Original Article

Clinical Implications of Assessing Coronary Flow Velocity Reserve and Plasma Endothelin-1 in Hypertensive Patients

Hideki FUJII^{*1,2}, Shin TAKIUCHI^{*1}, Kei KAMIDE^{*1}, Takeshi HORIO^{*1},
Shin-ichirou NIIZUMA^{*1}, Norio TANAKA^{*3}, Shuji HASHIMOTO^{*3},
Satoshi NAKATANI^{*4}, Masafumi FUKAGAWA^{*2}, and Yuhei KAWANO^{*1}

Previous reports have indicated that hypertensive patients who have angina-like chest pain and normal coronary arteriograms have reduced coronary flow velocity reserve (CFVR) levels. In addition, elevated plasma endothelin-1 (ET-1) levels have been reported to be associated with microvascular angina. The purpose of this study was to evaluate the plasma ET-1 levels and CFVR in patients with chest pain but without coronary artery disease (CAD). A total of 66 patients were included in this study. CAD was ruled out by exercise stress test or coronary angiogram. Plasma ET-1 and CFVR measurements were performed in patients with ($n=35$) and without ($n=31$) a history of angina-like chest pain. CFVR was measured using adenosine-triphosphate stress transthoracic Doppler echocardiography. The mean ET-1 level was significantly higher and the CFVR was significantly lower in patients in the symptomatic group than in those in the asymptomatic group (ET-1: 3.85 ± 1.24 pg/ml vs. 2.98 ± 1.27 pg/ml, CFVR: 2.26 ± 0.48 vs. 2.77 ± 0.11 , respectively). Plasma ET-1 level and CFVR were significantly correlated with each other ($-r=0.265$, $p=0.033$). Age, blood pressure, cardiovascular risk factors, and left ventricular mass index were similar between the two groups. The results of multiple regression analysis indicate that age ($p=0.008$) and plasma ET-1 concentration ($p=0.031$) had statistically independent associations with CFVR. Attenuated CFVR in the symptomatic hypertensive patients was associated with endothelial dysfunction, which results in elevated plasma ET-1 levels. The link between these two parameters may play a role in the genesis of chest pain in hypertensive patients without CAD. (*Hypertens Res* 2005; 28: 911–916)

Key Words: coronary flow velocity reserve, endothelin-1, endothelial function, hypertension, coronary artery disease

Introduction

The symptoms of angina or angina-like chest pain in the absence of significant coronary artery stenosis are frequently

seen in patients with hypertension (1–4). This kind of chest pain is sometimes indistinguishable from the pain observed in patients with obstructive coronary artery diseases. The mechanisms that are thought to underlie these symptoms are of both cardiovascular (vasospasm, cardiac syndrome X,

From the ^{*1}Division of Hypertension and Nephrology, ^{*3}Testing Division, Laboratory of Clinical Physiology, and ^{*4}Division of Cardiology, National Cardiovascular Center, Suita, Japan; and ^{*2}Division of Diabetes, Digestive, and Kidney Diseases, Department of Clinical Molecular Medicine, Kobe University Graduate School of Medicine, Kobe, Japan.

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Address for Reprints: Hideki Fujii, M.D., Division of Diabetes, Digestive, and Kidney Diseases, Department of Clinical Molecular Medicine, Kobe University Graduate School of Medicine, 7–5–2, Kusunoki-cho, Chuo-ku, Kobe 650–0017, Japan. E-mail: hideki-f@tg7.so-net.ne.jp

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impaired endothelial function) and non-cardiovascular origins (esophageal motility disorders, gastroesophageal reflux disease, psychogenetic mental disorder, *etc.*). Regardless of the actual mechanism, it is crucial to determine the cause of disease and administer appropriate medical treatment, since persistent symptoms may lead to disability and impose a substantial economic burden on the health care system.

