Elevated Endothelial Microparticles Following Consecutive Meals Are Associated With Vascular Endothelial Dysfunction in Type 2 Diabetes

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Type 2 diabetes is associated with prolonged and exaggerated postprandial hyperglycemia and hypertriglyceridemia, endothelial dysfunction, and cardiovascular disease (CVD) (1–7). Endothelial dysfunction may link postprandial dysmetabolism to CVD (5–7). Since the postprandial state unveils the full scope of metabolic abnormalities in type 2 diabetes, previous studies (1) in fasting subjects may have underestimated the true risk. Currently, endothelial functions can only be estimated from indirect measurements, such as flow-mediated dilation (FMD) (6,8).

Cell-derived microparticles are released by cells in response to stress. Increased numbers of microparticles of various cellular origin circulate in patients at risk of CVD (9,10). Recently, vascular-endothelial cadherin (CD144)-positive microparticles were demonstrated in type 2 diabetic patients with coronary artery disease and patients with end-stage renal disease (11,12). Since vascular-endothelial cadherin is exclusively expressed by endothelial cells, CD144-positive microparticles may be regarded as endothelium-derived microparticles (EMPs), directly reflecting endothelial damage. However, it is unknown whether circulating EMPs are cause or consequence of CVD, and whether their occurrence associates with CVD per se or, rather, with diabetes-related metabolic abnormalities.

We hypothesized that in patients with uncomplicated type 2 diabetes, exposure to three consecutive high-fat mixed meals, given during a 24-h period, will disclose the full scope of their compromised metabolism and subsequent endothelial dysfunction, measured as FMD and circulating EMPs.

RESEARCH DESIGN AND METHODS

— After obtaining informed consent, 27 nonsmoking Caucasian male subjects ($n = 15$ with uncomplicated type 2 diabetes and $n = 12$ healthy age-matched volunteers) were studied during a 24-h period. No drug use other than sulfonylureas and/or metformin was allowed. After an overnight fast, subjects received three consecutive, isocaloric (900 kcal) mixed meals (75 g carbohydrates, 50 g fat [60% saturated], and 35 g protein) at time points $t = 0$ h (breakfast), 4 h (lunch), and 8 h (dinner). The study was approved by the local ethics committee and conformed to the principles of the Declaration of Helsinki.

Venous blood samples were collected before and at fixed intervals following the first meal, as previously described (13). To avoid artifacts, no in-dwelling canulae were used, and for each blood collection a new collecting system (size 1.0 mm 19G; Microflex, Vygon, France) was employed. Plasma aliquots (250 μl) were obtained after centrifugation (1,550*g*, 20 min, 20°C), snap frozen in liquid nitrogen, and stored at −80°C until assay. Glucose, A1C, insulin, and lipids were measured as previously described (13).

Microparticles were isolated and analyzed as described (9,13) using a FACScan flow cytometer with CellQuest software (Becton Dickinson, San Jose, CA). Microparticles were identified on forward and sideward scatter, binding of annexin-V, and a monoclonal antibody directed against a cell type–specific antigen and quantified as described. Samples were analyzed by a blinded-for-grouping variable technician.

Before each blood collection, FMD was measured at the right brachial artery (8,13) by a single observer (coefficient of variation [CV] <2%) using an ultrasound (Wall-Track System; PieMedical, Maastricht, the Netherlands).

To estimate the overall changes during 24 h, the area under curve (AUC) of metabolic parameters, FMD and microparticles, plotted against time was calculated. Data were analyzed by repeated-measures ANOVA, with time of measurement as the within factor and group as the grouping factor. Non-normally distributed data were log transformed before analysis; otherwise, nonparametrical tests were performed. Post hoc tests were only performed when ANOVA revealed overall significant differences. Correlations were performed using Spearman's rank correlation test. $P < 0.05$ was considered statistically significant.

RESULTS

— At baseline, type 2 diabetic patients (mean ± SD age 55 ± 2 years) had higher BMI, blood pressure, A1C (7.1 ± 1.1%), plasma glucose, triglycerides, and insulin levels but lower

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Abbreviations: AUC, area under curve; CVD, cardiovascular disease; EMP, endothelium-derived microparticle; FMD, flow-mediated dilation.

