priate isotope-labeled internal calibrators. Amino acids containing amide and imine groups, such as citrulline, arginine, and asparagine, exhibited poor detection limits with the original formamidene derivatization method (6). The milder derivatization conditions and the use of isobutanol in this modified method improved MS/MS analysis of these amino acids dramatically.

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References

Exercise-induced Myocardial Ischemia Is Accompanied by Increased Serum Creatine Concentrations, Youyi E.C. Ties, Norbert H. Lameire, Marc L. De Buyzere, Amir Shoja, Guy De Backer, and Joris R. Delanghe* (1 Laboratory Clinical Chemistry, 2 Renal Division, Department of Internal Medicine, 3 Department of Internal Medicine, and 4 Cardiac Rehabilitation Centre, University Hospital Ghent, 9000 Ghent, Belgium; * address correspondence to this author at: Department of Clinical Chemistry 2P8, University Hospital Ghent, De Pintelaan 185, 9000 Ghent, Belgium; fax 32-9-240-4985, e-mail Joris.Delanghe@rug.ac.be)

Coronary artery disease remains the leading cause of mortality in adults in the westernized world (1, 2). Coronary artery disease can present itself as a wide range of diseases, from uncomplicated stable angina, to unstable angina, to myocardial infarction. The diagnosis of myocardial ischemia in patients with stable angina depends on the patient’s history and various technical investigations, such as electrocardiography (ECG) and stress testing. Exercise ECG is a simple and inexpensive investigation with diagnostic, prognostic, and functional information in selected populations (3). Exercise testing has an overall sensitivity of 66% and a specificity of 84% (4), but its diagnostic performance is much lower in asymptomatic individuals and females. The diagnosis and grading of unstable angina or myocardial infarction depend on biochemical markers together with clinical and electrocardiographic data (5). The currently used routine biochemical markers for the assessment of myocardial infarction or unstable angina require tissue necrosis to be detected in plasma. Serum and urine creatine concentrations have been described as markers for myocardial infarction. In contrast to other markers, creatine is a nonprotein nitrogenous compound, present in heart and skeletal muscle. It has a considerably lower molecular mass (131 Da) compared with conventional markers; release into the circulation therefore occurs at an early stage of myocardial ischemia, allowing rapid diagnosis (6, 7). We hypothesized that creatine could be released from ischemic myocardial tissue without the necessity of tissue necrosis. This study investigates the relationship between serum creatine and electrocardiographic indices during exercise testing.

We recruited 47 male Caucasians with a mean (SD) age of 61 (10) years from patients referred for exercise testing. Patients referred for detection of exercise-induced arrhythmias or with major arrhythmias (pacemaker rhythm, ventricular tachycardia, or conduction disorders) during exercise, excessive increase (>250 mmHg systolic, >120 mmHg diastolic) or decrease (>10 mmHg systolic blood pressure) in blood pressure during exercise, myocardial infarction <6 months, and symptomatic heart failure were excluded from the study. This study was approved by the local ethics committee. All participants underwent exercise testing on an electromagnetically braked bicycle ergometer with stepwise incremental workloads [50 W + 25 W/2 min (41 individuals); 50 W + 50 W/2 min (6 individuals)]. ECG recordings were acquired during exercise and during recovery. ST-segment depression was quantified 60 ms (ST60) after the J-point in lead V5. ST-segment depression ≥1.5 mm (0.15 mV) was considered to be diagnostic for myocardial ischemia. ST-segment depression was adjusted to heart rate (ST/HR index) (8). Exercise was stopped on achieving the age-matched maximal exercise level, severe ST-depression >2.0 mm (0.2 mV), or severe symptoms (incapacitating fatigue, grade III/IV angina, severe dyspnea).

The rate–pressure product [heart rate × systolic blood pressure (mmHg • beats • min⁻¹)] was determined at peak exercise. Fastig blood samples were taken at rest before exercise and 10 min after termination of exercise. Coronary angiography was performed as a clinical diagnostic procedure in 19 participants (40% of study population). Serum creatine concentration (CV = 2.2% at 230 μmol/L and 3.0% at 58 μmol/L) was determined by an enzymatic assay as described previously (6). Creatine kinase (CK) catalytic activity was determined according to the IFCC (9) at 37°C on a Modular analyzer using CK-NAC reagents (Roche Diagnostics). CK-MB was measured with an immunoinhibition assay. Total serum glutathione (CV = 2.4% at 1 μmol/L and 3.6% at 0.5 μmol/L) was determined according to the method of Griffith (10). Myoglobin was measured immunoturbidimetrically, and cardiac troponin T was measured with an electrochemiluminescence immunoassay (Roche Diagnostics). Plasma lactate was measured using commercial reagents on a
Modular analyzer (Roche Diagnostics). A one-sample Kolmogorov–Smirnov test was used to pretest gaussian distribution. The Student t-test was used to compare separate groups when appropriate, and the Fisher exact test was used to compare categorical data. Spearman rank correlation analysis was performed between variables.

