

# The Effect of Aspirin Dosing on Platelet Function in Diabetic and Nondiabetic Patients

## An Analysis From the Aspirin-Induced Platelet Effect (ASPECT) Study

Joseph DiChiara, Kevin P. Bliden, Udaya S. Tantry, Miruais S. Hamed, Mark J. Antonino, Thomas A. Suarez, Oscar Bailon, Anand Singla, and Paul A. Gurbel

**OBJECTIVE**— Diabetic patients may have a higher prevalence of platelet aspirin resistance than nondiabetic patients. Our goal was to analyze platelet aspirin responsiveness to various aspirin doses in diabetic and nondiabetic patients.

**RESEARCH DESIGN AND METHODS**— We examined the effect of aspirin (81, 162, and 325 mg/day for 4 weeks each) on platelet aspirin responsiveness in 120 stable outpatients (30 diabetic patients and 90 nondiabetic patients) with coronary artery disease (CAD) using light transmittance aggregometry (LTA), VerifyNow, platelet function analyzer (PFA)-100, and levels of urinary 11-dehydro-thromboxane B<sub>2</sub> (11-dh-TxB<sub>2</sub>).

**RESULTS**— In the total group, a low prevalence (0–2%) of aspirin resistance was observed with all aspirin doses as determined by arachidonic acid-induced LTA. Aspirin resistance was higher at the 81-mg dose in diabetic versus nondiabetic patients using collagen-induced LTA (27 vs. 4%,  $P = 0.001$ ), VerifyNow (13 vs. 3%,  $P = 0.05$ ), and urinary 11-dh-TxB<sub>2</sub> (37 vs. 17%,  $P = 0.03$ ). Diabetic patients treated with 81 mg exhibited higher platelet function measured by VerifyNow, collagen- and ADP-induced LTA, and 11-dh-TxB<sub>2</sub> levels ( $P \leq 0.02$  for all comparisons). Higher aspirin doses significantly inhibited platelet function and decreased aspirin resistance in diabetic patients ( $P < 0.05$ ).

**CONCLUSIONS**— Diabetic patients with CAD treated with 81 mg aspirin exhibit a higher prevalence of aspirin resistance and have significantly higher ADP- and collagen-induced platelet aggregation, 11-dh-TxB<sub>2</sub> levels, and aspirin reaction units measured by VerifyNow than nondiabetic patients. Increased aspirin dosing resulted in similar rates of resistance and platelet function levels between groups. These findings indicate that diabetic patients exhibit a global high platelet reactivity phenotype that may be partially overcome by higher aspirin doses. *Diabetes* 56: 3014–3019, 2007

From the Sinai Center for Thrombosis Research, Baltimore, Maryland.

Address correspondence and reprint requests to Paul A. Gurbel, MD, Sinai Center for Thrombosis Research, Hoffberger Building, Suite 56, 2401 W. Belvedere Ave., Baltimore, MD 21215. E-mail: pgurbel@lifebridgehealth.org.

Received for publication 24 May 2007 and accepted in revised form 7 September 2007.

Published ahead of print at <http://diabetes.diabetesjournals.org> on 11 September 2007. DOI: 10.2337/db07-0707.

P.A.G. has received research funding from Hemoscope, the National Institutes of Health, Schering, Millennium, and Bayer.

11-dh-TxB<sub>2</sub>, 11-dehydro-thromboxane B<sub>2</sub>; ACS, acute coronary syndrome; ARU, aspirin reaction unit; CAD, coronary artery disease; ELISA, enzyme-linked immunosorbent assay; LTA, light transmittance aggregometry; PFA, platelet function analyzer; PPP, platelet-poor plasma; PRP, platelet-rich plasma.

© 2007 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**D**iabetes is a "prothrombotic state" associated with accelerated atherosclerosis and inflammation (1). Despite the proven benefits of aspirin therapy in the prevention of cardiovascular complications in the diabetic patient, the diabetic patient has a greater risk for thrombotic complications than nondiabetic patients (2). It has been demonstrated that diabetic patients with acute coronary syndromes (ACSs) are at higher risk for recurrent events than nondiabetic ACS patients (3). Despite sharp declines in restenosis rates with the use of drug-eluting stents, diabetic patients with ACSs remain at the highest risk for recurrent ischemia (4). Moreover, recent studies have suggested that diabetes is an independent predictor of stent thrombosis and lower survival rates in patients treated with drug-eluting stents (5,6). It has been suggested that diabetic patients not treated with aspirin therapy generally display high platelet reactivity and elevated levels of platelet thromboxane synthesis and that aspirin treatment in diabetic patients is less effective in inhibiting thromboxane synthesis than in nondiabetic patients (7). However, few studies have evaluated the efficacy of different aspirin doses in the diabetic patient to determine the optimal regimen. Currently, existing guidelines recommend low-dose aspirin therapy in diabetic patients; however, there is little evidence to support this recommendation (8). In fact, in the Primary Prevention Project, a higher cardiovascular risk was observed in diabetic patients on low-dose aspirin than in nondiabetic patients, indicating that the benefits of aspirin therapy may be outweighed by aspirin-insensitive mechanisms of platelet activation (9). In addition, long-term treatment with high-dose aspirin resulted in similar rates of death and cardiovascular events in diabetic and nondiabetic patients (10). There are limited data quantifying the prevalence of platelet aspirin resistance in diabetic patients. In addition, no study has compared the effect of multiple aspirin doses on platelet function in both diabetic and nondiabetic patients using various methods. We hypothesized that diabetic patients with coronary artery disease (CAD) during therapy with 81 mg aspirin will exhibit a higher prevalence of aspirin resistance and higher platelet function than nondiabetic patients and that higher aspirin doses will result in similar rates of resistance and similar levels of platelet function between groups. In this investigation, we measured 11-dehydro-thromboxane B<sub>2</sub> (11-dh-TxB<sub>2</sub>) in urine and used conven-

