Premenopausal Women Exhibit an Inherent Protection of Endothelial Function Following a High-Fat Meal

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Abstract

Endogenous estrogens likely increase blood flow and subsequently shear stress but have also been associated with improved endothelial function and cardiovascular protection. In contrast, a high-fat meal is thought to reduce endothelial function and increase cardiovascular risk. Therefore, we tested the hypotheses that fluctuating hormones across the menstrual cycle (1) facilitate an increase in shear rate and explain phase-specific differences in flow-mediated dilation (FMD) and (2) provide vascular protection against the insult of a high-fat meal. Flow-mediated dilation was determined at baseline and 4 hours following a high-fat meal in young women during the menses (M), follicular (F), and luteal (L) phases of the menstrual cycle. Male control participants were studied once. 17 β -Estradiol was elevated ($P < .05$) during the F (5.3 ± 0.7 pg/mL) and L (5.2 ± 0.6 pg/mL) phases when compared to the M (3.9 ± 0.5 pg/mL) phase, and this was accompanied by an elevated FMD in the F and L phases ($12.4 \pm 1.4\%$ and $11.2 \pm 0.9\%$, respectively) compared to M (8.0 ± 0.9) with no change in shear rate. Female postprandial FMD was similar throughout the menstrual cycle, while men exhibited a 50% reduction (6.4 ± 1 to $3.3 \pm 1\%$; $P < .05$). Interestingly, the postprandial FMD response was not associated with concentrations of either 17 β -estradiol or progesterone. Despite acutely changing ovarian hormones across the menstrual cycle, shear stress is invariant and therefore does not account for the changes in FMD. Additionally, young women appear to have an inherent vascular protection from the insult of a high-fat meal, perhaps helping to explain sex-specific differences in cardiovascular risk.

Keywords

flow-mediated dilation, ovarian hormones, shear, postprandial

Introduction

Interestingly, the total incidence of morbidity and mortality in men is twice that of women until menopause.¹ This sex-linked difference in cardiovascular disease prevalence is likely due to different cardiovascular risk factors across the life span but may also be linked to ovarian hormone concentrations that are reduced at menopause.² Following menopause, this protective phenomenon is lost.

Endothelial dysfunction, an early manifestation of atherosclerotic cardiovascular disease, has been identified in many clinical populations,³⁻⁵ including postmenopausal women.⁶ Brachial artery flow-mediated dilation (FMD), a reliable noninvasive method to assess endothelium-dependent vasodilation,⁷ has been established as an independent predictor of future cardiovascular events.⁸ A pivotal investigation conducted by Hashimoto and colleagues⁹ revealed a distinct variation in FMD throughout the menstrual cycle, which coincided with the fluctuating concentrations of endogenous estradiol. These data support a beneficial effect of estrogen on vascular function; however, based on the tight link between shear forces and FMD, it is reasonable to suspect that the increases in blood flow and

corresponding shear stress throughout the menstrual cycle may have influenced the previously documented fluctuations in FMD.^{9,10}

There is convincing evidence that estrogens assist in the reduction of oxidative stress, in part, by restoring antioxidant capacity and increasing nitric oxide (NO) production and bioavailability.^{11,12} Conversely, a high-fat meal has been documented to attenuate vascular endothelial function¹³ through an

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increase in lipemia-induced oxidative stress.¹⁴ As a significant proportion of life is spent in the postprandial state, if estrogen-induced vascular protection does exist, this may play a key role in the recognized, but not well understood, sex-specific differences in atherosclerotic disease development.

Endothelial dysfunction is a strong, independent, risk factor for future cardiovascular disease and mortality,⁸ but the link between ovarian hormones and FMD is not well understood. Therefore, this study sought to test the hypotheses that fluctuating hormones across the menstrual cycle (1) facilitate an increase in shear rate and explain phase-specific differences in FMD and (2) provide vascular protection against the insult of a high-fat meal.

Methods

Study Population

A total of 25 healthy, nonsmoking, normally active, young men ($n = 10$) and women ($n = 15$) with no family history of cardiovascular disease participated in this study. All women reported having a regular menstrual cycle, no history of pregnancy, and never having used any type of hormonal birth control. Basal body temperature was measured for 3 months prior to the start of the study to ensure an accurate characterization of the menstrual cycle. Participants provided written and verbal consent prior to participation in the study and all procedures were approved by the University of California, San Diego Human Subject's Protection Program.

