

Aspirin Resistance in Healthy Drug-Naive Men Versus Women (from the Heredity and Phenotype Intervention Heart Study)

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This study was designed to determine the factors that contribute to interindividual variation in the antiplatelet effects of aspirin. We measured platelet response to aspirin in 745 (400 men and 345 women) drug-naive asymptomatic subjects of the Heredity and Phenotype Intervention (HAPI) Heart Study. Whole blood platelet aggregometry was performed to assess response to arachidonic acid, adenosine diphosphate, and collagen at baseline and after 14 days of aspirin 81 mg/day. There was wide interindividual variation in platelet aggregation in response to aspirin, with no clear biological threshold to define aspirin resistance. Variation in platelet function before and after aspirin was heritable. Women exhibited greater platelet aggregability in response to adenosine diphosphate and collagen at baseline and after aspirin administration. The degree to which aspirin inhibited collagen-induced platelet aggregation was also significantly less in women compared with men (mean \pm SD percent inhibition of collagen-induced [1 μ g/ml] platelet aggregation 49.9 ± 30.9 vs 57.5 ± 42.5 in women and men, respectively, $p = 0.005$). Using a cutoff $<70\%$ inhibition of collagen-induced platelet aggregation, 21% of the total population demonstrated aspirin resistance, which occurred in 30% of women and 16% of men ($p = 0.0002$). Aspirin-resistant subjects were older, had significantly higher total cholesterol and low-density lipoprotein cholesterol levels, lower hematocrit, and higher platelet count compared with aspirin-sensitive subjects. In conclusion, in this study group, platelet function is heritable. There is wide interindividual variation in platelet response to aspirin as defined by whole blood platelet aggregometry, with women having lower mean percent inhibition of platelet aggregation and greater prevalence of aspirin resistance than men. © 2009 Elsevier Inc. All rights reserved. (Am J Cardiol 2009;104:606–612)

The mechanisms underlying aspirin resistance or failure of aspirin therapy are largely unknown. Several studies have reported less effective inhibition of platelet function in response to aspirin in women than in men.^{1–3} In a recent study, women were found to have greater baseline platelet reactivity than men and women retained modestly more platelet reactivity than did men after aspirin therapy.⁴ Response to aspirin therapy may also be mediated by genetic variation, although specific polymorphisms definitively linked to aspirin responsiveness have yet to be identified. A better understanding of factors influencing aspirin responsiveness may help identify patients who may not benefit

from aspirin therapy and in whom substitution of an alternative therapy might prove more beneficial in decreasing cardiovascular disease (CVD) morbidity and mortality. In the present study, we have characterized the platelet response to low-dose aspirin in a large number of drug-naive patients asymptomatic for CVD from a population homogeneous with respect to genetics and lifestyle. We measured platelet aggregation in whole blood in response to several agonists before and after administration with aspirin. We also measured urinary 11-dehydrothromboxane as a marker of aspirin-induced inhibition of thromboxane generation. Our specific goals were to characterize the distribution of aspirin responsiveness across this population, estimate the heritability of platelet reactivity and aspirin responsiveness, and assess gender differences in aspirin responsiveness and its clinical correlates.

Methods

The Heredity and Phenotype Intervention (HAPI) Heart Study is part of the National Heart, Lung, and Blood Institute's program for genetic interaction (PROGENI) Network and was designed to identify genes that interact with environmental exposures to modify risk factors for CVD.⁵ From 2003 to 2006, Old Order Amish subjects from Lancaster,

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Pennsylvania, ≥ 20 years of age, and considered relatively healthy were recruited. Exclusion criteria included severe hypertension (blood pressure [BP] $>180/105$ mm Hg), malignancy, kidney, liver, or untreated thyroid disease, and inability to safely discontinue all vitamins, nutritional supplements, and prescription and nonprescription medications. For the aspirin-response substudy, subjects with a platelet count $<100,000$ or $>500,000/\mu\text{l}$ and a white blood cell count $>20,000/\mu\text{l}$ were excluded. Of the 868 total participants in the HAPI Heart Study, 756 completed the aspirin intervention, 745 of whom had evaluable whole blood platelet aggregometric measurements before and after aspirin. Because the Amish are a closed founder population,⁶ virtually all members can be connected into a single 14-generation pedigree. The 745 examined subjects included a large number of relative pairs suitable for estimating heritability; of these were 241 parent-offspring pairs, 493 sibling pairs, 11 grandparent-grandchild pairs, 368 avuncular pairs, and 159 first-cousin pairs.

