Platelet Isoprostane Overproduction in Diabetic Patients Treated With Aspirin

Roberto Cangemi,1 Pasquale Pignatelli,1 Roberto Carnevale,1 Carmen Nigro,1 Marco Proietti,1 Francesco Angelico,1 Davide Lauro,2 Stefania Basili,1 and Francesco Violi1

Aspirin modestly influences cardiovascular events in patients with type 2 diabetes mellitus (T2DM), but the reason is unclear. The aim of the study was to determine whether in T2DM patients aspirin enhances platelet isoprostanes, which are eicosanoids with proaggregating properties derived from arachidonic acid oxidation by platelet NOX2, the catalytic subunit of reduced NAD phosphate oxidase. A cross-sectional study was performed comparing T2DM patients, treated (n = 50) or not treated (n = 50) with 100 mg/day aspirin, with 100 nondiabetic patients, matched for age, sex, atherosclerosis risk factors, and aspirin treatment. A short-term (7 days) treatment with 100 mg/day aspirin also was performed in 36 aspirin-free diabetic and nondiabetic patients. Higher platelet recruitment, platelet isoprostane, and NOX2 activation was found in diabetic versus nondiabetic patients and in aspirin-treated diabetic patients versus nontreated patients (P < 0.001). Platelet thromboxane (Tx) A2 (P < 0.001) was inhibited in all aspirin-treated patients. In the interventional study, aspirin similarly inhibited platelet TxA2 in diabetic and nondiabetic patients (P < 0.001). Platelet recruitment, isoprostane levels, and NOX2 activation showed a parallel increase in diabetic patients (P < 0.001) and no changes in nondiabetic patients. These findings suggest that in aspirin-treated diabetic patients, oxidative stress-mediated platelet isoprostane overproduction is associated with enhanced platelet recruitment, an effect that mitigates aspirin-mediated TxA2 inhibition. Diabetes 61:1626–1632, 2012

A ccelerated atherosclerosis is a typical feature of type 2 diabetes mellitus (T2DM). Thus, patients with T2DM have a two- to fourfold increased risk of cardiovascular diseases (coronary artery disease) and a two- to sixfold increased risk of stroke (1–3).

Platelets play a major role in the etiology of atherosclerotic disease, as shown by the significant decrease of cardiovascular events in patients treated with aspirin, an inhibitor of cyclooxygenase (COX1) that prevents platelet thromboxane (Tx) A2 formation (4,5). Platelet TxA2 overproduction, combined with a significant decrease after aspirin administration, has been demonstrated in diabetic patients (6). Despite this, interventional trials with aspirin in diabetic patients failed to show a beneficial effect in primary prevention; reasons for this lack of response still are uncertain (7).

Isoprostanes are a family of eicosanoids derived from arachidonic acid interaction with reactive oxidant species (ROS). Thus, ROS generated by NOX2, the catalytic subunit of reduced NAD phosphate (NADPH) oxidase, play a crucial role in platelet isoprostane formation (8). In contrast to TxA2, isoprostanes are chemically stable compounds that serve to propagate platelet activation, amplifying platelet response to common agonists via glycoprotein (Gp)IIb/IIIa activation (9). Accordingly, patients with hereditary deficiency of NOX2 showed impaired isoprostane formation and GpIIb/IIIa activation, as well as a subnormal propagation of platelet thrombus (9).

