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# SUBOPTIMAL INHIBITION OF PLATELET CYCLOOXYGENASE-1 BY ASPIRIN IN METABOLIC SYNDROME

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## Abstract

Interindividual variation in the ability of aspirin to inhibit platelet cyclooxygenase-1 (COX-1) could account for some on-treatment cardiovascular events. Here, we sought to determine whether there are clinical phenotypes that are associated with a suboptimal pharmacological effect of aspirin. In a prospective, 2-week study, we evaluated the effect of aspirin 81 mg on platelet COX-1 in 135 patients with stable CAD by measuring serum thromboxane $B_2$ ($sTxB_2$) as an indicator of inhibition of platelet COX-1. A nested randomized study compared enteric-coated with immediate-release formulations of aspirin. We found that $sTxB_2$ was systematically higher among the 83 patients with metabolic syndrome than among the 52 patients without (median 4.0 ng/mL *vs.* 3.02 ng/mL, P=0.013). Twelve (14%) patients with metabolic syndrome, but none without metabolic syndrome, had $sTxB_2$ levels consistent with inadequate inhibition of COX ($sTxB_2$ ≥13 ng/mL). In linear regression models, metabolic syndrome (but none of its individual components) significantly associated with higher levels of log-transformed $sTxB_2$ (P=0.006). Higher levels of $sTxB_2$ associated with greater residual platelet function measured by aggregometry-based methods. Among the randomized subset, $sTxB_2$ levels were systematically higher among patients receiving enteric-coated aspirin. Last, urinary 11-dehydrothromboxane $B_2$ did not correlate with $sTxB_2$, suggesting that the former should not be used to quantitate aspirin's pharmacological effect on platelets. In conclusion, metabolic syndrome, which places patients at high risk for thrombotic cardiovascular events, strongly and uniquely associates with less effective inhibition of platelet COX-1 by aspirin.

### Keywords

aspirin; thromboxane; platelets; coronary disease; metabolic syndrome

## Introduction

Therapy with low-dose aspirin for secondary prevention of cardiovascular disease reduces the risk of major coronary events by 20–25%.[1, 2] Despite treatment with aspirin, however,

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approximately 10–20% of patients suffer recurrent arterial thrombotic events.[3] This led to consideration of whether interindividual variation in the effect of aspirin could contribute to those recurrent thrombotic events.

Aspirin exerts its anti-platelet effect by acetylating serine 529 of platelet cyclooxygenase-1(COX-1), which inhibits the enzyme and prevents formation of the platelet activator thromboxane (Tx) $A_2$.[4] We found that acetylation of the COXs by aspirin is inhibited by the redox cycling of the enzyme that occurs when hydroperoxides are reduced in the peroxidase active site of the COXs.[5] Accordingly, the response to aspirin varies considerably among different cell types, based on the hydroperoxide concentration of the cells. This suggested that the extent to which aspirin inhibits platelet COX-1 could vary among patients. Because activation of platelets and/or lipid peroxidation could lead to formation of several peroxides, platelet activation might attenuate aspirin's effect by this mechanism as well as by increased platelet turnover.

Although low-dose aspirin is almost uniformly effective in inhibiting platelet COX-1 among healthy, young individuals,[6] there could be pathophysiologic states that impair aspirin's effect. Here, we investigated the relationship of the pharmacologic effect of low-dose (81 mg) aspirin to a number of clinical phenotypic characteristics in a group of patients with documented coronary artery disease (CAD). Both obesity and diabetes have been associated with increased platelet activation *ex vivo* and *in vivo*[7–12] and with increased oxidative stress,[9, 13, 14] and the efficacy of aspirin in preventing cardiovascular events is marginal among diabetics compared with non-diabetics.[15] Body weight has been inversely correlated with suppression of $sTxB_2$. [1617,18] Metabolic syndrome constitutes a subset of both the diabetic and obese populations that confers a substantial increase in the risk of thrombotic cardiovascular events compared with both the general population and patients with diabetes.[19, 20] Accordingly, metabolic syndrome was a predetermined phenotype of interest in this study.

Drug formulation could also contribute to interindividual variation in response to aspirin. A summary of bioequivalence studies demonstrated that the enteric-coated formulations investigated were less potent in inhibiting $sTxB_2$ than immediate-release formulations among healthy volunteers.[16] Because these studies evaluated neither the McNeil formulation of enteric-coated aspirin nor the extent to which enteric coating affects platelet COX-1 inhibition among patients with CAD, we nested a randomized substudy within our cohort to study the effects of formulation in this population.

