minute walk distance; *DT*, deceleration time; *IVC*, inferior vena cava; *LV*, left ventricular; *PR*, pulmonary regurgitation; *RA*, right atrial; *RV*, right ventricular; *TR*, tricuspid regurgitation. Values are mean ± SD or number (percentage).
*Fifteen of 19 subjects.
†Twenty of 31 subjects.

with PAH. Recent studies have reported that RV regional isovolumic relaxation time measured by TDI was inversely correlated with mean RAP[18] and correlated strongly with invasively measured PASP.[19] This method was simple but was not routinely used because of a major impact of ventricular rate on the measurement.

This study examines another simple method for estimating mean RAP using Doppler and TDI. Previous studies have shown that the mitral E/E_a ratio had a good relationship to LV filling pressure by correcting the impact of relaxation on the mitral inflow E using mitral annular E_a.[9] Likewise, a similar approach has been applied to estimate RV filling pressure.[8,9] As RV filling pressure increases, tricuspid inflow E increases,[8] and as RV relaxation impairs, tricuspid inflow E decreases.[20] Sade et al[21] reported that the tricuspid E/E_a ratio, after correcting the effect of RV relaxation, related well to mean RAP in a wide range of clinical conditions. This method is simple and reliable but has only been validated in a small number of patients with PAH. The present study demonstrates the application of our method to patients with PAH with various underlying causes.

Predictive Value of Echocardiographic Measurements in Chronic Pulmonary Arterial Hypertension

In recent decades, a novel treatment, epoprostenol infusion, has changed the clinical course of PAH.[1] Thus, prognostic value of baseline hemodynamics seems to be less because those hemodynamic parameters are predictive for patients treated with conventional therapy. However, epoprostenol infusion is an invasive strategy, so we need to select patients with potential poor response to conventional therapy using the established prognostic predictors. Furthermore, recent studies have shown that increased mean RAP still had prognostic power in the era of prostanoid therapy.[7,22,23] This means patients with PAH refractory to these optimal medical therapies have worse outcome, and in such cases, intensive therapy including lung transplantation should be considered. In this study, mean RAP estimated by the tricuspid E/E_a remained a significant predictor of cardiac events in a multivariate analysis of echocardiographic data in patients with PAH with various causes. We believe our method is useful in assessing response to therapy noninvasively, and the cutoff value is predictive and helpful in considering more intensive therapy as a therapeutic option. Patients with pulmonary hypertension caused by congenital heart disease and evidence of an intracardiac shunt have a somewhat better prognosis than those with the other types of PAH[2] and are associated with higher CO and lower mean RAP despite similar PAP.[24] Therefore, we excluded these cases from this study.

TR pressure gradient is routinely measured to estimate PASP; however, this index had demonstrated no prognostic value among the patients with PAH in several studies.[5-7] The early response to vasodilator pharmacotherapy is a decrease in mean RAP and an increase in CO, whereas PASP does not change significantly.[25] Therefore, estimation of PASP by TR pressure gradient alone may underestimate the hemodynamic response to vasodilators. Several previous studies have demonstrated the relationships between echocardiographic parameters and clinical outcomes in patients with PAH, showing the predictive value of pericardial effusion size,[5] mitral inflow E/A,[5] RV myocardial performance index,[6] and indexed right atrial area.[7,26] In the present study, these echocardiographic parameters were significant predictors of cardiac events in a univariate analysis but not in a multivariate model. Among the echocardiographic parameters, only the tricuspid E/E_a ratio was significant and an independent predictor of adverse outcomes. Most recently, Shiina et al[27] reported the tricuspid E/E_a ratio was positively correlated with plasma BNP level, and both were associated with poor survival in patients with chronic thromboembolic PH.[27] Our study showed a significant correlation between the tricuspid E/E_a ratio and the plasma

Table 4 Results of univariate and multivariate Cox proportional hazards analyses in the final selected model

