The Alterations in Insulin Sensitivity During Angiotensin Converting Enzyme Inhibitor Treatment Are Related to Changes in the Calcium/Magnesium Balance

Arvo Haenni, Lars Berglund, Richard Reneland, Per-Erik Anderssson, Lars Lind, and Hans Lithell

The present analysis was undertaken to investigate the relations between alterations in mineral factors, especially the balance between serum calcium and magnesium concentrations (S-Ca and S-Mg, respectively), and variables reflecting glucose and lipid metabolism during angiotensin converting enzyme (ACE) inhibitor treatment. A total of 96 patients with essential hypertension, participating in four double-blind studies with four different ACE inhibitors and similar protocols, were included. At the end of the initial placebo period and at the end of the period of active drug treatment, a hyperinsulinemic euglycemic clamp test was carried out, lipoprotein status was assessed, and the concentrations of serum electrolytes were measured. The serum ACE activity was determined in the group treated with fosinopril. Changes in insulin sensitivity index (M/I) were directly correlated to alterations in S-Mg (r = 0.24, P < .02), and inversely correlated to changes in S-Ca (r = -0.19, P = .07) and the ratio between serum calcium and magnesium concentrations (Ca/Mg) (r = -0.27, P < .008). The change in total serum triglycerides (S-Tg) was inversely correlated to the change in S-Mg (r = -0.35, P < .0005), and directly correlated to the change in Ca/Mg ratio (r = 0.36, P < .0004). The reduction in serum ACE activity correlated to a more pronounced increase in S-Mg (r = -0.62, P < .002), and decrease in the Ca/Mg ratio (r = 0.73, P = .0002).

We conclude that the changes in the studied metabolic variables and serum ACE activity during ACE inhibitor treatment are related to alterations in mineral status and the balance between calcium and magnesium concentrations in serum. © 1997 American Journal of Hypertension, Ltd. Am J Hypertens 1997;1:145-151

KEY WORDS: Hypertension, magnesium, calcium, angiotensin converting enzyme inhibition, insulin.

In diabetes mellitus, as well as in essential hypertension, the serum magnesium concentration has been found to be decreased and inversely correlated to the glucose level.1-4 An ionic imbalance, implying an increased intracellular calcium/magnesium ratio, has been suggested as a possible mechanism in hypertension, glucose intolerance, and the insulin-resistant atherothrombogenic syndrome. Anti-hypertensive drugs have been shown to affect glucose tolerance, insulin sensitivity, and the lipoprotein status. Diuretics and β-blockers impair these metabolic variables,5,6 while angiotensin converting enzyme (ACE) inhibitors, α-blockers, and calcium antagonists have had neutral or positive effects.6-8 However, different effects on insulin sensitivity have been observed with different ACE inhibitors.6-11 The im-
improvement in insulin sensitivity during captopril treatment has been shown to be correlated to an increase in serum magnesium concentration.15 This report is focused on the question as to whether an altered calcium and magnesium status in the serum is related to changes in glucose and lipid metabolism in a larger group of patients with essential hypertension treated with different ACE inhibitors.

In a previous article on the metabolic effects of fosinopril treatment in essential hypertension it was reported that the reduction in serum ACE activity was correlated to a more pronounced increase in insulin sensitivity and decrease in serum triglycerides, although the changes in these metabolic variables did not reach statistical significance per se.16 Another aim of the present study was to investigate whether reduced serum ACE activity was associated with altered mineral status.

MATERIALS AND METHODS

A total of 96 patients participated, under approved informed consent, in four different double-blind studies on captopril, enalapril, fosinopril, and lisinopril, with similar protocols (Table 1). Included were previously treated or untreated male and female patients, aged 18 to 70 years, with a history of essential hypertension. Patients with secondary hypertension and those with malignant/accelerated hypertension were excluded, as were patients with diabetes mellitus, hepatic dysfunction. Hypertensive patients who had lipid disorders (total cholesterol > 8.0 mmol/L, plasma total triglycerides [Tg] > 5.0 mmol/L), renal impairment (plasma creatinine > 140 mmol/L), or hepatic dysfunction. Hypertensive patients who had had myocardial infarction during the past 3 months and those with a history of aortic outflow obstruction or unstable angina pectoris were also excluded.