Furthermore, we evaluated the validity of measuring the urinary metabolite of $TxA_2$, 11-dehydrothromboxane $B_2$ (Tx-M),[21] to assess aspirin's efficacy. Higher levels of Tx-M have been associated with adverse cardiovascular events in high-risk patients, [22] but we previously found that this biomarker reflects $TxA_2$ derived from both platelet and non-platelet (*e.g.* COX-2) sources in cigarette smokers.[23]

## Methods

**Study Population—**This study was approved by the Vanderbilt University Institutional Review Board and registered on ClinicalTrials.gov (NCT00753935). Participants provided written informed consent. Recruitment occurred between June 2006 and May 2009. Patients with known CAD were approached if they appeared to satisfy inclusion and exclusion criteria based on review of their medical record. Inclusion criteria included ≥ 40 year-old males or post-menopausal females who were receiving aspirin 81–325 mg as part of their outpatient regimen. Exclusion criteria included concurrent use of other antiplatelet drugs, NSAIDs or COX-2 inhibitors, coronary artery bypass grafting or percutaneous coronary

intervention within 6 months of enrollment, uncontrolled hypertension (systolic blood pressure > 180 mmHg), decompensated congestive heart failure, acute coronary syndrome within 6 months, significant GI bleeding, creatinine ≥ 176.8 μmol/L (2 mg/dL), hematocrit < 30%, or platelet count < 135,000/μL. Approximately 25% of patients approached declined participation, the majority citing an unacceptable travel distance to complete the study.

**Study design—**A prospective observational study was conducted to evaluate the phenotypic characteristics of patients with stable CAD in whom inhibition of platelet COX-1 by aspirin was suboptimal. Patients received a blister pack containing a 2-week supply of a daily dose of aspirin 81 mg (McNeil Pharmaceuticals) administered in the evening. The importance of strict adherence to therapy was emphasized and participants were contacted by the research coordinator mid-study to assess and encourage continued compliance. A pill count was performed at the conclusion of the study. After 2 weeks, patients returned for phlebotomy and provided a first-morning urine specimen.

We enrolled 181 patients with CAD in the observational study. Of these, 135 fulfilled the criteria for inclusion in the cohort for analysis (see http://hyper.ahajournals.com).

From the above 181 patients, 106 consecutive subjects were enrolled in a nested randomized controlled investigation of enteric-coated aspirin. Of the 54 patients randomized to enteric-coated aspirin, nine were withdrawn: three for unsuccessful phlebotomy, two for use of other antiplatelet agents mid-study, two for self-reported use of systemic anti-inflammatory medication, one for percutaneous coronary intervention with stent placement during the study, and one for an error in enrollment (CABG within 6 months). Of the 52 patients randomized to immediate-release aspirin, five were withdrawn: two for laboratory abnormalities discovered on the day of recruitment but after enrollment, one for self-reported NSAID use, one for loss to follow-up, and one for withdrawal for personal reasons. Therefore, the final analytic cohort of 135 patients in the observational study included 45 randomized to enteric-coated aspirin and 47 randomized to immediate-release aspirin. We assigned the metabolic syndrome phenotype in accord with the AHA/NHLBI criteria.[24] Additional prospectively selected phenotypic characteristics of interest were BMI, diabetes, smoking status, and age.

## Laboratory Measurements

**Serum $TxB_2$—**Serum $TxB_2$ was measured as an indicator of inhibition of platelet COX activity. Non-anticoagulated blood was incubated at 37°C for 45 minutes immediately after phlebotomy.[25] Serum was separated by centrifugation and stored at −80°C until analysis. Serum $TxB_2$ was assayed by stable isotope dilution gas chromatography/mass spectrometry (GC/MS) with selective ion monitoring.[26] Suboptimal inhibition of platelet COX, the primary endpoint of the study, was defined prospectively as failure to reduce $sTxB_2$ to less than 5% of the mean level obtained in normal individuals taking no anti-platelet drugs; using the analytical techniques described herein, this equated to ≤ 13 ng/mL. The rationale and supporting evidence for this criterion for a suboptimal effect of aspirin is presented in the online supplement (please see http://hyper.ahajournals.org).

**Urinary 11-dehydrothromboxane $B_2$ (Tx-M)—**Urine was stored at −80°C until analysis. Urinary Tx-M was assayed by stable isotope dilution GC/MS.[27]

**Platelet Aggregation Studies—**Citrated platelet-rich plasma (PRP), adjusted to $2.5\times10^8$ cells/mL with autologous platelet-poor plasma, was used to assess turbidimetric platelet aggregation induced by 2 μg/mL collagen (Chrono-log Corp., Havertown, PA) in a dual-channel aggregometer (Model 460VS, Chrono-log Corp.) as previously described.[28] As a

functional measure of residual platelet COX-1 activity in these patients treated with aspirin, we compared the extent of collagen-induced aggregation in PRP with or without pre-incubation with the highly selective thromboxane receptor antagonist SQ 29,548 (10 μM final concentration; Cayman Chemical, Ann Arbor, MI). These measurements were added to the protocol mid-study but then were performed on all subsequent patients except when difficulties with phlebotomy precluded aggregation studies.

### Statistical Analysis

Data are expressed as median (interquartile range [IQR]) or frequency (percentage). For bivariate group comparisons, Wilcoxon rank-sum tests were used for continuous data and chi-square or Fisher's exact tests were used for categorical data. Spearman rank correlations were used to study relationships between pairs of continuous variables. Linear models were used to study the relationships between log-$sTxB_2$ and aspirin formulation, age, sex, smoking status, platelet count, BMI, and metabolic syndrome (or each of its component criteria). In addition, a linear model was used to study the association between sTxB2 and residual platelet function as measured by the difference in collagen-induced aggregation with or without the addition of SQ 29,548. Analyses were conducted by using R 2.10.1 (r-project.org). Two-sided p-values less than 0.05 were considered statistically significant.

