Serum ionized magnesium in chronic alcoholism: is it really decreased?

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Chronic alcoholism is associated with a marked deficit in total magnesium (tMg). However, little is known about the status of the physiologically active form, ionized magnesium (iMg). We assessed serum iMg (measured with two ion-selective electrodes, AVL 988-4 and NOVA CRT) and tMg concentrations in chronic alcoholics at admission (n = 31) and after abstinence (n = 13) and compared these results with those for a control group (n = 40). At admission, the tMg and NOVA iMg concentrations in alcoholics (0.78 ± 0.020 and 0.38 ± 0.016 mmol/L, respectively) were significantly less (P < 0.001) than in the controls (0.85 ± 0.008 and 0.50 ± 0.006 mmol/L). The AVL iMg results, however, did not differ significantly between the two groups: 0.53 ± 0.013 vs 0.56 ± 0.006 mmol/L, respectively (P > 0.05). The mean iMg between the two analyzers differed significantly in both groups (P < 0.001). After 3 weeks of abstinence, the alcoholics showed a significant increase in tMg (P < 0.001) and in both NOVA and AVL iMg values (P < 0.01 for each). tMg concentrations were positively correlated with the AVL iMg values in both alcoholics and controls but correlated positively with the NOVA iMg results only in the controls. Thus, the altered status of iMg is instrument-dependent, and the usefulness of the measurement in alcoholics is yet to be determined.

INDEXING TERMS: ion-selective electrodes • electrolytes • ethanol • variation, source of

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Magnesium is currently a subject of major interest in biology and medicine. It is involved in many of the biochemical reactions that take place in the cell, particularly in those processes involving the formation and utilization of ATP [1]. A growing body of evidence links altered status of ionized magnesium (iMg) to many pathophysiological and disease states, e.g., long-term renal transplants, cardiac surgery (before and during), hypertension, asthma, and non-insulin-dependent diabetes mellitus [2–4]. Nevertheless, our understanding of the role of Mg ions in biological processes is still far from complete.

Since the first report in 1936 by Cline and Coleman [5], numerous studies have been published describing a marked deficiency of total magnesium (tMg) in chronic alcoholism [6,7]. Several mechanisms associated with alcoholism contribute to the magnesium deficiency, including urinary Mg wastage, malnutrition, gastrointestinal losses, phosphate deficiency, acidosis/alkalosis, vitamin D deficiency, and free fatty acidemia associated with alcohol (ethanol) withdrawal [6]. The potential detrimental effects of Mg deficiency are well established. Alcoholics, who are already at risk for multiple system failure because of malnutrition and the toxic effects of alcohol on all tissues, have the potential for exaggerated risk for morbidity and mortality in the presence of Mg deficiency [7]. Intracellular Mg concentration is the critical analyte in diagnosing Mg deficiency. Studies in vivo, as well as quantitative digital imaging microscopy of cultured cells, reveal that alcohol induces rapid concentration-dependent depletion of iMg [7]. However, the high level of expertise required for intracellular measurements of iMg precludes the determination of this analyte in routine clinical chemistry laboratories.

To complement the preceding studies, ion-selective electrodes (ISEs) for iMg recently introduced by various manufacturers allow the direct assessment of iMg in the extracellular space [8–11]. Given that serum iMg is...
thought to be in equilibrium with the intracellular compartment [12], determination of serum iMg may have clinical value in assessing disorders of Mg metabolism.

The aim of this investigation was to study serum iMg concentrations (determined with two different ion-selective analyzers) in subjects with chronic alcoholism; the relation of serum iMg to serum tMg in these subjects; and any possible changes in these analytes after a period of abstinence. Our previous experience with the ISEs used here indicated some unexplained discrepancies in results for iMg in healthy subjects and in a group of randomly selected patients [10]. In this study, therefore, we also examined the effect of ethanol, β-hydroxybutyrate, and acetoacetate on the Mg ISEs and performed routine biochemical determinations in an effort to relate potential between-analyzer differences in iMg to the biochemical profile in the subjects investigated.

