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Postprandial Endothelial Activation in Healthy Subjects and in Type 2 Diabetic Patients: Role of Fat and Carbohydrate Meals

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OBJECTIVES To compare the effect of a high-fat meal and a high-carbohydrate meal (pizza), with and without antioxidant vitamins, on endothelial activation in healthy subjects and in patients with type 2 diabetes mellitus.

BACKGROUND The postprandial state is becoming increasingly acknowledged to affect some early events of atherogenesis.

METHODS In a randomized, observer-blinded, crossover study, 20 newly diagnosed type 2 diabetic patients and 20 age- and gender-matched healthy subjects received two meals at one-week intervals: a high-fat meal (760 calories) and an isoenergetic high-carbohydrate meal (non-cheese pizza). In all subjects, the same meals were repeated immediately following ingestion of vitamin E, 800 IU, and ascorbic acid, 1,000 mg.

RESULTS In normal subjects, the high-fat meal increased the plasma levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), which were prevented by vitamins. No change in these parameters occurred after pizza ingestion or pizza ingestion with vitamins. In diabetic patients, basal concentrations of glucose, cytokines and adhesion molecules were significantly higher than in nondiabetic controls. Both meals significantly increased cytokine and adhesion molecule levels, but the increase was more sustained following the high-fat meal. There was no significant change from baseline when vitamin supplementation accompanied each meal. There was a relationship between changes in serum triglycerides and changes in TNF-α ($r = 0.39$, $p < 0.01$), IL-6 ($r = 0.28$, $p < 0.05$) and VCAM-1 ($r = 0.25$, $p < 0.05$), and between changes in plasma glucose and changes in IL-6 ($r = 0.36$, $p < 0.01$) and ICAM-1 ($r = 0.31$, $p < 0.02$).

CONCLUSIONS An oxidative mechanism mediates endothelial activation induced by post-meal hyperlipidemia and hyperglycemia. (J Am Coll Cardiol 2002;39:1145–50) © 2002 by the American College of Cardiology Foundation

A low-fat, high-fiber diet, rich in dietary antioxidants from fruits, vegetables and whole grains is recommended to help reduce cardiovascular risk in the general population (1). Coronary heart disease risk is increased by consumption of a high-fat diet (2); on the other hand, people following current dietary guidelines present a lower risk of coronary mortality (3). In this regard, there is evidence in human subjects that a single high-fat meal induces endothelial dysfunction (4), which, at least in coronary circulation, predicts adverse cardiovascular events and long-term outcome (5).

Increased inflammatory activity is believed to predispose established atherosclerotic plaques to rupture, which can lead to a coronary event (6). In diabetic patients, circulating levels of proinflammatory cytokines (7–9), C-reactive protein (7,8), and soluble adhesion molecules (10–12) are elevated, suggesting stimulation of proatherogenic inflammatory activity. In apparently healthy men and women, chronically high levels of C-reactive protein, interleukin-6 (IL-6), and soluble intercellular adhesion molecule-1 (ICAM-1) predict increased risk of future cardiovascular events (13–15).

The aim of the present study was to compare the effects of a high-fat meal and a high-carbohydrate meal on plasma concentrations of tumor necrosis factor (TNF)-α, IL-6, ICAM-1 and vascular cell adhesion molecule-1 (VCAM-1) in healthy subjects and in patients with type 2 diabetes mellitus. Because an oxidative mechanism seems to be implicated in endothelial dysfunction after a high-fat meal (16,17), we also investigated whether acutely induced changes in circulating levels of cytokines and adhesion molecules were influenced by administration of antioxidant vitamin supplements.

METHODS

Twenty healthy subjects were recruited from the medical and paramedical staff of the Department of Geriatrics and Metabolic Diseases at the Second University of Naples. They had no evidence of present or past hypertension, hyperlipidemia, diabetes or any systemic conditions. All subjects were following ad libitum diets, had no recent