## Results

### Study Population

Clinical characteristics for the patients included in the analysis are shown in Table 1. 28% had type 2 diabetes mellitus, and all but one patient carried a diagnosis of hypertension. Metabolic syndrome was present in 83 (61%) of the 135 patients. These patients were younger, had higher blood pressure at baseline, were more likely to have diabetes, and had larger median BMI and waist circumference. In addition, lower HDL, higher triglycerides, higher fasting glucose, and higher platelet count accompanied the metabolic syndrome (Table 1). Patients with metabolic syndrome were more likely to be taking antidiabetic drugs, but otherwise the use of medications was similar between those with and without metabolic syndrome (Table S1, http://hyper.ahajournals.com). There were no significant differences in clinical characteristics between patients in the randomized and nonrandomized cohorts.

### Metabolic Syndrome Associates with Inadequate Inhibition of Platelet Cyclooxygenase

To determine whether metabolic syndrome associates with apparent biochemical resistance of cyclooxygenase to aspirin, $sTxB_2$ was compared across metabolic syndrome status. Serum $TxB_2$ was systematically higher among the 83 patients with metabolic syndrome than among the 53 patients without (median 3.98 ng/mL [IQR 2.52–8.46 ng/mL] *vs.* 3.02 ng/mL [1.55–5.92 ng/mL], P=0.013) (Fig. 1).

It is generally accepted that at least 95% inhibition of platelet cyclooxygenase is required for aspirin to confer a significant antiplatelet effect.[6, 29, 30] Because we could not ethically discontinue aspirin in these patients with known CAD for this study, we estimated that a serum $TxB_2$ level of 13 ng/ml represented 95% inhibition of platelet COX based on the mean $sTxB_2$ in 83 healthy volunteers. Biochemical resistance to aspirin ($sTxB_2 \geq 13$ ng/ml) was significantly more common among patients with metabolic syndrome than without. In the absence of the metabolic syndrome, no patient had a $sTxB_2$ level ≥ 13 ng/mL after 2 weeks of monitored therapy whereas 12 (14%) patients with metabolic syndrome had $sTxB_2$ levels above this threshold (P=0.003).

Because setting an absolute $sTxB_2$ threshold to define biochemical resistance to aspirin could be considered arbitrary, log-transformed $sTxB_2$ was analyzed as a continuous variable in a linear regression model. Metabolic syndrome significantly associated with higher $sTxB_2$ even after adjusting for age, sex, smoking history, platelet count, and aspirin formulation (P=0.006). Replacing the metabolic syndrome variable with each of its component criteria in four otherwise identical models (hypertension could not be analyzed given its 99% prevalence in this cohort) demonstrated that none of the individual components significantly associated with $sTxB_2$ when considered alone (Table 2). The diabetic patients had higher $sTxB_2$ levels than non-diabetic patients (median 4.47 ng/mL [IQR 2.79–10.11 ng/mL] vs. 3.55 ng/mL [2.11–6.35 ng/mL]; P=0.061); although this difference was not statistically significant, this study was not powered for this comparison. With the adjustment of age, sex, smoking history, platelet count, and aspirin formulation in the linear regression model, the association of metabolic syndrome was significant (P=0.011) and the association of diabetes was not significant (P=0.82) (Fig. S1, http://hyper.ahajournals.com).

To further investigate the contributions of BMI and metabolic syndrome to inadequate suppression of cyclooxygenase, $sTxB_2$ levels were compared across metabolic syndrome stratified by BMI category. Serum TxB2 levels ≥ 13 ng/mL were only observed among patients who were both overweight/obese (BMI ≥ 25 $kg/m^2$) *and* had metabolic syndrome (16% of this population). Conversely, the 9 patients with metabolic syndrome and normal BMI (< 25 $kg/m^2$) had well-suppressed $sTxB_2$ levels (Fig. 2). Taken together, these data suggest that the metabolic syndrome associates with higher $sTxB_2$ levels among patients with CAD receiving aspirin 81mg daily, with 14% (95% CI: 7% – 22%) elevated to levels generally considered to reflect biochemical resistance to aspirin. This resistance is even more common among those who have metabolic syndrome and are also overweight/obese.

Because higher gastric pH could reduce the bioavailability of aspirin, we examined whether concomitant use of proton pump inhibitors (PPIs), $H_2$ antagonists, or antacids associated with higher levels of $sTxB_2$. Of the 37 patients taking PPIs, 3 (8%) had $sTxB_2$ > 13 ng/mL compared with 9 (9%) of the 98 patients not taking PPIs. Similarly, the use of neither $H_2$ antagonists nor antacids associated with $sTxB_2$ > 13 ng/mL. Additional data regarding concomitant medications are summarized in Table S2 (see http://hyper.ahajournals.com).

