Systolic Blood Pressure Alterations During Hyperinsulinemia Are Related to Changes in Ionized Calcium Status

Arvo Haenni, Andreas Fugmann, Lars Lind, and Hans Lithell

**Background:** A correlation between changes in ionized calcium status and changes in systolic blood pressure (BP) has previously been observed during induced euglycemic hyperinsulinemia in patients with essential hypertension. The objective of this study was to evaluate associations between alterations in ion status and BP changes during euglycemic hyperinsulinemia in healthy normotensive subjects.

**Methods:** Ion status in plasma and BP were measured before and at the end of euglycemic hyperinsulinemic clamp tests performed in 41 healthy normotensive volunteers.

**Results:** During euglycemic hyperinsulinemia plasma sodium increased by 1% (P < .0001), ionized calcium (iCa) by 5% (P < .0001), and ionized magnesium (iMg) by 4% (P < .01), whereas potassium decreased by 10% (P < .0001). The changes in plasma iCa and iMg correlated significantly to changes in systolic BP (r = 0.38, P < .02; r = −0.32, P < .05, respectively), but the correlation between changes in iMg and changes in systolic BP did not remain significant in a multiple regression model. The glucose infusion rate correlated inversely to the change in iMg (r = −0.39, P < .01).

**Conclusions:** The group mean systolic BP was unaltered during induced euglycemic hyperinsulinemia in healthy normotensive subjects; however, a more pronounced increase in the circulating iCa concentration was associated with a greater decline in systolic BP, which is in accordance with previous observations in patients with essential hypertension. The group mean diastolic BP was decreased; however, the lowered diastolic BP was not correlated to changes in ion status. Am J Hypertens 2001;14:1106–1111 © 2001 American Journal of Hypertension, Ltd.

**Key Words:** Calcium, magnesium, insulin, blood pressure, glucose clamp technique.
volunteers were recruited through local advertisements. At the initial screening visit the participants received information about the study and gave informed consent. Their medical history was taken and physical examination and laboratory tests were carried out. The study comprised 41 healthy volunteers, 27 men and 14 women. Because the clamp test procedure per se might induce hemodynamic changes, sham clamp tests were carried out in an additional group of 10 healthy volunteers, in 6 of whom the ion status was also assessed. The characteristics of the participants are presented in Table 1.

### Hyperinsulinemic Euglycemic Clamp Test

All investigations were carried out in the morning after 12 h of overnight fasting.

Insulin sensitivity was assessed by the hyperinsulinemic euglycemic clamp technique during a period of 120 min as previously described by DeFronzo et al. The insulin (Actrapid Human, Novo, Copenhagen, Denmark) was infused at a rate of 56 mU/m² body surface area/min in all subjects. The plasma insulin concentration was measured with a radioimmunoassay kit (Phadeseph Insulin RIA; Pharmacia, Uppsala, Sweden). The insulin concentrations in plasma attained during the insulin infusion in our study have been found at our laboratory to almost completely suppress hepatic glucose production, both in hypertensive and in diabetic subjects. The target plasma glucose concentration during the clamp test was 5.1 mmol/L, which was maintained by measuring the plasma glucose concentration every 5 min (Beckman Glucose Analyzer II; Beckman Instruments, Fullerton, CA) and adjusting the rate of glucose infusion (Glucose 20%, Pharmacia, Uppsala, Sweden) accordingly. The glucose uptake (M) per minute was calculated on the basis of the amount of glucose infused per minute and expressed per kilogram body weight. The insulin sensitivity index (M/I index) was calculated by dividing the mean glucose uptake, M, by the mean insulin concentration, I, during the steady state phase, that is, during the last 60 min of the 120-min clamp study, and is expressed as M/mU/L x100.

### Blood Pressure

The BP measurements were made semiautomatically (Omron 711; Omron Corp., Tokyo, Japan) in the right arm with a cuff of appropriate size. The cuff, also when deflated, was kept in the same position during the entire clamp test. The SBP and diastolic BP (DBP) were defined as Korotkoff phases I and IV, respectively. The BP was

### Table 1. Characteristics of the participants

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hyperinsulinemic Clamp Test</th>
<th>Sham Clamp Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>41</td>
<td>10</td>
</tr>
<tr>
<td>Age (y)</td>
<td>27 (5)</td>
<td>25 (3)</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>27/14</td>
<td>8/2</td>
</tr>
<tr>
<td>Body mass index</td>
<td>22.2 (2.1)</td>
<td>22.5 (2.4)</td>
</tr>
<tr>
<td>P-glucose (mmol/L)</td>
<td>5.1 (0.4)</td>
<td>5.0 (0.3)</td>
</tr>
<tr>
<td>P-insulin 0 min (mU/L)</td>
<td>9.9 (4.3)</td>
<td>9.5 (4.7)</td>
</tr>
<tr>
<td>M value (mg/kg BW/min)</td>
<td>7.9 (1.4)</td>
<td>—</td>
</tr>
<tr>
<td>P-insulin 120 min (mU/L)</td>
<td>91.7 (10)</td>
<td>8.2 (4.2)</td>
</tr>
<tr>
<td>M/I index (M value/mU/L x100)</td>
<td>9.0 (2.1)</td>
<td>—</td>
</tr>
</tbody>
</table>

Mean values (SD); P = plasma; M value = glucose uptake per min based on the glucose infusion rate during the euglycemic hyperinsulinemic clamp test; insulin 0 min = plasma insulin concentration at baseline, ie, before the euglycemic hyperinsulinemic or sham clamp tests, respectively; insulin 120 min = plasma insulin concentration at 120 min, ie, at the end of the clamp tests; BW = body weight; M/I index = insulin sensitivity index.