All participants underwent a medical history interview including assessment of CVD risk factors, vitamin, nutritional supplement, and prescription and nonprescription medication usage, and questions about previous CVD. Subjects taking aspirin (2.3%) were withdrawn from aspirin 14 days before initiation of the study. All other prescription or nonprescription medications and herbal or nutritional supplements or vitamins were discontinued 7 days before examination for the duration of the study. Physical examinations were conducted at the Amish Research Clinic in Strasburg, Pennsylvania, and blood samples were obtained after an overnight fast. Height and weight were measured using a stadiometer and calibrated scale with shoes removed and in light clothing, and body mass index (kilograms per meter squared) was calculated. Systolic BP (first phase) and diastolic BP (fifth phase) were obtained in triplicate using a standard sphygmomanometer with the subject sitting for ≥ 5 minutes. Hypertension was defined as a systolic BP ≥ 140 mm Hg and/or a diastolic BP ≥ 90 mm Hg and/or reported current use of BP-lowering medications. Diabetes was defined as a fasting glucose level ≥ 126 mg/dl or reported current use of prescription diabetes medications. Current smoking included use of a pipe, cigar, or cigarettes. Fasting serum lipid concentrations were assayed by Quest Diagnostics (Horsham, Pennsylvania). All subjects had triglyceride levels <400 mg/dl and low-density lipoprotein cholesterol levels were calculated.

At the initial clinic visit (visit 1), baseline whole blood platelet aggregometric studies were performed in the fasting state and a first morning urine sample was collected. The aspirin intervention began the day after clinic visit 1; the subject took aspirin 81 mg/day for 14 consecutive days. One day to 3 days before the second clinic visit (visit 2), a nurse and liaison performed a home visit to ensure compliance. On the fourteenth day the subject took aspirin shortly before arriving at the Amish Research Clinic for clinic visit 2 and collected the first morning urine sample. At clinic visit 2, fasting blood was drawn and whole blood platelet aggregometric studies were repeated in identical fashion to clinic visit 1. Compliance was assessed by a pill count and review of a subject's study diary. Subjects were permitted to miss up to 4 aspirin doses over the 2-week period and still be included in the final analysis, if the

Table 1
Clinical characteristics of study population

Variable	Men (n = 400)	Women (n = 345)	p Value
Age (years)	41.4 \pm 13.0	45.3 \pm 14.0	<0.0001
Body mass index (kg/m ²)	25.5 \pm 3.2	27.7 \pm 5.1	<0.0001
Systolic BP (mm Hg)	120.5 \pm 11.6	119.9 \pm 15.7	0.045
Diastolic BP (mm Hg)	77.3 \pm 8.7	75.2 \pm 8.0	<0.0001
Total cholesterol (mg/dl)	204.4 \pm 44.4	214.6 \pm 46.2	0.063
High-density lipoprotein cholesterol (mg/dl)	53.1 \pm 12.9	59.5 \pm 15.7	<0.0001
Low-density lipoprotein cholesterol (mg/dl)	138.7 \pm 40.9	140.3 \pm 42.8	0.534
Triglyceride (mg/dl)	62.5 \pm 36.0	73.9 \pm 45.9	0.034 [†]
White blood cell count (10 ³ / μl)	5.34 \pm 1.20	5.23 \pm 1.08	0.168
Hematocrit (%)	43.1 \pm 2.4	38.4 \pm 2.5	<0.0001
Platelet count (10 ³ / μl)	228.8 \pm 47.2	241.3 \pm 48.7	0.002
Hypertension medication	0	0.3%	0.28
Diabetes medication	0	0	N/A
Current smoker*	17.3%	0	<0.0001
Menopausal status	N/A	38.3%	N/A
Hormone use in postmenopausal women	N/A	2.0%	N/A

Values are means \pm SDs or frequencies. Adjusted for age (except for age).

