Ischemia-Modified Albumin Concentrations in Patients with Peripheral Vascular Disease and Exercise-Induced Skeletal Muscle Ischemia

DEBASHIS ROY,1† JUAN QUILES,1† RAJAN SHARMA,1 MANAS SINHA,1 PABLO AVANZAS,1 DAVID GAZE,2 and JUAN CARLOS KASKI1*

Background: Ischemia-modified albumin (IMA) is a new marker of myocardial ischemia, there is concern that IMA concentrations may be affected by ischemia occurring in tissues other than the myocardium.

Methods: We assessed 23 consecutive patients (15 males; mean age, 67 years) with typical leg claudication and documented peripheral vascular disease (PVD). All patients underwent both treadmill-exercise stress testing to induce leg ischemia and dobutamine stress echocardiography 1 week apart for the assessment of myocardial ischemia. Blood samples for IMA measurements were obtained at baseline, immediately after peak exercise/stress, and 1 h after exercise/stress. Statistical analysis was performed with the ANOVA repeated-measures test.

Results: Compared with baseline, mean (SD) IMA was significantly lower after the induction of skeletal muscle ischemia and returned to baseline values at 1 h: baseline, 74.6 (15.6) kilounits/L; peak stress, 69.5 (14.0) kilounits/L (P < 0.0001 vs baseline); 1 h after stress, 75.9 (15.7) kilounits/L (P < 0.0001 vs peak stress; P = 0.3 vs baseline). Baseline, peak stress, and 1-h poststress IMA concentrations were inversely correlated with the ankle-brachial index after exercise (r = −0.4; P < 0.05). None of the patients showed regional wall motion abnormalities during dobutamine stress echocardiography, and IMA concentrations remained unchanged from baseline. There were no differences in baseline [74.6 (15.6) vs 72.7 (11.5) kilounits/L; P = 0.6], peak stress, or poststress IMA concentrations when exercise testing and dobutamine stress echocardiography values were compared.

Conclusions: The relationship between disease severity (of a noncardiac origin) and baseline IMA values is an important and novel finding. IMA is significantly lower immediately after exercise-induced leg ischemia in patients with PVD and is related to disease severity. IMA concentrations can therefore be affected by the development of skeletal muscle ischemia, and this may have implications regarding the ability of IMA to detect myocardial ischemia in PVD patients.

Concentrations of ischemia-modified albumin (IMA),3 a new marker of myocardial ischemia, are increased after percutaneous coronary intervention (1–3). Moreover, in the percutaneous coronary intervention model, IMA production is higher in patients without collateral vessels than in those with collateral circulation; thus, IMA possibly reflects a protective effect of collateral vessels against percutaneous coronary intervention-induced myocardial ischemia (4). IMA also appears to be useful for ruling out acute coronary syndrome in patients attending the emergency department with chest pain suggestive of myocardial ischemia (5). However, concern exists that IMA concentrations may be affected by ischemia occurring in tissues other than the myocardium, e.g., leg claudication attributable to peripheral vascular disease (PVD). We therefore sought to assess whether changes in IMA concentrations occur after the induction of skeletal muscle ischemia in patients with PVD and intermittent claudication.

Departments of 1Cardiological Sciences and 2Chemical Pathology, St. George’s Hospital Medical School, London, UK.
†The first two authors contributed equally to this work.
*Address correspondence to this author at: Cardiological Sciences, St. George’s Hospital Medical School, Cranmer Terrace, London SW17 0RE, UK. Fax 44-208-725-3328; e-mail jkaski@sghms.ac.uk.
Received January 20, 2004; accepted June 28, 2004.
Previously published online at DOI: 10.1373/clinchem.2004.031690

3 Nonstandard abbreviations: IMA, ischemia-modified albumin; PVD, peripheral vascular disease; ABI, ankle-brachial index; and ECG, electrocardiography.
Materials and Methods
We assessed 23 consecutive patients (14 males; mean age, 65.7 years) with typical leg claudication and documented PVD. All patients satisfied the following inclusion criteria: (a) Fontaine stage II intermittent claudication (leg pain induced by exercise and relieved by resting for 10 min or less); (b) absence of leg pain at rest in the last 12 months; (c) ultrasound or angiographic evidence of obstructive PVD [i.e., arterial stenosis >50% in the lower limbs and resting systolic ankle-brachial index (ABI) <0.9]. We excluded patients with any form of angina or congestive heart failure. Similarly, patients were not included if they had had a myocardial infarction, unstable angina, or myocardial revascularization in the 6 months preceding the study. None of the patients had a history of ischemic or hemorrhagic stroke, transient ischemic attacks, kidney failure, shock, or coronary artery spasm. We also excluded patients with chronic obstructive airway disease and arthritis because these could have reduced their ability to exercise. All patients underwent both treadmill-exercise stress testing and dobutamine stress echocardiography. These tests were carried out 1 week apart and in random order to minimize the effects of training and ischemic preconditioning. The study was approved by the local research ethics committee, and all patients gave written informed consent for participation in the study.

