Native Platelet Aggregation and Response to Aspirin in Persons With the Metabolic Syndrome and Its Components

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Abstract

Background: Aspirin chemoprophylaxis for coronary artery disease (CAD) is recommended for persons with the metabolic syndrome. We determined the extent to which persons with increased risk for CAD with and without the metabolic syndrome accrued antiplatelet benefits from aspirin therapy.

Methods: We examined 2088 apparently healthy persons with a family history of CAD for the components that comprise metabolic syndrome and classified them according to national guidelines as having the metabolic syndrome or not. We assayed whole blood for *ex vivo* agonist-induced platelet aggregation (collagen, adenosine diphosphate, and arachidonic acid) and assessed a measure of *in vivo* platelet activation using urinary 11-dehydrothromboxane B2 (TxM), at baseline and after 2 weeks of treatment with 81 mg/day aspirin.

Results: At baseline, in multivariable analyses adjusted for race, age, sex, and risk factors, persons with metabolic syndrome had more aggregable platelets in response to all three agonists and higher levels of TxM ($P < 0.005$ for all) compared to those without metabolic syndrome. Postaspirin, although all individuals had lower platelet activation measures, subjects with metabolic syndrome retained higher platelet aggregation to adenosine diphosphate ($P = 0.002$) and higher TxM ($P < 0.001$), while aggregation to arachidonic acid ($P = 0.12$) and collagen ($P = 0.08$) were marginally different between those with and without the metabolic syndrome.

Conclusions: Among persons with an increased risk for CAD, metabolic syndrome was independently associated with overall greater platelet aggregation and activation at baseline and lesser, though significant, effect following aspirin, suggesting that low-dose aspirin therapy alone may not be sufficient to provide optimal antiplatelet protection in persons with metabolic syndrome.

Introduction

THE METABOLIC SYNDROME CONSISTS of a group of abnormalities related to insulin resistance and obesity^{1,2} that together create a proatherosclerotic,³ proinflammatory,⁴ and prothrombotic⁴ milieu in which coronary artery disease (CAD) events are expressed. Acute myocardial infarction is thought to occur due to activation and aggregation of platelets in atherosclerotic coronary arteries. Studies suggest greater levels of reactive P-selectin expression in platelets of persons with diabetes mellitus⁵ and greater levels of CD40 ligand expression in platelets of persons with metabolic syndrome.⁶ Due to the prothrombotic state in individuals with metabolic syndrome, the American Heart Association and the National Heart, Lung, and Blood Institute have recommended low-dose aspirin therapy to prevent cardiovascular

disease events.¹ However, to date, very little is known about functional platelet aggregation among persons with metabolic syndrome, either with or without aspirin therapy. To examine the extent to which metabolic syndrome and its components have an impact on *ex vivo* platelet aggregation and *in vivo* activation of platelets among high-risk persons, we examined apparently healthy individuals from families with a history of premature CAD.

Materials and Methods

Study sample

Subjects were recruited from 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. GeneSTAR is a part of the prospective Johns Hopkins Sibling and Family Heart Study, a 25-year prospective study of multiple aspects of cardiovascular risk among families identified from probands with CAD <60 years of age.⁷ Subjects with known CAD, stroke, any vascular disease, any bleeding or hematologic disorder, a history of hemorrhage, serious medical co-morbidity (e.g., renal or hepatic failure, acquired immunodeficiency disease [AIDS], cancer, or autoimmune disease), glucocorticosteroid use, or anticoagulant use were excluded from GeneSTAR. Family members were also excluded if they had a baseline platelet count <100,000/mL or >500,000/mL, hematocrit <30%, or white blood cell count >20,000/mL. The Johns Hopkins Institutional Review Board approved the study and all participants provided written informed consent.

Aspirin treatment

After baseline assessment, participants received a supply of aspirin (81 mg/day), the dosage recommended by the U.S. Preventive Services Task Force.⁸ Participants were asked to take one tablet per day for 14 days. At the postaspirin therapy visit, we assessed adherence to treatment using a modified Hill-Bone adherence questionnaire⁹ and pill counts.

