Elevated Circulating Free Fatty Acid Levels Impair Endothelium-Dependent Vasodilation

Helmut O. Steinberg,*‡ Manal Tarshoby,* Robert Monestel,* Ginger Hook,* Jessica Cronin,* Ann Johnson,* Basel Bayazeed,*‡ and Alain D. Baron*‡

**Department of Medicine, Indiana University Medical Center, Indianapolis, Indiana 46202; and ‡Richard L. Roudebush Veterans Affairs Medical Center, Indianapolis, Indiana 46202*

Abstract

We have recently shown that insulin-resistant obese subjects exhibit impaired endothelial function. Here, we test the hypothesis that elevation of circulating FFA to levels seen in insulin-resistant subjects can impair endothelial function. We studied leg blood flow responses to graded intrafemoral artery infusions of the endothelium-dependent vasodilator methacholine chloride (Mch) or the endothelium-independent vasodilator sodium nitroprusside during the infusion of saline and after raising systemic circulating FFA levels exogenously via a low- or high-dose infusion of Intralipid plus heparin or endogenously by an infusion of somatostatin (SRIF) to produce insulinopenia in groups of lean healthy humans. After 2 h of infusion of Intralipid plus heparin, FFA levels increased from 562±95 to 1,303±188 μmol, and from 350±35 to 3,850±371 μmol ($P < 0.001$) vs. saline for both low- and high-dose groups, respectively. Mch-induced vasodilation relative to baseline was reduced by ~ 20% in response to the raised FFA levels in both groups ($P < 0.05$, saline vs. FFA, ANOVA). In contrast, similar FFA elevation did not change leg blood flow responses to sodium nitroprusside. During the 2-h SRIF infusion, insulin levels fell, and FFA levels rose from 474±22 to 1,042±116 μmol ($P < 0.01$); Mch-induced vasodilation was reduced by ~ 20% ($P < 0.02$, saline vs. SRIF, ANOVA). Replacement of basal insulin levels during SRIF resulted in a fall of FFA levels from 545±47 to 228±61 μmol, and prevented the impairment of Mch-induced vasodilation seen with SRIF alone. In conclusion, (*a*) elevated circulating FFA levels cause endothelial dysfunction, and (*b*) impaired endothelial function in insulin-resistant humans may be secondary to the elevated FFA concentrations observed in these patients. (*J. Clin. Invest.* 1997. 100:1230–1239.) Key words: insulin resistance • nitric oxide • leg blood flow • arterial blood pressure • atherosclerosis

Introduction

Insulin causes nitric oxide–dependent vasodilation in skeletal muscle vasculature in lean insulin-sensitive man (1, 2). Interestingly, this insulin effect is either blunted or absent in obese insulin-resistant subjects with or without non-insulin-dependent diabetes mellitus (NIDDM)[1] (1, 3). We have recently reported that vasodilatory responses to the endothelium-dependent vasodilator methacholine chloride are also impaired in obese insulin-resistant subjects with and without NIDDM (4). Moreover, we found that insulin's normal ability to cause a shift to the left in the methacholine chloride dose-response curve (i.e., enhanced endothelium-dependent vasodilation) is also impaired in obese insulin-resistant subjects (4). Together, these data indicate that obesity/insulin resistance is associated with abnormalities in endothelium-dependent relaxation, and specifically suggest that, at a minimum, obesity/insulin resistance is associated with abnormalities in the endothelium-derived nitric oxide system.

The mechanism underlying impaired endothelium-dependent vasodilation in obese insulin-resistant patients is not known. Obesity/insulin resistance is associated with a number of abnormalities such as blood pressure and serum cholesterol elevations, which could contribute to this endothelial dysfunction. We found, however, that serum cholesterol and blood pressure elevations could account for only a small proportion of the endothelial dysfunction displayed in these subjects (4). A recent report by Davda et al. (5) has suggested that the FFA oleic acid reduces the activity of the endothelial nitric oxide synthase in vitro. Given that insulin resistance in man is characterized by elevated circulating levels of FFAs (6, 7) it is not unreasonable to suspect that FFAs may play a role in the endothelial dysfunction displayed by insulin-resistant patients.

Thus, the current study was designed to test the hypothesis that acute elevation of FFA in lean insulin-sensitive subjects results in impaired endothelial function. To this end, studies were performed in lean insulin-sensitive subjects in which experimental procedures were designed to raise circulating FFA concentrations two to ninefold that of basal levels. Endothelium-dependent vasodilation was studied before and after raising FFA concentrations with either a systemic infusion of a fat emulsion and sodium heparin (exogenous FFA administration) or an infusion of somatostatin designed to cause hypoinsulinemia and a resultant increase in lipolysis (endogenous FFA release).

Methods

Subjects

Demographic characteristics of the subject groups for each protocol are shown in Tables I, IV, and VI. All study subjects were healthy,

Address correspondence to Alain D. Baron, M.D., Division of Endocrinology and Metabolism, Indiana University Medical Center, 541 North Clinical Drive, Clinical Building 459, Indianapolis, IN 46202-5111. Phone: 317-274-1339; FAX: 317-278-0658; E-mail: abaron@mdep.iupui.edu

Received for publication 27 January 1997 and accepted in revised form 29 May 1997.

