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ORIGINAL ARTICLE

Postprandial hyperglycemia is a determinant of platelet activation in early type 2 diabetes mellitus

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Summary. *Background:* Chronic hyperglycemia is a major contributor to *in vivo* platelet activation in diabetes mellitus. *Objectives:* To evaluate the effects of acarbose, an α-glucosidase inhibitor, on platelet activation and its determinants in newly diagnosed type 2 diabetic patients. *Methods:* Forty-eight subjects (26 males, aged 61 ± 8 years) with early type 2 diabetes (baseline hemoglobin $A_{1c} \leq 7\%$ and no previous hypoglycemic treatment) were randomly assigned to acarbose up to 100 mg three times a day or placebo, and evaluated every 4 weeks for 20 weeks. The main outcome measures were urinary 11-dehydro-thromboxane $(TX)B_2$ (marker of *in vivo* platelet activation) and 8-iso-prostaglandin $(PG)F_{2\alpha}$ (marker of *in vivo* lipid peroxidation) excretion rate, 2-h postprandial plasma glucose (PPG) after a test meal, and assessment of glucose fluctuations by mean amplitude of glycemic excursions (MAGE). *Results:* Baseline measurements revealed biochemical evidence of enhanced lipid peroxidation and platelet activation. As compared with the placebo group, patients treated with acarbose had statistically significant reductions in urinary 11-dehydro-TXB_2 and 8-iso-$PGF_{2\alpha}$ excretion rate as early as after 8 weeks and at each subsequent time point (between-group $P < 0.0001$ at 12, 16 and 20 weeks), following earlier decreases in PPG and MAGE. Multiple regression analyses in the acarbose group revealed that PPG was the only significant predictor of 11-dehydro-TXB_2 urinary excretion rate ($\beta = 0.39$, $P = 0.002$) and MAGE the only predictor of 8-iso-$PGF_{2\alpha}$ urinary excretion rate ($\beta = 0.42$, $P = 0.001$). *Conclusions:* Postprandial hyperglycemia is associated with enhanced lipid peroxidation and platelet activation in early type 2 diabetes. A moderate decrease in PPG achieved with acarbose causes time-dependent downregulation of these phenomena, suggesting a causal link between early metabolic abnormalities and platelet activation in this setting.

Keywords: acarbose, platelet activation, postprandial hyperglycemia, type 2 diabetes mellitus.

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Introduction

Diabetes mellitus is associated with accelerated atherogenesis, resulting in premature ischemic manifestations of coronary, cerebrovascular and peripheral arterial disease [1]. Platelet activation has a pivotal role in the initiation and progression of atherothrombosis [2]. Abnormalities in the aggregation of diabetic platelets *ex vivo* were described by several groups [3], and chronic hyperglycemia was identified as a major determinant of *in vivo* platelet activation in diabetic patients [4].

We previously demonstrated enhanced thromboxane (TX) biosynthesis in type 2 diabetes mellitus and provided evidence that it is derived predominantly from platelets [4,5]. We also reported increased peroxidation of arachidonic acid to form biologically active F_2-isoprostanes, which may provide an important biochemical link between impaired glycemic control, enhanced oxidant stress and persistent platelet activation in this setting [6]. Tight metabolic control achieved by intensive insulin treatment, as well as metabolic improvement obtained by frequent reassessment of sulfonylurea therapy, reduced isoprostane formation and TX biosynthesis, as reflected by the urinary excretion of the TX metabolite 11-dehydro-TXB_2 [4,6,7]. However, prior studies evaluating lipid peroxidation and platelet activation in diabetes mellitus have been performed in patients at an advanced stage of the natural history of the disease, when the relative contribution of metabolic abnormalities with regard to accelerated atherogenesis to triggering platelet activation cannot be evaluated separately.

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Postprandial hyperglycemia, an early metabolic abnormality often preceding the clinical diagnosis of diabetes, is an independent risk factor for cardiovascular disease in type 2 diabetes [8]. The mechanism(s) by which glucose fluctuations during the postprandial period exert their deleterious effects may include enhanced oxidative stress and endothelial dysfunction, both of which contribute to platelet activation [3,9]. Treating patients with impaired glucose tolerance with acarbose, an α-glucosidase inhibitor that reduces postprandial hyperglycemia, prevents acute endothelial dysfunction [10] and may lower the risk of atherothrombotic events [11].

In the present study, we set out to evaluate biochemical indices of platelet activation and lipid peroxidation in patients with early type 2 diabetes mellitus and adequate metabolic control, naïve to hypoglycemic drugs, and with no evidence of microvascular or macrovascular complications. Moreover, in order to test the hypothesis that postprandial hyperglycemia is an important determinant of platelet activation in the early stage of diabetes, we performed a randomized, double-blind, placebo-controlled, multicenter study evaluating the effects of acarbose on platelet activation and its potential determinants in this setting.

Materials and methods

Setting and participants

We studied subjects with type 2 diabetes, diagnosed according to the American Diabetes Association criteria [12], in an early stage as defined by a known disease duration ≤ 6 months, presenting at the time of recruitment with hemoglobin $(Hb)A_{1c}$ values ≤ 7% in the absence of any treatment affecting glycemic control. Fifty-four caucasian subjects (30 males and 24 females, aged 61.4 ± 7.6 years) were recruited (from May 2005 through August 2007) at the Diabetes Clinics of the Universities of Chieti, Palermo, Pisa and Rome 'Tor Vergata'. Of these, 48 patients (26 males and 22 females, aged 60.7 ± 8.3 years) completed the randomized study and constitute the subject of this report.

