Brief Communications

Admission Proinsulin Is Associated with Mortality in Patients with Admission Hyperglycemia during Acute Coronary Syndrome: Results from a Pilot Observational Study

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BACKGROUND: Acute hyperglycemia (AHG) is associated with mortality in patients with acute coronary syndrome (ACS). The extent to which hyperproinsulinemia contributes to worse clinical outcomes for this specific patient population is unknown.

METHODS: We included 308 consecutive ACS patients who underwent coronary angioplasty in this pilot observational study. Patients were separated into 3 groups: patients with proven diabetes mellitus (DM group) (n = 55), nondiabetic patients with a normal glucose concentration at admission (NAG group) (n = 175), and nondiabetic patients with AHG at presentation (AHG group) (n = 78). Blood samples for glucose, insulin, and proinsulin measurements were obtained at admission. The primary end point of the study was all-cause mortality, which was assessed at a mean follow-up of 19 months (interquartile range, 12–28 months).

RESULTS: Patients in the AHG and DM groups had significantly (P = 0.048) higher all-cause mortality compared with the NAG group. A univariate Cox regression analysis revealed that the proinsulin concentration was significantly associated with all-cause mortality for all study participants (hazard ratio, 1.013; 95% CI, 1.002–1.024; P = 0.023). AHG patients with increased proinsulin concentrations showed a mortality rate similar to that of DM patients but had a significantly higher mortality rate than patients with AHG and a low proinsulin concentration (χ² = 7.57; P = 0.006) and patients with NAG (with or without increased proinsulin) [χ² = 7.66 (P = 0.006) and 13.98 (P < 0.001), respectively]. A multivariate regression analysis revealed that the concentrations of glucose and proinsulin at admission were significant (P = 0.002) predictors of all-cause mortality.

CONCLUSIONS: An increased proinsulin concentration may be a marker for mortality in ACS patients with hyperglycemia at admission and without known diabetes. Further studies are needed to evaluate the role of metabolic parameters such as proinsulin.

Hyperglycemia is a common sign of glucometabolic stress during acute coronary syndromes (ACSs) (1–3). Patients with acute hyperglycemia (AHG) during ACS show increased mortality compared with patients with normal glucose concentrations at admission (NAG), independent of the presence or absence of type 2 diabetes mellitus (DM) (1, 4–6). AHG in nondiabetic patients has a greater effect on the clinical course than in patients who have preexisting DM (7, 8). Hyperproinsulinemia is a common feature of the insulin resistance (IR) syndrome. Moreover, in the Hoorn Study population (a middle-aged nondiabetic cohort) (9), proinsulin was an independent predictor of all-cause mortality. Furthermore, proinsulin has been shown to activate procoagulatory markers, such as plasminogen activator inhibitor type 1 (PAI-1) (10). Therefore, we investigated the role of proinsulin in patients with AHG during ACS.

In this single-center observational study, we enrolled 308 consecutive ACS patients who underwent a diagnostic angiography evaluation followed by percutaneous coronary intervention and stent implantation. Patients who underwent emergency bypass surgery and ACS patients who did not receive reperfusion therapy were excluded. The diagnosis and treatment of ACS were performed according to the guidelines of the European Society of Cardiology (11, 12). Patients were subdivided into an NAG group, a group of patients with an increased glucose concentration on admission (AHG group), and patients with preexisting DM (DM group). All-cause mortality was assessed at a mean follow-up period of 19 months (interquartile range, 12–28 months). Mortality data were collected from

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6 Nonstandard abbreviations: ACS, acute coronary syndrome; AHG, acute hyperglycemia; NAG, normal glucose concentration at admission; DM, diabetes mellitus; IR, insulin resistance; PAI-1, plasminogen activator inhibitor type 1; CK, creatine kinase; GFR, glomerular filtration rate; HR, hazard ratio.
Statistik Austria. Because we assessed only all-cause mortality, there were no patients lost to follow-up. The local ethics committee approved the study, and all patients provided written informed consent.

Blood samples for glucose, creatine kinase (CK), troponin I, creatinine, insulin, and proinsulin measurements were obtained at hospital admission via an atraumatic forearm vein puncture. Serum samples for glucose, CK, troponin I, and creatinine measurements were obtained at the Central Laboratory of Wilhelminen Hospital. Samples collected for insulin and proinsulin measurements were centrifuged at 1500g for 15 min at 4 °C, and the EDTA-treated plasma was frozen at −80 °C until analysis. The glomerular filtration rate (GFR) was estimated via the Modification of Diet in Renal Disease equation. Blood samples for serum CK and troponin I measurements were drawn every 4 to 6 h until peak concentrations. AHG was defined as a blood glucose concentration at admission of >140 mg/dL (7.77 mmol/L). Insulin and proinsulin plasma concentrations were measured with a double-antibody RIA (Alpco). The inter- and intraassay CVs were 4.3% and 6.8%, respectively, for insulin and 2.5% and 4.7% for proinsulin. The proinsulin assay has the following cross-reactivities: 0.1% for insulin, <0.01% for C-peptide, and 0.5% for the recombinant form of insulin lispro. There is no cross-reactivity with insulin split products, insulin B chain/A chain, or des/split species of proinsulin.