All studies, except the captopril study, were designed as double-blind, randomized, parallel-group studies where one of the two groups on active antihypertensive treatment in each study were treated with an ACE inhibitor. The study including captopril was designed as a cross-over study; however, only the first period of active treatment is reported here, ie, only the results from the participating subjects who were treated with captopril during the first study period because of carry-over effects from the first to the second part of the period of active treatment. In all studies there was an initial single-blind placebo run-in phase of 2 to 6 weeks. If the diastolic blood pressure (DBP) in the sitting position, or, in the enalapril trial, in the sitting position, reached the inclusion level after this placebo run-in period, the patients were randomized to receive active treatment for 16 to 26 weeks, and a lower or higher dose of the active drug was given depending on the blood pressure response (Table 1). The aim in all studies was to reduce the supine DBP to at least 90 mm Hg.

The blood pressure was measured at least every 4th week. At the end of the placebo period and at the end of the period of active drug treatment, a hyperinsulinemic euglycemic clamp test was carried out, the lipoprotein status was assessed, and the concentrations of electrolytes in the blood were measured. All blood samples as well as the hyperinsulinemic euglycemic clamp test were performed after 12 h of fasting. The patients were seen at each visit at the same time of day by the same medical staff.

Insulin sensitivity was measured by the hyperinsulinemic euglycemic clamp technique as described by DeFronzo et al.15 The insulin (Actrapid Human, Novo, Copenhagen, Denmark) infusion rate was 56 mU/m2/min in all subjects. Plasma glucose was assayed immediately in duplicate in a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Immunoreactive insulin was assayed in plasma using a commercial radioimmunoassay kit (Phadeseph Insulin RIA, Pharmacia, Uppsala, Sweden). The plasma insulin concentrations attained during the insulin infusion in our study have been reported to suppress hepatic glucose production to a negligible rate both in hypertensive subjects and in diabetics, even in the presence of insulin resistance.16 The glucose uptake (M value) per minute was calculated on the basis of the amount of glucose infused per minute and expressed per kilogram body weight (mg/kg BW/min). The insulin sensitivity index (M/I ratio) was calculated by dividing the mean glucose uptake (M value) by the mean insulin concentration (I) during the steady state phase (the last 60 min) of the clamp study (M value/mU/L x 100).

TABLE 1. THE FOUR STUDIES ON ACE INHIBITOR TREATMENT IN ESSENTIAL HYPERTENSION INCLUDED IN THE PRESENT ANALYSIS

<table>
<thead>
<tr>
<th>Drug</th>
<th>Daily Dose (mg)</th>
<th>n</th>
<th>Initial Wash-Out Period (weeks)</th>
<th>Duration (weeks)</th>
<th>Inclusion Diastolic Blood Pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Captopril</td>
<td>50-100</td>
<td>23</td>
<td>4-6</td>
<td>16</td>
<td>95-119</td>
</tr>
<tr>
<td>Enalapril</td>
<td>10-20</td>
<td>22</td>
<td>4-6</td>
<td>26</td>
<td>95-114</td>
</tr>
<tr>
<td>Fosinopril</td>
<td>20</td>
<td>24</td>
<td>2-6</td>
<td>16</td>
<td>95-114</td>
</tr>
<tr>
<td>Lisinopril</td>
<td>10-20</td>
<td>27</td>
<td>4-6</td>
<td>24</td>
<td>95-115</td>
</tr>
</tbody>
</table>
Triglyceride (S-Tg) and cholesterol concentrations in serum were measured by enzymatic techniques (Boehringer Mannheim, Mannheim, Germany) in a Monarch 2000 (Instrumentation Laboratory, Lexington, MA) centrifugal analyzer.

