EXPEDITED RESEARCH

Cardiovascular Therapeutics

Deficiency of a New Protein Associated with Cardiac Syndrome X; Called Adropin

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SUMMARY

The pathophysiology of cardiac syndrome X (CSX) is still unclear, but most patients with CSX have endothelial dysfunction. It has been shown that adropin uniquely effects the regulation of endothelial function. The purpose of the study was to evaluate the role of adropin in CSX. Eighty-six consecutive cardiac syndrome X-diagnosed patients and 86 age-sex matched healthy subjects were enrolled into the study. Serum adropin levels, nitrite/nitrate levels were measured in each subject. The adropin levels were significantly lower in patients with CSX than healthy subjects (1.7 ± 0.8 ng/mL and 3.4 ± 1.8 ng/mL, respectively; $P < 0.001$). The BMI values of patients with CSX were significantly higher than control subjects (28.1 ± 2.4 kg/m^2 and 26.0 ± 3.7 kg/m^2, respectively; $P < 0.001$). Plasma nitrite/nitrate levels were lower in patients with CSX than control subjects (15.9 ± 1.6 μmol/L vs. 25.4 ± 2.8 μmol/L, respectively; $P < 0.001$), and they have a significantly positive correlation with plasma adropin levels ($r = 0.463$, $P < 0.001$). In the multiple linear regression analysis, nitrite/nitrate levels, BMI, and adropin were found to be independent risk factors for CSX. A ROC curve is used to identify the ability of adropin levels to predict the cardiac syndrome X. The area under the ROC curve was 0.854 for adropin levels ($P = 0.0001$). The sensitivity and specificity values of adropin levels were 90.7 and 70.9%, respectively (cut-off value 2.73). In conclusion, lower serum adropin levels were associated with CSX. Adropin is an independent risk factor for CSX.

Patients with a normal coronary angiogram, effort-induced angina pectoris, positive exercise stress test, and/or positive single-photon emission computed tomography study are considered to have cardiac syndrome X (CSX) [1]. The long-term prognosis of patients with CSX appears to be good, but these patients have recurrent chest pain attacks, a situation that does increase the rate of morbidity. The pathogenesis of CSX is still unclear. Although endothelial dysfunction is regarded as one of the main underlying reasons for CSX, the pathophysiology seems to be more complex. Motz et al. [2] first showed the impaired coronary endothelial function in patients with CSX. Patients with CSX have increased resistance in coronary flow which is attributed to endothelial dysfunction in microcirculation [3]. The endothelium has very important roles in the control of vascular tone, including regulation of both vasodilatory substances such as nitric oxide and prostacycline, and vasoconstrictive substances such as endothelin-1 and angiotensin II [4]. An adequate balance between these vasoconstrictors and vasodilators is essential for maintaining vascular homeostasis. Nitric oxide synthesized from the amino acid L-Arginine by the endothelial nitric oxide–synthetase (eNOS) enzyme plays a key role in the regulation of vascular tone and also inhibits the activation of plaque and leukocytes and has an antiproliferative effect on smooth vascular muscle [4]. L-arginine and tetrahydrobiopterin (an eNOS cofactor) normalize the vasodilator response to acetylcholine in patients with CSX; however, impaired nitric oxide function is a major cause of the reduced acetylcholine-mediated vasodilatation in these patients [5].

Adropin is a recently identified protein that has been implicated in the maintenance of energy homeostasis and insulin resistance [6]. It is also a product of the Energy Homeostasis Associated (*Enho*) gene, which was recently identified during an investigation of obese insulin-resistant mice as a novel factor linking signals of nutrient intake with metabolic homeostasis [6]. Synthetic peptide or transgenic over expression of adropin improves glucose homeostasis, fatty liver, and dyslipidemia observed with obesity. Lovren et al. [7] showed the functions of adropin to include regulating angiogenesis and increasing blood flow and capillary density in a model of hind limb ischemia, and reported its potential endothelial protective role. Adropin uniquely effects the regulation of endothelial function, by upregulating eNOS expression through the VEGFR2-PI3K-Akt and VEGFR2-ERK1/2 pathways [7]. According to these data, we hypothesized that adropin may

have a potential role in the pathophysiology of CSX, and these patients may have lower levels of adropin than healthy subjects, possibly contributing to the endothelial dysfunction associated with CSX.

