Female platelets have distinct functional activity compared with male platelets: Implications in transfusion practice and treatment of trauma-induced coagulopathy

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Ernest E. Moore, Editor: PI, research support and shared U.S. patents Haemonetics; PI, research support, Instrumentation Laboratory, Inc.; Co-founder, Thrombo Therapeutics. Associate Editors David Hoyt, Ronald V. Maier and Steven Shackford have nothing to disclose. Editorial staff and Angela Sauaia have nothing to disclose.

Author Disclosures

Julia Coleman, Women and Girls with Bleeding Disorders Foundation- Grant; Ernest Moore, Haemonetics and Instrumentation Laboratory- Grant.

Reviewer Disclosures

The reviewers have nothing to disclose.

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BACKGROUND: Females are hypercoagulable and have survival benefit in trauma-induced coagulopathy (TIC). The mechanism for this sex-specific hypercoagulability is unknown. Platelets and platelet function are central in providing hemostatic potential and are the largest contributor to clot strength. Ligands (adenosine diphosphate [ADP] and platelet-activating factor [PAF]) bind distinct platelet receptors to potentiate activation and aggregation. We hypothesize that female platelets have a differential response to ADP and PAF, resulting in greater aggregation and activation compared to males, and that estradiol pretreatment of male or female platelets enhances this activity.

METHODS: Platelets were collected from healthy volunteers: premenopausal/postmenopausal females (≤54 years, >54 years) and similarly aged males. Platelet aggregometry and flow cytometry (fibrinogen binding capacity) were examined. After treatment with ADP or PAF, platelet aggregation was assessed with Chronolog and activation assessed by CD41 receptor surface expression using flow cytometry. Aggregation and activation were again assessed after platelet pretreatment with estradiol.

RESULTS: Healthy volunteers included 12 premenopausal and 13 postmenopausal females and 18 similarly aged males. Female platelets (combined premenopausal and postmenopausal) had increased aggregation with ADP stimulation, as compared to male platelets. Male and female platelets had differential fibrinogen receptor expression, with female platelets (combined premenopausal and postmenopausal) demonstrating robust activation with ADP versus male platelets with PAF. In the presence of estradiol incubation, male platelets' activation with PAF approximated that of females (combined premenopausal and postmenopausal) and activation with PAF was enhanced in both male and female platelets.

CONCLUSION: Male and female platelets have differential response to stimuli, suggesting sex-dependent signaling and cellular activation. Female platelets have both increased aggregation and activation potential, and estradiol pretreatment feminizes male platelets to approximate female platelet activation with PAF. These findings offer potential explanation for sex-based differences in hemostatic potential in TIC and question whether donor sex of transfused platelets should be considered in resuscitation. Estradiol may also serve as a novel therapeutic adjunct in TIC. (*J Trauma Acute Care Surg.* 2019;87: 1052–1060. Copyright © 2019 Wolters Kluwer Health, Inc. All rights reserved.)

KEY WORDS: Sex dimorphisms; estradiol; trauma-induced coagulopathy; platelets.

Sex dimorphisms in coagulation are well-established, with females demonstrating a hypercoagulable profile.[1,2] On whole blood hemostatic assays, including thrombelastography (TEG), females have shortened phase of enzymatic clotting (reaction time), increased rate of clot propagation (angle), and increased clot strength (maximum amplitude [MA]) compared with males.[1–3] This hypercoagulability has clinical significance, with severely injured females demonstrating better physiologic response to similar degrees of shock and decreased transfusion requirements compared with their male counterparts in the setting of trauma-induced coagulopathy (TIC).[4] We have recently identified that female-specific hypercoagulability is present at baseline, persists following injury, and that when sex is evaluated as an experimental variable in severely injured trauma populations, female sex confers a survival benefit in the setting of depressed MA associated with TIC.[5]

The mechanism for female-specific hypercoagulability is unknown, but female sex appears to be protective against mortality, even when matched by injury severity, injury mechanism, and shock. It has been hypothesized that sex hormones and platelet biology may be the mechanistic drivers. This is suggested by a higher female MA (an effect predominantly driven by platelets) identified on whole blood clotting assessment (TEG), as compared to males. This is most pronounced in pregnant and peripartum females and those taking hormonal therapy, suggesting a potential sex hormone effect.[6–10] The presence of sex hormone receptors, including androgens and estrogens, on platelets, as well as estrogen- and androgen-responsive enzymes within platelets, further supports a sex hormone effect on platelets as a basis for female hypercoagulability.[11–13] The effect of sex hormones on platelet function and their mechanisms of action have not been fully elucidated.

