Effects of oral and intravenous fat load on blood pressure, endothelial function, sympathetic activity, and oxidative stress in obese healthy subjects

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Obesity has reached epidemic proportions in the US and worldwide. Data from the National Health and Nutrition Examination Survey obtained in 2007–2008 reported that the prevalence of obesity was 32.2% among adult men and 35.5% among adult women (14). High dietary fat intake is a major determinant of obesity that is frequently associated with hypertension, diabetes, and accelerated atherosclerosis (2, 7, 20). Understanding the mechanisms by which high fat intake mediates adverse cardiovascular effects is of great importance in formulating potential dietary interventions in obese subjects.

Obesity; endothelial dysfunction; hypertension; autonomic nervous system

Research Design and Methods

Participants. We studied 13 obese, normotensive, healthy subjects. Obesity was defined as a body mass index of ≥30 kg/m². All participants had a BP of <140/90 mmHg and had no prior history of diabetes mellitus, hypertension, or use of antihypertensive or lipid medications prior to the study. Diabetes mellitus was excluded with a 2-h glucose tolerance test and a fasting glucose of <126 mg/dl. Subjects with fasting triglyceride levels >250 mg/dl, hepatic disease (transaminases >3 times of ULN), renal insufficiency (creatinine >1.5 mg/dl), pregnancy, breast-feeding status, recent drug abuse (<3 mo) or with significant mental condition or smokers were excluded. The Institutional Review Board at Emory University approved the research protocol, and all subjects gave written and signed consent prior to participation in the study.
Protocol. Participants were admitted to the General Clinical Research Center at Grady Memorial Hospital in random order on five occasions to receive either 8-h iv or oral fat loads in either low or high doses or normal saline. After an overnight fast, iv catheters were placed in each forearm, one for infusion and one for blood sampling. After two fasting baseline blood samples were drawn, subjects received, in random order, on separate days, either an 8-h infusion of 1) normal saline at 40 ml/h, 2) 20% intralipid at 20 ml/h (32 g of fat, low iv fat), 3) 20% intralipid at 40 ml/h (64 g of fat, high iv fat), or oral fat load with either 4) 32 (low oral fat) or 5) 64 g of fat (high oral fat). The 20% intralipid solution is a long-chain triglyceride emulsion composed of 50% polyunsaturated fatty acids, 26% monounsaturated fatty acids, and 19% saturated fatty acids. During the intralipid and normal saline infusion studies, subjects remained fasting. For the low (32 g fat/8 h) and high (64 g fat/8 h, or ~100% daily fat intake based on 2,000-calorie diet) oral fat load studies, participants received fat with FFA composed of 33% polyunsaturated fatty acids, 34% monounsaturated fatty acids, and 22% saturated fatty acids. The oral fat load in either low or high dose was given in four equally divided doses at 0, 2, 4, and 6 h.

Patients remained in the supine position during the study. BP was measured with a manual cuff in triplicate on admission and at every 2 h. At baseline while fasting and then every 2 h during the study, blood samples were drawn at baseline (fasting) and every 2 h subsequently for FFAs and lipoprotein analysis. Blood samples at 0, 4, and 8 h were available for glucose, insulin, C-peptide, leptin, IL-6, C-reactive protein (CRP), TNFα, and markers of oxidative stress and lipid peroxidation. Endothelial function and heart rate variability were assessed at baseline and at 4 and 8 h.

Endothelial function. Endothelium-dependent brachial artery dilation was assessed as a measurement of endothelial function using established methodology (5). Briefly, ultrasound images of the brachial artery were obtained at baseline under standardized conditions and 60 s after induction of reactive hyperemia by 5-min cuff occlusion of the forearm. Image landmarks as well as surface markers were utilized to ensure anatomic consistency between serial imaging studies. All images were digitized online, and arterial diameters were measured with customized software (Medical Imaging Applications, Coralville, IA) by individuals blinded to the clinical and laboratory status of the subjects. Flow-mediated dilation (FMD) was expressed as the percentage increase in diameter from baseline. In our laboratory, the mean difference in FMD between two consecutive assessments ≥1 wk apart is 1.26 ± 0.76% (r = 0.75); the mean difference in the FMD between two readings of the same subjects is 0.82 ± 0.48% (r = 0.97).

Heart rate variability. Power spectrum analysis of R-R interval variability was used to assess the state of the autonomic nervous system (1). On the basis of a generated digital rhythm strip (Sphygmacor; AtCor Medical, West Ryde, Australia), we analyzed the absolute number of consecutive R-R intervals differing by >50 ms (NNS50) and heart rate (29).