It previously has been demonstrated that COX1 inhibition determines a shift in arachidonic acid metabolism toward other pathways, such as the lipooxygenase system (10). We speculated that COX1 inhibition also could be associated with an increased conversion of arachidonic acid to isoprostanes in platelets. The increase of platelet isoprostanes would counterbalance the inhibition of TxA2, therefore hampering the antiplatelet effect of aspirin. To explore this hypothesis, we performed a cross-sectional study comparing the behavior of platelet isoprostanes and TxA2 and their interplay with platelet NOX2 in diabetic and nondiabetic patients treated or not with aspirin. Platelet activation tests, including arachidonic acid–induced platelet aggregation, which is dependent upon TxA2 and their interplay with platelet NOX2 in diabetic and nondiabetic patients treated or not with aspirin. Platelet activation tests, including arachidonic acid–induced platelet aggregation, which is dependent upon TxA2 and their interplay with platelet NOX2 in diabetic and nondiabetic patients treated or not with aspirin. Platelet activation tests, including arachidonic acid–induced platelet aggregation, which is dependent upon TxA2 and their interplay with platelet NOX2 in diabetic and nondiabetic patients treated or not with aspirin. Platelet activation tests, including arachidonic acid–induced platelet aggregation, which is dependent upon TxA2 and their interplay with platelet NOX2 in diabetic and nondiabetic patients treated or not with aspirin. Platelet activation tests, including arachidonic acid–induced platelet aggregation, which is dependent upon TxA2 and their interplay with platelet NOX2 in diabetic and nondiabetic patients treated or not with aspirin. Platelet activation tests, including arachidonic acid–induced platelet aggregation, which is dependent upon TxA2 and their interplay with platelet NOX2 in diabetic and nondiabetic patients treated or not with aspirin. Platelet activation tests, including arachidonic acid–induced platelet aggregation, which is dependent upon TxA2 and their interplay with platelet NOX2 in diabetic and nondiabetic patients treated or not with aspirin. Platelet activation tests, including arachidonic acid–induced platelet aggregation, which is dependent upon TxA2 and their interplay with platelet NOX2 in diabetic and nondiabetic patients treated or not with aspirin. Platelet activation tests, including arachidonic acid–induced platelet aggregation, which is dependent upon TxA2 and their interplay with platelet NOX2 in diabetic and nondiabetic patients treated or not with aspirin. Platelet activation tests, including arachidonic acid–induced platelet aggregation, which is dependent upon TxA2 and their interplay with platelet NOX2 in diabetic and nondiabetic patients treated or not with aspirin. Platelet activation tests, including arachidonic acid–induced platelet aggregation, which is dependent upon TxA2 and their interplay with platelet NOX2 in diabetic and nondiabetic patients treated or not with aspirin. Platelet activation tests, including arachidonic acid–induced platelet aggregation, which is dependent upon TxA2 and their interplay with platelet NOX2 in diabetic and nondiabetic patients treated or not with aspirin.

RESEARCH DESIGN AND METHODS

Study design

Cross-sectional study. The study was performed in consecutive T2DM patients attending our metabolic outpatient clinic who were taking (n = 50) or not taking (n = 50) low-dose (100 mg/day) aspirin. T2DM was diagnosed according to the American Diabetes Association definition (12). As a control group, we selected nondiabetic outpatients taking (n = 50) or not taking (n = 50) low-dose aspirin who were matched to the diabetic group in terms of age, sex, and history of vascular disease.

Low-dose aspirin treatment was defined as a self-reported daily intake of 100 mg acetylsalicylic acid at least in the previous month. Exclusion criteria were 1) recent history (<3 months) of acute vascular events, 2) clinical diagnosis of type 1 diabetes (diagnosis of diabetes and insulin use before the age of 35 years), 3) serum creatinine level >2.5 mg/dL, 4) active infection or malignancy, 5) cardiac arrhythmia or congestive heart failure, and 5) use of nonsteroidal anti-inflammatory drugs, vitamin supplements, or other antiplatelet drugs, such as clopidogrel, in the previous 30 days. All participants provided written informed consent. The local ethical committee approved the study protocol (approval no. Prot. 403/09-Rif. 1621/07.05.09). Diabetic patients received different antidiabetes treatments: metformin (n = 57), subcutaneous insulin (n = 25), sulfonylureas (n = 14), glinides (n = 6), glitazones (n = 3), and dipeptidyl peptidase-4 inhibitor (n = 16).

Interventional study. We tested the effect of short-term treatment with 100 mg/day aspirin in diabetic (n = 18) and nondiabetic (n = 18) patients, not currently under aspirin treatment and with no clinical history of vascular diseases, who were matched for sex, age, and atherosclerotic risk factors. Aspirin was given after dinner between 8:00 and 8:30 P.M., and adherence was assessed by the pill-count method. Blood samples were collected before

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aspirin ingestion and after 3 and 7 days of treatment. The study was registered in August 2010 at clinicaltrials.gov (clinical trial reg. no. NCT01250340).

Laboratory analyses. All materials were from Sigma-Aldrich, unless otherwise specified. Blood analyses were performed in a blinded manner. All blood samples were taken after a 12-h fast. Between 8:00 and 9:00 a.m., subjects underwent routine biochemical evaluations, including fasting total cholesterol and glucose.