Among the various pathophysiological abnormalities that have been described in patients with angina-like chest pain but normal coronary angiographs, reduced coronary flow velocity reserve (CFVR) due to impairment of coronary endothelial function has received a great deal of attention (3, 5). The endothelium regulates vascular tone and growth by releasing both relaxation factors such as nitric oxide and vasoconstrictive factors such as endothelin. In the past decade, several studies demonstrated that the plasma concentration of endothelin-1 (ET-1) was significantly higher in patients with angina pectoris with and without epicardial coronary stenosis and suggested that ET-1 might play an active role in the disease process of microvascular angina (6–9). Although most of the investigators assumed that the link between ET-1 and chest pain was due to attenuated CFVR (10), there have been few reports examining their relationship in a clinical setting.

In this study, we measured coronary flow velocity noninvasively using transthoracic Doppler echocardiography (TTDE) (11–14) and evaluated the response of the diastolic components of coronary flow velocity in the left anterior descending (LAD) coronary artery to stepwise intravenous (IV) administration of the vasodilating agent, adenosine-triphosphate (ATP), in hypertensive patients with and without angina-like chest pain. The aims of our study were to compare the CFVR and plasma ET-1 levels, and to investigate the pathophysiological mechanisms underlying the impaired CFVR and angina-like chest pain observed in hypertensive patients without coronary artery disease.

Methods

Study Population

The study population consisted of 66 consecutive patients with essential hypertension but without coronary artery disease. All hypertensive patients had a well-established history of elevated casual blood pressure (BP) > 140/90 mmHg, with at least three sets of readings taken at 1-month intervals. The exclusion criteria for the present study included the presence of valvular heart disease, idiopathic cardiomyopathy, diabetes mellitus, renal insufficiency, and insufficient echo-imaging of the coronary arteries. Patients with arrhythmias, including atrioventricular blocks or atrial fibrillation, and bronchial asthma were also excluded because administration of ATP might have worsened their symptoms. Coronary artery disease was ruled out by negative findings on exercise-stress electrocardiography, scintigraphy or coronary angiography (if necessary).

For patients taking anti-hypertensive drugs, medication was withdrawn approximately 1 week before the examination to exclude direct effects on vasodilation. Individuals with a smoking habit abstained from smoking for 1 week before the examination. All patients abstained from caffeine the day before the examination. The experimental protocols were approved by the appropriate institutional review committee, and informed consent was obtained from all subjects. This study was performed prospectively and all examinations were performed by several investigators (HF, ST, SN, NT) who were blinded to the patients' characteristics.

We divided the patients into two groups based on their clinical history of angina-like chest pain. Patients with a history of chest pain were placed in the S group ($n=35$), while those without were placed in the N group ($n=31$). Individuals with episodes of chest discomfort (described as "viselike," "constricting," "suffocating," "crushing," "heavy," or "squeezing") during exercise or during several minutes of rest (15) and/or with effective prescriptions for sublingual nitroglycerine in recent clinical records were defined in the present study as suffering angina-like chest pain. All diagnoses were given by experienced cardiologists from the National Cardiovascular Center.

CFVR Measurement Using Transthoracic Doppler Echocardiography

CFVR measurement using TTDE has been previously described (11–14). Briefly, TTDE examinations were performed with the Siemens Sequoia digital ultrasound system at a frequency of 7.0 MHz (Siemens USA, Mountain View, USA). The ultrasound beam was transmitted toward the heart to visualize coronary blood flow in the distal portion of the LAD by color Doppler flow mapping. First, the left ventricle was imaged by cross-section along the longitudinal-axis, and then the ultrasound beam was inclined laterally. Next, coronary blood flow in the distal LAD was examined under the guidance of color Doppler flow mapping. After positioning a sample volume on the color signal in the distal LAD, Doppler spectral tracings of flow velocity were recorded by fast Fourier transformation analysis. All studies were recorded on 0.5-inch S-VHS videotapes for off-line analysis.

We first recorded baseline spectral Doppler signals in the distal portion of the LAD. ATP was administered ($140 \mu\text{g/kg/min}$ IV) for 3 min to record spectral Doppler signals during hyperemic conditions. All patients underwent continuous heart rate and BP monitoring throughout the study period.