Experimental Design

In a repeated measures design, FMD was performed at baseline and 4 hours following the ingestion of a high-fat meal. Women were studied on days 5 ± 1 , 13 ± 1 , and 21 ± 1 of a single menstrual cycle to correspond with the menses (M), follicular (F), and luteal (L) phases, respectively. Men participated as control participants and were studied only once. On each experimental day, participants reported to the laboratory following an overnight fast, having abstained from exercise and caffeine for 24 hours, and vitamin supplementation for 72 hours prior to the investigation. Upon arrival, all participants provided a saliva sample, achieved by the passive drool technique, to confirm the concentration of 17β -estradiol and progesterone.

Forearm Muscle Mass

Forearm circumferences, length, and ventral skin folds were taken to calculate limb and muscle volume as described previously.^{15,16}

Biochemical Analysis of 17β -Estradiol and Progesterone

Both 17β -estradiol and progesterone were detected in saliva using a highly sensitive competitive binding immunoassay according to the manufacturer's specifications (Salimetrics,

State College, Pennsylvania). Briefly, the bound estradiol and progesterone peroxidase was detected spectrophotometrically at 450 nm and was inversely proportional to the amount of free estradiol and progesterone present in the saliva sample. To assess the validity of salivary estradiol and progesterone concentrations, both the ovarian hormones were measured in a subsample of saliva and serum (total samples = 11) that was taken from both men and women. In the women, all phases of the menstrual cycle were validated. In addition to relationships between the 2 assessments, a Bland-Altman analysis for agreement (± 2 standard deviation) was used to compare the 2 specimens.

Flow-Mediated Dilation

Brachial artery FMD was performed in accordance with the recent tutorial on the Ultrasound Assessment of FMD¹⁷ at the baseline and 4 hours following the ingestion of a high-fat meal. Briefly, simultaneous B-mode and blood velocity profiles (duplex mode) of the brachial artery were obtained. FMD was expressed as a percentage increase in diameter from baseline. Although the investigator performing the FMD assessments was not blinded to either the phase of the menstrual cycle or prandial state of the participants, all data were randomly coded and stored for offline analysis that occurred at least 4 weeks following completion of each participant. This method ensured the investigator an unbiased approach to the automated edge detection analysis.

Shear Rate

Shear rate (s^{-1}) was calculated from the simultaneous measurements of blood velocity and brachial artery diameter, according to the following equation:

$$\text{Shear rate (s}^{-1}\text{)} = \frac{4 \times \text{mean blood velocity (cm/s)}}{\text{diameter (cm)}}$$

Cumulative shear rate (shear rate area under the curve [AUC]) was determined using the trapezoidal rule, every 4 seconds for the first 20 seconds following cuff release and every 10 seconds thereafter for the remainder of the 2 minute data collection period, as described previously.¹⁸ To normalize FMD for the vasodilatory stimulus (cumulative shear rate AUC, $s^{-1} \cdot s$), FMD was divided by the shear rate AUC defined by the attainment of peak dilation.¹⁹

High-Fat Meal

Following the baseline assessment of FMD, participants consumed a high-fat meal consisting of an Egg McMuffin, a Sausage McMuffin, and 2 hash brown patties (McDonald's Corporation, La Jolla, CA). Caloric composition of 48 g fat, 16.5 g saturated fat, 4.5 g trans fat, 280 mg cholesterol, 91 g carbohydrates, 33 g protein, and 2220 mg sodium for a total of 940 calories (3.93 kJ) was obtained from McDonalds food fact sheets. This high-fat meal has previously been used by our

group and others to induce vascular endothelial dysfunction.^{13,20} Water was provided ad libitum but was not measured. Due to dietary limitations and/or religious restrictions, only 10 of the 15 women participated in the high-fat meal portion of the study.