* Current smokers include pipe, cigar, and cigarette users.

[†] The p value for triglycerides is based on logarithm-transformed value.

subject took the aspirin for ≥ 3 consecutive days before clinic visit 2. The aspirin intervention could be extended for up to 3 days (17 days total) to meet this criterion.

Venous blood (9 ml) for platelet aggregation studies was collected from the antecubital vein by gentle aspiration using a 21-gauge butterfly cannula and a 10-ml sterile syringe charged with 0.105 M sodium citrate anticoagulant 1 ml. In addition, 1 ethylenediaminetetra-acetic acid Vacutainer tube (Becton Dickinson, Franklin Lakes, New Jersey) was drawn and sent to Quest Diagnostics where a complete blood cell count with differential was performed. Whole blood platelet impedance aggregometry was performed by the same technician at baseline and after aspirin therapy using a Chrono-Log 4-channel aggregometer (Chrono-Log, Havertown, Pennsylvania) within 3 hours after blood was drawn. Instrument incubation wells were set to 37°C, and stirring speed set at 1,000 rpm. Prewarmed cuvettes were filled with Hank's Balanced Salt Solution 0.5 ml (Sigma-Aldrich, St. Louis, Missouri), citrate anticoagulated whole blood 0.5 ml, and a stir bar. After a 5-minute incubation period, a prewarmed probe was inserted into each cuvette and the cuvettes were moved to reaction wells. The aggregation baseline was set to 0 and the impedance circuit was calibrated to 50%. Aggregation was initiated with the addition of 1 of 3 agonists, collagen, adenosine diphosphate (ADP), or arachidonic acid, purchased from Chrono-Log (Horsham, Pennsylvania). Each channel of the 4-channel aggregometer was dedicated to a particular agonist. A dose response was performed with collagen 0.5-, 1-, 2-, and 5- $\mu\text{g}/\text{ml}$ final concentrations in channels 1, 2, 3, and 4, respectively. After completion of collagen reactions, new reactions were performed containing ADP at a final concentration of 10 μM in channels 1 and 2, and arachidonic