Laboratory methods. Plasma glucose and lipids were measured on the CX7 Chemistry Analyzer (Beckman Diagnostics, Fullerton, CA) using reagents and calibrators from Beckman Diagnostics. Levels of LDL and HDL cholesterol were determined using homogeneous enzymatic kits (Genzyme Diagnostics, Elkhart, PA). FFA levels were determined by a colorimetric method (Genzyme Diagnostics). Levels of IL-6, TNFα, CRP, leptin, insulin, and C-peptide were measured in plasma using solid-phase, two-site, sequential chemiluminescent immunometric assays on the DPC Immulite analyzer (Diagnostic Products, Los Angeles, CA). Oxidative stress was measured by formation of dichlorofluorescein (DCF) and lipid peroxidation was determined by formation of thiobarbituric acid reactive substances (TBARS) (36). The coefficient of variance for the assays was <5%.

Statistical analysis. The primary end points of the studies included changes in FMD and BP from baseline during iv intralipid infusions, oral fat load, and normal saline. Comparison between baseline data and values during infusion or oral fat load were carried out using paired t-tests and one-way analyses of variance (ANOVA). Repeated-measures ANOVA were further used to evaluate differences in outcome changes over time between saline and lipid infusions and between saline and oral fat load. Statistical significance was defined as a P value <0.05. All data are expressed as means ± SD or means ± SE where indicated.

RESULTS

Patient characteristics. Nine subjects had family history of diabetes, and 12 subjects were African-Americans (Table 1). Despite the presence of obesity, no patients fulfilled American Heart Association/National Heart, Lung, and Blood Institute diagnostic criteria for metabolic syndrome (19).

BP. The infusion of normal saline resulted in no significant changes in systolic BP from baseline (P = 0.76). The administration of iv intralipid at both the low and high doses resulted in a significant rise in systolic BP (both P = 0.007) (Fig. 1A). The changes in systolic BP over time compared among the saline and both iv fat groups showed a trend toward difference (P = 0.07, by repeated-measures ANOVA). The low iv fat infusion raised systolic BP from baseline by 8.2 ± 12.8 mmHg (P = 0.04) at 4 h and 14.0 ± 10.4 mmHg (P = 0.0004) at 8 h, whereas high iv fat load increased systolic BP by 12.6 ± 7.6 mmHg (P < 0.0001) at 4 h and 12.1 ± 9.0 mmHg (P = 0.0005) at 8 h. Oral fat load was also associated with a significant increase in systolic BP (P < 0.01; Fig. 1A), with a mean increase in systolic BP from baseline of 13.4 ± 17.1 mmHg (P = 0.045) after low oral fat load and 10.6 ± 10.8 mmHg (P = 0.040) after high oral fat load. Similarly to iv fat load experiments, the effects of oral fat administration on systolic BP were not dose dependent (P = 0.07, by repeated-measures ANOVA). Diastolic BP did not change significantly during normal saline infusion, iv, or oral fat loads (Fig. 1B).

Endothelial function. Endothelial function, measured as FMD, was assessed at baseline and then after 4 and 8 h of iv and oral fat load. Brachial artery diameter was similar among study groups before cuff occlusion at 0, 4, and 8 h of study. We
observed a progressive dose-dependent decrease in FMD among saline and low and high iv intralipid ($P < 0.001$, by repeated-measures ANOVA; Fig. 1B). The baseline FMD before any intervention was $9.4 \pm 2.7\%$. FMD decreased by $3.8 \pm 2.1$ ($P = 0.002$) and $4.1 \pm 3.1\%$ ($P < 0.001$) after 8 h of low and high iv lipid infusion, respectively. These changes were equivalent to a relative decrease in FMD by $38\%$ after low iv and $49\%$ after high iv intralipid infusion. Similarly, oral fat resulted in a dose-dependent decrease in FMD by $3.8 \pm 1.8$ ($P = 0.002$) and $5.0 \pm 2.5\%$ ($P < 0.001$) after 8 h of low and high fat loads, respectively. For the oral fat administration, the relative FMD decrease was $38\%$ for low dose and $51\%$ for high oral load.

Heart rate and autonomic nervous system. We observed an increase in heart rate from baseline after iv ($P = 0.004$) and oral fat administration ($P = 0.083$) (Fig. 1C). Heart rate variability analysis showed a decrease in NN50 8 h after high oral fat load ($P = 0.05$), suggesting diminished parasympathetic activity.