The Journal of Clinical Investigation
Volume 100, Number 5, September 1997, 1230–1239
http://www.jci.org

1. *Abbreviations used in this paper:* LBF, leg blood flow; MAP, mean arterial blood pressure; Mch, methacholine chloride; NIDDM, non-insulin-dependent diabetes mellitus; NS, normal saline; SNP, sodium nitroprusside; SRIF, somatostatin.

and were not taking any medications. All had normal blood pressure determined by cuff measurements, and exhibited normal 75 g oral glucose tolerance tests. Studies were approved by the Indiana University Human Subjects Internal Review Board, and all volunteers gave informed consent.

Diet

All subjects were admitted to the Indiana University General Clinical Research Center 2 d before study, and were fed a weight-maintaining diet, the caloric content of which was distributed as 50% carbohydrate, 30% fat, and 20% protein.

Study drugs

All infusates were prepared under sterile conditions on the morning of the study. Heparin (10,000 U/ml; Elkins-Sinn Inc., Cherry Hill, NJ) and intralipid (20% Fat Emulsion; Pharmacia Inc., Clayton, NC) were infused into the antecubital vein to achieve elevation of systemic FFA levels. Heparin and Intralipid infusions were adjusted to achieve approximately three or ninefold elevations of circulating FFA levels according to the study protocol as described below. Methacholine chloride (Mch; Roche Laboratories, Division of Hoffman-La Roche, Nutley, NJ), was dissolved in normal saline (NS) to a concentration of 25 μg/ml, and sodium nitroprusside (SNP; Roche Labs), was dissolved in NS to a concentration of 7 μg/ml. Mch or SNP was infused directly into the femoral artery (Harvard programmable pump model 44; Harvard Apparatus, South Natick, MA). Somatostatin (SRIF; Bachem California, Torrance CA) was diluted in 148 cc NS with 2 cc of albumin added and administered via an antecubital catheter at a dose of 0.12 μg/kg/min. Regular insulin (Humulin Regular; Eli Lilly and Co., Indianapolis, IN) was prepared in NS with added albumin and infused via an antecubital catheter.

Protocol

To examine the effect of exogenously and endogenously increased prevailing FFA concentrations on endothelium-dependent vasodilation, separate groups of subjects were studied under distinct study protocols. In addition, the effect of exogenously increased FFA levels on endothelium-independent vasodilation was also assessed. Aspects of the protocol that are common to all studies are described below.

At ~ 7:00 am, after an overnight 14-h fast, a catheter was inserted into the antecubital vein for infusion of substances. Subsequently, the right femoral artery and vein were cannulated. A 5 French sheath (Cordis Laboratories, Inc., Miami, FL) was placed in the right femoral vein to allow the insertion of a custom-designed 5 French double lumen thermodilution catheter (Baxter Scientific, Edwards Division, Irvine, CA) to measure leg blood flow (LBF) as previously described (8). The right femoral artery was cannulated with a 5.5 French double lumen catheter (Arrow International, Reading, PA) to allow simultaneous infusion of substances through the proximal (most caudad), and invasive blood pressure monitoring through the distal port (most cephalad). Heart rate and mean arterial blood pressure (MAP) were monitored continuously via precordial leads and a pressure transducer connected to a vital signs monitor (Spacelabs Medical Inc., Redmond, WA).

Hemodynamic measurements

All hemodynamic measurements were obtained with the subjects in the supine position in a quiet, temperature-controlled room after the subject had emptied his/her bladder. Baseline measurements of LBF, MAP, and heart rate were obtained after allowing at least 30 min of rest after the insertion of the catheters. Rates of LBF were determined by injecting 1 ml of iced normal saline into the femoral vein via the thermodilution catheter. The thermodilution curves were recorded on a chart recorder, and were visually inspected for integrity. LBF was calculated by a cardiac output computer (Model 9520A; American Edwards Laboratories, Irvine CA) that integrates the area under the thermodilution curve and displays the flow rate in liter/min. The coefficient of variation for measuring LBF with the thermodilution catheter is 12%. During graded intrafemoral artery infusion of drugs (Mch or SNP), LBF measurements were begun 2 min after the onset of each dose. LBF measurements were performed approximately every 30 s for a total of 10 determinations at each drug dose. Invasively determined MAP and heart rate were recorded with every other LBF determination.

Protocol I: exogenously increased FFA levels

Intralipid 20% was infused into the antecubital vein at a rate of 45 cc/h with heparin 0.2 U/kg/min (low-dose FFA) or 90 cc/h with heparin 0.3 U/kg/min (high-dose FFA) to increase FFA levels to approximately three and ninefold that of basal levels. Intralipid and heparin were administered for a period of 2 h.