Assessment of risk factor covariates

At baseline, participants underwent a complete cardiovascular history and physical examination; all medications were reviewed by a nurse practitioner. Blood pressure was measured at rest using the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure Guidelines.¹⁰ Hypertension was defined as a blood pressure $\geq 140/90$ mmHg (average of four blood pressure measurements) and/or the use of an antihypertensive medication. Height and weight were measured and body mass index (BMI) was calculated as weight (kg)/height (m²). Waist circumference was measured in the standing position at the level midway between the lower rib margin and the iliac crest using a cloth tape. Serum glucose and total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were measured using a Cholestech LDX analyzer (Cholestech Corporation, Hayward, CA) after participants had fasted for 12 hours overnight. Low-density lipoprotein cholesterol (LDL-C) was estimated using the Friedewald formula.¹¹ Diabetes was defined as having a fasting glucose level ≥ 6.93 mmol/L and/or taking a hypoglycemic agent. Participants were classified as smokers if they reported smoking any cigarettes within the past month or had an expired carbon monoxide level of >8 ppm on the average of two readings.

Laboratory assessment

Blood and urine were sampled at baseline and 14 days later to assess preaspirin and postaspirin platelet activation *ex vivo* and *in vivo*, respectively. Blood was obtained from venipuncture and collected into vacutainer tubes containing 3.2% sodium citrate (for platelet function testing and fibrinogen levels) after discarding the first 4 mL. Platelet counts were determined by automated cell counter (ACT-Diff, Beckman-Coulter, Miami, FL). Platelet functional studies

were completed within 2 hours of blood drawing. Urine samples were stored at -80°C until batch analyzed.

Platelet reactivity *ex vivo* was assessed by whole blood impedance aggregometry using a Chrono-Log dual-channel lumi-aggregometer (Havertown, PA) after stimulating samples with arachidonic acid (0.5 mM), collagen (1 $\mu\text{g/mL}$), or adenosine diphosphate (ADP) (10 μM). Peak aggregation within 5 minutes of agonist stimulation was recorded in ohms.

Platelet thromboxane production *in vivo* was assessed by urinary excretion of 11-dehydrothromboxane B2 (TxM). TxM was quantified by commercially available enzyme-linked immunoassay (ELISA; Cayman Chemical Co., Ann Arbor, MI) and normalized to urinary creatinine levels.

Plasma fibrinogen was measured by the Johns Hopkins clinical coagulation laboratory on an automated optical clot detection device (Behring Coagulation System, Dade-Behring, Inc, Newark, DE). High-sensitivity C-reactive protein (hs-CRP) was measured by the University of Maryland Cytokine Core Laboratory from plasma using an ELISA kit.

Statistical analysis

Using the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) criteria, metabolic syndrome was defined as the presence of three or more of the following: (1) waist circumference ≥ 0.88 m in women, ≥ 1.02 m in men; (2) serum triglycerides ≥ 1.695 mmol/L; (3) HDL-C ≤ 1.295 mmol/L in women, ≤ 1.036 mmol/L in men; (4) systolic blood pressure ≥ 135 and/or diastolic blood pressure ≥ 85 mmHg; (5) fasting glucose ≥ 5.55 mmol/L, or taking antidiabetic medications. Differences in demographic variables between those with and without metabolic syndrome were compared using *t*-tests for continuous variables, and χ^2 tests for categorical variables.

The medians and interquartile ranges of platelet aggregation and activation were tabulated by the presence of metabolic syndrome, and differences were tested using rank sum tests. The nonparametric trend of the average percentile rank of platelet aggregation and activation measures by metabolic component count was assessed using the Kuskid nonparametric trend test for rank sums.

