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Impaired Endothelial Function Following a Meal Rich in Used Cooking Fat

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OBJECTIVES The purpose of this study was to test the hypothesis that intake of used cooking fat is associated with impaired endothelial function.

BACKGROUND Diets containing high levels of lipid oxidation products may accelerate atherogenesis, but the effect on endothelial function is unknown.

METHODS Flow-mediated endothelium-dependent dilation and glyceryl trinitrate-induced endothelium-independent dilation of the brachial artery were investigated in 10 men. Subjects had arterial studies before and 4 h after three test meals: 1) a meal (fat 64.4 g) rich in cooking fat that had been used for deep frying in a fast food restaurant; 2) the same meal (fat 64.4 g) rich in unused cooking fat, and 3) a corresponding low fat meal (fat 18.4 g) without added fat.

RESULTS Endothelium-dependent dilation decreased between fasting and postprandial studies after the used fat meal (5.9 ± 2.3% vs. 0.8 ± 2.2%, p = 0.0003), but there was no significant change after the unused fat meal (5.3 ± 2.1% vs. 6.0 ± 2.5%) or low fat meal (5.3 ± 2.3% vs. 5.4 ± 3.3%). There was no significant difference in endothelium-independent dilation after any of the meals. Plasma free fatty acid concentration did not change significantly during any of the meals. The level of postprandial hypertriglyceridemia was not associated with change in endothelial function.

CONCLUSIONS Ingestion of a meal rich in fat previously used for deep frying in a commercial fast food restaurant resulted in impaired arterial endothelial function. These findings suggest that intake of degradation products of heated fat contribute to endothelial dysfunction. (J Am Coll Cardiol 1999;33:1050–5) © 1999 by the American College of Cardiology

Endothelial dysfunction is regarded as an important initial event in atherogenesis (1) and has been associated with a range of cardiovascular risk factors including active (2) and passive smoking (3), hypercholesterolemia (4), homocystinuria (5) and diabetes mellitus (6). Increased oxidative stress has been postulated as a common pathway of endothelial injury and the subsequent development of atherosclerosis (7). In vitro studies have shown that oxidative stress is associated with impaired endothelium-dependent arterial dilation (8).

Diets containing high levels of lipid oxidation products accelerate the development of atherosclerosis in animals (9). It has been suggested that lipid oxidation products absorbed into the blood stream increase the susceptibility of atherogenic lipoproteins to oxidation, which is an important step in the formation of atherosclerotic lesions (10). Whether endothelial function is influenced by increased levels of lipid oxidation products in the human diet is uncertain.

Arterial endothelial function can be assessed noninvasively in the brachial artery with high frequency ultrasound (11). This technique measures the endothelium-dependent arterial dilation in response to an increase in blood flow. We used this method to assess the effects of a meal rich in fat that had been previously used for deep frying in a commercial fast food restaurant on endothelial function in healthy middle-aged men.

METHODS

The study group consisted of 10 nonsmoking men ages from 34 to 52 years. No subjects had hypertension, diabetes mellitus or hyperlipidemia, and none was taking cardiovascular medications or antioxidant agents. The subjects were recruited from university staff members. All subjects gave written informed consent and the study was approved by the regional health authority ethics committee.

After a 12h overnight fast, subjects had blood drawn into tubes containing disodium ethylenediaminetetraacetic acid and into a plain tube. Supine blood pressure was measured,

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Abbreviations and Acronyms

FLOP = fluorescent lipid oxidation products
GTN = glyceryl trinitrate
HDL = high density lipoprotein
TBARS = thiobarbituric acid reacting substances

and the baseline brachial artery reactivity was then assessed. Each subject received in random order a meal rich in fat that had previously been used for deep frying in a commercial fast food restaurant, a meal rich in the corresponding unused cooking fat and a low fat meal. The test meal was given and 4 h later a repeat specimen of blood was taken and the postprandial arterial reactivity study was repeated. Meals were consumed within 15 min, and there was at least 1 week between the test meals. Plasma lipids, lipoproteins, apolipoproteins, free fatty acid concentrations, serum concentration of thiobarbituric acid reacting substances (TBARS) and fluorescent lipid oxidation products (FLOP) were measured in fasting and postprandial samples. Serum TBARS and FLOP are indices of oxidative stress. Serum FLOP are partly derived from Schiff's bases formed by interaction of protein amino groups with carbonyls generated during lipid peroxidation.