tional light transmittance aggregometry (LTA) and two point-of-service analyzers.

## RESEARCH DESIGN AND METHODS

We studied 120 stable outpatients enrolled in the Aspirin-Induced Platelet Effect (ASPECT) study, a single-center, double-blind, double-crossover (Williams design) study evaluating the effect of 81, 162, and 325 mg/day aspirin therapy in patients with CAD (11). The study was approved by the Western Institutional Review Board (Olympia, WA), and all patients provided written informed consent before enrollment. Patients were randomly assigned to a drug treatment sequence of 81, 162, and 325 mg/day for 4 weeks each over a 12-week period. CAD was documented by coronary angiography or ultrafast computed tomography scan. Patients were >18 years of age. Patients were excluded from the study if they met any of the following criteria: bleeding diathesis or a history of gastrointestinal bleeding, hemorrhagic stroke, illicit drug or alcohol abuse, coagulopathy, major surgery within 6 weeks before randomization, platelet count <100,000/mm<sup>3</sup>, hematocrit <25%, creatinine >4 mg/dl, or current use of nonsteroidal anti-inflammatory drugs, anticoagulants, or antiplatelet drugs other than aspirin.

On completion of each dose, the platelet aspirin response was determined using 2 and 5 mmol/l arachidonic acid-, 2 µg/ml collagen-, and 5 µmol/l ADP-induced aggregation by LTA; VerifyNow using the arachidonic acid cartridge (Accumetrics, San Diego, CA); platelet function analyzer (PFA)-100 with the collagen/epinephrine cartridge (Dade-Behring, West Sacramento, CA); and urinary 11-dh-TxB<sub>2</sub> levels by the AspirinWorks method (Corgenix, Denver, CO). Patients were instructed to return their bottle of aspirin to count the remaining pills and calculate the percentage of compliance.

**Blood and urine collection.** In a fasting state, between 8:00 A.M. and 12:00 P.M. on the day of the last aspirin dose, blood was collected from the antecubital vein into Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). All patients were instructed to take morning medications as normal before blood draw. After discarding the first 2–3 ml of free flowing blood, the tubes were filled to capacity and gently inverted three to five times to ensure complete mixing of the anticoagulant. Tubes containing 3.2% trisodium citrate were used for LTA and PFA-100, and one tube containing 3.2% sodium citrate (Greiner Bio-One Vacuette North America, Monroe, NC) was collected for VerifyNow measurements. Urine samples were collected for measurements of 11-dh-TxB<sub>2</sub> at the end of each aspirin treatment period into tubes containing indomethacin (Corgenix) and stored at –70°C until analysis.

**LTA.** Platelet aggregation was assessed as described previously (12). In brief, the blood-citrate tubes were centrifuged at 120g for 5 min to recover platelet-rich plasma (PRP) and further centrifuged at 850g for 10 min to recover platelet-poor plasma (PPP). The PRP and PPP were stored at room temperature to be used within 30 min. Platelets were stimulated with 5 µmol/l ADP, 2 µg/ml collagen, and 2 and 5 mmol/l arachidonic acid. Aggregation was assessed using a Chronolog Lumi-Aggregometer (model 490-4D) with the Aggrolink software package (Chronolog, Havertown, PA). Aggregation was expressed as the maximum percent change in light transmittance from baseline, using PPP as a reference.

**VerifyNow aspirin assay.** VerifyNow is a turbidimetric-based optical detection assay designed to measure platelet aggregation that is based on the ability of activated platelets to bind to fibrinogen (13). The cartridge contains a lyophilized preparation of human fibrinogen-coated beads, arachidonic acid, preservative, and buffer. The fibrinogen-coated beads aggregate in whole blood in proportion to the number of unblocked platelet GPIIb/IIIa receptors. The instrument reports aggregation as aspirin reaction units (ARUs).

**PFA-100 assay.** In the PFA-100 point-of-care assay, platelets are exposed to high shear conditions within a cartridge containing a capillary, a sample reservoir, a collagen/epinephrine-coated membrane, and an aperture (14). Epinephrine and collagen activate platelets in whole blood, creating aggregate formation at the aperture, gradually diminishing and finally arresting blood flow. The PFA-100 records the time in seconds from the start of the test until the platelet aggregate occludes the aperture (closure time).