Plasma FFA and triglyceride concentrations. The iv administration of intralipid resulted in a dose-dependent increase in FFA concentration ($P < 0.001$; Fig. 2A). At the peak, mean FFA concentration was increased by $0.68$ ($P = 0.008$) and $1.03$ mmol/l ($P = 0.011$) after low and high iv load, respectively. In contrast, low and high oral fat load resulted in a mild but not significant increase in FFA concentration ($P = 0.724, P = 0.105$; Fig. 2A). Triglyceride levels increased significantly from baseline after iv intralipid compared with saline in both low- and high-fat load groups ($P < 0.001$; Fig. 2B). Intralipid raised triglycerides from baseline by 45 mg/dl ($P < 0.0001$) after 4 h and 34 mg/dl ($P < 0.0001$) after 8 h of infusion in the

![Fig. 1. Changes in systolic blood pressure (BP; A), diastolic BP (B), flow-mediated dilatation (FMD; C), and heart rate (D) at 4 and 8 h after intravenous (iv) infusion of saline, intralipid, or oral fat load in obese subjects. Baseline systolic BP was $110 \pm 3$ mmHg. Baseline brachial artery diameter was $3.4 \pm 0.11$ mm. Values are means $\pm$ SE. †$P < 0.05$, *$P < 0.01$ compared with baseline.](http://ajpendo.physiology.org/)

![Fig. 2. Changes in plasma free fatty acids (FFA; A) and triglyceride (B) concentrations during the 8-h iv infusion of saline and intralipid or oral fat load in obese subjects. †$P < 0.05$, *$P < 0.01$ compared with baseline.](http://ajpendo.physiology.org/)
low iv arm and by 119 mg/dl after 4 h ($P = 0.0006$) and 121 mg/dl after 8 h ($P = 0.0012$) of high iv fat load. Similarly, oral fat load resulted in a dose-dependent increase in triglyceride levels after low and high oral fat load ($P < 0.001$; Fig. 2B). Low oral fat increased triglycerides by 11 ($P = 0.026$) and 23 mg/dl ($P = 0.003$) after 4 and 8 h, respectively. The high oral fat intake increased triglycerides by 45 ($P < 0.001$) and 39 mg/dl ($P = 0.006$) after 4 and 8 h, respectively.

**Plasma glucose, insulin, and C-peptide concentrations.** Changes in plasma glucose, insulin, and C-peptide concentrations during the administration of fat are shown in Table 2. Compared with baseline levels, fasting during normal saline infusion led to blood glucose (BG) reduction by 9.8 mg/dl at 8 h ($P < 0.001$). Fasting during intralipid infusion was associated with BG decrement of 9.6 ($P = 0.002$) and 6.3 mg/dl ($P = 0.02$) at 8 h after low and high iv fat load, respectively. However, oral fat administration was not associated with significant glucose changes. Consistent with decreases in glucose levels, insulin and C-peptide were markedly reduced after normal saline infusion ($P < 0.01$). The low oral fat resulted in significantly higher insulin and C-peptide levels from baseline.

**Plasma levels of leptin and markers of inflammation and oxidative stress.** The mean fasting leptin levels were 46.4 ng/ml, with a higher level in females than males (58.4 vs. 18.4 ng/ml, $P = 0.025$). Interestingly, leptin levels decreased at 4 and 8 h by 8.2 and 13.0 ng/ml (both $P < 0.001$) in the normal saline group, and 8.7 and 12.5 ng/ml in the intralipid group (both $P < 0.001$), and 8.6 and 11.9 ng/ml in the oral fat group (both $P < 0.01$), respectively. Overall, there was no fat load type or sex-specific differences in leptin reduction among studied groups.

The levels of TBARS and DCF increased significantly in a dose-dependent manner with both low and high iv fat load groups ($P < 0.01$; Fig. 3). Only high but not low oral fat was associated with progressive increases in both TBARS and DCF ($P < 0.05$; Fig. 3). Plasma TNFα, CRP, and IL-6 (not shown) concentrations were unchanged during normal saline infusion or after iv and oral fat load at any time point during the study.

**DISCUSSION**

This is the first study to compare the effects of low (32 g) and high (64 g) fat load given by iv and oral route on BP, endothelial function, autonomic activity, and oxidative stress in
obese healthy subjects. We found that both oral and iv fat loads result in a rapid and significant increase in systolic blood pressure, endothelial dysfunction, and increased sympathetic nervous system activity in obese healthy individuals. The changes in endothelial function, the primary end point of the study, correlated with changes in systolic BP and in plasma triglyceride concentration but not with changes in FFA levels. These results indicate that both oral and iv fat load even in physiological amounts have acute detrimental effects on the cardiovascular system in obese subjects.