The low fat meal contained ice cream (100 g), trim milk (200 ml), evaporated milk (50 ml), yogurt (10 g), tinned apricots without syrup (50 g), egg yolk (12 g), egg white (30 g) and a chocolate flavor, presented as a milkshake. The used fat meal contained the same ingredients with the addition of 46 g of cooking fat that had been used for deep frying during a week in a commercial fast food restaurant. The cooking fat was stored in the dark at −20°C and used within 1 to 4 days. The quantity of fat in this meal approximated that which is found in an average "fast food" meal. The unused fat meal was prepared by adding 46 g of the corresponding unused cooking fat to the low fat meal. The composition of the high fat meals (used and unused fat) was: energy (3,754 kJ), fat (64.4 g), saturated fat (30 g), polyunsaturated fat (4 g), carbohydrate (62.5 g) and protein (20.5 g). The composition of the low fat meal was: energy (2,022 kJ), fat (18.4 g), saturated fat (8 g), polyunsaturated fat (2 g), carbohydrate (62.5 g) and protein (20.5 g). The peroxide value was 1.9 μmol/g, and the acid value was 117 μmol/g in the used cooking fat and 0.5 μmol/g and 7 μmol/g respectively in the unused cooking fat.

Brachial artery ultrasound studies were performed in a quiet temperature-controlled laboratory. The subjects rested in the supine position for 10 min before the study. Studies were performed using a Hewlett-Packard Sonos 2000 ultrasound machine and a high resolution 7.5-MHz linear array transducer. Longitudinal scans of the right brachial artery were obtained proximal to the antecubital fossa with the probe positioned so that the best images were obtained. Operating variables of the machine were kept constant during the study. The transmit focus zone was set at the depth of the anterior wall. Images were magnified and gain settings optimized to optimize the vessel wall and its interface with the vessel lumen. After obtaining adequate images the arm was marked and the arm kept in a constant position for the remainder of the study.

The method of assessing endothelium-dependent and -independent dilation was as previously described (11). Flow-mediated endothelium-dependent dilation was assessed by measuring the arterial diameter of the brachial artery before and during reactive hyperemia induced by deflation of a blood pressure cuff previously inflated to 300 mm Hg around the forearm for 5 min. Arterial flow velocity was measured using a pulsed Doppler signal at an angle of 60° in the center of the vessel at baseline and during the first 15 s of reactive hyperemia. The artery was allowed to recover for a period of 10 min, and a second baseline scan of brachial artery diameter was performed. The endothelium-independent response was assessed as the change in artery diameter after a 400 μg spray of sublingual glyceryl trinitrate (GTN).

Data analysis. Maximum arterial diameter after reactive hyperemia was assessed 50 to 60 s after cuff deflation and between 3 and 4 min after GTN administration. The ultrasound images were recorded on Super-VHS videotape for subsequent analysis. The recorded images were then digitized and stored using a Macintosh 8500 computer with built-in digitizer. Four images were digitized from each stage at end diastole, indicated by the R wave on the continuously recorded electrocardiogram. Subsequent analysis was performed using NIH Image 1.60, a public domain measurement program. The diameter of the vessel was measured by two independent investigators without knowledge of the scan sequence or test meal type. Arterial diameter was measured from a fixed anatomic marker in all scans of the same patient. Measurements were taken from the anterior "m" line (media–adventitia interface) anteriorly to the leading edge of the intima–lumen interface of the posterior wall. The four measurements for each stage were then averaged. The percent change in the brachial artery diameter was then calculated in response to reactive hyperemia and GTN by each observer, and the average results of the two observers were recorded.

Blood flow at baseline and during reactive hyperemia was calculated by multiplying the velocity–time integral of the Doppler flow signal by the heart rate and cross-sectional area of the blood vessel. Reactive hyperemia was calculated as the maximal flow in the first 15 s after cuff deflation divided by baseline flow. As the flow velocity was measured in the center of the vessel, absolute flow may be overestimated, but relative flow values before and after cuff inflation are accurate (11). Interobserver variability was determined by calculating the mean and standard deviation of the difference in the two observer's results from 40 arterial studies. The interobserver variability for measurement of flow mediated dilation was 0.2 ± 1.3%.

