Increase in Dialyzable Calcium Associated with Therapy with Lithium

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We measured total and dialyzable calcium concentrations in consecutive sera submitted for routine lithium analysis. Of 98 samples from 61 different individuals, six (6.1%) total calcium results and 32 (33%) dialyzable calcium results were above the respective reference intervals. By comparison, when both total and dialyzable calcium were measured on 50 different apparently healthy volunteers, no results were outside either reference interval (2.20–2.58 mmol/L for total and 1.30–1.47 mmol/L for dialyzable calcium). These increases were not due to age or sex differences between the patients and controls. From the dialyzable calcium data, there appears to be an even higher incidence of mild hypercalcemia in patients receiving oral lithium salts than is indicated by the total calcium concentration alone.

Additional Keyphrases: hypercalcemia • hyperparathyroidism • sex- and age-related differences

Although some studies have shown that lithium administration caused no statistically significant changes in serum total calcium, mild increases in calcium may actually have been present (1, 2). Other studies concerned with short-term use of lithium found either no detectable change in the concentration of calcium (3) or slightly lower calcium amounts in controls given lithium (4). Groehl et al. (3) found calcium to be increased by 10% over controls, and Christiansen et al., in a series of five reports on essentially the same study (6–10), observed a mean increase in total calcium of about 11%, but a mean increase in total calcium, corrected for variations in protein, of only 2%. This hypercalcemia appeared to be associated with lithium treatment and not the psychosis itself (9).

Hyperparathyroidism has also been noted in these patients (7–11). Christiansen et al. emphasize that this increase is of a mild biochemical nature (7). Christenson (11), while not mentioning the size of the population from which his group was drawn, found parathyroid adenomas in four of six patients on lithium who developed hypercalcemia.

Initially, we measured only total calcium on 50 consecutive samples submitted for routine serum lithium analysis. When it was apparent that the incidence of increased total calcium results was somewhat higher than expected (five of 50: 10%), we then began to collect samples for dialyzable calcium measurements as well. Although dialyzable (ultrafiltrable) calcium has not been a widely used routine clinical laboratory test, we now use a convenient continuous-flow method (12) on a daily basis because of its analytical dependability. These dialyzable calcium studies reveal a considerably higher incidence of increased dialyzable calcium values than total calcium in patients taking lithium, and this increase is not due to age or sex differences between the study and control groups.

Methods

Procedures for measuring dialyzable calcium (12), total calcium (13), and lithium (14) were used essentially as described in the references. Total protein was determined by refractometry.

The dialyzable calcium procedure was modified slightly by the use of a buffer at pH 7.40 and sera obtained with minimal loss of CO₂ as follows. Blood serum was collected into evacuated blood-collection tubes and allowed to clot, then centrifuged prior to removing the clot. Approximately 1 mL of serum was withdrawn immediately upon removing the top and then sealed into a 1-mL plastic syringe. To minimize atmospheric exposure, we added approximately 0.4 mL of this "anaerobic" serum to each analyzer cup 15 s or less before the sampling probe drew the serum into the analytical system. These modifications will be described more fully in a forthcoming report (15).

Our control results were obtained on sera collected from 50 apparently healthy volunteers, with the age and sex distributions shown in Table 1. These control subjects were seated for at least 2 min and a tourniquet was applied just prior to venipuncture. All stated they had not eaten for at least 1 h. Half of these results were obtained throughout the period in which data were concurrently being obtained on the patients receiving lithium.

Our reference interval for dialyzable calcium (1.30–1.47 mmol/L) is similar to that published initially (1.33–1.50 mmol/L) as mean ± 2 SD (12). Our experience over the last two years with dialyzable calcium measurements from hospital patients as well as from over 100 healthy individuals has demonstrated that our reference interval has been a useful guide to the interpretation of these results.

Results

Calcium, Age, and Sex Data

Overall, we analyzed 148 sera for total calcium from patients on lithium and found 11 (7.4%) to have above-normal values. Of the 98 samples for which we measured both total and dialyzable calcium, six had increased total calcium and 32 had increased dialyzable calcium. These results were from five and 22 individuals, respectively.

The age and sex distributions of each population are shown in Table 1. We feel that these distributions between patients on lithium and the control individuals are reasonably equivalent, and cannot be an explanation for the differences we have observed in calcium concentrations.