In this study, we researched the relation between the levels of adropin and patients with CSX. When the pathophysiology of CSX is revealed clearly, it should be possible to further develop the treatment choices for these patients. With this in mind, the goal of this study was to research the role of adropin in CSX and the relation between the circulating levels of adropin and patients with CSX.

Methods

A total of 86 consecutive patients with chest pain who were admitted to Elazig Education and Research Hospital (Turkey) Cardiology Department and diagnosed as CSX and 86 age-gender matched healthy subjects were enrolled between November 2010 and March 2012. All participants provided written informed consent to participate in the study. The study protocol was approved by the local Ethics Committee. Patients with CSX were included into the study according to the following definition of CSX [8]:

- Typical stable angina, exclusively or predominantly induced by effort;
- Findings compatible with myocardial ischemia/coronary microvascular disease on diagnostic investigation (including one or more of the following): (1) diagnostic ST segment depression during spontaneous or stress-induced typical chest pain; (2) reversible perfusion defects on stress myocardial scintigraphy; (3) documentation of stress-related coronary blood flow abnormalities by more advanced diagnostic techniques (e.g., cardiac magnetic resonance, positron emission tomography, Doppler ultrasound); and (4) metabolic evidence of transient myocardial ischemia (cardiac positron emission tomography or magnetic resonance, invasive assessment);
- Normal or near normal (vascular wall irregularities or discrete very mild stenosis (<20%) in epicardial vessels at angiography) coronary arteries on angiography;
- Absence of any other specific cardiac disease (e.g., variant angina, cardiomyopathy, valvular disease).

The exclusion criteria included the following: a history of coronary artery disease, heart failure, cardiomyopathy, acute or chronic renal failure, history of acute infection within the previous 7 days, acute or chronic hepatic failure, hematologic disorder, presence of any chronic inflammatory and autoimmune disease, and any known malignancy.

Exercise Stress Test

Patients underwent exercise treadmill testing using the modified Bruce protocol [9]. Predicted peak heart rate was calculated as (220 − age), with the aim being to reach at least 85% of the age-predicted heart rates. An electrocardiogram was continuously recorded during the exercise test. An exercise stress test was defined as positive in the presence of at least 1 mm horizontal or downsloping ST segment depression in at least two derivations and 60–80 ms after J point, typical angina, and/or ventricular arrytmias. After reaching the heart rate corresponding to the patient's age, or following development of the symptom, the exercise test was ended. Symptoms during exercise and the maximum stress capacity were recorded. A Duke treadmill score was calculated in each patient after stress test [10]. Patients with a Duke treadmill score of ≤ 4 were recognized as moderate- or high-risk patients and included in the study. Those with a Duke treadmill score of ≥ 5 were defined as low-risk patients and not subjected to coronary angiography and excluded from the study.

Single-Photon Emission Computed Tomography Imaging Protocol

To determine the myocardial viability, Technetium-99 m sestamibi scintigraphies were performed on patients. Miyocard perfusion scintigraphic images were analyzed by two experienced nuclear medicine physicians who had no knowledge of all the other data. All enrolled participants underwent a rest protocol using gated single-photon emission computed tomography myocardial perfusion imaging with technetium-99 m methoxyisobutylisonitrile (Tc-99 m MIBI). The positive myocardial perfusion imaging was defined according to the American Society of Nuclear Cardiology/American College of Cardiology/American Heart Association guidelines [11].

Coronary Angiographic Analysis

All patients with CSX underwent emergent coronary angiography using the Judkins technique with digitized coronary angiography equipment (Siemens, Medical Solutions 2007, Munchen, Germany). In all cases, the femoral artery was cannulated with a 6F sheath and coronary artery. Cannulation was performed using a 6F diagnostic catheter. Iohexol (Omnipaque, Nycomed Ireland, and Cork, Ireland) was used as a contrast agent during intervention in all patients.

