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Influence of Posture on Free Calcium and Related Variables

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We measured free calcium and related variables before and after the subject changed from the upright to the supine posture, doing 15 separate such experiments on 11 healthy men. After such a change, free calcium (1.7 ± 0.4%), total calcium (4.6 ± 0.7%), total protein (11.5 ± 1.4%), albumin (12.2 ± 2.0%), total magnesium (3.8 ± 0.9%), and the activity of hydrogen ion (2.9 ± 1.0%) decreased significantly (values are means ± SEM), but promptly reverted when three subjects assumed the alternative posture. Changes in lactate values were not rapidly reversible; sodium and potassium showed no significant change. The mechanism of the changes in free calcium is unclear, but they correlated only with the changes in total calcium and were notably less than the changes in total calcium, indicating that posture will have less effect on the interpretation of free calcium values than on values for total calcium.

Additional Keyphrases: *analytical error · intra- and interindividual variation · calcium binding · ion-selective electrodes*

Fluctuations in values for laboratory tests are often primarily the result of factors unrelated to the analysis itself (*1, 2*). For example, fluctuations caused by variation in the collection or storage of samples are particularly important to measurement of calcium and other analytes that are under close homeostatic control (*3, 4*).

Changes in posture are known to alter the concentration of total calcium in serum; by going from the supine to the standing or sitting position a person's total calcium may change by 2–6% (*5–10*). Although small, these changes exceed the biological variation in total calcium, which is 1.6 to 1.9% (*3, 4*), and the analytical variation of 2.5% for automated methods (*11*). These changes in total calcium with posture appear predominantly to be related to concomitant changes in the concentration of protein, particularly albumin (*5–10*), which in turn are due to alterations in the plasma volume resulting from changes in hydrostatic pressure when the posture is changed (*12, 13*).

Two studies (*6, 7*) have concluded that the concentration of free calcium, unlike that of total calcium, is unaltered when the posture is varied. We have re-investigated the influence of posture on free calcium and related variables and report here our findings that free calcium concentration is altered by changes in posture, although less than is total calcium.

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Materials and Methods

The subjects studied were 11 men, healthy laboratory personnel, who were on their usual diet. Of these 11 individuals, two were put through the entire protocol twice and one individual thrice, to yield 11 + 2 + 2 or 15 observation pairs. Each subject was seated in a blood-bank reclining chair, a pressure cuff placed on his upper arm, and a 19-gauge scalp-vein needle inserted into the antecubital vein. The needle was attached via a three-way stopcock to a sterile isotonic saline solution, which was constantly but slowly infused (0.5 mL/min) to ensure the patency of the sampling needle. This initial subject manipulation required no more than 15 min. The subject stood up, and 30 min later the first sample was obtained. The subject then lay down flat in a reclining chair, and after 30 min the second sample was collected.

We also tested, in three subjects, the reversibility of the influence of posture. We monitored several shifts in posture, all during a single controlled experiment. Thus the initial supine position after the indwelling catheter was inserted was extended to 30 min and a series of samples was obtained after 30-min intervals in each of the positions: supine, upright, supine, upright, and supine.

All samples were collected by use of the three-way stopcock. The saline flow was stopped and 5 to 10 mL of blood was removed and discarded, to eliminate any contamination and dilution with saline (*14, 15*), a 10-mL blood sample then being obtained in a plastic syringe and immediately transferred to a prelabeled evacuated blood-collection tube by placing a 19-gauge needle on the syringe, puncturing the top of the evacuated tube, and allowing the tube to fill by aspiration. In this fashion, the blood could be anaerobically transferred from the syringe to the tube for easier handling.

The blood sample was allowed to clot for 1 to 2 h at room temperature. The tubes were then centrifuged (1000 × *g*, 15 min) with the closures on. The closure was then removed, the tip of a prelabeled 5-mL plastic syringe was placed well under the surface of the serum, and 3 mL of serum was anaerobically transferred to the syringe for use in measurement of pH and free calcium (Ca_F). The serum remaining in the tube was removed, recentrifuged to eliminate any cellular contamination, combined with the excess serum from the syringe, and used to measure the other variables.

