Intravenous Intralipid-Induced Blood Pressure Elevation and Endothelial Dysfunction in Obese African-Americans with Type 2 Diabetes

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Objective: Increased free fatty acids (FFAs) are leading candidates in the pathogenesis of insulin resistance and hypertension in obese subjects. We evaluated the effect of sustained elevations of FFA on blood pressure, endothelial function, insulin secretion, inflammatory markers, and renin-angiotensin system.

Research Design and Methods: Twenty-four obese, African-American, normotensive diabetic subjects received a sequential 48-h infusion of Intralipid (20%, 40 ml/h) plus heparin (250 units/h) or normal saline (40 ml/h) plus heparin (250 units/h).

Results: Blood pressure was significantly increased within 4 h of lipid infusion and reached a peak increment of 13 mm Hg in systolic and 5 mm Hg in diastolic blood pressure at 24 h ($P < 0.01$). Compared to baseline, lipid infusion reduced flow-mediated dilatation by 11% at 24 h and 18% at 48 h ($P < 0.001$). FFA and triglyceride levels increased from a baseline of 0.5 ± 0.2 mmol/liter and 135 ± 76 mg/dl to 1.8 ± 1.0 mmol/liter and 376 ± 314 mg/dl at 48 h, respectively ($P < 0.01$). C-Reactive protein increased by 35% at 24 h and by 110% at 48 h of lipid infusion. There were no significant changes in plasma renin and aldosterone levels during lipid or saline infusions.

Conclusion: Increased FFA levels result in a rapid and sustained elevation in blood pressure, impaired endothelial function, and increased inflammatory markers in obese subjects with type 2 diabetes. The model of FFA-induced hypertension may be useful in examining disease mechanisms associated with the development of hypertension in obese subjects. **(*J Clin Endocrinol Metab* 94: 609–614, 2009)**

Recent studies suggest that increased free fatty acids (FFAs) (1) are leading candidates in the pathogenesis of insulin resistance, impaired glucose tolerance, and diabetes (2–5). Acute elevations of FFA produce insulin resistance dose-dependently in diabetic and nondiabetic individuals (5, 6). Increased FFA levels have also been associated with the development of hypertension and increased cardiovascular risk (7–9). In healthy volunteers, increases in FFAs with 4-h infusions of lipid emulsion impair endothelium-dependent vasodilatation (10) and increase vascular α_1-adrenoreceptor-mediated responses (11). At the cellular level, FFAs can reduce nitric oxide bioavailability by inhibiting nitric oxide synthase activity (12) and stimulate the production of reactive oxygen species (ROS) through activation of reduced nicotinamide adenine dinucleotide phosphate oxidase (13). Oxidative stress is known to alter vasomotor tone and other functions of the endothelium (14), but the mechanisms of FFA-induced hypertension and endothelial dysfunction are not clear.

While studying the effect of high FFA levels on insulin secretion and action (lipotoxicity) in obese African-Americans (15), we observed that the infusion of Intralipid/heparin to increase FFAs approximately 3-fold baseline levels for 48 h resulted in a significant and reproducible rise in systolic and diastolic blood

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Abbreviations: BMI, Body mass index; FFA, free fatty acid; FMD, flow-mediated dilatation; NS, not significant; ROS, reactive oxygen species.

pressure in obese African-American subjects with type 2 diabetes. In agreement with our observation, Bulow *et al.* (16) reported that raising FFAs in minipigs increased vascular resistance and raised blood pressure by nearly 30 mm Hg. Grekin *et al.* (7) reported that FFA infusion increased arterial pressure by 2–3 mm Hg in Sprague Dawley rats. Similarly, a 4-h infusion of 20% Intralipid at 0.8 $ml \cdot m^{-1} \cdot min^{-1}$ and heparin (200 U bolus, followed by 1000 U/h) was reported to induce a significant increase in systolic (13.5 ± 2.1 mm Hg) and diastolic (8.0 ± 1.5 mm Hg) blood pressure in lean, healthy adults (7). We hypothesized that the development of FFA-induced hypertension is associated with acute endothelial dysfunction and inflammatory response and/or activation of the renin-angiotensin system. Accordingly, we performed a systematic evaluation of sustained elevations of FFAs on blood pressure, endothelial function, renin-angiotensin system, insulin secretion, and cardiovascular inflammatory markers in obese subjects with type 2 diabetes.