**Urinary 11-dh-TxB<sub>2</sub>.** The AspirinWorks enzyme-linked immunosorbent assay (ELISA) has been previously described (15). In brief, assay buffer and urine were incubated with a monoclonal antibody followed by the addition of 11-dh-TxB<sub>2</sub>-alkaline phosphate tracer. Urinary 11-dh-TxB<sub>2</sub> concentrations were determined by measuring color development at 405 nm using an ELISA reader and expressed as picograms per milligram creatinine.

**Aspirin resistance.** Aspirin resistance was defined by previously reported criteria: ≥20% arachidonic acid-, ≥70% ADP-, and ≥70% collagen-induced aggregation by LTA (16); ≥550 ARUs by VerifyNow (13); ≤193 s by PFA-100 (14); and upper quartile (>420 pg) 11-dh-TxB<sub>2</sub>/mg creatinine during treatment with 81 mg daily aspirin (17).

**Diabetes.** Diabetes was present if at least one of the following criteria was present: a fasting glucose ≥126 mg/dl, treatment with oral hypoglycemic agents, or treatment with insulin (18).

**Statistical analysis.** Categorical variables are expressed as *n* (%) and continuous variables as mean ± SD. A one-way ANOVA for repeated measures was used to compare levels of platelet function between and within diabetic and nondiabetic patients. An unpaired *t* test was used to compare the prevalence of aspirin resistance between groups; *P* < 0.05 was considered significant. All statistical calculations were performed using SigmaStat software (Point Richmond, CA).

## RESULTS

**Patients.** One hundred twenty patients from the ASPECT study were analyzed with respect to diabetes status. Diabetes was observed in 30 patients (7 patients with type 1 diabetes and 23 patients with type 2 diabetes). Patient demographics are shown in Table 1. The majority of patients were elderly Caucasian men with a history of hyperlipidemia and hypertension receiving standard medical treatment for CAD, including statins, β-blockers, and ACE inhibitors. Diabetic patients had a higher prevalence of stroke and lower HDL levels than nondiabetic patients. Overall compliance with daily aspirin therapy was 98%.

**Arachidonic acid-induced platelet aggregation measured by LTA.** The effect of aspirin dose on arachidonic acid-induced platelet aggregation between and within diabetic and nondiabetic patients is shown in Tables 2 and 3, respectively. Arachidonic acid-induced aggregation (2 and 5 mmol/l) was low in both patient groups, and no significant effects of increased aspirin dosing on arachidonic acid-induced platelet aggregation were observed between or within groups.

Aspirin resistance was rare in both patient groups as measured by arachidonic acid-induced aggregation. One diabetic patient was resistant by 2 mmol/l arachidonic acid-induced LTA during treatment with 81 mg and by 5 mmol/l arachidonic acid-induced LTA during treatment with 81 and 162 mg. However, on administration of 325 mg aspirin, no resistance was observed. Among the nondiabetic patients, one patient was resistant by 2 mmol/l arachidonic acid-induced LTA while treated with 162 mg and by 5 mmol/l arachidonic acid-induced LTA during treatment with 81 mg aspirin. Similar to the diabetic group, no resistance was observed during 325-mg aspirin therapy. **VerifyNow aspirin assay.** During treatment with 81 and 162 mg aspirin, ARUs were significantly higher in diabetic patients than in nondiabetic patients (*P* = 0.02 and 0.04, respectively; Table 2). However, nearly identical ARU values were observed between groups during 325-mg aspirin therapy (Table 2). In nondiabetic patients, 162 and 325 mg aspirin provided significantly lower aggregation than 81 mg aspirin (*P* = 0.02 for both comparisons), whereas in diabetic patients, only 325 mg aspirin resulted in significantly lower aggregation than 81 mg (*P* = 0.004; Table 3).

A directional trend in reduced rates of aspirin resistance was observed in diabetic patients with doses >81 mg (*P* = 0.16); however, rates of resistance in nondiabetic patients were uninfluenced by increased aspirin dosing (Fig. 1A). Overall, diabetic patients during 81-mg aspirin therapy displayed significantly greater rates of aspirin resistance than nondiabetic patients during 81-mg aspirin therapy (*P* = 0.04), with no differences observed between groups at 162 or 325 mg aspirin (Fig. 1A).