For the lithium group, Table 2 shows that the increases in means of both total (2.46 vs 2.35 mmol/L) and dialyzable

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calcium (1.47 vs 1.37 mmol/L) in women was greater than the increases in total (2.44 vs 2.40 mmol/L) and dialyzable calcium (1.45 vs 1.39 mmol/L) in men. These differences were all significant, with \( p < 0.001 \) for all except the total calcium in men, where \( p < 0.1 \). Age factors cannot account for the differences observed because, in Table 2, for every age group where data were available, the dialyzable calcium was always higher in patients in lithium, and in all but two groups, the total calcium was also higher in these patients. The means of both the total and dialyzable calcium (2.45 and 1.46 mmol/L, respectively) from all patients receiving lithium was increased relative to the same measurements from all the healthy volunteers studied (2.37 and 1.38 mmol/L, respectively). The mean increases in total and dialyzable calcium were 0.08 mmol/L (3.4% increase) and 0.08 mmol/L (5.8%), respectively. Both of these differences were significant (\( p < 0.001 \)).

The total vs dialyzable calcium results are shown for the healthy group and for the patients administered lithium (Figure 1). It is noteworthy that, among our healthy controls, none of the results were outside our stated reference limits. The lithium group tended as a whole to have increased values, even though many were within the reference intervals.

**Quality Control**

The results from the patients on lithium and about half of our control group were obtained during September and October of 1978. During this period, as is routinely done, human serum matrix material stored frozen in individually sealed glass tubes was thawed and analyzed on each day of analysis. The variation for dialyzable calcium was low, as the mean ± SD of 1.32 ± 0.015 mmol/L (CV = 1.2%) on 16 different days indicates. This was also true for total calcium, where the mean ± SD was 2.24 ± 0.025 mmol/L (CV = 1.1%) during the same period.

**Effect of Lithium on Methods of Calcium Determination**

The serum pool was obtained and the pH was adjusted to 7.37 (just under 7.40) with 1 mol/L HCl, then aliquots were collected into 5-mL plastic syringes. While the serum was in the syringe, an appropriate volume of 100 mmol/L LiCl (from LiCO\(_3\)) was added to prepare serum samples containing 0, 0.5, 1.0, and 2.0 mmol of Li per liter. Although the pH change after this process was small, the pH of each specimen was checked and adjusted to 7.40 (at 37 °C) simply by allowing CO\(_2\) to escape, if necessary. The specimens were then analyzed in duplicate for dialyzable calcium. These results indicated that lithium had no discernible effect on the dialyzable calcium result, at least up to 2.0 mmol of Li per liter. Analysis of these samples for total calcium by atomic absorption spectrophotometry likewise showed no effect attributable to lithium.

**Correlation of Lithium with Total and Dialyzable Calcium**

Li concentrations were determined in all serum samples as part of the requested analyses. The correlation between lithium and dialyzable calcium (\( r = 0.30 \)) was better than that between lithium and total calcium (\( r = 0.08 \)). The correlation of lithium with dialyzable calcium was significant at the 99% confidence level (\( p < 0.01 \)); there was no significant correlation between lithium and total calcium.

**Measurement of Protein**

We measured total protein on 25 samples selected without conscious bias from the lithium-treated group and on 46 samples from our controls. The data, shown in Table 3, show that protein concentrations were nearly the same in both groups. The protein-bound calcium was the same for both groups studied, which was somewhat unexpected in view of the increased dialyzable calcium among the lithium-treated patients.

**Discussion**

In a study of this type, where one of two tests (dialyzable calcium) appears to be a more sensitive indicator of hypercalcemia, it was of utmost importance to establish that the limits set for each reference interval had nearly the same incidence of false positives. For example, if 1% false positives were found with use of our reference interval for total calcium

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**Table 1. Age and Sex Distribution of Subjects**

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Control group(^a)</th>
<th>Lithium treated(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>Under 20</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>20–29</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>30–39</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>40–49</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>50–59</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Over 59</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>28</td>
</tr>
</tbody>
</table>

\(^{a}\) Each person had only one result.

\(^{b}\) The number of different people is in the left portion of the column; the number of results obtained is shown in parentheses.