SKELETAL MUSCLE ISCHEMIA
All patients underwent symptom-limited treadmill-exercise electrocardiographic (ECG) stress testing with a standardized protocol (6). The treadmill speed (3.0 km/h) was kept constant with 2% increases in grade every 2 min. Patients were instructed to immediately report the onset of claudication pain (initial claudication time). All patients were exercised to their maximum claudication pain, and the test was stopped when they experienced peak claudication pain (absolute claudication time). Blood pressure was measured at regular intervals, and the 12-lead ECG was monitored continuously. None of the patients developed arrhythmias or ST-segment ischemic changes during testing. Serum samples for IMA were obtained at baseline before dobutamine stress, immediately after peak claudication pain, and 1 h after exercise. The ABI, a noninvasive method to assess the patency of the lower extremity arterial system and to detect occlusive arterial disease (7), was measured in every patient. The index was calculated as the ratio between ankle and brachial systolic pressures (both measured with a blood pressure cuff in the arms and the dorsalis pedis artery) before and immediately after exercise. Continuous wave Doppler ultrasound was used to detect flow in the arteries.

ASSESSMENT OF MYOCARDIAL ISCHEMIA
All patients underwent dobutamine stress echocardiography testing. Images were obtained with standard views and acquired at baseline, with each increment of dobutamine infusion, and during the recovery phase. Dobutamine was administered intravenously at incremental doses [5, 10, 15, 20, 30, and 40 μg · (kg of bodyweight) −1 · min −1] at 3-min intervals, and atropine was used (bolus dose, 0.6–1.2 mg) if 85% of the maximum age-predicted heart rate was not achieved at the maximum infusion rate. The 12-lead ECG and blood pressure were monitored. For left ventricular wall motion analysis, the standard 16-segment model of the left ventricle of the American Society of Echocardiography was used (8). A positive result was defined as the development of a new wall motion abnormality or worsening regional wall motion abnormality not present at baseline. Serum samples for IMA were obtained at baseline before dobutamine stress, immediately after peak stress, and 1 h after stress.

IMA MEASUREMENTS
Serum IMA was measured by the albumin cobalt binding test on a Roche Cobas MIRA PLUS instrument (ABX Ltd.). This method has been validated and described in previous studies (9, 10). According to the manufacturer, expected (normal) values determined in a population of 283 healthy individuals range from 52 to 116 kilounits/L with a 95th percentile at 85 kilounits/L. Samples were frozen at −70 °C within 2 h and stored until measurement. The total interassay CV was 4.9–7.5% at 72.54–140.16 kilounits/L for quality-control material.

STATISTICAL ANALYSIS
Results for gaussian-distributed continuous variables are expressed as the mean value (SD), and categorical data are shown as the value (percentage). The Kolmogorov–Smirnov tests for normality indicated that IMA values followed a gaussian distribution. Correlation between continuous variables was assessed with the Spearman test. We used the repeated-measures ANOVA to assess IMA values at every different time point (within-subject effects) and to test differences in time patterns of IMA after both stress tests (between-test effects). The change in ABI was tested with the Wilcoxon rank test. Differences were considered to be statistically significant if the null hypothesis could be rejected with >95% confidence. The SPSS 11.0 statistical software package (SPSS Inc.) was used for all calculations.

Results
The clinical characteristics of all patients included in the study are listed in Table 1. The mean initial claudication time was 316.5 (117.3) s, and the absolute claudication time was 467.4 (108.9) s. The baseline preexercise ABI was 0.64 (0.1) and decreased to 0.47 (0.1) immediately after the test ($P <0.0001$). There was a significant correlation between the baseline ABI and the final claudication time ($r = 0.47; P = 0.02$).