Categorical data are expressed as proportions, and continuous data are expressed as the mean (SE). Categorical variables were compared with the χ² test, and the Student t-test or the Mann-Whitney U-test was used when appropriate to compare continuous variables. Differences in survival rates were tested by Kaplan-Meier survival curve analysis and the log-rank test. Univariate and multivariate Cox proportional hazards models were used to identify significant predictors of all-cause mortality. Predictors statistically significant at the 0.2 level were included in the multivariate analysis by the backward-selection method. The following variables were tested in the univariate analysis: age; sex; estimated GFR; glucose concentration at admission; creatine kinase activity at admission; history of heart failure, atrial fibrillation, myocardial infarction, or previous percutaneous coronary intervention; and cardiovascular risk factors (hypertension, hyperlipidemia, smoking behavior, family history of coronary artery disease). Results of the Cox model analyses are presented as the hazard ratio (HR) and the 95% CI. All statistical tests were 2-sided, and statistical significance was accepted if the P value was <0.05. All statistical analyses were performed with the Software Package for the Social Sciences (IBM/SPSS).

The patients’ characteristics are summarized in Table 1. Patients in the AHG group were older and had lower estimated GFR values than patients in the NAG and DM groups. There were no differences between the study groups in plasma insulin and proinsulin concentrations.

Overall, 31 patients (10%) died during the follow-up period—11 (6.3%) in the NAG group, 11 (14.1%) in the AHG group, and 9 (16.4%) in the DM group [NAG group vs AHG group, χ² = 3.92 (P = 0.048); AHG group vs DM group, χ² = 0.145 (P = 0.70)].

The univariate Cox regression analysis showed that the proinsulin concentration at admission was a significant predictor of all-cause mortality (HR per 1 pmol/L increase, 1.013; 95% CI, 1.002–1.024; P = 0.023). When DM patients were excluded, the association between a higher proinsulin concentration and the risk of mortality was even more pronounced (HR, 1.019; 95% CI, 1.007–1.030; P = 0.001). Patients with proinsulin concentrations ≥31.4 pmol/L (75th percentile of the entire study population) exhibited a significantly higher risk of mortality than patients with lower proinsulin concentrations (HR, 2.46; 95% CI, 1.21–4.99; P = 0.013). By subdividing the nondiabetic population according to the glucose and proinsulin concentrations at admission (with the 75th percentile as the cutoff), the all-cause mortality of patients with AHG and a high proinsulin concentration was comparable to that of the DM patients (χ², 2.02; P = 0.12) but significantly higher than for the AHG patients with proinsulin concentrations below the 75th percentile (χ² = 7.57; P = 0.006) and for NAG patients with and without an increased proinsulin concentration, i.e., above or below the 75th percentile: χ² = 7.66 (P = 0.006) and 13.98 (P < 0.001), respectively (Fig. 1).

In the multivariable Cox regression analysis that included age, sex, body mass index, previous myocardial infarction and percutaneous coronary intervention, congestive heart failure, atrial fibrillation, cardiovascular risk factors, DM, renal function, glucose and proinsulin concentrations at admission, GFR, and cardiac enzymes, we found that an increasing proinsulin concentration to be a significant independent predictor of all-cause mortality (HR, 1.023; 95% CI, 1.009–1.037; P = 0.002). See the table in the Data Supplement that accompanies the online version of this brief communication at http://www.clinchem.org/content/vol57/issue10.

In contrast, although the insulin concentration for the entire study population was not associated with a higher risk of all-cause mortality (HR, 1.003; 95% CI, 0.999–1.006; P = 0.131), the insulin concentration for the DM group of patients was associated with a significantly higher risk of all-cause mortality [DM group: HR, 1.010...
In the present study, about one-quarter of the study population exhibited increased AHG concentrations, even those patients without a history of DM. There were no differences between the tested groups with respect to the insulin and proinsulin concentrations at admission. Despite these results, we found a strong independent association between proinsulin concentration and all-cause mortality, even when patients were separated according to their glucose values at admission. Furthermore, AHG patients with plasma proinsulin concentrations above the 75th percentile showed a significantly higher mortality rate during follow-up than patients below the 75th percentile. This result of our study is a new finding. Interestingly, AHG was associated with a higher mortality rate only when AHG was accompanied by an increased proinsulin concentration (Fig. 1). The strong effect of proinsulin concentration on all-cause mortality remained significant even in a multivariate analysis. In contrast, the insulin concentration at admission showed no influence on all-cause mortality among the patients with NAG and AHG. Pfützner et al. introduced the measurement of proinsulin as a surrogate marker of IR and β-cell stress (13). An increased proinsulin concentration has been shown to be associated with IR as estimated by a minimal model technique during an intravenous glucose tolerance test (13). In addition to the metabolic role of proinsulin, previous studies have reported an increased mortality rate or more severe macrovascular disease when patients display an increased fasting proinsulin concentration independent of the glucose tolerance status (9, 14, 15). In a phase II trial, subcutaneous proinsulin injections produced higher rates of cardiovascular events, which led to termination of the study (16). One possible mechanism by which proinsulin can be linked with higher mortality is that proinsulin increases the procoagulant milieu. Several studies found that proinsulin induces PAI-1 (10, 17). Furthermore, the fasting proinsulin concentration is a marker of deterioration in β-cell capacity (17–19). When we analyzed our data in view of previous findings, we assumed that patients with AHG experience a latent but evident defect in β-cell function that leads to increased concentrations of proatherogenic insulin precursors. This metabolic deficit can cause exhaustion of the β cells in a phase of increased stress and in turn lead to higher proinsulin secretion. On the other hand, we found no association between the insulin concentration at admission and all-cause mortality, with the exception of the DM population. A metaanalysis has revealed that insulin is associated with cardiovascular disease mor-