Materials and Methods

Subjects. We studied 29 men and 2 women between ages 31 and 65 (mean 43) years who fulfilled the diagnostic criteria for alcoholism (alcohol dependence) according to the Diagnostic and Statistical Manual of Mental Disorders IV [13]. The patients were admitted to an inpatient alcohol research unit at the National Institutes of Health under an abstinence protocol. All patients had been drinking until 24 h before admission and had consumed alcohol excessively for between 2 and 33 (mean 14) years. Their average alcohol intake during the last 6 months ranged from 67 to 517 g of absolute alcohol per occasion. Studied as a separate group were nine different subjects who met the criteria of alcohol dependence but who had not been drinking alcohol for the last several months. Finally, we studied a control group 40 presumably normal individuals (28 men and 12 women) between ages 23 and 66 years (mean 40), who abstained from alcohol.

All participants were volunteers under no legal constraints, and all gave a written informed consent before the study. All procedures were in accordance with the ethical standards laid down in the Helsinki Declaration of 1975, as revised in 1983.

Specimen collection and biochemical determinations. Initial blood specimens for routine biochemical determinations were drawn from all of the patients at the time of admission, before the administration of any medication. Using general clinical chemistry analyzers, we analyzed the samples for glucose, albumin, total protein, aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, alkaline phosphatase, total bilirubin, blood urea nitrogen, creatinine, uric acid, electrolytes, tMg, total and ionized calcium, phosphorus, lactate, hemoglobin, and prothrombin time. The specimens for iMg, obtained within 48 h of the time of admission, were analyzed by ISEs on two different instruments: NOVA CRT (NOVA Biomedical, Waltham, MA) and AVL 988-4 (AVL, Roswell, GA). The blood was drawn without forearm exercise into Vacutainer Tubes (Becton Dickinson, Rutherford, NJ; cat. no. 6397), under strictly anaerobic conditions. A description of both ISE instruments and of their analytical performance has been published previously [10].

We obtained additional samples for tMg and iMg determinations from 13 of the alcoholic patients who participated in standard inpatient detoxification treatment, after 3 weeks of abstinence. Again, their initial blood measurements were conducted before any medication had been administered.

Effect of ethanol and keto acids on the iMg electrodes. Because iMg determination in alcoholic subjects may involve assay of samples taken while the subjects are intoxicated or having metabolic disturbances (most commonly, metabolic acidosis), we studied the potential interference of ethanol, β-hydroxybutyrate, and acetoacetate on the performance of the two iMg electrodes used in the study. To test the effect of ethanol, we first prepared a series of working solutions at three different concentrations of Mg by adding MgCl₂·6H₂O to AVL Standard A (0.30, 0.98, and 1.65 mmol/L) and to NOVA Set C Level 1 (0.35, 1.02, and 1.68 mmol/L) calibrator solutions and then added increasing concentrations of ethanol (1.0, 2.0, and 4.0 g/L) to each solution. Similarly, for the other two potential interferents tested, we added increasing concentrations of β-hydroxybutyrate (2.0, 6.0, and 10 mg/L) or acetoacetate (1.0, 2.0, and 5.0 mg/L) to the NOVA and AVL calibrator solutions, which had been supplemented to contain tMg at 1 mmol/L.

All specimens were analyzed in triplicate on NOVA CRT and AVL 988-4 analyzers. Clinical significance was judged relevant if the difference in the results before and after addition of the interferent exceeded 10% of the iMg concentration in the working solutions.

Data and statistical analyses. We expressed results as mean ± SE and analyzed within- and between-group comparisons by using Student’s t-test and Deming (debiased) regression, as appropriate. Correlations were assessed with simple and multiple stepwise linear regression. All analyses were two-tailed and conducted with SAS software (Version 6.08 for Windows; SAS Institute, Cary, NC) and Microsoft Excel (Version 4.0; Microsoft, Redmond, WA). P <0.05 was considered statistically significant.

