

Exercise stress test and comparison of ST change with cardiac nucleotide catabolite production in patients with coronary artery disease

Sławomir Burakowski¹, Ryszard T. Smoleński², Jerzy Bellwon¹,
Andrzej Kubasik¹, Dariusz Ciećwierz¹ and Andrzej Rynkiewicz¹

¹1st Department of Cardiology, Medical University of Gdańsk, Poland

²Department of Biochemistry, Medical University of Gdańsk, Poland

Abstract

Background: *Uridine (Ur) and hypoxanthine (Hx) are the major end products of ischemic nucleotide breakdown in the human heart. Hypoxanthine is further metabolized to uric acid (UA). The aim of the study was the evaluation of whether changes in nucleotide concentrations during exercise correlate with electrocardiography (ECG) changes, and the severity of coronary artery disease (CAD).*

Methods: *Twenty-nine males with CAD and 11 controls without CAD (mean age 56.1 vs. 51.45) were subjected to treadmill exercise. The test was considered positive if ECG showed more than 1 mm ST segment depression. Venous blood samples taken before and 10 minutes after the exercise were analysed by high performance liquid chromatography.*

Results: *Twenty-two out of 29 patients with CAD and 6 of 11 in the control group had abnormal exercise stress tests according to ECG criteria only. Mean ΔUr was positive in the CAD group and negative in the control group ($0.45 \text{ SEM} \pm 0.09 \mu\text{M/L}$ vs. $-0.43 \text{ SEM} \pm 0.21 \mu\text{M/L}$, $p < 0.0001$). ΔUA was positive in the CAD group ($15.31 \text{ SEM} \pm 5.52 \mu\text{M/L}$) and negative in the control group ($15.31 \text{ SEM} \pm 5.52 \mu\text{M/L}$ vs. $-48.18 \text{ SEM} \pm 13.8 \mu\text{M/L}$, $p < 0.00001$); Hx increased in both groups, and the change was not significantly different. Correlations of CAD-index with ST depression, ΔUr and ΔUA , were: $r = 0.43$ ($p < 0.005$), $r = 0.62$ ($p < 0.001$), and $r = 0.39$ ($p < 0.01$), respectively. Sensitivity of any increase of uridine was superior to 1.5 mm ST depression during exercise.*

Conclusions: *Blood Ur and UA concentration changes during exercise correlate with severity of CAD. We observed slightly greater accuracy of uridine change in comparison to ST changes, thus being a possible new tool in diagnosis of CAD. (Cardiol J 2007; 14: 573–579)*

Key words: exercise stress test, coronary artery disease, uridine, hypoxanthine, uric acid, pyrimidine and purine catabolism in the human heart

Address for correspondence:

Dr. Sławomir Burakowski

1st Department of Cardiology

Medical University of Gdańsk

Dębinki 7, 80–211 Gdańsk, Poland

Tel: +48 58 349 16 13, fax: +48 58 349 27 01

e-mail: sbura@amg.gda.pl

Received: 2.08.2007 Accepted: 20.09.2007

Table 1. Clinical characteristics.

	Coronary artery disease group (n = 29)	Control group (n = 11)
Age (years)	56.14 ± 1.52	51.45 ± 2.44
Body mass index [kg/m ²]	28.09 ± 0.53	25.68 ± 0.48
Systolic blood pressure [mm Hg]	135.5 ± 3.86	137.27 ± 5.65
Diastolic blood pressure [mm Hg]	82.24 ± 2.17	83.18 ± 2.8
Total cholesterol [mg/dl]	237 ± 8.89	216 ± 7.62
Left ventricular ejection fraction (%)	57.17 ± 2.41	69.5 ± 1.01

Data shown are means ± SEM

Introduction

Despite the decreasing morbidity observed in developed countries in recent decades, the number of patients in Poland with angina pectoris suspected of coronary artery disease (CAD) is still high [1]. The number of deaths before arrival to hospital is very high, and in about 70% of them, CAD is the cause [2]. The symptomatology of this common disease is very complex, and noninvasive investigations still lack either sufficient sensitivity or specificity or are just too expensive for broad application. It is still important to develop easy to perform and inexpensive tests in order to meet the needs of current practice to decide whether patients should be referred to interventional cardiologists or start intensive medical treatment (including statins for life), even if total cholesterol is within the "normal" range.