The objective of this study was to compare the platelet function of healthy males and females including platelet aggregation (extent of shape change [ESC]) and activation (fibrinogen receptor binding capacity, i.e. CD41 receptor surface expression) in response to differential stimuli. Adenosine diphosphate (ADP) and platelet-activating factor (PAF) are both important activators of platelets, inducing aggregation and specifically causing robust fibrinogen receptor mobilization via distinct receptors and pathways.[14–16] We hypothesize that 1) female platelets have increased aggregation and activation with ADP and PAF stimulation compared to males and 2) treatment of platelets with estradiol will enhance male and female platelet aggregation and activation.

METHODS

Apheresis platelets from healthy volunteers, specifically from premenopausal and postmenopausal females (menopausal state determined by age cutoff of 54 years or younger, the average age of menopause[17]) and similarly aged males, were obtained under a Colorado Multiple Institute Review Board approved protocol (COMIRB00–004). As healthy males or females presented for voluntary donation to a blood donation center, platelets were acquired for each age group: 18 years to 54 years for males and females and older than 54 years for males and females. Platelet function was assessed by aggregometry and flow cytometry. For aggregation, 3×10^5 platelets/μL were placed in a Chronolog Aggrogometer (Model 490), activated with either 20 μM of

Submitted: January 15, 2019, Revised: May 3, 2019, Accepted: May 9, 2019, Published online: May 31, 2019.

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This work was presented at the 37th Annual Meeting of the Western Trauma Association, March 3–8, 2019 in Snowmass, Colorado.

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DOI: 10.1097/TA.0000000000002398

TABLE 1. Aggregation of Platelets after ADP or PAF Stimulation by sex

	ESC (%)			
Stimulant	**ADP**	***p* value**	**PAF**	***p* value**
By sex				
Females (n = 23)	35.5 (31.7–39.2)	0.03*	37.0 (31.0–40.0)	0.49*
Males (n = 28)	32.4 (27.5–36.6)		34.0 (29.9–40.2)	
By sex and age				
Premenopausal females (n = 11)	35.2 (31.7–38.2)	0.18†	33.2 (28.2–37.0)	0.05†
Postmenopausal females (n = 12)	36.0 (34.4–39.3)		39.6 (37.0–41.3)	
Young males (n = 15)	32.8 (28.5–36.7)		31.2 (30.3–40.2)	
Older males (n = 13)	29.3 (24.7–37.2)		37.2 (25.1–40.0)	

p Value comparing male and female aggregation using Mann–Whitney* or Kruskal-Wallis† tests as Appropriate.
Data Presented as Median (25–75 IQR).

ADP or 2 μM of PAF and monitored for ESC over time. Extent shape change was calculated as a percent (%) of change from baseline (platelet-rich plasma, no stimulant). For flow cytometry, platelets were diluted to a concentration of 60×10^3 platelets/μL, activated with 20 μM ADP (Chronolog) for 5 minutes or 2 μM PAF (Sigma Chemical Co.) for 10 minutes, and fixed with 1% paraformaldehyde. The fixed platelets were then incubated with either a PE-labeled isotype control or PE-labeled CD41 (fibrinogen receptor; BD Bioscience) to assess fibrinogen binding capacity by flow cytometry (BD FACSCanto II). Levels of CD41 were measured as mean fluorescent intensity (MFI) with the isotype control subtracted out.

Estradiol (Sigma Aldrich Co.) was dissolved in minimal quantity of 0.9% NaCl. Aggregation and flow cytometry were measured after incubating platelets in 105 pg/mL of estradiol or vehicle control for 15 minutes at 37°C, a physiologic level in healthy premenopausal females during mid-estrus.[18]

Statistical analysis was performed in R.[19] ESC (%) and CD41 (MFI) were compared between sexes with a Mann-Whitney test due to nonnormal distribution. ESC and CD41 MFI in the presence of estradiol or normal saline control were compared with the Wilcoxon signed rank test. Significance was determined at $p < 0.05$. Power analysis was conducted in R to determine a sufficient sample size using an alpha of 0.05, a power of 0.80, a large effect size, and two tails, with an equal allocation of participants into each group. Based on the aforementioned assumptions, the desired sample size was calculated as 20 per group (male or female).

RESULTS

Fifty-three healthy volunteers were included in this study: 12 premenopausal females, 13 postmenopausal females, and 18 similarly aged males (15 young male and 13 older males). The average ages were 30.3 years (range, 24–52 years) in the premenopausal females, 62.2 years (range, 58–72 years) in the postmenopausal females, 38.2 years (range, 25–53 years) in the younger males, and 64.9 years (range, 55–71 years) in the older males.

Platelet Aggregation

Platelet aggregation was assessed by ESC after stimulation with ADP or PAF. Compared with males, female platelets had significantly increased aggregation with ADP stimulation, with a median ESC of 35.5% (interquartile range [IQR], 32.7–39.2%) versus 32.4% (IQR, 27.5–36.6%) in males ($p = 0.03$) (Table 1, Fig. 1). There was no difference in platelet aggregation after PAF stimulation between females and males.