Variables	Univariate Hazard ratio (95% CI)	*P* value	Multivariate* Hazard ratio (95% CI)	*P* value
Age > 44 y	1.573 (0.631-3.923)	.331	1.060 (0.376-2.983)	.913
Gender female	0.923 (0.332-2.566)	.878	1.608 (0.553-4.670)	.383
Heart rate ≤ 76 beats/min	0.495 (0.194-1.261)	.140	0.866 (0.288-2.604)	.798
SBP ≤ 106 mm Hg	1.373 (0.551-3.424)	.496	1.428 (0.483-4.224)	.519
NYHA functional class, III or IV	5.600 (1.996-15.71)	.001	4.128 (1.241-13.73)	.021
6MWD ≤ 400 m	7.423 (2.152-25.60)	.002	5.799 (1.508-22.30)	.011
Tricuspid E/E_a ratio	1.231 (1.074-1.410)	.003	1.227 (1.042-1.444)	.014

CI, Confidence interval; *SBP*, systolic blood pressure; *NYHA*, New York Heart Association; *6MWD*, 6-minute walk distance.
*Goodness-of-fit test was performed using a chi-square test ($P < .01$).

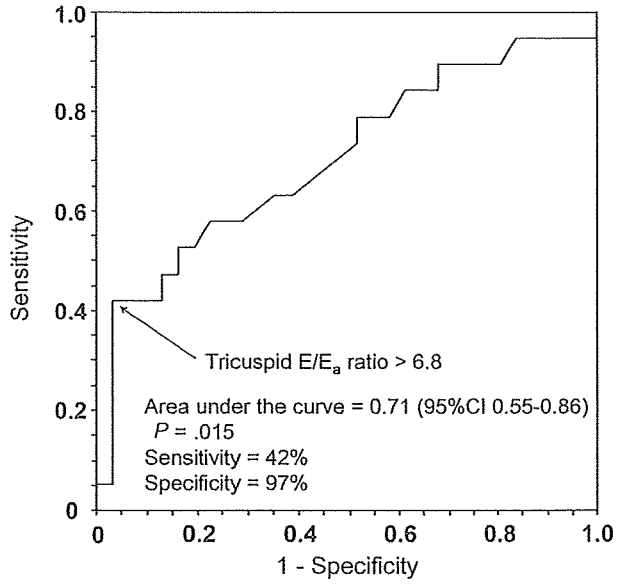

Figure 3 Receiver operating characteristic curve to identify patients with cardiac events. CI = confidence interval.

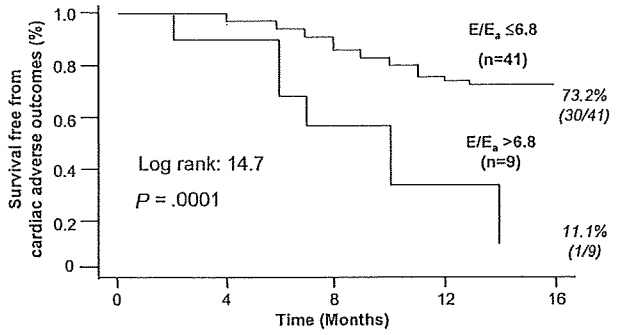

Figure 4 Event-free survival curves according to the tricuspid E/E_a ratio.

BNP level ($r = 0.39$, $P = .005$), and a higher BNP level was observed in the PAH subgroup with cardiac events ($P = .001$). Accurate, noninvasive estimation of mean RAP by the tricuspid E/E_a ratio may reduce the need for invasive right-sided catheterization and help identify patients suitable for earlier intensive therapy, including lung transplantation.