All samples from the same subject were analyzed in no particular order in the same analytical batch, to minimize analytical variability and bias. Ca_F was measured potentiometrically in duplicate with a Model SS-20 ionized calcium analyzer (Orion Biomedical, Inc., Cambridge, MA 02164). Rarely, if the values for duplicates did not agree to within 0.01 mmol/L, the sample was analyzed a third time and the median of the three values was used. Sample pH was determined in duplicate at 37 °C to the nearest 0.001 pH unit with a Radiometer E5021 microcapillary thermostated electrode system. If the results differed by more than 0.01 pH unit, a third assay was performed and the median used. The results for pH were converted to hydrogen ion activity (a_{H^+}) to provide relative changes comparable with the other parameters. Total calcium

(Ca_T) and total magnesium (Mg) were measured in duplicate or triplicate by an automated atomic absorption procedure (*16*).

Sodium and potassium were measured by automated flame photometry (KLINA Flame; Beckman Instruments, Inc., Fullerton, CA 92634). Total protein, albumin, and lactate were measured spectrophotometrically in a discrete analyzer (*aca;* DuPont Co., Wilmington, DE 19898); total protein was measured with the biuret reaction, albumin with bromcresol green, and lactate by use of lactate dehydrogenase (EC 1.1.1.27).

The significance of the difference in the values for the various analytes obtained for samples collected while the subject was upright and supine was tested by the paired *t*-test. All data for presentation in the figure and tables were combined from the individual's data and expressed as mean ± standard error of the mean (SEM).

Results

Figure 1 shows the percent decreases in Ca_F, Ca_T, Mg, total protein, albumin, lactate, and a_{H^+} when the subject went from the upright to the supine posture. As indicated, these changes were highly significant by the paired *t*-test. However, when we analyzed the results for Ca_F by the unpaired *t*-test (using mean values), the values for the upright posture (1.13 ± 0.01 mmol/L) did not differ significantly from those for the supine posture (1.11 ± 0.01 mmol/L). By the paired *t*-test, values for sodium and potassium were not significantly altered with changes in posture.

We assessed the relations between the decreases in the various analytes noted in Figure 1 by means of the correlation between the relative percent changes (Table 1). The relative changes in Ca_F correlated only with those of Ca_T, and then only moderately, while the changes in Ca_T were strongly correlated with those of total protein and albumin and only moderately correlated with lactate and Ca_F. Interestingly, the changes in total magnesium were not significantly correlated with protein or albumin but were with a_{H^+}. Changes in protein and albumin correlated very highly and the magnitude of changes in these two parameters were not different by the paired *t*-test. The changes in lactate were associated with the changes in protein, albumin, and Ca_T, and the changes in a_{H^+} only with those of Mg.

We examined the reversibility of the posture-induced changes in three subjects. The data indicate that the changes in all the analytes except lactate were reversible within the 30-min intervals as the postures were alternated (Table 2). Huckabee (*18*) has noted that it may take a few hours for lactate values to reach steady basal concentrations and this is no doubt responsible for the observed steady decreases in lactate values (Table 2), because our subjects were not required to be in a resting state for an extended period before the start of the experiment.