Biochemical Analysis

Venous blood samples were taken after a minimum eight-hour overnight fast and 20 min of supine rest and drawn into ethylenediaminetetraacetic acid tubes and promptly centrifuged at 4°C, and frozen at −80°C until analyses of adropin. The plasma adropin levels were measured using an Enzyme-Linked ImmunoSorbent Assay kit (Phoenix Pharmaceuticals, Belmont, CA, USA) according to the manufacturer's instructions. Adropin measurements were validated, as described previously [12]. The detection range of kit was 0.01–100 ng/mL. The sensitivity was 0.3 ng/mL. Linear range was 0.3–8.2 ng/mL. The intra- (within day) and interassay (between days) coefficients of variations were 7.4 and 9.2%, respectively. Glucose was evaluated in serum by the glucose-oxidase method (Konelab-60I). Serum insulin (Biosource, Nivelles, Belgium) was estimated by immunoradiometric assay. Insulin resistance was determined using the homeostasis model of assessment – insulin resistance index as [fasting insulin level (μIU/mL) × fasting plasma glucose (mmol/L)/22.5] [13]. Triglyceride, total cholesterol, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C)

concentrations were measured by automated chemistry analyzer (Roche Diagnostics, Indianapolis, IN, USA) with commercially available kits. Mean platelet volume was measured from tripotassium ethylenediaminetetraacetic acid-based anticoagulated blood samples and assessed by a *Sysmex* K-1000 (Block. Scientific, Bohemia, NY, USA) autoanalyzer within 30 min of sampling. Serum nitrite/nitrate levels were obtained as mentioned previously [14]. Nitrite levels were determined using a colorimetric method [15]. For this assay, the following reagents were added to 100 mL of serum in an assay tube: 250 mL of 100 mmol/L potassium phosphate buffer (pH 7.4), 50 mL of distilled water, 50 mL of 0.2 mmol/L FAD, and 10 mL of 12 mmol/L b-NADPH. The enzymatic reaction was initiated through the addition and subsequent mixing of 50 mL of 500 U/L nitrate reductase. After mixing, the reaction was allowed to develop in the dark because FAD is photo labile. After a 45-min reaction period conducted at room temperature, the absorbance at a wavelength of 340 nm was measured with a Techcomp, 8500 II spectrophotometer (Techcomp, Shangai, China). Absorbance was converted to concentration using a calibration curve prepared with sodium nitrite in distilled water. Serum nitrate was measured as nitrite after enzymatic reduction by an improved method [15–17]. Values obtained by this procedure represent the sum of nitrite and nitrate. Nitrate concentration was obtained by subtracting nitrite concentration from total nitrite + nitrate [15,17].

Statistics

Continuous variables were given as mean ± SD; categorical variables were defined as percentages. Comparisons between the two groups were carried out using an independent samples *t* test and chi-square test. A multiple linear regression model was used to identify independent predictors of CSX. Sensitivity and specificity values of adropin levels for predicting CSX were estimated using receiver operator characteristic curve analysis. The cut-off levels of adropin were determined using MedCalc 9.2.0.1 (MedCalc Software, Mariakerke, Belgium). Multiple linear regression analysis was applied to identify whether adropin was independently associated with CSX. Correlation analyses were performed using the Pearson coefficient of correlation. SPSS 15.0 software was used for basic statistical analysis (Version 15, SPSS Inc., and Chicago, IL, USA). A value of $P < 0.05$ was accepted as statistically significant.