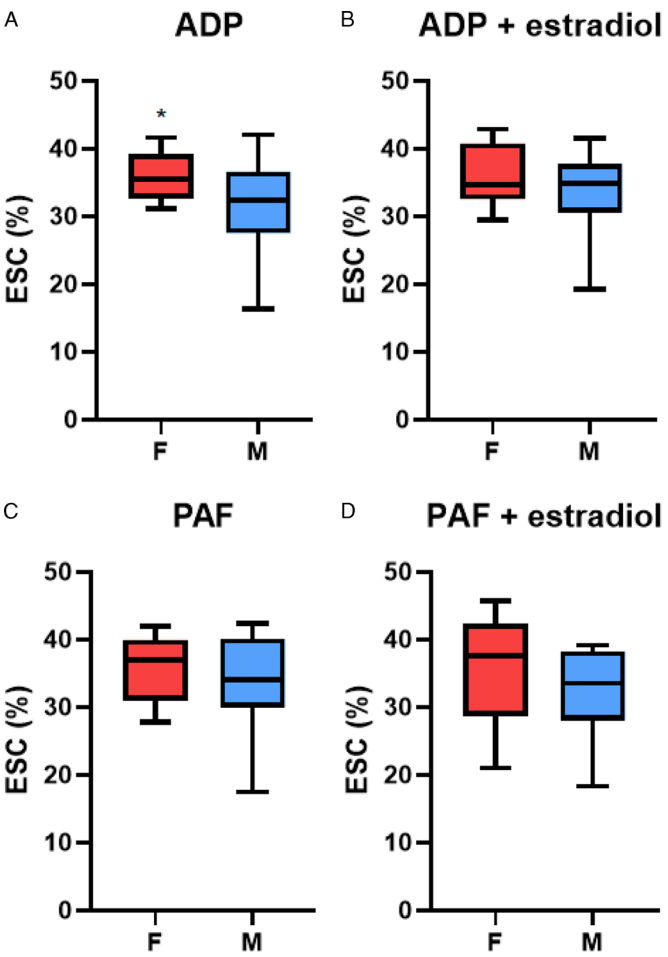

Figure 1. Platelet aggregation, as assessed by ESC, after adenosine diphosphate (ADP) stimulation (*A*) and PAF simulation (*C*) and estradiol pretreatment (*B, D*). Asterisks indicate $p < 0.05$.

TABLE 2. Platelet Activation, by CD41 Receptor Surface Expression, of Platelets After ADP or PAF Stimulation by sex

	CD41 Receptor Surface Expression (MFI)					
Stimulant	**Control**	**ADP**	***p* value**	**Control**	**PAF**	***p* value**
By sex						
Females (n = 23)	2,474 (1,842–3,935)	3,236 (2,267–5,050)	0.02	2,207 (1,508–3,895)	2,730 (1,888–4,093)	0.21
Males (n = 24)	3,123 (1,762–3,643)	3,203 (1,712–4,662)	0.08	2,957 (1,657–3,708)	3,045 (2,173–5,279)	0.04
By Sex and Age						
Premenopausal females (n = 11)	2,363 (1,920–3,881)	3,236 (2,498–3,860)	0.12	2,411 (1,834–3,895)	2,776 (1,706–4,249)	0.70
Postmenopausal females (n = 12)	2,633 (1,483–4,378)	2,853 (1,830–5,065)	0.13	2,192 (1,464–3,884)	2,492 (1,981–3,956)	0.11
Young males (n = 12)	2,876 (1,402–3,256)	2,429 (1,658–5,021)	0.15	2,471 (1,129–3,441)	4,200 (2,614–5,968)	0.002
Older males (n = 12)	3.632 (2.053–3.944)	3,623 (2,074–4,571)	0.30	3,632 (2,053–3,944)	2,490 (1,391–4,921)	0.73

Data presented as median (IQR,v). *p* Value reflective of Wilcoxon signed rank test.

To look at the effect of age and menopause, we stratified males by age and females by menopausal state. We did not identify an effect on platelet aggregation or activation by age or menopausal state (Table 1).

Platelet Activation

Platelets were assessed for activation by evaluating CD41 receptor surface expression. At baseline (unstimulated control platelets), there was no difference in CD41 receptor surface expression in male versus female platelets. Platelets were stimulated with both ADP and PAF. Female platelets had a robust activation in response to ADP and minimal response to PAF, as evidenced by CD41 receptor surface expression (increase from 2,474 [IQR, 1,842–3,935] to 3,236 [IQR, 2,267–5,050] after ADP [p = 0.02] vs 2,207 [IQR, 1,508–3,895] to 2,730 [IQR, 1,888–4,093] after PAF [p = 0.21]) (Table 2, Fig. 2). In contrast, male platelets had minimal activation with ADP stimulation and robust activation with PAF stimulation (CD41 receptor surface expression of 3,123 [IQR, 1,762–3,643] to 3,203 [IQR, 1,712–4,662] with ADP, p = 0.07; 2,957 [IQR, 1,657–3,708] to 3,045 [IQR, 2,173–5,279] with PAF, p = 0.04) (Table 2, Fig. 2).