<table>
<thead>
<tr>
<th>Variable</th>
<th>NAG group (n = 175)</th>
<th>AHG group (n = 78)</th>
<th>DM group (n = 55)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>61.3 (1.03)</td>
<td>66.7 (1.52)</td>
<td>63.1 (1.59)</td>
<td>0.013</td>
</tr>
<tr>
<td>Male patients, %</td>
<td>70.9</td>
<td>61.5</td>
<td>65.5</td>
<td>0.3</td>
</tr>
<tr>
<td>GFR, mL·min⁻¹·(1.73 m²)⁻¹</td>
<td>79.7 (1.9)</td>
<td>70.0 (2.6)</td>
<td>67.0 (3.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chronic renal failure, %b</td>
<td>3.5</td>
<td>2.6</td>
<td>5.6</td>
<td>0.6</td>
</tr>
<tr>
<td>Glucose concentration at admission, mg/dLc</td>
<td>111 (1.2)</td>
<td>175 (4.6)</td>
<td>211.4 (10.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.9 (0.38)</td>
<td>27.4 (0.28)</td>
<td>29.1 (0.69)</td>
<td>0.017</td>
</tr>
<tr>
<td>Family history of CAD, %b</td>
<td>16</td>
<td>5.2</td>
<td>5.6</td>
<td>0.015</td>
</tr>
<tr>
<td>Previous myocardial infarction, %b</td>
<td>8</td>
<td>1.3</td>
<td>14.8</td>
<td>0.014</td>
</tr>
<tr>
<td>Smoking, %b</td>
<td>36</td>
<td>28.2</td>
<td>35.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Hypertension, %b</td>
<td>71.4</td>
<td>64.1</td>
<td>64.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Hyperlipidemia, %b</td>
<td>72.6</td>
<td>66.7</td>
<td>64.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Insulin, μU/mLc</td>
<td>71.2 (6.3)</td>
<td>69.1 (11.13)</td>
<td>64 (9.94)</td>
<td>0.3</td>
</tr>
<tr>
<td>Proinsulin, pmol/Lc</td>
<td>25.4 (1.5)</td>
<td>23.2 (2.6)</td>
<td>25 (2.8)</td>
<td>0.3</td>
</tr>
<tr>
<td>Peak troponin I, pg/mL</td>
<td>36.42 (5.95)</td>
<td>43.22 (7.35)</td>
<td>39.04 (12.75)</td>
<td>0.2</td>
</tr>
<tr>
<td>Peak creatine kinase, U/L</td>
<td>1249.4 (129.4)</td>
<td>1796.8 (193.8)</td>
<td>1323.3 (212.3)</td>
<td>0.053</td>
</tr>
</tbody>
</table>

* Data are presented as the mean (SE) where indicated.
* Percentage of patients with history or condition.
* Concentrations in SI units are 6.16 (0.07) mmol/L, 9.71 (0.26) mmol/L, and 11.73 (0.59) mmol/L, respectively.
* BMI, body mass index; CAD, coronary artery disease.
* The factor for converting insulin data to SI unit of measure (picomoles per liter) is ×6.945.
In the Hoorn study population, insulin lost its predictive value when proinsulin was added into the multivariate analysis (9). These conflicting results could be due to differences in the patient populations.

Our study has several limitations. First, blood samples were collected from consecutive ACS patients at admission, and therefore we did not obtain fasting proinsulin concentrations. For this reason, we cannot establish a link between increased proinsulin concentrations and metabolic dysfunction, such as IR or β-cell exhaustion. Second, we did not perform oral glucose tolerance testing or measure glycohemoglobin to diagnose DM in detail. These tests could have identified more precisely patients with prediabetic conditions, such as an impaired glucose tolerance. Third, our investigation was performed with a small sample in a single-center manner, with its inherent limitations. The study therefore needs to be corroborated or rejected in future studies with large patient populations; however, to the best of our knowledge, this report is the first description of the prognostic relevance of proinsulin concentration in patients with ACS.

In conclusion, ACS patients with AHG experience glucometabolic abnormalities that are reflected by increased plasma glucose concentrations. Our data suggest that an increased proinsulin concentration may be a marker of higher mortality in such a population.

**Author Contributions:** All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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References


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