Analysis of coronary flow velocity was performed off-line by tracing the contour of the spectral Doppler signal using the ultrasound system computer. Mean diastolic velocity (MDV) was measured at baseline and peak hyperemic conditions. Measurements were averaged over three cardiac cycles (3). CFVR was defined as the ratio of hyperemic to basal MDV. We adopted a CFVR of less than 2.0 as the cut-off value for the presence of significant coronary arterial stenosis, as has

Table 1. Patients' Characteristics

	S group	N group
N	35	31
Age (years old)	62±9	60±11
Sex (M/F)	12/23*	19/12
BMI (kg/m ²)	25.1±4.0	24.2±2.7
SBP (mmHg)	148.9±22.7	155.3±23.3
DBP (mmHg)	85.7±12.0	87.2±15.2
Smoking (%)	8 (26%)	7 (20%)
T-Chol (mg/dl)	191.1±34.8	200.8±22.4
HDL (mg/dl)	49.2±12.7	51.5±19.9
TG (mg/dl)	110.6±47.1	123.2±50.4
HbA1c (%)	5.5±0.8	5.6±0.9
LVMI (g/m ²)	123.35±28.37	114.82±23.15
RWT (unitless)	0.450±0.015	0.459±0.014

M, male; F, female; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; T-Chol, total cholesterol; HDL, high density lipoprotein cholesterol; TG, triglyceride; HbA1c, hemoglobin A1c; LVMI, left ventricular mass index for body surface area; RWT, relative wall thickness. * $p < 0.05$ vs. N group. Values are mean±SD.

been done in published reports (14, 16).

Measurement of Left Ventricular Mass by M-Mode Echocardiography

Two-dimensional guided M-mode echocardiography was performed to measure the left ventricular wall mass. Left ventricular diastolic and systolic diameter (LVDd/LVDs), in addition to the diastolic thickness of the left ventricular posterior wall (LVPWT) and interventricular septum (IVST), were assessed in M-mode images in the parasternal longitudinal-axis view. M-mode analysis was performed according to the guidelines of the American Society of Echocardiography. The left ventricular mass index (LVMI) (g/m²) and relative wall thickness (RWT) were calculated using the following formula (17):

$$\text{LVMI (g/m}^2\text{)} = \frac{[1.04 \times \{(\text{IVST} + \text{LVPWT} + \text{LVDd})^3 - \text{LVDd}^3\} - 13.6]}{\text{body surface area}}$$

$$\text{RWT (unitless)} = 2 \times (\text{LVPWT}/\text{LVDd})$$

Measurement of Plasma ET-1 Level and Laboratory Determination

Venous blood was sampled from the antecubital vein after a period of 20 min supine rest in the morning after overnight fasting, on the same day as CFVR measurement. Blood was drawn into chilled citrate tubes on ice. Plasma was separated by centrifugation at $2,500 \times g$ for 10 min at 4°C and stored at -20°C until analysis. Plasma ET-1 levels were determined at

Table 2. Hemodynamic Data

	S group	N group
Baseline SBP (mmHg)	156.5±21.3	157.5±22.9
Baseline DBP (mmHg)	88.7±12.6	87.7±15.7
Hyperemia SBP (mmHg)	142.8±21.5	145.8±26.2
Hyperemia DBP (mmHg)	80.2±14.6	81.3±17.1
Baseline HR	67.0±9.4	63.2±11.2
Hyperemia HR	78.2±12.1*	71.1±11.2
Baseline MDV (cm/s)	17.6±4.7	15.8±4.5
Hyperemia MDV (cm/s)	38.7±11.0	42.6±11.3

SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; MDV, mean diastolic pressure. * $p < 0.05$ vs. N group. Values are mean±SD.