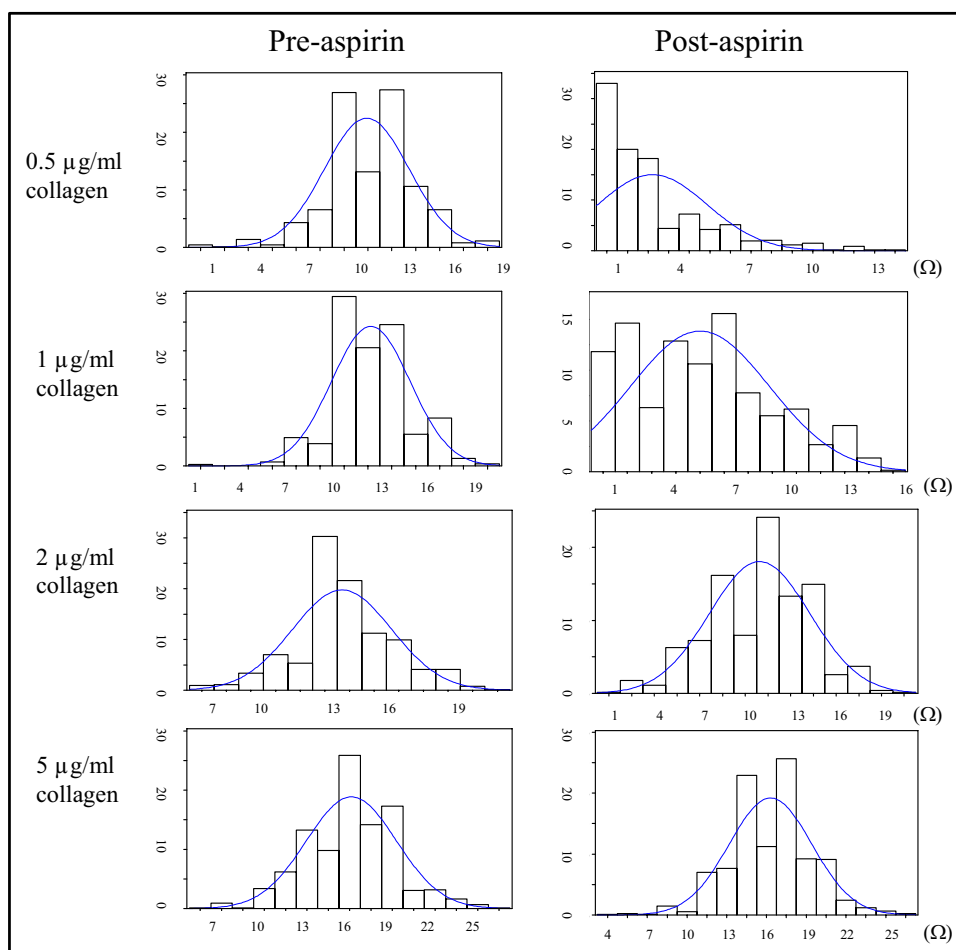


Figure 1. Distribution of whole blood platelet aggregation to collagen before and after aspirin administration.

Table 2

Age- and gender-adjusted correlation between platelet aggregation using different agonists before and after aspirin administration

	Collagen 0.5 µg/ml	Collagen 1 µg/ml	Collagen 2 µg/ml	Collagen 5 µg/ml	Adenosine Diphosphate 10 µM	Arachidonic Acid 0.5 mM
Collagen 0.5 µg/ml		0.636	0.585	0.505	0.487	0.351
Collagen 1 µg/ml	0.742		0.705	0.704	0.504	0.412
Collagen 2 µg/ml	0.539	0.786		0.669	0.421	0.386
Collagen 5 µg/ml	0.348	0.517	0.668		0.345	0.372
ADP 10 µM	0.280	0.408	0.455	0.389		0.654
Arachidonic acid 0.5 mM	0.337	0.308	0.242	0.142	0.274	

Pearson correlation coefficients (above diagonal before aspirin administration, below diagonal after aspirin administration).

All p values <0.0001.

acid at a final concentration of 0.5 mM in channels 3 and 4. Reactions were allowed to run for 10 minutes, but all calculations were based on a 5-minute test time.

First morning void urine samples, stored at -80°C , were thawed and assayed for urine 11-dehydro thromboxane B2 by an enzyme-linked immunoassay kit (Cayman Chemical Co., Ann Arbor, Michigan) according to the manufacturer's recommendation. Thromboxane levels were normalized to urinary creatinine.

Descriptive characteristics of the study participants were compared between men and women adjusting for age. Variables having non-normal distributions were logarithmically

(triglycerides) or inverse normally (urine 11-dehydro thromboxane B2) transformed before analysis. Categorical variables were compared between groups using chi-square statistic. Pearson correlations were estimated among the blood platelet aggregation measurements using different agonists, and partial correlations presented after adjustment for age and gender. We defined aspirin resistance as <70% inhibition of platelet aggregation to collagen 1 µg/ml, similar to the threshold reported by others.⁷ A p value (2-sided) ≤ 0.05 was considered statistically significant.