Table 1. Arterial Study Results in 10 Male Subjects

	Low Fat Meal		Unused Fat Meal		Used Fat Meal		
	Baseline	Postprandial	Baseline	Postprandial	Baseline	Postprandial	p*
Baseline vessel size (mm)	4.5 ± 0.8	4.6 ± 0.8	4.6 ± 0.5	4.6 ± 0.4	4.6 ± 0.7	4.7 ± 0.6	0.28
Baseline flow (ml/min)	169 ± 87	175 ± 95	188 ± 90	199 ± 62	188 ± 90	184 ± 71	0.93
Hyperemia (%)	598 ± 261	599 ± 240	584 ± 102	544 ± 233	587 ± 299	545 ± 222	0.91
EDD (%)	5.3 ± 2.3	5.4 ± 3.3	5.3 ± 2.1	6.0 ± 2.5	5.9 ± 2.3	0.8 ± 2.2	0.0003
GTN (%)	14.1 ± 4.0	12.8 ± 3.6	13.0 ± 3.5	13.5 ± 2.7	12.1 ± 5.7	11.0 ± 4.8	0.32

*p values for the interaction between the effects of meal type and time by two-way repeated measures analysis of variance.
EDD = endothelium-dependent dilation; GTN = glyceryl trinitrate induced dilation.

Laboratory methods. Plasma and serum were separated by low speed centrifugation at 4°C. Plasma high density lipoprotein (HDL) cholesterol was isolated in the supernatant after treatment of plasma with dextran sulfate/magnesium chloride (12), and HDL_3 was isolated from plasma by a precipitation method (13) and HDL_2 by the appropriate difference calculation. Cholesterol and triglycerides were measured in plasma and plasma fractions by enzymatic methods using commercial kits (Boehringer Mannheim, Germany). Plasma apolipoproteins A-I and B were measured by immunoturbidimetry (14). Plasma free fatty acid concentration was measured by an enzymatic method using a commercial kit (Boehringer Mannheim, Germany). Serum TBARS concentration was measured by fluorometry (15). Serum FLOP was measured by the method of Tsuchida and coworkers (16). Serum FLOP was expressed in arbitrary units in relation to readings obtained from a standard solution of quinine sulfate (16). Peroxide value and acid value were determined in the cooking fats using the methods of the American Oil Chemists Association (17,18).

Statistical analysis. The subject number for the study was calculated to have an 80% power at the 5% level of detecting a 2.5% reduction in arterial reactivity after the used fat meal (19). Descriptive data are presented as means ± SD. A two-way repeated measures analysis of variance was performed for all comparisons in the study. Spearman rank correlation analysis was used to test for a relationship between changes in endothelial function and changes in triglycerides, free fatty acids, serum TBARS concentration and serum FLOP. Statistical significance was defined as a two-sided p value of less than 0.05.

RESULTS

The 10 subjects were male with a mean age of 38 ± 6 years (range 34 to 52). Blood pressure in all subjects was within the normal range (systolic, 122 ± 7 mm Hg; diastolic, 86 ± 5 mm Hg). Body mass index for the group was 24.6 ± 2.9 kg/m^2. The baseline brachial artery size, baseline vessel flow and increase in flow after cuff deflation were similar for each of the six arterial studies (Table 1). In response to reactive hyperemia after cuff deflation the endothelium-dependent dilation was significantly different between the meals. There was a marked decrease in endothelium-dependent dilation after the used fat meal (5.9 ± 2.3% vs. 0.8 ± 2.2%, p = 0.0003) (Fig. 1). The low fat meal and unused fat meal were not associated with a change in endothelium-dependent dilation between fasting and postprandial studies (Table 1). The significance of the decrease in endothelium-dependent dilation after the used fat meal was unchanged when variation in baseline artery size, baseline flow and percent hyperemic flow were taken into account. The GTN-induced response was not significantly different before and after the low fat, unused fat or used fat meal.