Experimental studies performed in recent years have established that ischemia induces the release of purine and pyrimidine catabolites. They also identified several fundamental cell-specific, organ-related or species-dependent differences. It has been established that the main purines released from cardiomyocytes are adenosine and inosine. Further catabolic degradation of these nucleosides takes place in the endothelium, erythrocytes, liver and intestines. The uric acid is the main end product [3].

The breakdown of pyrimidines proceeds in parallel with purines and has a specific pattern in the human heart [4, 5]. Due to the high activity of cytidine deaminase and very low (or absent) activity of uridine phosphorylase, the main catabolite released from cardiomyocytes is uridine, which is fairly stable in the circulation (minutes) in contrast to hypoxanthine which is further catabolised to uric acid. Although uridine is not metabolized in the heart — human liver and intestines have some capacity for the breakdown of this nucleoside so that not uridine but its catabolite, uracil, is produced. In

addition, during vigorous exercise, blood flow in visceral circulation is reduced. Uridine could therefore be a relatively specific marker of ischemic nucleotide breakdown in the heart. In our former studies, we observed that arterial measurements of hypoxanthine and uridine were sensitive markers of cardiac ischemia produced during prolonged (3 min) occlusion of coronary arteries during percutaneous transluminal coronary angioplasty (PTCA) [6].

The aim of this study was to evaluate the change of concentration in venous blood uridine (Ur), hypoxanthine (Hx) and uric acid (UA) during exercise stress tests among patients with CAD confirmed by coronarography.

Methods

Patients

This study was approved by the local Ethics Committee of the Medical University of Gdansk (TKEBN/406/98). We investigated only men in order to make the groups as homogenous as possible. Table 1 presents the clinical characteristics of our subjects. Forty men participated in the study. Twenty-nine patients with angiographically confirmed CAD and 11 controls without CAD: either free of the symptoms of the disease without family history of CAD or truly excluded CAD in coronarography performed among 3 patients with modest ST depression and clinically inconclusive exercise stress test. Their age range was 35–70.

Coronarography and coronary artery disease index

Coronarography was performed in a typical way and we analysed stenoses on the basis of non quantitative assessment. Severity of coronary atherosclerosis was indexed according to a slightly modified Jenkins scale depending on the percentage narrowing of the vessel lumen [7]: 0–50% — 1 point; 51–75% — 2 points; 76–99% — 3 points; 100% — 4 points.

The index is a sum of points obtained for maximal stenosis in each of the three main coronary arteries. Maximum index for one patient is 12. Patients with left main disease were not included the study.

Study protocol

The patients fasted, and the exercise stress tests (EST) were performed in the morning. 0.8 ml venous blood sample was taken before EST for purine and pyrimidine catabolism end products. Symptoms-limited treadmill EST was performed according to Bruce (or modified Bruce protocol when necessary), with angina pectoris measured in 10 grade Borg scale (for safety reasons in our study, the maximum grade was 7) [8], ST deviation in mm, and workload in METs on a Marquette Case 15 treadmill. The test was regarded abnormal if ST was depressed more than 1 mm 80 ms from J point. Ten minutes after cessation of exercise, a 0.8 ml venous blood sample was taken. The blood was immediately deproteinised and the extracts were stored deep frozen for further analysis of purine and pyrimidine catabolism end products.

Metabolic determinations

End products of purines and pyrimidine catabolism were analysed using the high performance liquid chromatography (HPLC) system described in detail previously [9]. An analytic column (reverse phase) 0.49/15 cm packed with 3 μm BDS-Hypersil with 0.5 cm precolumn was used. Buffer A was 150 mM KH_2PO_4 with 150 mM KCl at pH 6.0. Buffer B was prepared by adding 15% acetonitrile to buffer A. Separation was carried out using the following linear step gradient: 0 min — 0% B, 0.1 min — 6% B, 3 min — 6% B, 5.5 min — 50% B, 8 min — 100% B, 9.4 min — 100% B, 9.5 min — 0% B, 13 min — 0% B. Time between injections was 13.5 min. The amounts in the samples were calculated using external standard calibration procedure.