Study Limitations

The study had a small sample size. The results from the Kaplan–Meier analysis in Figure 4 should be interpreted carefully because they might be different if the sample size were larger and a different cutoff were chosen. Thus, whether early detection of high RAP using the tricuspid E/E_a ratio in this population translates into better outcomes remains to be addressed in prospective, larger-scale, and longer-term studies. Our study population had various types of treatment, and approximately half of the patients had treatment with intravenously administered epoprostenol. However, several important conclusions were reached. In addition, patients with an E/E_a ratio > 6.8 had a worse prognosis than those with an E/E_a ratio ≤ 6.8 in subgroups of patients with (n = 22, log rank: 4.21, $P = .04$) or without (n = 28, log rank: 7.20, $P = .007$) epoprostenol infusion (data not shown). Another limitation is the lack of a simultaneous recording of invasive and noninvasive measurements. However, measurements of echocardiography during cardiac catheterization may be inaccurate because of suboptimal positioning of the patient. The time lag between TDI and cardiac catheterization (<24 hours) was thought to be acceptable.[19,28] Measurements of tricuspid inflow E and annular E_a may alter with respiration. However, mean values of 5 to 7 consecutive cardiac cycles have been shown to be similar to those obtained at end-expiratory apnea.[8,20] Similar results were observed in this study, with a mean percent E and E_a difference between averaged and end-expiratory values of 5% ± 4% and 5% ± 5%, respectively. Patients with irregular heart rhythm, such as atrial fibrillation and atrioventricular block, were excluded from this study because of the confounding effect on Doppler and TDI measurements. Thus, our results cannot be extrapolated to such cases. This study included patients with PAH with various causes. Thus, PAH may affect RV remodeling in different ways, and TDI parameters may be affected. However, other recent studies using TDI and strain rate imaging also included patients with various underlying causes of PAH.[29,30] Moreover, no study with a head-to-head comparison of these parameters in patients with different causes of PAH has been performed.

CONCLUSIONS

RV filling pressure can be well estimated using the tricuspid E/E_a ratio in patients with PAH with and without RV systolic dysfunction. This index is a powerful predictor of cardiac events in patients with chronic PAH as well as functional status and exercise capacity, suggesting the prognostic importance of RV diastolic dysfunction.