Subjects had similar baseline levels of lipoprotein, triglycerides and free fatty acids before all of the test meals (Table 2). Mean serum triglycerides increased significantly postprandially after all meals with the greatest increase in triglycerides after the unused fat meal (p = 0.001). Plasma cholesterol, lipoprotein and free fatty acid levels were not significantly changed after any of the meals. There were trends toward an increase in plasma TBARS and serum

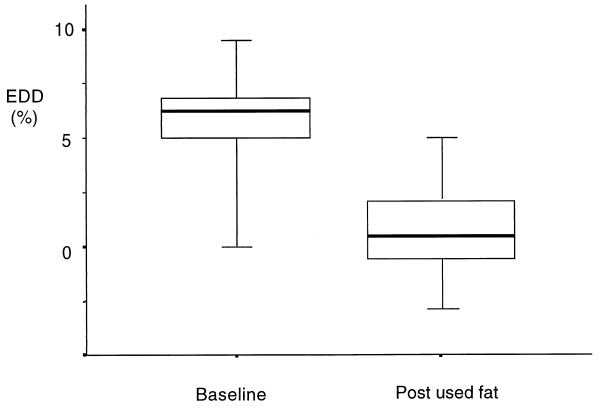

Figure 1. Endothelium-dependent dilation (EDD) before and after a used fat meal. Repeated measures analysis of variance showed that EDD was significantly lower after the used fat meal compared with the low fat meal and unused fat meal (p = 0.0003). In this box plot the **bottom** and **top** of the box represent the 25th percentile and 75th percentile, the **horizontal line** represents the median and the **vertical bars** encompass the entire range of values for all participants.

Table 2. Plasma Lipids, Lipoproteins, Triglycerides, Free Fatty Acids, and Oxidation Products Before and After Meals

	Low Fat Meal		Unused Fat Meal		Used Fat Meal		
	Baseline	Postprandial	Baseline	Postprandial	Baseline	Postprandial	p*
Total cholesterol (mmol/liter)	5.46 ± 0.74	5.55 ± 0.80	5.26 ± 0.64	5.37 ± 0.67	5.70 ± 0.93	5.80 ± 0.92	0.96
HDL cholesterol (mmol/liter)	1.31 ± 0.17	1.33 ± 0.18	1.25 ± 0.16	1.23 ± 0.18	1.34 ± 0.16	1.33 ± 0.17	0.14
HDL_2 cholesterol (mmol/liter)	0.25 ± 0.10	0.29 ± 0.11	0.33 ± 0.14	0.34 ± 0.13	0.28 ± 0.09	0.28 ± 0.08	0.26
HDL_3 cholesterol (mmol/liter)	1.06 ± 0.16	1.04 ± 0.17	0.92 ± 0.11	0.89 ± 0.11	1.06 ± 0.09	1.05 ± 0.12	0.67
Triglycerides (mmol/liter)	1.44 ± 0.48	1.95 ± 0.89	1.38 ± 0.46	2.67 ± 1.11	1.33 ± 0.39	2.05 ± 0.72	0.001
Free fatty acids (mmol/liter)	0.20 ± 0.15	0.24 ± 0.05	0.17 ± 0.09	0.22 ± 0.06	0.27 ± 0.17	0.29 ± 0.10	0.85
TBARS (μmol/liter)	1.82 ± 0.74	1.76 ± 0.60	1.66 ± 0.58	2.10 ± 0.79	1.43 ± 0.85	1.75 ± 0.87	0.14
FLOP (U/ml)	10.22 ± 1.52	10.16 ± 1.66	8.86 ± 1.79	9.09 ± 2.06	10.74 ± 2.46	11.30 ± 2.31	0.27

*p values for the interaction between the effects of meal type and time by two-way repeated measures analysis of variance.
FLOP = fluorescent lipid oxidation products; HDL = high density lipoprotein; TBARS = thiobarbituric acid reacting substances.

FLOP after the unused and used fat meals, but these did not reach statistical significance. There were no significant correlations between the change in endothelial function and change in levels of triglycerides, free fatty acids, oxidation products and other lipoprotein variables.

DISCUSSION

In this study, ingestion of a meal rich in fat that had been used for deep frying in a commercial fast food restaurant was associated with impaired arterial endothelial function in healthy men. These findings suggest that intake of degradation products of heated fat may contribute to endothelial dysfunction.