Comparisons between men and women in mean levels of platelet aggregation at baseline and after aspirin and urine

Table 3
Heritability of platelet aggregation and urinary 11-dehydro thromboxane B2 before and after aspirin administration

Variable	Before Aspirin Administration*				After Aspirin Administration*				After Aspirin Administration†			
	H ²	SE	p Value	Percent Variation Explained by Covariates	H ²	SE	p Value	Percent Variation Explained by Covariates	H ²	SE	p Value	Percent Variation Explained by Covariates and Baseline
Collagen 0.5 µg/ml	0.28	0.10	0.0005	0.07	0.10	0.09	0.12	0.05	0.06	0.09	0.24	0.08
Collagen 1 µg/ml	0.25	0.08	0.0006	0.05	0.22	0.10	0.01	0.09	0.22	0.10	0.01	0.12
Collagen 2 µg/ml	0.31	0.10	0.0005	0.04	0.22	0.10	0.01	0.10	0.14	0.10	0.08	0.16
Collagen 5 µg/ml	0.18	0.08	0.007	0.03	0.12	0.09	0.07	0.03	0.11	0.09	0.11	0.09
ADP 10 µM	0.42	0.10	0.000 004	0.05	0.42	0.10	0.000 003	0.11	0.24	0.10	0.004	0.32
Arachidonic acid 0.5 mM‡	0.28	0.09	0.0004	0.07								
Urinary 11-dehydro thromboxane B2 (ng/mg creatinine)§	0.23	0.12	0.02	0.03	0.15	0.09	0.03	0.03	0.10	0.08	0.09	0.14

* Adjusted for age, age², and gender.

† Adjusted for age, age², gender, and administration value before aspirin.

‡ Most administration values after aspirin for arachidonic acid were 0; hence, no heritability was calculated.

§ The p values for urinary thromboxane B2 values were based on inverse normally transformed value.

H² = heritability.

Table 4
Platelet aggregation and urinary thromboxane B2 before and after aspirin administration

Variable	Before Aspirin Administration				After Aspirin Administration				
	Men (n = 400)	Women (n = 345)	Unadjusted p Value	Covariate-Adjusted p Value*	Men (n = 400)	Women (n = 345)	Unadjusted p Value	Covariate-Adjusted p Value*	Covariate- and Baseline-Adjusted p Value†
Whole blood indirect COX-1 pathways									
Collagen 0.5 µg/ml (Ω)	10.6 ± 0.3	11.9 ± 0.2	<0.0001	0.13	1.9 ± 0.1	2.6 ± 0.2	0.0003	0.16	0.15
Collagen 1 µg/ml (Ω)	11.7 ± 0.3	12.7 ± 0.2	<0.0001	0.01	4.7 ± 0.4	6.2 ± 0.2	<0.0001	0.008	0.04
Collagen 2 µg/ml (Ω)	13.1 ± 0.3	14.0 ± 0.2	<0.0001	0.42	9.2 ± 0.4	10.9 ± 0.2	<0.0001	0.0003	0.0003
Collagen 5 µg/ml (Ω)	16.2 ± 0.4	17.1 ± 0.2	0.0003	0.12	14.9 ± 0.2	15.9 ± 0.2	<0.0001	0.05	0.09
ADP 10 µM (Ω)	9.2 ± 0.4	10.4 ± 0.2	<0.0001	0.09	9.0 ± 0.4	10.9 ± 0.2	<0.0001	0.0002	0.0006
Whole blood direct COX-1 pathway									
Arachidonic acid 0.5 mM‡ (Ω)	100.0	100.0	1	1	23.5	16.8	0.02	0.60	0.65
Urine direct COX-1 pathway									
Urinary thromboxane B2 (ng/mg creatinine)§	0.98 ± 0.12	1.15 ± 0.06	0.002	0.001	0.28 ± 0.04	0.32 ± 0.02	0.19	1.00	0.25

Values are means ± SEs.

* Covariate-adjusted p values were adjusted for age, body mass index, systolic BP, total cholesterol, triglyceride, hematocrit, platelet count, white blood cell count, smoking status, and family structure.

† Covariate- and baseline-adjusted p values were further adjusted for covariates above and baseline (before aspirin therapy) aggregation value (or thromboxane level).

‡ Most values after aspirin therapy for arachidonic acid were 0; values were stated as the percentage that failed to be inhibited entirely.

§ The p values for urinary thromboxane B2 values were based on inverse normally transformed value.