Statistical analysis

The data was analysed with Statistica 7.0 (StatSoft). Pre- and post-exercise differences in concentrations of Ur, Hx and UA were calculated and compared. We calculated the means of changes of hypoxanthine, uric acid and uridine and compared them between the investigated groups. Once we confirmed the statistically significant differences between the means of those changes, the Pearson's linear correlations were calculated separately for each biochemical and clinical parameter in both study groups. In order to evaluate the correlation of clinical parameters with the biochemical

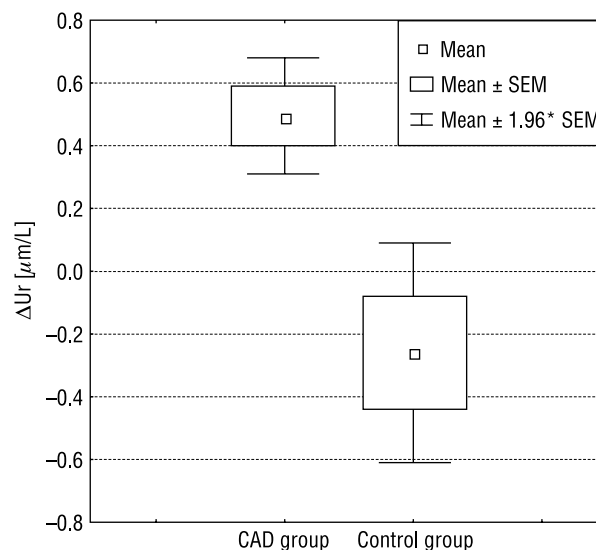


Figure 1. Comparison of uridine changes between coronary artery disease (CAD) group and control group ($p < 0.0001$).

changes, we performed multistep logistic regression analysis with sequential removal of the least important clinical parameters which influenced dependant biochemical variables (eg. Ur, Hx and UA). Finally, we also calculated the predictive (diagnostic) value of the measured clinical and biological parameters with different cut-off values.

Results

The mean CAD index in the study group was 6.34, which corresponds to the significant narrowing of at least two coronary vessels. Assumed (or true with negative coronarography) CAD index in the whole control group was zero. We found a significant increase of uridine during EST in the CAD group in comparison to the controls. The mean change in blood uridine (ΔUr) was positive in the CAD group after EST ($0.45 \text{ SEM} \pm 0.09 \mu\text{M/L}$) and negative in the control group ($-0.43 \text{ SEM} \pm 0.21 \mu\text{M/L}$, $p < 0.0001$; Fig. 1). After EST, hypoxanthine increased in both groups, and the change (ΔHx) was not significantly different between CAD and controls ($1.83 \text{ SEM} \pm 0.45 \mu\text{M/L}$ vs. $2.66 \text{ SEM} \pm 0.53 \mu\text{M/L}$; NS; Fig. 2). Uric acid change (ΔUA) differed statistically significantly: ΔUA was positive in the CAD group ($15.31 \text{ SEM} \pm 5.52 \mu\text{M/L}$) and negative among the controls ($-48.18 \text{ SEM} \pm 13.87 \mu\text{M/L}$; $p < 0.00001$; Fig. 3).

Mean ST change between the groups was significant ($-1.92 \text{ SEM} \pm 0.18 \text{ mm}$ vs. $-0.92 \text{ SEM} \pm 0.26 \text{ mm}$; $p < 0.0007$; Fig. 4).