For adjusted analysis, urinary thromboxane metabolite level corrected for urinary creatinine was analyzed on a log-transformed scale. Multivariable linear regression analyses were performed using the generalized estimating equations to account for any intrafamilial correlations, adjusting for age, sex, race, LDL-C levels, and current smoking. Using linear combinations of the model β coefficients, the platelet function measures in persons with metabolic syndrome and those without were tabulated and standardized for the pooled study population, i.e., 44 years of age, 58% women, 123 mg/dL of LDL-C, 25% smokers, 40% black, 0.5% other race. To assess mediation by fibrinogen or hs-CRP levels, these models were adjusted either for log(fibrinogen) or log(hs-CRP). In further models, the five individual components of metabolic syndrome were included to determine if they were related to platelet function measures independently of one another and the covariates detailed above. Hypothesis tests were considered statistically significant at the two-tailed $P < 0.05$. For independent association of components, $0.05 < P < 0.10$ was considered borderline significant. Data were analyzed using Stata (version 9.2, and version 10.0, College Station, TX).

The authors had full access to the data and take responsibility for its integrity.

Results

Table 1 shows the demographic characteristics of the 2088 participants, of whom 591 (28%) had metabolic syndrome. Persons with and without metabolic syndrome were similar with regard to sex, race, and smoking status, but were significantly older and more likely to have higher levels of cardiovascular risk factors not included in metabolic syndrome, including LDL-C, hs-CRP, and fibrinogen.

Baseline platelet aggregation and activation

The median and interquartile ranges of different measures of platelet function are shown by the presence and absence of metabolic syndrome in Table 2. All measures of platelet aggregation and activation are higher in those with metabolic syndrome as compared to those without. The average percentile ranks of these measures in the sample also tend to be higher with higher counts of co-prevalent metabolic abnormalities, with the trends being highly significant. Table 3 shows these associations of metabolic syndrome with platelet measures among persons with and without metabolic syndrome standardized to the mean age, sex, race, the mean LDL-C levels, and the distribution of current smokers in the study sample. *In vitro* platelet aggregation in all three cellular pathways (collagen, ADP, and arachidonic acid) was higher in persons with metabolic syndrome compared to those without. In addition, urinary excretion of TxM (geometric mean) was also higher among persons with metabolic syndrome. These differences remained significant on adjustment for fibrinogen levels for all measures, and on further adjustment for hs-CRP levels for all measures except Tx-M, which remained borderline significant.

We examined whether any of the specific individual components of metabolic syndrome were differentially related to the platelet function measures independently of all other components, by including the components simultaneously into models adjusted for sex, race, age, LDL-C levels, and

current smoking. The *P* values of the associations are tabulated in Table 4. All of the components were independently related to at least one of the platelet function assays. High TG levels were independently related to all four platelet assays, although statistically significant at the borderline level. A high glucose level or diabetes was independently associated only with aggregation to collagen, with borderline significance.

Platelet aggregation and activation after aspirin

After aspirin treatment, the differences between those with metabolic syndrome and those without in the pathway most directly affected by COX-1 inhibition, specifically arachidonic acid aggregation, are nearly eliminated (Table 5). Nonetheless, other proaggregatory differences remain among those with metabolic syndrome. A higher percentage retain nonzero aggregation to arachidonic acid, indicating incomplete suppression by aspirin. Those with a higher number of co-prevalent metabolic abnormalities demonstrated a significant trend to a higher percentile rank in the sample for aggregation induced by arachidonic acid and collagen. The association of ADP-induced aggregation and the number of co-prevalent syndrome components persisted after aspirin treatment. Similarly, although the levels of urinary thromboxane metabolites were much lower after aspirin treatment compared to baseline (*P* < 0.001, paired sign test), there was a significantly higher excretion of metabolites in those with metabolic syndrome than those without. This is a significant trend toward higher percentile ranks for thromboxane metabolite excretion among those with a larger number of co-prevalent metabolic components. For all variables, these trends are not as significant as prior to aspirin treatment.

