F-cell shift and protein loss strongly affect validity of PV reductions indicated by Hb/Hct and plasma proteins

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Lundvall, Jan, and Pär Lindgren. F-cell shift and protein loss strongly affect validity of PV reductions indicated by Hb/Hct and plasma proteins. J. Appl. Physiol. 84(3): 822–829, 1998.—Numerous studies have focused on alterations in plasma volume (PV) on interventions like quiet standing, exercise, or heat stress. However, no method seems capable of truly estimating the PV alteration. Therefore, an attempt was made to validate commonly used indexes of PV changes. Quiet standing was used to cause graded PV reductions estimated from hemoglobin and hematocrit (Hb/Hct) and from serum concentrations of total protein, albumin, and “large proteins” (LP; total protein minus albumin). Results indicated the following. 1) Hb/Hct, with the merit that the marker (erythrocyte) stays within the circulation, reflect accurately a small-to-moderate PV loss (< 10% of control PV). At large PV decrease (25–30% of control), however, F-cell shift can cause Hb/Hct to underestimate the response by up to 30%. 2) Albumin and total protein underate PV loss due to protein escape (mainly albumin) from the circulation. 3) LP also underestimate the PV decline due to protein escape but can often predict large PV reductions clearly better than Hb/Hct. 4) Prolonged standing can lead to pronounced 25% PV decline.

METHODS

THE RAPID CHANGES IN PLASMA VOLUME (PV) in response to interventions like exercise, heat stress, and quiet standing have attracted much interest. The PV alterations have sometimes been assessed by dilution technique [dye, isotope, CO-hemoglobin (Hb)]. However, these methods are often cumbersome and sometimes do not lend themselves to the repeated measurements at short intervals, which is often required. Consequently, changes in the hematocrit (Hct) and in the concentrations of Hb, and also of plasma protein, have frequently been used to obtain a measure of relative change in PV. The alteration in absolute terms can then be reached if control PV is known.

With proteins, however, there are studies indicating that transcapillary plasma fluid loss might lead to a simultaneous and significant net, mainly convective, loss of intravascular albumin (1, 10, 12; cf. also Refs. 21, 23). It follows that with albumin as test substance calculations of PV changes may be subjected to substantial errors. Furthermore, PV calculations based on total plasma protein or plasma density (e.g., Ref. 12) may also be problematic because albumin constitutes a large fraction of total plasma protein. With Hb/Hct, in turn, attention has been focused on factors that may influence these variables independently of transcapillary fluid shifts. Hence, even if Hb/Hct, by staying within the intravascular compartment without net loss or gain, fulfills a basic requirement for a marker of PV alterations, it may be affected by change in the ratio of the overall to the peripheral Hct (F-cell ratio; e.g., Refs. 11, 13) or by release of pooled erythrocytes from the spleen (e.g., Refs. 15, 22). Finally, a regional transcapillary fluid loss, even if rapid and large, might be reflected poorly in the overall circulation in states of uneven distribution and slow intravascular mixing of hemococoncentrated blood (17, 18). Sampled blood may then indicate a PV change deviating markedly from the true one.

Correct estimates of rapid PV changes may thus be difficult to obtain, since the commonly used indexes may be unreliable in predicting the volume responses. However, little is known about the magnitude of such errors, since the many studies in which the Hb/Hct and the plasma protein approaches have been compared apparently may be inconclusive for reasons indicated above. An attempt was, therefore, made to test the validity of Hb/Hct and of plasma protein as markers of PV reductions. In contrast to previous studies, the “protein approach” to PV changes emphasizes observations with large-molecular proteins to minimize errors due to protein escape from the circulation. Still, the analyses were based on common measurements of total plasma protein and albumin but with a novel treatment of the primary data. Hence, emphasis was placed on the protein fraction expressed by the difference between total protein and albumin (“large proteins” (LP)). Quiet standing was used as stimulus because, with this intervention, studies from our laboratory have elaborated procedures that allow valid hemoconcentration analyses (17, 18, 19). Furthermore, quiet standing permitted analyses over a quite wide range of PV changes deemed important for the interpretation of data.

