Sex Differences in Platelet Reactivity and Response to Low-Dose Aspirin Therapy

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Context Recent randomized trials suggest that women may not accrue the same cardioprotective benefits as men do from low-dose aspirin therapy used in primary prevention. Failure of aspirin to suppress platelet aggregation in women is one hypothesized mechanism.

Objective To examine differential platelet reactivity to low-dose aspirin therapy by sex.

Design, Setting, and Participants A clinical trial of aspirin at 81 mg/d for 14 days was conducted in 571 men and 711 women. Baseline and post–aspirin therapy measures included platelet aggregation to arachidonic acid, adenosine diphosphate, epinephrine, and platelet function analyzer closure time.

Main Outcome Measure Sex differences in cyclooxygenase 1 (COX-1) direct and indirect platelet activation pathways before and after administration of aspirin.

Results In 10 of the 12 platelet agonist exposures, women's platelets were significantly more reactive at baseline. However, after aspirin therapy, the percent aggregation to arachidonic acid (the direct COX-1 pathway) decreased more in women than in men ($P<.001$) and demonstrated near total suppression of residual platelet reactivity in both men and women. In COX-1 indirect pathways, women experienced the same or more platelet inhibition than men in 8 of the 9 assays yet retained modestly greater platelet reactivity after aspirin therapy. In multivariable analysis, female sex significantly predicted aggregation to 2 μM and 10 μM of adenosine diphosphate ($P=.02$ and $<.001$, respectively) and collagen at 5 μg/mL ($P<.001$) independent of risk factors, age, race, menopausal status, and hormone therapy.

Conclusions Women experienced the same or greater decreases in platelet reactivity after aspirin therapy, retaining modestly more platelet reactivity compared with men. However, most women achieved total suppression of aggregation in the direct COX-1 pathway, the putative mechanism for aspirin's cardioprotection.

JAMA. 2006;295:1420-1427 www.jama.com

LOW-DOSE ASPIRIN IS A MAINSTAY OF coronary heart disease secondary prevention because of its known antiplatelet effect.[1-3] Rates of recurrent myocardial infarction (MI) are consistently lower in both men and women with known coronary disease who are treated with low-dose aspirin therapy compared with placebo.[4] In a meta-analysis of 4 large primary prevention trials using low-dose aspirin therapy, a 15% reduction in total cardiovascular events and a 30% reduction in MI rates were observed. However, few women were included.[5]

In the recent randomized placebo-controlled trial of low-dose aspirin therapy in the Women's Health Study (WHS) in 39 876 women without coronary disease, a 24% reduction was observed in ischemic stroke risk with aspirin therapy compared with placebo, but no overall reduction was observed in MI or total cardiovascular disease events.[6] However, in women aged 65 years or older, aspirin therapy reduced overall cardiovascular disease by 26%, and the risk of MI was reduced by 34%.[6] In the Hypertension Optimal Treatment (HOT) study,[7] a 42% reduction in MI risk was observed in men, but no significant reduction was observed in women.

Aspirin's protective benefit is believed to result from its ability to inhibit platelet aggregation and to prevent platelet plug formation in atherosclerotic vessels.[8] Aspirin's primary effect on platelet aggregation is its capacity to irreversibly acetylate cyclooxygenase 1 (COX-1), which prevents the conversion of arachidonate to thromboxane A_2, a potent platelet activator.[9] Although aspirin strongly inhibits COX-1–dependent platelet acti-

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vation,[10-12] inhibition of platelet activation through pathways that are indirectly related to COX-1 is less robust. The ability of aspirin to protect against MI is thought to be predicted by ex vivo testing of platelet aggregation, particularly to arachidonic acid[13] and platelet function analyzer closure time.[14,15]

The extent to which aspirin may influence platelet reactivity differently in men and women remains unknown. To address this question, we examined the effects of aspirin at 81 mg/d for 14 days on platelet reactivity in a large population of men and women with known excess risk for cardiovascular disease because of family history, a group for whom primary prevention with aspirin therapy is particularly salient.