Patients and Methods

Participants

We studied 24 obese, normotensive African-American subjects with type 2 diabetes. Obesity was defined as a body mass index (BMI) of at least 30 kg/m^2. All participants had a blood pressure no greater than 130/80 mm Hg and had no prior history of hypertension or receiving antihypertensive drug therapy before the study. Diabetic subjects had a known history of diabetes of 3 yr or less and a hemoglobin A1C below 8%, and were controlled with diet alone or with stable doses of sulfonylureas for the preceding 2 months. Subjects with fasting triglyceride levels above 250 mg/dl or those treated with statins or lipid-lowering therapy were excluded. In addition, subjects with relevant hepatic disease (alanine aminotransferase 2.5x > upper limit of normal), serum creatinine at least 1.5 mg/dl for males or at least 1.4 mg/dl for females, pregnancy or breast-feeding status, or with significant medical or mental condition rendering the subject unable to consent were excluded. The institutional review board at Emory University approved the research protocol, and all subjects gave written and signed consent before participation in the study.

Protocol

Participants were admitted to the General Clinical Research Center at Grady Memorial Hospital, in random order, on two occasions for a 48-h infusion of Intralipid/heparin and saline/heparin. After an overnight fast, an iv catheter was placed in each forearm, one for infusion and one for blood sampling. After two fasting baseline blood samples were drawn, subjects received a 48-h infusion of Intralipid (20%, 40 ml/h) plus heparin (250 U/h) or normal saline (40 ml/h) plus heparin (250 U/h). Blood pressure and heart rate were measured on admission and every 2 h in supine position. Blood samples were drawn on admission for glucose, insulin, C-peptide, FFA, renin, aldosterone, C-reactive protein, and fasting lipoprotein analysis. During the infusion, glucose was measured every 2 h at the bedside and every 6 h for laboratory assays including glucose, insulin, C-peptide, FFA, renin, aldosterone, and C-reactive protein.

The Intralipid 20% long-chain triglyceride emulsion is composed of linoleic acid, 50%; oleic acid, 26%; palmitic acid, 10%; stearic acid, 9%; egg yolk; and phospholipids, 3.5%. During the study period, subjects consumed a 2000 calorie per day diet consisting of 20% of calories derived from protein, 30% from fat, and 50% from carbohydrate. Lipid and saline infusion started around 1200 h. During the first day, patients ate lunch (hour 0) and dinner (hour 6). During the second day of infusion, they ate breakfast (hour 20), lunch (hour 24), and dinner (hour 30). Thereafter, food and liquids were withheld until the completion of the 48-h infusion.

Endothelial function was measured noninvasively by using ultrasound to evaluate endothelium-dependent flow-mediated vasodilation (17) of the brachial artery. Measurements were performed according to the published guidelines (17) with a high-resolution vascular ultrasound before and after 24 and 48 h of Intralipid and saline infusions. Reactive hyperemia was produced by inflating a pneumatic tourniquet at least 50 mm Hg above systolic blood pressure to occlude arterial flow for 5 min. After cuff deflation, the longitudinal image of the artery was recorded continuously from 30 sec before and 2 min after cuff deflation. A mid-artery pulsed Doppler signal was obtained on immediate cuff release to assess hyperemic flow velocity. The average diameter was determined from at least three different diameter measurements determined along a segment of the vessel. Brachial artery diameter was measured at the same time in the cardiac cycle by use of electrocardiogram gating during image acquisition. The precision of our system is measured to be 0.004 mm.

Laboratory methods

Plasma glucose was measured using the glucose oxidase method. Triglycerides were measured as esterified glycerol with an enzymatic colorimetric kit (Roche Molecular Biochemicals, Mannheim, Germany). FFA levels were determined by a colorimetric method. Levels of C-reactive protein, insulin, and C-peptide were measured in plasma using a solid phase, two-site sequential chemiluminescent immunometric assay on the DPC Immulite analyzer (Diagnostic Products Corporation, Los Angeles, CA). The coefficient variance of all the assays was less than 5%. The instrument calibrations for the assays were performed as recommended by the manufacturers and were within the specifications.