IMA concentrations were above the cutoff point recommended by the manufacturer for the diagnosis of ischemia (85 kilounits/L) in 26% (6 of 23) and 48% (11 of
Table 1. Clinical characteristics of the study patients (n = 23).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) age, years</td>
<td>65.7 (8.5)</td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>15 (65.2%)</td>
</tr>
<tr>
<td>Smokers, n (%)</td>
<td>11 (47.8%)</td>
</tr>
<tr>
<td>Hypercholesterolemia, n (%)</td>
<td>14 (60.9%)</td>
</tr>
<tr>
<td>Systemic hypertension, n (%)</td>
<td>11 (47.8%)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Mean (SD) albumin, g/L</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>39.0 (27)</td>
</tr>
<tr>
<td>Immediately after exercise</td>
<td>38.9 (28)</td>
</tr>
<tr>
<td>1 h after exercise</td>
<td>38.5 (25)</td>
</tr>
<tr>
<td>Mean (SD) onset time of claudication, s</td>
<td>316.5 (117.3)</td>
</tr>
<tr>
<td>Mean (SD) duration of stress testing, s</td>
<td>467.4 (108.9)</td>
</tr>
<tr>
<td>Mean (SD) IMA concentrations during exercise testing, kilounits/L</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>74.6 (15.6)</td>
</tr>
<tr>
<td>At peak stress</td>
<td>69.5 (14)</td>
</tr>
<tr>
<td>1 h after stress</td>
<td>75.9 (15.7)</td>
</tr>
<tr>
<td>Mean (SD) IMA concentrations during dobutamine stress echocardiography, kilounits/L</td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>72.7 (11.5)</td>
</tr>
<tr>
<td>At peak stress</td>
<td>73.4 (11.5)</td>
</tr>
<tr>
<td>1 h after stress</td>
<td>74.3 (11.4)</td>
</tr>
</tbody>
</table>

* P <0.0001 compared with baseline.

23) of our PVD patients before and 1 h after exercise, respectively (Fig. 1). Compared with baseline, IMA concentrations decreased significantly at peak exercise stress [leg ischemia; 74.6 (15.6) vs 69.5 (14) kilounits/L; P <0.0001] and had returned to baseline concentrations at 1 h poststress measurement [75.9 (15.7) kilounits/L; after adjustment for confounding variables such as gender and smoking, P <0.0001 vs peak stress; P = 0.3 vs baseline; Table 1]. There was no correlation between peak stress IMA values and claudication time (r = −0.06; P = 0.9) or between peak stress IMA and baseline ABI values (r = −0.4; P = 0.09), we did find a significant inverse correlation between baseline (r = −0.43; P = 0.039), peak (r = −0.42; P = 0.048), and 1-h poststress (r = −0.44; P = 0.038) IMA concentrations and postexercise ABI values (Fig. 1).

None of the patients had chest pain or ischemic ECG changes, or showed regional wall motion abnormalities during dobutamine stress echocardiography. IMA concentrations remained unchanged from baseline [72.7 (11.5) kilounits/L] to both peak stress [73.4 (11.5) kilounits/L] and 1 h after stress [74.3 (11.4) kilounits/L; not statistically significant; Table 1].

When exercise ECG testing and dobutamine stress echocardiography were compared, there were no differences in baseline [74.6 (15.6) v 72.7 (11.5) kilounits/L; P = 0.6] or poststress IMA concentrations.

In the study patients, albumin concentrations were within the reference interval: mean albumin, 39 (2.7) g/L; minimum, 34 g/L; maximum, 43 g/L. Mean (SD) postexercise [38.9 (2.8) g/L] and 1-h poststress [38.5 (2.5) g/L] albumin concentrations did not differ from baseline concentrations. We found no correlation among albumin concentrations at any time point, IMA concentrations, and baseline or postexercise ABI.

Discussion

The results of the present study indicate that IMA concentrations are lower immediately after exercise-induced skeletal leg muscle ischemia and may be also affected (increased concentrations) by the presence of severe PVD.

Our study showed for the first time that in patients with documented PVD and intermittent claudication,
IMA concentrations decrease significantly immediately after exercise-induced skeletal muscle ischemia compared with baseline. IMA concentrations had returned to baseline when measured 1 h post stress. The ABI, a reliable measure of lower-extremity ischemia (11) attributable to occlusive arterial disease, was decreased in all patients. The transient decrease observed in IMA concentrations immediately after exercise is intriguing and consistent with observations from two previous studies that assessed the effect of skeletal muscle ischemia on circulating IMA concentrations in apparently healthy individuals (12, 13).