METHODS

Study Design and Population

This study was a planned part of the Genetic Study of Aspirin Responsiveness (GeneSTAR), an ongoing study designed to examine gene-environment determinants of platelet reactivity in response to low-dose aspirin therapy, with a prespecified juncture at which to determine phenotypic responses to aspirin therapy. We enrolled previously identified, unaffected, apparently healthy siblings of 403 unique patients with documented coronary heart disease events before 60 years of age, along with the index patients' and siblings' adult offspring and the offsprings' other parent (403 families). Eligible participants (N=1282) were aged 21 years or older and had no coronary disease, vascular thrombotic events, peripheral vascular disease, serious gastrointestinal disorders, autoimmune diseases, bleeding disorders, or hemorrhagic events. Participants were ineligible if they were taking anticoagulants or antiplatelet medications or nonsteroidal anti-inflammatory drugs that could not be safely discontinued for 4 weeks. Individuals were excluded if they had a history of aspirin intolerance or allergy or abnormal blood cell counts (platelet count <100 000/μL or >500 000/μL, hematocrit <30%, or white blood cell count >20 000/μL). Race was classified by the nurse practitioner by asking the person to state his or her race. Options were not provided. Race was assessed because platelet function is thought to differ by race, so that it was added as a potentially influential covariate in multivariable analyses.

The trial was approved by the institutional review board of the Johns Hopkins Medical Institutions and was monitored by an external data and safety monitoring board. Written informed consent was obtained from all participants.

Participants were given a 36-day supply of 81-mg aspirin tablets, the dose recommended for primary prevention by the US Preventive Services Task Force.[16] Participants were instructed to take 1 tablet each day for 14 days. After interviewer-administered assessment of diet and exercise with a modified 24-hour dietary recall and the Stanford 7-Day Physical Activity Recall, participants were instructed to maintain the same dietary and physical activity patterns during the 14 days of aspirin therapy. Foods (caffeine, chocolate, grapes, alcohol) or food supplements known to influence platelet function were proscribed for the week preceding the baseline measurement and during the 14 days of therapy. Aspirin therapy adherence was assessed at the post–aspirin therapy visit with a modified Hill-Bone compliance questionnaire[17] and pill counts.

Eligible participants were interviewed by a nurse practitioner and self-reported their age and race. They underwent a cardiovascular history and physical examination and assessment of cardiovascular risk factors. Blood pressure was measured at rest according to the American Heart Association guidelines. Hypertension was considered present if the average of 4 blood pressure measurements was at least 140/90 mm Hg or the patient was taking an antihypertensive medication. Current cigarette smoking was defined as any smoking within the past 30 days, verified by exhaled carbon monoxide levels. Height and weight were measured, and body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. All blood tests were performed after participants had fasted for 12 hours overnight. Serum glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglyceride levels were measured directly. Low-density lipoprotein cholesterol (LDL-C) level was estimated using the Friedewald formula,[18] and cut points for abnormal lipid levels were defined according to the guidelines of the National Cholesterol Education Program Adult Treatment Panel III.[19] Diabetes was defined as having a fasting plasma glucose level of at least 126 mg/dL (6.99 mmol/L) or taking a hypoglycemic agent.[20]

Platelet Function Testing

Platelet function testing was performed at baseline and after 14 days of aspirin therapy. Measurement variation was minimized by having the same technician process the same individual's samples on the same equipment for the pre–aspirin therapy and post–aspirin therapy visits. The platelet technicians were also blinded to the sex of participants.

Blood was obtained from venipuncture and collected into vacutainer tubes containing EDTA (for complete blood cell counts) or 3.2% sodium citrate (for platelet function testing, fibrinogen levels, and von Willebrand factor [vWF]) after the first 4 mL was discarded. Platelet counts were determined by automated cell counter (ACT-Diff; Beckman-Coulter, Miami, Fla). Platelet-rich plasma was prepared from whole blood by centrifugation at 180*g* for 15 minutes, and platelet-poor plasma was prepared by centrifugation at 2000*g* for 10 minutes. Platelet counts were adjusted to 200 000/μL by diluting platelet-rich plasma with platelet-poor plasma.

Plasma fibrinogen was measured on an automated optical clot detection device (Behring Coagulation System; Dade-Behring, Newark, Del). Plasma vWF was determined using a commer-

cially available enzyme-linked immunosorbent assay (DiaPharma, West Chester, Ohio).