REFERENCES

1. McLaughlin VV, McGoon MD. Pulmonary arterial hypertension. Circulation 2006;114:1417-31.
2. McLaughlin VV, Presberg KW, Doyle RL, Abman SH, McCrory DC, Fortin T, et al. Prognosis of pulmonary arterial hypertension: ACCP evidence-based clinical practice guidelines. Chest 2004;126:78S-92S.
3. Sandoval J, Bauerle O, Palomar A, Gomez A, Martinez-Guerra ML, Beltran M, et al. Survival in primary pulmonary hypertension. Validation of a prognostic equation. Circulation 1994;89:1733-44.
4. Chan KL, Currie PJ, Seward JB, Hagler DJ, Mair DD, Tajik AJ. Comparison of three Doppler ultrasound methods in the prediction of pulmonary artery pressure. J Am Coll Cardiol 1987;9:549-54.
5. Eysmann SB, Palevsky HI, Reichek N, Hackney K, Douglas PS. Two-dimensional and Doppler-echocardiographic and cardiac catheterization correlates of survival in primary pulmonary hypertension. Circulation 1989;80:353-60.
6. Yeo TC, Dujardin KS, Tei C, Mahoney DW, McGoon MD, Seward JB. Value of a Doppler-derived index combining systolic and diastolic time intervals in predicting outcome in primary pulmonary hypertension. Am J Cardiol 1998;81:1157-61.
7. Raymond RJ, Hinderliter AL, Willis PW, Ralph D, Caldwell EJ, Williams W, et al. Echocardiographic predictors of adverse outcomes in primary pulmonary hypertension. J Am Coll Cardiol 2002;39:1214-9.
8. Nageh MF, Kopelen HA, Zoghbi WA, Quinones MA, Nagueh SF. Estimation of mean right atrial pressure using tissue Doppler imaging. Am J Cardiol 1999;84:1448-51, A8.
9. Sundereswaran L, Nagueh SF, Vardan S, Middleton KJ, Zoghbi WA, Quinones MA, et al. Estimation of left and right ventricular filling pressures after heart transplantation by tissue Doppler imaging. Am J Cardiol 1998; 82:352-7.
10. Kircher BJ, Himelman RB, Schiller NB. Noninvasive estimation of right atrial pressure from the inspiratory collapse of the inferior vena cava. Am J Cardiol 1990;66:493-6.
11. Tribouilloy CM, Enriquez-Sarano M, Bailey KR, Tajik AJ, Seward JB. Quantification of tricuspid regurgitation by measuring the width of the vena contracta with Doppler color flow imaging: a clinical study. J Am Coll Cardiol 2000;36:472-8.
12. Masuyama T, Kodama K, Kitabatake A, Sato H, Nanto S, Inoue M. Continuous-wave Doppler echocardiographic detection of pulmonary regurgitation and its application to noninvasive estimation of pulmonary artery pressure. Circulation 1986;74:484-92.
13. Miller D, Farah MG, Liner A, Fox K, Schluchter M, Hoit BD. The relation between quantitative right ventricular ejection fraction and indices of tricuspid annular motion and myocardial performance. J Am Soc Echocardiogr 2004;17:443-7.
14. Ryan T, Petrovic O, Dillon JC, Feigenbaum H, Conley MJ, Armstrong WF. An echocardiographic index for separation of right ventricular volume and pressure overload. J Am Coll Cardiol 1985;5:918-27.
15. Tei C, Dujardin KS, Hodge DO, Bailey KR, McGoon MD, Tajik AJ, et al. Doppler echocardiographic index for assessment of global right ventricular function. J Am Soc Echocardiogr 1996;9:838-47.
16. Brennan JM, Blair JE, Goonewardena S, Ronan A, Shah D, Vasaiwala S, et al. Reappraisal of the use of inferior vena cava for estimating right atrial pressure. J Am Soc Echocardiogr 2007;20:857-61.
17. Nagueh SF, Kopelen HA, Zoghbi WA. Relation of mean right atrial pressure to echocardiographic and Doppler parameters of right atrial and right ventricular function. Circulation 1996;93:1160-9.
18. Abbas A, Lester S, Moreno FC, Srivathsan K, Fortuin D, Appleton C. Noninvasive assessment of right atrial pressure using Doppler tissue imaging. J Am Soc Echocardiogr 2004;17:1155-60.
19. Fahmy Elnoamany M, Abdelraouf Dawood A. Right ventricular myocardial isovolumic relaxation time as novel method for evaluation of pulmonary hypertension: correlation with endothelin-1 levels. J Am Soc Echocardiogr 2007;20:462-9.
20. Zoghbi WA, Habib GB, Quinones MA. Doppler assessment of right ventricular filling in a normal population. Comparison with left ventricular filling dynamics. Circulation 1990;82:1316-24.
21. Sade LE, Gulmez O, Eroglu S, Sezgin A, Muderrisoglu H. Noninvasive estimation of right ventricular filling pressure by ratio of early tricuspid inflow to annular diastolic velocity in patients with and without recent cardiac surgery. J Am Soc Echocardiogr 2007;20:982-8.
22. Sitbon O, Humbert M, Nunes H, Parent F, Garcia G, Herve P, et al. Long-term intravenous epoprostenol infusion in primary pulmonary hypertension: prognostic factors and survival. J Am Coll Cardiol 2002;40:780-8.
23. McLaughlin VV, Shillington A, Rich S. Survival in primary pulmonary hypertension: the impact of epoprostenol therapy. Circulation 2002; 106:1477-82.
24. Hopkins WE, Ochoa LL, Richardson GW, Trulock EP. Comparison of the hemodynamics and survival of adults with severe primary pulmonary hypertension or Eisenmenger syndrome. J Heart Lung Transplant 1996;15:100-5.
25. Barst RJ, Rubin LJ, Long WA, McGoon MD, Rich S, Badesch DB, et al. A comparison of continuous intravenous epoprostenol (prostacyclin) with conventional therapy for primary pulmonary hypertension. The Primary Pulmonary Hypertension Study Group. N Engl J Med 1996;334: 296-302.
26. Bustamante-Labarta M, Perrone S, De La Fuente RL, Stutzbach P, De La Hoz RP, Torino A, et al. Right atrial size and tricuspid regurgitation severity predict mortality or transplantation in primary pulmonary hypertension. J Am Soc Echocardiogr 2002;15:1160-4.
27. Shiina Y, Funabashi N, Lee K, Daimon M, Sekine T, Kawakubo M, et al. Doppler imaging predicts cardiac events in chronic pulmonary thromboembolism. Int J Cardiol 2009;133:167-72.
28. Arteaga RB, Hreybe H, Patel D, Landolfo C. Derivation and validation of a diagnostic model for the evaluation of left ventricular filling pressures and diastolic function using mitral annulus tissue Doppler imaging. Am Heart J 2008;155:924-9.
29. Kittipovanonth M, Bellavia D, Chandrasekaran K, Villarraga HR, Abraham TP, Pellikka PA. Doppler myocardial imaging for early detection of right ventricular dysfunction in patients with pulmonary hypertension. J Am Soc Echocardiogr 2008;21:1035-41.
30. Dambrauskaite V, Delcroix M, Claus P, Herbots L, D'Hooge J, Bijnens B, et al. Regional right ventricular dysfunction in chronic pulmonary hypertension. J Am Soc Echocardiogr 2007;20:1172-80.