In models adjusting for sex, race, age, LDL-C levels, and current smoking, postaspirin aggregation to collagen is not significantly associated with metabolic syndrome (Table 6). However, the odds of having nonzero aggregation induced by arachidonic acid, and the levels of aggregation due to ADP are associated with metabolic syndrome. These associations are slightly diminished when adjusted for baseline levels of

TABLE 1. CHARACTERISTICS OF THOSE WITH AND WITHOUT THE METABOLIC SYNDROME

	Metabolic syndrome present (n = 591)	Metabolic syndrome absent (n = 1497)	<i>P</i> value
Women (%)	56.2%	57.9%	0.469
White race (%)	61.9%	58.3%	0.286
Black race (%)	37.7%	41.1%	
Age (years)	48.2 ± 11.9	42.3 ± 12.9	<0.001
Current smokers (%)	27.4%	25.4%	0.341
Total cholesterol (mmol/L)	5.45 ± 1.11	5.09 ± 1.05	<0.001
LDL-C (mmol/L)	3.37 ± 1.02 (n = 567)	3.11 ± 0.94 (n = 1493)	<0.001
Non-HDL-C (mmol/L)	4.33 ± 1.11	3.63 ± 1.03	<0.001
BMI (kg/m ²)	34.5 ± 6.6	27.8 ± 6.2	<0.001
hs-CRP (mg/dL) ^a	2.88 (0.92–6.14) (n = 541)	0.86 (0.22–3.25) (n = 1360)	<0.001
Fibrinogen (μmol/L) ^a	407 (340–502) (n = 567)	354 (286–444) (n = 1442)	<0.001

^aMedian and interquartile range.

Abbreviations: LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; BMI, body mass index; hs-CRP, high-sensitivity C-reactive protein.

TABLE 2. NATIVE PLATELET AGGREGATION AND ACTIVATION MEASURES BY THE PRESENCE OF THE METABOLIC SYNDROME AND ITS COMPONENTS IN THE SAMPLE POPULATION ($n = 2088$)

	Measures of platelet aggregation or activation			
	Collagen (Ω)	ADP (Ω)	Arachidonic acid (Ω)	TxM ^a (ng/mmol creatinine)
	Median and interquartile range			
Metabolic syndrome present	20 (16–24)	13 (10–16)	16 (12–21)	155 (97–241)
Metabolic syndrome absent	19 (16–23)	12 (9–16)	15 (12–20)	137 (83–235)
<i>P</i> value ^b	0.002	<0.001	<0.001	0.004
	Average percentile rank			
<i>Number of components</i>				
0 ($n = 467$)	47.6%ile	47.5%ile	44.9%ile	46.2%ile
1 ($n = 525$)	46.5%ile	47.8%ile	47.8%ile	49.4%ile
2 ($n = 506$)	50.3%ile	51.1%ile	51.7%ile	50.7%ile
3 ($n = 349$)	54.4%ile	53.1%ile	54.1%ile	52.9%ile
4 ($n = 189$)	55.1%ile	53.1%ile	56.1%ile	52.3%ile
5 ($n = 53$)	57.0%ile	52.8%ile	53.4%ile	55.8%ile
Trend in ranks <i>P</i> value ^c	<0.001	<0.001	<0.001	<0.001

Whole blood aggregation measures: Aggregation responses in electrical impedance units in response to the agonists 1 μ g/mL collagen, 10 μ M ADP, 0.5 mM arachidonic acid.

^a $n = 1953$

^bWilcoxon rank sum test.

^cKusick nonparametric trend test.

Abbreviations: ADP, adenosine diphosphate; TxM, 11-dehydrothromboxane B2.

fibrinogen and are completely eliminated after adjustment for the baseline levels of hs-CRP. The geometric mean levels of the urinary thromboxane metabolites, however, remain significantly associated with metabolic syndrome in all of the adjusted models. This association remained significant even when additionally adjusted for BMI levels ($P = 0.039$). Although metabolic syndrome as a whole was associated with these postaspirin measures, no single component had significant independent association with postaspirin aggregation to collagen or ADP, or for urinary thromboxane metabolite. Only waist circumference was associated with significantly higher odds of nonzero aggregation induced by arachidonic acid after aspirin ($P = 0.002$).