Optical aggregometry in platelet-rich plasma was measured in a 4-channel PAP-4 Aggregometer (Horsham, Pa) after stimulating samples with collagen (1 and 5 µg/mL), adenosine diphosphate (ADP; 2 and 10 µM), arachidonic acid (0.5 mM), or epinephrine (2 and 10 µM). Whole blood impedance aggregometry was measured in a Chrono-Log dual-channel lumi-aggregometer (Havertown, Pa) after samples were stimulated with collagen (1 and 5 µg/mL), ADP (10 µM), or arachidonic acid (0.5 mM). In addition, 1 whole blood sample was incubated with aspirin (20 µM for 20 minutes) in vitro before stimulation with arachidonic acid to determine ability of pharmacologic doses to directly inhibit COX-1 in vitro. Peak platelet responses within 5 minutes of agonist stimulation were automatically recorded for aggregation (as percentage of aggregation for platelet-rich plasma and ohms for whole blood).

Whole blood was loaded into prefabricated proprietary cartridges (PFA-100, Dade-Behring) containing a combination of collagen and epinephrine as platelet agonists. Platelet function analyzer closure time (the time for cessation of flow caused by formation of a platelet plug) was recorded in seconds. The maximum time allowed for closure was 300 seconds.

Statistical Analysis

Data were analyzed using standard descriptive and multivariable methods. Distributions were examined for normality using the Kolmogorov-Smirnov statistic. Nonnormal continuous variables were appropriately transformed. Categorical variables were examined using contingency table arrays and the χ^2 statistic. Multivariable linear and logistic regression analyses were used to determine the independent impact of sex on post–aspirin therapy platelet function outcomes, controlling for potentially influential demographic and biological covariables, including age, race, BMI, smoking, blood pressure, glucose level, total cholesterol level, hematocrit, leukocyte and platelet counts, and fibrinogen level. All covariates were prespecified according to the published literature on the biological determinants of platelet aggregation. For analysis of platelet function analyzer closure time, the vWF level was an additional covariate because it is known to be a major determinant of platelet function analyzer closure times.

Adjustments for nonindependence within families were done using the generalized estimating equation.[21] Incremental multivariable regression analyses were also used to allow determination of the contribution of each variable to the total variance in each platelet reactivity outcome. In addition, analyses were modeled separately for women to determine the impact of menopausal status and oral contraceptive therapy or hormone therapy on platelet reactivity. All significance testing was 2-tailed, with an α of .05, and data were analyzed using SAS (version 9.1; SAS Institute Inc, Cary, NC) and SUDAAN (version 9.0.1, Research Triangle Institute, Research Triangle Park, NC).

Table 1. Baseline Sample Characteristics by Sex (N = 1282)

Variables	Men (n = 571)	Women (n = 711)	*P* Value*
Demographics			
Age, mean (SD), y	44.7 (13)	46.1 (13)	.06
Black, No. (%)	181 (31.7)	281 (39.5)	.002
Current smoker, No. (%)	157 (27.5)	169 (23.8)	.13
Obese (BMI ≥30), No. (%)	202 (35.4)	309 (43.5)	.003
Medical history			
Hypertension, No. (%)	197 (34.5)	257 (36.2)	.54
Diabetes, No. (%)	43 (7.5)	67 (9.4)	.23
Postmenopausal, No. (%)		353 (49.6)	
OCT use among premenopausal women, No. (%)		67 (18.7)†	
Hormone therapy use among postmenopausal women, No. (%)		53 (15.0)‡	
Laboratory parameters, mean (SD)			
White blood cell count, cells/µL	6210 (1900)	6490 (2000)	.01
Hematocrit, %	44.0 (3.1)	38.9 (2.9)	<.001
Platelet count, cells/µL	243 000 (58 000)	275 000 (62 000)	<.001
Total cholesterol, mg/dL	194 (39)	203 (41)	<.001
HDL-C, mg/dL	46.0 (13)	55.9 (15)	<.001
LDL-C, mg/dL	120 (35)	123 (37)	.12
Fibrinogen, g/dL	0.35 (0.09)	0.41 (0.12)	<.001
Von Willebrand factor, %	100 (61)	104 (61)	.28

Abbreviations: BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OCT, oral contraceptive therapy.
SI conversion factor: HDL-C and LDL-C to mmol/L, multiply by 0.0259.
*Differences by sex were examined using *t* tests performed on transformed variables when necessary to achieve normality; categorical variables were examined using contingency table arrays and the χ^2 statistic.
†Sixty-seven of 358 premenopausal women.
‡Fifty-three of 353 postmenopausal women.