Table 5
Patient characteristics stratified by aggregation test (<70% inhibition of platelet aggregation using collagen 1 $\mu\text{g/ml}$)

Variable	Aspirin Resistant (n = 156)	Aspirin Sensitive (n = 588)	p Value
Age (years)	47.9 \pm 1.2	41.9 \pm 1.4	<0.0001
Women	93 (59.6%)	252 (42.9%)	0.003
Body mass index (kg/m ²)	26.5 \pm 0.4	26.5 \pm 0.7	0.06
Systolic BP (mm Hg)	121.8 \pm 1.2	119.8 \pm 1.6	0.89
Diastolic BP (mm Hg)	75.7 \pm 0.7	76.5 \pm 1.1	0.14
Total cholesterol (mg/dl)	222 \pm 4	205 \pm 8	0.03
High-density lipoprotein cholesterol (mg/dl)	58 \pm 1	56 \pm 3	0.17
Low-density lipoprotein cholesterol (mg/dl)	149 \pm 8	137 \pm 4	0.07
Triglyceride (mg/dl)	76 \pm 4	66 \pm 7	0.99*
White blood cell count (10 ³ / μl)	5.32 \pm 0.1	5.28 \pm 0.2	0.84
Hematocrit	40.0 \pm 0.2	41.2 \pm 0.4	0.49
Platelet count (10 ³ / μl)	246.2 \pm 4.0	231.3 \pm 10.1	0.005
Urinary thromboxane B2 (ng/mg creatinine) (after aspirin administration)	0.33 \pm 0.02	0.27 \pm 0.04	0.02*
Hypertension medication	0	0.2%	0.40
Current smoker [†]	7.1%	9.9%	0.81
Menopausal status	47.3%	34.9%	0.89
Hormone use in postmenopausal women	1.6%	3.2%	0.17

Values are means \pm SEs, numbers of patients (percentages), or frequencies. Continuous variables were adjusted for age (other than age), gender, and family structure; chi-square test was used for categorical variables.

* The p values for triglycerides and thromboxane B2 were based on logarithmically or inverse normally transformed values.

[†] Current smokers include pipe, cigar, and cigarette users.

11-dehydro thromboxane B2 levels were adjusted for age, body mass index, BP, total cholesterol, triglyceride, hematocrit, white blood cell count, platelet count, and smoking. Administration values after aspirin were further adjusted for baseline value in addition to these covariates. To account for correlations in trait values owing to study subjects being related, we accounted for the nonindependence of study subjects using the variance components approach as implemented in the SOLAR (SFBR, San Antonio, Texas) software package (http://www.sfbr.org/Departments/genetics_detail.aspx?p=37).⁸ This approach allowed us to estimate the effect of variable of interest (e.g., gender) on the quantitative trait and simultaneously adjust for the effects of covariates and a polygenic component, computed as a function of the kinship matrix.

Heritability analyses were performed using the variance components method as implemented in SOLAR. Variation in platelet aggregation at baseline and after aspirin and urine 11-dehydro thromboxane B2 levels was modeled as a function of measured environmental covariates, additive genetic effects, and a residual error component. Heritability of those phenotypes was estimated as the proportion of the total phenotypic variation that could be attributable to additive genetic effects. Additive genetic effects were parameterized as a function of the kinship matrix.

Results

Characteristics of the study population by gender are presented in Table 1. Distributions of the platelet aggregator response to collagen in different concentrations are shown in Figure 1. With the exception of collagen 0.5 $\mu\text{g/ml}$, all distributions before and after aspirin administration were approximately normally distributed. Correlations of platelet aggregator response among different concentrations of collagen (before and after aspirin administration) were rela-

tively high (Table 2). The correlation was less when comparing the response among different agonists, e.g., collagen versus ADP or arachidonic acid.