Obese subjects are at risk for cardiovascular morbidity and mortality. Epidemiological studies suggest a possible link between high FFA levels and the increased incidence of hypertension in obese subjects (8, 13). We recently reported that high FFA levels after intralipid infusion for 48 h were associated with a rapid and sustained elevation in BP and endothelial dysfunction in obese diabetic individuals (39). The vascular abnormalities after chronic low-dose lipid infusion can also occur in nondiabetic individuals as well, as was demonstrated by Kashyap et al. (23) and Mathew et al. (28) in recent studies. Previous studies in humans (9, 27, 35, 37, 39) have shown that iv or oral fat load may result in vascular dysfunction; however, no previous studies have compared oral with iv fat load in a dose-dependent manner. Our study confirms previous reports that acute increases in plasma triglycerides are associated with increased BP and endothelial dysfunction (9, 27, 35, 37, 39). We also demonstrate that the dose-dependent relationship between endothelial function and plasma triglycerides reflects the maximum plasma triglyceride concentrations achieved and not whether triglyceride is delivered by diet or by direct iv infusion. The FMD studies performed in this investigation looked only at endothelial-dependent mechanisms of endothelial dysfunction. Our study cannot answer whether endothelial-independent pathways contributing to the global process of vasoreactivity were altered by the fat administration.

Systolic BP is a classic risk factor for stroke and coronary artery disease (22), and about one-half of strokes and ischemic heart disease cases are attributable to systolic BP 115 mmHg (25). Hypertension is associated with impaired endothelium-dependent vasodilation (16, 31). However, it is unknown whether BP elevation is triggered by endothelial dysfunction or vice versa. The results of our study show that oral fat intake but not iv fat infusion produced profound decrease in FMD before the increase in systolic BP. Our finding of fat load-induced high BP and altered FMD indicates that oral fat intake might play a direct role in the development of cardiovascular disease in obese subjects (12, 43).

The underlying mechanisms for the development of fat load-induced hypertension and endothelial dysfunction are not completely understood. Some have hypothesized that increased FFAs, including oleic and linoleic acids, can stimulate production of reactive oxygen species (21), increase lipid peroxidation (32), and regulate vascular tone and vascular smooth muscle cell growth contributing to endothelial dysfunction (10). Despite significant increases in BP and impaired endothelial function, our study failed to show a significant correlation between vascular changes after fat load and FFA changes and oxidative stress. Our experiments suggest that fat-induced changes in vascular and hemodynamic properties cannot be explained solely by acute changes in circulating FFA concentration. In contrast, we observed a strong inverse correlation between endothelial function and triglycerides during oral fat load, suggesting a potential role of prandial lipemia on blood vessel compliance. Recent human studies have reported that high triglyceride concentration is associated with endothelial dysfunction, prooxidative state, and formation of atherosclerotic lesions (26). The fact that these changes depend only on the plasma levels of triglycerides would suggest that interactions of triglyceride-rich particles with the arterial wall may be responsible for the fat-induced changes in vascular and hemodynamic functions.

The infusion of intralipid has been associated with sympathetic nervous system activation that, in turn, could affect both cardiac function and circulation (32, 33, 35). The autonomic nervous system modulates cardiovascular system via the interplay of sympathetic and parasympathetic activity (40). We found that both oral and iv fat load were associated with increased sympathetic activity, as indicated by increased heart rate, a simple but valuable clinical indicator of adrenergic activation (24), and altered R-R variability. Previous studies have demonstrated unfavorable vascular outcomes in subjects with autonomic dysfunction (1, 6). Leptin is proposed as one of the triggers of adrenergic hyperactivity and hypertension in obesity, although human data are inconsistent (38). The obese subjects in our study had high fasting leptin levels, and the normal saline infusion and both oral and iv fat load consistently decreased leptin concentration, suggesting that leptin is not a contributor to the vascular effects of fat load. A potential mechanism by which fat load in our experiments led to endothelial dysfunction, systolic BP elevation, and sympathetic nervous system activation can include the development of insulin resistance (17, 37). Indeed, oral fat administration resulted in elevation of insulin levels accompanied by steadystate blood glucose concentrations, suggesting reduced insulin-mediated peripheral glucose utilization (3, 34, 44). Mechanistic studies aiming to elucidate potential mechanisms mediating the cardiovascular effects of fat load in obese healthy subjects are currently underway (registry NCT00721617).

We acknowledge several limitations in this study. The study subjects were mostly African-Americans, precluding generalization of the findings to other ethnic groups. We studied obese insulin-sensitive subjects without metabolic syndrome, prediabetes, or diabetes; hence, the effects of the oral fat cannot be extrapolated to lean and overweight individuals or obese subjects who are already diagnosed with diabetes. Finally, our study was of short duration, and the composition of oral fat load was not identical to the intralipid composition.

In summary, our studies indicate that short-term oral and iv fat load in obese subjects is associated with elevation in BP and endothelial dysfunction with increased sympathetic activity and oxidative stress. Our study underscores the importance of fat intake in the development of hypertension and endothelial dysfunction in obese subjects.

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DISCLOSURES

The authors have nothing to disclose.

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