**Table 2. Calcium (mmol/L) by Age and Sex\(^a\)**

<table>
<thead>
<tr>
<th>Age, yr</th>
<th>Under 20</th>
<th>20–29</th>
<th>30–39</th>
<th>40–49</th>
<th>50–59</th>
<th>Over 59</th>
<th>All ages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total calcium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group, men</td>
<td>2.45</td>
<td>2.39 ± 0.11</td>
<td>2.41 ± 0.05</td>
<td>2.41</td>
<td>2.30</td>
<td>—</td>
<td>2.40 ± 0.08</td>
</tr>
<tr>
<td>Lithium group, men</td>
<td>2.47 ± 0.06</td>
<td>2.49 ± 0.08</td>
<td>2.39 ± 0.06</td>
<td>2.32 ± 0.05</td>
<td>2.46 ± 0.05</td>
<td>—</td>
<td>2.44 ± 0.08</td>
</tr>
<tr>
<td>Control group, women</td>
<td>2.40</td>
<td>2.34 ± 0.08</td>
<td>2.34 ± 0.06</td>
<td>2.39</td>
<td>2.35</td>
<td>—</td>
<td>2.35 ± 0.07</td>
</tr>
<tr>
<td>Lithium group, women</td>
<td>2.51 ± 0.14</td>
<td>2.51 ± 0.17</td>
<td>2.42 ± 0.11</td>
<td>2.41 ± 0.09</td>
<td>2.36 ± 0.07</td>
<td>2.40 ± 0.09</td>
<td>2.46 ± 0.14</td>
</tr>
<tr>
<td>Dialyzable calcium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control group, men</td>
<td>1.40</td>
<td>1.40 ± 0.02</td>
<td>1.38 ± 0.02</td>
<td>1.39</td>
<td>1.38</td>
<td>—</td>
<td>1.39 ± 0.03</td>
</tr>
<tr>
<td>Lithium group, men</td>
<td>1.46 ± 0.04</td>
<td>1.47 ± 0.05</td>
<td>1.42 ± 0.03</td>
<td>1.43 ± 0.04</td>
<td>1.48 ± 0.04</td>
<td>—</td>
<td>1.45 ± 0.05</td>
</tr>
<tr>
<td>Control group, women</td>
<td>1.37</td>
<td>1.37 ± 0.03</td>
<td>1.38 ± 0.03</td>
<td>1.35</td>
<td>1.39</td>
<td>—</td>
<td>1.37 ± 0.03</td>
</tr>
<tr>
<td>Lithium group, women</td>
<td>1.51 ± 0.07</td>
<td>1.50 ± 0.11</td>
<td>1.48 ± 0.07</td>
<td>1.44 ± 0.04</td>
<td>1.41 ± 0.02</td>
<td>1.42 ± 0.04</td>
<td>1.47 ± 0.09</td>
</tr>
</tbody>
</table>

\(^{a}\) Mean ± SD given whenever four or more results are available. Numbers of people and data in each group as in Table 1.
and 5% false positives with the dialyzable calcium reference interval, then we may have expected to get the type of result found here, namely, that a higher percentage of dialyzable calcium results apparently would be increased in patients on lithium. The data in Figure 1 (left) indicate that both reference intervals show few false positives, as all our data from healthy individuals fall within the intervals. The narrower limits for dialyzable calcium relative to total calcium appear justified because this measurement eliminates the protein-bound calcium, a major source of variation in the total calcium concentration.

Note that the means of both the total and dialyzable calcium were increased by the same net difference in concentration (0.08 mmol/L). Yet the mean increase in dialyzable calcium was 5.8%, whereas that for total calcium was 3.4%. These percent increases are less than the 11% increase in uncorrected total calcium observed by Christiansen et al. (10). These same investigators found only a 1.8% increase in "corrected" total calcium, implying that the ionic and dialyzable calciums were increased to a lesser degree than was total calcium. Our data indicate this is not the case, however, and that a "correction" of total calcium based upon the protein content often gives unsatisfactory results, as has been shown by Ladenson et al. (16).

Because total calcium has been shown to vary with age and sex (17), it was necessary to show that these factors did not account for the differences observed. The two groups studied were certainly not identical in their age and sex distributions, but we feel that age and sex differences were not reasons for the increased calcium. Support for this is seen with the data in Table 2, in which every sex and age group comparison showed the lithium group to have higher calcium concentrations than controls, except for total calcium in 30-39- and 40-49-year-old men. Furthermore, although neither the study nor the number of data allow us to reach a definite conclusion, we speculate that a person's sex, and possibly age, may be related to the calcium response during lithium treatment. Very distinct changes in both total and dialyzable calcium occurred in women under 30 years of age. These calcium changes were less dramatic in men, and overall the calcium changes of women as a whole were greater than those of men (Table 2).