Results

Comparisons with other biochemical markers. Alcoholics and controls gave significantly different results for most of the biochemical and hematological markers: P <0.001 (Student’s t-test) for aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, alkaline phosphatase, anion gap, and chloride; and P <0.03 for hemoglobin, total bilirubin, total protein, glucose, blood urea nitrogen, uric acid, and sodium.
iMg and tMg differences between chronic alcoholics and controls. The results for tMg, iMg, and %iMg in chronic alcoholics and controls are summarized in Table 1. Fig. 1 shows the distribution of iMg in both groups, as measured with a NOVA CRT and an AVL 988-4. All of the controls had tMg values within the reference interval, 0.65–1.05 mmol/L; for 13% of the alcoholics, however, tMg was <0.65 mmol/L.

For iMg, 42% (n = 13) of the alcoholics had a NOVA result of <0.39 mmol/L, but none had NOVA iMg >0.64 mmol/L (iMg reference interval on the NOVA CRT, 0.39–0.64 mmol/L) [10]. In contrast, only 6.5% (n = 2) of the AVL iMg results for the alcoholics were <0.39 mmol/L, whereas 19% (n = 6) exceeded 0.60 mmol/L (AVL reference interval, 0.44–0.60 mmol/L) [10]. None of the controls had iMg below the reference interval on either instrument, although 3 (7.5%) had an AVL iMg value >0.60 mmol/L. The lowest serum iMg concentration detected in an alcoholic was 0.13 mmol/L by the NOVA and 0.41 mmol/L by the AVL. For the controls, these values were 0.42 and 0.45 mmol/L, respectively.

There was a significant difference between the alcoholics and controls for tMg (P <0.001) and for the NOVA iMg (P <0.001) results but not for the AVL iMg results (P >0.05). The alcoholics had lower %iMg (i.e., iMg/tMg) calculated from the NOVA iMg results (P <0.001), but percentages were similar to those in the controls when calculated from the AVL iMg (P >0.05) (Table 1). The nine alcoholics who did not consume alcohol for several months had tMg of 0.86 ± 0.01 mmol/L and iMg (by AVL) of 0.58 ± 0.008 mmol/L, not significantly different from the values for the control group (P <0.001).

Effect of abstinence on tMg and iMg. After 3 weeks of abstinence, the serum concentrations of tMg and of iMg (by both NOVA and AVL) increased significantly (Table 1). Furthermore, mean tMg (0.85 mmol/L) and mean iMg by AVL (0.58 mmol/L) in these subjects were similar to the mean values in the controls (0.85 and 0.56 mmol/L, respectively). The mean NOVA iMg (0.41 mmol/L), however, was still well below the mean value for the control group (0.50 mmol/L). The increase in tMg and iMg serum concentrations was accompanied by normalization of the values for the liver enzymes.

Analyzer-dependent differences for iMg. The difference in mean iMg values between the two instruments was statistically significant in both alcoholics (paired t-test, P <0.001) and controls (P <0.001) (Table 1, Fig. 2). In the control group, the Deming (debiased) regression showed an acceptable slope of 1.007, an intercept of 0.057 mmol/L, and Syx = 0.01 mmol/L. The low correlation

