Effect of Venous Occlusion of the Arm on the Concentration of Calcium in Serum, and Methods for Its Compensation

Harry Husdan, Abraham Rapoport, Sandra Locke, and Dimitrios Oreopoulos

Data are presented on the effects on 12 normal subjects of a 3-min period of venous occlusion of the arm. Concentrations of both total calcium and total protein in serum are significantly increased, while serum magnesium and phosphorus and plasma ionic calcium concentrations remain unchanged. Concentrations of both total calcium and total protein are significantly greater in the serum of men than of women. The use of methods of adjusting calcium for changes in total protein concentration, as devised by Dent and by Parfitt, eliminate changes in these values because of venous occlusion and sex-related differences. Normal ranges for serum total calcium adjusted according to these methods are based on findings in 87 normal subjects (43 men and 44 women). The clinical usefulness of such adjustments in serum calcium measurements is discussed.

Additional Keyphrases: sex-related differences in serum protein, calcium • normal values • serum Mg, P, protein, Ca inter-relations

Although reports of the hemoconcentrating effect of venous occlusion on protein and protein-bound constituents in serum have previously appeared, surprisingly few have been well documented. Gerbrandy et al. (1) examined this effect for the purpose of determining in vivo the extent of protein binding of serum electrolytes. More recently, Dent (2) has re-emphasized the error in plasma calcium measurements in blood samples taken with use of a tourniquet. His evidence appears to be based on findings in five normal subjects, and, although no data are presented, the adjustment proposed by Dent is frequently quoted. Recently we (3) have examined the effect of changes in posture on plasma ionic calcium, and on serum total calcium, protein, and magnesium concentrations. The present paper re-examines in a statistical manner the hemoconcentrating effect of venous occlusion of the arm on the concentrations of serum total calcium, protein, magnesium, and phosphorus, and plasma ionic calcium. In addition, previously published means of adjusting1 the serum total calcium values for the effect of such occlusion are applied, and normal ranges of adjusted values are derived.

Methods

The experiments involved 12 normal subjects (seven men and five women) from the medical and laboratory staffs. Their ages varied from 26–50 years and, except in one case, they were all fasting. Each subject lay supine with arms resting horizontally throughout the entire experiment. During the first 30-min period, the subject's systolic blood pressure was measured with a sphygmomanometer. At the termination of this period, blood samples were withdrawn by a "no tourniquet" technique for serum total Ca, protein, magnesium, and phosphorus, and plasma ionic Ca measurements (baseline values). A sphygmomanometer cuff was then attached to an arm and rapidly pumped to a pressure equal to 10 mm of Hg (1330 Pa) less than the systolic pressure. This pressure was maintained for 3 min. Immediately thereafter, while the cuff was inflated, a needle and syringe was inserted into an arm vein and blood samples withdrawn for repeat estimations (3-min tourniquet values).

In the second experiment, designed to determine the normal range of serum total Ca adjusted for the effect of serum total protein, 87 normal fasting adults (43 men, 44 women) participated. Each subject sat down and blood was withdrawn immediately, by the "no tourniquet" technique, for serum total Ca and total protein measurements. Eighteen men and 31 women were supine 5 min before the blood withdrawal, whereas the remaining subjects had been carrying out their usual daily activities.

Each serum total Ca value was adjusted for its

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1. In this paper, "adjusted" replaces the term "corrected" used in previous studies (2–4) to indicate modification of the total calcium values for total protein concentration.

2. In a "no tourniquet" technique, blood is withdrawn without applying a tourniquet to the arm, or, if a tourniquet need be used, a 15-s interval is allowed after introduction of the needle and removal of the tourniquet, before the blood is withdrawn. No "pumping" of the hand is permitted.
total protein concentration by the methods of Dent (2) and Parfitt (4). Dent's equation requires the measurement of plasma relative density (specific gravity). For every 0.001 unit of relative density above 1.027, 0.25 mg of Ca per deciliter is subtracted from the measured total calcium, and conversely for changes in the reverse direction. Parfitt's formula, slightly modified for a nitrogen factor of 6.54 and applied to serum (3) is: Adjusted serum Ca (mg/dl) = measured serum Ca (mg/dl)/(0.6 + T.P./19.4), where T.P. is the serum total protein concentration (g/dl), as measured in our laboratory with a total solid (T.S.) Goldberg Refractometer.

Blood specimens for serum protein, calcium, magnesium, and phosphorus analysis were taken in disposable plastic syringes and allowed to clot in capped acid-washed Pyrex tubes. Serum was separated by centrifugation within 0.5 h of collection and stored in these tubes in the freezer. The specimens were thawed and well mixed before analysis. Blood for plasma ionic calcium measurement was taken anaerobically in a special 5-ml heparinized plastic syringe, capped, gently mixed, centrifuged, and treated as described previously (3).