**Urinary 11-dh-TxB<sub>2</sub>.** During treatment with 81 mg aspirin, urinary 11-dh-TxB<sub>2</sub> levels were significantly higher in diabetic patients than in nondiabetic patients (*P* = 0.02), whereas no significant differences were observed between

TABLE 1  
Patient demographics

	Total group	Diabetic group	Nondiabetic group	Diabetic vs. nondiabetic <i>P</i>
<i>n</i>	120	30	90	
Age (years)	65 ± 10	64 ± 9	66 ± 11	0.38
Sex and ethnicity				
Men	65 (65)	18 (60)	47 (67)	0.33
Caucasian	73 (73)	22 (73)	51 (73)	1.0
Weight (lbs)	195 ± 52	210 ± 55	192 ± 50	0.11
Risk factors/past medical history				
Current smoking	8 (8)	2 (7)	6 (9)	0.74
Previous smoking	30 (30)	10 (33)	20 (29)	0.69
Family history of CAD	38 (38)	13 (43)	25 (36)	0.51
Hypertension	65 (65)	22 (73)	43 (61)	0.25
Hyperlipidemia	83 (83)	26 (87)	57 (71)	0.09
Prior myocardial infarction	18 (18)	6 (20)	12 (17)	0.37
Prior CABG	31 (31)	9 (30)	22 (31)	0.13
Prior PTCA	33 (33)	10 (33)	23 (33)	1.0
Prior CVA	6 (6)	5 (17)	1 (1)	0.002
Baseline medications				
β-Blockers	56 (56)	18 (60)	38 (54)	0.58
ACE inhibitors	52 (52)	19 (63)	33 (47)	0.15
Calcium channel blockers	19 (19)	5 (17)	14 (20)	0.72
Lipid-lowering therapy	76 (76)	23 (77)	53 (76)	0.91
Laboratory data				
WBC (×1,000/mm <sup>3</sup> )	6.4 ± 2.2	6.3 ± 2.5	6.4 ± 1.9	0.82
Platelets (×1,000/mm <sup>3</sup> )	225 ± 67	208 ± 71	229 ± 63	0.14
Hemoglobin (g/dl)	13.3 ± 2.0	12.8 ± 3.6	13.6 ± 2.2	0.17
Hematocrit (%)	40.8 ± 5.1	38.2 ± 11.1	41 ± 5.2	0.09
Creatinine (g/dl)	1.1 ± 0.6	1.1 ± 0.2	1.2 ± 0.7	0.45
Total cholesterol	165 ± 41	155 ± 43	170 ± 44	0.12
LDL	95 ± 36	91 ± 37	98 ± 38	0.40
HDL	50 ± 12	45 ± 13	51 ± 11	0.02
TRG	123 ± 70	124 ± 59	122 ± 75	0.89

Data are means ± SD and *n* (%). ACE, angiotensin-converting enzyme; CABG, coronary artery bypass graft; CVA, cerebrovascular accident; PTCA, percutaneous transluminal coronary angioplasty; TRG, triglycerides; WBC, white blood cells.

groups during treatment with 162 or 325 mg aspirin (Table 2). Both 162 and 325 mg aspirin significantly reduced levels compared with 81 mg aspirin in diabetic patients ( $P = 0.01$  and  $0.002$ , respectively); however, a significant decrease in urinary thromboxane levels was not observed in nondiabetic patients with increased aspirin dosing (Table 3).

A directional trend in reduced rates of aspirin resistance was observed in diabetic patients from 81 to 162 mg ( $P = 0.24$ ), whereas 325 mg aspirin resulted in significantly decreased resistance rates compared with 81 mg in diabetic patients ( $P = 0.04$ ; Fig. 1B). The prevalence of aspirin resistance was significantly lower in nondiabetic patients than in diabetic patients at 81 mg aspirin ( $P = 0.02$ ), and

TABLE 2  
Group comparison

	Diabetic ( <i>n</i> = 30)			Nondiabetic ( <i>n</i> = 90)			Diabetic vs. nondiabetic <i>P</i>		
	81 mg	162 mg	325 mg	81 mg	162 mg	325 mg	81 mg	162 mg	325 mg
Arachidonic acid-stimulated assays									
2 mmol/l arachidonic acid-LTA (%)	7 ± 18	4 ± 2	5 ± 3	4 ± 2	4 ± 6	4 ± 1	NS	NS	NS
5 mmol/l arachidonic acid-LTA (%)	8 ± 22	7 ± 13	5 ± 4	5 ± 8	4 ± 2	5 ± 4	NS	NS	NS
Accumetrics (ARU)	470 ± 66	448 ± 57	426 ± 45	445 ± 48	429 ± 38	428 ± 43	0.02	0.04	NS
Other assays									
5 μmol/l ADP-LTA (%)	64 ± 13	60 ± 9	61 ± 11	57 ± 10	57 ± 12	57 ± 9	0.01	0.20	0.05
2 μg/ml collagen-LTA (%)	54 ± 23	33 ± 21	29 ± 16	31 ± 23	27 ± 20	27 ± 17	<0.001	NS	NS
PFA-100 (closure time)	208 ± 69	251 ± 67	231 ± 73	226 ± 74	257 ± 63	245 ± 65	NS	NS	NS
Urinary thromboxane (pg 11dhTxB <sub>2</sub> /mg creatinine)	413 ± 110	332 ± 106	302 ± 112	331 ± 136	315 ± 145	298 ± 148	0.02	NS	NS

Data are means ± SD.