Statistical Analysis

Descriptive statistics and independent *t* tests were performed to identify differences in participant characteristics between men and women. A repeated measures 1-way analysis of variance (ANOVA) was performed to identify differences in baseline diameter, peak diameter, absolute change in diameter, FMD, shear rate, FMD/shear, and time to peak dilation in women across the menstrual cycle. Additionally, hypothesis-driven planned comparisons of the same variables were performed between men and women during menses. To identify the effect of the high-fat meal on baseline diameter, peak diameter, absolute change in diameter, FMD, shear rate, FMD/shear, and time to peak dilation, a multifactorial 3 × 2 ANOVA (menstrual cycle × premeal/postmeal) was performed. When indicated by a significant *F*-ratio, Tukey post hoc comparisons were performed to identify differences. Regression analyses were performed to identify the relationships between ovarian sex hormones (17β-estradiol and progesterone) and the change in FMD following the high-fat meal. All statistics were performed using commercially available software (SPSS Inc, Chicago, Illinois: version 15.0). All data were analyzed for normality and are reported as mean ± standard error of the mean. Significance was set at *P* < .05.

Results

Participant characteristics and blood chemistry are presented in Table 1. There were differences in age (*P* = .001), height (*P* < .001), systolic blood pressure ([SBP] *P* = .023), hematocrit (*P* = .002), and hemoglobin (*P* = .001) between men and women; however, all values are within normal healthy respective ranges. Additionally, men had greater (*P* < .001) forearm muscle mass than women. More importantly, no differences (*P* > .05) in body mass index or baseline blood lipids were observed between the 2 groups.

17β-Estradiol and Progesterone

In women, average serum concentrations of estradiol (107 ± 25, 226 ± 46, and 371 ± 223 pg/mL) and progesterone (1.0 ± 0.3, 4.9 ± 4.0, and 7.6 ± 3.0 pg/mL) for the M, F, and L phases, respectively, were within reference limits. The Bland-Altman analysis indicates that 95% limits of agreement between the 2 specimens (blood and saliva) consistently provide similar measures. Additionally, the validation relationships between blood and saliva concentrations of estradiol and progesterone were *r* = .77, *P* = .006 and *r* = .82, *P* = .002, respectively. Salivary 17β-estradiol and progesterone detected in women during the M, F, and L phases and in men are illustrated in Figure 1. Estradiol data from 3 participants were

Table 1. Participant Characteristics and Blood Chemistry^a

N	Women 15	Men 10
Age (year)	20 ± 0.5	23 ± 0.9 ^b
Height (cm)	163 ± 1.3	177 ± 2 ^b
Weight (kg)	64 ± 4	73 ± 3
BMI (kg/m ²)	24 ± 1	23 ± 1
SBP (mm Hg)	105 ± 3	113 ± 2 ^b
DBP (mm Hg)	70 ± 3	68 ± 2
Average cycle length (day)	30 ± 1	—
Testing day during menses	5 ± 0.3	—
Testing day during follicular	13 ± 0.3	—
Testing day during luteal	22 ± 0.4	—
Forearm muscle mass (kg)	0.62 ± 0.02	0.96 ± 0.05 ^b
Blood chemistry		
TC (mg/dL)	162 ± 8	160 ± 9
HDL (mg/dL)	57 ± 3	53 ± 3
LDL (mg/dL)	88 ± 8	93 ± 7
Triglycerides (mg/dL)	87 ± 6	74 ± 7
TC: HDL	2.9 ± 0.2	3.1 ± 0.2
Hematocrit (%)	39 ± 1	44 ± 1 ^b
Hemoglobin (g/dL)	13 ± 0.3	15 ± 0.2 ^b

Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

^a Values are mean ± standard error of the mean.

^b Significant (*P* < .05) from women.

Figure 1. Concentrations of 17β-estradiol and progesterone in men and women throughout the menstrual cycle. a indicates significantly different (*P* < .05) from menses; b, significantly different (*P* < .05) from M and L phases.

unable to be analyzed (total *n* = 12) due to inadequate saliva samples. Ovarian sex steroid hormone concentrations were appropriate for each phase of the menstrual cycle (Figure 1). Estradiol was significantly elevated ($F_{2,22} = 4.57$, *P* = .022) during the F and L phases compared with the M phase. Additionally, progesterone was significantly elevated ($F_{2,28} = 11.28$, *P* < .001) during the L phase compared to both M and F phases. There was no significant difference (*P* > .05) in either estradiol or progesterone concentrations between men and women during the M phase.