## Functional Consequence of Incomplete $sTxB_2$ Inhibition

The functional consequence of incomplete inhibition of $sTxB_2$ was evaluated with light-transmission aggregometry. The extent of collagen-induced aggregation was compared with and without pre-incubation of the platelet-rich plasma with the highly selective thromboxane receptor antagonist SQ 29,548 (“SQ”). In the setting of complete inhibition of platelet COX-1 by aspirin, pre-incubation with SQ does not attenuate collagen-induced aggregation further, producing a “without SQ – with SQ” difference of 0. In the setting of incomplete inhibition of platelet COX-1 by aspirin, however, pre-incubation with SQ attenuates collagen-induced aggregation, producing a “without SQ – with SQ” difference greater than 0. By this functional measure, patients with sTxB2 ≥ 13 ng/mL had significantly greater residual platelet function (P=0.02) (Fig. 3). Furthermore, simple linear regression suggested that a 1 ng/mL increase in sTxB2 is associated with a 1.14 absolute percentage point increase in difference between collagen-induced aggregation without and with SQ pre-incubation, on average (P<0.0001).

## Enteric-coated Aspirin Affects Suppression of $sTxB_2$ by Aspirin

The effect of enteric-coating on the inhibition of $sTxB_2$ by aspirin was studied in a randomized subgroup of the patients. Serum $TxB_2$ levels were systematically higher and exhibited greater variability among patients randomized to enteric-coated aspirin compared

with those randomized to immediate-release aspirin (median 5.02 ng/mL [IQR 3.36–7.86 ng/mL] *vs.* 2.78 ng/mL [1.60–4.76]; P=0.005) (Fig. S2, http://hyper.ahajournals.com). Despite randomization, the median age was slightly lower and the median waist circumference was higher in the group receiving enteric-coated aspirin. Adjusting for these potential confounders in a linear model of log-transformed $sTxB_2$, enteric-coated aspirin remained associated with higher $sTxB_2$ levels (P=0.030).

### Urinary 11-dehydrothromboxane $B_2$ does not correlate with $sTxB_2$

Because $sTxB_2$ is the most direct measure of the pharmacological effect of aspirin, we compared each patient's $sTxB_2$ level with their urinary Tx-M to determine the extent to which Tx-M predicted inhibition of platelet COX-1. Urinary Tx-M did not correlate with sTxB2 in this population (Spearman's $\rho$=0.04, P=0.63) (Fig. 4).

## Discussion

The effect of aspirin on platelet COX-1 is substantially and significantly diminished in patients with the metabolic syndrome. Twelve (14%) patients with metabolic syndrome, but none without metabolic syndrome, had $sTxB_2$ levels consistent with inadequate inhibition of COX. These 12 poorest responders also were overweight/obese, constituting 16% of that BMI subgroup.

Several abnormalities in *ex vivo* platelet function have been demonstrated in patients with metabolic syndrome who are not on aspirin. Closure time in the flow-based clotting system, PFA-100®, is prolonged,[31] platelets exhibit increased P-selectin expression in response to ADP,[32] and aggregation in response to ADP, collagen and arachidonic acid is modestly but significantly increased.[33] Increased platelet surface expression of P-selectin and GP IIb/IIIa has been demonstrated,[31, 34] as well as elevated levels of circulating soluble P-selectin, soluble CD40 ligand,[35, 36] and conjugates of leukocytes with platelets and/or platelet microparticles.[34] Increased numbers of reticulated platelets suggest accelerated platelet turnover.[32] Furthermore, platelet count is higher in metabolic syndrome as demonstrated by both Jesri *et al.*[37] and the present study.

Increased *in vivo* platelet activation in metabolic syndrome can be hypothesized based on several observations. Platelet activation could be a basis for impaired acetylation of platelet COX-1 by aspirin. The biosynthesis of peroxides (*e.g.,* 12-HPETE, $PGG_2$, and peroxynitrite) during platelet activation would lead to redox cycling of COX-1, which we have shown to inhibit acetylation of COX-1 by aspirin.[5] Moreover, increased platelet turnover resulting from abnormal platelet activation *in vivo* would generate more young platelets with active COX-1 between the aspirin dosing interval, thus impairing accumulation of the effect of low-dose aspirin. If it can be shown that increased platelet activation and suboptimal effect of aspirin are associated in the same population, this would mean that aspirin's therapeutic effect is impaired in the patients who need it most. In this regard, it is of note that aspirin produces only a slight and non-significant reduction in thrombotic cardiovascular events in patients with diabetes, many of whom have metabolic syndrome.[1, 38, 39]

Although a pharmacokinetic effect of obesity is a conceivable basis for increased $sTxB_2$ levels in aspirin-treated patients with metabolic syndrome, direct evidence is elusive given the substantial acetylation of platelet COX-1 in the portal circulation and the extent of first-pass metabolism. Moreover, our data indicate that waist circumference, the parameter that measures central obesity, does not alone explain the relation of metabolic syndrome to elevated $sTxB_2$ (Table 2).