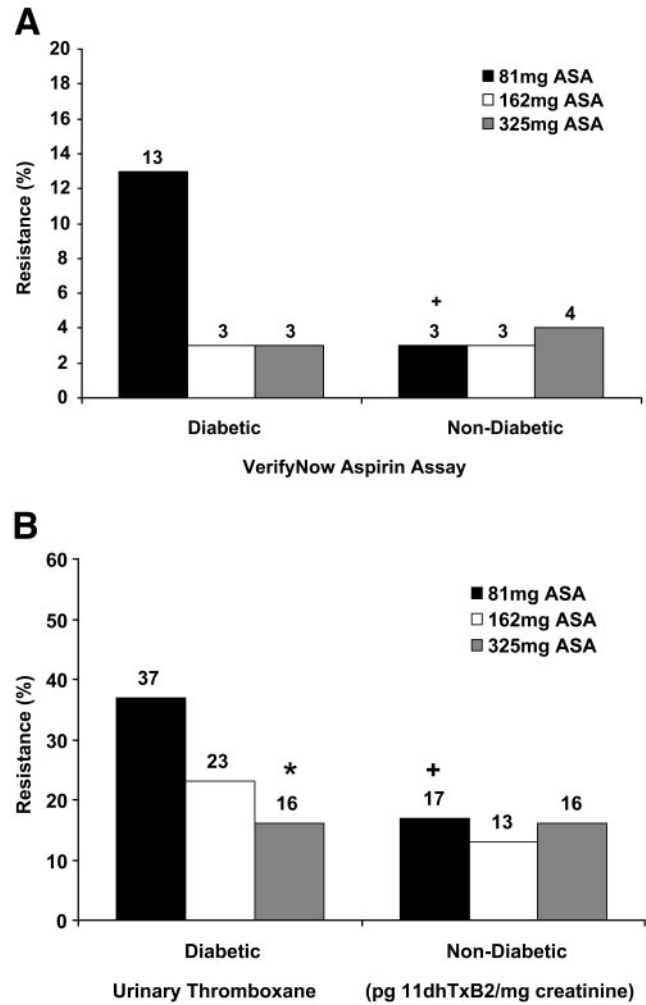


FIG. 1. A: Graph demonstrating the prevalence of aspirin resistance (%) measured by VerifyNow in diabetic and nondiabetic patients at three doses of aspirin. + $P \leq 0.05$  for difference between diabetic and nondiabetic patients treated with 81 mg aspirin. B: Graph demonstrating the prevalence of aspirin resistance (%) measured by urinary 11-dh-TxB<sub>2</sub> levels in diabetic and nondiabetic patients at three doses of aspirin. + $P \leq 0.05$  for difference in resistance between diabetic and nondiabetic patients treated with 81 mg aspirin. \* $P \leq 0.05$  for difference in resistance between 81 and 325 mg aspirin within the group of diabetic patients.

resistance rates were unaffected by increased aspirin dosing in nondiabetic patients (Fig. 1B).

**ADP-induced platelet aggregation measured by LTA.** ADP-induced platelet aggregation was significantly higher in diabetic patients than in nondiabetic patients at both 81- and 325-mg aspirin therapy ( $P \leq 0.05$  for both comparisons); however, no significance was observed between groups at 162 mg ( $P = 0.20$ ; Table 2). A dose-dependent effect of aspirin on ADP-induced aggregation was not observed in either patient group (Table 3).

No significant differences in aspirin resistance rates were observed with increased aspirin dosing in diabetic patients (Fig. 2). In contrast, 325 mg aspirin significantly reduced resistance rates in nondiabetic patients ( $P = 0.02$ ) (Fig. 3). Aspirin resistance rates were higher in diabetic patients than in nondiabetic patients at all aspirin doses; however, significance was observed between patient groups only at 325 mg ( $P = 0.002$ ) (Fig. 3).

TABLE 3  
Dose comparison

Arachidonic acid-stimulated assays	Diabetic (n = 30)				Nondiabetic (n = 90)			
	81 mg	162 mg	325 mg	P value	81 mg	162 mg	325 mg	P value
2 mmol/l arachidonic acid-LTA (%)	7 ± 18	4 ± 2	5 ± 3	NS	4 ± 2	4 ± 6	4 ± 1	NS
5 mmol/l arachidonic acid-LTA (%)	8 ± 22	7 ± 13	5 ± 4	NS	5 ± 8	4 ± 2	5 ± 4	NS
Accumetrics (ARU)	470 ± 66	448 ± 57	426 ± 45	0.18	445 ± 48	429 ± 38	428 ± 43	0.02
Other assays								
5 μmol/l ADP-LTA (%)	64 ± 13	60 ± 9	61 ± 11	NS	57 ± 10	57 ± 12	57 ± 9	NS
2 μg/ml collagen-LTA (%)	54 ± 23	33 ± 21	29 ± 16	<0.001	31 ± 23	27 ± 20	27 ± 17	NS
PFA-100 (closure time)	208 ± 69	251 ± 67	231 ± 73	0.02	226 ± 74	257 ± 63	245 ± 65	0.004
Urinary thromboxane (pg 11dhTxB <sub>2</sub> /mg creatinine)	413 ± 110	332 ± 106	302 ± 112	0.01	331 ± 136	315 ± 145	298 ± 148	NS

Data are means ± SD.