Results

The baseline characteristic properties of study patients showed no significant differences between the two groups with respect to sex distribution, age, frequencies of major coronary risk factors (hyperlipidemia, smoking, family history of coronary artery disease), fasting glucose, serum creatinine, total cholesterol, LDL-C, HDL-C, triglyceride, and mean platelet volume ($P > 0.05$ for all) (Table 1). The history rate of diabetes mellitus and hypertension was significantly higher in the CSX than the control group (respectively, 34.9–15.1%, $P = 0.002$ for diabetes mellitus and 46.5–29.1%, $P = 0.01$ for hypertension). The levels of serum adropin were significantly lower in the patients with CSX than the healthy subjects (1.7 ± 0.8 ng/mL and 3.4 ± 1.8 ng/mL, respectively; $P < 0.001$). The BMI values of the patients with CSX were significantly higher than the control subjects (28.1 ± 2.4 kg/m^2 and 26.0 ± 3.7 kg/m^2, respectively; $P < 0.001$). The levels of adropin were weakly negative correlated with BMI values ($r = -0.141$, $P = 0.06$).

Table 1 The baseline characteristic properties of study patients

	CSX Group (n = 86)	Control Group (n = 86)	*P*-value
Age (years)	54.8 ± 9.2	52.7 ± 8.5	0.1
Gender (female, %)	67.4	59.3	0.1
History of (%)			
Diabetes mellitus	34.9	15.1	0.002
Hypertension	46.5	29.1	0.01
Hyperlipidemia	25.6	18.6	0.1
Smoke	31.4	26.7	0.3
Family history of CAD	22.1	15.1	0.1
Fasting glucose (mg/dL)	110 ± 34	105 ± 20	0.3
Creatinin (mg/dL)	0.7 ± 0.2	0.7 ± 0.1	0.2
Triglyceride (mg/dL)	169 ± 118	146 ± 82	0.1
Total cholesterol (mg/dL)	191 ± 56	179 ± 39	0.09
LDL-C (mg/dL)	117 ± 50	110 ± 34	0.2
HDL-C (mg/dL)	38 ± 11	38 ± 9	0.9
MPV (fl)	10.1 ± 1.5	9.8 ± 1.3	0.2
BMI (kg/m^2)	28.1 ± 2.4	26.0 ± 3.7	<0.001
Adropin (ng/mL)	1.7 ± 0.8	3.4 ± 1.8	<0.001
Nitrite/Nitrate levels (μmol/L)	15.9 ± 1.6	25.4 ± 2.8	<0.001

CSX, cardiac syndrome X; CAD, coronary artery disease; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; MPV, mean platelet volume; BMI, body mass index. $P < 0.05$ was accepted as statistically significant.

Dividing the patients into two groups according to BMI values, the normal weight group consisting of patients with ≤25 kg/m^2 values (n = 48) and obesity group of patients with >25 kg/m^2 values (n = 123), the levels of adropin were found to be lower in the obesity group but not statistically significant (2.4 ± 1.4 to 2.9 ± 2.0 ng/mL, $P = 0.1$). The values of the homeostasis model of assessment – insulin resistance score were significantly higher in patients with CSX than in the control group (3.3 ± 1.2 to 3.0 ± 0.8, $P = 0.03$). Dividing the patients into two groups according to homeostasis model of assessment – insulin resistance score (≥2.5 = insulin-resistant group, <2.5 = insulin nonresistant group) showed the levels of adropin to be not different in the insulin-resistant than insulin nonresistant group (respectively; n = 119 and n = 53, adropin levels were 2.5 ± 1.6 to 2.7 ± 2.7 ng/mL, $P = 0.4$). Plasma nitrite/nitrate levels were lower in patients with CSX than control subjects (15.9 ± 1.6 μmol/L vs. 25.4 ± 2.8 μmol/L, respectively; $P < 0.001$). Plasma nitrite/nitrate levels have a significantly positive correlation with plasma adropin levels ($r = 0.463$, $P < 0.001$). In the linear regression analysis, nitrite/nitrate levels, BMI, and BMI were found to be independent risk factors for CSX (Table 2).

A receiver operator characteristic curve was used to identify the ability of adropin levels to predict the cardiac syndrome X (Figure 1). The area under the receiver operator characteristic curve was 0.854 for adropin levels ($P = 0.0001$). The sensitivity and specificity values of adropin levels were 90.7 and 70.9%, respectively (cut-off value 2.73).