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

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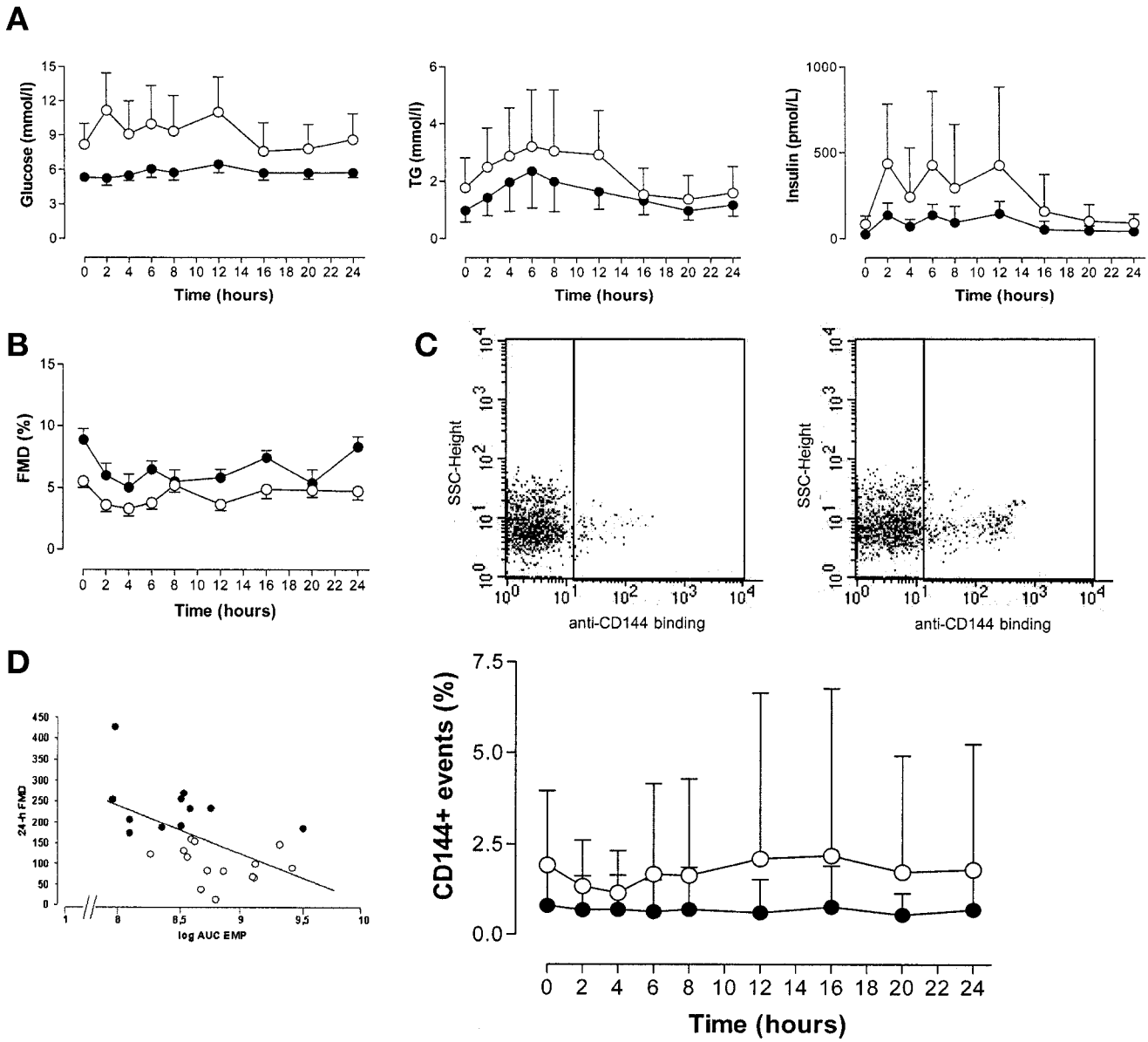