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Abbreviations and Acronyms

ICAM-1 = intercellular adhesion molecule-1
IL-6 = interleukin-6
TNF-α = tumor necrosis factor-alpha
VCAM-1 = vascular cell adhesion molecule-1

change in body weight or intercurrent illness and were taking no medication. Particular care was taken to ensure subjects had not recently used supplemental vitamins. None smoked. Twenty patients with newly diagnosed (within six months of diagnosis) type 2 diabetes mellitus were recruited from the Diabetes Clinic at the teaching hospital of the Second University of Naples. Exclusion criteria included presence of hepatic or renal disease; diabetic complications, both microvascular and macrovascular; cigarette smoking; hypertension; use of dietary antioxidant supplements; and treatment with lipid-lowering drugs. All patients were treated only by diet and were instructed not to change their usual dietary habits for the duration of the study. The protocol of the study was approved by the ethics committee of the Second University of Naples; healthy subjects and diabetic patients volunteered (without being paid) for the study and gave informed written consent before being tested. The clinical characteristics of the study populations are reported in Table 1.

Studies began at 8 AM, after a 12-h overnight fast. Fasting blood was drawn for determination of glucose levels, lipid (total, high-density lipoprotein cholesterol, and triglyceride levels), cytokine and soluble adhesion molecule count. Following this, subjects and diabetic patients ate, in random order and separated by a week interval, the following meals: 1) a high-fat meal; 2) an isoenergetic high-carbohydrate meal (pizza); and 3) the same meals following oral ingestion of antioxidant vitamin E (800 IU) and ascorbic acid (1,000 mg). The total energy content of the high-fat meal was 760 Kcal (3,180 J), with 58 g of carbohydrate, 50 g of fat, 20.4 g of saturated fat, 246 mg of cholesterol, 2.8 g of fiber and a total of 59.2 energy (%) from fat, 12.3 E% from protein and 28.5 E% from carbohydrates. It consisted of two sausages (80 g), six bread slices (90 g), a small egg (40 g), butter (15 g) and olive oil (5 g). The isoenergetic meal consisted of a pizza (300 g) with tomatoes (60 g), 144 g of carbohydrate, 17 g of fat, 2.2 g of saturated fat, no cholesterol, 4.5 g of fiber, with a total of 6.5 E% from protein, 20.6 E% from fat and 72.9 E% from carbohydrates. A person who was not involved in trial management randomly assigned the subjects using random numbers derived from published tables. The meals were prepared in one batch of the kitchen and consumed under supervision of a nurse. All parameters evaluated at baseline were repeated 2 and 4 h after eating.

Table 1. Characteristics of the Study Population

	Healthy Subjects	Diabetic Patients
Age, yrs	44 ± 5	46 ± 5
Gender, M/F	10/10	10/10
BMI, kg/m^2	26.8 ± 1.2	27.5 ± 1.3
Glucose, mmol/l	4.6 ± 0.6	8.5 ± 1.0*
HbA1c, %	4.7 ± 0.4	7.5 ± 0.8*
Cholesterol, mmol/l	4.8 ± 0.8	5.0 ± 0.9
HDL-cholesterol, mmol/l	1.4 ± 0.3	1.2 ± 0.3*
Triglycerides, mmol/l	0.8 ± 0.2	1.7 ± 0.5*
Blood pressure, mm Hg	115/72 ± 8/5	117/74 ± 9/6
ICAM-1, ng/ml	215 ± 37	325 ± 54*
VCAM-1, ng/ml	580 ± 95	705 ± 115*
IL-6, pg/ml	1.8 ± 0.8	3.6 ± 1.4*
TNF-α, pg/ml	2.9 ± 0.9	4.7 ± 1.3*

Data are presented as mean ± SD. *$p < 0.01$ compared with nondiabetic subjects.
BMI = body mass index; HDL = high-density lipoprotein; ICAM-1 = intercellular adhesion molecule-1; IL-6 = interleukin-6; TNF-α = tumor necrosis factor-α; VCAM-1 = vascular cell adhesion molecule-1.

Subjects were allowed to walk or sit, as they wished, during the interval between the first and second assessments. Blood was collected with minimal stasis by venipuncture after a brief rest in a supine position. Parameters were analyzed by independent investigators performing laboratory assays and blinded to the subject's identity, meal status and temporal sequence. Plasma was separated by centrifugation and stored at −80°C until analysis. Assays for serum total and high-density lipoprotein cholesterol, triglyceride, and glucose levels were performed in the hospital's chemistry laboratory. Plasma concentrations of TNF-α, IL-6, ICAM-1 and VCAM-1 were measured in duplicate using an enzyme-linked immunosorbent assay (R & D Systems, Minneapolis, Minnesota). All samples for a given subject were analyzed in the same series. The intra- and interassay coefficients of variation for the immunosorbent assays were below 5%.

