
CLIN. CHEM. 36/1, 142-144 (1990)

Albumin-Adjusted Calcium Concentration in Serum Increases during Normal Pregnancy

R. B. Payne, A. J. Little, and R. T. Evans

Concentrations of total calcium and albumin were measured in serum specimens from 41 women at intervals before, during, and after 42 pregnancies. The albumin concentration decreased but the calcium decreased more slowly, so that the albumin-adjusted calcium concentration increased from conception to term. These findings, taken in conjunction with published observations of hypercalcemia, increased concentrations of 1,25-dihydroxycholecalciferol and calcitonin in serum, and decreased concentrations of intact parathyrin in serum, strongly suggest that maternal ionized calcium increases throughout normal pregnancy.

Striking changes in maternal calcium metabolism take place during pregnancy. The plasma concentrations of 1,25-dihydroxycholecalciferol (1,25-DHCC) (1–3) and calcitonin (4) increase, the gastrointestinal absorption of calcium increases (2), and there is a marked increase in urinary calcium (2, 5, 6). Despite this, and despite evidence of some reversible bone resorption in early pregnancy (7), calcium balance is positive (5) and maternal bone structure (7) and bone mineral content (8) remain unchanged at term. Studies of changes in plasma concentrations of parathyrin (parathyroid hormone, PTH) during pregnancy have given conflicting results. Workers using older one-site radioimmunoassays that measure fragments as well as intact PTH have variously reported increased, normal, or decreased concentrations of the hormone. However, the first study involving a specific two-site radiometric assay for the intact PTH molecule has clearly shown lowered concentrations (9).

These changes suggest that 1,25-DHCC synthesis increases first, stimulating intestinal absorption of calcium and leading to hypercalcemia, increased calcitonin secretion, secondary hyperparathyroidism, and hypercalcuria. One link in this chain has not been confirmed by measurement: hypercalcemia. Because the albumin concentration in plasma decreases substantially by about 10 g/L during pregnancy (10, 11), total calcium decreases. Ionized calcium concentration measured in anaerobic specimens has, with one exception (12), been reported as unchanged or lowered (2, 9, 13–17). However, the instruments commonly used to measure ionized calcium report lower values when the albumin concentration is decreased (18–21), so that an increase in ionized calcium during pregnancy might be obscured by the decreasing albumin. We have therefore examined the relationship between total calcium and albu-
min in serum samples collected before, during, and after pregnancy, to seek evidence for hypercalcemia.

Subjects and Methods

Women who were attending family-planning clinics in and around Leeds during 1983–1986 and who expressed their intention to stop using contraception so as to become pregnant were invited by one of us (R.T.E.) to take part in a longitudinal study of changes in serum cholinesterase activity. Blood specimens were collected at four-week intervals before and during pregnancy. The volunteers came to the laboratory for venipuncture before conception and in the early stages of pregnancy, but were visited at home later and after delivery. Blood was obtained within 24 h of delivery and at varying intervals for 12 weeks subsequently. Further details of the subjects and the results of this study have been published (22).

We took the opportunity to analyze the serum samples for some common analytes on a Monitor Parallel discretionary analyzer (American Monitor UK Ltd., Burgess Hill, Sussex, U.K.). Total calcium was measured by using cresolphthalein complexone, and albumin by dye-binding with bromcresol green after a standard reaction time of 1.5 min. Analytical CVs at normal concentrations in serum, calculated from duplicate measurements, were 1.1% for calcium and 1.7% for albumin. Serum samples were stored at −15 °C before analysis, and all samples from one individual were thawed and analyzed in the same batch. Results were available for 42 normal pregnancies in 41 women.

The measured calcium concentration was adjusted for albumin in two stages. First, to estimate the concentration of non-protein-bound calcium in the sample, we subtracted from the total calcium the product of albumin concentration and the slope of the regression of calcium on albumin as determined in patients with a wide range of albumin concentrations but no obvious disturbance of calcium homeostasis. Then we added to that value a constant, the average concentration of protein-bound calcium in health (the difference between the intercept of the regression equation and the mean of the normal range). The equation we used was as follows: Adjusted calcium = total calcium − (0.025 × albumin) + 1.0, with calcium in mmol/L and albumin in g/L. The slope (0.025 mmol/g) and constant (1.0 mmol/L) have not differed significantly in assessments made on patients in this hospital over a period of 15 years (23–25).