At baseline, there was a significant heritable component to variation in each platelet aggregation phenotype to different agonists and in urinary thromboxane B2 excretion, with estimated heritabilities of 0.18 to 0.42 (Table 3). Age and gender explained only small proportions of the phenotypic variation and had little impact on heritability estimate. After aspirin administration, heritability decreased for all phenotypes ($h^2 = 0.10 - 0.22$), except for aggregation to ADP whose heritability remained 0.42. Baseline aggregation levels were significantly associated with aggregation levels after aspirin administration. After controlling for baseline aggregation levels, heritability of aggregation to ADP decreased from 0.42 to 0.24, whereas heritability of aggregation to collagen at 1 $\mu\text{g/ml}$ remained at 0.22 and heritability of aggregation to collagen at other concentrations decreased to the point where they were no longer significantly >0 .

At baseline (before aspirin), women exhibited consistently greater platelet aggregator responses to different agonists (Table 4). These included all 4 concentrations of collagen and ADP. Consistent with these observations, women had greater urinary thromboxane B2 excretion than men. After multivariate adjustment for age, body mass index, systolic BP, total cholesterol, triglyceride, hematocrit, platelet count, white blood cell count, and smoking status, women still exhibited a significantly greater platelet aggregator response to collagen at 1 $\mu\text{g/ml}$ and greater urinary thromboxane B2 excretion than men. After 2 weeks of daily low-dose aspirin ingestion, arachidonic acid-induced platelet aggregation was markedly decreased in women and men, with complete inhibition reached in most subjects. Further-

more, there was no difference in urinary 11-dehydro thromboxane B2 levels after aspirin administration between men and women. However, despite this similar degree of cyclooxygenase-1 (COX-1)-dependent pathway inhibition, women continued to show increased platelet aggregator response to collagen and ADP. Degree of inhibition was significantly less in women compared with men for collagen at the 1- and 2- $\mu\text{g}/\text{ml}$ doses and for the ADP 10- μM dose, even after adjusting for age, body mass index, systolic BP, total cholesterol, hematocrit, platelet count, white blood cell count, baseline aggregation value, and family structure. Mean percent inhibition of collagen-induced (1 $\mu\text{g}/\text{ml}$) platelet aggregation in response to aspirin in women was significantly lower than in men (mean \pm SD 49.9 \pm 30.9 vs 57.5 \pm 42.5, $p = 0.005$).

We also used a cutoff of $\geq 70\%$ inhibition of platelet aggregation with collagen 1 $\mu\text{g}/\text{ml}$ as an agonist to define aspirin resistance. Table 5 lists clinical characteristics of the 156 aspirin-resistant and 588 aspirin-sensitive subjects. By these criteria, 21% (156 of 744) of study subjects were aspirin resistant, including 30% of women and 16% of men ($p = 0.0002$ for gender difference). Using other threshold values to define aspirin resistance similarly resulted in an excess of women (data not shown). Aspirin-resistant subjects were older and had significantly higher total cholesterol levels, higher platelet number, and higher urinary 11-dehydro thromboxane B2 level after aspirin administration than aspirin-sensitive subjects.

Discussion

Large-scale epidemiologic studies of prevention of thromboembolic manifestations of CVD have generally shown benefit from long-term aspirin use. However, all subgroups appear not to enjoy the same benefits of aspirin therapy. The main finding in this study of a large number of asymptomatic drug-naïve subjects is that women have greater platelet aggregation at baseline and are less responsive to the platelet inhibitory activity of aspirin. Other factors that were associated with decreased aspirin response in our study include age, diastolic BP, cholesterol (total), and increased platelet number. Our results are consistent with large-scale trials of aspirin for primary prevention in which benefit occurs predominantly in men.⁹