Results are given as mean ± SD. Sample size was determined on the basis of two preliminary experiments with a high-fat meal, two with a pizza meal and two with a high-fat meal and vitamins. These experiments allowed us to estimate the SD and the difference between the means. For a desired p value of 0.05 and 80% power to detect an actual difference, a sample size of 10 per group was considered satisfactory (18). The statistical significance of differences between the experimental groups was determined by one-way analysis of variance (ANOVA). Scheffé's test method was used as a post hoc test if any differences were noted with ANOVA. The effect of order was tested with the analysis of variance. Linear regression analysis was used as appropriate. A p value of <0.05 was considered statistically significant.

RESULTS

Nondiabetic subjects. Lipid, glucose, blood pressure, cytokine and adhesion molecule parameters were similar in each of the study days, and there was no evidence of an order effect. Following the ingestion of the high-fat meal, mean serum triglyceride levels increased from 0.9 ± 0.2 to 1.2 ± 0.3 mmol/l at 4 h ($p = 0.009$). A similar increase was

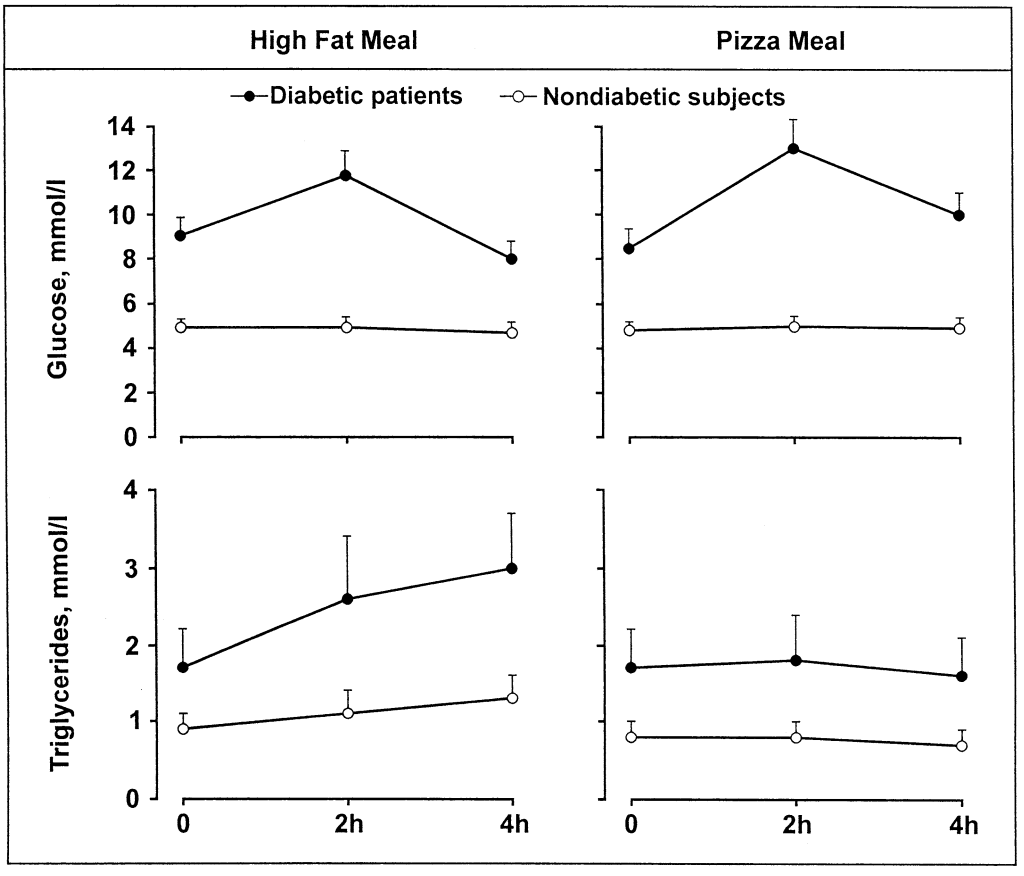

Figure 1. Changes in circulating glucose and triglyceride levels following high-fat and high-carbohydrate (pizza) meals in normal subjects and in patients with type 2 diabetes mellitus.