This represents the first evidence that the effect of aspirin on its pharmacologic target, platelet COX-1, is reduced in metabolic syndrome. Previously, investigations have found that inhibition of the function of platelets by aspirin is diminished in metabolic syndrome.[32, 33, 40, 41] However, platelet function assays (*e.g.,* ADP- and collagen-induced aggregation, PFA-100®, and VerifyNow®) indirectly measure the net effects of both thromboxane-dependent and thromboxane-independent pathways. Although these tests may provide information about platelet reactivity, they cannot determine the success or failure of aspirin to inhibit platelet COX-1 in an individual patient.[6] Indeed, a major predictor of hyper-function of platelets during aspirin treatment is elevated function in the absence of aspirin,[42] which is clearly the case in metabolic syndrome; therefore, it is not surprising that multiple studies have suggested that platelet reactivity in patients receiving aspirin is a harbinger of increased cardiovascular risk.[22, 43–45] It cannot be inferred that failure of aspirin to acetylate platelet COX-1 is the principal cause of this increased risk but COX-1-dependent residual platelet activity is certainly a contributor.[17]

The most effective treatment strategy for patients with metabolic syndrome who have an impaired response to low-dose aspirin remains to be determined. Certainly, an increase in the dose of aspirin is a consideration, and evidence supports an advantage of twice-daily dosing to compensate for the accelerated rate of entry of platelets with unacetylated platelet COX that results from the increased platelet turnover in diabetes[39, 46] and metabolic syndrome.[32] Higher doses, however, also would further inhibit prostacyclin biosynthesis,[30] which may be deleterious and could increase risk for gastrointestinal bleeding. Further research must identify the optimal anti-platelet therapy for patients with metabolic syndrome in whom aspirin's pharmacologic effect is suboptimal.

Enteric-coated formulations have several potential pharmacokinetic disadvantages: there is significant variability in the pill-coating process, possibly affecting bioavailability; delivery of aspirin to the more alkaline small bowel increases the likelihood of intra-intestinal deacetylation; and slower absorption allows for more efficient first-pass hepatic clearance. In healthy volunteers, Cox *et al.* demonstrated that 75-mg enteric-coated preparations had higher median $sTxB_2$ than a 75-mg immediate-release preparation.[16] The reduction in aspirin effect also varied between the different enteric-coated formulations, prompting us to study the McNeil 81-mg enteric-coated formulation in a target population for the drug, patients with CAD. We found that this formulation also has a diminished and more variable effect on platelet COX-1 compared with an immediate-release formulation. The cumulative evidence indicating a reduced pharmacological effect of enteric-coated formulations is cogent in light of the fact that the basis for the use of low-dose aspirin for prevention of thrombotic cardiovascular disease largely derives from a meta-analysis that comprised studies employing immediate-release formulations with the exception of two studies that used a 100-mg enteric-coated preparation.[1] Thus, no evidence exists to support the use of 81-mg enteric-coated aspirin to prevent cardiovascular events.

Interest in the possibility that urinary Tx-M could mark suboptimal suppression of platelet $TxA_2$ biosynthesis by aspirin followed the demonstration that higher levels of urinary Tx-M associate with an increased risk for cardiovascular events in a high-risk population.[22] Multiple subsequent studies used urinary Tx-M as an indicator of "aspirin resistance,"[45] even though urinary Tx-M could indicate resistance to aspirin's pharmacological effect, noncompliance, or non-platelet sources of thromboxane. For example, we found that 22% of Tx-M derives from COX-2 among smokers.[23] In healthy volunteers, Tx-M has not been found to correlate with $sTxB_2$.[42] In the present study of patients with CAD, we did not detect a correlation between these two measures, indicating that Tx-M is not a reliable biomarker of suboptimal inhibition of platelet COX-1 by aspirin. The concerted evidence,

therefore, indicates that urinary Tx-M should not be used to interpret whether a patient is resistant to the pharmacological effects of aspirin.

In conclusion, metabolic syndrome strongly and uniquely associates with suboptimal inhibition of platelet COX-1 by aspirin. The increasing prevalence of the metabolic syndrome and its association with greater risk for cardiovascular events highlight the importance of optimizing anti-platelet therapy to reduce cardiovascular risk for these patients.

