Impact of Posture on Reference Ranges for Serum Calcium and Protein

To the Editor:

These comments are not to be construed as criticisms of the excellent work by Humphrey et al. on the impact of posture on reference ranges for total serum calcium and proteins (1), but rather as shedding some light on the rationale for doing such studies and specifically on their utility in diagnostic medicine.

Since total serum calcium and protein cannot be meaningfully viewed separately, most clinicians consider both simultaneously. Even though the effects of posture on calcium and protein concentrations separately are demonstrable in this and other studies, it would nonetheless be highly instructive to see whether postural influences could be seen on the two concentrations jointly, that is, when they are expressed as an index or when one is “corrected” for the presence of the other.

The tables in that paper (1) did not provide us with the requisite data to calculate such an index (2). Our guess is that the perturbing effect of posture observed on each variable separately will be diminished or will even disappear when both variables are studied simultaneously. This does not deny the reality of the observations by Humphrey et al. but alleviates the rapidly mounting difficulties in interpreting reference ranges. Our view is that some of these difficulties may be reduced by developing reference values for multivariate indices, a first essay in this direction appearing last October (2).

We would wish to take this opportunity to correct an error in that paper. The formula for the index in S.I. units (footnote to Table 3) should read:

\[ \text{Calcium-protein index} = \frac{80 \text{ calcium} - 120}{\text{protein}} \]

total calcium being expressed in millimoles per liter and total protein as grams per liter. The equation in the report expressed as mg/dl and g/dl is not in error (2).

References


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Current “Corrected” Calcium Concept Rechallenged

To the Editor:

Because nearly half of serum total calcium is protein bound, it is current practice to “correct” the serum total calcium concentration for abnormal serum total protein or albumin concentrations by using an average binding factor (1-5). The validity of such manipulations depends on the assumption that there is little individual variation in calcium–protein binding factors. We have presented data from patients and from the literature which indicate that individual factors vary significantly (4, 5). Other workers have suggested that our findings can be explained by inaccuracies in serum calcium and albumin measurements (6-12). We present further evidence that there are statistically and clinically significant interindividual differences in “correction” factors.

In-dwelling venous cannulae were inserted into the antecubital veins of 17 apparently healthy members of our staff (13 men and four women; median age 28 years, range 19-46). The cannulae were kept patent by filling the dead space with 0.5 ml of a solution of sodium heparinate, 20 int. units/litre, and sodium chloride, 9 g/litre. A sphygmomanometer cuff was applied to the upper arm with the lower edge 7 cm above the entrance of the cannula and kept inflated at a constant pressure midway between the systolic and diastolic pressures (median 95 mmHg, range 85-110 mmHg). After discarding 2 ml of dead space, 10 ml venous samples were taken at 0, 5, 10, and 15 min. Samples were analyzed with an SMA 12/60 system (Technicon Instruments Corp., Tarrytown, N. Y. 10591) with between-batch coefficients of variation for serum albumin and total calcium determinations of 2.0 and 1.8%, respectively. The data were subjected to linear regression analysis to obtain the individual, overall, and median correction factors (regression coefficients). The individual correction factors were then tested for parallelism (i.e., for differences in regression coefficients) by analysis of variance (13).

There was considerable change in the serum albumin concentration (median 15 g/litre, range 7-22). Individual “correction” factors ranged from 0.013 to 0.044 (median 0.026 mmol/g). The correlation coefficients of the regression relationships of each individual’s four points were high (median 0.97, range 0.82 to >0.99). Use of all 68 pairs of measurements gave an overall correction factor of 0.029 (r = 0.85). The high correlation coefficients of the individual regression relationships imply statistically that technical factors are unimportant, because there is little residual variation unaccounted for by the regression relationship. The lower overall correlation coefficient compared to the high individual ones implies individual variation and this is borne out by the difference between individual regression coefficients (F ratio = 2.02; P <0.05).

It might be argued that using an average “correction” factor would obscure only small abnormalities in the serum ionized calcium concentration in those few patients with large abnormalities of serum albumin concentration and that clinically important abnormalities would still be detected. On the other hand, minor abnormalities are important in many diseases (for example renal calculous disease, hyperparathyroidism) and the maximum difference observed between individual correction factors was large (51 µmol of total calcium per litre of serum for a change of 1 g/litre in the serum albumin concentration). The differences in “correction” factors described were found in normal subjects; it is possible that larger differences occur in patients.

