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Ischemia-Modified Albumin during Skeletal Muscle Ischemia, *Edgar Zapico-Muñiz,[1] Miquel Santaló-Bel,[2] Javier Mercé-Muntañola,[1] José A. Montiel,[2] Antonio Martínez-Rubio,[3] and Jordi Ordóñez-Llanos[1,4*]* ([1] Biochemistry, [2] Emergency, and [3] Cardiology Departments, Hospital de la Santa, Creu i Sant Pau, Barcelona, Spain; [4] Biochemistry and Molecular Biology Department, Universitat Autònoma, Barcelona, Spain; * address correspondence to this author at: Servei de Bioquímica, Hospital de la Santa Creu i Sant Pau, Avinguda Sant Antoni Maria Claret 167, 08025 Barcelona, Spain; fax 34-93-2919196, e-mail jordonez@hsp.santpau.es)

Ischemia-modified albumin (IMA) has been proposed as a biological marker of myocardial ischemia *(1, 2)*. Exposure to ischemic myocardium modifies circulating albumin at its NH_2 terminus by different mechanisms, and this modification is the basis of IMA measurement by the albumin cobalt binding (ACB) test *(3)*. The tissue-specific nature of the mechanism by which ischemia modifies albumin remains undetermined. Together with a nondiagnostic electrocardiogram and negative troponin values, IMA concentrations within the reference interval have high negative predictive value of myocardial ischemia in patients with suspected acute coronary syndromes *(1, 2)*. However, IMA cardiospecificity has not been validated and needs an evidence base before routine clinical use. A recent report showed significant IMA increases 24–48 h after a marathon race, with exercise-promoted gastrointestinal and/or delayed skeletal muscle ischemia being evoked as possible causes of such increases *(4)*. However, because IMA has shown rapid kinetics of increase (in minutes) and return to baseline no longer than 12 h after angioplastic procedures *(5)*, long-duration skeletal muscle ischemia (i.e., occurring during marathons) does not appear to be the most appropriate model to investigate the effect of such ischemia on IMA values or the kinetics of IMA occurring during acute coronary syndromes. The aim of this work was to analyze the possible contribution of skeletal muscle ischemia to IMA by investigating its short-term kinetics in an isolated skeletal-muscle ischemia model. Because lactate and ammonia concentrations increase sharply after a forearm ischemia test, their possible influence in the ACB assay was studied.

Ten healthy volunteers (4 men and 6 women) from our laboratory staff (age range, 48–61 years; median, 53 years) with no personal or family history of cardiovascular disease and no known cardiovascular risk factors after a medical examination underwent a forearm ischemia test *(6)*. Briefly, after an overnight fast (10–12 h) and 30 min of previous rest, a preexercise (0 min) blood sample was drawn, and blood systolic pressure was recorded twice within a 5-min interval. Thereafter, forearm ischemia was produced by inflating the blood pressure cuff up to 20–30 mmHg higher than the maximum systolic pressure registered. Under these ischemic conditions, a hand-grip exercise at maximum possible strength was performed for 1 min. Thereafter, the cuff was removed, and serial blood samples were drawn at 1, 3, 5, 10, 15, and 30 min. Serum for IMA, creatine kinase, and potassium; EDTA plasma for ammonia; and fluoride plasma for lactate and glucose were collected at each time point into Vacutainer® Tubes (Becton Dickinson). To establish reference values, IMA was tested in a group of 86 fasting (10–12 h), ambulatory (median age, 57 years; 38 women) sedentary individuals who underwent blood sampling after health examinations or before minor surgical procedures. Individuals with cardiovascular risk factors or past or present signs or symptoms of cardiovascular disease recorded during the medical examination were excluded. Volunteers and reference individuals gave written informed consent. All procedures were in accordance with our Institutional Review Board protocols.