RESULTS

Population Characteristics

Women constituted 55% of the study population. Men and women were middle-aged, with some modest but statistically significant differences in risk factors and sociodemographic variables (TABLE 1). Women were more likely to be black and obese and were slightly older. White blood cell counts, platelet counts, fibrinogen levels, and HDL-C levels were all significantly higher in women, whereas hematocrit levels were higher in men. Levels of LDL-C were similar between men and women. More than a third of both men and women were hypertensive. Among women, 49.6% were postmenopausal, of whom 15% were receiving hormone therapy. Of the premenopausal

women, 18.7% were taking oral contraceptive agents.

All participants returned for follow-up. Pill counts and self-reported nonadherence showed that 7% of participants missed 1 of 14 doses, and 5.4% missed 2 doses. All participants had taken a dose in the 24 hours preceding the post–aspirin therapy measurements.

Baseline Platelet Aggregation

In unadjusted analyses by sex, platelet aggregation to all agonists was higher in women in whole blood and platelet-rich plasma. In 10 of the 12 agonist exposures, the differences were statistically significant but modest (TABLE 2). Measurable aggregation to arachidonic acid with 20 µM of aspirin added in vitro was significantly more prevalent in women.

Platelet Aggregation and Aspirin-Induced Change in Aggregation

For assays directly measuring the COX-1 pathway, most men and women demonstrated complete suppression (zero aggregation) after aspirin administration. For example, aggregation to 0.5-mM arachidonic acid in whole blood was not measurable in most participants (Table 2). Similarly, in platelet-rich plasma, after aspirin administration a marked decrement occurred in the percentage of both sexes with nonzero aggregation to arachidonic acid. The percentage with nonzero aggregation to this low dose of arachidonic acid at baseline does not indicate that this group was taking aspirin-containing drugs at baseline. Aggregation patterns to a more potent dose of arachidonic acid (1.6 mM) were similar, with fewer individuals demonstrating nonzero aggregation in platelet-rich plasma. When 20 µM of aspirin was added in vitro, slightly more women than men showed nonzero aggregation to arachidonic acid, although the difference was not statistically significant.

In platelet aggregation assays that are indirectly related to COX-1 (aggregation to collagen, ADP, and epinephrine), women again demonstrated more residual aggregation after aspirin therapy (Table 2). For example, aggregation to collagen at 1 µg/mL in whole blood and platelet-rich plasma was markedly reduced in men and women after aspirin therapy, with a similar change by sex, but the level of aggregation in women remained modestly but statistically significantly greater than in men. Relatively modest changes in ADP-induced aggregation occurred after aspirin therapy in either sex, but aggregation remained significantly higher in women. Aggregation to 2 µM and 10 µM of epinephrine in platelet-rich plasma was reduced by about 16% and 18% of total aggregable platelets in men and women, respectively, after aspirin therapy, with somewhat more suppression in women. Again, women's platelets remained slightly more reactive after aspirin therapy. Thus, in platelet activation pathways indirectly related to COX-1, women experienced the same or a greater amount of platelet inhibition in 8 of the 9 assays performed yet retained significantly but modestly greater platelet re-