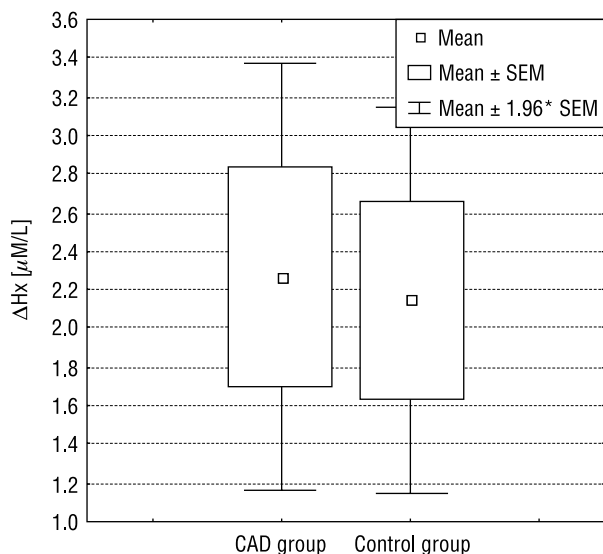


Figure 2. Comparison of hypoxanthine changes between coronary artery disease (CAD) group and control group (NS).

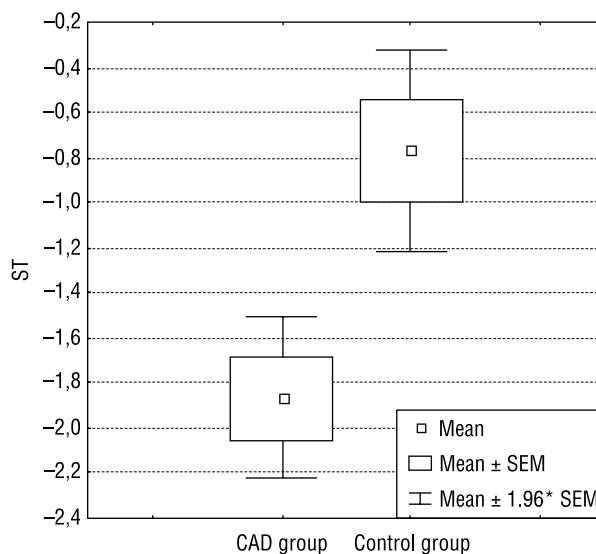


Figure 4. Comparison of ST changes between coronary artery disease (CAD) group and control group ($p < 0.0007$).

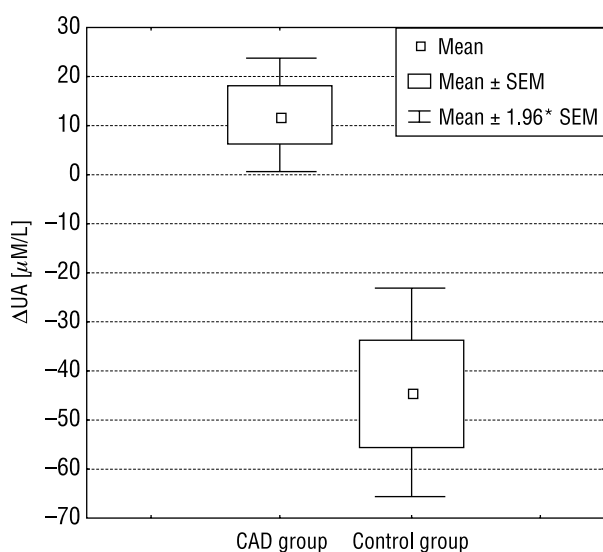


Figure 3. Comparison of uric acid changes between coronary artery disease (CAD) group and control group ($p < 0.0002$).

Table 2. Correlation of coronary artery disease index with ST change and biochemical parameter change.

Parameters	r	p
ST depression	0.43	< 0.005
ΔUr	0.62	< 0.001
ΔUA	0.39	< 0.01

change and the CAD index. Borderline statistical significance was observed for UA change.

The strongest correlation was observed for Angina score and CAD index ($r = 0.66$; $p < 0.05$; Fig. 5). ST change was not significantly related to UA change ($r = -0.26$; NS) and similarly uridine was not significantly related to ST changes ($r = -0.07$; NS).

Of note is the lack of a statistically significant correlation between ST depression and angina. In addition, correlation of uridine change with angina was rather weak ($r = 0.22$; NS).

In multiple regression analysis, we found a significant relation of uridine, ST depression and angina Borg score to the CAD index, and the strongest dependence was observed for uridine, as shown in Table 3.

The final calculations were made in order to assess the diagnostic value of measuring the uridine change in venous blood before and after EST.

The predictive (diagnostic) value of uridine change in venous blood is presented in Table 4.

