Impact of Posture on the "Reference Range" for Serum Proteins and Calcium

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The impact of water shifts due to changes in posture has been reinvestigated for serum protein and calcium concentrations in a young, healthy population. We found that generally accepted normal ranges for total serum protein, and to a lesser degree for total serum calcium, are not acceptable as a reference range for a hospitalized population that is predominantly in a supine position. Our data indicate a smaller biological variability for total protein and calcium for a reference population in the recumbent posture as compared to the same population in the upright position.

Additional Keyphrases: biological variation • methodologic variation • Bayesian statistics

The effect of recumbency on serum total protein and calcium has been studied before. In 1911, Böhme (1) demonstrated a rapid and marked decrease in serum protein concentrations in individuals who changed from an upright to a recumbent position. When they returned to an upright posture, the effect was reversed. Lowering either arm from a horizontal location to below the plane of the supine body resulted in an increased serum protein concentration in the blood in that arm. On the basis of earlier studies, Böhme concluded that such changes in protein concentrations were due to fluid shifts. These experiments were confirmed and expanded by others (2–12). According to Fawcett and Wynn (5), plasma protein concentration and hematocrit shift by about 15% in patients with edema, or abnormally low concentration of protein or albumin in their plasma when they change the body position, as compared to a shift of 10% in normal controls. Total serum calcium shows the same behavior, to a lesser extent. Ionized calcium does not exhibit posture dependent changes (6).

Unfortunately, the known interrelation between posture and fluid shift and the concentration of nondiffusible serum constituents has been disregarded in clinical laboratory practice, and we know of no studies of the impact on the reference range. Here, we demonstrate the importance of defining normal reference population ranges with respect to posture.

Materials and Methods

We studied 20 women and three men, 20 to 27 years old. All were medical technology students and all believed themselves to be healthy. Blood samples were collected at 0830 hours from fasting subjects, who were in an upright position. Thereafter, the students rested in a recumbent position for 1 h and blood was again sampled at 0900 and 0930 hours. From 0930 to 1030 hours, the students were in an upright position and were allowed either to sit or to stand. The last specimen was drawn at 1030 hours. A total of four 10-ml blood samples were collected from a vein of the antecubital fossa, into heparinized, evacuated blood-collection tubes. The plasma was separated from the cells without delay by centrifugation and analyzed. The experiments were carried out in five sessions during four weeks (July 1976). All specimens from the same individual were analyzed in the same batch.

We assayed total plasma protein manually by the biuret method and calcium with the Calcette (Precision Systems, Inc., Sudbury, Mass. 01776) using a titration method with [ethylenebis(oxyethylene)tetraacetic acid. Because plasma rather than serum was used for the determination of total protein, an average value for fibrinogen (3 g/liter) was subtracted to make the values for total protein for these subjects comparable to serum values for the reference and patient population.

The computer-derived data for the hospital inpatient population consisted of information on 681 individuals for total protein and 414 for calcium. The normal reference group consisted of 175 blood donors who were not regular ("professional") blood donors.

Results and Discussion

Our results for the corrected total serum proteins and for plasma calcium in the study group are summarized in Figure 1 and are compared with the data for the University Hospital
inpatient population. The median values for the reference group and for the students in the upright position are clearly greater than the inpatient median, while those for the students in the supine posture are nearer to it. The average shift for the student group from standing to resting in a recumbent position for 30 minutes was −5 g/liter (−7%) for total protein and only −50 μmol/liter (−2%) for total calcium.

For both total protein and calcium there was a statistically significant difference (P < .01) between the 0830-hour values (upright) and the 0900-hour values (30 min supine) and between the 0830- and 0930-hour values (60 min supine). There was no significant difference between 0900- and 0930-hour values for either total protein or calcium, which indicates that the shift in fluids is fairly complete after 30 min in the recumbent position.