During the screening evaluation, subjects were excluded from participating in the study if they presented any of the following: cigarette smoking; evidence of clinically significant hepatic, renal, cardiac or pulmonary insufficiency; history of malignant neoplasms (diagnosed and treated within the last 5 years); type 1 diabetes mellitus; microvascular complications (nephropathy, detected by the presence of persistent microalbuminuria between 30 and 300 mg per 24 h, in at least two of three consecutive 24-h urine collections; retinopathy, based on fundus oculi examination); macrovascular complications (negative clinical history of stroke, ischemic heart disease or peripheral arterial occlusive disease; negative electrocardiogram and arterial Doppler scanning); pregnancy or lactation; history of malabsorption; or regular (daily) consumption of alcohol. Patients requiring chronic non-steroidal anti-inflammatory drug therapy or low-dose aspirin were also excluded. Throughout the study, the only admitted analgesic was paracetamol (up to 500 mg d^{-1}). All patients with documented gastrointestinal disease and those taking medications likely to alter gut motility or absorption were also excluded. Patients with arterial hypertension or hypercholesterolemia were included if well controlled with stable drug therapy.

Study design

The study included a 3-week baseline period and a 20-week double-blind randomized treatment period. During the baseline period, patients were not treated with any drugs that could interfere with glucose metabolism.

Peripheral venous blood (at 9 a.m., after 12 h of fasting, and 2 h after a standard meal) and overnight urine samples were collected on the first and third weeks. Urine samples were additioned with the antioxidant 4-hydroxy-Tempo (1 mmol L^{-1}) (Sigma Chemical Co., St Louis, MO, USA) and stored at − 20 °C until extraction.

At the end of the baseline period, patients were randomly assigned to receive acarbose (up to 100 mg three times daily) or placebo in addition to dietary counseling. On the basis of the order of inclusion in the study, subjects were assigned a consecutive random number, and then allocated to one of the two treatment groups according to a permuted-block, 1 : 1 randomization list generated by the trial statistician with blocks of six participants, in which medication numbers 1–54 corresponded to either acarbose or placebo. These numbers were stored in opaque envelopes and opened by the enrolling clinic staff at the time of enrollment; opening the envelope constituted admission to the trial. Patients and all investigators were blind to the treatment allocation.

Acarbose was titrated over a period of 4 weeks, according to the following schedule: 50 mg once daily for the first 7 days (at dinner time); 50 mg twice daily (lunch and dinner) for the following 7 days; 50 mg three times daily (at breakfast, lunch and dinner) for another week; and finally, 100 mg three times daily. Down-titration to 50 mg three times daily for those who could not tolerate 100 mg three times daily was allowed in order to minimize the rate of drop-out.

During the double-blind treatment period, each subject received the randomized treatment every day with the first bite of each meal. Moreover, during this period, blood (at 9 a.m., after 12 h of fasting, and 2 h after a standard meal) and overnight urine samples were collected on the fourth, eighth, 12th, 16th and 20th weeks of treatment.

The standard meal consisted of measured portions of food containing 70 g of carbohydrates: 250 g of skimmed milk (about 234 kJ), five slices of toast (about 652 kJ), and two teaspoons of jam (about 292 kJ).

Patients were taught to self-monitor by fingerprick, and a glucometer was provided at the time of randomization. Subjects were asked to monitor their blood glucose at home once a week with nine determinations (before, 1 h after and 2 h after breakfast, lunch and dinner).

At randomization, patients were instructed to follow a low-fat and low-caloric diet (− 20% of estimated daily energy

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expenditure), and were encouraged to exercise regularly; these instructions were reinforced at each visit. Drug compliance was verified by pill counts.

Written informed consent was obtained from all participating subjects. The protocol was approved by the local Ethics Committees, and the study was performed in accordance with the principles of the Declaration of Helsinki, as revised in 2004.

Biochemical measurements

Blood glucose was measured by the glucose-oxidase method, and plasma insulin was measured by radioimmunoassay (Coat-A-Count Insulin kit; Diagnostic Products Corporation, Los Angeles, CA, USA). Incremental area under the curve was calculated using the trapezoidal rule after subtraction of the fasting glucose value.

Calculation of the mean amplitude of glycemic excursions (MAGE) was performed by measuring the arithmetic mean of the difference between consecutive peaks and nadirs, provided that the difference was greater than the standard deviation (SD) around the mean glucose values, using the nine-point glucose profiles obtained weekly during the double-blind treatment period. Homeostasis model assessment of insulin resistance (HOMA-IR) was performed as described by Matthews *et al.* [13].

Total cholesterol, triglyceride and high-density lipoprotein cholesterol concentrations were measured as previously described [14]. Low-density lipoprotein cholesterol was calculated using the Friedwald formula.

The urinary excretion rate of 8-iso-prostaglandin (PG)$F_{2\alpha}$, a marker of lipid peroxidation *in vivo*, and 11-dehydro-TXB_2 was measured by previously validated radioimmunoassay techniques [15,16]. Mean urinary excretion rates of these prostanoids in a non-diabetic population comparable for age and gender have been reported previously [8-iso-$PGF_{2\alpha}$, 208 ± 92 pg mg^{-1} creatinine (mean ± SD); 11-dehydro-TXB_2, 415 ± 244 pg mg^{-1} creatinine], using the same assay [6]. Plasma CD40 ligand (CD40L) was determined by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN, USA), according to recent recommendations [17]. Venous blood samples were drawn into plasma vacuum tubes containing one-tenth volume sodium citrate stock solution at 0.129 mM by atraumatic and sterile antecubital venipuncture at the study entry. To obtain platelet-poor plasma, the citrated blood was centrifuged (ROTANTA/TRC; Hettich, Tuttlingen, Germany) at 1500 × *g* for 15 min, and to obtain platelet-free plasma a second centrifugation step (Eppendorf, Milan, Italy) at 13 400 × *g* for 2 min was performed. Plasma aliquots were stored at − 80 °C until they were assayed for the determinations of interest. Samples were coded before laboratory analysis. The technicians were unaware of the patients' characteristics at all times. Soluble P-selectin (sP-selectin) levels were measured using a human sP-selectin Immunoassay (R&D Systems), following the manufacturer's instructions, as described previously [18]. We performed duplicate measurements with 100-μL aliquots of plasma diluted 20-fold into sample diluent included in the ELISA reagent set and measured the absorbance at 450 nm with a microplate reader (Spettra Max 190; Molecular Devices, Sunnyvale, CA, USA). We read the sP-selectin concentration from a calibration curve generated with SOFT-MAX PRO software (version 4.3 LS; Molecular Devices). The overall intra-assay and interassay coefficients of variation (CVs) were 2.4% and 5.2%, respectively, whereas the limit of detection was 1.06 ng mL^{-1}. Asymmetric dimethylarginine (ADMA) levels were measured in plasma samples by a commercially available enzyme-immunometric assay (DLD Diagnostika, Hamburg, Germany). C-reactive protein (CRP) plasma levels were measured by commercially available high-sensitivity immunoassay (Dade Behring Laboratories, Newark, DL, USA).

