Insulin Therapy Is Associated With Platelet Dysfunction in Patients With Type 2 Diabetes Mellitus on Dual Oral Antiplatelet Treatment

Dominick J. Angiolillo, MD, PhD, FACC,* Esther Bernardo, BSc,† Celia Ramírez, BSc,† Marco A. Costa, MD, PhD, FACC,* Manel Sabaté, MD, PhD,* Pilar Jimenez-Quevedo, MD,‡ Rosana Hernández, MD, PhD, † Raul Moreno, MD,§ Javier Escaned, MD, PhD, † Fernando Alfonso, MD, PhD, † Camino Bañuelos, MD, † Theodore A. Bass, MD, FACC, * Manel Sabaté, MD, PhD, † Celia Ramírez, BSc, † Antonio Fernandez-Ortiz, MD, PhD‡

Jacksonville, Florida; and Madrid, Spain

OBJECTIVES
This study sought to assess the influence of type 2 diabetes mellitus (T2DM) and the impact of hypoglycemic treatment (insulin vs. non-insulin) on platelet function profiles in patients treated with dual oral antiplatelet therapy.

BACKGROUND
Insulin inhibits platelet aggregation by suppressing the P2Y12 pathway. However, T2DM patients have a loss of responsiveness to insulin that leads to upregulation of the P2Y12 pathway, increased platelet reactivity, and reduced responsiveness to antiplatelet agents. Patients with insulin-treated diabetes mellitus (ITDM) have a more advanced disease status and higher atherothrombotic risk compared with non-ITDM (NITDM). However, the impact of insulin therapy on platelet dysfunction in patients treated with P2Y12 antagonists is unknown.

METHODS
A total of 201 T2DM and 65 nondiabetic patients with coronary artery disease in a steady phase of aspirin and clopidogrel treatment were studied. Platelet aggregation was assessed using agonists specific (6 and 20 μM adenosine diphosphate [ADP]) and nonspecific (6 μg/ml collagen and 20 μM epinephrine) for the P2Y12 pathway. High shear-induced platelet reactivity was assessed by means of the PFA-100 system (Dade-Behring International, Miami, Florida).

RESULTS
The T2DM patients had platelet aggregation and shear-induced platelet function significantly increased compared with nondiabetic patients using all assays. Platelet aggregation was increased in ITDM (n = 68) compared with NITDM (n = 133) patients after P2Y12-specific stimuli. Insulin treatment was the strongest predictor of ADP-induced aggregation. Platelet function profiles were similar between ITDM and NITDM using assays nonspecific to the P2Y12 pathway. Platelet dysfunction was independent of glycemic control and inflammatory status.

CONCLUSIONS
The P2Y12-dependent and -independent pathways of platelet reactivity are altered in T2DM compared with nondiabetic patients, and ITDM have greater ADP-induced platelet aggregation compared with NITDM. (J Am Coll Cardiol 2006;48:298–304) © 2006 by the American College of Cardiology Foundation

The platelet P2Y12 receptor plays a pivotal role in platelet aggregation (1). Its critical role on thrombosis has been emphasized by clinical trials showing improvement in long-term clinical outcomes of patients treated with the P2Y12 receptor antagonist clopidogrel (2–4). Patients with type 2 diabetes mellitus (T2DM) are characterized by a prothrombotic status (5–7) and seem to benefit most, particularly those requiring insulin therapy, from P2Y12 antagonism compared with aspirin (8).