Figure 2. Flow-mediated dilation (FMD), shear, and FMD/shear in men and women throughout the menstrual cycle. a indicates significantly different ($P < .05$) from all phases; b, significantly different ($P < .05$) from menses and men; c, significantly different ($P < .05$) from menses.

Vascular Endothelial Function

Vascular endothelial function in women varied throughout the menstrual cycle (Figure 2). Parameters of the FMD test are presented in Table 2. Men exhibited a greater ($P < .001$) baseline diameter (cm) and peak diameter (cm) than women during the M phase. In addition, the absolute change in brachial artery

diameter in women was significantly greater ($F_{2,28} = 3.54$, $P = .043$) during the F and L phases compared to the M phase. The time to peak vasodilation was greater ($F_{2,28} = 3.73$, $P = .037$) during the L phase compared to the M phase. Figure 2 illustrates FMD (Figure 2A), cumulative shear rate (Figure 2B), and FMD normalized for shear rate (Figure 2C) in women throughout the menstrual cycle and men on a single visit. Although FMD was significantly elevated ($F_{2,28} = 7.28$, $P = .003$) in women during the F and L phase compared to M, FMD was similar ($P = .248$) between men and women during M (Figure 2A). The baseline shear rates were similar ($P = .149$) among all phases of the menstrual cycle in women (M: $118 \pm 9 \text{ s}^{-1}$, F: $131 \pm 15 \text{ s}^{-1}$, and L: $148 \pm 17 \text{ s}^{-1}$) and in men ($100 \pm 14 \text{ s}^{-1}$). Furthermore, no differences ($P = .172$) in cumulative shear rate ($\text{s}^{-1} \cdot \text{s}$, AUC) were observed between the M, F, and L phases; however, men experienced approximately 35% less shear ($P = .016$) in response to reactive hyperemia than women in the M phase (Figure 2B). When FMD was normalized for shear (FMD/shear), endothelial function in women during the F phase was still greater ($F_{2,28} = 6.77$, $P = .004$) than the M phase (Figure 2C).

Endothelial Function Following a High-Fat Meal

No differences in baseline diameter ($P = .148$) were observed following the ingestion of a high-fat meal in either men or women (Table 3). Figure 3 illustrates vascular function prior to and 4 hours following a high-fat meal in men and women throughout the menstrual cycle. No differences ($P > .05$) were observed between premeal and postmeal FMD during any phase of the menstrual cycle in women; however, men exhibited a significant ($F_{1,36} = 6.83$, $P = .013$) reduction in FMD following the ingestion of the high-fat meal (Figure 3A). Additionally, there were no differences ($P = .256$) in shear rate in either men or women in response to the high-fat meal (Figure 3B). Moreover, when FMD was normalized for the shear rate stimulus (Figure 3C), the interpretation of the more traditional percentage FMD findings was not altered.

Relationships Between 17 β -Estradiol, Progesterone, and Change in FMD Following a High-Fat Meal

Figure 4 illustrates the collective relationship (both men and women) between change in FMD following a high-fat meal (delta FMD percentage) and both 17 β -estradiol (Figure 4A) and progesterone (Figure 4B). Specifically, no relationships were observed between delta FMD (%) and 17 β -estradiol concentrations (Figure 4A) in men ($r = -.08$, $r = .819$) or women during the M ($r = .04$, $P = .920$), F ($r = .191$, $P = .598$), and L phases ($r = .135$, $P = .710$). Moreover, no relationships were observed between delta FMD (%) and progesterone concentrations (Figure 4B) in men ($r = -.47$, $P = .176$) or women during the M ($r = .10$, $P = .781$), F ($r = .42$, $P = .228$), and L phases ($r = .42$, $P = .223$).

Table 2. Endothelial Function Parameters in Men and Women Throughout the Menstrual Cycle^a

Variable	Men (n = 10)	Women (n = 15)		
		Menses	Follicular	Luteal
Baseline diameter (cm)	0.361 ± 0.012	0.278 ± 0.008 ^b	0.275 ± 0.009	0.281 ± 0.008
Peak diameter (cm)	0.384 ± 0.012	0.301 ± 0.009 ^b	0.308 ± 0.009	0.312 ± 0.008
Absolute change (cm)	0.023 ± 0.004	0.022 ± 0.002	0.033 ± 0.003 ^c	0.032 ± 0.002 ^c
Time to peak vasodilation (s)	46 ± 6	45 ± 5	51 ± 3	57 ± 3 ^c

^a Values are presented as mean ± standard error of the mean.