To evaluate the effect of age and menopause, we again stratified males by age and females by menopausal state. No difference was detected in platelet aggregation or activation potential between premenopausal and postmenopausal females. Upon stratifying males by age, it became evident younger male platelets were the only group to respond with robust platelet activation after PAF stimulation (4,200 [IQR, 2,614–5,968] from 2,471 [IQR, 1,129–3,441]; p = 0.002). This activation was not observed in the older males (2,490 [IQR, 1,391–4,921] from 3,631 [IQR, 2,053–3,944]; p = 0.73) (Table 2).

Platelet Aggregation With Estradiol Pretreatment

In a second set of experiments with 21 consecutive donors, platelets were pretreated with estradiol, and aggregation and activation in response to ADP and PAF were measured, as above. This included four premenopausal females, five postmenopausal females, six younger males, and six older males.

There was no difference in aggregation of pretreated platelets after ADP or PAF stimulation between females and males (Table 3, Fig. 1). Compared with females, males had similar aggregation after ADP stimulation (ESC of 34.9% [IQR, 30.6–37.8] vs 34.7% [IQR, 32.6–40.8] in females) and PAF stimulation (33.5% [IQR, 28.0–38.2] vs 37.6% [IQR, 28.6–42.4] in females) (Table 3, Fig. 1).

Upon stratifying males and females by age and menopausal state respectively, we did not detect an effect by age or menopausal state on platelet aggregation or activation (Table 3).

Platelet Activation With Estradiol Pretreatment

After estradiol pretreatment, the robust activation of female platelets with ADP stimulation was diminished (3,533 [IQR, 2,422–4,682] from 2,641 [IQR, 1,682–3,782]; p = 0.43) (Table 4, Fig. 2). However, estradiol pretreated female platelet activation significantly increased after PAF stimulation (3,231 [IQR, 1,832–4,430] vs. 4,581 [IQR, 3,455–6,447]; p = 0.004) (Table 4, Fig. 2). Similarly, in male platelets pretreated with

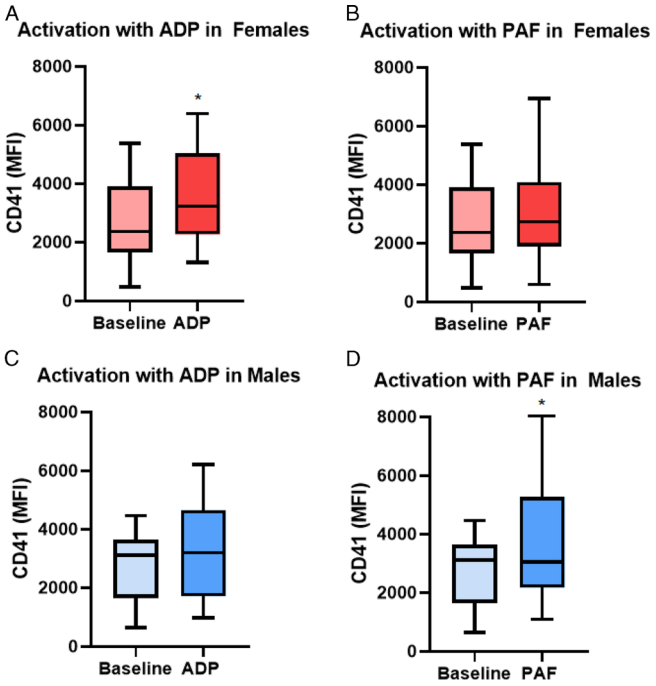

Figure 2. Activation, as assessed by CD41 receptor surface expression (in mean fluorescence intensity [MFI]), in females (red shades) and in males (blue shades) in platelets (*A*) and estradiol pretreated platelets (*B*) after ADP stimulation and PAF simulation. Asterisks indicate $p < 0.05$ as compared with baseline.

