

Effect of dietary antioxidants on postprandial endothelial dysfunction induced by a high-fat meal in healthy subjects^{1,2}

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ABSTRACT

Background: Persons following current dietary guidelines have a lower risk of mortality from coronary heart disease.

Objective: The objective was to compare the short-term effect of a high-fat meal and a high-carbohydrate meal, with and without dietary antioxidants, on vasomotor, antiplatelet, and hemostatic functions of the endothelium in healthy subjects.

Design: In an observer-blinded, randomized crossover study, 25 (13 men and 12 women) healthy subjects were given each of 3 meals in random order at 1-wk intervals: a high-fat meal (760 kcal), an isoenergetic high-carbohydrate meal, and a high-fat meal with dietary antioxidants from vegetables (865 kcal). Endothelial functions, as assessed by hemodynamic and rheologic responses to L-arginine—the natural precursor of nitric oxide—were evaluated before and 4 h after each meal.

Results: Unlike the high-carbohydrate meal, the high-fat meal increased the plasma concentrations of triacylglycerol ($P < 0.01$); both meals activated hemostasis. The high-carbohydrate meal did not modify blood pressure, and platelet aggregation decreased in response to the L-arginine load (-7.1 ± 2.3 mm Hg and $-8.5 \pm 4.5\%$, respectively). After the high-fat meal, the decrease in blood pressure in response to L-arginine was reduced (-1 ± 0.8 mm Hg), and platelet aggregation showed a paradoxical increase ($4.1 \pm 2.1\%$; $P < 0.01$ compared with the high-carbohydrate meal). The high-fat meal with antioxidants partially restored the vascular response to L-arginine.

Conclusion: Compared with a high-carbohydrate meal, a high-fat meal can modify endothelial functions toward a more atherogenic profile, which is partially prevented by dietary antioxidants. *Am J Clin Nutr* 2003;77:139–43.

KEY WORDS High-fat meal, high-carbohydrate meal, dietary antioxidants, endothelial functions, L-arginine

INTRODUCTION

Coronary heart disease risk increases with the consumption of a high-fat diet (1). The paradigm that dietary fats act exclusively via effects on serum lipids and lipoproteins has been challenged: the Lyon Heart Study (2) and the Indian Heart Study (3) have both shown in clinical trials that diet can prevent fatal and nonfatal cardiovascular events in patients with cardiovascular disease without significantly affecting plasma lipids. Supporting this challenging view are the findings that a single high-fat meal induces endothelial dysfunction in humans (4), and improvement of endothelial dysfunction is a potential mechanism by which antioxidants in the diet may prevent cardiovascular events (5).

Besides abnormal vasomotor responses (6), there is a growing perception that abnormal hemostatic processes of coagulation, fibrinolysis, and platelet aggregation contribute to cardiovascular disease etiology (7). Although the notion that diet may influence hemostasis is not new, the relation is far from clear (8): fibrinolysis is enhanced in the presence of coagulation activation, so it may be difficult to differentiate between primary and compensatory effects.

The aim of the present study in healthy subjects was to compare the effects of a high-fat meal with those of a high-carbohydrate meal on endothelial functions assessed by hemodynamic and rheologic responses to L-arginine, the natural precursor of nitric oxide. We also investigated the effect of the meals on coagulation and fibrinolysis indexes. Finally, the hypothesis that oxidative stress mediates the impairment of endothelial functions after a high-fat meal was tested by combining the high-fat meal with dietary antioxidants.

SUBJECTS AND METHODS

Experimental design

Twenty-five (13 men and 12 women) healthy subjects aged 23–40 y ($\bar{x} \pm$ SD: 27 ± 6 y) 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 systemic conditions, and were nonsmokers. The mean body mass index (weight in kilograms divided by the square of height in meters) was 23.7 ± 1.9 . All subjects were consuming weight-maintaining diets with 250 g carbohydrate/d, had no recent change in body weight or intercurrent illness, and were taking no medication. Particular care was taken to ensure against the recent use of supplemental vitamins or drugs that contain aspirin or similar products. The protocol of the study was approved by the ethical committee of the Second University of Naples; all subjects volunteered for the study and gave informed consent before being tested.