Table 2 The linear regression analysis of factors predicting cardiac syndrome X

	ß	t	*P*
Nitrite/nitrate	−0.826	−22.254	<0.001
BMI	0.098	2.952	0.004
Adropin	−0.104	−2.872	0.005

BMI, body mass index.

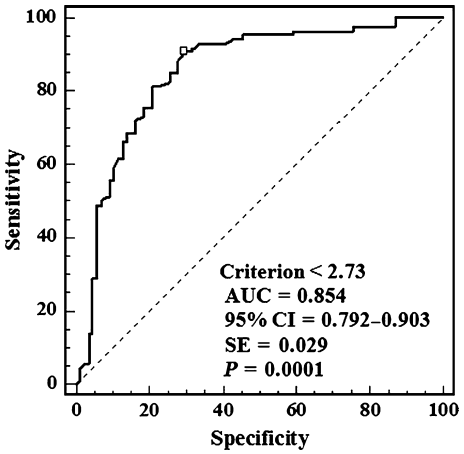

Figure 1 ROC curve of adropin levels in study patients for predicting cardiac syndrome X. AUC, area under curve; SE, standard error; CI, confidence interval.

Discussion

Up to 30% of patients with chest pain, who undergo coronary angiography, have normal coronary arteries. Patients with coronary arteries that appear normal on angiography who have typical angina and have a positive stress test are simply termed CSX [1]. Several causes and mechanisms have been investigated to explain both chest pain and ischemic angina-like ST depression in these patients. Among the suggested pathophysiological mechanisms, endothelial dysfunction of the coronary microcirculation has a very important role [4].

The protective role of adropin on the endothelium has been shown previously [7], and we hypothesized that adropin deficiency has a relationship with endothelial dysfunction which is a major cause of CSX. In the present study, we showed the lower circulating adropin levels to be associated with CSX. Adropin levels lower than 2.73 ng/mL predict CSX with a 90.7% sensitivity and 70.9% specificity. We believe that these results are very important to an understanding of the pathophysiology of CSX because this is the first study demonstrating the association between serum adropin levels and CSX. It was seen that the patients with CSX have lower levels of adropin, and so, we hypothesized that lower levels of adropin might have a crucial role in the pathophysiology of CSX.

The endothelium plays a crucial role in the maintenance of vascular homeostasis, and endothelial dysfunction contributes to the development and progression of cardiovascular diseases. Nitric oxide is a potent endogenous vasodilator which is formed in the endothelium by the endothelial isoform of eNOS; released in response to shear stress, it plays an important role in flow-mediated dilatation. Nitric oxide plays an important role in maintaining endothelial homeostasis. It also inhibits adhesion of monocytes and leukocytes to the endothelium, aggregation of platelets, oxidation of low-density lipoprotein, and smooth muscle cell proliferation [18–21]. Low levels of nitric oxide are associated with endothelial dysfunction. Although NO can be measured directly using NO-specific electrodes and chemiluminescence, the measurement of the stable end products of NO metabolism, nitrite, and nitrate is commonly used as a rapid and simple way to assess NO production. In the present study, we used nitrite and nitrate levels which is an indirect measure of NO bioavailability and found lower nitrite/nitrate levels in patients with CSX.

Adropin was recently identified as a secreted protein critically involved in energy homeostasis, metabolic adaptation to macronutrients, and modulation of insulin sensitivity and diabesity [6]. Even though increased adropin levels were also found in patients with heart failure [22], we found that the lower levels of adropin were associated with patients with CSX. It was found that adropin has a protective and regulator role on endothelial function (7). When considering the endothelial dysfunction has been widely reported in patients with heart failure, it was expected that patients with heart failure should have lower adropin levels. But Lian et al. showed higher adropin levels in patients with heart failure. So, further studies are needed to elucidate the role of adropin in heart failure. Overexpression or systemic administration of adropin in diet-induced obese mice resulted in a significant improvement in insulin sensitivity, reduction in diabetes, and weight loss. Ganesh Kumar et al. [23] have showed the association between adropin deficiency and increased adiposity and insulin resistance. Lower adropin levels were seen in diet-induced obesity. Lovren et al. recently showed that adropin is also expressed in the human umbilical vein and coronary artery endothelial cells. Adropin increases nitric oxide release and activates eNOS, so it directly affects the endothelium and has a protective role for endothelium via upregulating eNOS [7]. According to these studies, we hypothesize that higher adropin levels are protective for endothelium, insulin resistance, and glucose tolerance, while according to our study, low adropin levels are also associated with CSX. In the present study, we found that serum nitrite/nitrate levels were lower in patients with CSX like adropin levels. These results support our hypothesis.