Effect of FFA elevation on endothelium-dependent vasodilation (Mch dose response curves). To study the effect of elevated FFA levels on endothelium-dependent vasodilation, we assessed the LBF responses to intrafemoral artery infusions of Mch at sequential doses of 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 μg/min during infusion of saline (basal conditions), and after a 2-h infusion of intralipid and heparin to achieve low and high FFA concentrations. Each Mch dose was administered for ~ 8 min. The volume of Mch infusate delivered ranged from 0.1 to 0.6 ml/min. In this fashion, dose response curves for Mch were obtained under conditions of basal and approximately three and ninefold basal FFA levels.

Effect of FFA elevation on endothelium-independent vasodilation (SNP dose response curves). To study the effect of exogenously elevated FFA levels on endothelium-independent vasodilation, we evaluated LBF responses to graded intrafemoral artery infusions of SNP at sequential doses of 1.75, 3.5, 7.0, and 10.5 μg/min during infusion of saline (basal condition) and after 2 h of the low-dose intralipid and heparin infusion. Each SNP dose was administered for ~ 8 min. The volume of SNP delivered ranged from 0.25 to 1.5 ml/min. In this fashion, dose response curves for SNP were obtained under basal conditions, and during approximately threefold basal prevailing FFA levels.

Protocol II: endogenously increased FFA levels

To control for differences in FFA composition between endogenous and exogenous sources on endothelium-dependent vasodilation, circulating FFA levels were increased by increasing endogenous lipolysis. To this end, we infused SRIF via the antecubital catheter at a dose of 0.12 μg/kg/min to cause a relative hypoinsulinemia, which resulted in an approximately threefold increase in systemic FFA levels. SRIF was administered for a period of 2 h before assessment of endothelial function. To prevent the initial fall in prevailing glucose concentrations that accompanies SRIF infusion alone, and the fall in glucose concentrations that is associated with SRIF + insulin replacement, we used the glucose clamp technique to maintain the prevailing glucose concentration at basal levels as previously described (9).

Effect of endogenously elevated FFA on endothelium-dependent vasodilation (SRIF infusion alone). To assess the effect of endogenously increased FFA levels on endothelium-dependent vasodilation, we administered graded intrafemoral artery infusions of Mch during infusion of saline, and during the systemic infusion of SRIF designed to increase FFA levels approximately two to threefold. Mch was infused at sequential doses of 5.0, 10.0, and 15.0 μg/min. Each Mch dose was administered for ~ 8 min. The volume of Mch infusate delivered ranged from 0.2 to 0.6 ml/min. In this fashion, dose response curves for Mch were obtained under conditions of basal insulin and FFA levels, and under conditions of approximately threefold elevated FFA levels and decreased insulin levels.

Insulin replacement study. SRIF causes not only reduction of insulin secretion, but also the reduction of other hormones, i.e., glucagon. To determine whether the effect of SRIF on endothelium-dependent vasodilation was due to the decreased levels of insulin with its resultant increase in lipolysis, and not the effect of other hormone deficiencies, we studied LBF responses to intrafemoral artery infusions of Mch at sequential doses of 5.0, 10.0, and 15.0 μg/min during infusion of saline (basal conditions), and after a 2-h systemic infusion of SRIF with insulin replaced (4 mU/m^2/min). In this fashion, dose re-

Table I. Baseline Characteristics of the Groups Studied with Intralipid + Heparin Infusions

	Methacholine chloride		Sodium nitroprusside
	Low-dose FFA	High-dose FFA	Low-dose FFA
n	8	8	5
Male/female	5/3	8/0	5/0
Age (yr)	33.0±3.0	31.4±2.7	37.6±3.0
Body mass index (kg/m^2)	20.0±0.7	22.5±1.4	18.4±4.2
% body fat	17.2±2.9	16.9±1.4	18.3±3.4
MAP (mmHg)	87.6±1.9	84.9±3.2	88.2±2.3
Basal LBF (liter/min)	0.187±0.016	0.178±0.014	0.273±0.048
Cholesterol (mg/dl)	140±14	184±8	139±14
Triglyceride (mg/dl)	68±15	113±14	65±15
LDL-cholesterol (mg/dl)	83±12	107±6	87±13
HDL-cholesterol (mg/dl)	44±4	52±7	42±4
FFA levels (μmol)	562±95	350±35	391±74

sponse curves for Mch were obtained under basal conditions (basal insulin levels), and during a systemic SRIF infusion with basal insulin replaced.

Control studies

Time control study. To exclude the possibility that changes in endothelium-dependent vasodilation were the result of changes occurring over the time elapsed in the study (time course effect), we assessed the effect of time on endothelium-dependent vasodilation. To this end, we measured the LBF responses to intrafemoral artery infusions of Mch at sequential doses of 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 μg/min, 30 min after catheter placement (basal) and after a 2-h infusion of saline (45 cc/h). In this fashion, we obtained two Mch dose response curves over a time period of ~ 3 h.

Effect of elevated triglycerides (Intralipid without heparin). Infusion of Intralipid and heparin as well as insulin deficiency cause an increase in FFA, glycerol, and triglycerides. To exclude an effect of elevated triglyceride levels on endothelium-dependent vasodilation, we studied LBF responses to graded intrafemoral artery infusions of Mch at sequential doses of 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 μg/min, during infusions of saline (basal conditions), and during the infusion of Intralipid (45 cc/hr) without heparin. Each Mch dose was administered for ~ 8 min. In this fashion, dose response curves for Mch were obtained under basal conditions and during an approximately two-fold basal elevation of prevailing triglyceride levels.