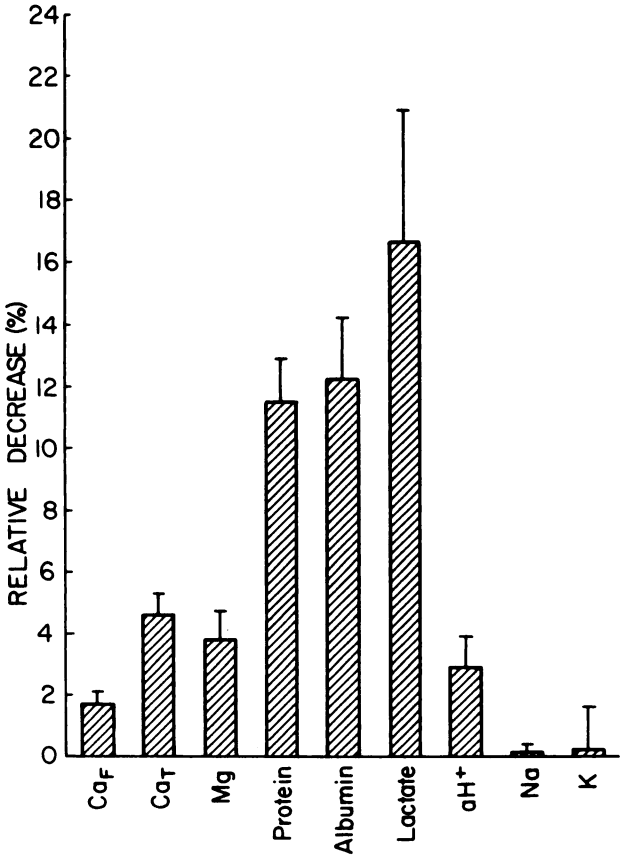

Fig. 1. Influence of posture on free calcium and related variables

The bars show the relative percentage decrease (mean ± SEM indicated by the error bars) in the values for free calcium (Ca_F), total calcium (Ca_T), total magnesium (Mg), total protein, albumin, lactate, hydrogen ion activity (a_{H^+}), sodium (Na), and potassium (K) in the serum on changing from the upright to the supine position. The decreases are significant at $p < 0.005$ by the paired *t*-test for Ca_F, Ca_T, Mg, protein, albumin, lactate, and a_{H^+}. Sodium and potassium were not significantly altered from baseline as judged by the same statistical test

Discussion

The decrease observed in Ca_F when a subject changed from the upright to the supine position was small (1.7 ± 0.4%) but real, because the paired *t*-test was highly significant and the changes evidently were reversible when the posture was alternated between upright and supine (Table 2). The changes in Ca_F with posture were not significant as judged by the unpaired *t*-test, probably owing to interindividual variation in the baseline values for Ca_F. These results appear to differ from those of others. Pedersen (*6*), using a spectrophotometric method applied to an ultra-filtrate of plasma, found no influence of posture on Ca_F values. Husdan et al. (*7*) also noted no effect of posture on Ca_F values; they used a protocol similar to ours in that a venous catheter was used to facilitate blood drawing and the Ca_F was measured directly in serum with a calcium-sensitive electrode. However, it is not clear whether they examined their data by use of paired or unpaired statistics. Visual inspection of their data (Figure 1 in reference 7) indicates a decrease in the mean values for Ca_F when the upright is compared to the supine posture that is similar in magnitude to that which we observed. Berry et al. (*8*) have also reported no significant changes in Ca_F with hemodynamic changes induced by venous stasis.

The mechanism by which Ca_F values change with posture is not clear. The changes in Ca_F correlated with changes in Ca_T but not with those in any of the other variables. The small decrease in a_{H^+} (2.9%, 0.018 increase in pH) when going from the upright to the supine position could account for a decrease of 0.01 mmol/L in Ca_F (*18*), or about half of the change actually observed. However, the lack of correlation of changes in Ca_F and a_{H^+} suggests that other factors are involved. The amount of calcium complexed to nonprotein species reportedly increases when a human goes from the upright to the supine position (*6*). This would imply an increase in a species of protein that can bind calcium and might explain the decrease in Ca_F under these conditions.

The changes in Ca_F with changes in posture appear to have some similarities to other situations in which Ca_F has been reported to change. After the administration of gastrin, betazole, or a steak meal, the Ca_F decreased and the pH increased (*19*). The increase in pH could only explain half of the change in Ca_F, and these authors felt that an increase in the calcium bound to bicarbonate accounted for the remainder of the decrease in Ca_F. We did not measure bicarbonate.

Table 1. Correlation between Those Variables That Changed with Posture[a]

	Ca_F	Ca_T	Mg	Prot	Alb	Lactate	a_{H^+}
Ca_F	—	0.62	0.19	0.25	0.40	0.23	0.23
		0.05	NS	NS	NS	NS	NS
Ca_T	0.62	—	0.48	0.82	0.87	0.60	0.42
	0.05		NS	0.001	0.001	0.05	NS
Mg	0.19	0.48	—	0.42	0.40	0.27	0.55
	NS	NS		NS	NS	NS	NS
Prot	0.25	0.82	0.42	—	0.94	0.74	0.30
	NS	0.001	NS	—	0.001	0.01	NS
Alb	0.40	0.87	0.40	0.94	—	0.68	0.22
	NS	0.001	NS	0.001		0.01	NS
Lactate	0.23	0.60	0.27	0.74	0.68	—	0.21
	NS	0.05	NS	0.01	0.01		NS
a_{H^+}	0.23	0.42	0.55	0.30	0.22	0.21	—
	NS	NS	0.05	NS	NS	NS	

[a] The correlation coefficient of the percent change in each variable is shown, as is the statistical significance of the correlation coefficient.