^b Significantly different ($P < .05$) from men.

^c Significantly different ($P < .05$) from menses.

Table 3. Endothelial Function Parameters 4 hours Following the Ingestion of a High-Fat Meal in Men and Women Throughout the Menstrual Cycle^a

Variable	Men (n = 10)		Women (n = 10)					
			Menses		Follicular		Luteal	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Baseline diameter (cm)	0.361 ± 0.012 ^b	0.373 ± 0.011 ^b	0.280 ± 0.011	0.276 ± 0.009	0.278 ± 0.012	0.282 ± 0.011	0.281 ± 0.011	0.283 ± 0.011
Peak diameter (cm)	0.384 ± 0.012 ^b	0.385 ± 0.012 ^b	0.303 ± 0.011	0.300 ± 0.009	0.313 ± 0.010	0.312 ± 0.011	0.312 ± 0.011	0.311 ± 0.009
Absolute change (cm)	0.023 ± 0.004	0.012 ± 0.004 ^c	0.036 ± 0.012	0.024 ± 0.002	0.035 ± 0.004	0.030 ± 0.003	0.031 ± 0.003	0.028 ± 0.003
Time to peak vasodilation (s)	46 ± 6	55 ± 5	47 ± 5	45 ± 3	50 ± 4	54 ± 5	58 ± 4	54 ± 3

^a Values are presented as mean ± standard error of the mean. Data include only the women who participated in the high-fat meal (HFM) part of the study (n = 10).

^b Significant ($P < .05$) main effect from women (collapsing for menstrual cycle phase) for both pre and post.

^c Significant ($P < .05$) from pre.

Discussion

Based on the limited understanding between ovarian hormones and vascular function, this study sought to determine whether fluctuating hormones across the menstrual cycle (1) facilitate an increase in shear rate and explain phase-specific differences in FMD and (2) provide vascular protection against the insult of a high-fat meal. It was determined that shear rate, the proposed stimulus for the FMD response, was unaffected by the change in ovarian hormones and, therefore, does not appear to be responsible for the varying FMD response throughout the menstrual cycle. Additionally, and perhaps, the most striking finding of this study was that young premenopausal women, independent of menstrual cycle phase, were protected against the insult of a high-fat meal whereas men were not.

Flow-Mediated Dilatation, Menstrual Phase, and Shear Rate

A primary methodological objective of the present study was to ensure that the measurements were made during the 3 distinct phases of the menstrual cycle, which are characterized by varied ovarian hormone concentrations. This was confirmed by the biochemical analysis of both 17 β -estradiol and progesterone, documenting that the studies occurred during M phase (low estradiol and progesterone), F phase (elevated estradiol

and low progesterone), and L phase (elevated estradiol and progesterone; Figure 1). As reported previously,^{9,10,21} we also observed fluctuations in FMD that not only coincided with the varied concentrations of 17 β -estradiol throughout the menstrual cycle (Figure 2) but may also be related to the estrogen-mediated fluctuations in plasma NO concentrations.²¹ It is noteworthy that although a statistical difference in age and SBP was observed between men and women (Table 1), there was no relationship between age and FMD ($r = -.056$, $P = .792$) or SBP and FMD ($r = .028$, $P = .894$), which is likely due to the narrow range of ages and blood pressures. Hence, these differences are likely physiologically insignificant and should not influence the strength of our findings. A novel aspect of the present study is the observation of the ovarian hormone/shear force interaction during an FMD test throughout different phases of the menstrual cycle. Following up on this gap in the literature, in contrast to our original hypothesis, shear rate was unaffected by changes in ovarian hormones and, therefore, is unlikely to be responsible for the fluctuations in FMD across the menstrual cycle. These findings are particularly important because they are, to our knowledge, the first to document the impact of both menstrual cycle phase and ovarian hormones on FMD assessment and reveal that this is independent of changes in shear rate.