Fujimoto Biomedical Laboratories, Inc. (Matsubara, Osaka, Japan) by a novel high-performance liquid chromatography (HPLC) method (Hitachi L-7480 system equipped with a fluorescence detector for 348 nm excitation and 450 nm emission, with an ODS column) using orthophthaldialdehyde (OPA) for fluorescence determination. Analysis of blood chemistry was carried out using an autoanalyzer.

Statistical Analysis

We used the computer software application, StatView (SAS Institute, Cary, USA) for all statistical analyses. Values are presented as the mean±SD. Differences before and after treatment were analyzed by ANOVA, followed by Fisher's protected least significant difference test for continuous variables and the χ^2 test for categorical variables. Univariate analysis of the plasma ET-1 concentration on CFVR was performed with the linear regression model. To exclude secondary effects and to find independent factors for CFVR, we performed multiple regression analysis. A p value of less than 0.05 was considered to represent statistical significance.

Results

Patient Characteristics

The main characteristics of the subjects are shown in Table 1. The percentage of females was significantly higher in the S group than in the N group. Systolic and diastolic BP levels were similar between the two groups. There were no differences in the serum cholesterol, high-density lipoprotein (HDL) cholesterol, or triglyceride levels, or in hemoglobin A1c between the two groups. There were no significant differences in LVMI and RWT between the two groups.

Coronary Flow Analysis between the Two Groups

No serious side effects occurred as a result of ATP infusion. Approximately 21.2% of subjects had mild palpitation; how-

Fig. 1. Average values of CFVR and plasma ET-1 concentration in the N group and S group. Values are shown as the mean $\pm$ SD.

ever, all of them were able to complete the entire study. Although ATP administration induced a decrease in BP and an increase in heart rate in both study groups (Table 2), the hemodynamic changes were similar to those previously observed in studies of CFVR measurements using the same protocol (11–14). Baseline MDV values were similar between the two groups; however, MDV during hyperemia tended to be lower in the S group than in the N group (Table 2). Thus, the CFVR for the S group was significantly lower than that for the N group (Fig. 1). Although most patients had CFVR values higher than 2.0, nine patients in the S group (25.7%) and four patients in the N group (12.9%) had CFVR values under 2.0 despite showing no coronary artery disease (Fig. 2).

Plasma ET-1 Concentration and Its Association with CFVR

The mean plasma ET-1 concentrations in the S and N groups are shown in Fig. 1 (S group: 3.85 ± 1.24 pg/ml, N group: 2.98 ± 1.27 pg/ml, respectively). The ET-1 concentration in the S group was significantly higher than that in the N group. Univariate analysis revealed that the plasma ET-1 concentration was significantly correlated with CFVR in hypertensive patients as a whole (Fig. 2). Multiple regression analysis was performed to define independent relationships between CFVR or ET-1, and other confounding coronary risk factors, such as age, systolic BP, body mass index, smoking status, total cholesterol, and LVMI. The results of this analysis indicated that age ($p=0.008$) and plasma ET-1 ($p=0.031$) concentration showed a statistically independent association with CFVR.

Discussion

Our study demonstrated that 1) CFVR was significantly lower and plasma ET-1 was significantly higher in hypertensive patients with chest pain syndrome, despite the fact that they showed no significant ischemic heart disease, and 2) an independent positive association between CFVR and plasma ET-

Fig. 2. Individual values of CFVR and plasma ET-1 concentration. The dotted lines represent the cut-off values (<2.0) of suspected coronary artery stenosis as reported in previous studies (14, 16).

1 level was evident in the hypertensive population as a whole.