Statistical analysis

Sample size calculation was based on the primary endpoint of the study, urinary 11-dehydro-TXB_2 excretion rate at 20 weeks. It was estimated that 22 patients would be required in each treatment group, for a two-tailed α of 0.05 and a power of 80%, to detect a mean difference in 11-dehydro-TXB_2 excretion rate of at least 40% between acarbose and placebo at the end of the treatment period. Allowing for a 15% drop-out rate, we estimated that 27 patients per group should be enrolled. Given the actually measured baseline values of 11-dehydro-TXB_2 excretion rate (1595 ± 975 pg mg^{-1} creatinine), and with 48 subjects completing the treatment period, the study had 86% power to detect a mean absolute difference in excretion rate of 650 pg mg^{-1} creatinine between the two groups.

Data from samples collected on the first and third weeks before randomization were averaged to obtain baseline values.

The intrasubject CV was the ratio of the SD to the mean value of each variable in one subject over six repeated measurements performed in the placebo group.

The Kolmogorov–Smirnov test and examination of residual distribution were used to determine whether each variable had a normal distribution. When necessary, log transformation was used to normalize the data, or appropriate non-parametric tests were used (Mann–Whitney *U*-test, Spearman correlation coefficient).

Comparisons of baseline data between the groups were performed by chi-squared statistics, Fisher exact tests, unpaired Student's *t*-tests or Mann–Whitney *U*-tests.

For the primary analysis, we used a linear mixed-effects model for repeated measures over time, with 11-dehydro-TXB_2 excretion rate as the dependent variable, week of visit, study group and time-by-group interaction as fixed effects, and patients and error as random effects. Compound symmetry was used to model the covariance structure within patients. Within the mixed model, we obtained least-squares estimates of the treatment differences and standard errors, and estimated 95% confidence intervals (CIs) and *P*-values for the two prespecified intergroup contrasts (acarbose and placebo) for change in 11-dehydro-TXB_2 excretion rate value between

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baseline and weeks 4, 8, 12, 16 and 20, and over time within each group.

For other continuous variables [fasting plasma glucose (FPG), postprandial plasma glucose (PPG), HbA_{1c}, MAGE, HOMA-IR, body mass index (BMI), 8-iso-$PGF_{2\alpha}$, CD40L, CRP, ADMA, P-selectin], we used the same procedure as in the primary analysis.

Results for the repeated measure analysis are expressed as (least square) means. Ninety-five per cent CIs were determined on the basis of the difference between acarbose and placebo. Data are represented as mean change from baseline (95% CI) (Figs 2 and 3).

Associations between changes in FPG, PPG and HbA_{1c} values and changes in prostanoid measurements were assessed by linear regression analysis. Multiple regression analyses with stepwise variable selection were also performed, with urinary 8-iso-$PGF_{2\alpha}$ or 11-dehydro-TXB_2 excretion rate throughout the study as the dependent variables.

Data are presented as mean $\pm$ SD or as median [interquartile range (IQR)]. *P*-values lower than 0.05 were regarded as statistically significant. All tests were two-tailed, and analyses were performed using the SPSS (v. 13.0; APSS, Chicago, IL, USA), and STATA 9 (Stata Corp., College Station, TX, USA) statistical packages.

Results

At baseline, BMI was 27.0 (24.7–30.2) kg m^{-2} [median (IQR)], HOMA-IR was 2.81 (2.04–3.91) mg dL^{-1}, FPG was 126.9 (120.6–138.8) mg dL^{-1}, PPG was 164.5 (130.1–194.6) mg dL^{-1}, and HbA_{1c} was 6.7% (6.3–7%) in the 54 diabetic patients. The urinary excretion rate of 11-dehydro-TXB_2 was 1263 (955–2006) pg mg^{-1} creatinine [median (IQR)]. Approximately 75% of diabetic patients had 11-dehydro-TXB_2 excretion rates in excess of 2SD above the mean value previously found in non-diabetic subjects using the same assay [6]. The median urinary excretion rate of 8-iso-$PGF_{2\alpha}$ was 464 (328–652) pg mg^{-1} creatinine [median (IQR)]. Fifty per cent of diabetic patients had 8-iso-$PGF_{2\alpha}$ excretion rates in excess of 2SD above the mean value previously reported in non-diabetic subjects [6].

After study drug titration, 94% of the subjects receiving acarbose had reached the full dose of 100 mg three times daily. In three patients, the drug was down-titrated to 50 mg twice daily during the last 4 weeks, owing to gastrointestinal intolerance. The overall drop-out rate was 11% (two on acarbose, and four on placebo). The most common reason for discontinuation was the development of gastrointestinal symptoms such as flatulence or diarrhea (Fig. 1). Compliance with weekly nine-point glucose monitoring was fairly good.