Experimental material. The subjects included 21 healthy men studied at a room temperature of 24.6–25.5°C. Subjects were aged 26–47 yr, weighed 77.7 ± 1.9 kg, and measured 183 ± 1.1 cm in height. Resting supine control PV averaged 3.678 ± 0.67 (SE) ml [determined separately within < 4 mo of the standing experiment by using 125I-radionated serum albumin (0.05 mCi/kg body wt)]. Originally, 26 subjects were recruited, but a pretest (end-point 15-min standing; no intravascular instrumentation and/or blood sampling) excluded five subjects who exhibited clear vasovagal reactions after 6, 9, 11, 12, and 13 min. Written consent for the procedures described below, approved by the Ethics Committee of the University of Lund, Sweden, was obtained from all subjects.

Experimental protocol and hemoconcentration analyses. The subjects arrived at the laboratory ~1 h after a light breakfast and were dressed in a short-sleeved shirts and shorts. They were instrumented with a radial artery cannula.
A blood-pressure cuff was fitted to the contralateral arm and electrocardiographic (ECG) electrodes to the chest to follow heart rate (HR). Subjects then rested supine on a tilt table for 60 min, covered by a blanket. Next, they were exposed to quiet standing accomplished by 85° head-up tilt (nearly maximal vertical displacement) completed in 6–10 s. A foot support carried the body weight to mimic normal standing. At the end of the standing period, subjects were tilted back to the supine position (“retilt”), completed within 5–8 s to promote rapid intravascular mixing of blood pooled in dependent regions as a result of standing; cf. Ref. 18 and METHODS below) and remained quiet and relaxed for 15 min.

A total of 58 standing experiments were performed. Seven of these experiments (in 7 subjects) have been used for other purposes (1). All subjects were exposed, on one separate day, to a 15-min standing period. Eighteen of the subjects were also exposed to two additional standing experiments lasting 3 or 5 min and 8 or 10 min, respectively. The latter experiments were performed on the same day (60 min of supine rest interspersed in between each experiment) separated by 6–12 wk from the 15-min experiment.

Arterial blood was sampled for Hb and Hct before standing and, repetitively, after standing. In the experiments with 8, 10, and 15 min, sampling was performed at 1.0, 1.5, 2, 3, 5, 7.5, 10, 12.5, and 15 min. Sampling was more frequent in the experiments with 3 and 5 min of standing (1.0, 1.5, 2.0, 3, 5, 7, 9, 11, 13, and 15 min). Arterial pressure (average of two consecutive measurements) and HR (60-s ECG in control and 15-s during and after standing) were measured repetitively before, during, and after standing. Mean arterial pressure was taken as diastolic pressure plus one-third of pulse pressure.

A three-syringe technique was employed for blood sampling. The first sample was discarded, the second utilized for Hb/Hct determination, and the third for plasma protein analyses. Heparin-isotonic saline ensured catheter potency. About 45 ml of blood were sampled in each experiment. No corrections were made for the sampling-induced decrease in intravascular erythrocyte and protein mass or PV. Hb and Hct in each sample were measured in triplicate (photometric analyzer, Coulter Electronics; methodological errors 0.5% for both Hb and Hct). The methodological error was reduced by using a mean value of the triplicate measurements in subsequent calculations. Total plasma (serum) protein was determined with the biuret reference method of Doumas et al. (4) and albumin with the bromocresol purple dye-binding method, according to Pinell and Northam (20). The methodological error was <2% for both total protein and albumin. The protein determinations were performed in quadruplicate. The mean value of the four determinations was used. The concentration of LP in the control state was taken as the difference between the control values for total serum protein and albumin. The LP concentration in response to experimental intervention was calculated as described below.

Determination of PV changes. Our previous investigations with quiet standing (17, 18) have demonstrated that 1) the hemoconcentration caused by plasma fluid loss by filtration is often markedly underestimated in blood sampled from the standing subject because of slow exchange of hemoconcentrated blood pooled in dependent regions; and 2) the overall circulatory hemoconcentration is revealed instead in blood sampled shortly after standing, because resumption of the supine body position facilitates mixing of blood between circulatory compartments. Figure 1 illustrates that such features were typical also in the present study. Note the small Hb rise (Fig. 1A) in blood sampled at the end of a standing period and that resumption of the supine position (retilt) caused a marked further rise. Hb then gradually declined toward control. The protein responses (Fig. 1, B and C) were similar to that of Hb, although they were disproportionally larger, being related to plasma rather than to blood volume.