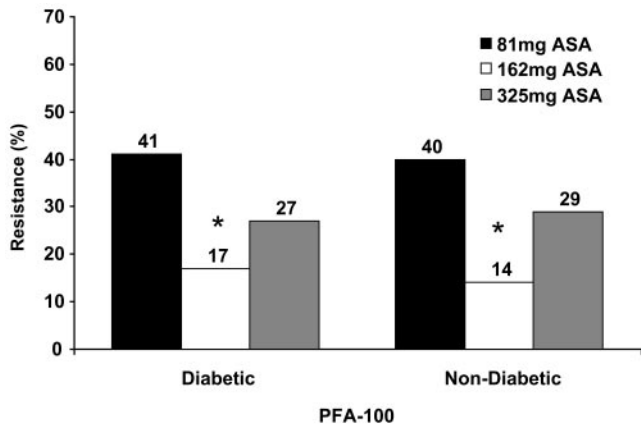


FIG. 2. Graph demonstrating the prevalence of aspirin resistance measured by PFA-100 in diabetic and nondiabetic patients at three doses of aspirin. \* $P \leq 0.05$  for difference between 81 and 162 mg in the diabetic and nondiabetic groups.

**Collagen-induced platelet aggregation measured by LTA.** Collagen-induced platelet aggregation was significantly higher in diabetic patients than in nondiabetic patients at 81 mg aspirin ( $P < 0.001$ ), whereas no significant differences were observed between groups during treatment with 162 or 325 mg aspirin (Table 2). Both 162 and 325 mg aspirin significantly reduced collagen-induced platelet aggregation compared with 81 mg aspirin in diabetic patients ( $P < 0.001$  for both comparisons); however, a significant decrease in collagen-induced platelet aggregation was not observed in nondiabetic patients (Table 3).

A significant decrease in aspirin resistance was observed in diabetic patients at doses  $>81$  mg ( $P = 0.01$ ); however, the prevalence of aspirin resistance was unaffected by aspirin dosing in nondiabetic patients (Fig. 3). Aspirin resistance rates were significantly higher in dia-

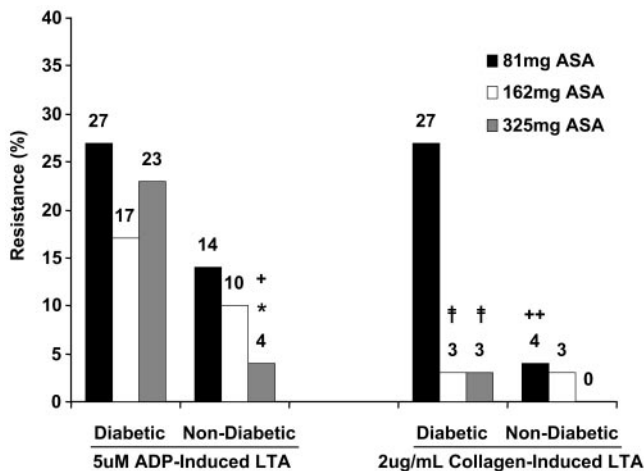


FIG. 3. Graph demonstrating the prevalence of aspirin resistance measured by 5  $\mu\text{mol/l}$  ADP-induced and 2  $\mu\text{g/ml}$  collagen-induced LTA in diabetic and nondiabetic patients at three doses of aspirin. + $P \leq 0.05$  for difference in resistance measured by 5  $\mu\text{mol/l}$  ADP-induced LTA between diabetic and nondiabetic patients treated with 325 mg aspirin. \* $P \leq 0.05$  for difference in aspirin resistance between 81 and 325 mg aspirin measured by 5  $\mu\text{mol/l}$  ADP-induced LTA in the nondiabetic patient group. ++ $P \leq 0.05$  for difference in resistance measured by 2  $\mu\text{g/ml}$  collagen-induced LTA between diabetic and nondiabetic patients treated with 81 mg aspirin. † $P \leq 0.05$  for difference in resistance between 81 and 162 mg, and 81 and 325 mg, as measured by 2  $\mu\text{g/ml}$  collagen-induced LTA in the diabetic group.

betic patients than in nondiabetic patients at 81 mg ( $P < 0.001$ ); however, similar rates of resistance were observed between groups at doses  $>81$  mg (Fig. 3).

**PFA-100 assay.** A significant effect of aspirin dosing on mean closure time was not observed between patient groups as determined by PFA-100 (Table 2). Although 325 mg aspirin did not significantly increase closure time measured by PFA-100, 162 mg aspirin significantly increased closure time in both diabetic and nondiabetic patients ( $P \leq 0.02$  for both comparisons) (Table 3).