Table 1 shows the demographic and clinical characteristics of the 48 patients who completed the randomized study. There were no statistically significant differences in any baseline variable between the two treatment groups (Table 1). Completers were comparable to non-completers with regard to baseline characteristics (data not shown).

Body weight, systolic and diastolic blood pressure and lipid profile were stable throughout the study in both groups (data not shown).

PPG

The effects of 20 weeks of treatment with acarbose or placebo on PPG in response to a test meal are illustrated in Fig. 2A,

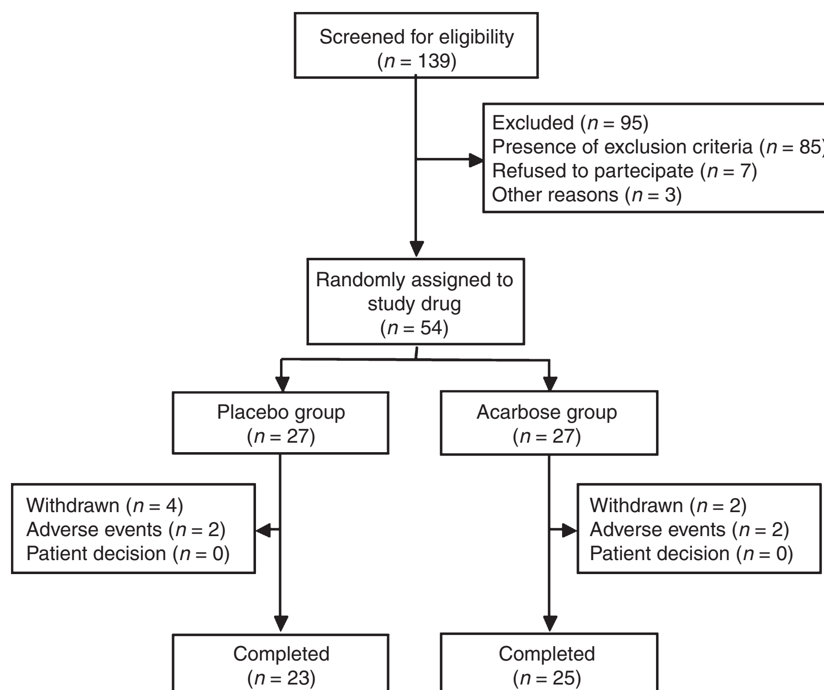

Fig. 1. Study flow diagram.

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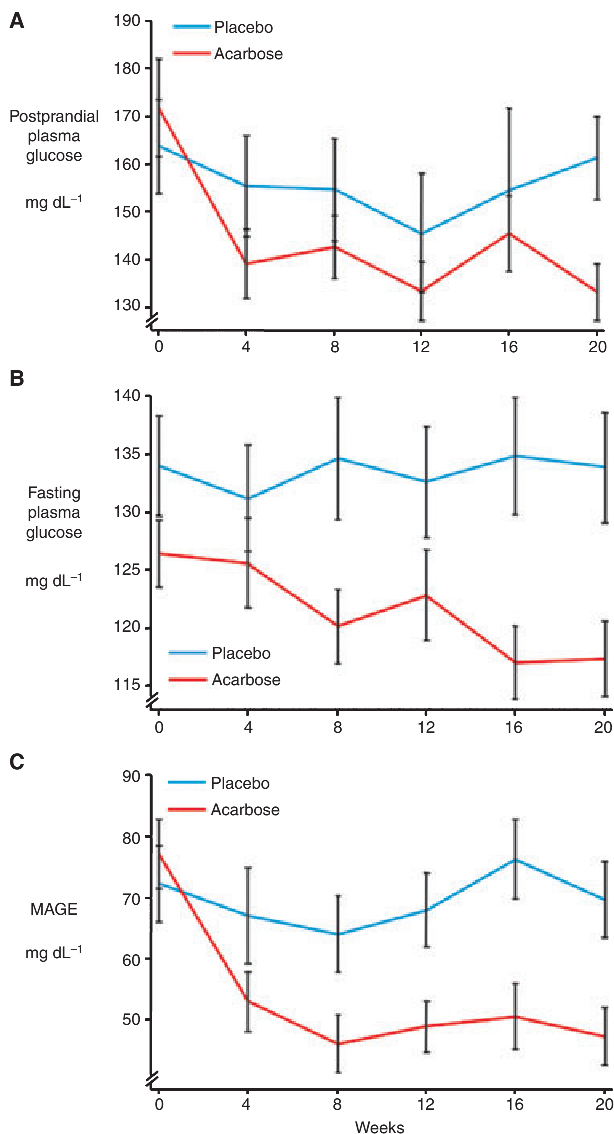

Fig. 2. Effects of 20 weeks of treatment with acarbose (red line) or placebo (blue line) on postprandial plasma glucose concentrations in response to a test meal (A), fasting plasma glucose (B) and mean amplitude of glycemic excursions (MAGE) (C). Data are represented as mean ± standard error of the mean.

and the results of the mixed-effects model repeated measures analysis are shown in Table S1. The acarbose group had a significantly greater reduction in mean PPG than the placebo group at weeks 4, 8 and 20. The mean change in PPG levels after 20 weeks was − 0.10 log mg dL^{-1} for the acarbose group and − 0.004 log mg dL^{-1} for the placebo group, giving a treatment difference of − 0.098 (95% CI − 0.15 to − 0.043) log mg dL^{-1} (P = 0.001) (Table S1; Fig. S1A).