Analytical methods

FFA were measured according to the method described by Novak (10). Total cholesterol and triglyceride levels were measured on a Kodak Ektachem 702 Analyzer with an enzymatic method (Eastman Kodak Co., Rochester, NY). HDL-Cholesterol was measured with the Magnetic HDL kit (Reference Diagnostics, Inc., Arlington, MA), and LDL cholesterol was calculated according to the Friedewald formula. Plasma glucose levels were determined by the glucose oxidase method with a YSI 2300 (Yellow Springs Instrument Co., Yellow Springs, OH). Insulin levels were measured with the Coat a Count kit (Diagnostic Products Corp., Los Angeles, CA). Body fat content was determined by DXA (dual energy X-ray absorptiometry, Lunar DPX-L; Lunar Corp., Madison, WI) with system software 1.2.

Statistical analysis

Results are shown as the mean±SEM. MAP is expressed in mmHg, and LBF is expressed in liter/min. Insulin levels are expressed in μU/ml. Changes in LBF are expressed as absolute (Δ) and percent change (%Δ) to adjust for differences at baseline. Conductance was calculated as LBF/MAP ([ml/min]/mmHg) and is given in arbitrary units (U). Two way ANOVA was used to compare the changes in LBF and conductance in response to the graded drug infusions during saline, and during elevation of FFAs. When significant differences between groups were found by ANOVA, this was followed by post hoc testing with Fisher's PLSD. The maximal response to the vasodilator drugs was defined as the highest LBF achieved regardless of the drug infusion rate. Correlational analysis between FFA levels and maximal LBF responses to Mch was performed. Because FFA concentrations were not normally distributed, FFA concentrations were transformed to a logarithmic scale, after which simple linear regression analysis was performed.

Statistical significance was accepted at a level of $P < 0.05$. Statistics were performed on a Macintosh computer with StatView IV (Abacus Concepts, Inc., Berkeley, Ca).

Results

Protocol I: exogenously increased FFA levels (Intralipid studies)

ENDOTHELIUM-DEPENDENT VASODILATION: METHACHOLINE CHLORIDE INFUSIONS

FFA, triglyceride, glucose, and insulin concentrations. FFA levels increased significantly in response to the systemic infusions of Intralipid and heparin in both the low- and high-dose FFA groups. In the low-dose group, FFA levels rose from 562±95 to 1,303±188 μmol ($P < 0.001$ vs. saline). In the high-

Table II. MAP, Systolic, and Diastolic Blood Pressure of the Various Study Groups Studied at Baseline (Saline Infusion) and During Heparin + Intralipid (FFA) or Somatostatin±Insulin Replacement (SRIF)

	Saline			FFA/SRIF		
	Blood pressure (mmHg)			Blood pressure (mmHg)		
Study groups	MAP	Systolic	Diastolic	MAP	Systolic	Diastolic
Mch (low-dose FFA)	87.6±1.9	118.0±2.8	69.1±2.1	87.6±2.4	121.3±2.4‡	68.8±2.3
Mch (high-dose FFA)	84.9±3.2	121.5±4.9	67.4±4.1	88.7±3.1‡	127.6±4.1‡	68.0±3.0
SNP (low-dose FFA)	88.2±2.2	122.6±2.9	69.0±2.5	90.6±2.1*	128.8±3.0‡	71.9±3.3
SRIF	86.3±2.7	119.5±3.6	69.6±2.5	86.3±3.8	122.4±4.9	68.7±3.0
SRIF + insulin	91.0±2.0	123.7±2.6	73.5±2.0	91.8±2.8	124.9±2.9	74.3±2.2

*$P = 0.07$ vs. saline; ‡$P < 0.05$ vs. saline.

dose group, FFA levels increased from 350±35 to 3,850±371 μmol ($P < 0.001$ vs. saline). Triglyceride levels in the low-dose FFA group increased from 68.2±7.2 to 120.9±17.3 mg/dl ($P < 0.01$) in response to the infusion of Intralipid and heparin. Glucose levels declined slightly from 91.9±2.5 to 85.6±2.1 ($P < 0.05$), and from 92.5±2.2 to 89.6±2.0 (P = NS) after 2 h of Intralipid and heparin infusion in the low- and high-dose FFA groups respectively. Basal insulin levels during saline infusion were 7.82±0.71 and 5.6±0.63 μU/ml in the low- and high-dose FFA groups, respectively. After 2 h of Intralipid and heparin infusion, insulin levels were 6.85±0.88 and 5.06±0.67 μU/ml in the low- and high-dose FFA groups, respectively, with insulin levels unchanged from baseline ($P = 0.3$ and $P = 0.6$, low- and high-dose FFA, respectively).