A number of reports published in the past decade have described a reduction in CFVR in hypertensive patients (3, 12, 18, 19) that was thought to arise during advanced stages of disease, when left ventricular hypertrophy (LVH) becomes obvious (20, 21). However, recent reports have suggested that CFVR is decreased even at early disease stages in hypertensive patients without LVH (18, 19). This hemodynamic index has been useful for explaining the phenomena of silent ischemia and microvascular angina, which occur in patients with hypertensive heart disease, when occlusive atherosclerotic epicardial coronary disease cannot be demonstrated cineangiographically (22). Disturbance of coronary microcirculation is based on impaired blood flow in small (<200 μ m) intramural resistance vessels or in the coronary capillary system. Opherk *et al.* have suggested that the reduced CFVR in hypertensive individuals may be attributed to abnormalities in small intramyocardial vessels that cannot be visualized by coronary angiography (1). According to their model, the underlying cause of the reduced CFVR can be traced to small vessel disease and an increase in the extravascular component of coronary resistance due to elevated myocardial tissue pressure during diastole. However, Kozáková *et al.* have reported that reduced CFVR may be associated with inadequate myocardial neoangiogenesis or dysfunction of the microvascular endothelium (19). Moreover, Palombo *et al.* have suggested that endothelial dysfunction of coronary arterioles may be one of the key elements underlying the reduced CFVR in patients with hypertension (18). Indeed, a decrease in the release of endothelium-derived nitric oxide has been well documented in experimental animal models of hypertension as well as in hypertensive patients (23). Recently, we reported a significant correlation between CFVR, forearm endothelial function and asymmetric dimethylarginine, which is an endogenous

competitive inhibitor of nitric oxide synthases (24). Although ET-1 may play a role in angina-like chest pain, nitric oxide and other vasoactive substances related to endothelial function may also be involved.

Angina pectoris results from a mismatch between the myocardial supply and demand, due to a significant epicardial atherosclerotic lesion. Myocardial ischemia may also occur when, even in the absence of significant epicardial stenosis, there is diffused or focal vasoconstriction, which may result from such conditions as coronary spasm and coronary endothelial dysfunction. ET-1 is a potent vasoconstrictor peptide derived from endothelial cells and vascular smooth-muscle cells, and has mitogenic and possible algogenic properties (25, 26). McCarthy *et al.* demonstrated that a specific ET-1 receptor antagonist was able to cause significant coronary vasodilation in normal coronary arteries, suggesting that endogenous ET-1 plays an important role in the physiological regulation of basal coronary vasomotor tone (27). Many investigators have also reported that ET-1 may play an important role in coronary microcirculation and may be an important pathophysiologic mediator in microvascular angina. For example, Cox *et al.* reported that elevated endothelin activity might be correlated with reduced CFVR responses during rapid atrial pacing (28). In addition, Huelmos *et al.* reported an inverse relationship between plasma ET-1 levels and scintigraphically determined myocardial perfusion reserve values in a group of patients with cardiovascular risk factors (29). Our data also indicate an inverse correlation between plasma ET-1 levels and CFVR, further supporting the hypothesis that increased ET-1 activity may contribute to microvascular endothelial dysfunction, leading eventually to pathogenesis, including cardiac syndrome X or chest pain syndrome without epicardial coronary stenosis.

The gender specificity of chest pain syndrome, and of microvascular angina in particular, has been widely recognized (30). According to the Women's Ischemia Syndrome Evaluation (WISE) study (31), up to 50% of patients with chest pain and normal or near normal coronary angiograms have reduced CFVR values. In the present study, the percentage of female subjects in the symptomatic group was also higher than that of male subjects. The hypothesis that estrogen deficiency is associated with coronary endothelial dysfunction and abnormal vasomotor control in such women has been supported by evidence from a variety of studies, although the underlying mechanisms are not clearly understood (32).

Our study has several limitations. First, TTDE does not measure the absolute volumetric flow in the LAD but only flow velocity, although it has become an established method to assess coronary flow response. A mild increment of coronary artery diameter after ATP infusion might decrease the accuracy of the assessment of coronary flow reserve by the present method, leading to a slight underestimation of the flow response to the vasodilator. Second, we did not verify direct evidence of myocardial ischemia during chest pain in

the S group. It is possible that some patients in the S group did not have myocardial ischemia during their symptomatic period, although most patients showed effective response to sublingual nitroglycerin.