Serum IMA was measured with the ACB test (Ischemia Technologies Inc.) adapted to a Roche Cobas Mira analyzer (ABX Diagnostics) according to the manufacturer's instructions for specimen and reagent handling. The principle of the test has been described previously *(3)*. In individuals undergoing the forearm ischemia test, ammonia, lactate, and glucose (all samples), and creatine kinase and potassium (basal and 5 min postexercise) were measured in a Vitros 250 analyzer (Ortho Diagnostics). Concentrations of L-(+)-lactic acid (lactic acid free acid; 300 g/L solution in water; Sigma, cat. no. L-1875; lot no. 052K1278; M_r 90.08) ranging from 50 to 900 mmol/L and ammonia (from ammonium chloride salt; Merck; cat. no. 1145; lot no. 7448183; M_r 53.49) ranging from 2 to 18 mmol/L were dissolved separately in 20 mmol/L MOPS buffer and added to a serum pool (IMA = 106 kilounits/L) at a constant ratio of 1/100 of the final sample volume. Lactate and ammonia concentrations of the enriched pool were measured by the above-described methods. All enriched samples were measured in quadruplicate. The Wilcoxon paired *t*-test and correlation equations were calculated with GraphPad Prism, Ver. 3.0 (GraphPad Software Inc.).

Between-batch imprecision (CV) of the ACB test was assessed at IMA concentrations of 69 and 114 kilounits/L and was <3% (n = 8) for both concentrations. The 95th percentile for IMA in the reference population was 101 kilounits/L (nonparametric). Results of the forearm ischemia test are shown in Table 1. Increases in lactate and ammonia were five- and sevenfold over basal values, respectively, during the first 5 min after exercise, demon-

Table 1. Mean (SD) lactate, ammonia, albumin, and IMA values and IMA:albumin ratios measured before (0 min) and after (1–30 min) the forearm ischemia test in 10 healthy volunteers.[a]

Time, min	Lactic acid, mmol/L	Ammonia, mmol/L	Albumin, g/L	IMA, kilounits/L	IMA:Albumin, kilounits/g
0	1.10 (0.30)	20 (10)	39.9 (2.3)	94.5 (9.1)	2.37 (0.27)
1	5.17 (1.14)[b]	116 (39)[b]	40.9 (2.8)	87.5 (8.9)[b]	2.14 (0.27)[b]
3	5.26 (1.14)[b]	136 (55[b]	40.3 (2.1)	87.1 (7.9)[b]	2.16 (0.25)[b]
5	4.35 (1.02)[b]	133 (60)[b]	39.8 (2.1)	89.1 (6.3)[b]	2.24 (0.22)[b]
10	2.77 (1.14)	90 (54)	39.4 (2.5)	90.7 (7.9)	2.30 (0.26)
15	2.07 (0.83)	62 (35)	39.2 (2.3)	91.8 (8.1)	2.34 (0.29)
30	1.22 (0.35)	32 (23)	39.2 (2.4)	93.8 (7.3)	2.29 (0.25)

[a] The test is described in detail in the text.
[b] $P < 0.05$ with respect to basal (0 min) values.

strative of ischemic-performed exercise (Table 1 and Fig. 1A). Creatine kinase, glucose, and potassium concentrations remained unchanged after exercise (data not shown). A significant, negative correlation between IMA values and albumin concentrations was found both in forearm ischemia and in reference samples [IMA (kilounits/L) = −2.366albumin (g/L) + 186.4; $r = -0.756$; $P < 0.001$; n = 152; Fig. 1B]. The ratio of IMA values to albumin (IMA:albumin) was also assessed, and the nonparametric 95th reference percentile was 2.59 kilounits/g. Mean IMA and IMA:albumin values obtained during the forearm ischemia test were below the respective 95th reference percentiles, although 6 of the 70 samples were above these limits. A significant ($P < 0.05$) decrease in both IMA and IMA:albumin at 1, 3, and 5 min was observed, with a return to baseline thereafter (Table 1 and Fig. 1A). Albumin concentrations did not change significantly. In the enriched serum pool, IMA concentrations decreased, whereas lactate increased, with negative mean differences of −9% at a lactic acid concentration of 5 mmol/L and −25% at the maximum concentration of 11 mmol/L (Fig. 1C). A significant correlation ($r = -0.98$; $P < 0.001$) was found between IMA and lactate values. After MOPS buffer addition in the proportion used for the assay, the pH range of the enriched pools varied only by 0.04 units. IMA values remained unaffected by ammonia