Hemodynamic data. Basal blood pressure measured invasively was in the normal range in both study groups (Table I). MAP increased slightly in both groups (Table II), but the rise in MAP reached statistical significance only in the high-dose FFA group. Further analysis of the blood pressure data revealed that during the FFA infusion, diastolic blood pressure did not change, and that the systolic blood pressure increased moderately three to five percent (Table II).

Basal LBF was 0.187±0.016 and 0.178±0.014 liter/min in the low- and high-dose FFA groups, respectively, (P = NS). LBF rose moderately but significantly in both groups in response to the systemic infusions of Intralipid and heparin. After 2 h of FFA elevation, LBF had increased to 0.237±0.024 and 0.219±0.023 liter/min in the low- and high-dose FFA groups, respectively (P = NS between groups, and $P < 0.05$ vs. basal in both groups).

LBF increased in a dose-dependent manner ($P < 0.001$, ANOVA) in response to the graded intrafemoral artery infusions of Mch in the low- and high-dose FFA groups during both saline and Intralipid plus heparin infusions. Increments in LBF, however, were reduced by elevation in FFA levels. Maximum response to Mch was 0.771±0.099 and 0.692±0.069 liter/min during saline and low-dose FFA, respectively ($P = 0.08$), and 0.617±0.052 and 0.500±0.080 liter/min during saline and high-dose FFA, respectively ($P < 0.05$). The overall absolute increments in LBF above baseline (ΔLBF) were on average 20% higher during saline as compared to FFA in the low FFA group ($P = 0.06$, ANOVA) and 25% higher in the high FFA group ($P < 0.05$, ANOVA), respectively (Table III). Maximum increments in LBF above baseline in response to Mch during saline and FFA conditions were 0.573±0.092 liter/min and 0.440±0.042 liter/min, and 0.453±0.049 liter/min and 0.281±0.077 liter/min in low- and high-dose FFA groups ($P < 0.05$ saline vs. FFA, both groups). LBF changes expressed relative to baseline (%Δ to adjust for baseline differences) were higher at each Mch infusion rate (Fig. 1, *A* and *B*) with maximal Δ%LBF of 291±36 and 193±15% in the low-dose, and 253±25 and 121±29% in the high-dose group during saline and FFA respectively, $P < 0.001$ saline vs. FFA, both groups.

Basal conductance was 2.1±0.2 and 2.1±0.2 U in the low- and high-dose FFA groups, respectively. Changes in conductance reflected the changes in LBF. Conductance rose

Table III. LBF, MAP, Conductance, and Absolute Changes Above Baseline of LBF (Δ LBF) and Conductance (Δ Conductance) in Response to Graded Intrafemoral Artery Infusions of methacholine Chloride During Systemic Infusion of Saline or 20% Intralipid Emulsion Plus Heparin

	Saline					FFA (low dose)				
	LBF	MAP	Conductance	Δ LBF	Δ conductance	LBF	MAP	Conductance	Δ LBF	Δ conductance
	liter/min	*mmHg*	*U*	*liter/min*	*U*	*liter/min*	*mmHg*	*U*	*liter/min*	*U*
Basal	0.187±0.016	87.5±1.9	2.1±0.2			0.237±0.029*	87.6±2.4	2.7±0.4*	‡	‖
Mch (2.5 μg/min)	0.277±0.037	83.9±1.3	3.3±0.5	0.090±0.027	1.2±0.3	0.334±0.030	88.2±2.8	3.8±0.4	0.096±0.014	1.1±0.2
Mch (5.0 μg/min)	0.445±0.046	85.7±2.8	5.2±0.6	0.258±0.038	3.1±0.5	0.428±0.047	87.2±3.3	5.0±0.6	0.191±0.027	2.2±0.3
Mch (7.5 μg/min)	0.518±0.087	86.5±2.9	6.0±1.0	0.331±0.041	3.9±1.0	0.532±0.063	87.4±2.9	6.1±0.8	0.294±0.041	3.4±0.5
Mch (10.0 μg/min)	0.605±0.104	88.6±2.9	6.8±1.2	0.418±0.096	4.7±1.1	0.597±0.075	87.0±2.9	6.9±0.9	0.360±0.057	4.2±0.7
Mch (12.5 μg/min)	0.704±0.108	88.8±3.7	8.0±1.3	0.517±0.099	5.9±1.2	0.636±0.099	87.6±3.0	7.4±1.0	0.399±0.049	4.6±0.7
Mch (15.0 μg/min)	0.755±0.099	87.3±2.9	8.6±1.1	0.573±0.092	6.5±1.0	0.677±0.067	87.3±3.1	7.8±0.9	0.440±0.042	5.1±0.6
	Saline					FFA (high dose)				
Basal	0.178±0.014	84.8±3.2	2.1±0.2			0.219±0.023*	87.7±2.8*	2.5±0.3*	§	¶
Mch (2.5 μg/min)	0.236±0.032	84.2±3.1	2.8±0.4	0.057±0.019	0.7±0.2	0.268±0.042	87.0±2.7	3.2±0.6	0.049±0.026	0.6±0.3
Mch (5.0 μg/min)	0.321±0.041	83.2±3.1	3.9±0.5	0.143±0.031	1.8±0.4	0.327±0.042	87.7±3.0	3.8±0.5	0.108±0.025	1.3±0.3
Mch (7.5 μg/min)	0.357±0.040	84.0±3.3	4.3±0.6	0.179±0.029	2.2±0.4	0.372±0.057	87.8±2.9	4.3±0.7	0.153±0.040	1.8±0.5
Mch (10.0 μg/min)	0.424±0.044	83.9±3.4	5.1±0.5	0.245±0.038	2.9±0.4	0.398±0.053	89.2±2.9	4.5±0.6	0.179±0.035	2.0±0.4
Mch (12.5 μg/min)	0.500±0.066	84.3±3.4	6.0±0.8	0.322±0.056	3.8±0.7	0.461±0.074	89.4±3.0	5.2±0.9	0.242±0.058	2.7±0.7
Mch (15.0 μg/min)	0.617±0.052	84.8±3.8	7.6±0.8	0.453±0.049	5.4±0.6	0.494±0.083	90.6±3.0	5.6±1.1	0.281±0.077	3.1±0.9