Discussion

This is the largest study to date of the relationship of pre- and postaspirin platelet aggregation and activation and the impact of metabolic syndrome in healthy but high-risk individuals. The results show that aspirin has an effect in all persons. Those with metabolic syndrome had greater *in vitro* platelet aggregability in three platelet function measures representing different cellular pathways and a higher degree of platelet activation *in vivo* as measured by TxM in individuals with metabolic syndrome. These increased levels of platelet activation are seen even on adjustment for fibrinogen and for hs-CRP as a measure of systemic inflammation. Our

TABLE 3. STANDARDIZED ADJUSTED PLATELET ACTIVATION AND AGGREGATION MEASURES IN THE NATIVE STATE BY METABOLIC SYNDROME PRESENCE

Platelet activation measure	Metabolic syndrome present	Metabolic syndrome absent	<i>P</i> value (model 1) $n = 2082$	<i>P</i> value (model 2) $n = 2002$	<i>P</i> value (model 3) $n = 1822$
Collagen (Ω)	20.4 $\pm$ 0.3	19.0 $\pm$ 0.2	<0.001	<0.001	<0.001
ADP (Ω)	13.0 $\pm$ 0.2	12.2 $\pm$ 0.2	0.003	0.024	0.020
Arachidonic acid (Ω)	16.7 $\pm$ 0.3	15.3 $\pm$ 0.2	<0.001	<0.001	0.002
TxM (ng/mmol creatinine) ^a	148.0 (134.4–162.9)	131.2 (122.7–140.3)	0.042	0.019	0.095

Whole blood aggregation measures: Aggregation responses in electrical impedance units in response to the agonists collagen, 1 μ g/mL collagen; ADP, 10 μ M ADP; arachidonic acid, 0.5 mM arachidonic acid. Fibrinogen and hs-CRP were log-transformed prior to inclusion as covariates.

TxM indicated urinary excretion of thromboxane metabolite 11-dehydro thromboxane B2, a measure of *in vivo* platelet activation. All platelet measures are presented standardized for the average of the study sample 44 years of age, 58% women, 123 mg/dL of LDL, 25% smokers, 40% black, 0.5% other race (model 1), further adjustment for fibrinogen (model 2) and hs-CRP (model 3).

^aPresented as the geometric mean and its 95% confidence interval (model 1, $n = 1953$; model 2, $n = 1877$; model 3, $n = 1706$).

Abbreviations: ADP, adenosine diphosphate; TxM, 11-dehydrothromboxane B2.

TABLE 4. SIGNIFICANCE LEVELS^a (*P* VALUES) FOR THE INDEPENDENT ASSOCIATION OF EACH COMPONENT OF THE METABOLIC SYNDROME WITH MEASURES OF NATIVE PLATELET ACTIVITY PRIOR TO ASPIRIN (*n* = 2082)

	Collagen (Ω)	ADP (Ω)	Arachidonic acid (Ω)	TxM (ng/mmol creatinine) (<i>n</i> = 1951)
Waist circumference	0.066	0.297	0.005	0.060
Low HDL-C	0.027	0.064	<0.001	0.416
High triglycerides	0.010	0.028	0.075	0.047
IFG/diabetes	0.040	0.426	0.182	0.525
Pre/hypertension	<0.001	0.597	0.003	0.881

Whole blood aggregation measures: Aggregation responses in electrical impedance units in response to the agonists 1 μ g/mL collagen, 10 μ M ADP, 0.5 mM arachidonic acid.

^aAll associations are adjusted for all other metabolic components, age, sex, race, LDL-C levels, and current smoking.