Cigarette Smoking Abolishes Ischemic Preconditioning-Induced Augmentation of Endothelium-Dependent Vasodilation

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Abstract—We have shown recently that repetition of ischemic preconditioning stimulus augments endothelium-dependent vasodilation in forearm circulation of healthy subjects through increases in NO production and the number of circulating progenitor cells under a local condition. The purpose of this study was to evaluate the "late" effect of ischemic preconditioning on endothelial function in smokers. Ischemic preconditioning was induced by upper-limb ischemia 6 times a day for 1 month. We evaluated forearm blood flow responses to acetylcholine and sodium nitroprusside before and after ischemic preconditioning stimulus in 15 male smokers (27±7 years) and 15 male nonsmokers (26±5 years). Forearm blood flow was measured by using a strain-gauge plethysmography. The ischemic preconditioning stimulus resulted in significant increases in the circulating level of circulating progenitor cells from 1029±261 to 1232±341 mL (P=0.02), cell migration response to vascular endothelial growth factor from 38±16 to 52±17 per high-power field (P=0.02), and forearm blood flow response to acetylcholine from 25.1±5.2 to 32.4±6.6 mL/min per 100 mL of tissue (P=0.002) in nonsmokers, but these did not change in the smoker group. The forearm blood flow responses to sodium nitroprusside before and after the ischemic preconditioning stimulus were similar. Intra-arterial infusion of N^G-monomethyl-L-arginine, an NO synthase inhibitor, completely eliminated the ischemic preconditioning stimulus-induced augmentation of forearm blood flow responses to acetylcholine in nonsmokers. These findings suggest that repetition of ischemic preconditioning stimulus may be a simple, safe, and feasible therapeutic technique for endothelial protection of peripheral vessels. However, smoking abolishes ischemic preconditioning stimulus-induced augmentation of endothelium-dependent vasodilation. (***Hypertension*. 2009;53:674-681.**)

Key Words: preconditioning ■ endothelial function ■ NO ■ vascular endothelial growth factor ■ circulating progenitor cells ■ smoking

Several studies have shown that prodromal angina pectoris occurring shortly before the onset of infarction reduced infarct size and improved left ventricular function.[1,2] A brief ischemic period, followed by episodes of reperfusion, increases the resistance to further ischemic damage, a phenomenon known as ischemic preconditioning (IPC). IPC has been observed in the heart, liver, brain, and other organs.[3–8] IPC is an important mechanism by which tissues protect themselves from impending ischemic damage. IPC has protective effects against myocardial infarction and myocardial stunning.

It is thought that IPC is a multifactorial phenomenon that includes components of endothelium-derived NO and adenosine. Endothelial function, especially NO function, plays a critical role in the development and maintenance of cardiovascular diseases.[9–13] Therefore, from a clinical perspective, it is important to select an appropriate intervention that is effective in improving endothelial dysfunction in patients with cardiovascular diseases. Under the condition of hypoxia, vascular endothelial growth factor (VEGF) gene expression is upregulated by induction of hypoxia-inducible factor-1 (HIF-1), resulting in an increase in migration of endothelial progenitor cells (EPCs). Interestingly, endothelial function has been found to be associated with the number of circulating EPCs in humans.[14] Recently, we have shown that repetition of IPC stimulus augments endothelial function through an increase in circulating progenitor cells.[15]

Cigarette smoking is a well-established independent risk factor of cardiovascular diseases. Smoking is associated with endothelial dysfunction.[16,17] It is postulated that increase in oxidative stress and decrease in EPCs contribute to vascular failure in smokers. On the other hand, several lines of evidence have shown that smoking has paradoxical beneficial effects on immediate mortality and prognosis in patients with acute myocardial infarction.[18,19] However, there is no infor-

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mation on the effects of smoking on IPC effects, especially IPC effect on endothelial function.