There are other problems associated with correction of the serum total calcium concentration. If correction is based on a single pair of measurements of serum total calcium and albumin concentrations, the “corrected” value will have a greater error than the original calcium value. Even if several measurements are used, the “corrected” value should be compared to a reference range for serum “corrected”—not uncorrected—total calcium concentration. A further difficulty arises with low serum albumin concentrations, because the generally used bromcresol green methodology overestimates serum albumin because of nonspecific dye
binding by many proteins (14). If total protein were used for "correction," the technical problems associated with albumin measurement would be avoided, but the relationship between total protein and calcium is less satisfactory than that between albumin and calcium (15). Either an expensive, immunological measurement could be used for albumin or the simple modification of the bromocresol green method proposed by our laboratory (16). Lastly, the basic assumption in "correcting" may not be valid; that is, the relationship between serum albumin and total calcium concentrations may not be strictly linear. This might explain the results of Payne et al. (6), who used our published range of "correction factors" and calculated that the range of serum non-protein-bound calcium concentration was 0.07-2.37 mmol/litre!

For the above reasons, any "correction" may be misleading. A direct measurement of serum ionized calcium concentration (for example serum ultrafiltrable calcium estimation) should be performed whenever there is a clinical suspicion of an abnormality of serum ionized calcium concentration.

References

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Application of a Dye-Binding Method to the Determination of Protein in Urine and Cerebrospinal Fluid

To the Editor:
Bradyd (1) showed that the dye Coomassie Brilliant Blue G250 binds a wide variety of proteins and used it to determine protein concentration in column eluates. This prompted me to apply the method to the determination of protein in urine and cerebrospinal fluid. The Bradford method (1) was used without modification, with human serum albumin as a standard. The method was linear over the range 0.1-1.5 g/liter. Daily analysis of the same batch of diluted serum for 20 days established the method's precision, giving a standard deviation of 60 mg/liter for a concentration of 0.99 g/liter. The analytical recovery of albumin added (0.5 g/liter) to normal urine was 102% (range 95-106%).

I examined the specificity of the method by comparing its results with those of accepted methods applied to samples of urine and cerebrospinal fluid. Eighteen samples of cerebrospinal fluid, ranging in protein concentration from 0.1 to 12 g/liter were analyzed by the turbidimetric method of Henry et al. (2) and the dye-binding method; the correlation coefficient was 0.91. Thirty-one samples of urine, ranging in protein concentration from 0.1 to 5 g/liter, were analyzed by an automated turbidimetric method as used at Palmerston North Public Hospital, the Sephadex-biuret method of Doetsch and Gadaden (3), and the dye-binding method. The correlation coefficient between the Sephadex-biuret method and dye-binding methods was 0.91; that between the turbidimetric and dye-binding methods was 0.65. The dye-binding and Sephadex-biuret methods gave similar color yields for Tamm-Horsfall mucoprotein and proteins of low molecular weight isolated from human urine. These values were two- to threefold higher than those obtained by the turbidimetric method.

With the dye-binding method, physiological variations in the pH of urine (4-5.5) may result in a maximum variation of 10% in apparent protein concentration; however, extreme variations in pH such as an acidified urine at pH 1 or staled urine at pH 11, will introduce errors as much as -12% and +21%, respectively. Common urine preservatives such as boric acid and toluene do not interfere, but thymol gives a 20% increase in the apparent protein concentration. Drugs such as penicillin and roentgenographic contrast media ("Angiografin") do not interfere with the method, but salicylates in concentration of 5 g/liter cause a positive interference of 12%.

The control range for protein in cerebrospinal fluid was determined from analysis of 58 routine specimens. Samples that were cloudy or colored were not used. The range, calculated by the percentile method (10 to 90), was 100 to 600 mg/liter.

The control range for 24-h urinary protein excretion was determined by analysis of specimens from 97 hospital outpatients. The distribution of results showed a nonuniform population, and it was difficult to distinguish a "normal" population, but a tentative control range of 0-255 mg of urinary protein per 24 h was established for this method.

The Coomassie Blue dye-binding method for determining protein in urine and cerebrospinal fluid has improved in specificity and speed and requires a smaller sample volume than do the present turbidimetric methods, and it is simple enough for routine clinical use.

References
3. Doetsch, H., and Gadaden, R. H., Determination of total urine protein combining