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The studies began at 0800 after the subjects had fasted overnight for 12 h. Fasting blood was drawn for the measurement of glucose, lipids (total cholesterol, HDL cholesterol, and triacylglycerol), coagulation, and fibrinolysis variables. Vasomotor and antiplatelet functions of the endothelium, in terms of blood pressure and platelet aggregation responses to L-arginine, were then assessed. The subjects then ate in random order and separated by a 1-wk interval the following meals: 1) a high-fat meal, 2) an isoenergetic high-carbohydrate meal, and 3) the same high-fat meal with dietary antioxidants.

The total energy content of the high-fat meal was 760 kcal (3180 kJ); the meal provided 58 g carbohydrate, 50 g fat, 20.4 g saturated fat, 246 mg cholesterol, 2.8 g fiber, and 59.2% of energy from fat, 12.3% from protein, and 28.5% from carbohydrate. It consisted of 2 sausages (80 g), 6 bread slices (90 g), a small egg (40 g), butter (15 g), and olive oil (5 g).

The high-carbohydrate meal consisted of a pizza (300 g) with tomatoes (60 g); the meal provided 144 g carbohydrate, 17 g fat, 2.2 g saturated fat, no cholesterol, 4.5 g fiber, and 20.6% of energy from fat, 6.5% from protein, and 72.9% from carbohydrate.

The high-fat meal with antioxidants consisted of the same high-fat meal described above plus vegetable foods: 100 g tomatoes, 200 g carrots, and 100 g peppers (184 mg vitamin C, 19.65 mg vitamin E, 15 mg β -carotene, and 9.2 g fiber). The total energy content of the meal was 865 kcal. Ten of the 25 subjects (5 men and 5 women) also ingested a high-carbohydrate meal with antioxidants. A person who was not involved in trial management randomly assigned the subjects by using random numbers derived from published tables. The meals were prepared in one batch and were consumed under supervision. All variables evaluated at baseline were reassessed 4 h after meal consumption, including the L-arginine test.

Assessment of endothelial functions

Endothelial functions in the form of hemodynamic and rheologic responses to L-arginine (9) were assessed in the fasting state (between 0800 and 0900) and again 4 h after ingestion of each meal. In brief, the subjects were placed in a comfortable supine position with a room temperature of 20–24 °C. After the cannulation of an antecubital vein with an intravenous line kept open with a 0.9% saline drip, the subjects' blood pressure was automatically recorded with a noninvasive technique (model 2300; Ohmeda, Englewood, CA). After a 10-min equilibration period, an intravenous bolus of 3 g L-arginine (10 mL of a ready-to-use 30% solution of L-arginine monochloride) was injected within 60 s. Blood pressure and the platelet aggregation response to adenosine diphosphate were measured before L-arginine injection and 10 min thereafter. The test was repeated again 4 h later (postmeal). The subjects were allowed to walk or sit, as they wished, during the interval between the first and second assessments. Variables were analyzed by independent investigators blinded to the subject's identity, meal status, and temporal sequence. The overall reproducibility of the L-arginine test in the same subject (the 3 baseline L-arginine tests) was 0.75% (SD of the difference). Blood pressure and platelet aggregation variations after L-arginine administration were confirmed as having returned to pretest values by 20 min.

Analytic methods

Blood was collected with minimal stasis after the subjects rested briefly in a supine position. Assays for serum total and

HDL cholesterol, triacylglycerol, and glucose concentrations were performed in the hospital's chemistry laboratory. Blood for the assessment of hemostatic variables was collected through a silicone-treated needle and was allowed to flow freely into silicone-treated glass tubes, where it was mixed with 1/10 of its volume of 0.1 mol/L sodium citrate. Coagulation variables were measured with commercially available immunosorbent kits: prothrombin fragments 1 and 2 (Dade Behring, Marburg, Germany), D-dimer (Diagnostica Stago, Asmières, France), plasminogen activator inhibitor 1 (PAI-1), and tissue plasminogen activator (tPA; Byk-Sangtek Diagnostica, Dietzenbach, Germany). Activated factor VII (FVIIa) was measured with a one-stage prothrombin time-based assay with the use of a truncated soluble form of recombinant tissue factor that reacts with FVIIa but not with the zymogen FVII (STACLOT VIIa-rTF; Diagnostica Stago). Platelet aggregation was determined according to the method of Born (10) by using a final concentration of 1.25 μ mol adenosine diphosphate/L.