seen 4 h after the high-fat meal with vitamins ($p = 0.008$), whereas no increase in plasma triglyceride levels occurred after the pizza meal (Fig. 1). No significant change in glucose levels was observed after the meals. Plasma levels of TNF-α ($p = 0.009$), IL-6 ($p = 0.01$), ICAM-1 ($p = 0.011$) and VCAM-1 ($p = 0.01$) rose significantly after the high-fat meal, but not the pizza meal (Figs. 2 and 3); when vitamin supplementation accompanied the high-fat meal, there was a significant reduction of the rise of cytokine (TNF-α, $p = 0.04$; IL-6, $p = 0.045$) and adhesion molecule parameters (ICAM-1 $p = 0.03$, VCAM-1 $p = 0.035$) with values not significantly different from baseline (Table 2). There was a significant correlation between changes in serum triglyceride levels after the high-fat meal and changes in TNF-α ($r = 0.23$, $p < 0.05$).

Diabetic patients. The diabetic patients enrolled in the study were well-matched with the nondiabetic subjects for age, gender, body mass index and blood pressure values (Table 1). Plasma concentrations of glucose, HbA1c, TNF-α, IL-6, ICAM-1 and VCAM-1 were significantly higher in diabetic patients as compared with nondiabetic controls, whereas the plasma concentration of HDL-cholesterol was lower in diabetic patients than in controls (Table 1).

Triglyceride, glucose, cytokine and adhesion molecule parameters were similar in each of the study days and there was no evidence of an order effect. The rise in serum triglyceride levels in the diabetic patients was significantly greater ($p = 0.009$) than that recorded in nondiabetic subjects following the same meals (Fig. 1).

Mean plasma glucose rose from a basal value of 9.1 ± 0.8 to 11.8 ± 1.1 mmol/l ($p = 0.01$) 2 h after the high-fat meal and returned to below baseline at 4 h (Fig. 1). Following the pizza meal, the rise in plasma glucose levels was more sustained and lasted longer ($p = 0.008$ vs. baseline, $p = 0.02$ vs. high-fat). The rise in serum triglyceride and plasma glucose levels following the high-fat meal and pizza meal with vitamins were not significantly different ($p = 0.53$, $p = 0.47$, respectively) from the meals ingested without vitamins (Table 2).

Following the ingestion of high-fat meal, mean plasma concentrations of TNF-α ($p = 0.008$), IL-6 ($p = 0.009$), ICAM-1 ($p = 0.008$) and VCAM-1 ($p = 0.007$) rose steadily, reaching a plateau at 4 h (Fig. 2). These elevations from basal values were significantly higher than those recorded in nondiabetic subjects after the same high-fat meal ($p = 0.01$, $p = 0.016$, $p = 0.026$, $p = 0.016$, respectively). Moreover, the increase in plasma cytokine and adhesion molecule levels following the high-fat meal was also greater than that obtained after the pizza meal ($p =$