Table 2. Platelet Aggregation Before and After Aspirin Therapy (N = 1282)*

	Before Aspirin Therapy			After Aspirin Therapy			Change		
Aggregation	Men (n = 571)	Women (n = 711)	*P* Value	Men (n = 571)	Women (n = 711)	*P* Value	Men (n = 571)	Women (n = 711)	*P* Value
Whole blood indirect COX-1 pathways									
Collagen 1 µg/mL, Ω	20.9 (5.3)	22.0 (5.7)	<.001	6.3 (5.4)	7.1 (5.6)	.004	−14.6 (6.8)	−14.9 (7.2)	.38
Collagen 5 µg/mL, Ω	28.2 (6.5)	28.8 (6.2)	.11	25.8 (6.8)	26.4 (6.2)	.13	−2.40 (7.2)	−2.42 (6.8)	.97
ADP 10 µM, Ω	10.7 (6.1)	14.7 (5.4)	<.001	10.5 (6.2)	14.5 (6.2)	<.001	−0.19 (5.2)	−0.21 (5.5)	.94
Whole blood direct COX-1 pathways									
Arachidonic acid 0.5 mM, %†	94.9	98.0	.002	4.38	3.80	.60	−90.5	−94.2	.02
Nonzero aggregation to arachidonic acid 0.5 mM incubated with 20-µM aspirin, %	0.55	2.14	.02	0.18	0.29	.99	−0.4	−1.5	.03
Platelet-rich plasma indirect COX-1 pathways‡									
Collagen 1 µg/mL, %	37.4 (34)	41.9 (35)	.02	6.03 (8.3)	7.79 (9.7)	.01	−31.4 (31)	−34.1 (32)	.12
Collagen 5 µg/mL, %	81.7 (17)	83.0 (17)	.19	27.2 (21)	31.8 (22)	<.001	−54.2 (23)	−51.1 (23)	.01
Epinephrine 2 µM, %	52.8 (35)	59.8 (34)	<.001	21.2 (13)	22.8 (14)	.03	−31.5 (29)	−36.9 (29)	.001
Epinephrine 10 µM, %	69.0 (29)	73.4 (28)	.007	28.1 (15)	29.7 (16)	.07	−40.8 (25)	−43.7 (24)	.04
ADP 2 µM, %	36.9 (26)	46.6 (28)	<.001	31.1 (17)	36.6 (17)	<.001	−5.62 (21)	−10.2 (23)	<.001
ADP 10 µM, %	77.5 (15)	81.0 (13)	<.001	65.6 (13)	70.3 (12)	<.001	−11.8 (15)	−10.8 (14)	.25
Platelet-rich plasma direct COX-1 pathway									
Nonzero aggregation to arachidonic acid 0.5 mM, %	62.2	69.2	.008	2.65	3.24	.53	−59.8	−65.9	.03

Abbreviations: ADP, adenosine diphosphate; COX-1, cyclooxygenase 1.

*Data are presented as nontransformed mean (SD). Significance testing was performed on appropriately transformed variables using *t* tests for continuous variables and contingency table arrays and the χ^2 statistic for categorical variables.

†The majority of after aspirin therapy values for arachidonic acid in men and women were zero; values are thus expressed as the percentage who failed to be inhibited completely. Change with aspirin (negative) indicates the percentage of individuals who converted from noninhibition to full inhibition after aspirin therapy.

‡The units of measure of aggregation in platelet-rich plasma are the percentage of all platelets that aggregate. The change measures refer to the change in aggregation in units and do not represent a fractional change from baseline.

activity in 7 of 9 assays and near-significant greater reactivity in the remaining 2 assays.

Platelet Function Analyzer Closure Time

Men and women had similar platelet function analyzer closure times at baseline (mean [SD], 125 [27] and 127 [31] seconds, respectively, $P=.36$). At follow-up, 32.4% of men and 50.8% of women had platelet function analyzer closure times of less than 300 seconds, the maximum value for the assay ($P<.001$), which is consistent with more residual platelet reactivity after aspirin therapy in women compared with men.

Multivariable Analyses

Multivariable analyses could not be performed on platelet reactivity to arachidonic acid after aspirin therapy because most participants demonstrated zero aggregation. In multivariable analyses on other aggregation outcomes, post–aspirin therapy values were adjusted for pre–aspirin therapy values and for risk factors associated with platelet aggregation, including age, race, sex, blood cell counts, fibrinogen levels, and vWF for platelet function analyzer closure time. TABLE 3 shows these analyses in whole blood for collagen at 5 µg/mL and ADP at 10 µM as examples. In these models, as in all of the others, the greatest contribution to the total variance in aggregation outcomes was the baseline level of aggregation. Sex was significant in model 2 (ADP) after adjustment for baseline aggregation. In model 1 (collagen), sex was borderline significant. However, in other models for the remaining agonists, there was no statistical significance by sex after adjustment for baseline aggregation level. In all cases, risk factors and blood cell counts contributed little to the total model variance, even when statistically significant.

Multivariable models in platelet-rich plasma aggregation produced similar results. The percentage of the variance explained by the different variables was similar to that of whole blood for aggregation to collagen at 1 and 5 µg/mL. For aggregation to 2 µM of epinephrine, 36% of the variance was explained by the total model, with 32% of that contributed by the pre–aspirin therapy value ($P<.001$). In platelet-rich plasma, female sex was statistically significant for aggregation to 10 µM of ADP ($P<.001$), 2 µM of ADP ($P=.02$), and 5 µg/mL of collagen ($P<.001$). There were no risk factor, demographic, or blood cell count patterns associated with the outcomes, and few variables made meaningful contribution to the variance in the outcomes except the pre–aspirin therapy values. Adjustments for regimen adherence did not alter any results.