Calculations and statistical analyses

Sample size was determined on the basis of 2 preliminary experiments with a high-fat meal, 2 with a high-carbohydrate meal, and 2 with a high-fat meal with antioxidants. 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 (11). Mean blood pressure was calculated as diastolic blood pressure plus one-third of the pulse pressure. Individual responses to L-arginine were calculated as the difference between the values found at 10 min and baseline values. Analysis of covariance with the fasting values as covariate was used to assess changes over time in response to each different meal and L-arginine. Differences in the responses to each meal and L-arginine were compared with analysis of covariance and Tukey's test. The effect of order was tested with analysis of variance. Linear regression analysis was used as appropriate. A *P* value < 0.05 was chosen as the level of significance. SPSS (version 10.0; SPSS Inc, Chicago) was used for the analyses.

RESULTS

Lipid, glucose, blood pressure, and hemostatic variables before and after the 3 meals are shown in **Table 1**. Premeal (fasting) variables were not significantly different between each of the 3 study days, and no evidence of an order effect was observed. After the ingestion of the high-fat meal, mean serum triacylglycerol concentrations increased from 0.9 ± 0.2 to 1.4 ± 0.3 mmol/L at 4 h ($P < 0.01$). An increase in serum triacylglycerol concentrations, which was not significantly different from that observed after the high-fat meal, was seen 4 h after the high-fat meal with antioxidants, whereas no increase in serum triacylglycerol concentrations occurred after the high-carbohydrate meal. No significant changes in cholesterol, HDL cholesterol, glucose, blood pressure, or platelet aggregation were observed after the meals. All hemostatic indexes increased significantly after ingestion of the 3 different meals, except for FVIIa after the high-carbohydrate meal.

The vascular responses to the control L-arginine test obtained in the fasting (premeal) state were -7.5 ± 2.1 mm Hg for mean blood pressure and $-9.7 \pm 5.3\%$ for platelet aggregation ($P < 0.01$). After the high-carbohydrate meal, L-arginine produced a significant decrease in mean blood pressure and platelet aggregation,

TABLE 1Lipid, glucose, blood pressure, and hemostatic variables in 25 healthy subjects in the fasting state and 4 h after the 3 meals¹

Variable	High-carbohydrate meal		High-fat meal		High-fat meal with antioxidants	
	Fasting	Change from fasting	Fasting	Change from fasting	Fasting	Change from fasting
Total cholesterol (mmol/L)	4.4 ± 0.7	-0.1 ± 0.1	4.5 ± 0.7	0.0 ± 0.1	4.6 ± 0.8	-0.1 ± 0.1
HDL cholesterol (mmol/L)	1.2 ± 0.3	0.1 ± 0.2	1.2 ± 0.3	-0.1 ± 0.1	1.2 ± 0.3	-0.1 ± 0.1
Triacylglycerol (mmol/L)	0.8 ± 0.1	-0.1 ± 0.1	0.9 ± 0.2	0.5 ± 0.2 ²	0.8 ± 0.2	0.4 ± 0.1 ²
Glucose (mmol/L)	4.8 ± 0.4	0.1 ± 0.1	4.9 ± 0.5	-0.2 ± 0.2	4.7 ± 0.4	0.1 ± 0.1
Blood pressure (mm Hg)						
Systolic	105 ± 7	3.0 ± 2.5	107 ± 8	0.5 ± 1.1	108 ± 8	-2 ± 2
Diastolic	70 ± 6	-2.1 ± 1.4	68 ± 6	-2.0 ± 2	67 ± 7	1 ± 1
Platelet aggregation (%)	31 ± 10	2.8 ± 2.4	35 ± 9	-2.5 ± 3	30 ± 8	3 ± 4
FVIIa (U/L)	80 ± 10	10 ± 8.2 ³	90 ± 12	42 ± 15 ⁴	89 ± 11	30 ± 13 ⁴
F1+2 (nmol/L)	0.5 ± 0.1	0.3 ± 0.1 ⁴	0.4 ± 0.1	0.6 ± 0.2 ²	0.7 ± 0.2	0.5 ± 0.2 ⁴
D-Dimer (µg/L)	165 ± 35	104 ± 45 ⁴	179 ± 39	135 ± 52 ^{2,5}	168 ± 32	120 ± 49 ⁴
PAI-1 (µg/L)	15 ± 4.5	7.0 ± 3.4 ⁴	22 ± 6.7	20 ± 9.1 ^{2,3}	21 ± 5.3	13 ± 5.9 ⁴
tPA (µg/L)	1.8 ± 0.5	3.4 ± 1.2 ⁴	3.0 ± 0.7	7 ± 2.9 ^{2,3}	2.6 ± 0.6	4.5 ± 2.1 ⁴