Correlation coefficients of CAD-index with ST depression, change in Ur and change in UA were: $r = -0.43$ ($p < 0.005$), $r = 0.62$ ($p < 0.001$) and $r = 0.39$ ($p < 0.01$), respectively (Table 2).

Significant (negative) correlation was observed between ST depression during EST and CAD-index and significant (positive) between uridine

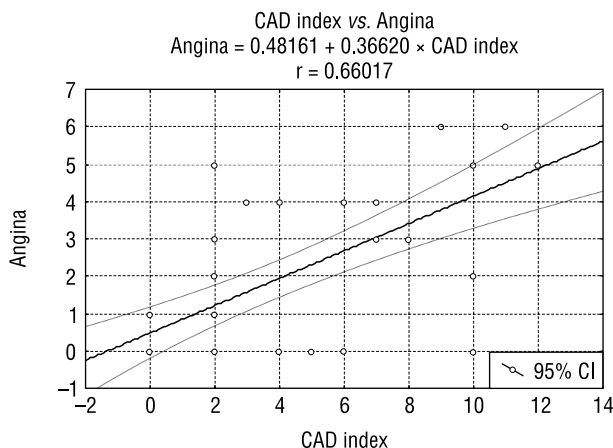


Figure 5. Correlation of coronary artery disease (CAD) indices with Borg scale.

Table 3. Multiple regression analysis of association of clinical and biochemical variables and coronary artery disease index.

Independent variable	Statistical significance (p)
ΔUr	< 0.0001
ST	< 0.004
Angina (Borg)	< 0.003
ΔUA	NS
MET	NS
Age	NS
Body mass index	NS
Ejection fraction	NS

Twenty-two out of 29 patients with CAD had positive exercise stress tests according to standard electrocardiography (ECG) criteria indicating a sensitivity of 79% and specificity 36%. The mean change in blood uridine was positive in the CAD group ($0.45 \pm 0.09 \mu\text{M/L}$, mean \pm SEM) and negative in the control group ($0.43 \pm 2.16 \mu\text{M/L}$; $p < 0.0001$). Any positive change in uridine concentration indicates CAD with sensitivity of 75% and specificity 72%. Setting cut-off values of ST depres-

sion to 1.5 mm and ΔUr to $0.1 \mu\text{M/L}$ resulted in both becoming more valuable for suspecting or ruling out CAD but still in favour of uridine.

Discussion

The major finding of this study is the demonstration of the potential diagnostic value of monitoring uridine concentration changes during treadmill exercise stress test for detection or ruling out of coronary artery disease. Among patients with angiographically confirmed CAD, a statistically significant change of concentration in peripheral blood of uridine was observed after EST in comparison to the control group. Uridine concentration change after EST showed a correlation with the CAD index, comparable to the correlation of ST depression during exercise.

Some differences between patients and controls were observed for age, body mass index (BMI) and ejection fraction (EF). The study group was older, but this minor age difference would not change the probability of having CAD according to assessment based on gender, age and presence of angina [10, 11]. Body mass index was higher in the study group, which corresponds to the risk factors. Lower EF was a result of CAD and previous myocardial infarction among some patients. Of note is the lack of statistical difference in total cholesterol between the groups.

About 50% of patients referred for a submaximal (at 85% of heart limit) EST with ECG assessment do not obtain a definite answer e.g. test negative or positive for ischemia, mostly because of beta adrenergic blockade, poor exercise tolerance or hypertonic reaction. Another subset of patients had nonspecific ECG changes at rest with borderline ST shift during exercise, without angina or with atypical chest pain. In our study, only the ECG criteria were analysed. In the majority of patients (approx 70% — our own data), the treadmill exercise stress is clinically appropriate and provides sufficient data even if the heart limit was not obtained. As well as ST changes, the same attention is attributed

Table 4. Diagnostic accuracy.