In a study of marathon runners, a decrease in IMA concentrations was observed immediately after exercise, and this was followed by a delayed increase after 24–48 h. It was speculated that this delayed increase was attributable to skeletal muscle ischemia or the occurrence of gastrointestinal ischemia (12). Similarly, in a study of 10 healthy volunteers, an immediate and transient decrease in IMA concentrations was observed after the induction of forearm ischemia (13). The authors hypothesized that this immediate decrease could have been attributable to interference in the IMA measurement by lactate produced during skeletal muscle exercise. Unfortunately, lactate concentrations could not be measured in our patients because of logistic problems, and this represents a limitation of the study. However, release of lactate after a standardized treadmill exercise stress test in patients with stage II PVD has been reported previously by Duprez et al. (14), who described a twofold increase in lactate concentrations immediately after exercise testing and a return to baseline concentrations after 30 min. Furthermore, compared with healthy controls, lactate concentrations in patients with stage II PVD have been shown to be significantly increased after exercise, and this increment appears to be independent of the work load achieved (15). It is likely that lactate concentrations were also increased in our patients after exercise testing, and it can therefore be speculated, as proposed previously by Zapico-Muñiz et al. (13), that the decrease in IMA concentrations observed immediately after exercise in our study could have been attributable to lactate interference with IMA measurements.

Whether this transient decrease in IMA concentrations after exercise-induced skeletal muscle ischemia may lead to false-negative results in angina patients who also have PVD requires investigation. Moreover, the exact timing and duration of the IMA changes after exercise-induced skeletal muscle ischemia need to be characterized in different patient populations.

None of our patients developed regional wall motion abnormalities during dobutamine stress echocardiography testing, which is a sensitive and specific method for the detection of myocardial ischemia (16, 17). Accordingly, IMA concentrations remained unchanged compared with baseline.

Our study showed a statistically significant inverse correlation between postexercise ABI values and baseline, peak, and 1-h postexercise IMA concentrations (i.e., higher IMA concentrations in patients with lower ABIs). This finding suggests that IMA concentrations are related to the severity of induced skeletal muscle ischemia and that conceivably, this relationship could be potentiated in patients with more advanced PVD. To the best of our knowledge, ours is the first report of a link between the severity of PVD and IMA concentrations. It has been proposed that albumin modification by reactive oxygen species produced during ischemia leads to IMA formation. Thus it is possible that in PVD patients with more severe leg ischemia than that observed in our patients, greater free radical production could occur, leading to higher IMA concentrations.

From a clinical perspective, the association found in the present study between PVD and IMA concentrations also has potential implications for the diagnosis of myocardial ischemia in patients with PVD. There is some concern regarding the specificity of a circulating protein such as albumin for the diagnosis of cardiac ischemia because noncardiac ischemia and/or oxidative stress could conceivably increase IMA concentrations and therefore limit the usefulness of this marker for the diagnosis of myocardial ischemia. These two conditions often coexist, and changes in IMA concentration caused by skeletal muscle ischemia may affect the specificity of the biomarker. This is particularly important when IMA measurements are used for assessing acute chest pain suggestive of acute coronary syndrome. In the present study, a proportion of PVD patients had increased IMA concentrations at baseline (26%) and 1 h (48%) after exercise testing despite the absence of cardiac ischemia (negative results of dobutamine stress echocardiography testing).

Low albumin concentrations had been linked to vascular disease, atherosclerosis (18, 19), and cardiovascular mortality (20). Plasma volume expansion after intense exercise is also associated with an increase in plasma albumin content and selective expansion of the intravascular compartment (21). In our study, albumin concentrations were within the reference interval and did not significantly change after exercise. Our patients were a highly selected group with only stage II PVD; therefore, extrapolation of our results regarding albumin concentrations to other patients with more severe PVD may not be straightforward.

In summary, our results suggest that complex mechanisms determine circulating IMA concentrations in patients with PVD and that these are likely to be modulated by numerous, as yet unknown variables. Studies are required to identify these mechanisms and to determine the optimum IMA cutoff values in various disease states, including PVD with or without accompanying cardiac ischemia. Furthermore, it will be necessary to establish the diagnostic and prognostic implications of a positive IMA test in these patient groups.
We are grateful to J.M. Bellón from the Department of Preventive Medicine of Gregorio Marañón General Hospital (Madrid, Spain) for expert statistical advice. This study was partially funded by an unrestricted educational grant from Ischemia Technologies Inc. (Denver, CO).

References