### Table 2. Plasma ion status and blood pressure during the euglycemic hyperinsulinemic clamp test

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Hyperinsulinemia</th>
<th>Δ %</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-Na (mmol/L)</td>
<td>139.8 (1.7)</td>
<td>141.1 (1.6)</td>
<td>+1%</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>P-K (mmol/L)</td>
<td>3.85 (0.19)</td>
<td>3.46 (0.17)</td>
<td>−10%</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>P-iCa (mmol/L)</td>
<td>1.14 (0.08)</td>
<td>1.19 (0.05)</td>
<td>+5%</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>P-iMg (mmol/L)</td>
<td>0.50 (0.05)</td>
<td>0.52 (0.04)</td>
<td>+4%</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>113 (12)</td>
<td>114 (9)</td>
<td>+1%</td>
<td>NS</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>71 (8)</td>
<td>68 (8)</td>
<td>−4%</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

iCa = ionized calcium concentration; iMg = ionized magnesium concentration; SBP = systolic blood pressure; NS = not significant; DBP = diastolic blood pressure.

Mean values (SD) for plasma ion concentrations and blood pressure immediately before the euglycemic hyperinsulinemic clamp test (baseline) and during hyperinsulinemia at the end of the steady state period of the clamp test, and the changes in these variables (Δ %).
measured to the nearest mm Hg after 30 min in the supine position immediately before the start of the insulin and glucose infusions, and at frequent intervals during the clamp test. The same equipment was used in all participants.

**Ion Status**
The ion status (Na, K, iCa, and iMg concentrations and pH in plasma) was determined by the KONE microlyte analyzer (KONE Instruments, Espoo, Finland). Blood samples were taken with a syringe from an antecubital vein without stasis. The blood samples were heparinized in accordance with the guidelines given in the instructions for the KONE microlyte analyzer. Silicon-free laboratory equipment was used. The blood samples were centrifuged for 1 min and the ion status in the plasma was then assessed. The adequacy of the free ion analysis was checked by using prefabricated solutions with known ion concentrations (KONE). The coefficients of variation for the different plasma ion measurements were less than 5% in consecutive measurements from the same sample. Although the blood samples were treated as anaerobically as possible, total anaerobic conditions are difficult to establish, and for this reason a theoretical correction for pH-dependent changes in ionized calcium and magnesium concentrations is integrated into the KONE microlyte system. The iCa and iMg values at pH 7.4 (corrected) were used in the statistical calculations.

**Body Mass Index**
Body mass index was calculated as body weight in kilograms divided by height in meters squared.

**Statistics**
Descriptive measurements used were mean and standard deviation. Continuous variables were tested for normality (Shapiro-Wilk’s test). Mean changes in variables induced by 120 min of euglycemic hyperinsulinemia were tested for statistical significance by Student’s t test, or with the Wilcoxon signed-rank test when the data were not normally distributed (change in DBP), and the results are presented as mean change and P value for the change. Bivariate associations are expressed as Pearson’s correlation coefficient. Nonparametric measures of associations (Spearman’s rank-order correlations) were used in cases of non-normally distributed variables. To elucidate the interdependence of different variables, two different stepwise multiple regression analyses were applied, in the first model the change in SBP was chosen as dependent variable and changes in iCa and iMg concentrations as candidate regressors; in another model the change in iMg was chosen as dependent and M/I index and I as independent variables. The statistical analyses were performed with the statistical software package JMP (SAS Institute Inc., Cary, NC).

**Results**
During the hyperinsulinemic euglycemic clamp test the plasma Na, iCa, and iMg concentrations increased significantly, whereas the K concentration decreased (Table 2). The changes in plasma iCa and iMg were closely correlated ($r = 0.73, P < .0001$). During hyperinsulinemia the group mean DBP decreased significantly, but there was no significant change in group mean SBP (Table 2).

The individual changes in the plasma iCa and iMg concentrations correlated significantly to the changes in
SBP ($r = -0.38$, $P < .02; r = -0.32$, $P < .05$, respectively; Figs. 1A and 2A); however, the correlation between the changes in plasma iMg and the changes in SBP did not remain significant ($P = .55$) in a stepwise regression model where the change in plasma iMg was entered in a model already including the change in SBP as dependent and the change in iCa as independent variables. The changes in plasma Na and K did not correlate significantly to alterations in BP variables. There were no significant correlations between alterations in ion variables and DBP (Figs. 1B and 2B).