Intralipid emulsion was administered at a rate of 45 cc/h (low-dose FFA) with heparin 0.2 U/kg/min designed to increase systemic FFA levels approximately two to threefold, or at a rate of 90 cc/h with heparin 0.3 U/kg/min (high-dose FFA) designed to increase systemic FFA levels approximately six to ninefold (see text for detailed analysis). *$P < 0.05$ vs. saline. ‡$P = 0.06$, §$P < 0.05$ for overall dose response for LBF increments (Δ LBF) above baseline, saline vs. FFA (ANOVA). ‖$P = 0.08$, ¶$P < 0.01$ for overall dose response for increments in conductance (Δ conductance) above baseline, saline vs. FFA (ANOVA).

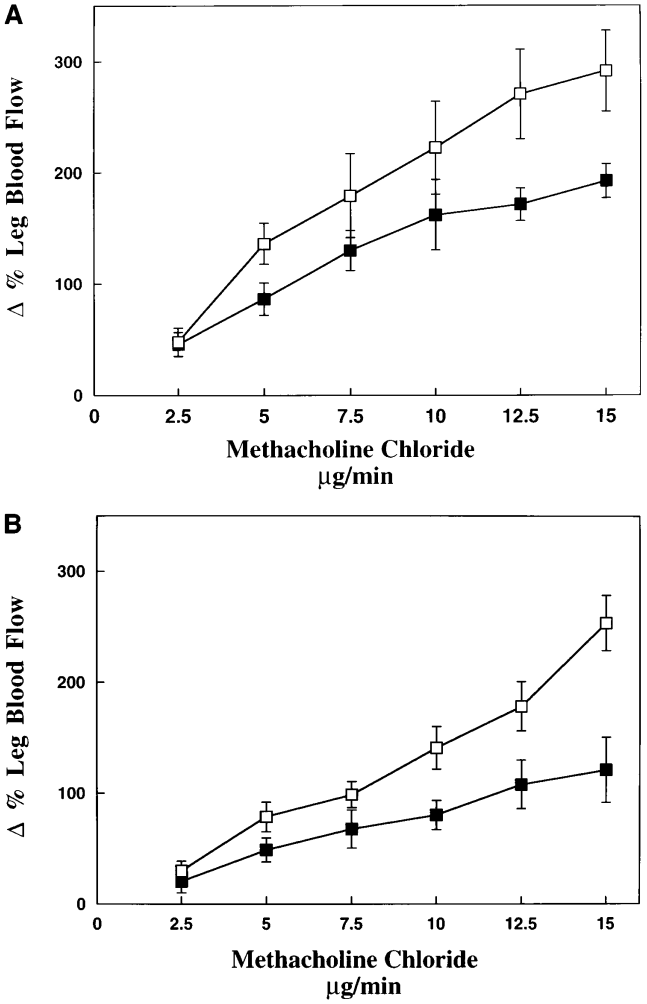

Figure 1. (*A*) LBF increments relative to baseline (Δ%LBF) in response to graded intrafemoral artery infusions of Mch during infusion of saline or 20% intralipid emulsion at a rate of 45 cc/h combined with Heparin 0.2 U/kg/min (low-dose FFA) designed to increase systemic levels of FFA two or threefold; (*B*) Δ%LBF in response to graded intrafemoral artery infusions of Mch during infusion of saline or 20% intralipid emulsion at a rate of 90 cc/h combined with Heparin 0.3 U/kg/min (high-dose FFA) designed to increase systemic levels of FFA approximately six to ninefold. *White squares*, saline; *black squares*, FFA.