Developing reliable bioassays of aspirin's effectiveness has the potential to identify a subpopulation at risk for aspirin failure or a subpopulation in which the use of aspirin is more likely to harm than to benefit. However, this has been difficult because there is a multiplicity of nonstandardized methods to measure platelet function and there is no consensus with regard to how to define aspirin resistance. We chose to use whole blood platelet aggregometry and urinary thromboxane, methods that have been used previously to identify subjects less responsive to aspirin.^{2,10,11} We found that these platelet aggregation traits measured before and after aspirin therapy and calculated changes in response to aspirin were approximately normally distributed in our sample of relatively healthy drug-naïve subjects, suggesting that aspirin response is a complex trait in which there is no clear cutoff to define aspirin resistance. Like others,¹² we found a moderate correlation in platelet aggreg-

ator response to collagen, ADP, and arachidonic acid and, to a lesser extent, urinary thromboxane B2 levels (data not shown). These relations held before and after aspirin therapy. This moderate degree of correlation among these platelet function traits are likely because stimulators of platelet aggregation act through different, albeit converging, pathways and thus query common and different aspects of the platelet aggregation cascade. Measurement error is also a factor because technical and other variables can affect the strength of correlations.

Our study also confirmed the contribution of heritable factors to the variability in platelet function before and after aspirin therapy and the heritability estimates we observed were consistent with those previously reported from Caucasian subjects from the Genetic Study of Aspirin Responsiveness (GeneSTAR) study.¹³ Baseline platelet function contributed substantially to heritability of platelet function after aspirin therapy. Further studies are needed to identify the genetic determinants of those platelet functions.

The best understood effect of aspirin is its direct inhibition of COX-1. Urinary thromboxane levels and whole blood platelet aggregation in response to arachidonic acid may reflect variation that is directly attributable to inhibition of COX. In contrast, aspirin-related effects on whole blood aggregation in response to collagen and ADP, although also important mediators of arterial thrombosis, may involve biochemical pathways that are not directly related to COX inhibition. It is likely that aspirin does not have a single mechanism of action with regard to its antiplatelet effect and, more broadly, with regard to its clinical effect. In addition, 1 mechanism of action may be more important in some subgroups than others or may be more important in some disease states than others. For example, 1 mechanism may be more important in women than in men and this could explain why aspirin used for primary prevention in women may be more effective in preventing stroke than in preventing myocardial infarction. In our study, the gender difference in platelet function in response to aspirin was most pronounced with respect to collagen-induced aggregation, an indirect COX pathway, and less so (if at all) with respect to arachidonic acid-induced platelet aggregation, a direct COX pathway. These findings suggest that the mechanism of aspirin resistance in women may be independent of its direct effect on COX inhibition. An example of a gender-specific mechanistic effect was found in recent work by Chiang et al.¹⁴ This study demonstrated that low dose aspirin has a gender-dependent impact on anti-inflammatory 15-epi-lipoxin A4 production, which may contribute in part to the gender-dependent clinical benefits of aspirin.

Previous studies of gender differences in platelet aggregation-related phenotypes have yielded mixed results. One study, using whole blood aggregometry, reported a relative higher prevalence of aspirin nonresponders in women compared with men²; another study, using optical platelet aggregometry, similarly reported a higher incidence of aspirin resistance in women.¹ In contrast, in a large study by Becker et al,⁴ women demonstrated increased baseline (before aspirin) platelet reactivity with similar or greater absolute changes in response to aspirin compared with men. Our study demonstrates increased baseline platelet activity and decreased response to aspirin in women. However, our

study and the 1 reported by Becker et al showed that women retained more platelet reactivity compared with men. Possibly, these contrasting results are due to different demographics among studied populations. For example, the study by Becker et al comprised subjects at higher risk of CVD who were first-degree relatives of patients with CVD, slightly older than our subjects, and with higher body mass index. All these factors increase the likelihood for the presence of atherosclerotic vascular disease, and the presence of atherosclerotic vascular disease may heighten platelet reactivity.

Our study has several strengths, including the large number of subjects, all recruited from a homogenous population. We minimized confounding influences by performing these studies in asymptomatic drug-naïve subjects using a well-controlled prospective study design. However, there are also some limitations. Subjects were relatively healthy and the intervention was performed for only 2 weeks. Furthermore, we studied only Caucasian subjects. Longer-term studies in more mixed populations examining CVD events and mortality will be required to understand further the clinical relevance of aspirin resistance relevant to disease.

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