However, the early retilt data also underestimate responses at the end of the standing period because of rapid recovery of hemoconcentrations shortly on retilt (18). This problem was possible to circumvent, since restitution of Hb/Hct as well as total protein and albumin always fitted neatly a monoexponential function (regression coefficient from 0.975 ± 0.004 to 0.984 ± 0.003 for the different variables). This allowed, by extrapolation, definition of hemoconcentration at the very end of standing (“zero-time” retilt hemoconcentration; cf. Refs. 1, 18, and 19).

The zero-time retilt hemoconcentration data show PV changes in percentage of control: 1) from Hb/Hct [Hct multiplied by 0.91 (F-cell correction)]; [(Hbcontrol/Hctcontrol) × (100 − Hdttest)/(Hbcontrol/Hctcontrol − 1)] × 100 (see Refs. 3, 10); and 2) from total serum protein, albumin, or LP protein as [1 − (proteincontrol/protein) test].

Presentation. PV changes are given as %control (means ± SE). Two-tailed Students’ t-tests (paired and unpaired, as applicable) were used; P < 0.05 was considered significant.

RESULTS
In the vast majority of experiments (85%), subjects tolerated standing without adverse reactions or significant change in mean arterial pressure (control 82.3 ± 0.58 mmHg). There was an initial increment in HR (from 57.1 ± 0.77 to 65.7 ± 1.1 beats/min) followed often by a gradual small further increase. In nine experiments, however, subjects exhibited symptoms and signs of marked circulatory stress (rapidly developed pallor, dizziness, and nausea). These vasovagal reactions, occurring at 3, 4.5, 5, 7, 9, 11, 12.5, 13, and 13.5 min of standing, prompted rapid retilt. Repetitive blood sampling was then performed as described in METHODS. Retilt caused quick relief of adverse reactions in seven experiments. The two remaining subjects temporarily lost consciousness with an associated marked bradycardia. Below, for simplicity, the time spent in the erect posture in the nine experiments described is denoted as 3–5 min (3 experiments), 8–10 min (3 experiments), and 15 min (3 experiments), although standing ceased at times that deviated somewhat from these ones.

In the supine control state, Hb averaged 142.4 ± 0.8 g/l, Hct 42.6 ± 0.3%, total serum protein 65.9 ± 0.7 g/l, albumin 40.6 ± 0.3 g/l, and LP 24.8 ± 0.2 g/l. The hemoconcentration at the end of the standing period (zero-time retilt, see METHODS) showed a rough relation to the duration of standing for all five parameters. The protein responses (%concentration rise above control), however, were generally larger than those for Hb/Hct, being related to plasma rather than to blood volume. Hb and Hct responded very similarly (P > 0.05 for the whole material), indicating that the mean erythrocyte volume remained constant. The PV changes reported below thus reflect reduction of intravascular volume per se, without alteration of erythrocyte water.

Figure 2 shows that Hb/Hct indicated PV reductions
of 9.51 ± 0.5, 14.5 ± 0.6, and 17.4 ± 0.8% as a result of 3–5, 8–10, and 15 min of standing, respectively. The corresponding reductions reached from total plasma protein and from albumin, respectively, were 7.9 ± 0.4, 12.7 ± 0.5, 16.2 ± 0.7% and 7.3 ± 0.4, 11.5 ± 0.4, 14.7 ± 0.6%. In the overall material (n = 58), there were significantly smaller PV reductions calculated from total protein and from albumin than from Hb/Hct (P < 0.001 for both parameters), indicating a net loss of plasma protein during standing. This net loss seemed confined mainly to albumin, since the LP data gave PV reductions that, in the collected material, were quite similar to those of Hb/Hct (Fig. 2, A-C; P > 0.05).

Figure 3 provides more detailed analyses of PV responses reached from Hb/Hct on the one hand and from the different plasma protein fractions on the other. Here, PV reductions in individual experiments (n = 58) calculated from LP (Fig. 3A), from total protein (Fig. 3B), and from albumin (Fig. 3C) are plotted against the corresponding PV reductions calculated from Hb/Hct. Lines of identity and regression lines are given. Note that in the great majority of experiments the albumin PV decline (Fig. 3C) was smaller than the Hb/Hct one. Total protein exhibited a similar trend, but PV reductions here tended to be larger than for albumin, therefore showing more close resemblance to those of Hb/Hct. The LP responses, in turn, seem to deviate clearly from those of albumin and total plasma protein. Hence, the LP responses tended clearly to be lower than those of Hb/Hct at small PV reductions and, conversely, larger than those of Hb/Hct at large PV decline. The latter tendency was most evident in the experiments in which subjects exhibited more or less marked vasovagal reactions (Fig. 4).