Serum electrolyte concentrations were assayed at the Department of Clinical Chemistry, University Hospital, Uppsala, Sweden. The blood samples were collected in vacuum tubes for serum analysis (Vacutainer 7609BN324ZZN7, Becton Dickinson Vacutainer Systems, Rutherford, NJ) in the morning after 12 h of fasting, and transported to the department of clinical chemistry where the electrolyte concentrations were assessed during the same day. Total serum calcium (S-Ca) was measured by a cresolphthalein complexone method (Boehringer Mannheim) automatic analysis for Hitachi 717 (Hitachi Ltd., Tokyo, Japan). The serum magnesium (S-Mg) was measured by atom absorption photometry (Video 22, Instrumentation Laboratory). The coefficients of variation is <2% for both of these methods.

The plasma aldosterone concentration was assayed by a commercial radioimmunoassay kit (Aldosterone-RIA, DPC Diagnostic Products Corp., Los Angeles, CA).

The ACE activity in serum was determined fluorometrically using hippuryl-histidyl-leucine as substrate. All procedures were performed by the same investigator (RR).

The body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

### STATISTICS

For variables with a skewed distribution, logarithmic transformation was performed. An analysis of variance model was used to test changes within groups and between groups over time. Least squares means constitute the basis of the tests and estimates in the analysis, and the reported significance levels correspond to these calculations. All comparisons were made against the results from the end of the placebo period. Relationships between variables were analyzed by calculations of Pearson's correlation coefficients. A preliminary test using Bartlett's method was carried out to analyze equality of variance in the different drug groups. The correlations included in the analysis with combined data were found not to be significantly different in the different treatment groups. Adjustments were made for different means in the different treatment groups and joint within-group correlations were calculated. To elucidate the interdependence of different variables, stepwise multiple regression analysis was applied. Values of $P < .05$ were considered statistically significant. All statistical analyses were performed with the statistical pro-

### TABLE 2. METABOLIC CHARACTERISTICS OF THE HYPERLIMINIC PATIENTS AFTER THE INITIAL PLACEBO RUN-IN PERIOD (0), AND CHANGES ($\Delta$) AFTER TREATMENT WITH FOUR DIFFERENT ACE INHIBITORS