¹ $\bar{x} \pm SD$. FVIIa, activated factor VII; PAI-1, plasminogen activator inhibitor 1; tPA, tissue plasminogen activator; F1+2, prothrombin fragments 1 and 2.^{2,4}Significantly different from fasting (ANCOVA with fasting value as covariate); ² $P < 0.01$, ⁴ $P < 0.05$.³Significantly different from the response to the other 2 meals, $P < 0.05$.⁵Significantly different from the high-carbohydrate meal, $P < 0.05$.

which was not significantly different from that observed with the premeal L-arginine test (Table 2). After the high-fat meal, the vascular responses to L-arginine were completely absent for blood pressure (-1.0 ± 0.8 mm Hg) or showed a paradoxical increase ($4.1 \pm 2.1\%$) for platelet aggregation ($P < 0.01$ compared with the premeal L-arginine test and the high-carbohydrate meal). The high-fat meal with antioxidants partially restored the responses of blood pressure and platelet aggregation to L-arginine (Table 2). Thus, the reduction of endothelial function in terms of impairment of blood pressure and platelet aggregation responses to L-arginine after the high-carbohydrate meal differed significantly from the responses after the other 2 meals. After the high-fat meal, individual changes in plasma triacylglycerol concentrations correlated with changes in mean blood pressure ($r = -0.26$, $P < 0.05$) and platelet aggregation ($r = -0.23$, $P < 0.05$) responses to L-arginine.

Hemostatic variables before and after the postmeal L-arginine tests are shown in Table 3. L-Arginine caused a decrease in all hemostatic variables after the high-carbohydrate meal and the high-fat meal with antioxidants, except for FVIIa and tPA, which

were significantly lower after the high-fat meal than after the other 2 meals.

The vascular responses to L-arginine in the 10 subjects who ingested the high-carbohydrate meal with antioxidants did not differ from the results when the same subjects were given the high-carbohydrate meal alone (data not shown).

DISCUSSION

The novel findings of the present study are that 1) a single high-fat meal in healthy subjects impairs the antiplatelet function of the endothelium and activates both coagulation and fibrinolysis; 2) although to a lesser extent, a high-carbohydrate meal also activates hemostasis that, unlike after the high-fat meal, returns to prestimulatory values after increased nitric oxide availability by L-arginine; and 3) food antioxidants partially prevent endothelium dysfunction acutely induced by the high-fat meal. Taken together, these results indicate that a high-fat meal modifies the relaxing, antiplatelet, and hemostatic properties of the endothelium toward

TABLE 2Vascular responses to L-arginine in 25 healthy subjects after the 3 meals¹