The 95% range was narrower when the students were in a supine posture than while standing up, more so for total protein than for total calcium (Figure 1, Table 1). Pedersen (6) reported a statistically smaller variance for total protein in men, and more so in women, in the recumbent position than when they were standing up. This effect was also demonstrable for protein-bound calcium in women, but not in men. Recalculation of the data of Aull and McCord (8) also shows a narrower range for individuals in the supine than in the erect posture.

For clinical decision-making it is of interest to analyze by Bayesian statistics (13) the probabilities with which a certain result will fall within or outside of the various ranges given in Table 1, taking also into consideration the impact of the analytical error. A healthy individual in the recumbent position with an analytical value of 58 g of total protein per liter of serum and with a method that has an analytical standard deviation (S_m) of 1.5 g/liter of serum will only have a 15% probability of being within the normal reference range of 61 to 77 g, a 49% probability of being within the range for the upright student population (60 to 74 g), and a 99% probability of being in the supine-student range (56 to 68 g). If the "normal" range becomes narrower because of a smaller biological variation, S_b, while the day-to-day analytical error, S_m, remains the same, the ratio of S_b to the overall SD, S_o, changes. S_o, S_m, and S_b are related by the expression:

\[ S_b = \sqrt{S_o^2 - S_m^2} \]  

(1)

S_b is one-fourth of the reference range and S_m is easily obtained from quality-control data (14).

Table 1 demonstrates the varying relationship between S_m and S_b in percent of S_o for serum protein and calcium with change in posture. An S_o/S_b ratio of 50% just fulfills Tonk's criterion (15, 16) and results in a desirably high S_o/S_b ratio of 87% for total protein. The biological variability (S_b) of the data for the reference group and for the students lying down is 3.7 and 2.6 g/liter, respectively. A method with an S_m of 1.5 g does not require greater precision for the narrower range (2.6 g) than for the wider range (3.7 g), because the ratio of S_m/S_o is still within the allowable 50% criterion.

We anticipated that the variability of calcium would be larger for the student group in the upright position than in the recumbent posture, because about half of the serum calcium is protein bound and should therefore follow the same pattern as total protein. Figure 1 and the data in Table 1 show that this is indeed the case. If Tonk's criterion is accepted, then the analytical standard deviation is too large for the calcium method for all groups except the hospital population. It follows that with a decrease in biological variability of calcium, a simultaneous improvement in analytical precision is ultimately required if useful clinical data are to be provided.

In the foregoing it has been assumed that the variation in S_o is the composite of two well-defined variables, S_m and S_b.

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2 We recognize that subtracting an average value of 3 g of fibrinogen per liter from total protein to convert values for plasma protein into those for serum protein is only a first approximation. The normal range for fibrinogen in our laboratory is between 2 and 4 g/liter of plasma. The correction for fibrinogen has no impact on the dispersion of the student ranges.

Dilution of serum by erythrocyte water during clotting might contribute an error of up to 1.5% when plasma and serum protein values are compared (18).
One can obtain a reasonable estimate of $S_m$ from quality-control data for blind studies. At best, we are able to estimate $S_p$ from equation 1 and to define conditions under which we can maintain basal-state conditions that minimize such external influences as circadian rhythm, posture, or improper stasis during venipuncture.

The generally agreed-upon normal range for serum protein of somewhere between 60 to 85 g/liter is in conflict with data derived from populations exclusively or predominantly in a supine position (6, 10). The data provided here and cited above, as well as the wide range of 46-80 g of protein per liter found for the hospital population, indicate that this group is made up of a mix of at least two subgroups, those from whom specimens were collected while the subject was erect, the other while supine. A plot of the inpatient population on probability paper yields a straight line for the interval 6 to 96 cumulative percent (Figure 2), corresponding to a protein range of 50 to 78 g/liter of serum. In contrast, total calcium demonstrates two overlapping subgroups on probability paper. One subgroup, representing the interval 2.5 to 63 cumulative percent of the population, has values between 1.80 and 2.50 mmol of calcium per liter while the interval 64 to 92 cumulative percent of the second subgroup has values from 2.50 to 2.85 mmol of calcium per liter of plasma. The distribution curve for calcium in the hospital population is nongaussian (Figures 1 and 2); that for the reference group is gaussian (13).