## Perspective

In patients with metabolic syndrome, platelet hyperactivity likely contributes to the risk for acute coronary syndrome. This emphasizes the need for effective anti-platelet therapy in the very patients in whom we found the effect of low-dose aspirin to be suboptimal. Inhibition of platelet cyclooxygenase by aspirin may be substantially inadequate in approximately 14% of patients with metabolic syndrome and coronary artery disease. Because a growing proportion of the 18 million patients in the United States with coronary artery disease have metabolic syndrome, this suggests that hundreds of thousands of patients may be receiving inadequate treatment for their hyperactive platelets.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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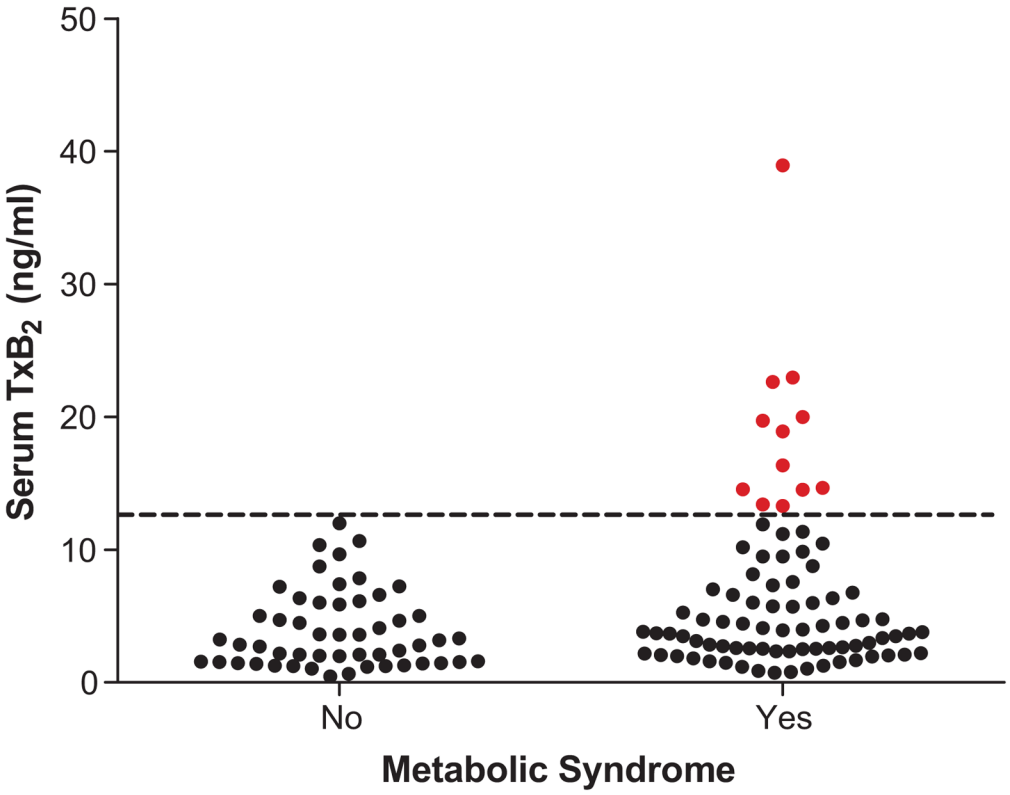


**Figure 1.**
Comparison of serum $TxB_2$ levels between patients with and without metabolic syndrome (P=0.013), all of whom had known coronary artery disease. The dashed line (13 ng/mL) represents an estimate of 95% inhibition of cyclooxygenase.

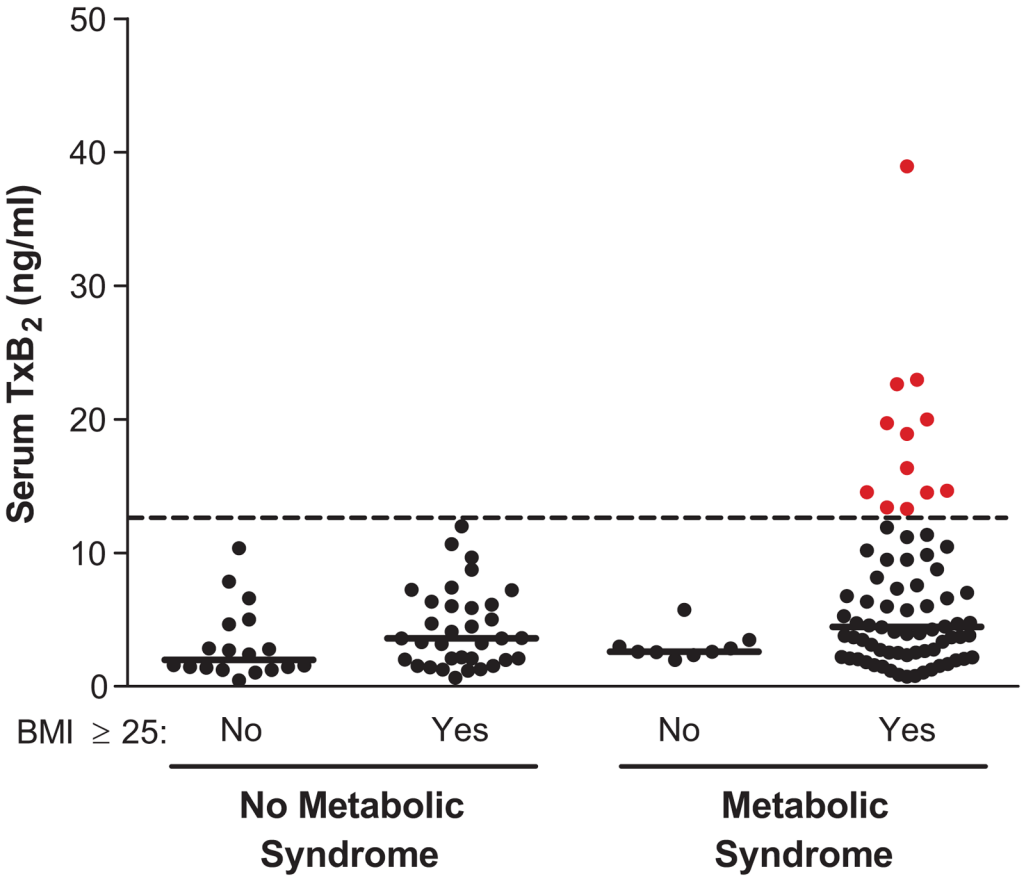


**Figure 2.**
Serum $TxB_2$ levels by body mass index (BMI) across metabolic syndrome (MetSyn) status. Solid lines indicate medians. No significant differences were detected between BMI categories among patients with or without metabolic syndrome (P=0.08 and P=0.11, respectively).