<table>
<thead>
<tr>
<th></th>
<th>Captopril</th>
<th>Enalapril</th>
<th>Fosinopril</th>
<th>Lisinopril</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI</strong></td>
<td>26(4)</td>
<td>26(4)</td>
<td>26(4)</td>
<td>26(4)</td>
</tr>
<tr>
<td><strong>SBP</strong></td>
<td>165(14)</td>
<td>164(18)</td>
<td>158(18)</td>
<td>162(16)</td>
</tr>
<tr>
<td><strong>DBP</strong></td>
<td>101(5)</td>
<td>104(5)</td>
<td>94(4)</td>
<td>102(7)</td>
</tr>
<tr>
<td><strong>M</strong></td>
<td>5.7(2.5)</td>
<td>4.5(1.9)</td>
<td>5.9(1.6)</td>
<td>6.0(3.1)</td>
</tr>
<tr>
<td><strong>Mg</strong></td>
<td>5.6(3.3)</td>
<td>4.4(2.9)</td>
<td>6.0(1.9)</td>
<td>6.2(3.8)</td>
</tr>
<tr>
<td><strong>FDP</strong></td>
<td>5.7(1.4)</td>
<td>6.0(1.4)</td>
<td>5.4(1.6)</td>
<td>5.5(1.6)</td>
</tr>
<tr>
<td><strong>FDP INR</strong></td>
<td>9.0(5.7)</td>
<td>13.1(6.6)</td>
<td>7.6(4.2)</td>
<td>8.4(5.9)</td>
</tr>
<tr>
<td><strong>S-Tg</strong></td>
<td>1.9(1.0)</td>
<td>2.3(1.2)</td>
<td>1.4(0.9)</td>
<td>1.6(0.9)</td>
</tr>
<tr>
<td><strong>S-Chol</strong></td>
<td>6.0(0.9)</td>
<td>6.4(1.5)</td>
<td>5.7(1.1)</td>
<td>5.9(1.1)</td>
</tr>
<tr>
<td><strong>S-Ca</strong></td>
<td>3.95(0.23)</td>
<td>4.03(0.58)</td>
<td>3.90(0.29)</td>
<td>3.94(0.24)</td>
</tr>
<tr>
<td><strong>S-Mg</strong></td>
<td>2.32(0.09)</td>
<td>2.30(0.07)</td>
<td>2.30(0.09)</td>
<td>2.33(0.08)</td>
</tr>
<tr>
<td><strong>S-Mg</strong></td>
<td>0.81(0.05) #</td>
<td>0.83(0.07)</td>
<td>0.83(0.07)</td>
<td>0.84(0.04)</td>
</tr>
<tr>
<td><strong>S-Ca/Mg ratio</strong></td>
<td>2.90(0.22)</td>
<td>2.79(0.22)</td>
<td>2.83(0.28)</td>
<td>2.76(0.15)</td>
</tr>
<tr>
<td><strong>S-Ace activity</strong></td>
<td>na</td>
<td>0.04(0.01)</td>
<td>0.04(0.01)</td>
<td>na</td>
</tr>
<tr>
<td><strong>P-alcohol</strong></td>
<td>na</td>
<td>1.37(55)</td>
<td>1.37(55)</td>
<td>na</td>
</tr>
</tbody>
</table>

Adjusted mean values (± SD) for body mass index (BMI), systolic and diastolic blood pressures (SBP, DBP, mm Hg), glucose (mg/dl), and insulin sensitivity index (S-I) are given at the hyperinsulinemic euglycemic clamp test. Fasting plasma glucose (S-Gl), insulin (S-Inr), triglyceride, and total cholesterol concentrations (S-Tg, S-Chol, respectively, mmol/L), serum potassium, calcium, and magnesium concentrations (S-K, S-Ca, S-Mg, respectively, mmol/L), plasma aldosterone (S-Ace), plasma aldosterone (S-P-alcohol) and the changes in these variables after treatment with captopril, enalapril, fosinopril, or lisinopril.

*P < .05, †P < .01, ‡P < .001, compared to placebo. †P = .001, captopril v lisinopril.

RESULTS

Ninety-six patients completed the four studies with the different ACE inhibitors (Table 1).

The blood pressure decreased significantly during treatment with all four ACE inhibitors. The mean values and changes in metabolic and mineral variables in the four ACE inhibitor treated groups are shown in Table 2. The group of hypertensives treated with captopril showed an increased glucose infusion rate at the clamp test, and improved insulin sensitivity index. Lisinopril treatment, on the other hand, resulted in decreased M value; however, the impairment of M/I was not statistically significant. No significant changes appeared in these variables in the enalapril or fosinopril treatment groups.

The fasting plasma glucose concentration, serum potassium, and serum total cholesterol and triglycerides did not change significantly during treatment with any of the four drugs tested (Table 2).

Serum magnesium increased in the captopril treated group while there were no significant changes in S-Mg (ΔS-Mg) or S-Ca (ΔS-Ca) in the other three groups (Table 2). There was no significant correlation between ΔS-Mg and ΔS-Ca.

When the different ACE inhibitor treated groups were analyzed separately it was found that in each group the changes in the Ca/Mg ratio (ΔCa/Mg) and M/I (ΔM/I) were inversely correlated (r = -0.17 to -0.41, P between 0.41 (NS) and < .05) (Figure 1A). When all the included ACE inhibitor treated subjects were combined a significant inverse correlation between ΔCa/Mg and ΔM/I was found (r = -0.27, P < .008) (Figure 1B).