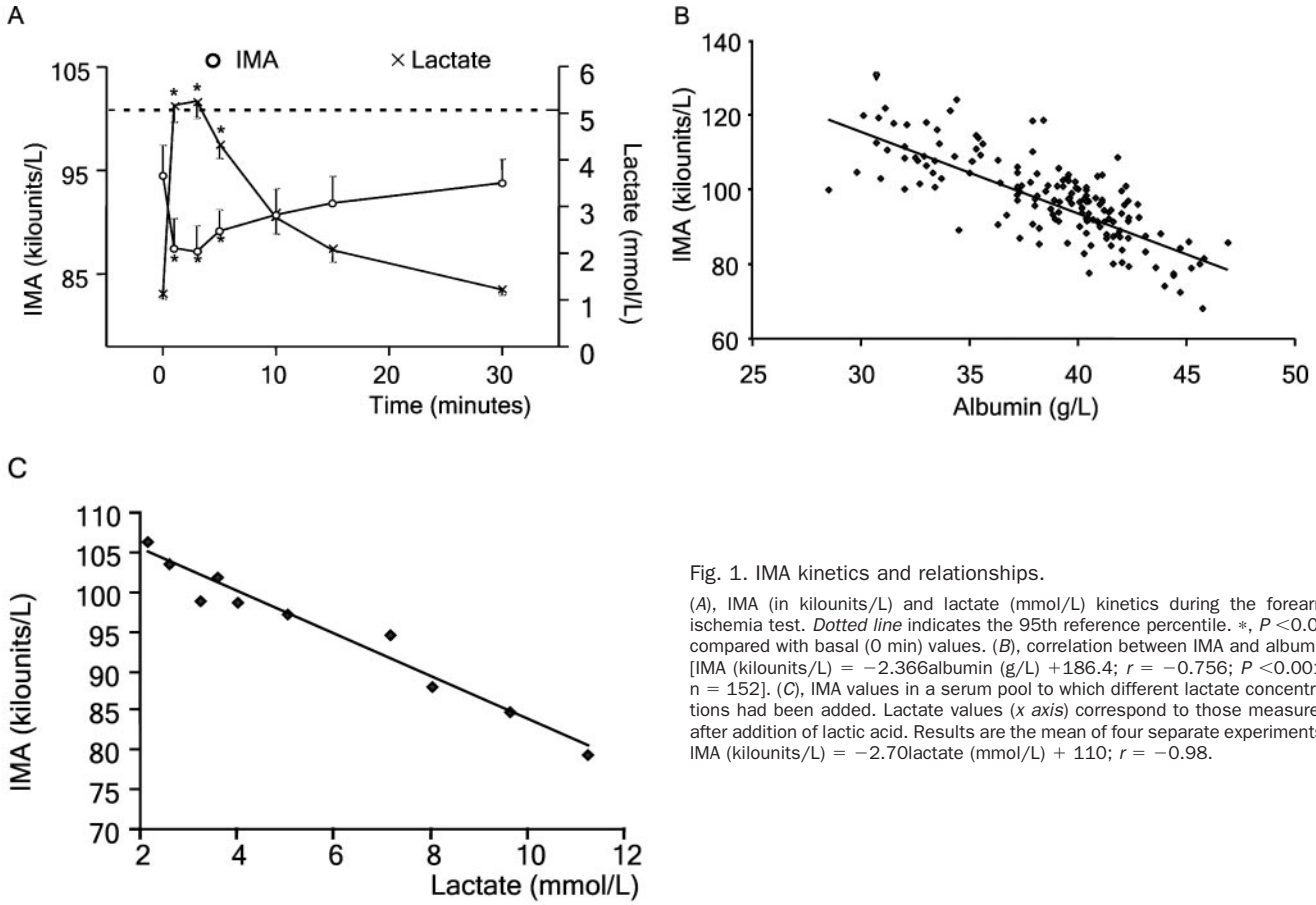

Fig. 1. IMA kinetics and relationships.

(*A*), IMA (in kilounits/L) and lactate (mmol/L) kinetics during the forearm ischemia test. *Dotted line* indicates the 95th reference percentile. ∗, $P < 0.05$ compared with basal (0 min) values. (*B*), correlation between IMA and albumin [IMA (kilounits/L) = −2.366albumin (g/L) +186.4; $r = -0.756$; $P < 0.001$; n = 152]. (*C*), IMA values in a serum pool to which different lactate concentrations had been added. Lactate values (*x axis*) correspond to those measured after addition of lactic acid. Results are the mean of four separate experiments. IMA (kilounits/L) = −2.70lactate (mmol/L) + 110; $r = -0.98$.

concentrations up to 250 μmol/L (mean difference ranging from −1% to 5%).

