Food Intake Enhances Thromboxane Receptor–Mediated Platelet Activation in Type 2 Diabetic Patients but Not in Healthy Subjects

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Diabetes is associated with platelet hyperactivity (1,2), increases in circulating activated platelets and platelet-leukocyte aggregates (PLAs), and platelet hyperreactivity to in vitro stimulation (2,3). Thromboxane-mediated platelet activation is of special interest since diabetes is associated with increased platelet-dependent thromboxane A_2 (TxA_2) production (2), and diabetic patients appear to benefit less from prevention with aspirin than nondiabetic patients (4,5).

Insufficient insulin production and/or insulin resistance in diabetes leads to hyperglycemia, especially after food intake. Accumulating evidence indicates that postprandial hyperglycemia contributes to diabetes cardiovascular complications and may even predict complications more closely than fasting glucose levels (6). We recently reported (7) the effects of food intake and oral antidiabetes treatment on platelet function in type 2 diabetic patients. We found that food intake augmented platelet activation stimulated by ADP but not by thrombin (7). Responses to the TxA_2 analog U46619 were also studied but have not been reported. Healthy control subjects were only studied in the fasting state in the original study design (7). We therefore recalled them to test if the meal causes similar enhancement of platelet activation in subjects with normal blood glucose regulation, and we currently focus on the responsiveness to U46619.

RESEARCH DESIGN AND METHODS

— Fifteen patients with fairly well-controlled type 2 diabetes (mean ± SE age 53 ± 6 years, 11 male and 4 female, BMI 28.1 ± 3.8 kg/m^2, A1C 6.8 ± 1.7% [reference <5.2%]). The study originally recruited 15 matched healthy control subjects, but only 10 (age 51 ± 7 years, 7 male and 3 female, BMI 25.9 ± 3.3 kg/m^2, A1C 4.5 ± 0.2%) could be restudied with food intake for various reasons. All subjects gave informed consent to participate in the study, which was approved by the ethics committee of the Karolinska Institute. All subjects denied taking any platelet-active medication during the 2 weeks before each experiment.

After a 7- to 10-day wash-out period with dietary intervention only (baseline), the patients were randomized to 6 weeks of treatment with repaglinide or glibenclamide, followed by a 2-week wash-out and cross-over to the alternative treatment. They paid three visits to our laboratory: at baseline and after treatment with either drug (7). Measurements were performed before and 90 min after a standardized meal (54% carbohydrate, 30% fat, 16% protein; 621 kcal) on each occasion. Repaglinide (3.5 ± 1.4 mg daily) or glibenclamide (3.9 ± 1.9 mg daily) was given immediately before the meal (7). Platelet responses to food intake in the recalled healthy control subjects were compared with those of the patients at baseline.

Whole blood flow cytometry was used to determine platelet P-selectin expression, leukocyte CD11b expression, and PLA formation (3). U46619 and fMLP (*N*-formyl-methionyl-leucyl-phenylalanine) were used to stimulate platelets and leukocytes, respectively. Plasma elastase was determined by immunoassay. Data are presented as means ± SE. Paired *t* tests and repeated-measures ANOVA were used for data analyses.

RESULTS

— The carbohydrate-rich meal increased blood glucose from 10.5 ± 2.7 to 13.6 ± 3.6 mmol/l ($P < 0.05$) in the type 2 diabetic patients at baseline and from 9.7 ± 2.5 to 12.3 ± 3.2 mmol/l ($P < 0.05$) and 9.2 ± 2.0 to 11.2 ± 2.9 mmol/l ($P < 0.05$) with repaglinide and glibenclamide treatment, respectively. The meal did not increase blood glucose in the healthy control subjects (4.5 ± 0.2 mmol/l before and 4.2 ± 0.2 mmol/l after the meal).

The TxA_2 analog U46619 (0.3 μmol/l) enhanced platelet P-selectin expression similarly in patients and control subjects in the fasting state. Food intake markedly enhanced U46619-induced platelet P-selectin expression in the patients but not in the healthy subjects (Fig. 1*A*). Treatment with repaglinide or glibenclamide did not significantly attenuate U46619-induced platelet activation either before or after the meal (Fig. 1*A*).

Platelet activation by U46619 increased PLA formation more markedly among patients than among control subjects ($P < 0.05$) before the meal (Fig. 1*B*). U46619-induced PLA formation was markedly enhanced after food intake in the type 2 diabetic patients ($P < 0.05$) but not in the healthy control subjects (Fig.