In conclusion, this study suggests that ET-1 plays an important role in attenuation of CFVR, leading to angina-like chest pain in patients with hypertension. In the clinical setting, hypertensive patients often complain of chest pain resulting from a variety of causes. The present findings may provide useful information for distinguishing between the cases of microvascular angina and those of non-cardiac origin that present similar symptoms. Furthermore, our data suggest that specific ET-1 antagonists are beneficial for treating patients with microvascular angina. Further pharmacological interventional studies are necessary to test their potential benefit.

References

1. Opher D, Mall G, Zebe H, *et al*: Reduction of coronary reserve: a mechanism for angina pectoris in patients with arterial hypertension and normal coronary arteries. *Circulation* 1984; **69**: 1–7.
2. Brush JE Jr, Cannon RO 3rd, Schenke WH, *et al*: Angina due to coronary microvascular disease in hypertensive patients without left ventricular hypertrophy. *N Engl J Med* 1988; **319**: 1302–1307.
3. Hamasaki S, Al Suwaidi J, Higano ST, Miyauchi K, Holmes DR Jr, Lerman A: Attenuated coronary flow reserve and vascular remodeling in patients with hypertension and left ventricular hypertrophy. *J Am Coll Cardiol* 2000; **35**: 1654–1660.
4. Panza JA: Myocardial ischemia and the pains of the heart. *N Engl J Med* 2002; **346**: 1934–1935.
5. Bugiardini R, Pozzati A, Ottani F, Morgagni GL, Puddu P: Vasotonic angina: a spectrum of ischemic syndromes involving functional abnormalities of the epicardial and microvascular coronary circulation. *J Am Coll Cardiol* 1993; **22**: 417–425.
6. Cox ID, Botker HE, Bagger JP, Sonne HS, Kristensen BO, Kaski JC: Elevated endothelin concentrations are associated with reduced coronary vasomotor responses in patients with chest pain and normal coronary arteriograms. *J Am Coll Cardiol* 1999; **34**: 455–460.
7. Hoffmann E, Assennato P, Donatelli M, Colletti I, Valenti TM: Plasma endothelin-1 levels in patients with angina pectoris and normal coronary angiograms. *Am Heart J* 1998; **135**: 684–688.
8. Kaski JC, Cox ID, Crook JR, *et al*: Coronary Artery Disease Research Group: Differential plasma endothelin levels in subgroups of patients with angina and angiographically normal coronary arteries. *Am Heart J* 1998; **136**: 412–417.
9. Kaski JC, Elliott PM, Salomone O, *et al*: Concentration of circulating plasma endothelin in patients with angina and normal coronary angiograms. *Br Heart J* 1995; **74**: 620–624.
10. Artigou JY, Salloum J, Carayon A, *et al*: Variations in plasma endothelin concentrations during coronary spasm. *Eur Heart J* 1993; **14**: 780–784.

