

**REPLY TO LETTERS FROM
FALL AND BAILEY, AND
MULDOON**

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 previous work (2) and ours, which deserve consideration. They also used the same correction technique of Dill and Costill (3) after maximal

exercise, although it yielded different results to their study and one by Darlington and colleagues (4). Darlington and colleagues found that progressive dilutions from 0 to 90% of citrated plasma elongated coagulation times, which is in agreement with the results of the experiment of Fall et al. (2). However, we feel that the design of the study of Darlington et al. is not very useful to explain our findings. Although 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 (PV) shift or reconstituting plasma samples from stressed participants.

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 PV shifts, and the equation should incorporate a plus sign (+) rather than a minus sign (−), as reported in our study. 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 activated partial thromboplastin time (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 ΔPV from baseline to poststress ($3.5\% \pm \approx 2.3\%$) is used and apply the correction method incorporating a (+), the aPTT only reduces by 0.6 seconds (33.8 ± 0.8 seconds from 34.4 ± 0.8 seconds), thereby removing the reduction. Please note that we actually reported that the uncorrected aPTT at stress was 33.1 seconds and not 33.8 seconds. We alert

the readers to an erratum related to our study, which 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 PV 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 study, is that the analyses for aPTT that we reported in the study only included 26 participants, whereas the values for PV included all participants. 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 because the stress aPTT and percentage of prothrombin time (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 1 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. Although 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 PT 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 who 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 PV shift. Fall and Bailey suggest that we should have added a norepinephrine spike to post-stress samples, and indeed, catecholamine infusion results in coagulation activation (e.g., increase in factor VIII clotting activity [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 such as 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

poststress (14), although a few studies have documented increases in interleukin-6 within 5 minutes after 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 2 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 PV 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 correction method of Dill and Costill is not necessarily appropriate for functional measures of hemostasis. We agree because 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 PV changes also have been applied in previous studies on the effects of physical exercise on hemostasis (16). Moreover, because the correction of Dill and Costill is the gold

standard for correcting for PV shifts, we found it appropriate to compare it with the reconstitution techniques we used. Additionally, 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 time course of changes in PV 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 PV and hemostasis measures might be an alternative way to look at stress-hemoconcentration effects on functional measures of hemostasis, that is, activities of individual 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 PV from baseline to immediately poststress and corresponding changes in aPTT ($r = 0.09$, $p = .64$), FVIII:C ($r = -0.13$, $p = .51$), and PT% ($r = -0.36$, $p = .058$). 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 the 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 seem to be a consequence of PV 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 not significant) 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 PV changes remains a controversial issue. More research in this area is clearly warranted.

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REFERENCES

1. Austin AW, Wirtz PH, Patterson SM, Stutz M, von Känel R. Stress-induced alterations in coagulation: assessment of a new hemoconcentration correction technique. *Psychosom Med* 2012;74:288–95.
2. Fall L, Evans KA, Lewis MH, Bailey DM. Haemostatic response to hypoxaemic/exercise stress: the dilemma of plasma volume correction. *J Clin Pathol* 2011;64:269–71.
3. Dill DB, Costill DL. Calculation of percentage

- changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 1974;37:247–8.
4. Darlington DN, Delgado AV, Kheirabadi BS, Fedyk CG, Scherer MR, Pusateri AE, Wade CE, Cap AP, Holcomb JB, Dubick MA. Effect of hemodilution on coagulation and recombinant factor VIIa efficacy in human blood in vitro. *J Trauma* 2011;71:1152–63.
 5. von Känel R, Kudielka BM, Haerberli A, Stutz M, Fischer JE, Patterson SM. Prothrombotic changes with acute psychological stress: combined effect of hemoconcentration and genuine coagulation activation. *Thromb Res* 2009;123:622–30.
 6. Leung RS, Liesner R, Brock P. Coagulopathy as a presenting feature of Wilms tumour. *Eur J Pediatr* 2004;163:369–73.
 7. Lippi G, Favaloro EJ. Activated partial thromboplastin time: new tricks for an old dogma. *Semin Thromb Hemost* 2008;34:604–11.
 8. Hron G, Eichinger S, Weltermann A, Quehenberger P, Halbmayr WM, Kyrle PA. Prediction of recurrent venous thromboembolism by the activated partial thromboplastin time. *J Thromb Haemost* 2006;4:752–6.
 9. von Känel R, Preckel D, Zraggen L, Mischler K, Kudielka BM, Haerberli A, Fischer JE. The effect of natural habituation on coagulation responses to acute mental stress and recovery in men. *Thromb Haemost* 2004;92:1327–35.
 10. de Boer D, Ring C, Wood M, Ford C, Jessney N, McIntyre D, Carroll D. Time course and mechanisms of mental stress-induced changes and their recovery: hematocrit, colloid osmotic pressure, whole blood viscosity, coagulation times, and hemodynamic activity. *Psychophysiology* 2007;44:639–49.
 11. von Känel R, Vökt F, Biasiutti FD, Stauber S, Willemin WA, Lukas PS. Relation of psychological distress to the INR in Patients with venous thromboembolism with and without oral anticoagulant therapy. *J Thromb Haemost* 2012;10:1547–55.
 12. von Känel R, Dimsdale JE. Effects of sympathetic activation by adrenergic infusions on hemostasis in vivo. *Eur J Haematol* 2000;65:357–69.
 13. Wirtz PH, Ehlert U, Emini L, Rüdüsüli K, Groessbauer S, Gaab J, Elsenbruch S, von Känel R. Anticipatory cognitive stress appraisal and the acute procoagulant stress response in men. *Psychosom Med* 2006;68:851–8.
 14. Steptoe A, Hamer M, Chida Y. The effects of acute psychological stress on circulating inflammatory factors in humans: a review and meta-analysis. *Brain Behav Immun* 2007;21:901–12.
 15. Kop WJ, Weissman NJ, Zhu J, Bonsall RW, Doyle M, Stretch MR, Glaes SB, Krantz DS, Gottdiener JS, Tracy RP. Effects of acute mental stress and exercise on inflammatory markers in patients with coronary artery disease and healthy controls. *Am J Cardiol* 2008;101:767–73.
 16. van den Burg PJ, Hospers JE, van Vliet M, Mosterd WL, Bouma BN, Huisveld IA. Changes in haemostatic factors and activation products after exercise in healthy subjects with different ages. *Thromb Haemost* 1995;74:1457–64.
 17. Zraggen L, Fischer JE, Mischler K, Preckel D, Kudielka BM, von Känel R. Relationship between hemoconcentration and blood coagulation responses to acute mental stress. *Thromb Res* 2005;115:175–83.