**Serum and platelet-poor plasma sampling.** After overnight fasting (12 h) and supine rest for at least 10 min, blood samples were taken into tubes containing either 3.8% sodium citrate (ratio 9:1) or anticoagulant-free tubes and centrifuged at 3000 for 10 min to obtain supernatant (plasma or serum), which was stored at −80°C until use.

**Platelet-rich plasma preparation.** To obtain platelet-rich plasma (PRP), samples were centrifuged for 15 min at 180g. To avoid leukocyte contamination, only the top 75% of the PRP was collected; leukocyte contamination was verified as reported below.

**Washed-platelet procedure.** Platelet pellets were obtained by double centrifugation (2 × 5 min, 300g) of PRP after the addition of acid/citrate/dextrose (1:7 vol/vol) to avoid cell activation during processing. Samples were suspended in HEPES buffer in the presence of 0.1% albumin, pH 7.35 (2 × 10⁸ platelets/mL, unless otherwise noted).

**Platelet sizing.** To assess if there were differences between T2DM patients and control subjects in platelet size and function, we stratified in two portions (50% upper phase, 50% lower phase) the top 75% of the PRP. After the washing procedure of the two portions, we analyzed TXA₂ and 8-iso-PGF₂α-III production.

**Cytometric analysis of platelets and leucocytes.** Leukocyte content in the top 75% of the PRP was analyzed using the specific fluorochrome isothiocyanate–labeled monoclonal antibody (Mab) anti-CD4 (BD International). Platelets were analyzed using the specific phycoerythrin-labeled monoclonal antibodies anti-CD61 (Mab) (BD International).

All assays included samples to which an irrelevant isotype-matched antibody (fluorescein isothiocyanate–labeled monoclonal antibody (Mab) anti-CD4) (BD International). Platelets were analyzed using the specific phycoerythrin-labeled monoclonal antibodies anti-CD61 (Mab) (BD International).

All materials were from Sigma-Aldrich, unless otherwise specified. Blood analyses were performed in a blinded manner. All blood samples were taken after a 12-h fast. Between 8:00 and 9:00 a.m., subjects underwent routine biochemical evaluations, including fasting total cholesterol and glucose.

**Platelet NOX2, TXA₂, 8-iso-PGF₂α-III, and ROS.** The platelet isoprostane 8-iso-PGF₂α-III was measured by the enzyme immunoassay (EIA) procedure described by Pignatelli et al. (13). The peptide was rec-}
TABLE 1

Clinical and anthropometric characteristics of study participants

<table>
<thead>
<tr>
<th></th>
<th>Diabetic patients (n = 100)</th>
<th>Nondiabetic patients (n = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ASA treatment</td>
<td>Yes (n = 50)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>66 ± 10</td>
<td>67 ± 14</td>
</tr>
<tr>
<td>Smoking habit (%)</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.1 ± 4.1</td>
<td>27.7 ± 4.2</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>192.8 ± 63</td>
<td>192.8 ± 63</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>51.5 ± 50.8</td>
<td>51.5 ± 50.8</td>
</tr>
<tr>
<td>Previous coronary artery disease (%)</td>
<td>31%</td>
<td>16%</td>
</tr>
<tr>
<td>Previous stroke (%)</td>
<td>8%</td>
<td>8%</td>
</tr>
<tr>
<td>Arterial hypertension (%)</td>
<td>86%</td>
<td>76%</td>
</tr>
<tr>
<td>8-iso-PGF2α (pmol/L)</td>
<td>7.1 ± 6.2</td>
<td>7.1 ± 6.2</td>
</tr>
<tr>
<td>TxA2 (pmol/L)</td>
<td>134 ± 82</td>
<td>134 ± 82</td>
</tr>
<tr>
<td>SPM (pmol/L)</td>
<td>27.7 ± 19</td>
<td>27.7 ± 19</td>
</tr>
<tr>
<td>Lipoprotein (A) (µg/mL)</td>
<td>2.15 ± 1.21</td>
<td>2.15 ± 1.21</td>
</tr>
</tbody>
</table>

Data are means ± SD, unless otherwise indicated. ASA, aspirin; TxA2, thromboxane A2; platelet TxA2, platelet TxA2 levels; SPM, 2-series lipoyzyloxyoctanes; HbA1c, hemoglobin A1c; 8-iso-PGF2α, 8-iso-prostaglandin F2α; FXA2, thromboxane A2 receptor; ANOVA, analysis of variance.