The methods used for measuring serum total protein, relative density, and plasma ionic calcium have been described previously (3). Serum total calcium and magnesium were measured with the Model A.A.120 atomic absorption spectrophotometer (Varian Techtron Pty Ltd., Melbourne, Australia). The day-to-day precision of the serum calcium measurements was based on 72 replicate measurements of a serum control. The latter gave a mean value of 9.60 ± 0.10 mg/dl (SD) and a CV of 1.05%. Phosphorus was measured with a basic AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N.Y. 10591) by the procedure of Fiske and SubbaRow (N-4b methodology).

**Results**

Table 1 presents data on the serum total calcium, protein, magnesium, phosphorus, and plasma ionic calcium concentrations in 12 subjects before and after 3 min of venous occlusion. Clearly, there is a significant rise in the concentrations of both total calcium (P <0.01) and protein (P <0.001), with mean increases of 0.22 mg/dl and 0.5 g/dl, respectively. However, serum magnesium, phosphorus, and plasma ionic calcium were unchanged. However, when the concentration of serum total calcium is adjusted for total protein concentration by either the Dent (2) or the Parfitt (4) methods, no significant change in the value of the adjusted total calcium after venous occlusion is noted. The mean values of the total calcium adjusted by either the Parfitt or Dent methods are in agreement, 10.11 and 10.08 mg/dl, respectively, in the baseline period, and 10.09 and 10.03 mg/dl, respectively, in the 3-min tourniquet period. This agreement is in spite of a somewhat different basis in the construction of the formulas (3).

Table 2 compares data on the serum total calcium, total protein, and serum total calcium values adjusted by both the Dent and Parfitt methods for men and women. These data confirm the fact (3, 5) that total calcium and total protein concentrations in serum are significantly greater (P <0.001 and P <0.005, respectively) in men than in women. Of particular interest, however, is the fact that adjusted serum total calcium values, by either method, show no sex-related difference.

Because of the absence of a sex-related difference in the adjusted serum total calcium values, the values for both sexes are combined and the statistics based on a total of 87 values. The mean adjusted values agree closely: 9.88 ± 0.29 (SD) mg/dl (Par-
Table 2. Relationship of Adjusted and Unadjusted Serum Calcium, Serum Proteins, and Sex of the Subject

<table>
<thead>
<tr>
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<th>Mean ± SD values (mg/dl)</th>
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<tr>
<td></td>
<td>Men (n = 43)</td>
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<tr>
<td>Calcium (mg/dl)</td>
<td>9.84 ± 0.34</td>
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<td></td>
<td>Women (n = 44)</td>
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<tr>
<td>Calcium (mg/dl)</td>
<td>9.58 ± 0.27</td>
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<tr>
<td>Protein (g/dl)</td>
<td>7.6 ± 0.5</td>
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<tr>
<td>Adjusted calcium (mg/dl)</td>
<td>9.92 ± 0.29</td>
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<tr>
<td>Parfitt (1)</td>
<td>9.84 ± 0.29</td>
<td>N.S.</td>
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<tr>
<td>Dent (2)</td>
<td>9.83 ± 0.28</td>
<td>N.S.</td>
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</tbody>
</table>

The normal adjusted serum total calcium range, based on a 95% confidence limit, is 9.30–10.46 mg/dl (Parfitt) and 9.22–10.40 mg/dl (Dent). No correlation with age was observed with either the Parfitt (r = 0.12) or Dent adjusted values (r = 0.15).

Discussion

The results of our experiments confirm the earlier findings of Gerbrandy et al. (1) and Dent (2) that venous occlusion of the arm, even for 3 min, results in a significant increase in serum calcium and total protein concentrations. Serum magnesium and phosphorus and plasma ionic calcium concentrations, however, remain unchanged. This is to be expected with ionic calcium (which is considered not bound to protein), and is in agreement with the finding of Sibryan et al. (6), but the reasons for lack of change in magnesium and phosphorus, which are considered to be partly bound to protein (1, 7–9), are not clear. Some published reports (10, 11), however, indicate that phosphorus in normal serum is essentially all ultrafilterable and hence not protein-bound. Our finding of no change in the concentration of serum phosphorus after venous occlusion is consistent with this. These latter findings are contrary to those of Gerbrandy et al. (1), who observed an increase in both plasma magnesium and phosphorus concentrations (expressed as mmol/liter of plasma water) in 14 subjects after a 7-min occlusion of the arm veins at a compression of 90 mm Hg (120 kPa). These subjects, however, did not have a baseline (30-min supine) determination before their venous occlusion. Our findings in venous occlusion, nonetheless, are identical to those we found (3) in postural changes. It would thus appear that both assumption of sitting or standing posture and venous occlusion of the arm have similar effects, namely, an increase in the concentrations of total calcium and protein in serum, but no change in the concentrations of serum phosphorus, magnesium, or ionic calcium. This similarity is unsurprising, in that compression of the arm veins by a tourniquet would be expected to have the same effect on capillary filtration pressure and ultrafiltration of plasma water in the arms as would the assumption of the erect posture on the vessels in the legs. Thus the assumption of the erect posture might be considered a “whole body” tourniquet in regards to hemoconcentration. The mean unadjusted Ca values for men and women (Table 2) are slightly higher than those previously reported (3), because almost half of our subjects were not supine immediately before the blood sampling.