Eleven participants (23%) met the ECG diagnostic criteria for myocardial ischemia. The clinical characteristics of the study groups with and without ECG changes are summarized in Table 1.

Clinical characteristics were comparable among individuals who met and did not meet the ECG criteria for myocardial ischemia. Individuals from both groups attained the same exercise level [164 (40) W vs 164 (47) W; P not significant] and had comparable rate-pressure products [27 300 (7900) vs 28 200 (5700) mmHg·beats·min⁻¹; P = 0.73]. Serum creatine concentrations before and after exercise are also given in Table 1. Participants who met the ECG criteria for ischemia had higher serum creatine concentrations after exercise. The absolute increase in serum creatine concentrations after exercise (Δ creatine) was larger in individuals meeting the ECG criteria for ischemia than in individuals who did not meet the criteria [9.36 (10.55) vs −0.01 (5.34) μmol/L; P = 0.0002]. Individuals who did not meet the ECG criteria had comparable serum creatine concentrations before and after exercise. Δ creatine correlated significantly with maximal ST depression in lead V5 on ECG during exercise (r = −0.34; P = 0.02).

Patients who met the ECG criteria had higher plasma lactate concentrations [9.0 (2.9) vs 6.9 (2.3) mmol/L; P = 0.001] than did patients who did not meet the criteria at a comparable exercise intensity level. Individuals with high postexercise lactate (>7.6 mmol/L) had significantly higher Δ creatine values [5.7 (9.8) vs −0.2 (5.0) μmol/L; P < 0.01] compared with individuals with lower postexercise lactate (<7.6 mmol/L). Individuals with coronary lesions on coronary arteriography had higher increments in serum creatine after exercise [46.1 (25.1) μmol/L (post) vs 39.2 (20.8) μmol/L (pre); P = 0.03] compared with individuals with a normal coronary arteriography [44.0 (16.8) μmol/L (post) vs 43.0 (19.0) μmol/L (pre); P not significant]. CK activities in the ECG criteria-positive group were significantly lower before and after exercise. CK-MB activity was comparable between both groups and was unaltered by exercise in both groups (Table 1). Serum total glutathione was significantly lower in the ECG criteria-positive group compared with the group that did not meet the criteria [0.83 (0.13) vs 0.90 (0.08) μmol/L; P =0.05] and correlated positively with CK activity (r = 0.34; P = 0.02). We found no relationship between CK-MB activity and total serum glutathione.

Myoglobin concentrations were comparable between groups, and cardiac troponin T concentrations were below the detection limit (<0.01 μg/L) in all samples, documenting the absence of preexisting cardiac damage.

Several possible mechanisms can be proposed to explain the relationship between myocardial ischemia and creatine release from the myocardium. Muscle cells take up creatine from the bloodstream and maintain a steep concentration gradient, which is 100- to 1000-fold higher than serum creatine concentrations, by means of a high-affinity creatine transporter (11, 12). The creatine transporter is Na⁺-driven and ATP-dependent. Localized ischemia during exercise testing could lower the intracellular pH and diminish the myocyte ATP concentration and the ability to maintain the steep concentration difference between the myocyte and serum creatine concentrations by inhibiting the sarcolemmal creatine transporter. Our data demonstrated a relationship between ST depression on the ECG during exercise and changes in serum creatine concentrations. The ECG changes during exercise most

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**Table 1. Comparative clinical characteristics and pre- and postexercise biochemical values in patients who did or did not meet the ECG criteria for myocardial ischemia.**