Figure 5 displays more prominently the differences in PV responses indicated by different protein fractions. Note again that the albumin PV responses especially (Fig. 5C), but also those of total plasma protein (Fig. 5B), usually were clearly smaller than those of Hb/Hct over the entire range of PV decline along the abscissa. Hence, the albumin response (all data) averaged 79.2 ± 0.9% (P < 0.001) and the total protein response 87.6 ± 1.2% (P < 0.001) of the Hb/Hct response. Still, the spread of data was considerable, and quite a number of the total protein and albumin responses indicated PV reductions of similar magnitude as Hb/Hct. Figure 5A emphasizes the tendency for the LP responses to exceed those of Hb/Hct at large PV changes and, conversely, to be smaller than the Hb/Hct ones at small-to-moderate PV alterations, although the relationship between the LP and Hb/Hct PV reductions showed considerable variation. On the average (all data), however, LP indicated a similar (99.1 ± 1.5%; P > 0.05) PV decline as Hb/Hct.

The above data (Figs. 2–5) show that Hb/Hct and the different plasma protein fractions, among themselves and compared with Hb/Hct, can indicate quite dissimi-
lar values for the PV decline induced by quiet standing. Hence, Hb/Hct and LP indicated a PV decline that was on average 25% larger than that suggested by the albumin concentration changes. Furthermore, LP quite often indicated a 10–25% larger PV reduction than Hb/Hct. One phenomenon that can contribute to clarify these findings is the above-postulated net loss of intravascular protein (mainly albumin) during standing. However, such protein loss seems capable of explaining only part of the findings. Further interpretation of data may be aided significantly by the presentation in Fig. 6. These diagrams, like Figs. 3–5, show the relationship (ratio) between PV change calculated from plasma protein (LP, total plasma protein, albumin) and from Hb/Hct. Here, however, data have been grouped according to the LP response, i.e., LP PV reductions of >115% (Fig. 6A), 100–115% (Fig. 6B), 85–100% (Fig. 6C), and <85% (Fig. 6D) of the Hb/Hct reduction.

Note that with this presentation the PV responses indicated by LP, by total plasma protein, and by albumin exhibited a common pattern. Hence, when LP indicated a PV reduction significantly smaller than that of Hb/Hct (82.9 ± 0.7%; see Fig. 6D), there were also, in all experiments, quite small PV reductions calculated from total protein (76.6 ± 1.2%) and from albumin (72.1 ± 1.4%). However, when LP, in relation to Hb/Hct, indicated gradually larger PV reductions, there was a similar trend for total plasma protein and albumin. Note thus in Fig. 6A that, when LP gave a PV decline of 119.3 ± 1.6% of the Hb/Hct decline (P < 0.001), the PV reduction calculated from total protein was very similar to that of Hb/Hct (101.3 ± 1.4%; P > 0.05). Furthermore, the albumin response was also large (92.1 ± 1.3%), although still different (P < 0.05) from that of Hb/Hct. The possible physiological significance of this pattern of response is discussed below.

DISCUSSION

Numerous studies have focused on alterations in PV to interventions like quiet standing, exercise, and heat stress and on the secondary consequences for fluid balance and cardiovascular function. However, the reported effects of a given stimulus often differ markedly. Such differences have been ascribed to a large extent to the widely differing experimental protocols that have been adopted (2, 9, 17). However, very little detailed attention has been paid to possible errors inherent in the methods used to measure the PV changes.

It appears, in fact, that all commonly used indexes, Hct, Hb, and plasma proteins, might provide unreliable data. First, with changes in PV by transcapillary fluid exchange the protein content of plasma may change as well, because convection is an important mechanism for protein transfer across the capillaries (cf. Refs. 21, 23). This may be true for all plasma proteins and, in particular, for albumin, with its tendency to pass through the capillary walls. Nevertheless, a number of studies have suggested that shift in posture fails to alter plasma protein content (e.g., Refs. 2, 6, 9, 24; see

Fig. 3. PV reduction evoked by 3–15 min of quiet standing as reached from plasma concentration changes of LP (A), total protein (B), and albumin (C). Protein PV responses plotted vs. PV reductions calculated from Hb/Hct. Lines of identity and regression lines are given. For further details see text.