The Dent equation was designed to adjust plasma Ca for changes in plasma protein secondary to venous occlusion, whereas Parfitt’s equation was applied to plasma protein changes without regard to their cause. Both methods are suitable for adjusting serum calcium for changes in protein, whether caused by changes in posture (3) or by the use of an arm tourniquet. The normal adjusted (Dent and Parfitt) ranges for serum total calcium permit us to modify serum total calcium measurements for the effect of changes in total protein, whether these be due to postural changes, venous occlusion of the arm, or both, and for sex-related differences. In these respects it simplifies the conditions of taking blood specimens. The use of the Parfitt equation in conjunction with the Goldberg T.S. Refractometer for measuring protein, makes it easy clinically to correct serum calcium measurements. Furthermore, inspection of Table 1 indicates that the adjusted total calcium ranges have a smaller span than the unadjusted range. The span associated with the Parfitt equation appears smaller than that with the Dent equation. This fact suggests that the use of an adjustment, especially Parfitt’s, may well improve the sensitivity of serum calcium measurements as a diagnostic aid.

In our laboratory, the ranges for the adjusted total calcium in serum were found to be 9.30–10.46 mg/dl (Parfitt method) and 9.22–10.40 mg/dl (Dent method), based on 87 subjects and measured by atomic absorption spectrophotometry and refractometry. The latter range is similar to that reported for plasma by Davies et al. (12), in Dent’s laboratory, of 8.9–10.2 mg/dl based on 73 subjects and measured by emission flame photometry and copper sulfate relative density standards.

Our adjusted values for serum total calcium values, with the modified Parfitt equation (see Methods section), are about 0.1–0.2 mg/dl higher than would be the case if plasma were used in the original Parfitt equation (4). This is because the original Parfitt’s equation is based on plasma having a mean protein concentration of 7.4 g/dl and a nitrogen factor of 6.25. This equation was modified by basing the mean plasma protein concentration on the more recently accepted nitrogen factor of 6.54 (i.e., on 7.4 × 6.54/6.25, or 7.7 g/dl). We then elected to adjust our serum calcium values with the use of this modified equation designed for plasma.

The actual protein concentration of the baseline to which one adjusts all the serum calcium values is of no particular consequence, because this is taken into account in the derivation of the normal adjusted
serum total calcium range. This will permit one to make valid diagnostic interpretations based on the adjusted data.

The close agreement between the range that we obtained for the normal adjusted serum total calcium by the Dent method and that found by Davies et al. (12) reflects the similarity in the relative densities of serum and plasma.

It should be emphasized that the Parfitt or Dent equations are not applicable to situations in which there is an abnormal albumin/globulin ratio—such as myeloma, nephrotic syndrome, and cirrhosis—because these equations assume normal calcium bindings and a normal albumin/globulin ratio.

Moore (13), reports that 81% of the protein-bound calcium in normal serum is bound to albumin and the remaining 19% to globulins. The concentration of albumin, rather than total protein, is thus of prime importance in the protein-binding of serum calcium. Linear relationships, however, are known to exist between total serum calcium and albumin (14) and between total serum calcium and total protein (15). Both Dent and Parfitt based their equations on the concentration of plasma proteins rather than albumin. Total proteins are much more reproducibly measured clinically (by the T.S. Refractometer) than is albumin (by photometry and electrophoresis), and our previous study (3) showed that substitution of albumin for total protein values did not improve the calcium adjustment in situations in which protein concentration changes were caused by a similar mechanism (namely, postural changes rather than venous occlusion).

In conclusion, our data confirm both the observations of Dent on the hemoconcentrating effect of venous occlusion of the arm on plasma calcium and protein as well as the applicability of Parfitt's formula. Normal ranges for such adjustments have been derived with use of equipment that is generally available in the clinical laboratory. Finally, the application of these adjustments obviates the requirement of standardizing posture and the avoidance of an arm tourniquet, thus greatly simplifying blood-collection procedures. Furthermore, by narrowing the range of normal values, these modifications may enhance the sensitivity of serum calcium measurements in the diagnosis of disease.

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References