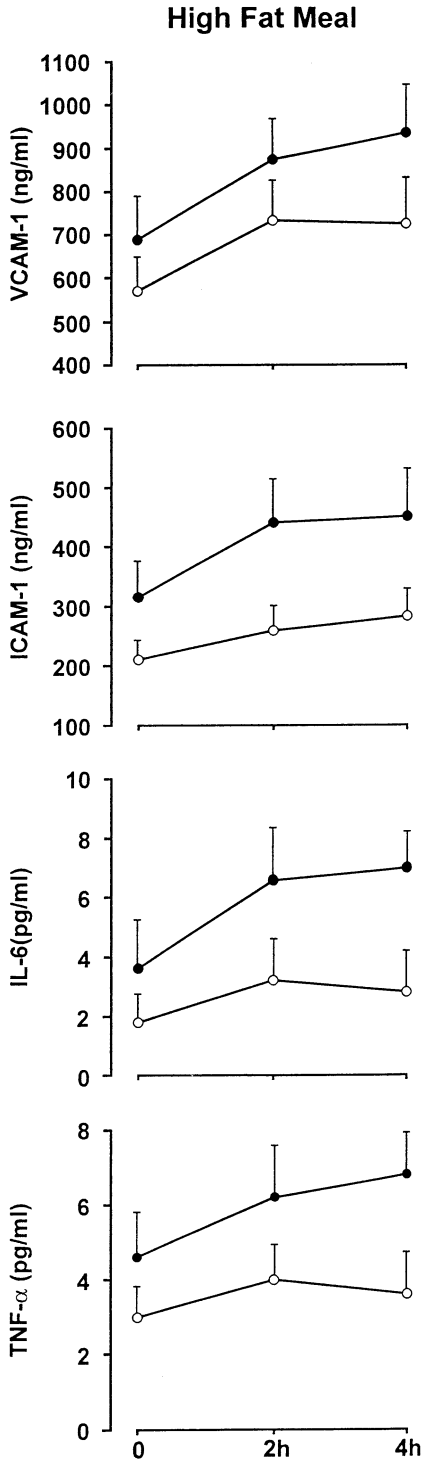

Figure 2. Effects of a high-fat meal on plasma concentrations of proinflammatory cytokines (TNF-α, IL-6) and adhesion molecules (ICAM-1, VCAM-1) in healthy subjects **(open circles)** and diabetic patients **(filled circles)**. ICAM-1 = intercellular adhesion molecule-1; IL-6 = interleukin-6; TNF-α = tumor necrosis factor-α; VCAM-1 = vascular cell adhesion molecule-1.

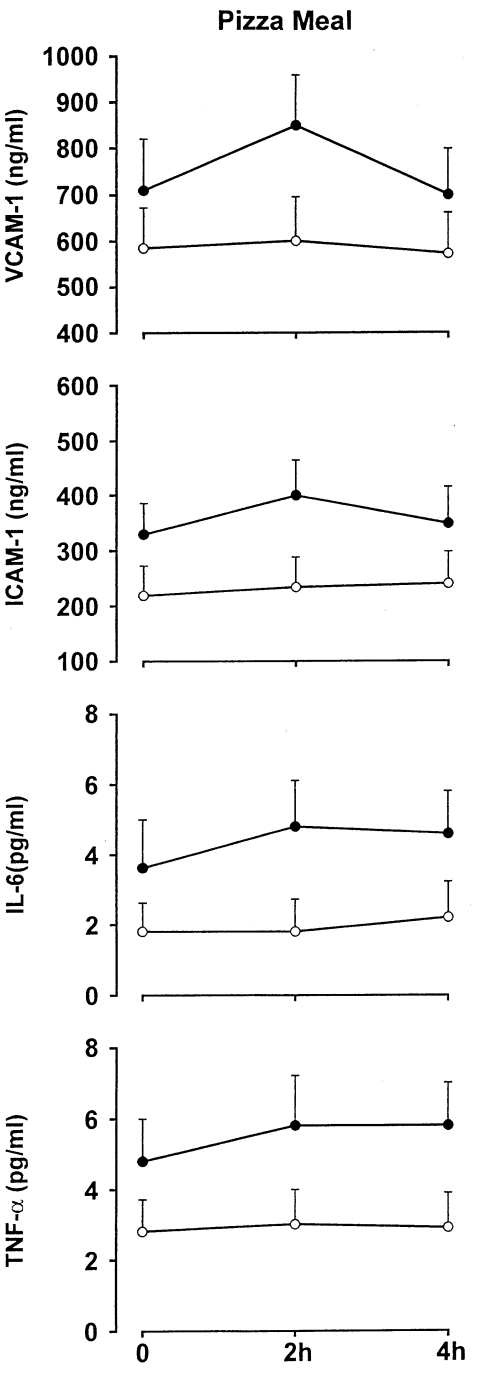

Figure 3. Effects of a high-carbohydrate meal (non-cheese pizza) on plasma concentrations of proinflammatory cytokines (TNF-α, IL-6) and adhesion molecules (ICAM-1, VCAM-1) in healthy subjects **(open circles)** and diabetic patients **(filled circles)**. ICAM-1 = intercellular adhesion molecule-1; IL-6 = interleukin-6; TNF-α = tumor necrosis factor-α; VCAM-1 = vascular cell adhesion molecule-1.