Although the mortality of patients with CSX is considered favorable [24], it is, nevertheless, still higher as related to the recurrent chest pain in these patients [25,26]. The main aim of clinical management in patients with CSX is the control of symptoms. Medical therapy includes beta-blockers, nitrates, calcium channel blockers alone or in combination, statins in patients with hyperlipidemia, angiotensin-converting enzyme inhibitors in hypertensive patients, and nicorandil therapy; these are reported to be helpful in a significant proportion of patients [27]. It was thought that insulin resistance and increased serum insulin levels have a key role in coronary microvascular dysfunction in CSX

[28]. Ganesh Kumar et al. [6] showed that systemic adropin administration or transgenic overexpression of adropin reduces insulin resistance and glucose intolerance in diet-induced obese mice. We believe that future studies should research the effect of adropin-based therapies in the treatment for CSX by improving endothelial function.

The main limitation of the present study was the small number of patients with CSX. It will be very useful to see the effect of adropin-based therapies in these patients. Therefore, large and long-term follow-up studies are needed with patients with CSX after adropin administration. Other limitation was using nitrite/nitrate levels for determining NO which is an indirect measure of NO bioavailability and the lack of a measure for endothelial dysfunction in the subjects investigated.

Conclusions

Serum adropin levels were lower in patients with CSX as compared with age-sex-matched control subjects. The present findings will be useful for new studies in the future aimed at the development of a new treatment for patients with CSX.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Vermeltfoort IA, Raijmakers PG, Riphagen II, Odekerken DA, Kuijper AF, Zwijnenburg A, Teule GJ. Definitions and incidence of cardiac syndrome X: review and analysis of clinical data. *Clin Res Cardiol* 2010;**99**:475–481.
2. Motz W, Vogt M, Rabenau O, Scheler S, Lückhoff A, Strauer BE. Evidence of endothelial dysfunction in coronary resistance vessels in patients with angina pectoris and normal coronary angiograms. *Am J Cardiol* 1991;**68**:996–1003.
3. Epstein SE, Cannon RO III. Site of increased resistance to coronary flow in patients with angina pectoris and normal epicardial coronary arteries. *J Am Coll Cardiol* 1986;**8**:459–461.
4. Vázquez-Rey E, Kaski JC. Cardiovascular syndrome X and endothelial dysfunction. *Rev Esp Cardiol* 2003;**56**:181–192.
5. Cooke JP, Rossitch E Jr, Andon NA, Loscalzo J, Dzau VJ. Flow activates an endothelial potassium channel to release an endogenous nitrovasodilator. *J Clin Invest* 1991;**88**:1663–1671.
6. Kumar KG, Trevaskis JL, Lam DD, et al. Identification of adropin as a secreted factor linking dietary macronutrient intake with energy homeostasis and lipid metabolism. *Cell Metab* 2008;**8**:468–481.
7. Lovren F, Pan Y, Quan A, et al. Adropin is a novel regulator of endothelial function. *Circulation* 2010;**122**(11 Suppl):S185–S192.
8. Lanza GA. Cardiac syndrome X: a critical overview and future perspectives. *Heart* 2007;**93**:159–166.
9. Bruce RA, Blackmon JR, Jones JW, Strait G. Exercising testing in adult normal subjects and cardiac patients. *Pediatrics*. 1963;**32**(Suppl):742–756.
10. Mark DB, Hlatky MA, Harrell FE Jr, Lee KL, Califf RM, Pryor DB. Exercise treadmill score for predicting prognosis in coronary artery disease. *Ann Intern Med*, 1987;**106**:793–800.