TABLE 3. Aggregation of Native and Estradiol Pretreated Platelets After ADP or PAF Stimulation by sex

	ESC (%)					
Stimulant ± Estradiol	**ADP (Untreated)**	**ADP (Estradiol)**	***p* value**	**PAF (Untreated)**	**PAF (Estradiol)**	***p* value**
By sex						
Females (n = 9)	35.6 (34.9–39.6)	34.7 (32.6–40.8)	0.30	37.0 (30.6–40.3)	37.6 (28.6–42.4)	0.82
Males (n = 12)	35.8 (32.7–39.4)	34.9 (30.6–37.8)	0.42	34.0 (29.9–40.2)	33.5 (28.0–38.2)	0.13
By sex and age						
Premenopausal females (n = 4)	35.6 (31.4–38.8)	35.0 (30.8–38.6)	0.12	33.2 (28.2–37.0)	32.8 (22.8–38.5)	0.89
Postmenopausal females (n = 5)	35.9 (34.9–40.0)	33.7 (32.6–42.4)	0.62	39.6 (35.2–41.6)	41.1 (33.4–44.7)	0.31
Young males (n = 6)	35.0 (31.0–39.4)	34.0 (29.9–36.2)	0.44	31.2 (30.2–40.8)	31.7 (29.0–37.8)	0.16
Older males (n = 6)	37.0 (30.8–40.1)	37.2 (28.8–39.2)	0.84	37.2 (23.2–40.0)	36.4 (22.5–38.6)	0.56

Data presented as median (IQR, 25–75). *p* Value reflective of Wilcoxon signed rank test.

estradiol, activation did not change with ADP stimulation (2,469 [IQR, 1,828–4,185] from 3,641 [IQR, 1,677–4,274]; $p = 0.99$), but significantly increased with PAF stimulation as compared to untreated platelets (2,490 [IQR, 1,391–4,921] vs. 3,872 [IQR, 2,195–5,691]; $p = 0.01$) (Table 4, Fig. 2).

Upon stratifying by age and menopausal state in males and females respectively, there were no differences in activation of female platelets by menopausal state with ADP or PAF stimulation. Younger male platelets treated with estradiol had a robust activation with PAF stimulation compared with platelets without estradiol pretreatment (3,034 [IQR, 2,000–4,223] vs. 4,544 [IQR, 3,322–6,320], $p = 0.03$), whereas estradiol pretreatment did not affect platelet activation with PAF in older male platelets (Table 4).

DISCUSSION

This investigation characterizes sex dimorphisms in platelet function, specifically platelet aggregation (as reflected by ESC) and activation (as reflected by fibrinogen receptor surface expression). The results indicate that female and male platelets have sex-specific aggregation and activation potentials. Females had increased platelet aggregation with ADP stimulation as compared with males. Female platelets had robust activation with ADP stimulation, in contrast to male platelets which had increased activation with PAF stimulation, suggesting sex-dependent activation and receptor responses. Estradiol treatment effectively "feminized" the male platelet response to stimulation by PAF, enhancing activation (CD41 receptor surface expression) (schematic for visual representation, Fig. 3).

This differential aggregation of platelets may be related to sex hormones, as suggested by both estrogen and testosterone receptors on megakaryocytes and platelets.[12] The topic of sex dimorphisms in platelet activity has been an area of controversy in the cardiovascular literature, with some studies indicating increased aggregation in females,[20,21] while others describe the opposite.[22] Part of this discrepancy may be explained by the stimuli used, as the results of our investigation indicate that platelets behave differently by sex depending on the stimulating agent. In a study of platelet aggregation over the menstrual cycle in 16 healthy women, there was significant variation in the level of aggregation based on the agonist alone, from ADP to arachidonic acid to thrombin-receptor activating peptide (TRAP), regardless of the stage of menstrual cycle.[23] This suggests that circulating sex hormone levels alone do not explain the differential platelet responses, rather type of stimulation, in conjunction with hormones.[23] In a study of 32 women on estrogen-based hormone replacement therapy, investigators found stimulation of female platelets with ADP, but not thrombin, caused increased intracellular calcium levels.[24] Our results agree with previous reports of similar platelet aggregation between premenopausal and postmenopausal females and increased platelet aggregation in females versus males.[20,21,25] Part of the disagreement in the

TABLE 4. Activation, as Assessed by CD41 Receptor Surface Expression, in Native and Estradiol-Incubated Platelets After ADP or PAF Stimulation by Sex

	CD41 Receptor Surface Expression (MFI)					
Stimulant ± Estradiol	**ADP (Untreated)**	**ADP (Estradiol)**	***p* value**	**PAF (Untreated)**	**PAF (Estradiol)**	***p* value**
By sex						
Females (n = 9)	3,860 (2,842–5,385)	3,533 (2,422–4,682)	0.25	3,231 (1,832–4,430)	45,81 (3,455–6,447)	0.004
Males (n = 12)	3,623 (2,074–4,571)	2,469 (1828–4,185)	0.52	2,490 (1,391–4,921)	3,872 (2,195–5,691)	0.009
By sex and age						
Premenopausal females (n = 4)	4,585 (3,392–5,435)	3,946 (3,243–4,615)	0.25	2,355 (1,266–4,251)	4,959 (3,110–6,784)	0.12
Postmenopausal females (n = 5)	3,644 (1,866–5,267)	2,786 (1,712–5,163)	0.81	3,452 (2,472–5,531)	4,581 (3,111–6,080)	0.06
Young males (n = 6)	3,870 (1,848–4,882)	2,088 (1,680–5,147)	0.99	2,490 (1,233–5,863)	4,544 (3,322–6,320)	0.03
Older males (n = 6)	3,203 (1,949–4,536)	2,977 (1,870–3,985)	0.44	2,434 (1,635–4,349)	2,443 (2,038–5,434)	0.31

Data presented as median (IQR, 25–75). *p* Value reflective of Wilcoxon signed rank test.