11. Hozumi T, Yoshida K, Akasaka T, *et al*: Noninvasive assessment of coronary flow velocity and coronary flow velocity reserve in the left anterior descending coronary artery by Doppler echocardiography: comparison with invasive technique. *J Am Coll Cardiol* 1998; **32**: 1251–1259.
12. Takiuchi S, Rakugi H, Fujii H, *et al*: Carotid intima-media thickness is correlated with impairment of coronary flow reserve in hypertensive patients without coronary artery disease. *Hypertens Res* 2003; **26**: 945–951.
13. Takiuchi S, Rakugi H, Masuyama T, *et al*: Hypertension attenuates the efficacy of hypoglycemic therapy for preserving coronary flow reserve in patients with type 2 diabetes. *Hypertens Res* 2002; **25**: 893–900.
14. Hozumi T, Yoshida K, Ogata Y, *et al*: Noninvasive assessment of significant left anterior descending coronary artery stenosis by coronary flow velocity reserve with transthoracic color Doppler echocardiography. *Circulation* 1998; **97**: 1557–1562.
15. Branwald E: The history. in Branwald E, Zipes DP, Libby P (eds): Heart Disease, 6th ed. Philadelphia: W.B. Saunders Co., 2001, pp 30–31.
16. Matsumura Y, Hozumi T, Watanabe H, *et al*: Department of internal cut-off value of coronary flow velocity reserve by transthoracic Doppler echocardiography for diagnosis of significant left anterior descending artery stenosis in patients with coronary risk factors. *Am J Cardiol* 2003; **92**: 1389–1393.
17. Devereux RB, Reichek N: Echocardiographic determination of left ventricular mass in man. Anatomic validation of the method. *Circulation* 1977; **55**: 613–618.
18. Palombo C, Kozakova M, Magagna A, *et al*: Early impairment of coronary flow reserve and increase in minimum coronary resistance in borderline hypertensive patients. *J Hypertens* 2000; **18**: 453–459.
19. Kozáková M, Palombo C, Pratali L, Pittella G, Galetta F, L'Abbate A: Mechanisms of coronary flow reserve impairment in human hypertension. An integrated approach by transthoracic and transesophageal echocardiography. *Hypertension* 1997; **29**: 551–559.
20. Antony I, Nitenberg A, Foulst JM, Aptekar E: Coronary vasodilator reserve in untreated and treated hypertensive patients with and without left ventricular hypertrophy. *J Am Coll Cardiol* 1993; **22**: 514–520.
21. Sasaki O, Hamada M, Hiwada K: Effects of coronary blood flow on left ventricular function in essential hypertensive patients. *Hypertens Res* 2000; **23**: 239–245.
22. Maseri A, Crea F, Kaski JC, Crake T: Mechanisms of angina pectoris in syndrome X. *J Am Coll Cardiol* 1991; **17**: 499–506.
23. Piatti P, Fragasso G, Monti LD, *et al*: Endothelial and metabolic characteristics of patients with angina and angiographically normal coronary arteries: comparison with subjects with insulin resistance syndrome and normal controls. *J Am Coll Cardiol* 1999; **34**: 1452–1460.
24. Takiuchi S, Fujii H, Kamide K, *et al*: Plasma asymmetric dimethylarginine and coronary and peripheral endothelial dysfunction in hypertensive patients. *Am J Hypertens* 2004; **9**: 802–808.
25. Yanagisawa M, Kurihara H, Kimura S, *et al*: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature* 1988; **332**: 411–415.
26. Miyauchi T, Tomobe Y, Shiba R, *et al*: Involvement of endothelin in the regulation of human vascular tone. Potent vasoconstrictor effect and existence in endothelial cells. *Circulation* 1990; **81**: 1874–1880.
27. McCarthy PA, Pegge NC, Prendergast BD, Shah AM, Groves PH: The physiological role of endogenous endothelin in the regulation of human coronary vasomotor tone. *J Am Coll Cardiol* 2001; **37**: 137–143.
28. Cox ID, Salomone O, Brown SJ, Hann C, Kaski JC: Serum endothelin levels and pain perception in patients with cardiac syndrome X and in healthy controls. *Am J Cardiol* 1997; **80**: 637–640.
29. Huelmos A, Garcia Velloso MJ, Maceira A: Myocardial perfusion reserve and endothelin plasmatic levels in asymptomatic patients with cardiovascular risk factors. *Eur Heart J* 1997; **18** (Suppl): 435 (Abstract).
30. Cannon RO 3rd, Balaban RS: Chest pain in women with normal coronary angiograms. *N Engl J Med* 2000; **342**: 885–887.
31. Merz CN, Kelsey SF, Pepine CJ, *et al*: The Women's Ischemia Syndrome Evaluation (WISE) study: protocol design, methodology and feasibility report. *J Am Coll Cardiol* 1999; **33**: 1453–1461.
32. Rosano GM, Collins P, Kaski JC, Lindsay DC, Sarrel PM, Poole-Wilson PA: Syndrome X in women is associated with oestrogen deficiency. *Eur Heart J* 1995; **16**: 610–614.