FPG

The effects of 20 weeks of treatment with acarbose or placebo on FPG are illustrated in Fig. 2B. The mean change in FPG levels was significantly different between the two treatment groups after 16 weeks [− 0.036 (95% CI − 0.067 to − 0.004) log mg dL^{-1}, P = 0.028], whereas at week 20 the difference was not significant (P = 0.058) (Table S1; Fig. S1B).

HbA_{1c}

The mean change in HbA_{1c} value was different between the two treatment groups as early as after 8 weeks (Table S1). After 20 weeks, the difference between treatments was − 0.028 (95% CI − 0.044 to − 0.011) log percentage points ($P < 0.0001$).

MAGE

The effects of 20 weeks of treatment with acarbose or placebo on MAGE are illustrated in Fig. 2C. The mean change in glycemic instability as assessed by MAGE was different between the two treatment groups as early as after 4 weeks, and at each subsequent time point (Table S1). After 20 weeks, the difference between treatments was − 0.23 (95% CI − 0.35 to − 0.11) log mg dL^{-1} ($P < 0.0001$) (Table S1; Fig. S1C).

HOMA-IR was not affected by either treatment throughout the study period (Table S1).

Urinary 11-dehydro-TXB_2

The intrasubject CV of urinary 11-dehydro-TXB_2 excretion rate averaged 15% ± 7%, as determined on the basis of six repeated measurements performed in the placebo group throughout the study.

The mean change in urinary 11-dehydro-TXB_2 excretion rate was significantly different between the two treatment groups as early as after 8 weeks, and at each subsequent time point. After 20 weeks, the acarbose group had a significantly greater decrease in 11-dehydro-TXB_2 levels (by 40% vs. baseline) than did the placebo group (mean change − 0.23 vs. 0.031 log pg mg^{-1} creatinine); the difference between treatments was − 0.26 (95% CI − 0.33 to − 0.18) log pg mg^{-1} creatinine, $P < 0.0001$) (Table S1; Fig. 3A).

Plasma P-selectin

The mean change in plasma P-selectin levels was significantly different between the two treatment groups as early as after 8 weeks, and at each subsequent time point. After 20 weeks, the acarbose group had a significantly greater decrease in P-selectin levels than did the placebo group (mean change − 0.19 vs. 0.041 log ng mL^{-1}); the difference between treatments was − 0.23 (95% CI − 0.33 to − 0.12) log ng mL^{-1} ($P < 0.0001$) (Table S1; Fig. 3B).

Urinary 8-iso-$PGF_{2\alpha}$

The intrasubject CV of urinary 8-iso-$PGF_{2\alpha}$ excretion rate averaged 18% ± 6%. The mean change in urinary 8-iso-

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$PGF_{2\alpha}$ excretion rate was significantly different between the two treatment groups as early as after 8 weeks, and at each subsequent time point. After 20 weeks, the acarbose group had a significantly greater (by 33% vs. baseline) decrease in 8-iso-$PGF_{2\alpha}$ excretion rate than did the placebo group (mean change − 0.19 vs. 0.013 log pg mg^{-1} creatinine); the difference between treatments was − 0.20 (95% CI − 0.27 to − 0.13) log pg mg^{-1} creatinine ($P < 0.0001$) (Table S1; Fig. 3C).

Urinary 8-iso-$PGF_{2\alpha}$ and 11-dehydro-TXB_2 excretion rates were significantly related in the placebo group throughout the study (ρ 0.41, $P < 0.0001$). In addition, the percentage of baseline 8-iso-$PGF_{2\alpha}$ excretion rate was related to the percentage of baseline 11-dehydro-TXB_2 excretion rate (ρ 0.58, $P < 0.0001$) in the whole group.

Plasma CD40L

The mean change in plasma CD40L levels (intrasubject CV: 35% ± 13%) was not significantly different between the two treatment groups at any time point, except at 16 weeks ($P = 0.015$), despite a statistically significant median reduction by 31% vs. baseline after 20 weeks of acarbose (Table S1; Fig. 3D).

Serum CRP

CRP levels were not affected by either intervention to any statistically significant extent ($P = 0.15$) (Table S1).

Plasma ADMA

The mean change in plasma ADMA levels (intrasubject CV: 19% ± 10%) was not significantly different between the two treatment groups at any time point (Table S1).

Statistically significant correlations were found between MAGE and 8-iso-$PGF_{2\alpha}$ (ρ = 0.387, $P < 0.0001$; Fig. 4A) over the 20-week acarbose treatment period. PPG, but not FPG, was significantly related to both 11-dehydro-TXB_2 excretion rate (rho = 0.26, $P = 0.001$) and 8-iso-$PGF_{2\alpha}$ excretion rate (ρ = 0.30, $P = 0.0002$) during acarbose treatment.

Multiple regression analysis performed by pooling the data from both treatment groups revealed that urinary 8-iso-$PGF_{2\alpha}$ excretion rate [standardized regression coefficient (β) = 0.314, standard error of the mean (SEM) = 0.31, $P < 0.0001$] and HbA_{1c} ($\beta = 0.273$, SEM = 135.6, $P = 0.001$) were the only significant predictors of urinary 11-dehydro-TXB_2 excretion rate, independently of acarbose treatment, FPG, PPG, MAGE, CD40L, P-selectin and ADMA used as covariates. In the 25

Table 1 Baseline characteristics of the 48 type 2 diabetic patients who completed the randomized study, subdivided according to treatment group