Insulin has been shown to inhibit platelet aggregation by suppressing the P2Y12 pathway in healthy volunteers (9). However, such antithrombotic protection is hampered in T2DM patients because their platelets have reduced sensitivity to insulin (7,10). In addition, the P2Y12 pathway per se is upregulated in patients with T2DM (10). Overall, these findings may explain the reduced responsiveness to P2Y12 antagonists in patients with T2DM compared with nondiabetic patients (10,11). Insulin-treated diabetes mellitus (ITDM) patients may represent a diabetic subpopulation with more advanced stages of insulin resistance and biological disorders (12,13). However, the impact of insulin therapy on platelet dysfunction in patients with T2DM treated with P2Y12 antagonists remains to be demonstrated. In the present study we assessed platelet function profiles in a large cohort of T2DM patients treated with aspirin and the P2Y12 antagonist clopidogrel, and hypothesize that: 1) T2DM patients have increased platelet aggregation compared with nondiabetic patients; and 2) platelet P2Y12 dysfunction is more pronounced in ITDM compared with non-ITDM (NITDM) patients.

METHODS

Patient population. A total of 201 patients with T2DM were studied. We defined T2DM according to World Health Organization criteria, and patients were classified as...
ITDM or NITDM (14). Diet-controlled diabetic patients were excluded. A control group composed of 65 nondiabetic patients was also studied. All patients had previously undergone percutaneous coronary intervention and were treated with dual oral antiplatelet therapy (aspirin plus clopidogrel). Because pharmacokinetic and pharmacodynamic profiles vary in the early stages of dual antiplatelet therapy and several days or weeks may be required to achieve full antiplatelet effects (15,16), only patients in their steady phase of treatment (≥1 month) were included. All patients were treated with the same maintenance doses of aspirin (100 mg/day) and clopidogrel (75 mg/day). Patient compliance with antiplatelet treatment was assessed by interview and/or pill counting.

Exclusion criteria were diet-controlled diabetes mellitus, combined aspirin and clopidogrel treatment <1 month, concomitant use of other antithrombotic drugs (oral anticoagulants, dipyridamole, ticlopidine, cilostazol) or nonsteroidal anti-inflammatory drugs, occurrence of an acute ischemic episode (unstable angina/myocardial infarction/ cerebrovascular event) since the time of last coronary intervention, platelet count <125,000/mm³, hematocrit <25%, or creatinine levels >2.5 mg/dl.

This study complied with the Declaration of Helsinki, it was approved by the Ethical Committee of the San Carlos University Hospital, and all patients gave their informed consent.

Blood sampling. Blood samples for platelet function assays were collected from an antecubital vein using a 21-gauge needle 2 to 4 h after antiplatelet therapy intake. The first 2 to 4 ml of blood were discarded to avoid spontaneous platelet activation. Platelet function assessments included platelet aggregation and high shear-induced platelet reactivity.

Platelet aggregation was performed using light transmittance aggregometry according to standard protocols (11). In brief, blood was collected in tubes containing 3.8% trisodium. Platelet aggregation was assessed using platelet-rich plasma (PRP) by the turbidimetric method in a 2-channel aggregometer (Chrono-Log 490 Model, Chrono-Log Corporation, Havertown, Pennsylvania). The PRP was obtained as a supernatant after centrifugation of citrated blood at 800 rpm for 10 min. The isolated PRP was kept at 37°C before use. Platelet-poor plasma (PPP) was obtained by a second centrifugation of the blood fraction at 2,500 rpm for 10 min. The platelet count in PRP was adjusted to the range of 250,000/µl by dilution with autologous plasma when the platelet count was out of range. Light transmission was adjusted to 0% with PRP and to 100% for PPP for each measurement. Platelet aggregation was assessed within 2 h from blood sampling. Curves were recorded for 5 min, and platelet aggregation was determined as the maximal percent change in light transmittance from baseline using PPP as a reference. Because clopidogrel is a P2Y12 adenosine diphosphate (ADP) receptor antagonist, ADP stimuli (6 and 20 µM ADP) were used to assess individual response to clopidogrel (11,17) and test our study hypotheses: ADP-induced platelet reactivity will be higher in T2DM compared with nondiabetic patients (hypothesis 1) and in ITDM compared with NITDM patients (hypothesis 2). Agonists not specific to the P2Y12 pathway (6 µg/ml collagen and 20 µM epinephrine) were also used to assess P2Y12-independent pathways leading to platelet aggregation (17,18).