For the platelet function analyzer closure time assay, multivariable logistic regression analysis was used to predict achievement of maximum closure time. Only female sex was significant (relative odds, 0.66; 95% confidence interval, 0.47-0.93). This finding indicates that women were 34% less likely to reach the maximum closure time compared with men, independent of all other variables and adjustment for baseline levels, intrafamily clustering, and regimen adherence.

Because pre–aspirin therapy platelet reactivity was the primary determinant of variance in post–aspirin therapy platelet function, we conducted additional multivariable analyses of the pre–aspirin therapy assay results, incorporating the same prespecified variables used in the post–aspirin therapy models. For most pre–aspirin therapy platelet reactivity tests, white blood cell count and platelet counts were statistically significantly and positively associated with the outcomes but explained little of the variance. Although female sex was significantly associ-

Table 3. Multivariable Linear Regression Models Predicting Platelet Aggregation After Aspirin Therapy in Whole Blood (N = 1282)

	Model 1: Aggregation to Collagen at 5 µg/mL			Model 2: Aggregation to ADP at 10 µM		
	β (SE)	r^2*	*P* Value	β (SE)	r^2*	*P* Value
Age, y	0.022 (0.013)	0.003	.09	–0.006 (0.013)	0.0001	.64
Female sex	–0.905 (0.510)	0.003	.09	0.923 (0.404)	0.008	.02
White race	0.844 (0.443)	0.002	.06	–1.491 (0.373)	0.01	<.001
Body mass index	0.027 (0.030)	0.0008	.37	0.045 (0.026)	0.003	.09
Current smoker	0.745 (0.434)	0.002	.09	–0.252 (0.342)	0.0002	.46
Systolic blood pressure	0.006 (0.013)	0.0001	.63	0.013 (0.012)	0.0005	.26
Glucose	–0.001 (0.005)	0.000	.88	–0.005 (0.006)	0.0005	.42
Total cholesterol	–0.003 (0.004)	0.0004	.41	0.004 (0.004)	0.0008	.23
White blood cell count	0.764 (0.116)	0.04	<.001	0.393 (0.089)	0.01	<.001
Hematocrit	–0.208 (0.060)	0.004	.001	–0.074 (0.055)	0.001	.18
Platelet count	–0.001 (0.003)	0.0001	.69	0.011 (0.003)	0.02	<.001
Fibrinogen	0.001 (0.002)	0.000	.69	–0.001 (0.001)	0.0004	.55
Baseline aggregation value†	0.317 (0.033)	0.17	<.001	0.543 (0.028)	0.40	<.001
Total model R^2		0.23			0.46	

*r^2 Is the portion of the total variance in the model explained by each variable; total model R^2 is the total variance in each outcome explained by all variables in the model.
†The baseline aggregation value represents the agonist and dose-specific aggregation for each individual. Thus, all postvalues in the regression analyses are adjusted for the prevalues.

ated with most platelet aggregation outcomes in both whole blood and platelet-rich plasma before aspirin therapy, it explained only a modest amount of the baseline total variance.

Subgroup Analysis in Women

To address whether menopausal status, the use of extrinsic hormones, age, race, or risk factors influenced baseline or post–aspirin therapy platelet reactivity differently in women (n=711), we also conducted all analyses separately for women. In unadjusted bivariable analyses, all aggregation tests in whole blood and platelet-rich plasma at baseline and post–aspirin therapy showed no significant differences by menopausal status. Premenopausal women receiving oral contraceptive therapy had a modestly shorter platelet closure time at baseline (greater platelet reactivity) compared with those not receiving oral contraceptive therapy (mean [SD], 118 [27] vs 127 [30] seconds, $P=.01$) but no differences in other platelet reactivity measures. TABLE 4 shows few differences in any measure of platelet aggregation among postmenopausal women before or after aspirin therapy by the use of hormone therapy in unadjusted bivariable analysis.

In multivariable analyses in women only, menopausal status and hormone therapy were not significant predictors of any baseline or post–aspirin therapy aggregation outcomes. However, hormone therapy was significantly predictive of baseline platelet function analyzer closure time ($P=.003$), with a 1% contribution to the 14% total variance explained by all variables. Menopausal status alone was not predictive ($P=.15$). The strongest predictor of baseline platelet function analyzer closure time was vWF (3% of the 14% total variance, $P<.001$). After aspirin therapy, postmenopausal status ($P=.03$) and use of hormone therapy ($P=.04$) became significantly predictive of platelet function analyzer closure time, independent of all other variables, and of the pre–aspirin therapy value. No other variables were significant, except for hematocrit ($P=.01$) and the pre–aspirin therapy value ($P=.001$).