moderately but significantly in both groups in response to the systemic infusions of Intralipid and heparin (Table III). Conductance increased in a dose-dependent manner in response to the graded Mch infusions in both the low- and high-dose groups ($P < 0.001$). Increments in conductance, however, were reduced by elevation of FFA levels (Table III). Maximum conductance in response to Mch was 8.9±1.2 and 8.2±0.9 U during saline and low-dose FFA, respectively ($P = 0.08$), and 7.6±0.7 and 5.6±1.0 U during saline and high-dose FFA, respectively ($P < 0.05$). The overall increments in conductance above baseline (Δ conductance) was on average 20% higher during saline as compared to FFA in the low-dose FFA group ($P < 0.08$, ANOVA) and 30% higher in the high-dose FFA group ($P < 0.01$, ANOVA). Maximum increments in conductance above baseline in response to Mch during saline and FFA conditions, respectively, were 6.8±1.0 and 5.5±0.6 U, and 5.2±0.6 and 3.1±0.7 U in low and high FFA groups ($P < 0.05$, saline vs. FFA, both groups). Changes in conductance expressed relative to baseline (%Δ to adjust for baseline differences) were higher at each Mch infusion rate in both the low- and high-dose groups during saline versus FFA respectively ($P < 0.001$ saline vs. FFA, both groups). Thus, increments in LBF and conductance in response to graded intrafemoral artery infusions of Mch were impaired by elevation of FFA, showing that both low and high FFA doses caused marked reductions in endothelium-dependent vasodilation relative to saline infusion.

ENDOTHELIUM-INDEPENDENT VASODILATION: SODIUM NITROPRUSSIDE INFUSIONS

FFA, triglyceride, glucose, and insulin levels. Basal FFA increased significantly in response to the systemic infusions of Intralipid and heparin from 391±74 to 921±100 μmol ($P < 0.01$). Triglyceride levels rose from 117±11 to 219±20 mg/dl ($P < 0.01$) in response to the infusion of Intralipid and heparin. Glucose levels fell slightly from 93.4±2.7 to 90.2±2.4 mg/dl over the 2-h infusion period ($P = 0.07$). Insulin levels were 6.74±0.55 and 6.31±0.39 μU/ml during the infusion of saline, and after 2 h of Intralipids and heparin, respectively ($P = 0.1$).

Hemodynamic data. Basal blood pressure was in the normal range and comparable to the MAP in the Mch study groups (Table I). MAP increased slightly in response to the systemic infusions of Intralipids and heparin (Table II). Similar to the methacholine studies, systolic blood pressure increased moderately by ~5% ($P < 0.05$) without any change in diastolic blood pressure.

Basal LBF was 0.273±0.048 liter/min and rose to 0.313±0.055 liter/min in response to the systemic infusions of Intralipid and heparin ($P = 0.2$). LBF increased in a dose-dependent manner ($P < 0.001$, ANOVA) in response to the graded intrafemoral artery infusions of SNP (Fig. 2) during infusions

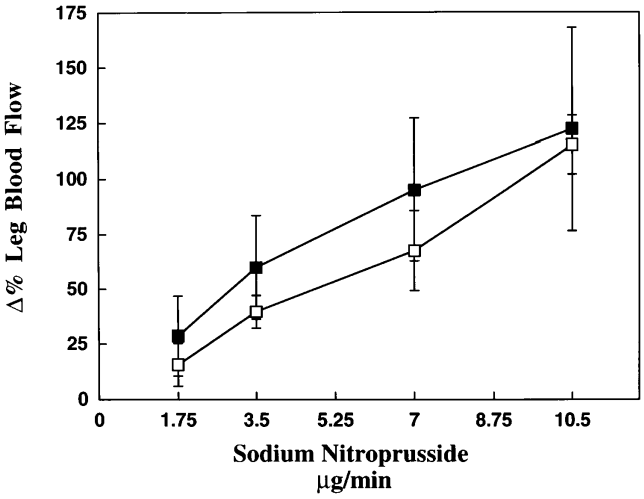

Figure 2. LBF increments relative to baseline (Δ%LBF) in response to graded intrafemoral artery infusions of SNP during a systemic infusion of saline, or 20% intralipid emulsion at a rate of 45 cc/h combined with Heparin 0.2 U/kg/min designed to increase systemic levels of FFA two to threefold. *White squares*, saline; *black squares*, FFA.

Table IV. Baseline Characteristics of the Groups Studied with SRIF±Insulin Replacement

	SRIF	SRIF + insulin replacement
n	8	8
Male/female	7/1	7/1
Age (yr)	35.0±1.9	31.4±2.7
Body mass index (kg/m^2)	22.4±0.6	22.6±0.9
% body fat	17.1±1.7	18.2±2.0
Mean arterial blood pressure (mmHg)	86.3±2.7	91.0±2.0
Basal LBF (liter/min)	0.211±0.018	0.266±0.039
Cholesterol (mg/dl)	159±15	152±9
Triglyceride (mg/dl)	85±9	64±10
LDL-cholesterol (mg/dl)	93±13	90±5
HDL-cholesterol (mg/dl)	46±5	50±6
FFA levels (μmol)	451±75	545±47