Shear stress is thought to play a fundamental role in mediating vasodilation following forearm occlusion-induced reactive hyperemia.²² Accordingly, women typically have smaller

Figure 3. Postprandial effect on flow-mediated dilation (FMD), shear, and FMD/shear in men and women throughout the menstrual cycle. a indicates significantly different ($P < .05$) from pre meal. Note: differences between sex and phase are presented in Figure 2.

arteries than men which, with all else held constant, would lead to greater shear. Thus, although the male participants had greater forearm muscle mass (Table 1), which as expected resulted in a greater reactive hyperemia, the shear stimulus following cuff release was actually greater in women compared with men. Consequently, controlling for baseline diameter did not influence the overall results (Figure 3C).

Figure 4. Relationship between the postprandial change in flow-mediated dilation ([FMD] delta FMD) and ovarian hormone concentrations.

Ovarian Hormones and Postprandial FMD

Concentrations of estrogen and progesterone are cyclical in nature and appear to have vasodilatory properties that are both dependent and independent of the endothelium.²³ In an attempt to perturb vascular endothelial function and investigate the role of ovarian hormones on postprandial FMD, the present study utilized the ingestion of a high-fat meal. This same meal has been shown to elicit a reduction in FMD^{13,24} through the transient postprandial increase in oxidative stress.¹⁴ Interestingly, majority of the studies that originally observed the postprandial reduction in FMD utilized a mixed sex design^{13,24}; however, sex related differences were not presented. The results of the present study are in agreement with and extend beyond the findings of a single report, which only investigated postprandial sex differences in women during the follicular phase.²⁵ As expected, when estrogen is elevated (follicular phase), vascular protection against a high-fat meal is observed. However, perhaps the most striking finding of the present investigation was that even during menses, when no differences in 17 β -estradiol were observed between men and women, FMD in the women was protected against the insult

of the high-fat meal, whereas men experienced approximately a 50% reduction (Figure 3). Additionally, concentrations of both estradiol and progesterone did not correlate with the change in FMD following the high-fat meal (Figure 4). The absence of the concentration dependent association aforementioned is consistent with a previous report that indicated no effect of estradiol on postprandial triglyceride or markers of oxidative stress.²⁶

Collectively, the present data question a concentration specific role of ovarian sex-steroid hormones in affording women greater vascular protection from the insult of a high-fat meal, but also support the concept of extended vascular protection in women even when concentrations of ovarian hormones are acutely suppressed (ie, menses). The ingestion of a high-fat meal acutely changes the blood lipid profile and has previously been documented to reduce endothelial function for many hours following the meal.¹³ Therefore, as a significant proportion of life is spent in the postprandial state, the ability to avoid this transient impairment in endothelial function may very well play a key role in protecting premenopausal women from the atherosclerotic disease process.

There is extensive literature to support the attenuated postprandial lipemia in women when compared to men.²⁶⁻²⁸ Moreover, Bloomer and colleagues²⁹ have recently observed significantly lower postprandial oxidative stress in women compared to men. In contrast to the present study, Bloomer and colleagues²⁹ provided the percentage of dietary fat in proportion to body mass (kilograms), such that the high-fat load was similar (1.2 g of fat per kg of body mass) between sexes. In theory, a high-fat load would result in more oxidative stress and contribute to a reduction in both NO bioavailability and FMD. Thus, it is important to note that in the current study, the men were, as expected, taller and heavier than the women (Table 1). Therefore, it is conceivable that the fat load (grams fat per kilogram body mass) was actually greater in women, but still their endothelial function appeared to be protected (Figure 3).

Conclusion

The present study determined that shear rate, the proposed stimulus for the FMD response, was unaffected by the changing ovarian hormones and, therefore, does not appear to be responsible for the fluctuations in FMD throughout the menstrual cycle in young premenopausal women. Additionally, and perhaps the most striking finding of this study was that women, in contrast to men, were protected against the insult of a high-fat meal equally across the menstrual cycle. Recognizing that a significant proportion of life is spent in the postprandial state with the potential for endothelial dysfunction and the incomplete understanding of the reduced risk of cardiovascular disease in women when compared to men, this finding may have important clinical implications when assessing dietary intake coupled with the disparity in cardiovascular risk between men and women.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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