Fig. 4. Standing-induced PV reductions indicated by concentration changes of plasma LP are clearly larger than those calculated from Hb/Hct in subjects exhibiting pronounced circulatory stress and vasovagal reactions (difference between regression line and line of identity).
also Ref. 10). However, the protein (albumin in particular) approach to PV changes may apparently give rise to large errors. Hence, a recent study (1) provided strong evidence indicating that standing in fact leads to quite large net albumin loss (cf. also Ref. 12 and RESULTS). Note that net loss (gain) of albumin by necessity also affects PV estimates from concentration changes of total plasma protein. It also affects estimates using plasma density [which correlates closely to plasma total protein and has gained popularity because it can be determined with precision on a small plasma sample (e.g., Refs. 12, 13, 16)]. The discussed errors will be apparent when aiming to reveal relative (using native protein as marker of PV alterations) as well as absolute changes in volume (using, e.g., Evans blue or radioiodinated albumin).

The use of Hct and Hb has the advantage that the marker, the erythrocyte and its content, stays within the intravascular compartment without net loss or gain. Hct only often follows, but this can give erroneous data if the erythrocyte volume changes. However, this problem can be circumvented by calculating PV from both Hb and Hct (3, 5, 10). Yet, Hb/Hct may vary independently of the loss or gain of fluid by the intravascular space. Most immediate perhaps is the possibility of a change in the F-cell ratio (e.g., 11). In fact, clear-cut F-cell shift caused by compensatory mobilization of blood from the micro- to the macrocirculation has been demonstrated in response to bleeding in animals (13), suggesting that similar changes might occur in other states of hypovolemia, e.g., after transcapillary PV loss. Such events serve to underestimate, more or less, PV changes calculated from Hb/Hct. On the other hand, Hb/Hct might possibly overestimate PV changes, since in humans, as in animals, the spleen might release pooled erythrocytes, as indicated by several exercise studies (7, 8, 15, 22). It appears, however, that the quantitative importance of such an effect, if present, still remains to be settled. Yet, Laub et al. (15) suggested that this overestimation may be quite marked during heavy exercise, arguing that the reported >50% reduction in spleen erythrocyte volume could account for 25% of the simultaneously observed circulatory hemoconcentration. Their study also suggested that the spleen can release erythrocytes in response merely to shift from the supine to the erect body posture. Note, finally, that F-cell shift or mobilization of erythrocytes

![Fig. 5. PV decline resulting from quiet standing (3–15 min) calculated from plasma concentrations of LP (A), total protein (B), and albumin (C) in %PV decline calculated from Hb/Hct. Overall, LP indicated similar (99.1 ± 1.5%) PV loss as Hb/Hct, whereas total protein (87.6 ± 1.2%) and albumin (79.2 ± 0.9%) indicated clearly smaller responses than Hb/Hct. In each diagram, however, there was fairly large spread of data, and LP responses especially seemed to vary with the degree of PV reduction.](image)

![Fig. 6. PV decline on quiet standing (3–15 min) calculated from plasma concentrations of LP (solid bars), total protein (hatched bars), and albumin (open bars) in %PV decline calculated from Hb/Hct. Data classified with regard to magnitude of LP vs. Hb/Hct response, i.e., LP reduction of PV >115% (A), 100–115% (B), 85–100% (C), and <85% (D) of that indicated by Hb/Hct. Total protein-Hb/Hct and albumin-Hb/Hct PV reductions correlated with that of LP-Hb/Hct (P < 0.01 and P < 0.001, respectively).](image)
from the spleen might affect relative as well as absolute (tagged erythrocytes) determinations of PV alterations.

The complexity indicated above illustrates that attempts to validate Hb/Hct and protein as markers of PV changes are associated with great difficulties. In fact, this problem has resisted a solution for almost a century. Yet, the present study might provide new information by virtue of some special features in the experimental approach and design. Still, the conclusions that can be drawn must necessarily be based on indirect grounds, since, as emphasized above, there is no reference method that can provide truly valid PV data.