The correlations between ΔS-Mg, ΔS-Ca, and ΔCa/Mg, on the one hand, and the changes in M value (ΔM), plasma insulin concentration at the end of the steady-state phase at the hyperinsulinemic euglycemic clamp test (ΔIns 120 min), ΔM/I, and ΔS-Tg, on the other hand, are shown in Table 3.

Because the S-Mg during the initial placebo period in the captopril treated group was significantly lower (P < .007) than that in the lisinopril treated patients, another statistical analysis was performed with all captopril treated subjects excluded. However, the correlations between ΔCa/Mg, on the one hand, and ΔM (r = -0.27, P < .02), ΔM/I (r = -0.24, P < .05), and ΔS-Tg (r = 0.35, P < .003), on the other, remained significant in the lisinopril, fosinopril, and enalapril treated patients combined. The correlation between ΔS-Mg and ΔS-Tg was also significant in this subgroup (r = -0.30, P < .01).

When the combined data from the four studies were analyzed, it was found that the inverse correlation between the ΔM/I and ΔCa/Mg remained significant (P < .05), even when treatment, the changes in BMI, fasting blood glucose, insulin, and triglyceride concentrations were included in a stepwise multiple regression model.

The change in ΔS-Tg was correlated to the ΔCa/Mg (r = 0.36, P < .0004), and inversely correlated to ΔM/I (r = -0.30, P < .003) (Figure 2A, B). These correlations remained at a significant level also when all three variables were included in a multiple analysis.

There were no significant correlations between the ΔCa/Mg and blood pressure levels. No significant correlations were observed between the changes in serum potassium concentration and M, M/I, or lipid levels.

The serum ACE activity and plasma aldosterone concentration were assayed in the fosinopril treated subjects, and were found to be significantly decreased by 60% and 34%, respectively (Table 2). The reduction in serum ACE activity, was observed to be inversely correlated to ΔS-Mg (r = -0.62, P < .002) and correlated to ΔS-Ca (r = 0.45, P < .05) and ΔCa/Mg (r = 0.73, P = .0002) (Figure 3A, B, C). There were no
TABLE 3. CORRELATIONS BETWEEN THE CHANGES IN MINERAL STATUS AND METABOLIC VARIABLES

<table>
<thead>
<tr>
<th></th>
<th>$\Delta M$</th>
<th>$\Delta \text{ins 120 min}$</th>
<th>$\Delta M/I$</th>
<th>$\Delta T_g$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta Mg$</td>
<td>$r = 0.21$</td>
<td>$p &lt; 0.04$</td>
<td>$r = 0.24$</td>
<td>$p &lt; 0.05$</td>
</tr>
<tr>
<td>$\Delta Ca$</td>
<td>$r = 0.20$</td>
<td>$p &lt; 0.01$</td>
<td>$r = 0.19$</td>
<td>$p &lt; 0.0005$</td>
</tr>
<tr>
<td>$\Delta Ca/Mg$</td>
<td>$r = 0.29$</td>
<td>$p &lt; 0.05$</td>
<td>$r = 0.27$</td>
<td>$p &lt; 0.005$</td>
</tr>
</tbody>
</table>

The changes in serum magnesium ($\Delta Mg$) and calcium ($\Delta Ca$) concentrations and the ratio between calcium and magnesium ($\Delta Ca/Mg$), on the one hand, and the changes in insulin-mediated glucose disposal ($\Delta M$), plasma insulin concentration at 120 min of the hyperinsulinemic euglycemic clamp test ($\Delta \text{ins 120 min}$), insulin sensitivity index ($\Delta M/I$), and total triglyceride concentrations ($\Delta T_g$), on the other hand.

significant correlations between changes in plasma aldosterone level and mineral status.

DISCUSSION

In the present analysis the changes in insulin-mediated glucose disposal and the insulin sensitivity index as evaluated by the hyperinsulinemic euglycemic clamp test were directly correlated to the change in serum Mg concentration and inversely correlated to the Ca/Mg ratio. Also when the change in plasma insulin level, a variable previously shown to be closely related to M/I, was included in that analysis, the correlation between the changes in M/I and Ca/Mg ratio remained significant.