Cut-off criteria	Sensitivity	Specificity	PPV	NPV	OR
ST depression 1 mm	79%	36%	76%	40%	1.24
ST depression 1.5 mm	68%	81%	91%	50%	3.79
ΔUr > 0.0 $\mu\text{M/L}$	75%	72%	88%	53%	2.78
ΔUr > 0.1 $\mu\text{M/L}$	82%	91%	96%	66%	9.1

PPV — positive predictive value; NPV — negative predictive value; OR — odds ratio (positive likelihood ratio)

to exercise capacity, presence of angina and blood pressure behaviour during exercise, including the incidence of arrhythmia or abnormalities of conduction, according to guidelines [11].

The next step in diagnosing CAD usually includes either single photon emission computed tomography (SPECT) cardiac examination (Thallium 99) or dobutamine stress echocardiography. High resolution computed tomography (Cardio-CT) or magnetic resonance imaging investigation could be other possibilities of noninvasive assessment of the presence of coronary arteriosclerosis.

Over the past 20 years, we have observed an improvement in the understanding of the basic metabolism of the human heart. This may provide the basis for distinguishing the true heart ischemia from other benign causes of chest pain. Measurement of cardiac troponins, I or T, among patients experiencing short-term heart ischemia seems to be of limited value in this setting, as these large molecules can only be released if the cell membrane is damaged after prolonged severe ischemia. They serve, therefore, as the confirmation of the need for rapid intervention in acute syndromes, not as the alternative for inconclusive exercise ECG test [12]. There were, however, investigations showing that short ischemia can induce the release of small amounts of troponins into coronary sinus blood [13, 14].

In contrast to protein markers, the heart releases nucleotide catabolites immediately after discordance of blood supply, and demand occurs well before any cellular membrane damage starts. The measurements of changes in catabolite levels in coronary sinus, arterial blood or in peripheral vein blood could be clinically useful, if interpreted in combination with clinical events. The most convenient in the setting of the exercise stress test seems to be venous blood taken at peak exercise (or immediately after it) with consecutive samples taken in a timely manner within 5–10 or maximum 20 minutes. In earlier studies, we investigated the release of hypoxanthine, uric acid and uridine in arterial and venous blood during PTCA, which served as the model of ischemia, in order to assess the value of sampling venous blood. We noticed that venous blood samples in resting patients within 10 minutes after controlled 2–3 minute ischemia were equally valuable as arterial samples only for uridine [6].

This work was undertaken assuming that uridine metabolism is relatively slow in circulation, and our results confirmed this among the exercised patients. The release of uridine seems to be an early and specific catabolite indicative for human heart

ischemia. The presented results show that exercise induced increase in uridine concentration correlated better with severity of arteriosclerosis than ST depression. Not unexpectedly, ST depression during EST was significantly greater in patients with CAD, and ST depression correlated significantly (negative correlation) with severity of coronary atherosclerosis (CAD index). However, there was no correlation between uridine concentration change and ST change. This is a consequence of different mechanisms responsible for both effects, as ST was depressed significantly also among patients without any signs of CAD in the control group where CAD was excluded in angiography among patients in doubt, and not all patients in the CAD group experienced angina. Simultaneous ST depression and the occurrence of typical angina pectoris are probably the most sensitive and specific noninvasive signs of heart ischemia. In our study, the correlation of angina with CAD was strong.

Hypoxanthine increased in both groups, and this change was not significantly different between them. It is possible that skeletal muscle release of hypoxanthine during exercise masked the cardiac hypoxanthine release, and therefore no difference was observed. This contrasts to the uric acid changes where, in the control group, uric acid change decreased statistically significantly 10 minutes after cessation of exercise in comparison to CAD patients. The mismatch in uric acid and hypoxanthine changes is difficult to explain, as uric acid is a metabolite of hypoxanthine. However, uric acid concentration change may not only indicate its increased production but also its redistribution between intracellular and vascular space. Lower pH in the ischemic regions would trigger release of uric acid from cardiac cells related to changes in the ionization status of uric acid.

Conclusions

In summary, among patients with CAD confirmed in angiography, a statistically significant change of concentration of uridine in peripheral blood was observed after treadmill exercise in comparison to the control group. Uridine concentration change after EST shows a correlation with the severity of coronary arteriosclerosis, comparable to the correlation of ST depression during exercise. Analysing uridine concentration changes could be a useful diagnostic investigation for evaluating the presence of CAD, but further studies are needed to ascertain its clinical value. The lack of correlation of uridine change with ST depression is not surprising, as ST depression is sometimes present

without arteriosclerosis, and in contrast we often do not observe ST depression among patients with severe CAD (e.g. triple vessel disease).