The PFA-100 (Dade-Behring International, Miami, Florida) was used to assess shear-induced platelet reactivity (19). The PFA-100 system is a microprocessor-controlled instrument/test cartridge system used to assess platelet function simulating platelet-based primary hemostasis in vitro. A syringe aspirates citrated whole blood under high shear flow conditions (5,000 to 6,000 s⁻¹) through a small aperture (150 µm) cut into a membrane placed in the test cartridge. The membrane is coated with type I collagen and either 10 µg epinephrine bitartrate (CEPI) or 50 µg ADP (CADP). The time necessary for the occlusion of the aperture is defined as closure time (CT). Reduced CTs are indicative of increased platelet reactivity and vice versa. After 300 s, the process automatically terminates. Any CEPI-CTs ≥300 s are indicative of an optimal response to antiplatelet therapy (19).

In the cohort of patients with T2DM, hemoglobin A1C (HbA1C) and high-sensitivity C-reactive protein were assessed and correlated with platelet function assessments.

Statistical analysis. Variables were analyzed for a normal distribution with the Kolmogorov-Smirnov test. Normally distributed variables are presented as mean ± standard deviation. Variables that did not follow a normal distribution are represented as median and interquartile range. Categorical variables are expressed as frequencies and percentages. Categorical variables were compared by means of the chi-square test or the Fisher exact test when at least 25% of values showed an expected cell frequency below 5. The Student t test and one-way analysis of variance were used to compare continuous variables when these were normally distributed and the Mann-Whitney U test or Kruskal-Wallis test if not normally distributed. Correlation analyses were performed according to the Spearman or the Pearson correlation coefficient. A multivariate linear regression analysis was performed to identify the independent determinants of platelet aggregation. Variables included in the
multivariate model were those that were significant (p < 0.1) in a univariate model that included age (>65 years), gender, obesity (body mass index [BMI] ≥30 kg/m²), smoking, hyperlipidemia, hypertension, prior myocardial infarction, prior coronary artery bypass surgery, peripheral vasculopathy, and medical therapy (beta-blockers, nitrates, angiotensin-converting enzyme inhibitors, lipid-lowering agents, calcium–channel blockers). A p value < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 11.0 software (SPSS Inc., Chicago, Illinois).

RESULTS

Patient demographics. A total of 201 patients with T2DM with stable coronary artery disease on sustained (≥1 month) aspirin and clopidogrel treatment were analyzed: 68 ITDM and 133 NITDM. Demographics of IT2DM and nondiabetic patients are shown in Table 1. There were no differences between groups, except for a higher prevalence of female patients, increased age, and higher BMI in patients with ITDM. The NITDM patients were treated with either metformin or sulfonylurea derivatives; insulin-sensitizing agents appertaining to the family of peroxisome proliferator-activated receptor-gamma receptor agonists were not used in these patients.

T2DM versus nondiabetic patients. Platelet aggregation (expressed as percentage) profiles presented a normal distribution and were significantly higher in patients with T2DM compared with nondiabetic patients after ADP 6 μM (41.5 ± 14.7 vs. 31.8 ± 14.8; p < 0.0001), ADP 20 μM (52.9 ± 13.8 vs. 43.0 ± 17.6; p = 0.001), 6 μg/ml collagen (42.8 ± 18.5 vs. 31.5 ± 18.4; p < 0.0001) and 20 μM epinephrine (31.8 ± 14.9 vs. 26.4 ± 11.0; p = 0.0015) stimuli. Platelet function values assessed by PFA-100 did not follow a normal distribution and are presented as median and interquartile range. The CT’s (expressed in seconds) of CADP (81.0 [70.0 to 101.0] vs. 91.0 [77.5 to 106.2]; p = 0.02) and CEPI (182.5 [129.5 to 300.0] vs. 300.0 [157 to 300.0]; p = 0.001) cartridges were significantly lower in T2DM, indicative of increased platelet reactivity. The CEPI values above 300 s were observed less frequently in T2DM (42.5% vs. 66.2%; p = 0.001). Overall, nondiabetic patients had the lowest platelet aggregation and highest CT values compared with NITDM and ITDM (Table 2).