Our study with dialyzable calcium indicates that this measurement may be more sensitive than total calcium for detecting hypercalcemia in patients receiving lithium. Christiansen et al. (6), using total calcium "corrected" to a constant protein concentration, found hypercalcemia in 12% of those patients taking lithium, but did not state the percentage of increased, uncorrected results.

Neither analytical variation nor interference in the calcium measurements is a likely explanation for our results. The day-to-day precision was good (CV ~ 1%) and lithium could not be shown to interfere with the analytical method (Figure 3). Further, there was a poor correlation between lithium and total calcium. The correlation between lithium and dialyzable calcium, while statistically significant, nevertheless indicates

![Figure 1](image1.png)

**Fig. 1.** Dialyzable vs total calcium concentrations from apparently healthy individuals (left) and from patients taking lithium (right). One lithium patient, not shown, had values of (mmol/L) dialyzable calcium, 1.91, and total calcium, 2.96.

![Figure 2](image2.png)

**Fig. 2.** Effect of lithium concentration on amount of dialyzable calcium.

<table>
<thead>
<tr>
<th>Table 3. Protein and Calcium Data (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Total protein, g/L</td>
</tr>
<tr>
<td>Total calcium, mmol/L</td>
</tr>
<tr>
<td>Dialyzable calcium, mmol/L</td>
</tr>
<tr>
<td>Protein-bound calcium, mmol/L</td>
</tr>
</tbody>
</table>
there was no agreement in 91% (1 - 0.30²) of the paired results.

This statistically significant correlation (confidence level 0.99) may, however, indicate that a possible physiologic relationship exists between lithium and dialyzable calcium. One may speculate that an even higher correlation might be found between dialyzable calcium and erythrocyte lithium, this intracellular parameter having been suggested to be a better index of the physiologically functional concentration of lithium (18, 19).

The increase in dialyzable calcium without an increase in either protein or protein-bound calcium (Table 3) may be explainable by an increased complexed calcium fraction, a decreased affinity of the proteins for calcium or both. Without simultaneous measurement of the free ionized calcium, no conclusion can be reached about an increase in the complexed fraction. A decreased affinity of the protein for the calcium could be caused by lower pH in the serum, but only if a severe acidois had been present (pH < 7.1). This is because pH is controlled at around 7.40 in the dialyzable calcium procedure, to the extent that a change in serum pH of 0.1 alters the final dialyzable calcium result by only 0.02 mmol/L. Our results also indicate that addition of lithium to pooled serum does not appreciably alter the proportion of dialyzable calcium. Therefore, our present study does not provide sufficient information to satisfactorily explain the changes. A more direct study of the protein binding, with gel filtration (20), may provide more insight into this area.

Two of the patients in our study were of particular interest regarding possible hyperparathyroidism. One was a 71-year-old woman who developed hypercalcemia after about eight months of lithium therapy for depression and after one year was referred to an endocrinologist at this hospital. The lithium therapy was considered to be ineffective and was discontinued, but the hypercalcemia persisted. Parathyrin was increased, and later a parathyroid adenoma was found at surgery. The other case was a 21-year-old woman on lithium for about three years, who had a very high dialyzable calcium (1.91 mmol/L) and subsequently increased parathyrin. She has not had any other symptoms of hyperparathyroidism, and determination of dialyzable calcium several months later was much lower, although still above normal (1.54 mmol/L). Christiansen (11) described six patients on lithium who developed hypercalceemia, with four of these six eventually developing parathyroid adenomas. Christiansen et al. (7) criticized this work as not having given the size of the population from which the six patients were drawn. This is important to consider; our patient with an adenoma was referred to this hospital for endocrine evaluation and therefore was selected from a much larger population than the typical patient receiving lithium at this hospital. The other, more typical, patient is doing well on lithium and no intervention seems warranted.

Our data are consistent with the hypothesis that lithium is an agent capable of inducing mild hypercalcemia, either transiently or persistently, in most individuals being administered lithium. There is also a possibility that lithium therapy uncovers latent hyperparathyroidism in a few individuals. Obviously, careful long-term study of patients before and after repeated courses of lithium will be required to provide more understanding of these possible relationships. Finally, dialyzable calcium has been an informative tool for studying calcium metabolism and, in this application, appears to be more sensitive to changes than is total calcium.

References