Biological evidence of myocardial ischemia remains a diagnostic challenge. Detection of IMA appears to be a promising tool for myocardial ischemia detection in patients with no increases in troponin *(1, 2)*. However, before IMA can be routinely used in clinical practice several questions, including its cardiospecificity, upper reference limit, and albumin relationship, must be answered. This work attempted to answer some of these questions. Release of biological markers from skeletal muscle is a major concern for specific myocardial damage detection. Thus, IMA tested under skeletal muscle ischemia conditions is an appropriate model. To date, only Apple et al. *(4)* have analyzed IMA in such conditions, showing increased basal IMA values in 31% of marathon runners, a drop to baseline values immediately after a marathon, and a return to increased values in 63% of cases 24–48 h thereafter. Skeletal muscle and gastrointestinal ischemia was implicated as a cause of such delayed increases in IMA values. However, this model of extensive, long-duration skeletal muscle ischemia likely does not reproduce the events occurring during myocardial ischemia.

The forearm ischemia test model, in which transient but complete blood flow occlusion is produced and skeletal muscle works under such conditions, produces skeletal muscle ischemia and is more analogous of myocardial ischemia. In the current study, during the forearm ischemia test, a significant decrease in IMA occurred 1–5 min after exercise, with recovery to basal values thereafter. Mean IMA values were always below our laboratory's 95th reference percentile, and only 9% of the analyzed samples were above this limit. However, our 95th reference limit of 101 kilounits/L was 20% higher than the 85 kilounits/L stated by the manufacturer. This is in accordance with the manufacturer's recommendation indicating the need for reference values derived from populations with the same characteristics as patients to be evaluated with the test. However, it should be noted that the small size of our reference population could also have influenced our 95th percentile.

Forearm exercise in ischemic conditions promoted sharp increases in lactate (fivefold) and ammonia (sevenfold). Because these increases occurred simultaneously with IMA decreases, possible interference of both metabolites on the ACB test was assessed. Addition experiments using a serum pool with both lactate and ammonia added produced different results. IMA values remained unchanged throughout increasing concentrations of ammonia. However, as lactate concentrations increased, IMA values decreased. Final lactate concentrations of 3–11 mmol/L reduced the initial IMA value by 7–25%, whereas concentrations of 4 and 5 mmol/L, which can be observed in clinical practice, decreased IMA values by 8% and 9%, respectively. Although an effect of lactate on the ACB test at plasma lactate concentrations within reference values could be negligible, our data suggest that lactate could interfere in the ACB test. In patients with increased lactate concentrations, decreasing true IMA values might decrease the diagnostic sensitivity. The potential of an interesting finding was the strong negative association between albumin and IMA values. Each 1 g/L change in albumin within the physiologic range of albumin (35–45 g/L) produced an opposite change of 2.6% in IMA values. This could partly explain IMA differences between populations, such as those observed between our reference value and that stated by the manufacturer. However, the contribution of interinstrument differences cannot be ruled out as a reason for such a difference. It could also suggest the need to evaluate IMA values together with those of albumin to avoid possible false-positive or -negative values in individuals with hypo- or hyperalbuminemia.

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DNA Methylation Changes in Sera of Women in Early Pregnancy Are Similar to Those in Advanced Breast Cancer Patients, *Hannes M. Müller,[1†] Lennart Ivarsson,[1†] Hans Schröcksnadel,[1] Heidi Fiegl,[1] Andreas Widschwendter,[1] Georg Goebel,[2] Susanne Kilga-Nogler,[3] Horst Philadelphy,[4] Wolfgang Gütter,[4] Christian Marth,[1] and Martin Widschwendter[1*]* (Departments of [1] Obstetrics and Gynecology, [2] Biostatistics and Documentation, and [3] Central Blood Transfusion and Immunology, Medical University Innsbruck, Innsbruck, Austria; [4] Institutes of Laboratory Medicine in Innsbruck and Wörgl, Tirol, Austria; † H.M. Müller and L. Ivarsson contributed equally to this work; * address correspondence to this author at: Department of Obstetrics and Gynecology, Medical University Innsbruck, Anichstrasse 35, A-6020 Innsbruck, Austria; fax 43-512-504-23112, e-mail martin.widschwendter@uibk.ac.at)

In normal human pregnancy, the uterus and its arterial system, including the decidua and the adjacent third of