**ITDM versus NITDM.** The ADP-induced platelet aggregation was significantly higher in ITDM compared with NITDM patients (Fig. 1). Similarly, shear-induced platelet reactivity using ADP-containing cartridges (CADP) was higher in ITDM compared with NITDM as reflected by their shorter CTs (75.0 [66.4 to 93.5] vs. 84.0 [71.5 to 103.0]; p = 0.02). No differences were observed in collagen-induced (43.0 ± 17.6 vs. 42.7 ± 18.9; p = 0.9) and epinephrine-induced (32.0 ± 13.9 vs. 31.2 ± 15.4; p = 0.9) platelet aggregation between ITDM and NITDM patients. The CEPI-CT (178.0 [122.5 to 300.0] vs. 190.0 [135.5 to 300.0]; p = 0.6) were also similar in ITDM and NITDM patients, as well as the number of patients with CEPI-CT above 300 s (39% vs. 44%; p = 0.9).

The HbA1C levels were significantly higher in ITDM compared with NITDM patients (7.9 ± 1.5 vs. 6.9 ± 1.0; p < 0.0001). The HbA1C levels were not correlated with any of the platelet function assays performed (r < 0.3 for all assessments). The CRP levels were not normally distributed and were significantly higher (p = 0.007) in ITDM (0.39 mg/dl [0.24 to 0.75]) compared with NITDM (0.28 mg/dl [0.15 to 0.50]). The CRP levels were not associated with platelet function. The ITDM had a higher BMI compared with NITDM (30.2 ± 4.8 vs. 28.8 ± 3.7; p = 0.04);

### Table 1. Demographics of Study Population

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>ITDM (n = 68)</th>
<th>NITDM (n = 133)</th>
<th>NDM (n = 65)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>68 ± 9</td>
<td>66 ± 9</td>
<td>62 ± 11</td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>Gender (male), n (%)</td>
<td>34 (50)</td>
<td>100 (75)</td>
<td>51 (79)</td>
<td>0.001</td>
</tr>
<tr>
<td>Race (Caucasian), n (%)</td>
<td>68 (100)</td>
<td>133 (100)</td>
<td>65 (100)</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index</td>
<td>30.2 ± 4.8</td>
<td>28.8 ± 3.7</td>
<td>27.2 ± 3.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Risk factors/past medical history, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>7 (10)</td>
<td>17 (13)</td>
<td>11 (17)</td>
<td>NS</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>47 (69)</td>
<td>92 (69)</td>
<td>43 (66)</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension</td>
<td>48 (71)</td>
<td>82 (62)</td>
<td>34 (52)</td>
<td>NS</td>
</tr>
<tr>
<td>Prior myocardial infarction</td>
<td>29 (43)</td>
<td>66 (50)</td>
<td>29 (45)</td>
<td>NS</td>
</tr>
<tr>
<td>Prior coronary artery bypass surgery</td>
<td>3 (5)</td>
<td>4 (3)</td>
<td>1 (2)</td>
<td>NS</td>
</tr>
<tr>
<td>Peripheral vasculopathy</td>
<td>16 (24)</td>
<td>18 (13)</td>
<td>9 (14)</td>
<td>NS</td>
</tr>
<tr>
<td>Treatment, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>48 (70)</td>
<td>84 (63)</td>
<td>44 (59)</td>
<td>NS</td>
</tr>
<tr>
<td>Nitrates</td>
<td>32 (47)</td>
<td>55 (41)</td>
<td>36 (55)</td>
<td>NS</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme inhibitors</td>
<td>47 (69)</td>
<td>92 (69)</td>
<td>43 (66)</td>
<td>NS</td>
</tr>
<tr>
<td>Lipid-lowering agents</td>
<td>51 (75)</td>
<td>97 (73)</td>
<td>44 (68)</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium-channel blockers</td>
<td>18 (27)</td>
<td>35 (26)</td>
<td>26 (40)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are presented as the mean value ± SD or number (%). Continuous variables were compared by means of the one-way analysis of variance test and categorical variables by means of the chi-square test or Fisher exact test when appropriate (*). ITDM = insulin-treated diabetes mellitus; NDM = non-diabetes mellitus; NITDM = non-insulin-treated diabetes mellitus.
however, BMI was not correlated with any of the platelet function assays performed (r < 0.3 for all assessments). In a multivariate linear regression analysis, insulin treatment was the strongest variable associated with both 6 and 20 μM-ADP induced platelet aggregation (Table 3).