Variable	Baseline ²	10 min after L-arginine	Response ³
Mean blood pressure (mm Hg)			
Fasting	80.5 ± 6.2	73.0 ± 5.4 ⁴	-7.5 ± 2.1
After high-carbohydrate meal	81.0 ± 6.0	73.8 ± 5.1 ⁴	-7.1 ± 2.3
After high-fat meal	80.0 ± 5.8	79.0 ± 5.9	-1.0 ± 0.8 ⁵
After high-fat meal with antioxidants	79.0 ± 6.0	76.0 ± 5.8 ⁴	-3.0 ± 1.7 ⁵
Platelet aggregation (%)			
Fasting	35.0 ± 8.0	25.3 ± 7.2 ⁴	-9.7 ± 5.3
After high-carbohydrate meal	34.0 ± 8.5	25.0 ± 6.2 ⁴	-9.0 ± 4.5
After high-fat meal	32.0 ± 8.3	36.0 ± 9.4 ⁴	4.1 ± 2.1 ⁵
After high-fat meal with antioxidants	33.0 ± 7.9	29.0 ± 8.1 ⁴	-4.0 ± 1.9 ⁵

¹ $\bar{x} \pm SD$.²Baseline indicates values recorded 4 h after ingestion of meals, immediately before the postmeal L-arginine test.³Differences between the 10-min and baseline values.⁴Significantly different from baseline, $P < 0.01$ (ANCOVA with baseline value as covariate).⁵Significantly different from each other meal, $P < 0.05$.

TABLE 3
Hemostatic variables in 25 healthy subjects after the postmeal L-arginine test¹

Variable	High-carbohydrate meal		High-fat meal		High-fat meal with antioxidants	
	Baseline ²	Change from baseline	Baseline ²	Change from baseline	Baseline ²	Change from baseline
FVIIa (U/L)	90 ± 14	-12 ± 13	132 ± 19	-30 ± 13 ³	110 ± 15	-30 ± 11 ³
F1+2 (nmol/L)	0.8 ± 0.2	-0.4 ± 0.2 ³	1.0 ± 0.2	-0.1 ± 0.1 ⁴	1.2 ± 0.3	-0.6 ± 0.3 ³
D-Dimer (μg/L)	270 ± 64	-85 ± 41 ³	315 ± 52	16 ± 15 ⁴	291 ± 57	-109 ± 45 ³
PAI-1 (μg/L)	22 ± 5.7	-3.0 ± 1.5 ³	42 ± 11	-7 ± 7 ⁴	34 ± 9	-14 ± 8 ³
tPA (μg/L)	5.3 ± 2.1	-3.1 ± 1.3 ³	11 ± 2.7	-3 ± 2.8	7.0 ± 2.8	-4.2 ± 2.1 ³

¹ $\bar{x} \pm$ SD. FVIIa, activated factor VII; PAI-1, plasminogen activator inhibitor 1; tPA, tissue plasminogen activator; F1+2, prothrombin fragments 1 and 2.

²Baseline indicates values recorded 4 h after ingestion of meals, immediately before the second L-arginine test.

³Significantly different from baseline, $P < 0.05$ (ANCOVA with baseline value as covariate).

⁴Significantly different from each other meal, $P < 0.05$.

a more atherogenic profile; this switch is partially prevented by dietary antioxidants.

Impairment of endothelium-dependent vasodilation after a high-fat meal was previously reported (12, 13). Postprandial hypertriglyceridemia has been linked to endothelial dysfunction (4, 12, 14), a finding consistent with our data. We found an inverse relation between the magnitude of decrease in blood pressure after L-arginine and the increase in triacylglycerol concentrations after the high-fat meal. On the other hand, not all studies have found such a relation. In particular, Chowienzyk et al (15) reported the integrity of endothelial function in patients with severe hypertriglyceridemia, whereas 2 other reports found endothelial functions unchanged after acute increases in plasma triacylglycerol concentrations after infusion of a lipid emulsion (16, 17). The vascular responses to L-arginine are thought to be the consequence of increased synthesis of endogenous nitric oxide, either as a direct consequence of increased substrate availability for nitric-oxide synthase (EC 1.14.13.39) or mediated, at least in part, by the simultaneously released insulin from the pancreatic β cells (18). Although we did not measure insulin in the present study, circulating insulin concentrations are known to return to baseline within 30 min after a 3-g arginine load (19), well before the time of postmeal testing. Moreover, the vascular effects of insulin are also attributed to stimulation of endogenous nitric oxide synthesis (18).