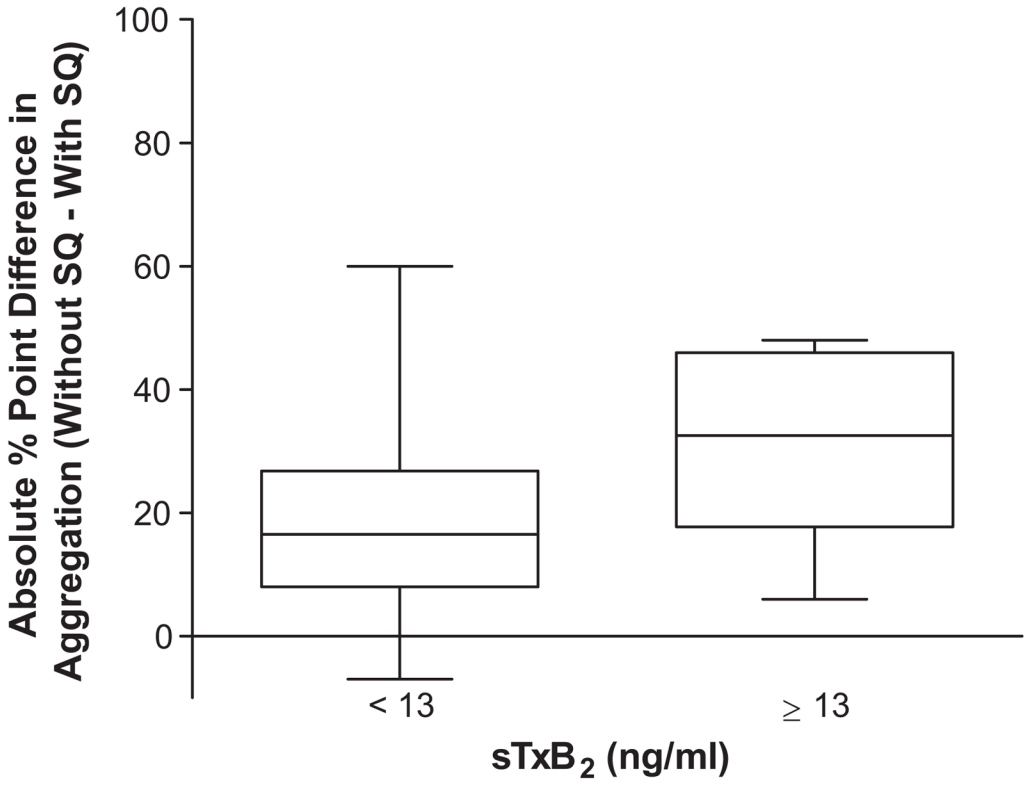


**Figure 3.**
Positive values of "without SQ – with SQ" suggest greater residual thromboxane receptor-mediated platelet aggregability. The bounds of the boxes indicate the 1st and 3rd quartiles; the line within the box indicates the median.

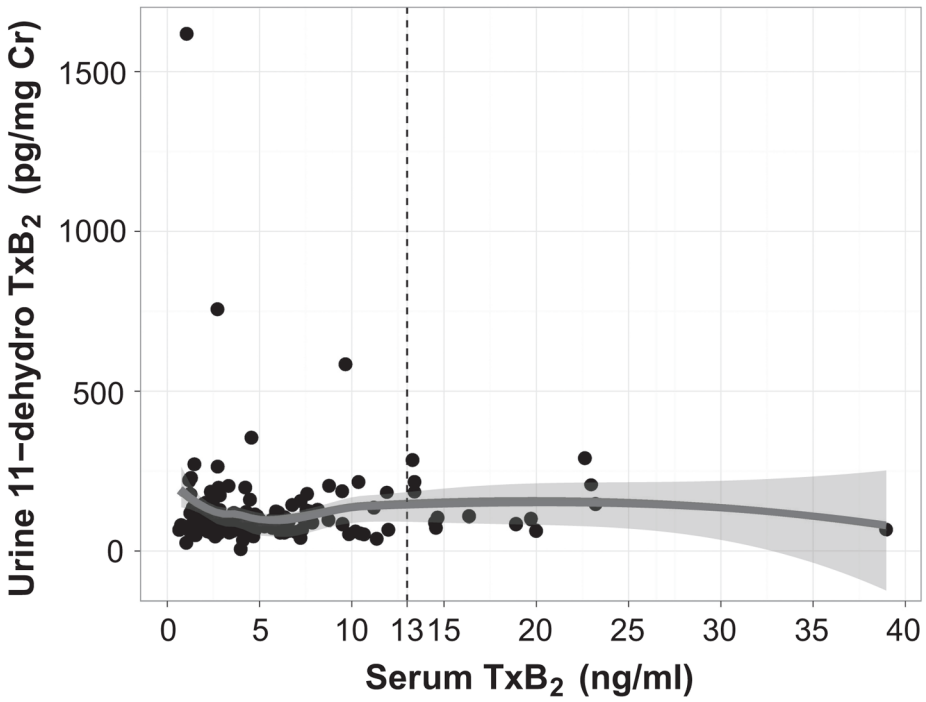


**Figure 4.**
Serum TxB2 did not correlate with urinary 11-dehydrothromboxane $B_2$ among patients with coronary artery disease taking aspirin 81mg daily (Spearman's $\rho$=0.04, P=0.63).