Statistical analysis

The primary analysis for this research study targeted changes in blood pressure and endothelial function during 48-h Intralipid plus heparin and normal saline plus heparin infusions. Comparisons between baseline data and values during infusion were carried out using paired *t*-tests. Cross-sectional comparisons between different infusion groups were conducted by two-sample *t*-tests. Repeated measures linear model or repeated measures ANOVA were further used to evaluate differences in outcome changes between saline and lipid infusions, while adjusting for subject's age, BMI, and gender. Statistical significance was defined as a type 1 error of 0.05. All data are expressed as mean ± SD.

Results

Patient characteristics

Patients were African-Americans (17 males and seven females). Their age (mean ± SD) was 41 ± 8 yr (range, 26 to 56 yr) and BMI was 37 ± 6 kg/m^2 (range, 31 to 54 kg/m^2), and they had a mean duration of diabetes of 3.6 ± 3 yr (range, 1 to 10 yr). Fourteen patients were treated with diet alone, and 10 patients were treated with low-dose sulfonylureas. The mean hemoglobin A1C was 6.2 ± 1%, and the fasting plasma glucose was 127 ± 55 mg/dl. None of the patients had a history of hypertension or of receiving antihypertensive agents.

Blood pressure changes during Intralipid and saline infusion

The infusion of Intralipid (20% solution at 40 ml/h) and heparin (250 U/h) resulted in a significant and reproducible rise in blood pressure (Fig. 1). The mean systolic and diastolic blood pressure readings before the lipid and saline infusion were 116 ±

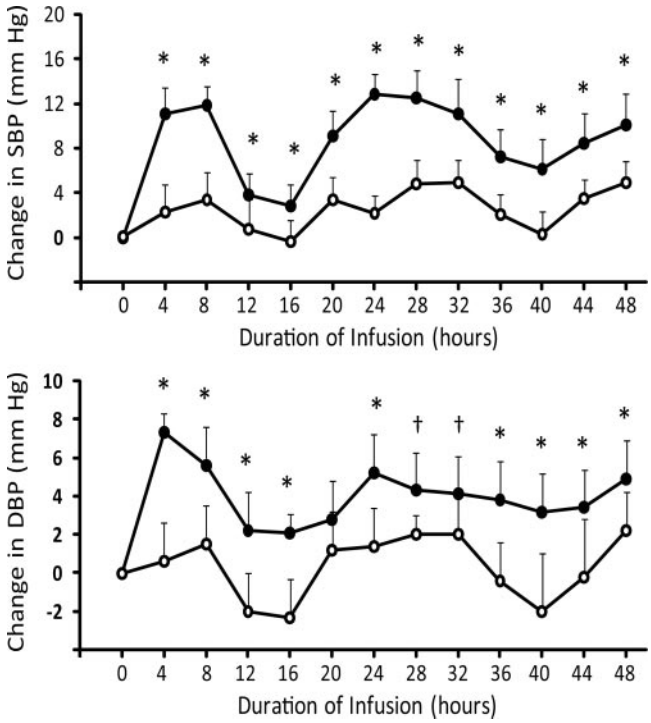

FIG. 1. Changes in systolic blood pressure (*top*) and diastolic blood pressure (*bottom*) during 48-h Intralipid/heparin (*closed circles*) and saline/heparin (*open circles*) infusion in obese subjects with type 2 diabetes. Values are mean ± SEM. *, $P < 0.01$. †, $P < 0.05$.

15 and 120 ± 12 mm Hg and 71 ± 9 and 72 ± 11 mm Hg, respectively. Intralipid infusion raised systolic and diastolic blood pressure from baseline within 4 h (11 ± 11 and 5 ± 2 mm Hg), and the blood pressure effects persisted throughout the 48 h of infusion ($P < 0.01$). In contrast, infusion of normal saline at 40 ml/h resulted in no significant blood pressure changes from baseline [P = not significant (NS)].