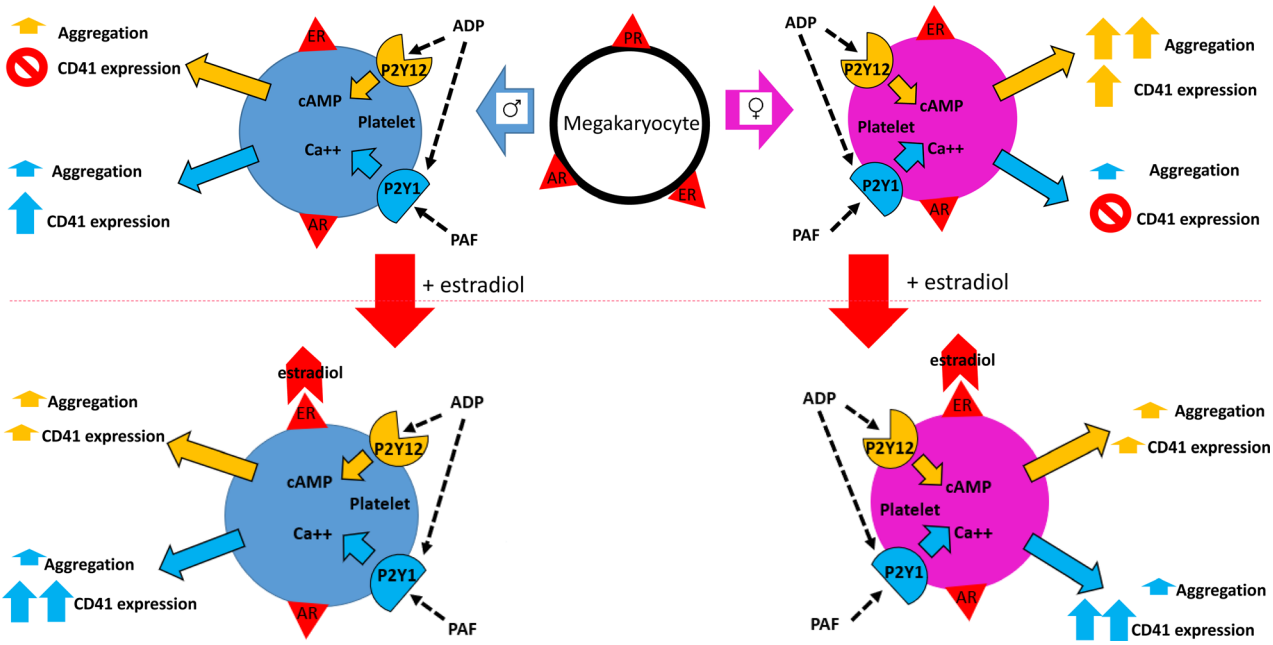

Figure 3. Sex dimorphisms in platelet aggregation and activation potentials and feminization of the male platelet. ER, estrogen receptor; AR, androgen receptor; PR, progesterone receptor, Ca^{++}, calcium.

literature likely lies in the complexity of estradiol's effects, from genomic to nongenomic, the latter which is of particular interest in anucleate platelets.

In this study, female platelets had increased activation with ADP, whereas male platelets had markedly increased activation with PAF. This differential behavior is likely due to ADP and PAF acting on different receptors and downstream intracellular cascades.[14] Platelet-activating factor, released from endothelial cells, platelets, and other cellular players, stimulates the P2Y1 receptor, a G_q-coupled receptor, which leads to an increase in intracellular calcium levels through PIP2/IP3 signaling.[26] The consequent increase in intracellular calcium potentiates shape change and platelet aggregation. In contrast, ADP, released from the dense granules of platelets, stimulates both the P2Y1 receptor and the P2Y12 receptor, the latter of which is a G_i-coupled receptor related to modulation of intracellular cyclic adenosine monophosphate (cAMP) levels.[27,28] Activation of the P2Y12 receptor results in repressing baseline tonic inhibition, causing a decrease in cAMP levels and a cascade leading to thromboxane A_2 production, alpha and dense granule release, expression of P-selection, cross-linking of fibrin, and platelet aggregation. Differential activation of these distinct pathways in male and female platelets may be the reason increased aggregation was observed in the female platelets with ADP stimulation, whereas there was no sex-difference with the less robust platelet stimulation of PAF. While ADP stimulation of P2Y12 is well established, it is less well-understood about the effects of ADP on other similarly structured class P2Y receptors, including P2YT, which has been shown to be required for full aggregation potentiation and may be involved in the differential effects observed between males and females.[28] Ultimately, the differential response to ADP and PAF in male and female platelets in this study imply the activation and intracellular signaling of the P2Y1 and P2Y12 are complex and sex-specific. This is also suggested by the literature describing a distinct response to clopidogrel (a P2Y12 receptor inhibitor) by sex, such that females have higher resistance to clopidogrel compared with males.[29]