<table>
<thead>
<tr>
<th></th>
<th>Did not meet ECG criteria</th>
<th>Met ECG criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Preexercise</td>
<td>Postexercise</td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>36</td>
<td>60 (10)</td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td>60 (10)</td>
<td>7.6 mmol/L</td>
</tr>
<tr>
<td><strong>Body mass index, kg/m²</strong></td>
<td>27 (4)</td>
<td>39.6 (16.3)</td>
</tr>
<tr>
<td><strong>Blood pressure, mmHg</strong></td>
<td></td>
<td>135 (20)</td>
</tr>
<tr>
<td>Systolic</td>
<td>81 (12)</td>
<td>61 (10)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Heart rate at rest, beats/min</td>
<td>164 (40)</td>
<td>164 (40)</td>
</tr>
<tr>
<td>Personal history of myocardial infarction, %</td>
<td>0.69 (0.90)</td>
<td>1.66 (0.87)</td>
</tr>
<tr>
<td><strong>Exercise level, W</strong></td>
<td>125 (76)</td>
<td>127 (76)</td>
</tr>
<tr>
<td><strong>CK, U/L</strong></td>
<td>15 (7)</td>
<td>13 (4)</td>
</tr>
<tr>
<td><strong>CK-MB, U/L</strong></td>
<td>125 (76)</td>
<td>127 (76)</td>
</tr>
<tr>
<td><strong>Myoglobin, nmol/L</strong></td>
<td>1.69 (0.90)</td>
<td>1.66 (0.87)</td>
</tr>
</tbody>
</table>

*a All values except number of individuals and personal history of myocardial infarction are given as the mean (SD).

*b P = 0.015 compared with preexercise concentrations in individuals who met the ECG criteria.

*c P < 0.05 compared with individuals who did not meet the ECG criteria.
indicative of myocardial ischemia are mainly ST changes. These are the result of changes in myocyte action potential duration and in the repolarization process. In the presence of ischemia, the action potential duration is shortened, and electrical gradients are created, leading to ST displacement (13). Ischemia-triggered membrane destabilization could also influence the Na\(^+\)-dependent creatine transporter, leading to a net creatine loss from the myocyte.

CK remains a major biochemical marker of myocardial or skeletal muscle damage, but it can be inactivated by oxidation of sulfhydryl groups, with apparent lower serum CK activities. Our group has previously demonstrated significant correlations between serum glutathione and CK activity (14). Individuals who met the ECG criteria had lower CK activities and total glutathione concentrations, suggesting higher oxidative stress and lower in vivo antioxidant capacity (14, 15).

High concentrations of creatine are found in myocardial and skeletal muscle (11, 12). Creatine release during bicycle ergometry could theoretically elicit creatine release from the exercising skeletal muscle. High-intensity cycling exercise in young athletes with high skeletal muscle mass did not produce increased serum creatine concentrations [Δ creatine, −3.0 (6.8) μmol/L] (16). Serum creatine changes in patients with ECG changes are small and correspond to a limited muscle area, from which creatine is leaking. However, a limitation is that creatine release can be derived from either skeletal or cardiac tissue; the observed creatine increase with exercise could therefore have been from cardiac muscle, skeletal muscle, or both. The fact that the individuals had ECG changes associated with ischemia indicates that cardiac muscle was probably an important source in these patients. Further research is necessary to elucidate the underlying mechanism of creatine release from cardiac and skeletal muscle or both. The availability of an early biochemical marker for myocardial ischemia, without the need of tissue necrosis, could have important clinical application. Diagnosis of stable coronary artery disease is based on medical history, ECG data, and various imaging techniques. In situations in which ECG data are difficult to interpret, biochemical markers could have additional value.

In conclusion, this study demonstrates the potential use for creatine measurements as a marker of myocardial ischemia in the setting of diagnostic exercise testing. Clinical studies are needed to examine the diagnostic performance of serum creatine measurements in a large population for the detection of coronary artery disease.

Y.E. Taes is Research Assistant of the Fund for Scientific Research–Flanders (Belgium; F.W.O.–Vlaanderen).

References


Population Distribution of High-Sensitivity C-reactive Protein among US Men: Findings from National Health and Nutrition Examination Survey 1999–2000, Earl S. Ford," Wayne H. Giles,2 Gary L. Myers,3 and David M. Mannino3 (Divisions of 1 Environmental Hazards and Health Effects and 3 Laboratory Sciences, National Center for Environmental Health, and 2 Division of Adult and Community Health, National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention, Atlanta, GA 30341; * address correspondence to this author at: Centers for Disease Control and Prevention, 4770 Buford Hwy., MS K66, Atlanta, GA 30341; fax 770-488-8150, e-mail esf2@cdc.gov)

C-reactive protein, an acute-phase reactant, is produced in the liver and belongs to the pentraxin family of proteins (1). This protein is very sensitive to inflammation, and its concentration can increase rapidly in response to a wide range of stimuli. Originally described in 1930 (2), C-reactive protein measurements served mostly in a diagnostic, albeit a nonspecific one, and in a monitoring role in such fields as infectious diseases and rheumatology. In the past decade, as the role of inflammation in cardiovascular disease became appreciated, interest turned to C-reactive