### Table 1. Serum iMg and tMg (mean ± SE) in alcoholics at admission and after 3 weeks of abstinence and in controls.

<table>
<thead>
<tr>
<th>At admission</th>
<th>tMg, mmol/L</th>
<th>NOVA CRT</th>
<th>AVL 988-4</th>
<th>NOVA CRT</th>
<th>AVL 988-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcoholics (n = 31)</td>
<td>0.78 ± 0.020</td>
<td>0.38 ± 0.016</td>
<td>0.53 ± 0.013</td>
<td>49.8 ± 2.4</td>
<td>68.2 ± 1.0</td>
</tr>
<tr>
<td>Controls (n = 40)</td>
<td>0.85 ± 0.008</td>
<td>0.50 ± 0.006</td>
<td>0.56 ± 0.006</td>
<td>58.8 ± 0.8</td>
<td>65.9 ± 0.5</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&gt;0.05</td>
<td>&lt;0.001</td>
<td>0.04</td>
</tr>
<tr>
<td>Abstinence effect (n = 13)</td>
<td></td>
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<td></td>
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<tr>
<td>At admission</td>
<td>0.75 ± 0.025</td>
<td>0.37 ± 0.019</td>
<td>0.51 ± 0.025</td>
<td>49.07 ± 2.5</td>
<td>67.08 ± 1.9</td>
</tr>
<tr>
<td>After 3 weeks</td>
<td>0.85 ± 0.022</td>
<td>0.41 ± 0.019</td>
<td>0.58 ± 0.016</td>
<td>48.08 ± 2.1</td>
<td>67.78 ± 1.0</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>0.005</td>
<td>0.002</td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

* P <0.001, NOVA vs AVL in alcoholics and controls (paired t-test).  
* Significance of the comparison between alcoholics and controls (Student’s t-test).
Coefficient \((r = 0.272)\) was attributed to the narrow iMg range among those subjects. The alcoholic group, however, had a slope of \(-0.382\), an intercept of 0.676 mmol/L, and \(S_{\text{uix}} = 0.02\) mmol/L. When we compared only values for the alcoholics with iMg \(<0.38\) mmol/L by NOVA \((n = 13)\), the mean between-analyzer difference for iMg was 0.23 mmol/L. For comparison, the mean difference between the alcoholics for whom iMg was \(>0.39\) mmol/L by NOVA was 0.09 mmol/L \((n = 18)\); for the control group, that difference was only 0.06 mmol/L.

**Correlation**. The correlation between serum tMg and the AVL iMg was significant and positive in both alcoholics \((r = 0.864, P <0.001)\) and controls \((r = 0.740, P <0.001)\). However, NOVA iMg results were significantly and positively correlated to tMg only in the control group \((r = 0.389, P <0.03)\), not in the alcoholic group \((r = -0.058, P >0.7)\) (Fig. 3). Application of stepwise linear multiple regression analysis to evaluate the relation between the serum iMg and each of the following—tMg, total and ionized calcium, albumin, total protein, electrolytes, phosphorus, and anion gap—gave the best fit for the relation between iMg and tMg. Because addition of these other variables did not improve the fit, we concluded that they did not have an independent effect on iMg differences between instruments and between groups.

**Analytical interference of ethanol and keto acids**. Our interference study with ethanol, \(\beta\)-hydroxybutyrate, and acetoacetate did not detect any clinically significant changes (differences exceeding \(\pm 10\%\)) in the iMg results measured by either ISE instrument.

**Discussion**

Our finding of significantly decreased mean serum iMg in chronic alcoholics \((0.38\) mmol/L\), as measured on NOVA CRT, is in good agreement with the iMg results published recently by Wu and Kenny [14], using a similar instrument: \(0.35 \pm 0.12\) mmol/L. Results of the AVL analyzer, however, were not significantly different between alcoholics and controls \((P >0.05)\). The comparison between the NOVA CRT and AVL 988-4 analyzers indicated that the two instruments agreed very well for iMg in the control group (slope 1.007, intercept 0.057 mmol/L), despite the fact that the mean difference between the instruments, 0.06 mmol/L, was statistically significant \((P <0.001)\). In contrast, the comparison between results of the two analyzers for the alcoholic group showed a negative slope and no significant correlation (slope \(-0.382\), intercept 0.676 mmol/L, mean difference 0.15 mmol/L, and \(r = -0.201\)).
We suggest two possible explanations for the observed discrepancy: First, most of the NOVA iMg results for alcoholics were at or below the instrument’s lower reference limit of 0.39 mmol/L, the area in which the NOVA and AVL disagreed the most (mean difference 0.23 mmol/L). Several methodological factors such as differences in the manufacturers’ calibration solutions, reference electrode construction, and the liquid-junction potential have been associated with this lack of agreement and have already been described [10, 15]. Second, potential interferences in the alcoholics’ specimens would have to affect the analytical performance of the iMg electrodes or at least the performance of the ion-selective membrane. The results from our interference study and other recently published data [14] lead us to conclude that ethanol—and β-hydroxybutyrate and acetoacetate—are unlikely to affect the ISE measurements of iMg.