The glucose infusion rate, M, correlated inversely to the change in plasma iMg ($r = -0.39$, $P < .01$; Fig. 3), whereas the correlation between M/I index and plasma iMg change did not reach statistical significance ($r = -0.18$, $P = .21$). This discrepancy might be explained by the variability in plasma insulin concentrations during the steady-state phase of the clamp test (I). When I was entered in a stepwise regression model where the change in iMg had been chosen as dependent variable and M/I index as independent, the correlation between M/I and change in iMg turned out to be significant ($P < .05$). No significant correlations were found between changes in plasma Na, K, or iCa concentrations, SBP, or DBP, on the one hand, and the M value or M/I index, on the other.

The sham clamp test did not induce any significant changes in the ion status or in SBP or DBP (Table 3).

**Discussion**

The group mean DBP decreased during euglycemic hyperinsulinemia in healthy normotensive subjects, which is in conformity with previous observations. The group mean SBP, on the other hand, showed a small tendency toward the increase. The opposite changes in SBP and DBP during induced euglycemic hyperinsulinemia in healthy subjects have previously been reported. However, the range of observed SBP changes indicates differences in the circulatory response to induced hyperinsulinemia in different experimental subjects. Although the
group mean SBP did not change significantly there was an inverse correlation between the changes in plasma iCa and iMg concentrations, on the one hand, and SBP changes, on the other. An inverse relationship between the changes in circulating iCa and changes in SBP during induced hyperinsulinemia has previously been observed in patients with essential hypertension. Similarly, Resnick et al. reported an inverse relationship between changes in serum iCa and changes in SBP during a 2-h intravenous calcium chloride infusion in hypertensive patients. Natriuretic effects, which have been seen during hypercalciemia induced by calcium infusion in healthy subjects, may help to explain the reduction in SBP observed in healthy subjects.

A high calcium diet has been shown to produce an increase in renal sodium excretion and a decrease in SBP. In two recent meta-analyses calcium supplementation has been shown to result in small but significant reductions in SBP but not in DBP. In accordance with previous findings in patients with essential hypertension there were no correlations between changes in DBP and ion status.

The changes in the plasma Na and iCa concentrations did not correlate to the glucose infusion rate or insulin sensitivity, which may indicate that the changes in ion status might be independent of the glucose disposal rate. Experiments on platelets led to the conclusion that insulin directly affects calcium metabolism, resulting in a decreased intracellular concentration of ionized calcium. Insulin, by stimulating the Na\(^{-}\)–K\(^{+}\) pump and thereby hyperpolarizing the cell, reduces intracellular influx of ionized calcium through voltage-operated calcium channels. Insulin incubation has been found to result in a dose-dependent increase in ionized calcium efflux from vascular smooth muscle mediated by stimulation of plasmalemmal Ca\(^{2+}\)-ATPase activity.

The change in the plasma iMg level was inversely related to glucose uptake. Paolissio et al. reported that insulin increased the intracellular magnesium content in incubated erythrocytes. However, cells from insulin-resistant subjects showed an impaired magnesium uptake, with a dose–response curve shifted to the right and a reduction in the maximum magnesium uptake. Reduced intracellular magnesium concentrations have been observed in different tissues in patients with conditions associated with insulin resistance. Thus, impaired intracellular magnesium uptake may contribute to the inverse correlation between alterations in serum magnesium and the rate of glucose disposal. However, the increase in plasma iMg during hyperinsulinemia still has to be explained. An inverse correlation between circulating iMg and free fatty acids has previously been demonstrated. Because lipolysis is reduced during induced hyperinsulinemia, it may be hypothesized that a decreased level of circulating free fatty acids might help to explain the increased iMg concentration.

In contrast to observations in hypertensive patients, no significant correlation was found between changes in plasma Na and changes in SBP during euglycemic hyperinsulinemia. High salt intake was recently reported by Alam et al. to induce a significant increase in BP in patients with isolated systolic hypertension, but it did not significantly alter the BP in normotensive individuals. Previously, Beretta-Piccoli and Weidmann had reported an association between exchangeable sodium and SBP in hypertensive but not in normotensive diabetics. These observations might reflect alterations in salt sensitivity in different experimental subjects. Another reason for reported divergences regarding the insulin-mediated effects on the ion status may be that the changes in circulating ion concentrations might possess nonlinear qualities that are not taken into account when changes in the ion status are calculated from measurements made before and after 120 min of induced euglycemic hyperinsulinemia.

In conclusion, the group mean SBP was not altered during induced euglycemic hyperinsulinemia in healthy normotensive subjects; however, a more pronounced increase in the circulating ionized calcium concentration was associated with a greater decline in SBP, which is in accordance with previous observations in patients with essential hypertension. The group mean DBP was decreased; however, the lowered DBP was not correlated to changes in ion status.

### References