Abbreviations: ADP, adenosine diphosphate; HDL-C, high-density lipoprotein cholesterol; IFG, impaired fasting glucose >100mg/dL.

data demonstrate that each of the components of metabolic syndrome likely partially and independently contribute to increased native platelet activation before aspirin. Although aspirin effects on the odds of retained *ex vivo* platelet aggregation to arachidonic acid were primarily related to waist circumference, suggesting a dependence on overall body size, this was not so for TxM, which represents *in vivo* platelet activation. Metabolic syndrome (rather than each individual component) is associated with an impaired aspirin effect on TxM independent of BMI, suggesting that our results are not explained by any relationship between aspirin pharmacokinetics and body size.

Urinary TxM, an estimate of *in vivo* activation of platelets and endothelial cells, is increased in persons with

coronary artery thrombosis.¹² Additionally, persistently high TxM after aspirin therapy has been shown to predict recurrent coronary artery disease events, in a graded manner within the range <15, 15–22, 22–34, and >34 ng/mmol creatinine, with an overall 80% difference in odds.¹³ In this context, the persistently higher TxM after aspirin therapy among persons with metabolic syndrome (36 vs. 32 ng/mmol creatinine) that we observed may suggest inadequate primary prevention that would be particularly important in a high-susceptibility group with a family history of premature CAD. Similarly, increased platelet aggregation in response to collagen, ADP, and arachidonic acid^{14,15} is also associated with increased risk of both myocardial infarction and stroke in aspirin-treated patients with atherosclerosis.

TABLE 5. POST-ASPIRIN PLATELET AGGREGATION AND ACTIVATION MEASURES BY THE PRESENCE OF THE METABOLIC SYNDROME AND ITS COMPONENTS IN THE SAMPLE POPULATION (*n* = 2007)

	Measures of platelet aggregation or activation			
	Collagen (Ω)	ADP (Ω)	Arachidonic acid (Ω)	TxM (ng/mmol creatinine)
Metabolic syndrome present	5 (2–9)	13 (9–16)	Median (interquartile range) 0 (0–0) (8% non-zero)	36 (24–59)
Metabolic syndrome absent	5 (2–9)	12 (8–16)	0 (0–0) (6% non-zero)	32 (20–51)
<i>P</i> value ^a	0.076	0.002	0.116	<0.001
<i>By number of components</i>		<i>Average percentile rank</i>		
0 (<i>n</i> = 451)	48.4	46.3	48.6	46.3
1 (<i>n</i> = 511)	48.9	48.2	49.5	46.7
2 (<i>n</i> = 500)	50.5	51.7	51.1	51.5
3 (<i>n</i> = 342)	51.6	53.9	50.4	54.6
4 (<i>n</i> = 187)	52.7	53.1	51.2	53.1
5 (<i>n</i> = 50)	49.8	49.2	50.8	59.7
Trend in ranks <i>P</i> value ^b	0.046	<0.001	0.003	<0.001

Whole blood aggregation measures: Aggregation responses in electrical impedance units in response to the agonists 1 μ g/mL collagen, 10 μ M ADP, 0.5 mM arachidonic acid.

^aWilcoxon rank sum test.

^bKusick nonparametric trend test.

Abbreviations: ADP, adenosine diphosphate; TxM, 11-dehydrothromboxane B2.

TABLE 6. STANDARDIZED ADJUSTED PLATELET ACTIVATION MEASURES BY METABOLIC SYNDROME PRESENCE

Platelet activation measure	Metabolic syndrome present	Metabolic syndrome absent	P value (model 1) n = 2036	P value (model 2) n = 1958	P value (model 3) n = 1821
Collagen (Ω)	6.2 $\pm$ 0.3	6.3 $\pm$ 0.1	0.764	0.322	0.594
ADP (Ω)	12.6 $\pm$ 0.2	12.2 $\pm$ 0.1	0.059	0.060	0.123
Arachidonic acid ^a	1.53 (1.02–2.30)	1.0 (REF)	0.038	0.090	0.294
TxM (ng/mmol creatinine) ^b	34.8 (32.5–37.3)	31.4 (29.9–33.0)	0.009	0.002	0.004

Whole blood aggregation measures: Aggregation responses in electrical impedance units in response to the agonists Col—1 μ g/mL collagen; ADP, 10 μ M ADP; AA, 0.5 mM arachidonic acid. Fibrinogen and hs-CRP were log-transformed prior to inclusion as covariates.