Characterization of sex dimorphisms in receptor biology and the roles of sex hormones is essential to understanding the differential performance of severely injured male and female patients in TIC. Recently, our group observed that following severe injury, females tolerate depressed clot strength, an effect of platelet and fibrinogen interactions, better than males, conferring a survival benefit for females following trauma.[5] This differential performance of platelets may be related to distinct responses to ADP and PAF and P2Y receptor signaling. In addition to the platelets themselves, the downstream players from platelet signaling, specifically fibrinogen, may also play a role in the sex-specific performance in TIC and be affected by sex hormones. In a multicenter study of severely injured patients admitted to the intensive care unit, estradiol was positively correlated with rate of fibrin deposition and cross-linking and overall clot strength in both men and women, effects which may be due to augmentation of P2Y1- and P2Y12-stimulated pathways.[30] These works underline the critical importance of evaluating sex as a variable in biologic response, clinical research and basic scientific mechanisms. Ultimately, our therapies directed at resuscitation and attenuation of TIC and postinjury inflammation may best be achieved by differential approaches to sex-specific cellular capacities in male and female trauma patients.

Estradiol incubation feminized male platelet activation with PAF stimulation. These findings suggest estrogen and the estradiol receptor, known to be present and active on the surface of both male and female platelet membranes, have an important role in platelet behavior.[12] Upon activation, the platelet estradiol receptor ERβ can provoke several intracellular cascades, including involving the PIP2/IP3 signaling pathway, which is the same cascade downstream of P2Y1.[31] Therefore, estradiol may

augment the PAF-initiated calcium signaling that ultimately causes increased platelet activation through convergence on and augmentation of the PIP2/IP3 signaling. The reported experiments demonstrate, however, that the signaling pathways are complex (unlikely related to single signaling cascade point) and sex-specific. In this investigation, estradiol pretreatment of female platelets did not change the activation potential with ADP, perhaps because there is a limitation to the additive effects of the P2Y and estrogen receptor beta (ERβ) receptors or due to the chronicity of estradiol exposure in females at baseline. Literature has described a lack of responsiveness (and increased bleeding time) to *ex vivo* estradiol in platelets chronically exposed to the female sex hormones.[32] In addition, the circulating estradiol in female donors may be relevant. Specifically, there may be a dose response effect with estradiol, such that circulating levels and/or receptor number and type dictate the extent of profibrinogen binding effect of estrogen (whether through changes in levels of fibrinogen receptor, threshold of activation, extent of downstream signaling or augmentation of the fibrinogen receptor effect through amplification of the P2Y receptors). This dose-response effect is suggested in that platelets in premenopausal females are known to have differential fibrinogen receptor activation over the menstrual cycle, glycoprotein IIb-IIIa activation increase during the luteal phase as compared with the follicular phase, the biphasic periodicity of platelet adhesion to type I collagen over the menstrual cycle, and increased fibrinogen contribution to clot strength with increasing doses of in vitro fertilization sex hormones.[11,33,34]

This investigation of platelet response to female sex hormones (estradiol) is limited in that we have not similarly evaluated for a sex-specific platelet response to androgens. In a similar investigation focused on the effects of sex hormones, Banerjee et al.[35] added supraphysiologic levels of testosterone to platelets of healthy male and female donors and found that testosterone increased male ADP-induced platelet aggregation and nitric oxide synthase and thromboxane A_2 production. However, testosterone had no effect on female platelets. Taken together, findings from our current investigation and those from Banerjee et al. support the concept that sex hormones have differential effects on platelets and suggest that balance of hormonal stimuli should be evaluated.