**DISCUSSION**

This is the first report on platelet dysfunction in a large cohort of patients with T2DM treated with the P2Y<sub>12</sub> receptor antagonist clopidogrel and aspirin. Our results confirm the hypotheses that: 1) patients with T2DM have increased platelet function compared with nondiabetic patients, and 2) ITDM patients have reduced clopidogrel effects on ADP-induced platelet aggregation compared with NITDM. Further, our findings suggest that both P2Y<sub>12</sub>-dependent and -independent pathways are altered in patients with T2DM compared with nondiabetic patients, as shown by increased platelet reactivity to the multiplicity of agonists used in this study. Pathways leading to platelet aggregation independent of the P2Y<sub>12</sub> receptor, although dysfunctional in the overall T2DM patients, seem to be comparable between ITDM and NITDM, because platelet reactivity to epinephrine and collagen, which do not stimulate the P2Y<sub>12</sub> receptor, was similar between the diabetic subgroups. However, ITDM and NITDM differed with regards to the response to ADP, more specific to the P2Y<sub>12</sub> pathway.

In vitro studies assessing the impact of insulin on platelets of healthy volunteers have led to ambiguous findings (9,20). In a recent report, Ferreira et al. (9) observed that insulin reduces platelet aggregation by inhibiting the P2Y<sub>12</sub> pathway. In fact, human platelets are the target of the action of insulin through specific platelet membrane receptors that lead to loss of G<sub>i</sub> activity (9). This reduces cyclic adenosine monophosphate suppression, thus inhibiting P2Y<sub>12</sub> signaling and reducing platelet reactivity. However, these in vitro studies evaluated the effects on platelet function of a single dose of insulin, whereas in our study we assessed two groups of T2DM patients with and without chronic insulin treatment. Notably, platelets of T2DM patients have decreased sensitivity to insulin, leading to reduced P2Y<sub>12</sub> inhibition and increased platelet reactivity (10). Because patients with ITDM are likely to be at a more advanced state of insulin resistance, this may explain the enhanced platelet reactivity to ADP, used to test the responsiveness to the P2Y<sub>12</sub> receptor antagonist clopidogrel, in these patients. Patients who have poor glycemic control on oral hypoglycemic medication require exogenous insulin therapy (13). This generally occurs in patients with a longer history of diabetes and a more advanced stage of insulin resistance (12,13). Importantly, women are more intrinsically insulin resistant.

**Table 2. Platelet Function Profiles of the Study Population**

<table>
<thead>
<tr>
<th></th>
<th>ITDM (n = 68)</th>
<th>NITDM (n = 133)</th>
<th>NDM (n = 65)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP 6 μM (%)</td>
<td>44.4 ± 15.4</td>
<td>39.9 ± 14.1</td>
<td>31.8 ± 14.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ADP 20 μM (%)</td>
<td>56.3 ± 14.7</td>
<td>51.2 ± 13.1</td>
<td>43.0 ± 17.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Collagen 6 μg/ml (%)</td>
<td>43.0 ± 17.6</td>
<td>2.7 ± 18.9</td>
<td>31.5 ± 18.4</td>
<td>0.0001</td>
</tr>
<tr>
<td>Epinephrine 10 μM (%)</td>
<td>32.0 ± 13.9</td>
<td>31.2 ± 15.4</td>
<td>26.4 ± 11.0</td>
<td>0.03</td>
</tr>
<tr>
<td>CADP-CT (s)</td>
<td>75.0 [66.4–93.5]</td>
<td>84.0 [71.5–103.0]</td>
<td>91.0 [77.5–106.2]</td>
<td>0.005</td>
</tr>
<tr>
<td>CEPI-CT (s)</td>
<td>178.0 [122.5–300.0]</td>
<td>190.0 [135.5–300.0]</td>
<td>300.0 [157–300.0]</td>
<td>0.006</td>
</tr>
</tbody>
</table>