TxM is urinary excretion of thromboxane metabolite 11-dehydrothromboxane B2, a measure of *in vivo* platelet activation. All platelet measures are presented standardized for the average of the study sample 44 years of age, 58% women, 123 mg/dL of LDL, 25% smokers, 40% black, 0.5% other race (model 1), further adjustment for fibrinogen (model 2), and hs-CRP (model 3).

^aPresented as the odds of nonzero aggregation (95% CI).

^bGeometric mean and its 95% confidence interval (model 1, *n* = 1900; model 2, *n* = 1827; model 3, *n* = 1687).

Abbreviations: ADP, adenosine diphosphate; TxM, 11-dehydrothromboxane B2; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; CI, confidence interval.

Platelet activation has also been shown to be correlated with impaired metabolic states: Glycoproteins associated with platelet activation are elevated in persons with diabetes^{5,16,17} and metabolic syndrome,⁶ and diabetics have higher thromboxane biosynthesis.¹⁸ In our study, both *in vivo* and *ex vivo* measures of platelet aggregation are higher in persons with metabolic syndrome, providing support for the proposition that people with metabolic syndrome may be at higher risk of future acute coronary disease events due to an overall increase in platelet aggregability. Furthermore, the persistence of higher aggregability to ADP and higher levels of TxM suggests that this risk may not be fully nullified by preventive low-dose aspirin therapy.

Greater platelet aggregability among those with metabolic syndrome was not attributable to any single syndrome component in our study. Indeed, many of metabolic syndrome components have independent associations with one or the other aggregability and activation measure, but very few retain independent associations with postaspirin measures. Thus, it is extremely important to note that a constellation of metabolic syndrome components rather than any single component constitutes a particularly potent risk factor for increased platelet aggregability either in the native state or after aspirin therapy. Metabolic syndrome is thought to be a proxy diagnosis for insulin resistance, which, in later stages, results in β -cell failure and the inability to maintain normal fasting glucose levels. In this population, 2 of every 5 persons with metabolic syndrome were normoglycemic, presumably because the insulin resistance had not advanced to the point of β -cell failure. This suggests that even early insulin resistance may be related to increased platelet aggregability.

The primary limitation of our study, as in most others where platelet aggregation has been measured, is the lack of long-term follow-up data to determine the extent to which these *ex vivo* tests confer excess risk for a thrombotic cardiovascular event, and we cannot directly estimate the extent of aspirin resistance conferred by metabolic syndrome. Thus, as have others, we have had to rely on intermediate phenotypes that reflect a prothrombotic milieu and on the ability of these measures to predict CAD events. We have not defined any threshold for aspirin resistance, although

previous studies have defined aspirin resistance using different levels of platelet aggregation and activation measures. However, the differing definitions have led to wildly differing prevalence estimates from 6% to 26% (reviewed by Gasparyan et al.¹⁹) making any comparisons difficult.

In conclusion, the constellation of symptoms that comprise the ATP III-defined metabolic syndrome is associated with greater *in vivo* and *ex vivo* native platelet reactivity in persons from families at high risk for CAD. Although platelet function may be only one of many pathophysiological pathways to disease, the ability of antiplatelet therapy to provide improved protection for high-risk persons with metabolic syndrome would be important to determine so that tailored therapeutic primary prevention approaches can be refined.

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Author Disclosure Statement

No competing financial interests exist.

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