**Table 1**

Demographics of Study Population

| | All Subjects (N=135) | | | Randomized Cohort Only (N=92) | |
|---|---|---|---|---|---|
| **Patient Characteristic** | **MetSyn (N=83)** | **No MetSyn (N=52)** | **P*** | **Immediate-release (N=47)** | **Enteric-coated (N=45)** |
| Age (y) | 64[58, 71] | 68[61, 76] | 0.007 | 68[59, 75] | 62[56, 69] |
| Male sex | 58 (70%) | 36 (69%) | 0.94 | 33 (70%) | 33 (73%) |
| Race – White | 69 (83%) | 49 (94%) | 0.082 | 42 (89%) | 36 (80%) |
| Black | 13 (16%) | 2 (4%) | | 4 (9%) | 8 (18%) |
| Other | 1 (1%) | 1 (2%) | | 1 (2%) | 1 (2%) |
| SBP (mmHg) | 126[119,136] | 117[110, 131] | 0.008 | 126[112, 133] | 124[116, 136] |
| DBP (mmHg) | 74[70, 80] | 70[62, 78] | 0.003 | 72[64, 78] | 74[70, 80] |
| Diabetes | 33 (40%) | 5 (10%) | <0.001 | 9 (19%) | 12 (27%) |
| Hypertension | 83 (100%) | 51 (98%) | 0.21 | 47 (100%) | 45 (100%) |
| Current or Former Smoker | 52 (63%) | 26 (50%) | 0.15 | 33 (70%) | 24 (53%) |
| BMI ($kg/m^2$) | 31.2[27.7, 34.6] | 26.2[23.7, 29.2] | < 0.001 | 28.8[25.8, 32.9] | 30.3[27.3, 33.3] |
| Waist circumference (cm) | 109.2[100.3, 118.1] | 99.1[90.8, 105.7] | < 0.001 | 100.3[94.0, 111.8] | 108.0[99.1, 114.3] |
| Total cholesterol (mmol/L) | 3.67[3.23, 4.19] | 3.74 [3.43, 4.24] | 0.28 | 3.67 [3.34, 4.09] | 3.67 [3.41, 3.98] |
| LDL (mmol/L) | 1.99[1.63, 2.28] | 1.89 [1.68, 2.33] | 0.82 | 1.84 [1.66, 2.28] | 1.99 [1.55, 2.30] |
| HDL (mmol/L) | 0.98[0.83, 1.22] | 1.29 [1.11, 1.62] | < 0.001 | 1.11 [0.93, 1.40] | 1.03 [0.91, 1.32] |
| Triglycerides (mmol/L) | 1.22[1.00, 2.05] | 1.03 [0.76, 1.26] | < 0.001 | 1.14 [0.86, 1.66] | 1.14 [0.91, 1.60] |
| Platelet count ($x10^3/mL$) | 223[190, 256] | 184[168, 219] | 0.0002 | 191[176, 244] | 222[184, 243] |
| Fasting glucose (mmol/L) | 5.94[5.44, 6.94] | 5.16 [4.88, 5.44] | < 0.0001 | 5.50 [5.17, 6.22] | 5.55 [5.11, 6.16] |
| Metabolic Syndrome | - | - | - | 27 (57%) | 30 (67%) |
| Enteric-coated formulation | 56 (67%) | 32 (62%) | 0.48 | - | - |

Values are median[IQR] or counts (column %). MetSyn = metabolic syndrome; SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index; LDL = low-density lipoprotein; HDL = high-density lipoprotein.

*P values compare patients with and without metabolic syndrome.

## Table 2

Comparison of metabolic syndrome with its components in separate multivariable linear models of log-transformed $sTxB_2$

| Model* | Estimate | P |
|---|---|---|
| Model 1: Metabolic Syndrome† | 0.441 | 0.006 |
| Model 2: Low HDL‡ | 0.172 | 0.26 |
| Model 3: Elevated triglycerides§ | 0.118 | 0.52 |
| Model 4: Elevated fasting glucose// | 0.244 | 0.09 |
| Model 5: Elevated waist circumference¶ | 0.282 | 0.09 |

*In addition to the variable listed, all models adjust for age, sex, smoking status (current/former vs. never), platelet count, and aspirin formulation (enteric-coated vs. immediate-release). The P value corresponds to the listed variable in the model.

†AHA/NHLBI criteria.[24]

‡HDL < 1.03 mmol/L (40mg/dL) for men, < 1.29 mmol/L (50 mg/dL) for women.

§Triglycerides ≥ 1.69 mmol/L (150 mg/dL)

//Fasting glucose ≥ 5.56 mmol/L (100 mg/dL) or diagnosis of diabetes

¶Waist circumference ≥ 102 cm (40 inches) for men, ≥ 88 cm (35 inches) for women