Thus, both bivariable and multivariable analyses suggest that women experience only minimal effects of menopause and extrinsic hormones on most platelet reactivity tests, except for platelet function analyzer closure time, in which women taking extrinsic hormones seem to have modestly shorter closure times at baseline and after aspirin therapy.

COMMENT

In this study of low-dose aspirin therapy in unaffected individuals from families with premature coronary disease, we found that women had consistently more reactive platelets compared with men to multiple agonists in both whole blood and platelet-rich plasma. Increased platelet reactivity in women was present at baseline, as shown in previous studies,[22-24] and generally persisted after aspirin therapy, particularly in aggregation assays that were indirectly dependent on COX-1. In pathways directly dependent on COX-1, men and women demonstrated a marked and quantitatively similar inhibition by aspirin therapy. Pre–aspirin therapy platelet function contributed the most to the variance in platelet reactivity after aspirin therapy. Thus, aspirin therapy effectively inhibited platelet reactivity in women and men, fully suppressing the direct COX-1 platelet activation pathway but

Table 4. Platelet Aggregation Before and After Aspirin Therapy in Postmenopausal Women by Hormone Therapy Status*

	Before Aspirin Therapy			After Aspirin Therapy			Change		
Aggregation	Hormone Therapy (n = 53)	No Hormone Therapy (n = 300)	*P* Value	Hormone Therapy (n = 53)	No Hormone Therapy (n = 300)	*P* Value	Hormone Therapy (n = 53)	No Hormone Therapy (n = 300)	*P* Value
Whole blood indirect COX-1 pathways									
Collagen 1 µg/mL, Ω	21.5 (6.7)	21.6 (5.6)	.93	7.1 (4.8)	7.2 (5.8)	.70	–14.4 (7.6)	–14.4 (7.5)	.98
Collagen 5 µg/mL, Ω	29.5 (6.4)	28.2 (6.0)	.14	27.0 (7.4)	26.0 (6.4)	.32	–2.47 (7.6)	–2.22 (6.7)	.80
ADP 10 µM, Ω	16.2 (5.9)	14.5 (5.2)	.04	15.2 (6.4)	14.1 (5.9)	.31	–1.04 (5.2)	–0.37 (5.4)	.41
Whole blood direct COX-1 pathway									
Nonzero aggregation to arachidonic acid 0.5 mM, %	98.1	98.3	.91	0	3.3	.18	–98.1	–95.0	.18
Nonzero aggregation to arachidonic acid 0.5 mM incubated with 20-µM aspirin, %	1.89	2.70	>.99	0	0	>.99	–1.9	–2.7	.71
Platelet-rich plasma indirect COX-1 pathways									
Collagen 1 µg/mL, %	39.3 (36)	44.8 (35)	.31	9.6 (9.1)	8.7 (9.6)	.32	–29.7 (33)	–36.2 (32)	.18
Collagen 5 µg/mL, %	81.4 (18)	83.7 (16)	.43	33 (20)	33.7 (21)	.87	–48.2 (22)	–50.0 (22)	.58
Epinephrine 2 µM, %	66.4 (33)	63.0 (34)	.63	28.4 (14)	24.3 (13)	.06	–38.1 (30)	–38.7 (28)	.88
Epinephrine 10 µM, %	75.7 (27)	74.5 (27)	.87	35.0 (15)	31.5 (16)	.16	–40.6 (24)	–43.0 (23)	.50
ADP 2 µM, %	53.1 (28)	48.0 (28)	.30	42.1 (19)	38.5 (17)	.20	–11.4 (24)	–10.2 (23)	.75
ADP 10 µM, %	81.7 (10)	81.1 (13)	.89	72.3 (12)	70.7 (11)	.34	–9.21 (10)	–10.7 (14)	.36
Platelet-rich plasma direct COX-1 pathway									
Nonzero aggregation to arachidonic acid 0.5 mM, %	73.6	71.0	.70	7.55	3.00	.11	–66.0	–68.0	.79

Abbreviations: ADP, adenosine diphosphate; COX-1, cyclooxygenase 1.
*Data are presented as mean (SD).

also beneficially affecting COX-1 indirect pathways. Overall, although sex differences were often statistically significant, the magnitude of the differences was small.