Repetition of IPC stimulus in forearm circulation is an ideal model for evaluating effects of IPC on the coronary artery, leading to protection of myocardiocytes against damage caused by severe ischemia. To determine the validity of the hypothesis that smoking diminishes the beneficial effect of "late" IPC on endothelial function, we measured forearm blood flow (FBF) responses to acetylcholine (ACh), an endothelium-dependent vasodilator, and sodium nitroprusside (SNP), an endothelium-independent vasodilator, in the presence and absence of N^G-monomethyl-L-arginine (L-NMMA), an inhibitor of NO synthase, and we also investigated circulating levels of VEGF and the number of and function of circulating progenitor cells.

Methods

Subjects

We studied 15 young nonsmoker men (mean age: 27.5±4.5 years) and 15 young smoker men (mean age: 28.2±4.1 years). All of the subjects had no history of cardiovascular or cerebrovascular disease, hypertension, hypercholesterolemia, diabetes mellitus, liver disease, renal disease, or other diseases. The results of physical and routine laboratory examinations of the subjects were normal. None of the subjects were taking oral antioxidant vitamins or vasoactive drugs. Current smokers were defined as smokers who had smoked ≥1 pack-year, 1 pack-year being defined as 20 cigarettes per day for 1 year. All of the smokers (28.7±7.4 pack-years) had a current cigarette smoking history of >5 years and abstained from smoking for ≥3 hour before the measurement of vascular function. We defined nonsmokers as subjects who had never smoked. The ethical committee of Hiroshima University Graduate School of Biomedical Sciences approved the study protocol. Written informed consent for participation in the study was obtained from all of the subjects.

Study Protocol

None of the subjects received any drugs for ≥24 hours before the study. An upper-arm cuff was inflated to 200 mm Hg for 5 minutes 6 times a day for 4 weeks using a rapid cuff inflator (EC-20, Hokanson, Inc) to obtain repetition of transient ischemia as a strategy of IPC. All of the subjects underwent 4 weeks of follow-up without any lifestyle modification. Forearm vascular responses to ACh (Daiichi Pharmaceutical Co) and to SNP (Maruishi Pharma Co) were evaluated before and after 4 weeks of IPC repetition stimulus. The studies began at 8:30 AM after 14 hours of the last IPC stimulus. Subjects were kept in a supine position in a quiet, dark, and air-conditioned room throughout the study. A 23-gauge catheter was inserted into the brachial artery for infusion using 1% lidocaine to record arterial pressure with an AP-641G pressure transducer (Nihon Koden Co). Another catheter was inserted into the deep antecubital vein to obtain blood samples. Total volume of the blood sample was 20 mL. After 30 minutes in the supine position, blood samples were obtained, and baseline FBF, heart rate, and arterial blood pressure were measured. Then, ACh (3.75, 7.50, and 15.00 μg/min) or SNP (0.75, 1.50, and 3.00 μg/min) was infused intra-arterially for 5 minutes at each dose with a constant-rate infusion pump (Terfusion ETG-523, Terumo Co). FBF during the final 2 minutes of each infusion was measured. The infusions of ACh and SNP were carried out in a random order. Each study proceeded after the FBF had returned to the baseline level. After a 30-minute rest period, L-NMMA (CLINALFA Co) was infused intra-arterially at a dose of 8 μmol/min for 5 minutes while the baseline FBF and arterial blood pressure were recorded. After L-NMMA infusion was complete, ACh (3.75, 7.50, and 15.00 μg/min) was administered. Please see the online data supplement at http://hyper.ahajournals.org for infusion protocol (Figure S1).