The feminization of male platelet activation with estradiol highlights a potential role for therapeutic hormone receptor targets and consideration of estradiol therapy in males with TIC and hemorrhagic shock. Administration of estradiol has been linked to abrogation of hemorrhagic shock in female murine models[35–38] and in a study of murine hemorrhagic shock in males, estradiol treatment was associated with improved cardiovascular performance and hepatocellular function.[39] The concept of sex hormones as a therapeutic adjunct in humans has been described in other specialties, including orthotopic liver transplantation in which conjugated estrogen has been shown to reduce transfusion requirements, and in neurotrauma, in which progesterone was attempted as a therapeutic adjunct in traumatic brain injury.[40,41] The effects of estradiol on coagulation require further evaluation in the *in vivo* setting to establish efficacy, but the differential platelet receptor performance in males versus females offers novel hypotheses for future mechanistic and clinical investigations. In addition to future resuscitation considerations, these results suggest that current transfusion practices might be enhanced to exploit the differential performance of female platelets. The sex of donor platelets may need to be considered, and preferentially selected for, in blood component resuscitation of TIC after severe injury.

Limitations of our work include a lack of granularity in assessment of oral hormonal therapy and biochemical confirmation of menopausal state. Future investigations could be enhanced by precise documentation of oral contraception or hormone replacement therapy, as well as consideration of biochemical confirmation of estradiol levels. This investigation focused on a singular sex hormone (estradiol) for treatment of platelets. Future investigations to fully characterize sex hormone response of platelets should include expanded sex hormone stimulation with multiple estrogen and androgen players (testosterone, progesterone, estradiol, dihydroepiandrosterone). While our study was adequately powered for a comparison of platelets from males versus females, the smaller sample size to compare the response of platelets from males versus females after estradiol pretreatment may be underpowered and will be further examined in a larger sample size. Lastly, given our platelets were received from the donor center, we were unable to assess basic demographic factors such as race, which is known to have a relationship with platelet biology. A larger sample size and inclusion of demographic data will be included in future prospective studies.

In sum, male and female platelets have sex-specific aggregation and activation potentials and responses to stimuli, which can be augmented with estrogen pretreatment and feminize male platelet activation response. These data offer a potential explanation and generate novel hypotheses for sex-based differential performance of platelets in TIC, suggesting that cellular and sex hormone biology impart thrombotic potential and may contribute to patient outcomes following severe injury. Sex dimorphisms in receptor function and platelet behavior offer potential therapeutic targets and ultimately question whether donor sex of transfused platelets should be considered in blood component resuscitation strategies. Future experiments are required to delineate the dose-response of estradiol effects, any potential role of testosterone given the concomitant presence of androgen receptors on platelets, biochemical confirmation of hormonal state in females alongside platelet function testing, and establish normal ranges for ADP- and PAF-induced platelet responsiveness by sex of donor. Estradiol may also serve as a novel therapeutic adjunct in platelet dysfunction and requires additional rigorous *in vitro* and animal model investigation.

AUTHORSHIP

J.R.C., C.C.S., and E.P. originated experimental hypothesis, developed experimental design, performed data interpretation, and composed the article. J.R.C. also performed the platelet experiments and statistical analysis. E.E.M., A.S., M.J.C., and A.B. assisted with experimental conduct, data interpretation, and article editing. M.R.K. performed the platelet experiments with J.R.C. and assisted in data organization and interpretation, as well as article editing. J.M.S. was responsible for data interpretation and article editing.

ACKNOWLEDGMENT

Research reported in this publication was supported by the National Institute of General Medical Sciences of the National Institutes of Health (T32 GM008315 and P50 GM049222) and Department of Defense (USAMRAA, W81XWH-12-2-0028), as well as the Foundation for Women and Girls with Bleeding Disorders. The content is solely the responsibility of the authors

and does not necessarily represent the official views of the National Institutes of Health or other sponsors of the project.

DISCLOSURE

There are no conflicts of interest to report.

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COMMENTARY

Coleman et al. recently demonstrated that severely injured females have a more hypercoagulable profile than males.[1] To further investigate this finding, the authors now examined sex-specific hypercoagulabilty as it relates specifically to platelet function.[2] Using donor plasma from healthy male and pre-and postmenopausal female donors, they demonstrated a differential response of platelets to known platelet activators. Female platelets had increased aggregation and activation potential, regardless of menopausal status. Interestingly, they also demonstrated that estradiol pre-treatment feminized male platelets to approximate the response of female platelets to stimulation. This raises an interesting question and implication for precision medicine, should female platelets be preferentially transfused to injured males with trauma-induced coagulopathy (TIC)? Although not likely feasible at the current time with the known, albeit low, risk of transfusion related acute lung injury (TRALI) with administration of female platelets, as blood products become safer this practice may become a viable option. Results of the current study also suggest a potential role for estradiol therapy in males with TIC. This builds upon the preclinical work of Chaudry et al demonstrating that estradiol may prolong survival following hemorrhagic shock and suggests estradiol as a novel therapeutic adjunct.[3] Like most good studies, the work by Coleman et al raises questions and paves the way for future investigations. Should female platelets be considered for use in TIC? I say, go girl.

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