

## CHALLENGES IN CONTROLLING FOR THE EFFECTS OF HEMOCONCENTRATION REPLY

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### **To the Editor:**

Two letters were recently submitted to the editor regarding our recent publication (1), both of which raised important issues. Fall and Bailey identified discrepancies between their work (2) and ours, which deserve consideration. They also used the same Dill and Costill correction technique (3) after maximal exercise, though it yielded different results to their study and one by Darlington and colleagues (4). Darlington and colleagues found that progressive dilutions from 0-90% of citrated plasma elongated coagulation times, which is in agreement with the results of Fall and Bailey's experiment (2). However, we feel that the design of the Darlington et al. study is not very useful to explain our findings. While the dilution of citrated

plasma resulted in an elongation of coagulation time in that study, this is not the same technique as correcting mathematically for plasma volume shift or reconstituting plasma samples from stressed subjects.

Fall and Bailey suggest that our divergent results could be explained by mathematical error. According to Fall and Bailey, an “increase” in coagulation results in a decrease in coagulation time across the extrinsic and intrinsic pathways and the common pathway. Thus, time needs to be “given back” to correct for plasma volume shifts and the equation should incorporate a plus sign (+) rather than a minus sign (-), as reported in our paper. This idea of “giving time back” is intriguing and does make intuitive sense. In a previous study (5), we were to our knowledge the first group to attempt to arithmetically correct for plasma volume changes when examining clotting time. We used the equation that has been used extensively to correct for plasma volume changes when examining concentrations, which incorporates a (-). In this study, we also reported a significant shortening of APTT when arithmetically correcting for plasma volume changes. We agree that this equation may not be appropriate for the correction of coagulation times, an issue also brought up by Muldoon (cf. below). This is why we used the novel reconstitution techniques.

Fall and Bailey go on to say that if the reported  $\Delta PV$  from baseline to post stress ( $3.5 \pm \approx 2.3\%$ ) is used and apply the correction method incorporating a (+), the aPTT only reduces by 0.6 s ( $33.8 \pm 0.8$  from  $34.4 \pm 0.8$ ), thereby removing the reduction. Please note that we actually reported that the uncorrected aPTT at stress was 33.1 s and not 33.8 s. We alert the readers to an erratum related to our paper (the erratum is published in this issue of Psychosomatic Medicine). For illustrative purposes we recalculated the data based on the corrected equation presented in

the erratum using the published means. The reported results do correspond to the published data.

The formula for calculating percent change in plasma volume is as follows:

$$\% \Delta PV = 100(PV_A - PV_B)/PV_B.$$

Using our values for PV, we get

$$\% \Delta PV = 100(53.3 - 56.8)/56.8$$

$$\% \Delta PV = -6.16.$$

Then, if this value is used in the established equation for the corrected value, we get:

$$C_C = C_U/[1 - (\% \Delta PV/100)]$$

$$C_C = 33.1/[1 - (-6.16/100)]$$

$$C_C = 33.1/1.0616$$

$$C_C = 31.2$$

The reason this is 31.2 instead of 31.1, as we reported in our paper, is that the analyses for aPTT that we reported in the paper only included 26 subjects, whereas the values for PV included all subjects. Thus, the values do correspond to the published data.

However, if the equation incorporated a (+), then the corrected APTT would be:

$$C_C = C_U/[1 + (\% \Delta PV/100)]$$

$$C_C = 33.1/[1 + (-6.16/100)]$$

$$C_C = 33.1/.9384$$

$$C_C = 35.3$$

Fall and Bailey also challenge whether the shortening from baseline to uncorrected stress values is clinically significant, as the stress aPTT and PT% are still within the boundaries of normal (6). The shortening from baseline to stress was statistically significant. Nevertheless, this is a good point raised by Fall and Bailey. However, such a shortening reflecting

hypercoagulability, even if only by one second, could potentially be harmful in those with atherothrombotic disease. To compare, it is known that even within normal reference ranges elevated blood pressure and high-sensitive C-reactive protein levels both are associated with a relatively greater risk of cardiovascular morbidity and mortality. While the role of an abnormally short aPTT in atherothrombotic disease, including acute coronary events (that may be triggered by acute mental stress) is only emerging, an aPTT value below the lower normal reference limit is now acknowledged to have predictive value for venous thrombotic events (7, 8). Moreover, there was a significant continuous relationship between a shorter aPTT and an increased risk of recurrent venous thromboembolism in patients after discontinuation of oral anticoagulant therapy (8).

Fall and Bailey also criticize the expression of prothrombin time as PT% and suggest that the International Normalized Ratio (INR) would be more accessible to the clinical reader. However, few previous studies have examined PT changes during acute psychological stress. Of those that have, PT has been expressed as PT% (5) and PT as raw times (9, 10). We indicated that an increase in PT% corresponds to an acceleration, or shortening, of clotting time of the extrinsic pathway. The INR is typically used in clinical settings to monitor chronic anticoagulant therapy which was not the focus of our experimental stress study. Moreover, there are no previous reports that examined acute psychological stress-induced changes in INR (11). However, it may be fruitful for future investigations to incorporate the INR to help clinical interpretation.