As stated above, PV estimates based on concentration changes of plasma proteins may be in error because proteins undergo transcapillary exchange. However, proteins can be expected to respond differently, with albumin showing perhaps the greatest and larger proteins the smallest tendency to cross the microvascular walls. Yet, capillary protein permeability is not a simple function of molecular size, and a complex interplay between various factors that determine permeability still remains to be elucidated (14, 21, 23). Therefore, it does not appear to be possible at present to designate one particular protein as the most suitable marker of PV. Instead, determination of total plasma protein and albumin enabled definition of an LP fraction, as opposed to the albumin fraction. Analysis could then be made of PV changes calculated from LP, from total plasma protein, as well as from albumin.

Furthermore, for the purpose of the present study, it was important to obtain data on concentration changes of blood constituents that reflected the overall circulation and not merely individual, circumscribed circulatory regions. Quiet standing was used to change PV, because with this stimulus the experimental procedures described in METHODS seem to ensure that the induced hemoconcentration, within limits, is evenly distributed among various circulatory compartments at the time of blood sampling. For similar reasons, hemoconcentration analyses were performed on arterial rather than on antecubital venous blood (cf. Ref. 18). In addition, with quiet standing, it was possible to induce PV reductions ranging from relatively small to quite pronounced ones. Such detailed data, based on quite a large number of experiments, were considered important because it can apparently be foreseen that the magnitude of PV change in itself might be crucial for the possibility to reveal whether Hb/Hct and plasma proteins might fail to adequately describe the induced volume change (see further below).

The albumin PV changes were usually much smaller than those derived from Hb/Hct, reflecting, in all likelihood, quite a large net loss of intravascular albumin (e.g., Fig. 5C). The PV change calculated from total protein was also, in general, clearly smaller than that indicated by Hb/Hct (Fig. 5B), as an inevitable consequence of the net loss of albumin. LP predicted, as an average for all observations, similar PV changes as Hb/Hct (P > 0.05) but, still, there were consistent differences on detailed scrutinization of responses. Hence, with small PV alterations, Hb/Hct usually indicated a larger volume change than LP (P < 0.01). Conversely, with large PV changes, the Hb/Hct response was usually smaller than that of LP (P < 0.01). Even if this complex pattern of response admittedly may resist incontestable interpretation, these data, when taken together, seem capable to offer reasonable explanations for the observations.

First, with small PV changes, it seems unlikely that shift of blood from the micro- to the macrocirculation (F-cell change) or release of erythrocytes from the spleen should have affected Hb/Hct in sampled blood. Hence, with limited circulatory stress, there seems to be no need for such compensatory reactions. Therefore, Hb/Hct may have reflected the PV alteration accurately. The fact that LP demonstrated a more limited PV decline seems to indicate a small but significant net loss of large-molecular proteins. With large PV changes, on the other hand, the smaller PV alteration indicated by Hb/Hct than by LP seems to strongly indicate that, first, spleen release of pooled erythrocytes into circulating blood was not present or, at least, was not a prominent feature, and, second, that the Hb/Hct response in sampled blood was blunted by a change in the F-cell ratio caused by redistribution of blood with low Hct from the micro- to the macrocirculation. Hence, it appears that the large PV reduction present in the recumbent subject early after more prolonged 10–15 min of standing (~15–20% PV loss) might have constituted a sufficient stimulus for significant compensatory shift of blood from the micro- to the macrocirculation. This may be more than likely because the intravascular volume change was similar to that reported to cause a marked F-cell shift in animals (13).

This interpretation was supported by the observation that when subjects exhibited marked circulatory stress (vasovagal reactions) Hb/Hct indicated a clearly smaller PV reduction than LP (Fig. 4). However, the main support for an F-cell shift was provided by Fig. 6. Without F-cell shift, but with decline in plasma protein content, protein would indicate a PV loss smaller than Hb/Hct, as in Fig. 6D. Conversely, shift of blood with low Hct from micro- to macrocirculation should imply that not only LP but also plasma total protein and albumin would indicate a relatively large PV loss in relation to Hb/Hct, despite the net loss of intravascular protein. The data in Fig. 6A exhibited such a pattern of response. These findings illustrate that meaningful interpretation of the present data must focus on the integrated overall patterns of PV responses, indicated by different plasma protein fractions and by Hb/Hct in conjunction, considering also that the magnitude of PV decline affected the responses. Note also that, with large PV change, an F-cell shift seemed present in many but not in all experiments (LP data in Fig. 5A). Apparently, the hemodynamic stress of a rapid and large PV decline, and the need for compensatory reactions, varied significantly among individuals. Such
differences help to explain the sometimes large variation in responses (e.g., Figs. 3 and 5).