Our data indicate that a meal rich in previously used fat impairs endothelium-dependent dilation but has no effect on endothelium-independent dilation in the postprandial period. Similar meals with an equivalent amount of the corresponding unused fat or without added fat did not alter arterial function. These findings suggest that a component(s) of the used cooking fat, and not the fat content or other constituents of the meal, was responsible for the postprandial reduction in endothelial function. This effect of heat-modified fat may contribute to the reported postprandial decrease in endothelium-dependent dilation following a high fat McDonald's meal (20,21).

An oxidative mechanism appears to be responsible for the impaired endothelial function after a high fat McDonald's meal (21). Plotnick and coworkers have reported that pretreatment of subjects with antioxidant vitamins C and E blocks this decrease in endothelial function (21). Increased oxidative stress appears to cause endothelial dysfunction by superoxide-induced degradation of nitric oxide (8,22). In the present study, lipid peroxides and free fatty acids levels in the used fat were nearly fourfold higher than those in the corresponding unused fat, but serum TBARS and FLOP levels were not different during these meals. However, TBARS and FLOP are relatively nonspecific indices of oxidative stress and may not have responded to the factors in used cooking fat that are responsible for the impaired endothelial function.

Fats that have been used for deep-frying contain numerous products derived from oxidative modification of polyunsaturated fatty acids and from other types of degradative processes. The fat in the present study contained a portion of polyunsaturated fatty acids that could be vulnerable to oxidative degradation. One or more of these products may be absorbed from the gut (23,24) and may impair endothelial function. A recent study has reported that transalkenals reach the systemic circulation and react with glutathione when rats are fed thermally stressed polyunsaturated cooking oils (24). Depletion of glutathione may increase oxidative stress and impair endothelial function, which can be ameliorated by infusion of reduced glutathione (25). However, future studies are required to identify the active component(s) in the heated fat and the mechanism underlying its effect on endothelial function.

Postprandial hypertriglyceridemia (20,21) and elevated levels of free fatty acids (26,27) have previously been linked with endothelial dysfunction. Vogel and coworkers reported an inverse relationship between the 4-h change in postprandial flow-mediated vasoactivity and the 2-h change in plasma triglycerides after a high fat meal (20). They suggested that postprandial hypertriglyceridemia reduced endothelial function by an oxidative mechanism (21). In the present study, the postprandial reduction in endothelium-dependent dilation after a meal rich in used cooking fat seemed to be independent of plasma levels of triglycerides. The increase in plasma triglycerides 4 h after the unused fat meal was greater than the increase after the used fat meal, but endothelium-dependent dilation was unaffected. These findings are consistent with those of Chowienczyk and coworkers, who reported that endothelial function is preserved in patients with severe hypertriglyceridemia (28). In addition, two recent studies have reported that endothelial function is unchanged by an acute increase in plasma triglycerides after infusion of a lipid emulsion (Intralipid) (26,29). However, Intralipid infusion under conditions that increase

plasma free fatty acids leads to abnormal endothelial function (26,27). In the present study plasma free fatty acids did not influence endothelial function, as levels did not change during the meals. A previous study has also reported unchanged levels of plasma free fatty acids repeatedly measured during the 12 h following a fatty meal (30).

There are limitations to the study. The number of subjects investigated was relatively small. Thus, care must be taken in the extrapolation of these findings to other populations. Measurements were made at only one time point during the postprandial period. We cannot exclude the possibility that levels of some variables of interest may have been different earlier in the postprandial period, and this may have affected their relationships with endothelium-dependent dilation. It is unlikely, however, that measurement of endothelium-dependent dilation only at the 4-h point appreciably influenced our findings, as previous studies have reported similar impairment of endothelial function at 2 and 3 h after a fatty meal (20,21).

In conclusion, the results of the present study suggest that components of heat-modified cooking fat are associated with impaired endothelial function. Since endothelial dysfunction is an important early step in the development of atherosclerosis, these components could further increase the atherogenic potential of saturated fat rich fast foods. We suggest that a reduction in saturated fat intake as a means of preventing and treating coronary artery disease should particularly focus on decreasing the fast food content of the diet.

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