One might conclude from the foregoing and the data provided in Table 1 that there is a need for a more rigorous definition of normal reference ranges for both calcium and total protein. The upper normal range of these two constituents is of clinical importance when screening for hypoproteinemia or for hypercalcemia. Large fluctuation of calcium in hyperparathyroidism has been reported (17) and care should be taken not to enhance the fluctuations by disregarding the posture-dependence of total calcium concentrations. Indeed, lower and upper limits of normal ranges for protein are ill-defined at present. If, for instance, 56 g of protein per liter of serum is set as the lower normal limit, 20% of the hospital inpatient population would be hypoproteinemic by definition.

It is not uncommon for the clinician to be confronted with laboratory results that are in conflict with his clinical observations on a patient. Often the source of this conflict is not laboratory imprecision, as exemplified by these calcium and protein determinations it rather may be due to the physician's and the analyst's lack of awareness of the sampling requisites. In the case of variability caused by differences in posture, the phlebotomist should help minimize unnecessary errors by reporting the subject's posture at the time the blood-specimen was collected.

References

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Heparin Pretreatment Suppresses Norepinephrine Concentrations in Dogs in Endotoxic Shock

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Mongrel dogs were treated intravenously with either 1000 units of beef-lung heparin per kilogram of body weight or with isotonic saline, before intravenous administration of E. coli endotoxin. We found significant differences in circulating norepinephrine concentrations between a heparin-pretreatment group (1.89 ± 0.39 µg/liter) and the control group (9.83 ± 4.64 µg/liter), but none with respect to epinephrine. Systolic blood pressures at 360 min were also significantly (P < 0.05) different, 148 ± 8 mmHg as compared with 118 ± 13.4 mmHg. Evidently heparin pretreatment can decrease circulating norepinephrine concentrations in the endotoxic state and changes in circulating catecholamine concentrations can affect physiological variables.

Plasma catecholamine concentrations (1, 2) increase markedly in endotoxic shock. It has been suggested (3) that this plays a major role in physiological responses to endotoxic shock. We wished to determine if suppression of catecholamine release would affect the magnitude of certain physiological responses.

Agents such as steroids and fluids affect certain physiological variables in experimentally induced endotoxic shock (4), but it has not been determined what effect, if any, an alteration in endogenous catecholamine concentrations will have on these same variables. Steroids and fluids are effective secondarily, in that they affect the organism's response after the endotoxic insult. We suggest that a decrease in circulating catecholamines may affect physiological responses directly rather than secondarily.

Methods and Materials

Five control and five preheparinized mongrel dogs weighing 18 to 28 kg were anesthetized with 23 mg of intravenous pentobarbital per kilogram of body weight. A carotid artery and the superior vena cava were cannulated to monitor pressures and pulse rate and for blood sampling. Lactated Ringer's solution, 15 ml/kg body weight, was administered rapidly through a peripheral venipuncture, to expand the circulating volume before endotoxin administration. We allowed 10 min for intravascular equilibration. Systolic, diastolic, and central venous pressures were monitored continuously (E for M. Instruments, Houston, Tex.). Additional variables monitored, intermittently were pulse rate, temperature, respiratory rate, and urine output. To five animals, 1000 units of beef-lung heparin per kilogram was given intravenously and 20 min later, just before endotoxin administration, samples of arterial blood were drawn for use in measuring hematocrit, epinephrine, and norepinephrine (Bio-Science Labs., Van Nuys, Calif). E. coli endotoxin (Difco Labs, Detroit, Mich.), 5 mg/kg body weight, in 10 ml of physiological saline, was then given during 5 min via a peripheral vein. Hematocrit was deter-

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