During Intralipid infusion, the heart rate increased from a baseline of 68 ± 11 beats/min to 72 ± 12 and 74 ± 12 beats/min at 24 h and 48 h of infusion, respectively (P = NS). The heart rate was 67 ± 11 beats/min at baseline, 70 ± 11 beats/min at 24 h, and 66 ± 11 beats/min at 48 h of saline infusion (P = NS).

Plasma FFAs, triglycerides, renin, aldosterone, and C-reactive protein concentrations

Changes in plasma FFA, renin, and aldosterone concentrations during the 48-h lipid and saline infusions are shown in Fig. 2. Intralipid infusion resulted in rapid and sustained elevations of FFA levels compared with normal saline. From a fasting FFA of 0.5 ± 0.2 mmol/liter, FFA levels increased to 1.8 ± 0.8 mmol/liter at 24 h ($P < 0.001$) and remained at this level during the 48-h infusion. Compared with baseline levels, saline plus heparin infusion resulted in no significant changes in FFA concentrations. Similarly, triglyceride concentration increased significantly in response to Intralipid infusion compared with saline. Triglyceride levels rose from 134 ± 76 to 468 ± 353 mg/dl at 24 h and 376 ± 314 mg/dl at 48 h of Intralipid infusion ($P < 0.01$ *vs.* baseline). Plasma triglyceride concentrations were 130 ± 54 mg/dl at baseline, 158 ± 84 mg/dl at 24 h, and 147 ± 47 mg/dl at 48 h of saline infusion.

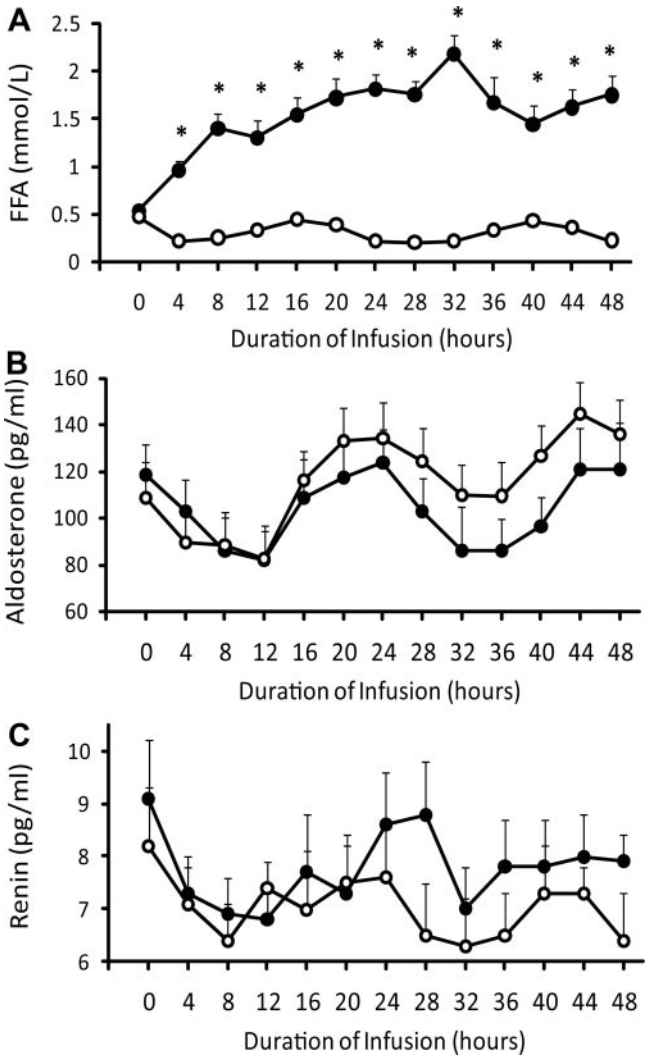

FIG. 2. Changes in plasma FFA (A), aldosterone (B), and renin (C) concentration during 48-h Intralipid/heparin and saline/heparin infusion in obese subjects with type 2 diabetes. Values are mean ± SEM. *, $P < 0.01$.