Extracellular fluid expansion in animals also produces decreases in Ca_F that have some similarities to the posture-induced changes we observed in humans. Expansion of the extracellular fluid volume of dogs with Ringer's solution containing 1.5 mmol of $CaCl_2$ per liter caused the total protein to decrease by 17 g/L, the Ca_T by 12.4%, and the Ca_F by 3.5% (*20*). The change in Ca_F from this study (*20*) was 28% of the change in Ca_T. This relative change is similar to the 37% we found in humans changing their posture. Extracellular fluid volume expansion in rats (*21*) or dogs (*22*) via saline infusion led to a decrease in both ultrafiltrable calcium and total calcium, the decrease in ultrafiltrable calcium being only 50–60% as great as that in total calcium. Moreover, expansion of the extracellular fluid volume of rats with hypertonic saline was reported to cause a decrease in total calcium but an increase in ultrafiltrable calcium (*23*), suggesting an increase in calcium complexed to nonprotein species. Thus extracellular fluid expansion in animals by means other than posture results in changes in calcium values with some similarity to those we observed due to posture in humans.

Regardless of the mechanism of the changes in Ca_F with changes in posture, these changes were less than the corresponding changes in Ca_T (1.7 ± 0.4% for Ca_F, as compared to 4.6 ± 0.7% for Ca_T). Therefore, changes in posture will have less influence on the interpretation of Ca_F values than of Ca_T values.

The changes with posture that we document in Ca_T (*5–10*), total protein and albumin (*6–8, 24*), and a_{H^+} (*6*) are similar to those observed by others. The changes in Ca_T were 36.9% of those of total protein, in excellent accord with the percentage of calcium reportedly bound to protein in serum (*25*). The magnitude of the changes in the various analytes varied much from subject to subject (e.g., Table 2), but was quite reproducible in any given subject.

The influence of posture on magnesium concentration has not been studied as often as calcium. Husdan et al. (*7*) reported little if any change in magnesium values with changes in posture, although magnesium is known to be bound to albumin in serum to a similar extent as calcium (*26*). The changes we observed in total magnesium values (3.8 ± 0.9%) were slightly less than those for Ca_T but, unlike Ca_T, did not correlate with changes in protein or albumin but did correlate with changes in a_{H^+}. The correlation with a_{H^+} (pH) suggests that pH-dependent shifts in magnesium between plasma and cells may be involved in the changes in magnesium values with changes in posture. The changes in lactate values we observed

Table 2. Relative Changes (%) in Some Variables from Values at Previous Posture[a]

Posture	Ca_F	Ca_T	Mg	Protein	Albumin	Lactate	a_{H^+}
			Subject 1				
Upright	1.8	7.1	2.9	20.3	21.9	−4.0	−0.3
Supine	−1.3	−5.8	−3.2	−17.8	−16.8	−16.7	−2.9
Upright	2.2	8.3	7.8	21.9	26.1	35.0	6.7
Supine	−2.9	−8.4	−4.2	−17.9	−17.9	−33.3	−5.8
			Subject 2				
Upright	1.9	3.2	9.9	9.7	9.6	−17.0	2.7
Supine	−0.4	−2.7	−3.8	−9.1	−3.7	−33.3	−7.9
Upright	1.9	1.6	3.0	10.5	2.8	−3.8	5.7
Supine	−0.2	−3.6	−1.0	−8.5	−8.3	−24.0	−1.1
			Subject 3				
Upright	0	1.1	0.6	7.5	5.5	−13.0	−3.6
Supine	1.2	0.1	−0.9	−7.8	−5.0	−10.0	−3.8
Upright	3.8	5.3	3.8	15.5	13.0	−2.8	9.5
Supine	−2.6	−5.1	−2.1	−11.8	−11.2	−5.7	−2.0

[a] Samples were obtained after 30 min in each posture.

are not considered to be related to posture because they were not reversible when the posture was alternated.

In conclusion, these studies indicate that values for Ca_F, Ca_T, total protein, albumin, magnesium, and pH will be altered when an individual changes posture. The magnitude of these changes will vary from individual to individual, but will be relatively constant in any given individual. The mechanism of some of these changes is not clear, but they can be a source of problems in the interpretation of laboratory data and should be considered whenever the laboratory values on a given patient are monitored serially or compared to a reference range derived from subjects in a different posture.

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