Student’s paired t-test was used to assess changes in concentration from shortly before conception to shortly before delivery.

Results

The mean concentrations of total calcium and albumin in serum were determined in specimens collected within each four-week period of pregnancy and at intervals afterwards, and expressed as a percentage of the mean values before conception (Figure 1). The total calcium concentration decreased less steeply than would be predicted from the relationship between calcium and albumin if ionized calcium were unchanged. In the last trimester of pregnancy, the total calcium concentration began to increase while the mean albumin concentration was still declining. However, adjusted calcium increased linearly from conception to delivery (r = 0.962; P < 0.001). Quantitative differences between concentrations in the specimens taken shortly before conception and before delivery are summarized in Table 1. Mean bicarbonate concentration decreased by 3.1 mmol/L from conception to delivery (P < 0.001). The anion gap did not change significantly.

Immediately after delivery there was an abrupt decrease in both total calcium and albumin concentration, presumably because of redistribution of protein and fluid. However, adjusted calcium remained unchanged. Total calcium and albumin values then increased and the adjusted calcium values decreased exponentially to reach preconception values by eight weeks post partum.

Discussion

The gradual increase in albumin-adjusted calcium we observed during pregnancy could be due to (a) an increasing underestimation of albumin concentration as pregnancy progressed, (b) a gradual increase in protein-bound or complexed calcium with no change in ionized calcium, so that the slope of the regression we used to adjust calcium for albumin became increasingly inappropriate, or (c) a true increase in ionized calcium.

Albumin can be underestimated in the presence of metabolites that interfere with the chemistry of the method used for its measurement. For example, substances retained during renal failure result in spuriously low albumin values when measured by a dye-binding method involving bromcresol purple (26, 27). However, there have been no reports of metabolite interference with the bromcresol green method we used. Protein-bound calcium increases in pregnancy as a consequence of the mild compensated respiratory alkalosis, with an increase of pH of about

Table 1. Change In Serum Concentrations in 42 Normal Pregnancies from Shortly before Conception to Shortly before Delivery

<table>
<thead>
<tr>
<th>Albumin, g/L</th>
<th>Calcium, mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Measured</td>
<td>Adjusted</td>
</tr>
<tr>
<td>Mean change</td>
<td>−9.7*</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>2.79</td>
</tr>
<tr>
<td>Lower 95% limit</td>
<td>−15.3</td>
</tr>
<tr>
<td>Upper 95% limit</td>
<td>−4.1</td>
</tr>
</tbody>
</table>

*Significantly (P < 0.001) different from zero.
0.02 unit and a decrease in bicarbonate of about 3 mmol/L. However, such binding can account, at most, for a change of only 1% compared with the 7% we observed (Figure 1). An increase in complexed calcium is improbable because the concentration of bicarbonate, the principal complexing anion, decreased and the anion gap did not change. Thus, the largest part of the increase in adjusted calcium is most probably due to an increase in ionized calcium.

Why has the concentration of ionized calcium been reported to be unchanged or lowered during pregnancy (2, 9, 12–17)? The commonly used ionized calcium analyzers show a positive relationship between the calcium result and the albumin concentration in a sample (18–21), almost certainly an effect of reference electrode design and composition (28). Because the apparent ionized calcium decreases as albumin does (20), a true increase during pregnancy could be obscured.

The gradual linear increase in adjusted calcium during pregnancy accompanies a similar linear increase in serum 1,25-DHCC (1). It seems likely that the sequence of events leading to hypercalcemia and hypercalciuria is initiated by hormonal changes that increase renal (29) or placental (30, 31) synthesis of 1,25-DHCC. Fetal PTH-related protein may be responsible, animal experiments having shown this protein to be present in placenta as well as fetal parathyroid; this protein is also thought to control calcium transport from mother to fetus (32). It may well be that the activity of placental 1-α-hydroxylase is controlled by PTH-related protein, by analogy with the control of the renal enzyme by PTH.

The present coherent interpretation of changes in calcium homeostasis during pregnancy has depended on recognizing the nonspecificity of two clinical laboratory methods: ionized calcium measurement and single-antibody radioimmunoassay for PTH.

Clinically, it is worthwhile remembering that an increase in albumin-adjusted calcium in serum by up to about 0.40 mmol/L is normal in pregnancy (Table 1), as is urinary calcium excretion as high as 15 mmol/day (6); neither is an indication for intensive investigation of calcium homeostasis.

References