Plasma aldosterone and renin concentration were not significantly different between Intralipid and saline infusion (P = NS). C-Reactive protein concentration increased from a baseline of 1.57 mg/dl to 2.01 mg/dl at 24 h and 2.66 mg/dl at 48 h of lipid infusion, on average 35 and 110% increase, respectively (both $P < 0.001$). C-Reactive protein concentration levels remained unchanged during saline infusion (baseline, 1.65 mg/dl; 24 h, 1.76 mg/dl; and 48 h, 1.63 mg/dl).

Assessment of endothelial function

Endothelial function, as measured by percentage change in flow-mediated dilatation (FMD), decreased significantly during Intralipid compared with saline infusion (Fig. 3). Starting at a baseline mean of 6.4 ± 4.1, FMD decreased to 5.9 ± 2.7 (−11%) at 24 h and to 5.4 ± 2.9 (−18%) at 48 h of Intralipid infusion. During normal saline infusion, the FMD was 6.4 ± 2.3 at baseline, 6.7 ± 2.7 at 24 h (18%), and increased to 8.2 ± 2.8 (42%) at 48 h (P = 0.01 for change pattern with time *vs.* Intralipid infusion). Of interest, we observed significant gender differences

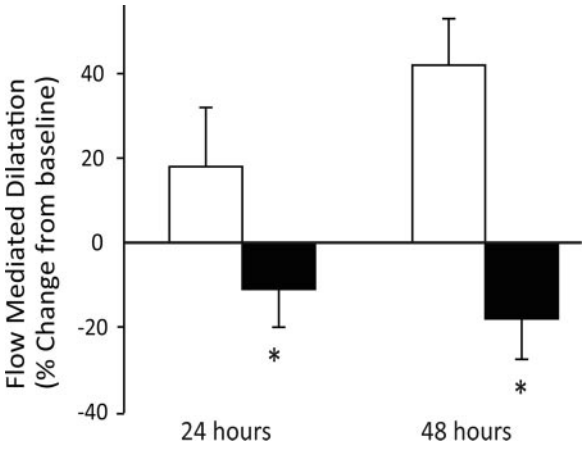

FIG. 3. Changes in FMD during 48-h saline/heparin infusion (*open bars*) and Intralipid/heparin (*closed bars*) in obese subjects with type 2 diabetes. Values are mean ± SEM. *, $P < 0.01$.

in FMD, with more severe impairment in FMD in male patients compared with female subjects ($P = 0.049$).

Serum glucose and C-peptide concentrations

Compared with baseline levels, changes in glucose values significantly increased during lipid infusion compared with saline infusion (Fig. 4). Glucose levels rose from 111 ± 37 to 148 ± 48 and 136 ± 40 mg/dl after 24 and 48 h of Intralipid infusion ($P < 0.01$ from baseline). In contrast, during saline infusion, the mean glucose level was 109 ± 27 mg/dl at baseline, 118 ± 43 mg/dl at 24 h, and 93 ± 22 mg/dl after 48 h of saline infusion. C-Peptide levels were similar at baseline between the Intralipid and saline group (3.8 ± 2.3 *vs.* 4.2 ± 1.6 ng/ml; P = NS). Starting at comparable baseline levels, C-peptide concentration was higher during Intralipid infusion (8.3 ± 4.6 ng/dl at 24 h, and 7.9 ± 4.7 ng/dl at 48 h; both $P < 0.001$ *vs.* baseline) than during saline infusion (7.0 ± 2.6 ng/dl at 24 h, and 5.9 ± 3.8 ng/dl at 48 h; both $P < 0.05$ *vs.* baseline; both P = NS *vs.* saline). Insulin release was estimated by the C-peptide glucose ratio [C-peptide (ng/ml)/glucose (mg/dl) × 100] and by differences in area under the curve during Intralipid and saline infusions. We observed no significant differences in the C-peptide/glucose ratio during the 48-h Intralipid and saline infusions (Fig. 2) or in the area under the curve for insulin (P = NS), indicating that increased FFA was not associated with impaired insulin secretion.