Baseline fasting serum concentrations of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein (LDL) cholesterol, malondialdehyde-modified LDL, triglycerides, glucose, insulin, electrolytes, interleukin 6, and high-sensitivity C-reactive protein (hs-CRP) and plasma concentrations of VEGF were obtained after a 30-minute rest period before the study. The 24-hour urinary excretion levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) and nitrite/nitrate were determined.

Measurement of FBF

FBF was measured using a mercury-filled Silastic strain-gauge plethysmography (EC-5R, Hokanson, Inc), as described previously.[11,12] Please see the online data supplement for additional details.

Measurement of the Number of Circulating Progenitor Cells

The number of circulating progenitor cells was analyzed by flow cytometry. Please see the online data supplement for additional details.

Characterization of Progenitor Cells

Mononuclear cells were isolated by Ficoll density-gradient centrifugation of human blood buffy coats from 50 mL of peripheral blood. Please see the online data supplement for additional details.

Migration Assay

Progenitor cell migration was evaluated using a modified Boyden chamber assay, as described previously.[20] Please see the online data supplement for additional details.

Analytical Methods

Samples of venous blood were placed in tubes containing sodium EDTA (1 mg/mL) and in polystyrene tubes. Please see the online data supplement for additional details.

Statistical Methods

Values are expressed as the means±SDs. The Mann–Whitney U test was used to evaluate differences between before and after the IPC stimulus with respect to baseline parameters. Two-tailed Student's paired *t* test was used to evaluate differences before and after IPC stimulus. The FBF responses to ACh and SNP before and after IPC stimulus were analyzed by 2-way ANOVA for repeated measures, followed by Scheffe's F test. Results were considered significant at $P<0.05$.

Results

Clinical Characteristics

The baseline clinical characteristics of the 15 nonsmokers before (0 weeks) and after (4 weeks) IPC and the 15 smokers before (0 weeks) and after (4 weeks) IPC are summarized in the Table. Serum concentrations of interleukin 6 and hs-CRP, indices of systemic inflammation, were significantly higher in smokers than in nonsmokers. Urinary excretion of 8-OHdG was significantly higher in smokers than in nonsmokers before and after IPC. Serum concentration of malondialdehyde-modified LDL showed a tendency to be high, but not significantly, in smokers compared with that in nonsmokers before and after IPC ($P=0.06$ and $P=0.07$, respectively). There were no significant differences in other parameters between the 2 groups. The plasma concentration of VEGF was increased significantly in both the nonsmoker group and the smoker group by 4 weeks of IPC. The plasma concentration of VEGF was similar in the 2 groups after the IPC stimulus. IPC stimulus did not alter systemic hemodynamics, including blood pressure, heart rate, lipid profile, inflammation markers (interleukin 6 and hs-CRP),