Figure 1—A: *The 24-h course of plasma glucose, triglyceride, and insulin concentrations in type 2 diabetic patients (○) and healthy subjects (●).* B: *Changes in FMD in type 2 diabetic patients (○) and healthy subjects (●) during the 24-h study day.* C: *Representative fluorescence-activated cell sorter dot plots of EMPs of a type 2 diabetic patient before* (left) *and after* (right) *three high-fat mixed meals and the 24-h course of EMPs in type 2 diabetic patients (○) and healthy subjects (●).* D: *Scatter plot representing the relationship between FMD and EMPs during 24 h in type 2 diabetic patients (○) and healthy subjects (●). Data in* A–C *are means ± SD.*

HDL cholesterol than control subjects (data not shown).

Plasma glucose, triglyceride, and insulin concentrations rose postprandially in patients, relative to baseline and healthy control subjects (all $P < 0.05$) (Fig. 1*A*). AUC glucose, triglycerides, and insulin concentrations were significantly higher in patients versus control subjects (all $P < 0.02$).

At baseline, FMD was decreased in patients versus control subjects (5.5 vs. 8.9%; $P < 0.01$), and deteriorated postprandially in both groups (Fig. 1*B*). FMD during 24 h was reduced in patients versus control subjects ($P < 0.01$) and was inversely associated with AUC glucose, triglycerides, and insulin ($r = -0.77$, $r = -0.52$, and $r = -0.59$, respectively, all $P < 0.01$).

Total microparticles at baseline and AUC of total microparticles were similar in patients and control subjects (3.2×10^9/l vs. 3.5×10^9/l, $P = 0.32$). In patients, the CD144-EMP fraction at baseline was higher than in control subjects ($1.9 \pm 2.0\%$ vs. $0.8 \pm 1.1\%$, $P < 0.05$) and rose significantly to $2.2 \pm 4.6\%$ (vs. control subjects $0.6 \pm 0.9\%$, $P < 0.05$) at $t = 12$ h (i.e., after consumption of all three meals) (Fig. 1*C*).

AUC CD144 EMPs were positively correlated with AUC glucose, triglycerides, and insulin ($r = 0.51$, $r = 0.38$, and $r = 0.57$, respectively, all $P < 0.05$) and negatively associated with FMD during 24 h ($r = -0.60$, $P < 0.01$) (Fig. 1*D*). When five subjects per group were additionally studied during a 10-h fast, no in-

crease of CD144 EMPs was found (data not shown).

CONCLUSIONS — CD144 microparticles circulate in patients with uncomplicated type 2 diabetes and associate with postprandial metabolic derangements and impaired FMD. We mimicked a real-life situation by giving type 2 diabetic patients three consecutive meals to unveil the true burden of meal-induced metabolic disturbances during a 24-h period (1). These derangements coincided with the highest levels of CD144 microparticles, which are only derived from endothelial cells. Other investigators used CD31 (i.e., PECAM-1) to identify EMPs (14). However, CD31 is not only present on endothelial cells but also on platelets and platelet-derived microparticles, the latter being the most abundant microparticles in the circulation (10,15). Therefore, previously reported EMP numbers may be an overestimation of the actual EMPs.

Under physiological conditions, the ability of cells to release microparticles may reflect their capacity to cope with stress (9,10). By shedding caspase 3–containing microparticles, cells may prevent apoptosis and maintain homeostasis (9). In disease, however, the ability to handle cell stress and to release microparticles may be altered. This may explain the difference between our present and earlier findings, showing that in well-trained, young, healthy male subjects, the total number of circulating microparticles rose postprandially, whereas in type 2 diabetic patients no diurnal changes in total microparticles were observed, and only a trend toward total microparticle elevation was observed in control subjects (data not shown). Thus, in type 2 diabetic patients, the ability of (endothelial) cells to respond to excessive metabolic stress may be impaired.

To summarize, in patients with uncomplicated type 2 diabetes, consumption of high-fat meals results in dysmetabolic changes and subsequent endothelial stress and injury, thereby contributing to atherogenesis and CVD risk. The possible value of CD144 microparticles as a marker to quantify endothelial dysfunction and/or injury needs further exploration.

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