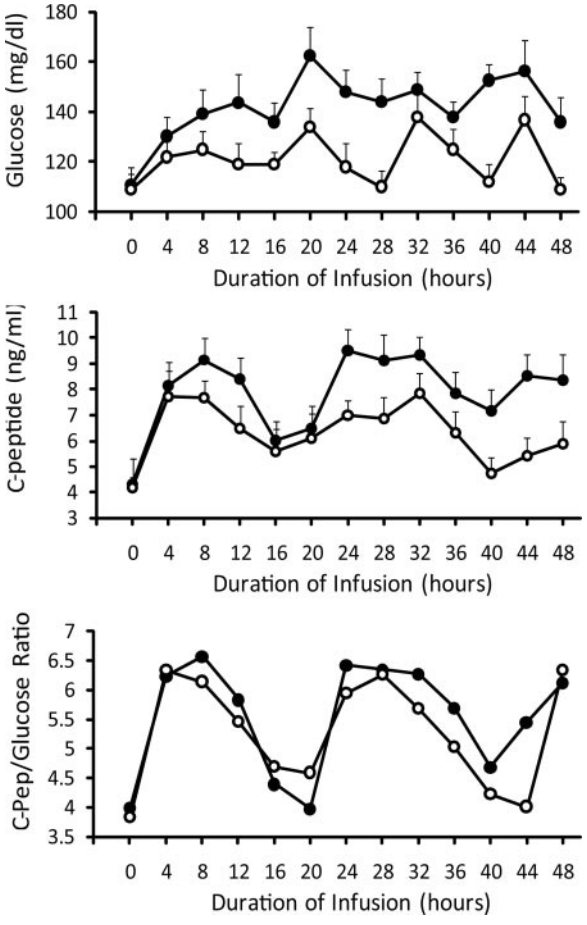

FIG. 4. Changes in glucose, C-peptide, and C-peptide/glucose ratio during 48-h Intralipid/heparin and saline/heparin infusion in obese subjects with type 2 diabetes. Values are mean ± SEM.

Discussion

We have shown that FFA increase due to the infusion of Intralipid/heparin for 48 h resulted in a rapid and sustained elevation in blood pressure, increased markers of inflammation, and endothelial dysfunction. Elevated plasma fatty acids have been independently associated with the risk of developing hypertension (7, 8, 18). In the Paris prospective study (18), among the 2968 nonhypertensive and nondiabetic middle-aged Caucasian men, high fasting and 2-h FFA levels were risk factors for hypertension over 3 yr of follow-up after controlling for age, family history of hypertension, alcohol consumption, and BMI. More directly, animal and human studies have shown that short-term Intralipid infusion (2–4 h) increase vascular resistance and raised blood pressure (7, 10, 11, 16). Our study confirms the rapid increase in blood pressure, but in addition, it demonstrates that the elevation of blood pressure is sustained and lasted throughout the 48-h lipid infusion.

Several factors may confound the relationship between FFAs and hypertension in obese subjects. FFA may increase the neurovascular tone by enhancing α_1-adrenoreceptor sensitivity (11) and by increasing baroreflex sensitivity (19). Increased FFA levels also impair endothelium-dependent vasodilatation (10) and can lead to sympathetic nervous system activation (20, 21). Sympathetic activation can result in vasoconstriction (21), impaired natriuresis (22), enhanced oxidative stress (23), and neural pressor effects (24). Exogenous FFAs also regulate aldosterone secretion (25) and renin-angiotensin system in obese subjects (26). Obesity is strongly related to elevated blood pressure and is associated with higher plasma renin activity (27), tissue-specific angiotensin-converting enzyme (28), and plasma aldosterone (26, 27). In this study, we observed no significant changes in circulating levels of plasma renin and aldosterone during Intralipid and saline infusion, indicating that activation of renin-angiotensin system may not be a primary mechanism for the observed blood pressure changes during lipid infusion.