It can be argued, however, that the larger PV loss indicated by LP than by Hb/Hct simply reflects a net gain of protein into the circulation, e.g., by lymph flow. Hence, rather than postulating that Hb/Hct underestimates the PV loss due to F-cell shift, the possibility should be considered that LP might overestimate the PV reduction instead. This is a very remote possibility, however, because it would require that fluid with a protein concentration exceeding that of plasma enter the circulation. Instead, it appears that the induced PV loss is no smaller than that indicated by the LP concentration change. First, the overall net protein loss from the circulation during standing (albumin as well as LP), as calculated from total protein data, should be no smaller than the net albumin loss. Simple calculations show that a PV change as large as that indicated by the LP data by definition implies that the calculated net albumin loss will be exactly equivalent to the net loss of total plasma proteins. However, with a PV decline smaller than that indicated by LP, as often suggested by Hb/Hct (Figs. 3 and 5), loss of total plasma protein would be smaller than that of albumin. A priori, this should not occur. Furthermore, not only albumin but also LP proteins can probably escape the circulation (cf. 21, 23) as also indicated by the data at small PV change. Therefore, the PV loss indicated by LP proteins seems not to overestimate but rather to underestimate the true PV changes that were induced.

That LP was measured in serum instead of in plasma might contribute to an underestimation, because fibrinogen, with its large molecule, may exhibit less tendency than most other LP proteins to leave the intravascular space. With measurement in plasma, LP might thus indicate a larger PV loss than in the present experiments. Still, the difference would probably be small, because the fraction of LP in plasma that is fibrinogen is limited. In fact, the LP response was similar in serum and in plasma (P > 0.05) in test experiments.

The LP underestimation of PV loss in the present study might be roughly assessed if the net transcapillary escape of LP proteins occurred preferentially by convection [secondary to PV loss (cf. Refs. 21, 23)]. Calculations showed in fact that the LP (and albumin) loss exhibited a direct relation (P < 0.001) to the magnitude of plasma fluid loss. Figure 6D showed that, after small-to-moderate PV decline, when Hb/Hct correctly estimated the PV change (no F-cell shift, see above), LP indicated a PV loss only 83% of that of Hb/Hct [i.e., true PV loss 20% larger (1.00/0.83) than the LP one]. However, a similar error should prevail also after more pronounced PV reduction, since LP escape from the circulation correlated to plasma fluid loss. It follows that the true PV alteration, regardless of whether small or large, may be reached by increasing the LP PV reduction by 20%. Such data are depicted in Fig. 7. Note that the PV loss after prolonged standing (15 min) in a majority of subjects may have been in the order of 25% instead of the maximal 17–21% change indicated by Hb/Hct (>900 ml instead of <700 ml). Such pronounced PV decline (hypovolemia) supports the hypothesis stated above that standing may be associated with a hemodynamic need for compensatory mobilization of blood from peripheral vascular regions to the central circulation. However, such blood mobilization seems attended by F-cell shift (13), which, in turn, can explain that Hb/Hct in many experiments underestimated the PV loss by 20–30% (i.e., true PV reduction 25–35–40% larger than indicated by Hb/Hct). Clearly, however, there is a need to support these quantitative estimates by further research.

In summary, Hb/Hct and LP proteins, in contrast to albumin and total plasma protein, are capable of predicting roughly the PV loss induced by quiet standing over a wide range of volume changes. LP, however, seems always to underestimate (by some 15%) the induced PV loss due to net transcapillary escape of proteins. Hb/Hct, in turn, can reflect quite accurately a small-to-moderate PV loss. However, with large PV decrease (≥15% of control PV) and secondary large circulatory stress, Hb/Hct seem often to underestimate markedly the PV alteration (by as much as 20–30% or >200 ml) due to F-cell shift. Therefore, quiet standing seems to evoke an even more rapid and large PV decline than suggested by our recent Hb/Hct studies (17–19). In fact, PV seems to be reduced by no less than 25% as a result of prolonged standing still.

Fig. 7. PV decline as a result of quiet standing (3–15 min), calculated from plasma concentration changes of LP and from Hb/Hct alterations. LP data corrected for transcapillary loss of protein. Note that LP indicate very large, up to ~25%, PV reduction. LP, rather than Hb/Hct, might reflect induced PV reductions because Hb/Hct seemed blunted by F-cell shift.
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