Acknowledgments

This work was supported by the Medical University of Gdańsk from research resources. Dr. S. Burakowski performed EST and collected blood samples, performed and was responsible for analysing coronarograms. This paper is part of his doctoral thesis. Dr. R.T. Smoleński was responsible for biochemical analysis. Dr. J. Bellwon helped with statistical analysis. Dr A. Kubasik and Dr. D. Ciećwierz coordinated the work, and Prof. A. Rynkiewicz supervised the study and reviewed the manuscript.

References

1. Broda G, Davis CE, Pajak A et al. Poland and the United States Collaborative Study on Cardiovascular Epidemiology. A comparison of HDL cholesterol and its subfractions in populations covered by the United States Atherosclerosis Risk in Communities Study and the Pol-MONICA Project. *Arterioscler Thromb Vasc Biol*, 1996; 6: 339–349.
2. Fornes P, Lecomte D, Nicolas G. Sudden coronary death outside of hospital; a comparative autopsy study of subjects with and without previous cardiovascular diseases. *Arch Mal Coeur Vaiss*, 1994; 87: 319–324.
3. Smolenski RT, Lachno DR, Yacoub MH. Adenine nucleotide degradation in human myocardium during heart and heart-lung transplantation. *J Mol Cell Cardiol*, 1991; 23 (suppl. 5): S102.
4. Rossi A, Olivares J. Basis of pyrimidine nucleotide metabolism in the myocardium. *Cardiovasc Drugs Ther*, 1998; 12 (suppl.) 2: 171–177.
5. Smolenski RT, de Jong JW, Janssen M et al. Formation and breakdown of uridine in ischemic hearts of rats and humans. *J Mol Cell Cardiol*, 1993; 25: 67–74.
6. Burakowski S, Smolenski RT, de Jong JW et al. Uridine and hypoxanthine concentrations during cardiac surgery operations, heart transplantations, PTCA and exercise stress testing. *J Mol Cell Cardiol*, 1999; 31: A85.
7. Jenkins PJ, Harper RW, Nestel PJ. Severity of coronary atherosclerosis related to lipoprotein concentration. *Br Med J*, 1978; 2: 388–391.
8. Borg G, Holmgren A, Linblad I. Quantitative evaluation of chest pain. *Acta Med Scand*, 1981; 64: 43–45.
9. Smolenski RT, Skladanowski AC, Perko M. Simultaneous separation of purine nucleotides and their degradation products by HPLC step gradient elution and the application of this procedure to the examination of heart biopsies and cardioplegic fluid. *Klin Wochenschr*, 1987; 65: 17.
10. Diamond GA, Forrester JS. Analysis of probability as an aid in the clinical diagnosis of coronary artery disease. *N Eng J Med*, 1979; 300: 1350–1356.
11. Gibbons RL, Balady GJ, Beasley JW et al. ACC/AHA guidelines for exercise testing: a report of the American College of Cardiology/American Heart Association Task force on Practice Guidelines (Committee on Exercise Testing). *J Am Coll Cardiol*, 1997; 30: 260–315.
12. Zarich SW, Bradley K, Mayall ID, Bernstein LH. Minor elevations in troponin T values enhance risk assessment in emergency department patients with suspected myocardial ischemia: analysis of novel troponin T cut-off values. *Clin Chim Acta*, 2004; 343: 223–229.
13. Suleiman MS, Luchetti V, Caputo M, Angelini GD. Short periods of regional ischemia and reperfusion provoke release of troponin I from the human hearts. *Clin Chim Acta*, 1999; 284: 25–39.
14. Conti A, Gallini C, Costanzo E et al. Early detection of myocardial ischemia in the emergency department by rest or exercise (99m)Tc tracer myocardial SPECT in patients with chest pain and non-diagnostic ECG. *Eur J Nucl Med*, 2001; 28: 1806–1810.