An acute increase in FFAs causes an inflammatory response, as reflected in an increase in ROS generation by mononuclear cells and an increase of intranuclear nuclear factor-κB binding and nuclear factor-κB expression by mononuclear cells (9, 29). In addition, high FFA levels result in abnormal vascular reactivity because FFAs induce a state of inflammation and reduce nitric oxide release by the endothelium (9, 29). FFAs also reduce vascular prostacyclin secretion as well as rendering it unstable (30). In healthy volunteers, increases in FFAs with 4-h infusions of lipid emulsion impair endothelium-dependent vasodilatation (10). Unsaturated FFAs directly activate both typical and atypical isoforms of protein kinase C, which is involved in regulation of vascular tone and vascular smooth muscle cell growth (26). At the cellular level, FFA can reduce nitric oxide bioavailability by inhibiting nitric oxide synthase activity (12) and stimulating production of ROS through activation of reduced nicotinamide adenine dinucleotide phosphate oxidase (13). ROS are associated with activation of MAPKs, transcription factors, matrix metalloproteinases, increases in IGF-I levels, and endothelial dysfunction (26), and oleic acid induces protein kinase C-dependent production of ROS in vascular smooth muscle cells (31). Our results indicate that high FFA was associated with sustained endothelial dysfunction. FMD decreased by 11% at 24 h and by 18% at 48 h of Intralipid infusion.

Clinical and experimental data indicate that changes in lipid metabolism may contribute to the development of type 2 diabetes. Acute elevations of FFA produce insulin resistance dose-dependently in diabetic and nondiabetic individuals (5, 6, 32). In addition to inhibiting insulin action, recent evidence indicates that FFAs also have an important role in the regulation of pancreatic β-cell function (33, 34). The acute or short-term stimulatory effect of FFAs on glucose-stimulated insulin secretion has been well described both *in vitro* and *in vivo* (35). Recent studies, however, have shown that prolonged (48 h) exposure of rat (36) and human islets (37) to fatty acids decreases glucose-stimulated insulin secretion. Mason *et al.* (33) showed in the rat model that a prolonged *in vitro* elevation of FFAs with an iv infusion of heparin/Intralipid decreases insulin secretion in response to hyperglycemic stimulus. The effect of prolonged elevation of FFAs on insulin secretion in humans remains controversial. Several investigators have reported that 24- to 48-h elevation of FFAs either increased (6) or decreased insulin secretion (38, 39). In our study, increased FFA levels by Intralipid infusion caused a significant increase in plasma glucose concentration without a significant rise in C-peptide levels compared with saline infusion. Although the increased blood glucose levels can be the result of FFA-induced insulin resistance, the C-peptide response during lipid infusion (Fig. 4) suggests that the amount of insulin secreted was inadequate to maintain normal glucose homeostasis. In agreement with these results, Kashyap *et al.* (39) recently reported that a 4-d lipid infusion impairs insulin secretion but does not worsen insulin resistance in already insulin-resistant subjects.

We acknowledge the following limitations in this study. Intralipid emulsion is a soybean oil-based lipid emulsion rich in ω-6 polyunsaturated fatty acids (40) that is different from African-American dietary intake. Due to its high content of linoleic acid, soybean-based lipid emulsions might promote the generation of arachidonic acid-derived eicosanoids and exaggerate the inflammatory response (40). In addition, the high levels of polyunsaturated fatty acids have been shown to impair endothelial function and nitric oxide production (40). It should be noted that we infused Intralipid 20% at 40 ml/h, which resulted in supraphysiological FFA levels. It is not known whether more physiological FFA levels may also increase blood pressure and cause endothelial dysfunction, and/or whether comparable increases in FFAs by repeated oral fat load result in similar blood pressure and vascular effects as iv lipid infusion. In this study, we only included obese African-Americans with type 2 diabetes, so the effect of FFAs in lean, nondiabetic subjects from different ethnic populations needs to be determined in future studies. Finally, in this study we assessed vascular reactivity by changes in FMD during reactive hyperemia. The technique provokes the release of nitric oxide, resulting in vasodilation that can be quantitated as an index of vasomotor function. The noninvasive nature of the technique allows repeated measurements over time to study the effectiveness of various interventions that may affect vascular health. However, despite its widespread use, there are technical and interpretive limitations of this technique. Recently, assessment of forearm blood flow during intraarterial infusion of acetylcholine or nitroprusside has been shown to represent a more accurate and a more reproducible assessment of endothelium-dependent and endothelium-independent dilation.

In summary, our studies indicate that sustained elevation in FFAs by Intralipid infusion is associated with a rapid and sustained increase in blood pressure, increased levels of inflammatory markers, and endothelial dysfunction. Our model should be useful to examine disease mechanisms and test new therapeutic interventions to correct the different disorders associated with hypertension and insulin resistance.

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