There was no evidence that any differential distribution of risk factors, age, race, blood cell counts, or aspirin adherence accounted for sex differences or that menopausal status, the use of oral contraceptives, or hormone therapy played a major role in the increased residual platelet reactivity observed in women after aspirin therapy, except for platelet function analyzer closure time. The use of extrinsic hormone therapy resulted in statistically significant but only slightly shorter times to platelet plug formation, representing greater residual platelet reactivity in women. There were no differences in platelet responses to aspirin therapy by age (decade) in women.

In the largest randomized, placebo-controlled, primary prevention trial of low-dose aspirin therapy in women to date, the WHS followed up 39 876 healthy women for 10 years for incident cardiovascular events.[6] The trial resulted in a small reduction in total cardiovascular disease that was not statistically significant, a significant reduction in total stroke and ischemic stroke rates, and no overall effect on the risk of MI with aspirin therapy. In an accompanying meta-analysis of low-dose aspirin therapy primary prevention studies, no overall benefit was seen for MI in women in 3 randomized controlled trials, but a significant reduction of 32% ($P=.001$) was observed in the 5 trials conducted in men.[6] The difference between men and women overall in the meta-analysis was significant at the $P=.01$ level for MI. The editorial[25] that accompanied publication of the WHS suggested that it may be reasonable to consider avoiding low-dose aspirin as preventive therapy for coronary disease in women younger than 65 years unless there is a particularly high global risk score. However, a marked benefit was observed for stroke, with no significant increment in other cardiovascular end points.

In a recent meta-analysis of randomized controlled trials in 51 342 women, aspirin therapy was associated with a 12% reduction in total cardiovascular events and a 17% reduction in stroke, which primarily reflected a reduction in ischemic stroke because there was no significant effect of aspirin therapy on MI.[26] However, the majority of women in this analysis were participants in the WHS, so these overall observations primarily represent the findings of the WHS. In the WHS, because expected reductions in thromboxane and prostacyclin concentrations were of similar magnitude in men and women with low-dose aspirin therapy, the investigators concluded that "resistance to aspirin" was an unlikely explanation for aspirin therapy's failure to reduce MI risk in women.[6] In support of this conclusion, we found a notable impact of aspirin therapy in women on the COX-1 platelet pathways that are thought to be most protective against MI, even with increased residual platelet reactivity in COX-1 indirect pathways.

The major limitation of our study is the lack of prospective data linking measures of platelet reactivity to aspirin to subsequent cardiovascular disease. Furthermore, the extent to which these ex vivo tests of platelet function represent in vivo platelet activity remains unknown, as does their overall predictive value for incident cardiovascular events.

In conclusion, we have shown that low-dose aspirin therapy in women seems to produce a similar or even greater reduction in platelet reactivity in women compared with men. Although women in our study still retained a modest residual platelet reactivity that exceeds that of men, regardless of age, there is virtually total suppression of the COX-1 direct platelet function pathways in most women.

Author Contributions: Dr D.M. Becker had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
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Obtained funding: D.M. Becker, Bray, L.C. Becker.
Administrative, technical, or material support: D.M. Becker, Moy.
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Financial Disclosures: None reported.
Funding/Support: This study was supported by the National Heart, Lung, and Blood Institute (Genotypic Determinants of Aspirin Response in High Risk Families grant U01 HL72518, PROGENI) and National Center for Research Resources grant M01-RR000052. McNeil Consumer and Specialty Pharmaceuticals supplied aspirin to the study.
Role of the Sponsor: The NHLBI provided peer review and approval of the design, analysis, and implementation plan. The National Institutes of Health has been involved in the design of the study but not in data collection or preparation of the manuscript. The National Institutes of Health, the National Center for Research Resources, Johns Hopkins General Clinical Research Center, was involved in the review of design and analysis, conduct, and management of the study but not the data collection or preparation of the manuscript. McNeil Consumer and Specialty Pharmaceuticals has not been involved in the design, conduct, management, interpretation, preparation, or review of the manuscript.

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> Belief is desecrated when given to unproved and unquestioned statements for the solace and private pleasure of the believer. . . . It is wrong always, everywhere, and for every one, to believe anything upon insufficient evidence.
> —William James (1842-1910)