

# Serum Chelatable Redox-Active Iron Is an Independent Predictor of Mortality After Myocardial Infarction in Individuals With Diabetes

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As a result of its strong oxidative activity, iron (1) has been hypothesized to be of importance in morbidity and mortality from atherosclerotic cardiovascular disease (CVD) (2). Numerous studies (3) have failed to show a relationship between total body iron and CVD. However, total iron may not be reflective of the risk of oxidative damage mediated by iron. A linkage between iron and CVD is more likely to be found in the amount of iron available for participating in oxidative reactions (4). Labile serum iron or labile plasma iron (LPI) represents iron bound to serum albumin, citrate, and other undefined, negatively charged ligands (5). Iron bound as LPI is associated with reactive oxygen species formation and increased oxidative stress (6).

LPI is elevated in only a small fraction of ambulatory diabetic individuals (<3%) and has not been seen in individuals without diabetes. However, LPI may become increased when large fluxes in iron occur, as in acute myocardial infarction (AMI) (7,8). We have therefore proposed that LPI is increased in the setting of AMI and that it is associated with mortality. We tested this hypothesis prospectively in individuals presenting with AMI.

## RESEARCH DESIGN AND METHODS

All patients presenting to the coronary care unit with AMI from July 2001 to July 2003 were eligible for the study ( $n = 1,156$ ). The investigational review committee on human research approved the study protocol. The primary end point of this study was death occurring within the first 30 days. Following hospital discharge, mortality data were acquired by reviewing the national death registry. Patients were categorized as having diabetes if they had any of the following: self-reported history of diabetes, diabetes medication use on admission, or a measured fasting glucose >126 mg/ml documented at least twice during the hospitalization.

## Blood collection and measurement of LPI

Serum was obtained within 24 h of symptom onset. LPI levels were measured using a previously described methodology (9) from the above-described study population in all diabetic subjects ( $n = 329$ ) and in a similar number in randomly selected nondiabetic subjects ( $n = 322$ ).

## Statistical analysis

Stratification of study participants with an LPI concentration above or below 0.4  $\mu\text{mol/l}$ , indicating the presence or absence of significantly elevated LPI, was established before all statistical analyses. This significance level for LPI was based on published reference values for LPI (no normal individuals have an LPI value  $\geq 0.4 \mu\text{mol/l}$ ) and on the reliability of the assay to determine that LPI values >0.3  $\mu\text{mol/l}$  are reproducibly >0 (5,6,9). Survival curves were estimated by the Kaplan-Meier method and were compared with the log-rank test.

## RESULTS

### LPI in nondiabetic versus diabetic patients with and without AMI

In the 322 study participants without diabetes for whom LPI was determined, 43 patients (13.5%) showed significant LPI levels. However, 35.3% (116 of 329) of the diabetic individuals had significant LPI ( $P < 0.0001$ ). The mean ( $\pm$ SD) LPI level in diabetic subjects was significantly greater than in nondiabetic subjects ( $0.43 \pm 0.7$  vs.  $0.14 \pm 0.23 \mu\text{mol/l}$ ,  $P < 0.001$ ). There was no significant difference in the baseline demographic and clinical characteristics in study participants with or without LPI in either the presence or absence of diabetes (age, sex, history of prior myocardial infarction, smoking, hypertension, hypercholesterolemia, prior medications, anterior infarction, ST elevation infarction, systolic blood pressure on admission, heart rate on admission, killip class [a clinical measurement of the severity of heart failure with myocardial infarction at the time of admission], and the use of thrombolytic or primary angioplasty therapy).