Variable	Placebo (n = 23)	Acarbose (n = 25)	P-Value
Male gender, n (%)	12 (52)	14 (56)	0.98
Age (years)	61 (54–65)	62 (51–68)	0.76
BMI (kg m^{-2})	26.7 (23.7–30.1)	27.0 (24.6–31.2)	0.46
Waist-to-hip ratio	0.96 (0.88–0.99)	0.98 (0.94–1.03)	0.27
Systolic blood pressure (mmHg)	130 (120–146)	130 (120–135)	0.78
Diastolic blood pressure (mmHg)	79 (70–80)	80 (71–87)	0.27
Hypertension, n (%)	13 (56.5)	15 (60)	0.79
Total cholesterol (mg dL^{-1})	213.9 (173.9–221.9)	191.9 (151.9–225.9)	0.29
HDL cholesterol (mg dL^{-1})	53.9 (46.9–61.9)	49.9 (43.9–57.9)	0.34
Triglycerides (mg dL^{-1})	109.1 (91.1–150.2)	108.1 (84.1–152.2)	0.78
Hemoglobin A_{1c} (%)	6.5 (6.1–6.8)	6.7 (6.5–7.0)	0.12
Fasting plasma glucose (mg dL^{-1})	126.8 (121.8–140.8)	125.87 (115.8–136.8)	0.25
Postprandial plasma glucose (mg dL^{-1})	160.8 (126.9–180.8)	178.8 (122.9–207.8)	0.49
HOMA-IR	2.66 (2.12–3.46)	2.84 (1.55–4.76)	0.96
MAGE (mg dL^{-1})	72.3 (46.8–103.1)	81.9 (51.3–101.3)	0.86
Antihypertensives, n (%)	13 (56.5)	15 (60)	0.96
ACE-Is or ARBs, n (%)	10 (43)	12 (48)	0.98
Ca^{2+} channel blockers, n (%)	5 (21.7)	2 (8)	0.24
β-Blockers, n (%)	1 (4.3)	2 (8)	1.00
Diuretics, n (%)	3 (13)	6 (24)	0.46
Statins, n (%)	4 (17)	8 (32)	0.40
8-iso-$PGF_{2\alpha}$ (pg mg^{-1} creatinine)	481 (243–656)	475 (344–664)	0.38
11-dehydro-TXB_2 (pg mg^{-1} creatinine)	1126 (735–2127)	1509 (1035–2241)	0.43
sCD40L (ng mL^{-1})	0.61 (0.31–1.72)	0.86 (0.58–1.32)	0.17
P-selectin (ng mL^{-1})	184 (129–226)	125 (90–200)	0.07
ADMA (μmol L^{-1})	0.69 (0.58–1.05)	0.76 (0.62–0.85)	0.98

Data are median (interquartile range) unless otherwise specified. ACE-I, angiotensin-converting enzyme inhibitor; ADMA, asymmetric dimethylarginine; ARB, angiotensin receptor blockers; BMI, body mass index, calculated as weight in kilograms divided by the square of the height in meters; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; MAGE, mean amplitude of glycemic excursions; PG, prostaglandin; sCD40L, soluble CD40 ligand. SI conversions: to convert total and HDL cholesterol to mmol L^{-1}, divide by 38.6; to convert triglycerides to mmol L^{-1}, divide by 88.6; to convert glucose to mmol L^{-1}, divide by 18.0.

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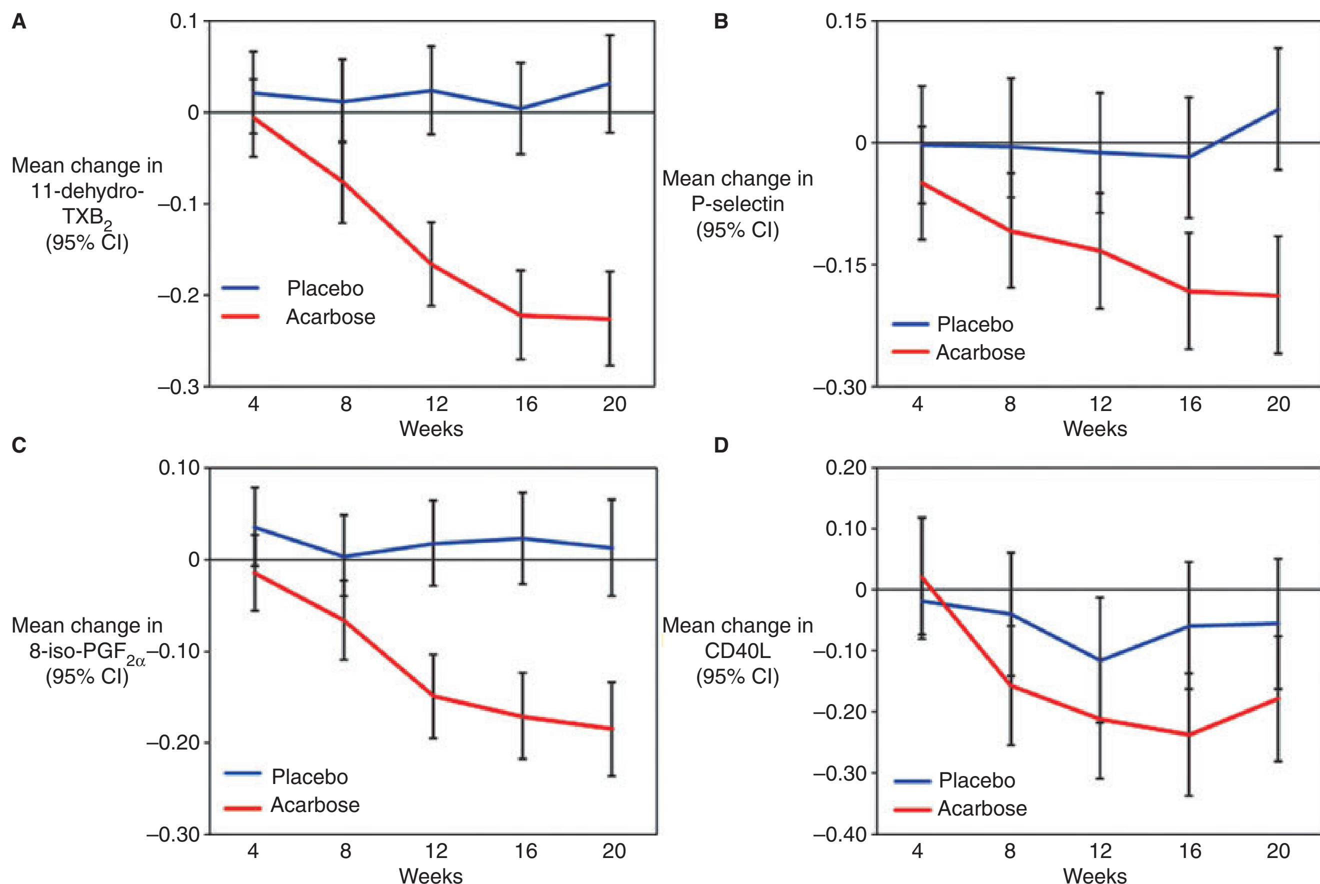