Fall and Bailey have biochemical concerns as well. Clearly, there is a range of stress-induced biochemical changes that also may affect coagulation activity above and beyond the concomitant plasma volume shift. Fall & Bailey suggest that we should have added a norepinephrine spike to post-stress samples and indeed catecholamine results in coagulation

activation (e.g., increase in FVIII:C) in a dose-response manner within minutes (12). Moreover, there also was a direct association between stress-induced increases in norepinephrine and D-dimer levels in one of our previous studies (13). It is unlikely that stress-responsive proinflammatory cytokines like interleukin-6 evidently contributed to our coagulation measures. In terms of stress effects on inflammation processes, studies show that proinflammatory changes to acute psychosocial stress occur with a delay of up to 60 minutes post-stress (14), although a few studies have documented increases in interleukin-6 within 5 minutes following mental and physical challenge tests (15). Although there is an association between oxidative stress and coagulation at steady state conditions, it has not been investigated whether these two systems influence each other at times of acute stress and whether mutual influences would emerge within minutes of an acute psychosocial stress task.

Moreover, Fall and Bailey question whether the flavanol intervention actually does not influence the present data. As mentioned in our study, we actually controlled statistically for flavanol exposure which showed no association with our coagulation measures. We acknowledge that there remains a possibility that a flavanol effect (although minor in this case) might have gone undetected because of insufficient power.

Fall and Bailey further ask about potential clinical consequences of stress-hemoconcentration effects on hypercoagulability in patients taking medications which contract plasma volume in their own right. Although the outcome of such patient care is currently unknown, our answer is “yes” that it might seem prudent to carefully monitor the hydration status of patients with PV contracting drugs who also are repeatedly exposed to acute bouts of psychosocial stress. The reason is that an additive effect of the drug- and stress-induced contraction of PV might theoretically increase their thrombotic risk. Moreover, encouraging

distressed patients to drink more water and isotonic beverages may help to offset some of the drug-induced contracted PV.

Dr. Muldoon raised the point that the Dill and Costill correction method is not necessarily appropriate for function measures of hemostasis. We agree, as this was more or less the rationale for trying to find an alternative way of adjusting for hemoconcentration effects. However, it should also be mentioned that similar correction of functional hemostasis measures, including FVIII:C, aPTT, and PT, for plasma volume changes also have been applied in previous studies on effects of physical exercise on hemostasis (16). Moreover, as the Dill and Costill correction is the gold standard for correcting for plasma volume shifts, we found it appropriate to compare it to the reconstitution techniques we used. Moreover, Muldoon questions the appropriateness of reconstitution techniques themselves given the dynamic nature of the blood. Adding saline simply dilutes the plasma, whereas adding baseline plasma puts in additional quantities of clotting factors. We presumed that saline closely represents the filtrate that is lost through capillary pores. However, the filtrate also includes smaller substances such as catecholamines. Thus, it may not be an entirely appropriate fluid for reconstitution. Nevertheless, it is still more appropriate than baseline plasma reconstitution or arithmetic correction.

Finally, Muldoon suggests an alternative, indirect approach to this issue. Specifically, examining the correlations between, and time course of, changes in plasma volume and changes in functional hemostasis may provide evidence that acute changes in hemostasis are driven by hemoconcentration. We agree with Dr. Muldoon that computation of a correlation between changes in plasma volume and hemostasis measures might be an alternative way to look at stress-hemoconcentration effects on functional measures of hemostasis, i.e. activities of individuals clotting factors and global coagulation tests that are sensitive to levels of several

clotting factors of the intrinsic and extrinsic coagulation pathways. Accordingly, we computed the correlation coefficients between the change in plasma volume from baseline to immediately post-stress and corresponding changes in aPTT ( $r=0.09$ ,  $p=0.64$ ), FVIII:C ( $r=-0.13$ ,  $p=0.51$ ), and PT% ( $r=-0.36$ ,  $p=0.058$ ). From these results, we may imply that there is little evidence for hemoconcentration having a substantial effect on changes in functional hemostasis regarding the intrinsic pathway of coagulation. In contrast, stress-induced hemoconcentration might have a comparably stronger effect regarding function of the extrinsic coagulation pathway, as the variance in PT% change that was explained by change in PV reached almost 13%. Nevertheless, when applying this technique to “control” for stress-hemoconcentration, most of the variance in the stress-induced change in functional hemostasis measures does not appear to be a consequence of plasma volume shift. This finding actually concurs with a previous study from our group in a different sample of healthy men, where the stress-induced changes in PV explained between 4% and 10% (all n.s.) of the variance in changes of FVII:C, FVIII:C, and FXII:C (17).

Given the points raised in these two letters to the editor, how to handle the problem of plasma volume changes remains a controversial issue. More research in this area is clearly warranted.

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