**Correlation analysis.** In the overall population, platelet isoprostanes significantly correlated with platelet recruitment (r = 0.43, P < 0.001) and sNOX2-dp (r = 0.49, P < 0.001). To further define the predictors of the platelet isoprostane level, multiple regression analysis including clinical and laboratory characteristics, presence of diabetes, and concomitant use of aspirin and statins was performed. Stepwise linear regression yielded a model in which only T2DM (β = 0.276, P < 0.001), aspirin treatment (β = 0.158, P = 0.009), and sNOX2-dp (β = 0.229, P < 0.001) predicted platelet isoprostane levels, independently from the other included variables.

**Interventional study.** Clinical and anthropometric characteristics of interventional study participants are reported in Table 2.

At baseline, platelets from diabetic and nondiabetic patients showed similar platelet TxB2 values (Fig. 2) and platelet aggregation percentages (94 ± 2.2 vs. 97 ± 3 LT %; P = 0.640). Conversely, platelet recruitment, platelet isoprostanes, platelet ROS, and sNOX2-dp were significantly higher in diabetic compared with nondiabetic patients. Platelet TxB2 formation (Fig. 2A) and platelet aggregation (not shown) were significantly inhibited after aspirin intake in both groups. Platelet isoprostanes, sNOX2-dp, platelet ROS, and platelet recruitment increased after aspirin intake only in diabetic patients (Fig. 2).

MANOVA analysis showed a significant influence of aspirin treatment in diabetic patients on platelet isoprostanes (F = 11.9, P < 0.001), platelet ROS production (F = 171.6, P < 0.001), sNOX2-dp (F = 32.5, P < 0.001), and platelet recruitment (F = 9.6, P < 0.001). No significant interaction with covariates was found. Incubation of platelets from aspirin-treated diabetic patients with the NOX2-blocking peptide was associated with a significant inhibition of ROS (−3.3 S.I. at 3 days, P = 0.006, and −7.9 S.I. at 7 days, P = 0.003, respectively) and platelet isoprostane (−134 pmol/L at 3 days, P < 0.001, and −137 pmol/L at 7 days, P = 0.005) production. Conversely, in aspirin-treated nondiabetic patients, NOX2-blocking peptide had a less significant effect on platelet ROS (−0.8 stimulation index at 3 days, P = 0.023, and −0.9 S.I. at 7 days, P = 0.021) and isoprostane (−6 pmol/L at 3 days, P = 0.048, and −7 pmol/L at 7 days, P = 0.043) production.

The Tx/isoprostane inhibitor SQ29548 significantly inhibited recruitment of platelets from aspirin-treated diabetic patients but not from aspirin-treated nondiabetic patients (Fig. 2E and F).

**In vitro study.**

**Effect of 8-iso-PGF2α on platelet activation.** TRAP (0.01–10 µmol/L) dose-dependently increased GpIIb/IIIa activation (Fig. 3). Compared with TRAP, GpIIb/IIIa activation by 8-iso-PGF2α (25–350 pmol/L) was weaker and significantly inhibited by platelet coinubation with SQ29548. GpIIb/IIIa activation by ADP was dose dependent; it almost was undetectable with doses <0.1 µmol/L and became detectable with values >1 µmol/L. The incubation of 8-iso-PGF2α (300 pmol/L) with lower ADP doses (0.01–0.1 µmol/L) amplified GpIIb/IIIa activation with values similar to those achieved with higher ADP doses.

**Influence of platelet size in eicosanoids formation.** The top 75% of the PRP of the diabetic and control subjects was stratified in two portions (50% upper and 50% lower phases). Arachidonic acid-induced platelet TxB2 and isoprostanes were measured in both phases. Platelets from diabetic patients were greater in the upper 50% (Fig. 4A...
and B), whereas no difference was detected in the lower phase. The increase in eicosanoids formation between diabetic patients and control subjects persisted in the two phases, suggesting that platelet size was not a determinant in the above-reported differences (Fig. 4C and D).

**Leukocyte contamination.** To ensure the quality of platelet preparation, samples also were tested for leukocyte contamination. The contamination found was <1% in all samples (an exemplificative image of flow cytometric analysis is shown in Fig. 4A and B).