Fig. 3. Mean changes from baseline in urinary 11-dehydro-thromboxane (TX)B_2 excretion rate (A), plasma P-selectin (B), urinary 8-iso-prostaglandin (PG)$F_{2\alpha}$ excretion rate (C) and plasma CD40 ligand (CD40L) (D), by treatment group, over the 20-week treatment period. Data are logarithmically transformed and represented as least square mean with 95% confidence intervals (CIs).

patients randomized to acarbose, PPG remained the only significant predictor of urinary 11-dehydro-TXB_2 excretion rate (β = 0.47, SEM = 0.27, P < 0.0001), independently of FPG, MAGE, HbA_{1c} and 8-iso-$PGF_{2\alpha}$ excretion rate. In contrast, in the same group, MAGE (β = 0.42, SEM = 0.83, P = 0.001) was the only predictor of urinary 8-iso-$PGF_{2\alpha}$ excretion rate, independently of the other covariates. Among the 16 patients with 8-iso-$PGF_{2\alpha}$ and 11-dehydro-TXB_2 percentage of baseline in the first and second quartiles, 13 (81.2%) had a percentage of baseline PPG below the median. In contrast, only five (31%) patients with 8-iso-$PGF_{2\alpha}$ and 11-dehydro-TXB_2 percentage of baseline in the third and fourth quartiles had a percentage of baseline PPG below the median (Fig. 4B).

Discussion

In newly detected type 2 diabetes, postprandial but not fasting glucose is associated with an increased risk of myocardial infarction and mortality [19].

In the present study, we have demonstrated persistent *in vivo* platelet activation and enhanced lipid peroxidation in response to postprandial hyperglycemic spikes in patients with early type 2 diabetes mellitus, with HbA_{1c} levels ≤ 7%, and free of detectable microvascular and macrovascular complications. Biochemical evidence of persistent oxidative stress and platelet activation was obtained from seven repeated measurements throughout the 23-week study period in patients treated with placebo. Interestingly, TX biosynthesis was at least as high in newly diagnosed diabetic patients as previously reported in patients with longer-standing disease [6], supporting the hypothesis that platelet activation is related to the metabolic disorder and not to vascular disease *per se* [5].

The contribution of postprandial glucose excursions to overall diurnal hyperglycemia is predominant in well-controlled patients, whereas the contribution of fasting hyperglycemia increases with diabetes worsening [20,21]. Because *in vivo* lipid peroxidation and platelet activation were downregulated by a 20-week acarbose treatment, this effect could be largely attributed to the reduction in postprandial glycemia and acute glucose fluctuations, as shown by the multiple regression analyses.

In our study, only PPG (upward glycemic spikes) predicted thromboxane biosynthesis, consistently with our previous finding that acute, short-term hyperglycemia induced by hyperglycemic clamping enhances urinary 11-dehydro-TXB_2 excretion in patients with type 2 diabetes mellitus [22].

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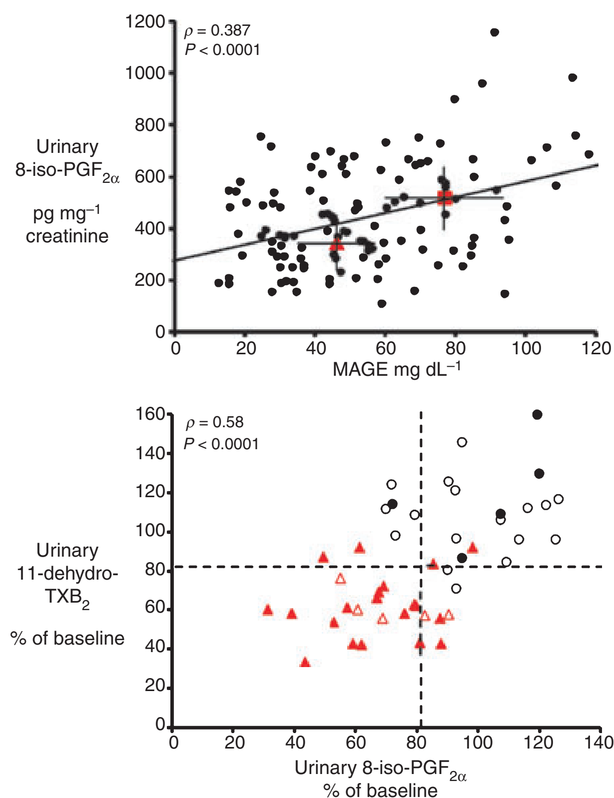

Fig. 4. Relationship between the extent of glycemic instability and lipid peroxidation and between the changes in PPG levels, lipid peroxidation and platelet activation throughout the treatment period. (A) Linear relationship between individual values of mean amplitude of glycemic excursions (MAGE) and urinary 8-iso-prostaglandin (PG)$F_{2\alpha}$ excretion rate measured in the 25 type 2 diabetic patients who completed the 20-week randomized treatment with acarbose. Mean ± standard deviation values of both parameters measured before (square) and after (triangle) acarbose treatment are also represented. (B) Correlation of individual percentage of baseline urinary 8-iso-$PGF_{2\alpha}$ and 11-dehydro-thromboxane (TX)B_2 excretion rates after the 20-week treatment with placebo (circles) or acarbose (triangles). Closed symbols represent subjects in the first and second quartiles for percentage of baseline postprandial glycemia. Open symbols represent subjects in the third and fourth quartiles for percentage of baseline postprandial glycemia. Vertical and horizontal dotted lines mark the boundaries of median values of percentage of baseline for both urinary metabolite excretion.