In a previous study, Rehak et al. [15] found that the responses of both AVL and NOVA ISEs for iMg were affected by an increase in the measured concentration of ionized calcium. The mean ionized calcium in our alcoholic group, however, as measured on either instrument, did not differ significantly from that in the controls (NOVA: 1.21 vs 1.19 mmol/L, respectively (P > 0.05); AVL: 1.28 vs 1.25 mmol/L, respectively (P > 0.05)). Even after analysis of a possible relation between the difference in the iMg results and the unique biochemical profile of the alcoholics, we were not able to verify that any of the tested analytes had affected the performance of the instruments. In our opinion, therefore, the difference in the results between the two analyzers is primarily attributable to differences in the design, construction, and calibration of these two ISEs.

A low tMg is expected in subjects with chronic alcoholism [6, 7]. The mean tMg of 0.78 mmol/L in our alcoholics is a little higher than but in agreement with the results reported by De Marchi et al. [16], 0.70 ± 0.15 mmol/L (n = 61). Among the factors likely to contribute to our higher results could be differences in the proportion of alcoholics studied with tMg < 0.65 mmol/L (13% in our study vs 30% reported by De Marchi et al.), methodological differences for tMg determinations, and sample selection bias (0.85 mmol/L mean tMg in our control group vs 0.90 mmol/L in theirs).

Recently published data by Wu and Kenny [14] showed that acute alcohol consumption is more likely to influence serum iMg than tMg. Mean iMg was 0.349 mmol/L in their ethanol-positive specimens—significantly below normal and not correlated with the mean tMg, which was 0.865 mmol/L and not significantly different from the tMg in their controls. In our alcoholic group, the iMg measured by the NOVA also did not correlate significantly with the tMg (r = 0.058, P > 0.05); however, our group of chronic alcoholics had years of excessive alcohol consumption and, unlike the ethanol-positive group studied by Wu and Kenny, also had decreased tMg.

The correlation between the tMg and NOVA iMg was positive and significant in our controls, as was the correlation between tMg and AVL iMg in both controls and alcoholics. Further, an increase in the serum tMg in our group of alcoholics was associated with a decrease in %Mg measured with the NOVA (Fig. 3). The negative slope (-0.051) was mainly attributable to the results for three alcoholics who had both very low NOVA iMg values (0.13, 0.16, and 0.24 mmol/L) and normal tMg (0.82, 0.91, and 0.93 mmol/L, respectively). Excluding these three results from the analysis gave a correlation between tMg and NOVA iMg in the alcoholics that was close to that found for the control group (slope 0.206, intercept 0.247 mmol/L, r = 0.344). After examining the hospital records of the three alcoholics, however, we could find no clinical reasons to exclude their results from the study.

After 3 weeks of abstinence, the concentrations of tMg and iMg in the alcoholics (n = 13) increased. Only tMg and AVL iMg values, however, reached the mean concentrations of the control group. Recent publications have indicated that acute alcohol consumption is associated with some electrolyte and metabolite disturbances, which tend to normalize after even a short period of abstinence [16]. In support of this observation are our results for the nine alcoholics who were abstinent at the time of admission. Their tMg and AVL iMg concentrations did not differ significantly from those of the controls (P > 0.05); their NOVA iMg (0.37 mmol/L), however, was almost identical with the mean iMg in those alcoholics who had been drinking until the time of admission (0.38 mmol/L).

In conclusion, this and earlier studies show that determination of iMg activity in biological fluids is influenced by several factors and that the values found for it may differ somewhat when measured with different instruments. In particular, the status of serum iMg in alcoholics cannot be assessed with the necessary degree of certainty by current ISE methodologies; accordingly, the usefulness of iMg determinations is yet to be established.

References