**TABLE 2**
Clinical and anthropometric characteristics of interventional study participants

<table>
<thead>
<tr>
<th></th>
<th>Diabetic patients</th>
<th>Nondiabetic patients</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>18</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>62.3 ± 9.4</td>
<td>61.6 ± 10.1</td>
<td>0.886</td>
</tr>
<tr>
<td>Male (%)</td>
<td>61</td>
<td>56</td>
<td>0.735</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.3 ± 4.3</td>
<td>27.0 ± 2.8</td>
<td>0.789</td>
</tr>
<tr>
<td>Arterial hypertension (%)</td>
<td>77</td>
<td>72</td>
<td>0.700</td>
</tr>
<tr>
<td>Previous coronary artery disease (%)</td>
<td>0</td>
<td>0</td>
<td>1.000</td>
</tr>
<tr>
<td>Previous stroke (%)</td>
<td>0</td>
<td>0</td>
<td>1.000</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>188.1 ± 36.4</td>
<td>186.8 ± 37.2</td>
<td>0.385</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>51.4 ± 16.6</td>
<td>50.8 ± 15.8</td>
<td>0.472</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>144.5 ± 63.2</td>
<td>134.2 ± 51.8</td>
<td>0.239</td>
</tr>
<tr>
<td>Blood glucose (mg/dL)</td>
<td>140.2 ± 35.4</td>
<td>91.5 ± 9.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.1 ± 1.4</td>
<td>5.5 ± 0.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Statin therapy (%)</td>
<td>11</td>
<td>6</td>
<td>0.546</td>
</tr>
<tr>
<td>ACE/angiotensin receptor blockers (%)</td>
<td>67</td>
<td>61</td>
<td>0.729</td>
</tr>
<tr>
<td>Antidiabetes drugs (%)</td>
<td>89</td>
<td>0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are means ± SD, unless otherwise indicated.
DISCUSSION
This study provides evidence that, in diabetic patients treated with low-dose aspirin, platelets overproduce isoprostanes, an effect that is dependent upon enhanced formation of ROS generated by NOX2 activation. Platelet ROS plays a central role in the propagation of platelet aggregation by inactivating platelet nitric oxide, releasing ADP, and producing isoprostanes (9,18). Previous studies
showed that diabetic patients have a systemic isoprostane overproduction (19,20), but the role of platelets has never been examined. The cross-sectional study showed enhanced platelet isoprostane formation and platelet recruitment along with NOX2 activation in diabetic versus nondiabetic patients without aspirin treatment.

Comparing diabetic and nondiabetic patients treated or not with aspirin, a great behavioral difference between the two platelet eicosanoids was detected. Thus, in nondiabetic patients, aspirin inhibited platelet TxB2 without affecting two platelet eicosanoids was detected. Thus, in nondiabetic patients, aspirin treatment showed platelet TxB2 inhibition but a higher isoprostanes production compared with aspirin-untreated diabetic patients. The increase in isoprostane levels was associated with enhanced platelet NOX2 activation. This reinforces the hypothesis that NOX2 is crucial for the ROS overproduction observed in T2DM (21) and suggests a role for NOX2 in platelet isoprostane overproduction in T2DM. Furthermore, our data reinforce previous reports showing that NOX2 upregulation occurs in diabetes not only in the endothelial cells but also in platelets (22). Hyperglycemia is likely to play a major role as also indicated by the direct correlation between HbA1c and sNOX2-dp, but the underlying mechanism still is controversial (23).

The different effect of aspirin on TxB2 and isoprostanes likely accounts for the divergent behavior of platelet function tests in aspirin-treated diabetic patients. Although arachidonic acid–induced platelet aggregation was inhibited, platelet recruitment was enhanced. To further support these findings, we measured platelet TxB2 and isoprostanes in diabetic and nondiabetic patients before and after short-term aspirin treatment. Although both groups disclosed a similar platelet TxB2 inhibition, platelet isoprostanes showed a different behavior. In nondiabetic patients, platelet isoprostane formation was not influenced by aspirin, and in diabetic patients isoprostanes increased 3 and 7 days after aspirin treatment. This change in isoprostane levels was coincidental with enhanced platelet ROS formation and NOX2 activation, suggesting a cause-and-effect relationship between NOX2-generated ROS and platelet isoprostane overproduction. This hypothesis was supported by ex vivo experiments where platelets were incubated with a specific NOX2 inhibitor. The NOX2 inhibitor significantly inhibited platelet ROS and isoprostanes in both diabetic and nondiabetic patients with a reduction of the two variables that was, however, much higher in T2DM patients.