The MAGE, which includes both upward (postprandial) and downward (interprandial) acute fluctuations of glucose around a mean value [9], predicted *in vivo* lipid peroxidation, as previously reported by Monnier *et al.* [9], in a group of 21 patients with type 2 diabetes (mean HbA_{1c}, 9.6%) on oral antidiabetic drugs.

The statistically significant correlation between the urinary excretion rates of 11-dehydro-TXB_2 and 8-iso-$PGF_{2\alpha}$ supports the hypothesis that persistent platelet activation may, at least in part, be related to enhanced formation of biologically active products of arachidonic acid peroxidation [6], although this association may simply reflect a common trigger of both phenomena.

Acarbose treatment for 20 weeks induced a time-dependent reduction of the *in vivo* markers of oxidative stress and platelet activation that followed a progressive improvement of postprandial glucose fluctuations and long-term glycemic control. Thus, both abnormal lipid peroxidation and persistent platelet activation are reversible, at least in part, by an intervention aimed at flattening acute glucose fluctuations and PPG, at a time of the natural history of diabetes when metabolic control as assessed by HbA_{1c} and FPG is not yet substantially impaired. Indeed, percentage changes in F_2-isoprostane formation and TX biosynthesis were significantly related to MAGE and PPG level changes induced by acarbose.

Reduction of postchallenge hyperglycemia by acarbose has been shown to prevent acute endothelial dysfunction [10,23], which prompted us to measure ADMA, an inhibitor of NO synthase causing reduced NO availability and thus endothelial dysfunction [24]. The time-dependent pattern of inhibition of sP-selectin by acarbose, in the face of unaltered levels of ADMA, is consistent with the hypothesis that the enhanced sP-selectin in this setting largely originates from platelets rather than endothelial cells, and may therefore be considered an additional platelet activation marker. In this regard, our findings confirm and extend recent observations reporting inhibition of soluble selectins as well as platelet-derived microparticles by acarbose in diabetic patients [25].

CD40L is rapidly upregulated during platelet activation and promotes further platelet activation [2]. The hypothesis of CD40L release during TXA_2-dependent platelet activation in type 2 diabetes [26] was confirmed in the present study by the significant reduction in circulating levels of CD40L after acarbose treatment.

One limitation of the study is that analyses were not performed on an intention-to-treat basis. However, it should be emphasized that this was not a therapeutic trial with clinical endpoints, but rather a mechanistic study with biochemical endpoints that used acarbose as an investigative tool.

We also acknowledge that assessment of platelet activation was limited to two *in vivo* assays. However, it should be emphasized that measurement of platelet aggregation *ex vivo* is merely a capacity index that by no means reflects the extent and duration of platelet activation in response to stimuli operating *in vivo*.

Moreover, one may argue that, by collecting overnight urine samples, we evaluated lipid peroxidation and platelet activation during the fasting state that is characterized by a relative stability in plasma glucose. This may have weakened the strength and tightness of the correlations observed between variables throughout the study.

The simplification of the MAGE index determination based on a 1-day, nine-point glucose profile may not accurately reflect all of the glucose nadirs and peaks, as compared with the method previously described [9], using continuous glucose monitoring over 2 days. However, our aim was to test a relatively simple, 'point of care' index of glycemic instability in an early phase of the disease, and evaluate its modulation by acarbose.

In conclusion, this study provides biochemical evidence of persistently enhanced lipid peroxidation and platelet activation

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in the early phase of the natural history of type 2 diabetes, characterized by adequate metabolic control, but glucose fluctuations during postprandial periods, as assessed by MAGE and PPG. A decrease in PPG and MAGE achieved with acarbose was associated with downregulation of oxidative stress and platelet activation in this setting. These findings may suggest a causal relationship between early metabolic derangement and persistent platelet activation, thereby providing a mechanistic explanation for the cardioprotection associated with acarbose in a meta-analysis of long-term randomized studies [27].

Addendum

F. Santilli was involved in the acquisition, analysis and interpretation of data, drafting of the manuscript, and critical revision of the manuscript for important intellectual content. G. Davì shared with C. Patrono the study concept and design, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, obtaining funding, and study supervision. A. Consoli, M. Averna, R. Miccoli, P. Sbraccia and G. Ciabattoni were involved in the analysis and interpretation of data, as well as in the critical revision of the manuscript for important intellectual content. P. Sbraccia was also involved in the acquisition of data, together with G. Formoso, P. Di Fulvio, A. Ganci, and N. Pulizzi. S. Lattanzio performed the biochemical analyses and participated in the interpretation of data. All authors approved the final version of the manuscript.

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Disclosure of Conflict of Interests

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Mean changes from baseline in postprandial plasma glucose concentrations in response to a test meal (A), fasting plasma glucose (B) and mean amplitude of glycemic excursions (MAGE) (C), by treatment group, over the 20-week treatment period.

Table S1. Estimates of mean changes from baseline in the levels of biochemical variables under study with acarbose and placebo.

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