Platelet aggregation (ADP, collagen, and epinephrine) data were normally distributed and presented as the mean value ± SD; p values were calculated using one-way analysis of variance testing. PFA-100 data (CADP-CT and CEPI-CT) were not normally distributed and are presented as the median and interquartile range; p values were calculated using Kruskal-Wallis testing.

ADP = adenosine diphosphate; CADP = collagen/ADP-coated cartridges; CEPI = collagen/epinephrine-coated cartridges; CT = closure time; other abbreviations as in Table 1.

![Figure 1. Platelet aggregation after 6 and 20 μM adenosine diphosphate (ADP) stimuli in noninsulin-treated diabetes mellitus (NITDM; open bars) and insulin-treated diabetes mellitus (ITDM; solid bars).](image)

**Table 3. Multivariate Linear Regression Analysis of ADP-Induced Platelet Aggregation**

<table>
<thead>
<tr>
<th></th>
<th>Coefficient β</th>
<th>95% CI</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP 6 μM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin treatment</td>
<td>10.0</td>
<td>4.6–15.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Female gender</td>
<td>3.4</td>
<td>0.2–8.1</td>
<td>0.06</td>
</tr>
<tr>
<td>Obesity</td>
<td>3.7</td>
<td>0.3–7.7</td>
<td>0.07</td>
</tr>
<tr>
<td>ADP 20 μM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin treatment</td>
<td>11.1</td>
<td>5.7–16.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Obesity</td>
<td>3.8</td>
<td>0.2–7.9</td>
<td>0.02</td>
</tr>
</tbody>
</table>

In this model, ADP (6 and 20 μM)-induced platelet aggregation was used as the dependent variable, and the independent variables included age, gender, obesity, smoking, hyperlipidemia, hypertension, prior myocardial infarction, prior coronary artery bypass surgery, peripheral vasculopathy, and medical therapy (beta-blockers, nitrates, angiotensin-converting enzyme inhibitors, lipid-lowering agents, calcium-channel blockers). Age, gender, obesity, and insulin-treatment (p < 0.1 in univariate analysis) were included in the final multivariate model. CI = confidence interval; other abbreviations as in Table 2.
than are men (21,22). Overall, these observations support the increased age and higher incidence of female patients with ITDM in our study. Similar findings have been reported previously (23–25).

In addition to platelet aggregation, shear-induced platelet function was also increased in T2DM platelets. Although no differences in platelet reactivity were observed between ITDM and NITDM patients using cartridges containing epinephrine, this was increased with cartridges containing ADP in ITDM. Of note, the PFA-100 cartridges have reduced sensitivity to thienopyridines (19). Therefore it may be argued whether or not responsiveness to clopidogrel may be truly revealed by the CADF cartridges. However, to date, similar assessments have been primarily performed in nondiabetic subjects. Because patients with T2DM have a dysfunctional status of the P2Y$_{12}$ pathway, they may be more sensitive to this assay compared with nondiabetic patients, and therefore the assay may not only reveal functional differences between T2DM and nondiabetic patients, but, in agreement with the platelet aggregation assays, also between ITDM and NITDM. Regardless of the causes, the overall findings of our multiple platelet function assays strongly support the differential responsiveness to antiplatelet agents in T2DM compared with nondiabetic patients.