0.025, p = 0.036, p = 0.016, p = 0.026, respectively) (Fig. 3). When vitamin supplementation accompanied the high-fat or pizza meal, there was a significant reduction of the rise of cytokine and adhesion molecule parameters, with values that were significantly lower as compared with those recorded following the meals without vitamins (TNF-α, p = 0.025; IL-6, p = 0.035; ICAM-1, p = 0.016; VCAM-1, p = 0.04) and not significantly different from baseline (Table 2).

There was a significant correlation between changes in

Table 2. Effects of Vitamin Supplementation on Cytokine and Adhesion Molecule Parameters in Normal Subjects and Type 2 Diabetic Patients

	Baseline		2 h		4 h	
Parameters	High-Fat With Vitamins	Pizza With Vitamins	High-Fat With Vitamins	Pizza With Vitamins	High-Fat With Vitamins	Pizza With Vitamins
Normal subjects						
Glucose, mmol/l	4.9 ± 0.4	4.8 ± 0.4	4.8 ± 0.4	5.0 ± 0.4	4.8 ± 0.4	4.8 ± 0.4
Triglycerides, mmol/l	0.9 ± 0.2	0.8 ± 0.2	1.2 ± 0.3*	0.8 ± 0.2	1.3 ± 0.3*	0.8 ± 0.2
TNF-α, pg/ml	2.9 ± 0.8	2.8 ± 0.8	3.1 ± 0.9	2.9 ± 0.7	2.8 ± 0.7	2.8 ± 0.6
IL-6, pg/ml	1.7 ± 0.7	1.8 ± 0.8	2.2 ± 0.9	1.9 ± 0.8	2.1 ± 0.9	1.8 ± 0.8
ICAM-1, ng/ml	329 ± 56	341 ± 60	345 ± 62	327 ± 53	345 ± 70	359 ± 62
VCAM-1, ng/ml	575 ± 83	597 ± 90	592 ± 30	582 ± 92	609 ± 91	601 ± 97
Diabetic subjects						
Glucose, mmol/l	9.2 ± 0.7	8.8 ± 0.9	12 ± 1.2*	13.3 ± 1.3*	8.4 ± 0.9	10.9 ± 1.2*
Triglycerides, mmol/l	1.6 ± 0.5	1.7 ± 0.5	2.5 ± 0.8*	1.8 ± 0.5	3.1 ± 0.8*	1.7 ± 0.5
TNF-α, pg/ml	4.7 ± 1.2	4.9 ± 1.3	5.2 ± 1.4	5.0 ± 1.2	5.4 ± 1.5	5.1 ± 1.3
IL-6, pg/ml	3.5 ± 1.2	3.7 ± 1.2	4.3 ± 1.6	4.0 ± 1.3	4.4 ± 1.8	4.0 ± 1.2
ICAM-1, ng/ml	330 ± 60	321 ± 57	357 ± 65	333 ± 58	361 ± 71	319 ± 65
VCAM-1, ng/ml	689 ± 100	705 ± 104	724 ± 115	735 ± 115	735 ± 121	730 ± 111

Data are presented as mean ± SD. *$p < 0.01$ compared with baseline.
ICAM-1 = intercellular adhesion molecule-1; IL-6 = interleukin-6; TNF-α = tumor necrosis factor-α; VCAM-1 = vascular cell adhesion molecule-1.

serum triglyceride levels following the high-fat meal and changes in TNF-α ($r = 0.39$, $p < 0.01$), IL-6 ($r = 0.28$, $p < 0.05$), and VCAM-1 ($r = 0.25$, $p < 0.05$). Moreover, there was also a correlation between changes in plasma glucose levels following the pizza meal and changes in IL-6 ($r = 0.36$, $p < 0.01$), and ICAM-1 ($r = 0.31$, $p < 0.02$).

DISCUSSION

We found that a single high-fat meal acutely affects the level of markers of cardiovascular risk in normolipidemic healthy subjects. To our knowledge, this is the first demonstration that a fat-enriched meal, in contrast to a high-carbohydrate meal, is associated with endothelial activation, as indicated by the increased circulating levels of the adhesion molecules ICAM-1 and VCAM-1.