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Abbreviations: PLA, platelet-leukocyte aggregate; TxA_2, thromboxane A_2.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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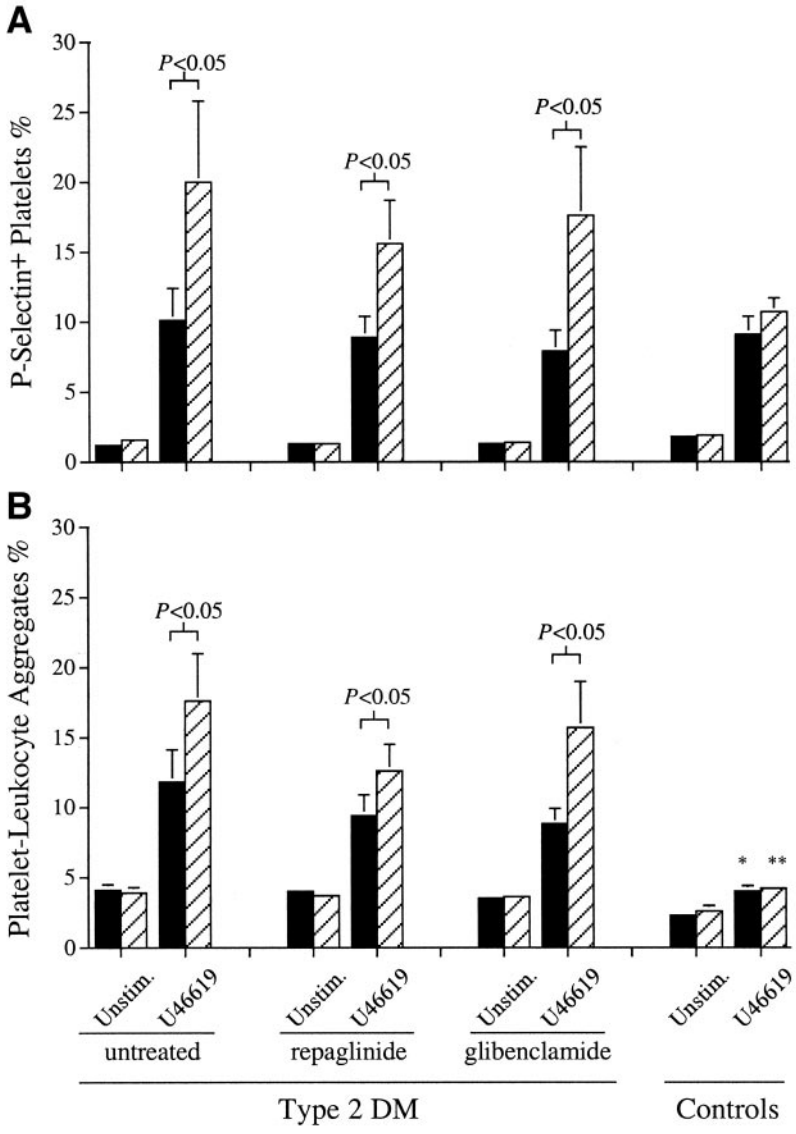

Figure 1—*Effects of food intake on platelet reactivity and platelet-leukocyte aggregation on TxA_2 receptor stimulation by U46619. Venous blood was obtained before (■) and 90 min after a standardized carbohydrate-rich meal (▨). Blood samples were incubated without (Unstim.) or with U46619 in the presence of fluorescent antibodies. Platelet P-selectin expression* (A) *and PLAs* (B) *were measured using whole blood flow cytometry. U46619 enhanced the PLA formation in both patients and control subjects before the meal* ($P < 0.05$)*; this effect was less pronounced in the control subjects* (*$P < 0.05$, **$P < 0.01$ *vs. the patients). Data plotted are means ± SE from 15 diabetic patients and 10 control subjects. DM, diabetes.*

1*B*). Enhancements were seen among platelet-neutrophil and platelet-monocyte, but not platelet-lymphocyte, aggregates. For example, U46619 stimulation increased platelet-neutrophil aggregates from 3.9 ± 0.6 to 14.3 ± 4.0% before and from 3.5 ± 0.5 to 22.8 ± 5.3% after the meal among patients ($P < 0.05$). PLA responses to U46619 stimulation were not significantly attenuated by repaglinide or glibenclamide treatment (Fig. 1*B*).

Plasma elastase, reflecting granulocyte/monocyte activation in vivo, was elevated in type 2 diabetic patients compared with control subjects (43.3 ± 2.8 vs. 33.3 ± 4.0 ng/ml; $P < 0.05$). Basal or fMLP (0.1 μmol/l)-stimulated leukocyte CD11b expression did, however, not differ between patients and control subjects ($P = 0.27$ by ANOVA). Neither food intake nor hypoglycemic drug treatment influenced plasma elastase or leukocyte CD11b expression (data not shown).

CONCLUSIONS — Food intake markedly enhanced platelet activation and PLA formation induced by TxA_2 receptor stimulation in type 2 diabetic patients but not in healthy subjects. This difference is probably related to postprandial hyperglycemia, which was seen in the diabetic group only. Indeed, hyperglycemia may augment platelet activation through elevated osmolality, and the effect is related to enhanced protein kinase C signaling and superoxide anion synthesis (8,9). Of interest, the present patients were relatively well controlled (mean A1C 6.8%), and their urinary excretion of the stable thromboxane metabolite 11-dehydro-TxB_2 was not elevated (7). Plasma elastase was elevated and platelet-leukocyte aggregation enhanced in our type 2 diabetic patients. Together with previous reports that glucose intake enhances leukocyte superoxide anion production (10) and causes proinflammatory changes in vivo (11), our data support the idea that increased inflammatory activity may contribute to hemostatic activation in diabetes (12). Increased sensitivity to thromboxane stimulation (the present findings) and an increased platelet turnover leading to less complete platelet cyclooxygenase inhibition at the end of a dosing interval (13) may contribute to the lesser efficacy of aspirin among diabetic patients (4,5).

The addition of oral antidiabetes treatment with repaglinide or glibenclamide only slightly reduced the fasting and postprandial blood glucose levels in our type 2 diabetic patients and influenced the postprandial platelet hyperreactivity little. Our results suggest that antidiabetes treatment with better effects on postprandial hyperglycemia may help to prevent diabetic platelet hyperreactivity.

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