11. Cerqueira MD, Weissman NJ, Dilsizian V, et al. American Heart Association Writing Group on Myocardial Segmentation and Registration for Cardiac Imaging. Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart: a statement for healthcare professionals from the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American Heart Association. *Circulation* 2002;**105**:539–542.
12. Aydin S. A comparison of ghrelin, glucose, alpha-amylase and protein levels in saliva from diabetics. *J Biochem Mol Biol* 2007;**40**:29–35.
13. Bonora E, Targher G, Alberiche M, et al. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity. *Diabetes Care* 2000;**23**:57–63.
14. Aydin S, Aydin S, Croteau AG, Sahin I, Citil C. Ghrelın, nitrite and paraoxonase/arylesterase concentrations in cement plant workers. *J Med Biochem* 2010;**29**:78–83.
15. Bories PN, Bories C. Nitrate determination in biological fluids by an enzymatic one-step assay with nitrate reductase. *Clin Chem* 1995;**41**:904–907.
16. Gutman SI, Hollywood CA. Simple, rapid method for determining nitrates and nitrites in biological fluids. *Clin Chem* 1992;**38**:2152.
17. Nithipatikom K, Pratt PF, Campbell WB. Nitro-Larginine interferes with the cadmium reduction of nitrate/griess reaction method of measuring nitric oxide production. *Eur J Clin Chem Clin Biochem* 1996;**34**:133–137.
18. Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc Natl Acad Sci USA* 1991;**88**:4651–4655.
19. Wolf A, Zalpour C, Theilmeier G, et al. Dietary L-arginine supplementation normalizes platelet aggregation in hypercholesterolemic humans. *J Am Coll Cardiol* 1997;**29**:479–485.
20. Hogg N, Kalyanaraman B, Joseph J, Struck A, Parthasarathy S. Inhibition of low-density lipoprotein oxidation by nitric oxide. Potential role in atherogenesis. *FEBS Lett* 1993;**334**:170–174.
21. Boger RH, Bode-Boger SM, Kienke S, Stan AC, Nafe R, Frölich JC. Dietary L-arginine decreases myointimal cell proliferation and vascular monocyte accumulation in cholesterol-fed rabbits. *Atherosclerosis* 1998;**136**:67–77.
22. Lian W, Gu X, Qin Y, Zheng X. Elevated plasma levels of adropin in heart failure patients. *Intern Med* 2011;**50**:1523–1527.
23. Ganesh Kumar K, Zhang J, Gao S, et al. Adropin deficiency is associated with increased adiposity and insulin resistance. *Obesity (Silver Spring)* 2012;**20**:1394–1402. Doi: 10. 1038/oby.2012. 31.
24. Kaski JC, Rosano GM, Collins P, Nihoyannopoulos P, Maseri A, Poole-Wilson PA. Cardiac syndrome X: clinical characteristics and left ventricular function. Long-term follow-up study. *J Am Coll Cardiol* 1995;**25**:807–814.
25. Bugiardini R, Bairey Merz CN. Angina with 'normal' coronary arteries: a changing philosophy. *JAMA* 2005;**293**:477–484.
26. Atienza F, Velasco JA, Brown S, Ridocci F, Kaski JC. Assessment of quality of life in patients with chest pain and normal coronary arteriogram (syndrome X) using a specific questionnaire. *Clin Cardiol* 1999;**22**:283–290.
27. Fox K, Garcia MA, Ardissino D, et al. ; Task Force on the Management of Stable Angina Pectoris of the European Society of Cardiology; ESC Committee for Practice Guidelines (CPG). Guidelines on the management of stable angina pectoris: executive summary: the task force on the management of stable angina pectoris of the European society of cardiology. *Eur Heart J* 2006;**27**:1341–1381.
28. Vestergaard H, Skøtt P, Steffensen R, Wroblewski H, Pedersen O, Kastrup J. Insulin-resistant glucose metabolism in patients with microvascular angina–syndrome X. *Metabolism* 1995;**44**:876–882.