Similarly, a significant decrease in rates of aspirin resistance was observed in both diabetic and nondiabetic patients with the administration of 162 mg aspirin ( $P \leq 0.04$  for both comparisons); however, 325 mg aspirin did not significantly affect rates of resistance compared with 81 mg aspirin (Fig. 2). Similar rates of aspirin resistance were observed between patient groups at all aspirin doses (Fig. 2).

## DISCUSSION

The current study was designed to compare the effect of aspirin dosing on platelet function in diabetic and nondiabetic patients from the ASPECT study, a randomized, double-blind, double-crossover trial using multiple methods to assess aspirin responsiveness. During treatment with all aspirin doses, there was no difference in platelet function or rates of aspirin resistance as measured by arachidonic acid-induced aggregation between diabetic and nondiabetic patients. However, during 81-mg daily aspirin therapy, diabetic patients had higher platelet function than nondiabetic patients measured by VerifyNow, urinary 11-dh-TxB<sub>2</sub> levels, and ADP- and collagen-induced LTA. Similarly, except for platelet aggregation measured by ADP-induced LTA and PFA-100, aspirin resistance was more common in diabetic patients than nondiabetic patients during 81-mg aspirin therapy as measured by all other assays. In general, increasing the dose of aspirin in the diabetic patient to  $>81$  mg/day reduced platelet function and the prevalence of resistance to levels observed in the nondiabetic patient, suggesting that low-dose aspirin therapy may not provide adequate platelet inhibition in selected diabetic patients and that higher aspirin dosing reduces the prevalence of outliers above the cut points for resistance.

These data agree with previous studies, in which increased aspirin resistance and normal platelet function have been demonstrated in patients receiving low doses of aspirin (19,20). Lee et al. (19) demonstrated that 150- and 300-mg daily aspirin doses were associated with decreased rates of aspirin resistance compared with daily doses  $\leq 100$  mg in patients with CAD measured by VerifyNow. Moreover, Alberts et al. (20) demonstrated that a significant portion of patients on low-dose aspirin therapy display normal platelet function measured by the PFA-100.

The exposure of collagen in the subendothelial matrix after endothelial disruption is an important primary activator of platelets that precedes thrombotic vessel occlusion. The observed dose-related effects of aspirin on collagen-induced aggregation in diabetic patients, despite inhibition of COX-1 as measured by arachidonic acid-induced aggregation, suggest that aspirin may exert antiplatelet effects through non-COX-1 mediated pathways. Because collagen-induced aggregation was significantly higher in diabetic patients than in nondiabetic patients during 81-mg aspirin therapy, our data suggest that diabetic patients may require higher aspirin doses to achieve equivalent inhibition of collagen-induced aggregation.

Our data demonstrate that the diabetic patient's platelet response to ADP stimulation is unaffected by aspirin dose compared with that of the nondiabetic patient, in whom we observed a lower prevalence of resistance at the higher dose. Recent studies have suggested an association between aspirin resistance and clopidogrel resistance in patients with diabetes, implying a global high platelet reactivity state in diabetic patients that can be detected by stimulation with multiple agonists (21). In the current study, although arachidonic acid-induced aggregation was equally suppressed in both patient groups, diabetic patients displayed greater ADP-induced aggregation at low and high doses than nondiabetic patients. These data suggest a potential mechanism to explain the benefit of dual antiplatelet therapy using an ADP receptor blocker in addition to aspirin in selected diabetic patients and may serve as important laboratory data for future studies of targeted antiplatelet therapy. Moreover, the reason for increased ischemic risk in the diabetic compared with the nondiabetic patient as demonstrated in previous studies may in part be explained by higher global platelet reactivity (22).

Finally, we demonstrated a dose-dependent effect of aspirin on the formation of 11-dh-TxB<sub>2</sub> in diabetic patients. A COX-2 source has been previously postulated as a mechanism to explain aspirin-insensitive thromboxane production at low doses (23). Our data provide support that higher doses of aspirin may significantly affect this source in diabetic patients.

Pretreatment studies could not be conducted because all patients had CAD and were therefore on aspirin therapy at the time of enrollment. In addition, because subjects were studied retrospectively, patient groups were not evenly distributed, and comparisons between patients with type 1 and type 2 diabetes could not be performed because of the small sample size. However, the Williams design is a robust method to determine treatment effects using small numbers of patients. Moreover, determination of diabetes status was assessed by clinical criteria rather than laboratory measurements of A1C. Thus, comparisons between the degree of glucose control and measurements of platelet function were not conducted. Future large-scale studies are needed to evaluate the clinical efficacy of higher aspirin dosing in diabetic patients in relation to glucose control.

In conclusion, diabetic patients with CAD during therapy with 81 mg have a higher prevalence of aspirin resistance by selected tests and greater ADP- and collagen-induced aggregation and in vivo production of thromboxane than nondiabetic patients. In general, increasing the dose of aspirin in the diabetic patient reduces platelet function and the prevalence of resistance to a level observed in the nondiabetic patient. The dose-related effects of aspirin on platelet function and thromboxane production in diabetic patients, despite near complete COX-1 blockade as determined by arachidonic acid-induced aggregation, suggest that the antithrombotic properties of the drug may not all be explained by COX-1 inhibition. The observation that ADP-induced aggregation was unaffected by aspirin dosing in diabetic patients suggests a potential benefit of treating selected diabetic patients with ADP receptor blockers in addition to higher dose aspirin.