It is unclear whether the change in mineral status represents a cause or an effect of the improved insulin sensitivity. Recently, Kawahara et al reported a beneficial effect of oral magnesium supplementation on insulin resistance, evaluated by OGGT, in subjects with essential hypertension. Previously, Paolisso et al observed an increased acute insulin response and improved insulin sensitivity after chronic magnesium supplementation in NIDDM subjects, and reported that both the plasma magnesium concentration and the erythrocyte magnesium content increased, while the fasting plasma glucose decreased. Hypomagnesemia may lead to an increased vasomotor tone in many types of blood vessels. Magnesium administration results in vasodilatation, reduced response to different vasoconstrictor agents, decreased vascular resistance, increased local blood flow, and higher cardiac output. Calcium ions have been reported to induce vasoconstriction and may also potentiate the arterial response to sympathetic activity and catecholamines. Infusion of calcium increases the blood pressure and the concentrations of both epinephrine and norepinephrine. Although there were no correlations found between changes in the Ca/Mg ratio and blood pressure levels, this does not rule out changes in peripheral circulation. Thus, it may be hypothesized that the increased serum magnesium concentration, and, consequently, the decreased Ca/Mg ratio during ACE inhibitor medication may, through improved microcirculatory supply or by influence on cellular metabolic processes, enhance glucose and lipid transportation to and disposal in the target tissue, the skeletal muscle, which may partly explain the inverse correlation between $\Delta S-T_g$ and $\Delta S-Mg$ and between $\Delta S-T_g$ and $\Delta M/I$. These findings are in accordance with those of previous studies, where oral magnesium supplementation has been shown to decrease the triglyceride concentration in serum and improve insulin sensitivity.

On the other hand, Lindemann et al observed that insulin administration increased urinary magnesium excretion in non-diabetic individuals. Thus, if the plasma insulin concentration decreases as a consequence of improved insulin sensitivity, it may have a magnesium-sparing effect, increasing the serum magnesium concentration.

A larger reduction in serum ACE activity, shown in the fosinopril treated hypertensives, was associated with...
with more pronounced mineral changes, implying a decreased Ca/Mg ratio in serum. The results indicate that the alteration in serum ACE activity is more closely correlated to the change in the balance between calcium and magnesium concentrations in serum, expressed as the Ca/Mg ratio, than to each ion concentration separately.

ACE is responsible for the inactivation of the potent vasodilator bradykinin, a substance that has been shown to improve glucose disposal. Thus, a decrease in ACE activity might result in a higher bradykinin concentration. Uehara et al observed that ACE inhibitors with a sulphydryl group had a more potent action on the plasma bradykinin concentration and produced greater improvement in insulin sensitivity than those without. This might help to explain why the sulphydryl containing, serum magnesium elevating captopril was found to have an improving effect on insulin sensitivity. So far, there has been only scant information about the direct effects of magnesium on bradykinin metabolism. Another aspect regarding the different effects on metabolic variables of the different ACE inhibitors might be that the recommended doses of the drugs lie on different parts of the sigmoid log dose response curve for ACE inhibition.

In conclusion, the results of this analysis of correlations indicate that the changes in the metabolic variables and serum ACE activity analyzed in the present study are correlated to changes in magnesium and calcium concentrations in serum, and the balance between these minerals, expressed as the Ca/Mg ratio. An increase in serum magnesium in relation to serum calcium is associated with improved insulin sensitivity during treatment with ACE inhibitors in essential hypertension. The reduction in serum ACE activity is associated with a more pronounced increase in serum magnesium concentration and decrease in the serum Ca/Mg ratio. However, the question as to whether the change in mineral status represents a cause or an effect, or both, of improved insulin sensitivity still remains.

REFERENCES