### Mortality and LPI

A total of 64 deaths (9.8%) occurred within the 30-day study period. In diabetic patients, the unadjusted mortality

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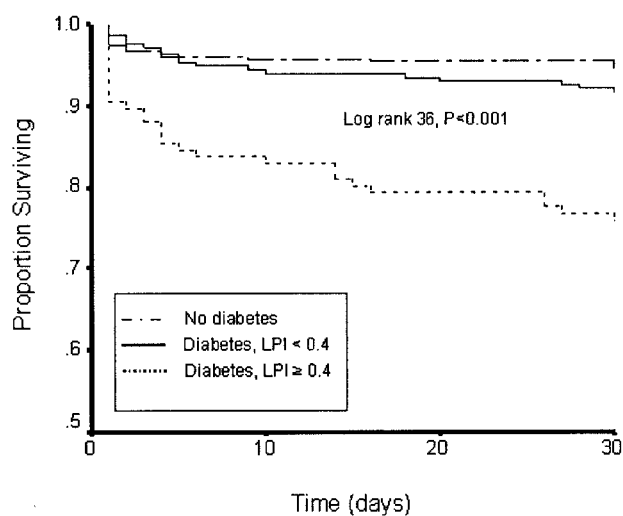
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**Abbreviations:** AMI, acute myocardial infarction; CVD, cardiovascular disease; LPI, labile plasma iron. A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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**Figure 1**—Kaplan-Meier survival curves by diabetes status and the presence or absence of significant LPI (LPI  $\geq 0.4$ ).  $P < 0.001$  for the comparison of diabetic patients with and without elevated LPI and for the comparison of diabetic patients with elevated LPI and nondiabetic patients. There was no significant difference between nondiabetic and diabetic patients without elevated LPI.

rate was 2.5-fold higher compared with that of the nondiabetic patients (i.e., 13.9 vs. 5.5%, respectively,  $P < 0.001$ ). In diabetic patients but not in nondiabetic patients, LPI was associated with an increase in mortality (24.1 vs. 8.5%, odds ratio [OR] 3.4 [95% CI 1.8–6.6],  $P < 0.001$  in diabetes, as opposed to 4.7 vs. 5.7%,  $P = 0.7$  in nondiabetic patients). After adjustment for all covariates found to be significant predictors of 30-day mortality in univariate analysis (age  $> 65$  years, sex, previous myocardial infarction, hypertension, smoking, prior aspirin treatment, anterior infarction, ST elevation infarction, systolic blood pressure  $< 100$  mmHg, heart rate  $> 100$  bpm, killip class  $> 1$ , and the use of thrombolytic or primary angioplasty therapy), elevated LPI was found to be an independent determinant of mortality at 30 days in the diabetic cohort (2.7 [1.2–6.2],  $P = 0.02$ ). The elevated mortality rate in the diabetic compared with the nondiabetic cohort could be attributed in large part to those diabetic patients with elevated LPI (Fig. 1) because those diabetic patients without elevated LPI had a mortality rate that was not significantly different from that of the study participants in the nondiabetic cohort.

**CONCLUSIONS**—Diabetes is associated with a two- to threefold increase in mortality following AMI that cannot be entirely explained by differences in infarct

size or recurrent ischemia. We have demonstrated here that LPI is elevated in over one-third of all diabetic individuals presenting with AMI and that it is an independent and powerful predictor of mortality in diabetic individuals presenting with AMI.

Previous work (9) has shown that normal individuals do not have elevated LPI levels. Moreover, LPI is elevated in only a small fraction of ambulatory diabetic individuals ( $< 3\%$ ). Elevated LPI has previously only been demonstrated (9,10) in patients with iron overload or in patients receiving intravenous iron. AMI is associated with a large flux in serum and intracellular iron stores (7,8). The elevated LPI seen in patients with AMI is not due to iron overload, as evidenced by the lack of elevated iron, transferrin saturation, and ferritin in these patients (M.S., R.A., Z.I.C., W.B., D.A., A.S., R.M.-L., H.H., A.P.L., unpublished observation). LPI may be elevated to a greater extent in AMI patients with diabetes due to both a change in the proteins that normally sequester iron and an increase in glycation of serum proteins such as albumin, thereby increasing their affinity for iron (11–15). However, this study clearly cannot be used to support a hypothesis concerning the role of protein glycation in LPI due to the lack of data on the degree of glycation in these patients (i.e., HbA<sub>1c</sub>).

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