A crucial issue of the study was to establish if platelet isoprostane increase observed after aspirin treatment was functionally relevant in diabetic patients (i.e., it was associated with platelet activation). We found no changes in platelet recruitment in nondiabetic but an increase in diabetic patients. Of note, platelet incubation with the TxA2/isoprostane receptor antagonist prevented platelet recruitment increase in diabetic but not in nondiabetic patients. We speculated that this different behavior could depend upon the 8-iso-PGF2α concentration expressed by platelets from the two groups. To evaluate this, we conducted in vitro experiments to analyze the interplay between isoprostane concentration and GpIIb/IIIa activation (9). A dose-response curve with scalar concentration of 8-iso-PGF2α demonstrated that GpIIb/IIIa was activated in a range of 250–350 pmol/L, which is close to the level of isoprostanes produced by platelets from aspirin-treated diabetic patients (~300 pmol/L) but higher than that expressed by platelets from aspirin-treated nondiabetic patients (~200 pmol/L). Together, these data indicate that in diabetic patients aspirin enhances the platelet production of isoprostanes up to functionally relevant concentrations, so enhancing platelet recruitment via GpIIb/IIIa activation.

The study has pathophysiologic and clinical implications. The upregulation of NOX2 in T2DM has important functional consequences, as it is associated with enhanced ROS and isoprostane production and ultimately with platelet activation. This finding supports and extends previous data indicating that platelet ROS formation are implicated in the
thrombus formation process (18) and provides further insight into the association between T2DM and thrombosis.

The upregulation of NOX2-generated ROS is likely to have a negative impact on the antiplatelet effect of aspirin in diabetic patients. In T2DM, the inhibition of COX1 by aspirin could favor a shift of arachidonic acid toward other metabolic pathways with ensuing enhancement of ROS-mediated nonenzymatic oxidation of arachidonic acid and subsequent isoprostane overproduction. Accordingly, previous studies showed that arachidonic acid activates NADPH oxidase via interaction with p47phox, a subunit of NADPH oxidase (24,25).

Such effect represents a plausible explanation for the lower efficacy of aspirin in preventing cardiovascular events in diabetic patients, as the divergent effect on platelet TxA2 and isoprostanes has a different impact on platelet activation. The suppression of the early phase of platelet aggregation by TxA2 may be counterbalanced by the increased propagation of platelet aggregation elicited by isoprostane overproduction. The inhibition of this last effect by in vitro addition of TxA2/isoprostane receptor antagonist suggests a potential benefit of drugs that antagonize TxA2/isoprostane receptors in patients with T2DM. Prevention of isoprostane overproduction via inhibition of NOX2-generated ROS is another attractive option to be considered. Statins have been reported to downregulate systemic isoprostanes with a mechanism that may involve inhibition of NADPH oxidase (13,26). Therefore, it could be interesting to examine if statins improve the antiplatelet effect of aspirin via inhibition of platelet isoprostanes.

A limitation of the study is the lack of analysis of aspirin compliance in the cross-sectional study. However, both diabetic and control groups had similar values of platelet TxB2, indicating that a scarce adherence to aspirin treatment, if any, was well balanced between the two. Furthermore, platelet function analysis was performed in vitro and ex vivo, which may not reflect platelet activation in vivo. Pharmacologic study with an inhibitor of isoprostane receptors could be useful to explore our study hypothesis in vivo.

In conclusion, we provide evidence that, in T2DM patients, low-dose aspirin enhances platelet isoprostanes as a consequence of NOX2-generated ROS upregulation. This effect mitigates the antiplatelet effect of aspirin and may account for its lower clinical efficacy in T2DM compared with other atherosclerotic settings.

ACKNOWLEDGMENTS

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No potential conflicts of interest relevant to this article were reported.

R.Car., C.N., and M.P. researched data. R.Can. and P.P. wrote the manuscript and researched data. F.A. and D.L. reviewed and edited the manuscript. S.B. researched data. F.V. designed the study and wrote the manuscript. F.V. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

REFERENCES