An oxidative mechanism seems to be responsible for the impaired flow-mediated brachial artery vasodilation after a high-fat meal. In one study (12), pretreatment with vitamins C and E blocked the decrease in endothelial function. In our study, a mixture of food antioxidants, which are more representative of dietary patterns than are supplements, partially restored the endothelium-dependent vascular responses during the high-fat meal. This seems to give support, at least indirectly, to the hypothesis that a fat-generated oxidative stress mediates the impairment of endothelial functions. In contrast, other mechanisms might also be implicated in the attenuation of endothelial dysfunction by food antioxidants, including gastric emptying and gut hormone release, which were not evaluated in the present study.

Unlike the high-fat meal, the high-carbohydrate meal did not produce any significant modification of the responses to L-arginine. Although acute and postprandial hyperglycemia are increasingly being seen as toxic to endothelial functions (20, 21), the glycemic excursion after the high-carbohydrate meal was very small and had returned to baseline concentrations at the time of postmeal testing. In general, studies looking at an independent effect of one

component of the diet should control for the amount of the other component. This is difficult because a high-carbohydrate meal, for example, is necessarily low in fat and vice versa; therefore, the putative effect of a high-carbohydrate meal may be that of a low-fat meal. However, it may be unnecessary to elucidate every mechanism of a single nutrient or food: current recommendations for disease prevention emphasize the simultaneous change in several dietary behaviors, such as a decrease in fat and an increase in grains and greens (22).

The relation of hemostatic variables with the dietary fat content and type has received more attention than has the relation with other nutrients, probably because of the established association of fat intake with coronary heart disease. There is agreement among the results of epidemiologic surveys and both acute and long-term studies that high total fat intakes, rather than the type of fat, are associated with increased FVIIa during fasting or postprandial states (23). Humans are usually in a nonfasting state, and nonfasting deviations in pathogenetic factors may be of particular importance in the risk of developing ischemic heart disease. Little is known about the effects of total fat intake on other hemostatic variables or on the acute perturbations of these variables after different meals. The present results suggest that a high-fat meal may activate thrombin formation and consequently fibrinolysis. When coagulation is activated, cleavage products—including prothrombin fragments 1 and 2 and D-dimer—are released into the circulation (24, 25). D-Dimer is the primary degradation product of cross-linked fibrin and therefore may serve as a direct marker of ongoing fibrinolysis (26). Moreover, the ratio of PAI-1 to tPA decreased by $\approx 50\%$ after the meals, indicating augmentation of fibrinolytic potential.

Activation of hemostasis after the high-carbohydrate meal was unexpected. However, the few studies reported thus far have focused mainly on FVIIa and showed an increase after a fat-rich meal (23). Although the absolute increase in FVIIa was significantly lower after the high-carbohydrate meal than after the high-fat meal, coagulation activation occurred after both meals. This suggests that the process is independent, at least in part, of the type of food. However, we did document a difference among the responses of hemostatic variables to L-arginine after the different meals: unlike the responses to the high-carbohydrate meal and the high-fat meal with antioxidants, activation of hemostasis, as indicated by the persisting elevated indexes, was still evident after L-arginine in the group consuming the high-fat meal. This seems to indicate that L-arginine may have a role, at least after acute administration, to reset activated hemostasis, and that this putative

effect, specific or nonspecific (27), may be impaired during a high-fat meal. What seems clear is that both procoagulant and anticoagulant events may be influenced by the same food or nutrient. This is possibly the result of the interrelations between different components of the system, and the compensatory adjustments in one part when another is affected, in an attempt to maintain hemostatic balance.

The present study did not attempt to differentiate the effect of a 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 nonnutrients.

The results from this study indicate that a high-fat meal can switch the endothelium toward a more atherogenetic profile and that dietary antioxidants partially prevent the activation of endothelial cells. The high-carbohydrate meal does not cause any significant change in the vasomotor and antiplatelet functions of the endothelium, which may contribute to the healthier cardiovascular outlook of persons consuming a Mediterranean diet (28, 29). 

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