Foodstuffs and endothelial activation. Soluble forms of cellular adhesion found in plasma are considered an index of endothelial activation and even a molecular marker of early atherosclerosis (15). The term endothelial activation describes the functional changes that endothelia may undergo under the influence of various stimuli, the best studied of which are inflammatory cytokines and bacterial endotoxin (19). In this context, the increase of circulating levels of TNF-α and IL-6 following the high-fat meal might have induced endothelial activation and upregulation of adhesion molecule expression. Tumor necrosis factor-α functions within a complex and tightly regulated cytokine network activating multiple signal transduction pathways and inducing or suppressing a wide variety of genes, including those encoding for other cytokines and adhesion molecules (20).

Postprandial lipidemia is strongly associated with a risk of development of atherosclerotic lesions (21). It is noteworthy that IL-6 may induce endothelial expression of chemokines and adhesion molecules (22), and its plasma concentrations are predictive of future myocardial infarction among apparently healthy men (14). Thus, postprandial hypertriglyceridemia may be the link between high-fat meal and endothelial activation in normal subjects.

Unlike the high-fat meal, the pizza meal does not activate endothelium in normal subjects. Although acute and postprandial hyperglycemia are increasingly being seen as toxic for endothelial functions (23,24), the glycemic excursions following the pizza meal in normal subjects were very small and had returned to basal levels at the time of testing. This did not happen in type 2 diabetic patients in whom plasma glucose levels remained elevated 4 h after ingestion of the pizza meal. In this condition, circulating levels of cytokines and adhesion molecules were significantly elevated above baseline, with a direct relationship with blood glucose levels. Moreover, the diabetic patients who ate the high-fat meal presented with the greatest endothelial activation, as indicated by the highest circulating levels of TNF-α, IL-6, ICAM-1 and VCAM-1.

The diabetic patients herein studied presented endothelial activation in the basal state as indicated by the circulating cytokine and adhesion molecule levels, which were significantly higher than those of age-matched nondiabetic subjects. This is consistent with current evidence indicating that in diabetic patients circulating levels of some proinflammatory cytokines (7–9), C-reactive protein (7,8), and soluble adhesion molecules, such as ICAM-1 (10,11) and VCAM-1 (11,25), are significantly higher compared with the nondiabetic population. Because increased inflammation activity is believed to predispose established atherosclerotic plaques to rupture, endothelial activation occurring in diabetes may help explain why type 2 diabetic patients are at increased risk of developing coronary heart disease compared with the general population (26).

The effect of antioxidant vitamins. Impairment of endothelium-dependent vasodilation following a high-fat meal has previously been reported (16,17) and attributed to an oxidative mechanism. In our study, pretreatment with

antioxidant vitamin supplements normalized endothelial activation following the high-fat meal in normal subjects and strongly reduced circulating levels of cytokine and adhesion molecules in diabetic patients following high-fat or pizza meals. Supplementation with high doses of vitamin E markedly reduces susceptibility of isolated LDL to oxidation and inhibits secretion of proinflammatory cytokines (27). Our data seem to indicate that an oxidative mechanism mediates the effect of a high-fat meal on cytokine and adhesion molecule levels; this hypothesis is strengthened by the evidence that another structurally unrelated antioxidant, such as glutathione, prevents the increased plasma ICAM-1 levels seen after postprandial hyperglycemia in humans (28).

Study limitations. Although the present study utilized familiar foodstuffs rather than laboratory diet, it did not attempt to differentiate the effect of single nutrient in the meals, or to determine the healthiest mixture of foods for the endothelium. However, diets consumed by individuals consist of a combination of foods containing multiple nutrients and non-nutrients. Moreover, an attempt was not made to differentiate the effects of vitamin C and vitamin E. Finally, this study was based on surrogate end points.

Conclusions. The results from this study indicate that a high-fat meal in healthy subjects is able to switch the endothelium towards a more atherogenetic profile, which is amplified in patients with type 2 diabetes mellitus. The pizza meal causes endothelial activation in diabetic subjects only, although to a lesser extent than the high-fat meal does. Endothelial activation is prevented by antioxidant vitamins, suggesting an oxidative mechanism mediated by post-meal hyperlipidemia and hyperglycemia. The lack of endothelial activation after the pizza meal, at least in nondiabetic subjects, may contribute to the healthier cardiovascular outlook of people consuming a Mediterranean-type diet (29,30).

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