Poor glycemic control is another important cause of increased platelet reactivity (6,7). Hyperglycemia leads to nonenzymatic glycation of platelet glycoproteins, causing changes in their structure and conformation, as well as alterations of membrane lipid dynamics (26). Importantly, platelet aggregation can be reduced with tight control of glucose levels (27). However, no correlation was observed between HbA1C levels and platelet function in our study. Notably, our study was conducted in a tightly controlled diabetic population (the coefficient of variation of HbA1C levels was only 6%), which led to a limited variability in HbA1C levels. Such a narrow window of HbA1C levels was only 6%), which led to a limited variability in HbA1C levels. Such a narrow window of HbA1C levels was only 6%), which led to a limited variability in HbA1C levels. Such a narrow window of HbA1C levels was only 6%), which led to a limited variability in HbA1C levels. Such a narrow window of HbA1C levels was only 6%), which led to a limited variability in HbA1C levels. Such a narrow window of HbA1C levels was only 6%), which led to a limited variability in HbA1C levels. Therefore, patients with T2DM may be more susceptible to the effects of P2Y$_{12}$ antagonists because of their enhanced inflammatory status (35). Although the mechanisms have not been fully elucidated, the P2Y$_{12}$ receptor per se has been invoked as a mediator of anti-inflammatory responses achieved with clopidogrel (35). In our study we observed an enhanced inflammatory status in ITDM compared with NITDM, which may be associated with dysfunction of the P2Y$_{12}$ pathway. However, the lack of correlation between CRP levels and platelet reactivity in the present study does not support such a hypothesis. Therefore, high CRP levels observed in the ITDM patients are more likely related to the more advanced disease status of these patients and the intrinsic proinflammatory effects of insulin (36).

**Therapeutic implications.** Treatment with insulin is typically considered a surrogate of increased atherothrombotic risk. Previous studies from our group and others were unable to show differences in platelet reactivity between ITDM and NITDM likely because of limited sample sizes, which typically characterize platelet function studies (11). The prognostic implications associated with enhanced platelet reactivity, even in patients treated with clopidogrel, are noteworthy (37–39). Importantly, in this study we assessed posttreatment platelet reactivity, which has shown to be a better predictor of ischemic risk (39). The seminal
findings from our present functional study may provide a mechanistic explanation to the higher ischemic risk observed in T2DM patients, especially ITDM, within largescale clinical studies (24,25). In particular, the proinflammatory and prothrombotic status that characterizes ITDM may explain why these patients have higher rates of restenosis, even after drug-eluting stent implantation, as well as stent thrombosis (40). Aggressive and/or tailored antithrombotic regimens for these patients may be warranted. Landmark results showing a mortality benefit in diabetic patients and in particular in ITDM with platelet glycoprotein Iib/IIIa receptor inhibitors are supportive of the need for more aggressive antithrombotic treatment regimens in these patients (25). Whether more potent P2Y$_{12}$ antagonism using a higher maintenance dose of clopidogrel or novel oral P2Y$_{12}$ antagonists (prasugrel, AZD6140) will be able to inhibit more efficiently the upregulated P2Y$_{12}$ pathway in platelets of T2DM patients is currently under investigation (41). Ultimately, the use of novel insulin-sensitizing agents that exert their effects on peroxisome proliferator-activated receptor-$\gamma$ receptors as a therapeutic measure of glycemic control may also represent an important adjunctive approach because of their pleiotropic (anti-inflammatory and antithrombotic) effects (42). This is related to the ubiquity of insulin resistance, which affects multiple cell lines, and underscores how pleiotropic benefits may be achieved by overcoming this phenomenon with these agents. In the future, individualized and more aggressive therapeutic approaches with multifaceted properties should be considered to provide further protection to these high-risk patient subsets.

**Study limitations.** The present study was not designed to evaluate long-term outcomes. Therefore, despite the known prognostic implications associated with increased platelet reactivity, its clinical impact in our study population warrants further investigation.

**REFERENCES**