## ACKNOWLEDGMENTS

The study has received support from Bayer HealthCare (Morristown, NJ) and Sinai Hospital of Baltimore.

This study was presented at the American College of Cardiology Annual Scientific Session, New Orleans, Louisiana, 24–27 March 2007.

## REFERENCES

- Purushothaman KR, Meerarani P, Moreno PR: Inflammation and neovascularization in diabetic atherosclerosis. *Indian J Exp Biol* 1:93–102, 2007
- Evangelista V, Totani L, Rotondo S, et al.: Prevention of cardiovascular disease in type-2 diabetes: how to improve the clinical efficacy of aspirin. *Thromb Haemost* 1:8–16, 2005
- Antithrombotic Trialists' Collaboration: Collaborative meta-analysis of randomized trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *Br Med J* 324:71–86, 2002
- Roffi M, Topol EJ: Percutaneous coronary intervention in diabetic patients with non-ST-segment elevation acute coronary syndromes. *Eur Heart J* 25:190–198, 2004
- Kuchulakanti PK, Chu WW, Torguson R, et al.: Correlates and long-term outcomes of angiographically proven stent thrombosis with sirolimus- and paclitaxel-eluting stents. *Circulation* 113:1108–1113, 2006
- Moussa I, Leon MB, Baim DS, et al.: Impact of sirolimus-eluting stents on outcome in diabetic patients: a SIRIUS (SIrolImUS-coated Bx Velocity balloon-expandable stent in the treatment of patients with de novo coronary artery lesions) substudy. *Circulation* 109:2273–2278, 2004
- Watala C: Blood platelet reactivity and its pharmacological modulation in (people with) diabetes mellitus. *Curr Pharm Des* 11:2331–2365, 2005
- American Diabetes Association: Aspirin therapy in diabetes (Position Statement). *Diabetes Care* 26 (Suppl. 1):S87–S88, 2003
- Sacco M, Pellegrini F, Roncaglioni MC, et al.: Primary prevention of cardiovascular events with low-dose aspirin and vitamin E in type 2 diabetic patients: results of the Primary Prevention Project (PPP) trial. *Diabetes Care* 12:3264–3272, 2003
- ETDRS Investigators: Aspirin effects on mortality and morbidity in patients with diabetes mellitus: Early Treatment Diabetic Retinopathy Study report 14. *JAMA* 10:1292–1300, 1992
- Gurbel PA, Bliden KP, DiChiara J, et al.: Evaluation of dose-related effects of aspirin on platelet function: results from the ASpirin-Induced Platelet EffeCT (ASPECT) Study. *Circulation* 25:3156–3164, 2007
- Gurbel PA, Bliden KP, Zaman KA, et al.: Clopidogrel loading with eptifibatide to arrest the reactivity of platelets: results of the Clopidogrel Loading with Eptifibatide to Arrest the Reactivity of Platelets (CLEAR-PLATELETS) Study. *Circulation* 111:1153–1159, 2005
- Chen W-H, Lee P-Y, Ng W, et al.: Aspirin resistance is associated with a high incidence of myonecrosis after non-urgent percutaneous coronary intervention despite clopidogrel pretreatment. *J Am Coll Cardiol* 43:1122–1126, 2004
- Jilma B, Fuchs I: Detecting aspirin resistance with the platelet function analyzer (PFA-100). *Am J Cardiol* 88:1348–1349, 2001
- Kearney D, Byrne A, Crean P, et al.: Optimal suppression of thromboxane A<sub>2</sub> formation by aspirin during percutaneous transluminal coronary angioplasty: no additional effect of a selective cyclooxygenase-2 inhibitor. *J Am Coll Cardiol* 43:532–533, 2004
- Gum PA, Kottke-Marchant K, Welsh PA, et al.: A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease. *J Am Coll Cardiol* 41:961–965, 2003
- Eikelboom JW, Hirsh J, Weitz JI, et al.: Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. *Circulation* 105:1650–1655, 2002
- Alberti KG, Zimmet PZ: Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 15:539–553, 1998
- Lee PY, Chen WH, Ng W, et al.: Low-dose aspirin increases aspirin resistance in patients with coronary artery disease. *Am J Med* 118:723–727, 2005
- Alberts MJ, Bergman DL, Molner E, et al.: Antiplatelet effect of aspirin in patients with cerebrovascular disease. *Stroke* 35:144–145, 2004
- Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al.: Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. *Diabetes* 54:2430–2435, 2005
- Colwell JA, Nesto RW: The platelet in diabetes: focus on prevention of ischemic events. *Diabetes Care* 26:2181–2188, 2003
- Patrignani P: Aspirin insensitive eicosanoid biosynthesis in cardiovascular disease. *Thromb Res* 110:281–286, 2003