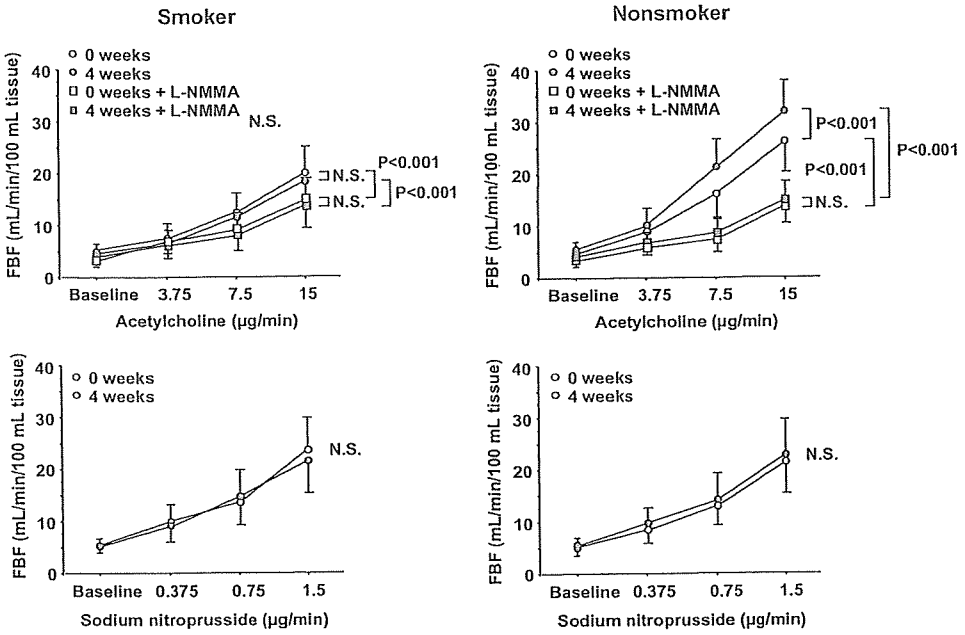

Figure 1. Comparison of FBF responses to ACh and SNP and ACh in the presence of L-NMMA at 0 weeks and 4 weeks of ischemic preconditioning in nonsmokers and smokers.

and urinary excretion of 8-OHdG. Other parameters before and after IPC stimulus were similar in the 2 groups.

Effects of IPC on FBF Responses to ACh and SNP

Intra-arterial infusion of ACh and SNP increased FBF in a dose-dependent manner in all of the subjects. The response of FBF to ACh was significantly less in smokers than in nonsmokers ($P<0.001$; Figure 1). Vasodilatory responses to SNP were similar in the 2 groups (Figure 1). There was a significant relationship between maximal FBF response to ACh and urinary excretion of 8-OHdG ($r=-0.42$; $P=0.02$), whereas SNP-induced vasodilation did not correlate with any parameters. There were no significant relationships among the vascular responses to ACh and SNP and serum concentrations of interleukin 6 and hs-CRP.

IPC stimulus did not alter baseline FBF in the nonsmoker group or smoker group (Table). The response of FBF to infusion of ACh was increased significantly from 25.1±5.2

Table. Baseline Clinical Characteristics Before and After 4 Weeks of Preconditioning in the Nonsmoker and Smoker Groups

	Nonsmoker		Smoker	
Variable	Before (0 wk)	After (4 wk)	Before (0 wk)	After (4 wk)
Body mass index, kg/m^2	22.8±0.9	22.8±0.9	23.0±0.8	23.0±0.8
Systolic blood pressure, mm Hg	120.4±4.2	119.6±3.9	119.8±4.4	120.1±3.8
Diastolic blood pressure, mm Hg	63.5±3.0	62.0±2.8	62.9±3.1	63.1±2.9
Heart rate, bpm	68.2±3.2	67.4±2.9	69.1±2.8	68.7±2.6
Total cholesterol, mmol/L	4.86±0.22	4.84±0.33	4.79±0.26	4.78±0.37
Triglycerides, mmol/L	1.37±0.19	1.34±0.17	1.36±0.28	1.35±0.26
HDL cholesterol, mmol/L	1.29±0.09	1.37±0.11	1.22±0.12	1.25±0.14
LDL cholesterol, mmol/L	2.94±0.21	2.86±0.19	2.88±0.22	2.82±0.18
Glucose, mmol/L	3.61±0.19	3.86±0.29	3.74±0.26	3.69±0.24
Insulin, pmol/L	53.6±5.8	59.1±6.9	54.8±5.3	56.4±6.1
VEGF, pg/mL	88.2±7.3	118.1±11.5*	90.2±10.1	129.2±9.7*
Interleukin 6, ng/L	1.2±2.1	1.3±2.2	2.1±2.4†	2.0±2.5†
Hs-CRP, mg/L	1.1±1.3	1.0±1.4	1.9±2.1†	1.9±2.0†
MDA-LDL, U/L	53.2±22.9	52.7±27.3	63.9±30.1	64.8±31.6
Urinary 8-OHdG, ng/mg of Cr	7.8±3.4	7.7±3.6	12.3±4.8†	12.1±4.2†
FBF, mL/min per 100 mL of tissue	4.9±0.4	5.2±0.6	5.0±0.7	4.9±0.6

HDL indicates high-density lipoprotein; CRP, C-reactive protein; MDA, malondialdehyde; Cr, creatinine. All of the results are presented as mean±SD.

*$P<0.05$ vs before (0 wk) in the same group.

†$P<0.05$ vs nonsmoker at the same follow-up period.