of both saline and Intralipid and heparin. In contrast to the methacholine studies, elevation of systemic FFA had no effect on the LBF response to the endothelium-independent vasodilator. Maximum LBF in response to SNP was 0.573±0.082 and 0.646±0.096 liter/min ($P = 0.6$) during saline and low-dose FFA, respectively. The absolute increments in LBF above baseline (Δ LBF) were slightly higher during FFA as compared to saline, but the difference was not significant ($P = 0.5$). Maximum increments in LBF above baseline (Δ LBF) in response to SNP were 0.299±0.038 and 0.328±.0112 liter/min, and were not different ($P = 0.8$). Expressing the LBF changes in response to SNP in percent to adjust for changes in baseline revealed no differences between saline and FFA conditions (Fig. 2). Maximal percent increments in LBF were 115±13 and 122±46% during saline and FFA, respectively ($P = 0.8$). These data indicate that both relative and absolute increments in LBF were comparable during infusions of saline or Intralipids and heparin, suggesting that in contrast to the effect of elevated FFA on endothelium-dependent vasodilation, elevated FFA levels do not impair the LBF response to the endothelium-independent vasodilator SNP.

Protocol II: studies with endogenously elevated FFA levels (SRIF infusions)

SOMATOSTATIN INFUSION ALONE

FFA, glucose, and insulin levels. Basal FFA levels were comparable to the levels in the studies with exogenously elevated FFA levels (Table I) and increased significantly from 451±75 to 1,006±107 μmol ($P < 0.0001$) in response to the systemic infusions of SRIF. FFA levels during SRIF (1,006±107 μmol) were similar to the FFA levels achieved by low-dose infusion of Intralipids and heparin (1,303±188 μmol), both representing an approximate 150% increase from baseline. Glucose levels were 95.3±2.2 and 93.7±6.3 mg/dl during saline, and after the 2-h infusion of SRIF, respectively ($P = 0.8$). As expected, insulin levels fell from 9.1±0.6 to 3.7±0.6 μU/ml ($P < 0.001$) in response to the systemic SRIF infusion.

Hemodynamic data. Basal MAP, systolic, and diastolic blood pressure were in the normal range (Table IV) and did not change during the SRIF infusion (Table II).

Basal LBF was 0.211±0.018 and increased to 0.261±0.025 liter/min ($P < 0.05$) after 2 h SRIF infusion. LBF increased in a dose-dependent manner ($P < 0.001$, ANOVA) in response to the graded intrafemoral artery infusions of Mch during both saline and SRIF infusions. Increments in LBF, however, were significantly reduced in the SRIF infused group (Table V). Maximum increments in LBF (Δ LBF) in response to Mch were 0.275±0.028 and 0.215±0.036 liter/min during saline and SRIF, respectively ($P < 0.05$ vs. SRIF). The absolute increments above baseline (Table V) were on average 30% higher during saline as compared to SRIF ($P < 0.05$). When LBF changes in response to Mch were expressed as percent changes above baseline to adjust for differences at baseline, Δ% LBF was higher at each Mch infusion rate during saline as compared to SRIF (Fig. 3 *A*). Elevation of systemic FFA levels caused reductions in the maximum Δ% LBF, 136±17 and 100±16% during saline and SRIF, respectively ($P < 0.05$).

Basal conductance was 2.5±0.2 and rose slightly but significantly ($P < 0.05$) to 3.1±0.4 U during SRIF infusion (Table V). Conductance increased in a dose-dependent manner in response to the graded Mch infusions (Table V) during both the saline and the SRIF infusion ($P < 0.001$). Increments in conductance, however, were reduced by elevation of FFA levels (Table V). The overall increments in conductance above base-

Table V. LBF, MAP, Conductance, and Absolute Changes Above Baseline of LBF (Δ LBF) and Conductance (Δ conductance) In Response to Graded Intrafemoral Artery Infusions of Methacholine Chloride During Systemic Infusion of Saline or SRIF

	Saline					SRIF				
	LBF	MAP	Conductance	Δ LBF	Δ conductance	LBF	MAP	Conductance	Δ LBF	Δ conductance
	liter/min	*mmHg*	*U*	*liter/min*	*U*	*liter/min*	*mmHg*	*U*	*liter/min*	*U*
Basal	0.211±0.018	85.1±2.6	2.5±0.2			0.261±0.025*	86.3±3.8	3.1±0.4*	‡	§
Mch (5.0 μg/min)	0.361±0.036	86.0±2.3	4.2±0.5	0.150±0.033	1.8±0.4	0.332±0.033	85.4±4.0	4.0±0.5	0.071±0.023	0.9±0.3
Mch (10.0 μg/min)	0.473±0.045	86.3±2.8	5.6±0.7	0.262±0.044	3.1±0.6	0.416±0.055	85.4±3.9	5.0±0.8	0.155±0.039	1.4±0.5
Mch (15.0 μg/min)	0.546±0.030	88.4±2.1	6.2±0.3	0.335±0.032	3.7±0.4	0.477±0.046	85.2±3.8	5.7±0.6	0.215±0.036	2.6±0.4

See text for detailed analysis. *$P < 0.05$ vs. saline; ‡$P < 0.05$ for overall dose response for LBF increments (Δ LBF) above baseline, saline vs. FFA (ANOVA); §$P < 0.01$ for overall dose response for increments in conductance (Δ conductance) above baseline, saline vs. FFA (ANOVA).

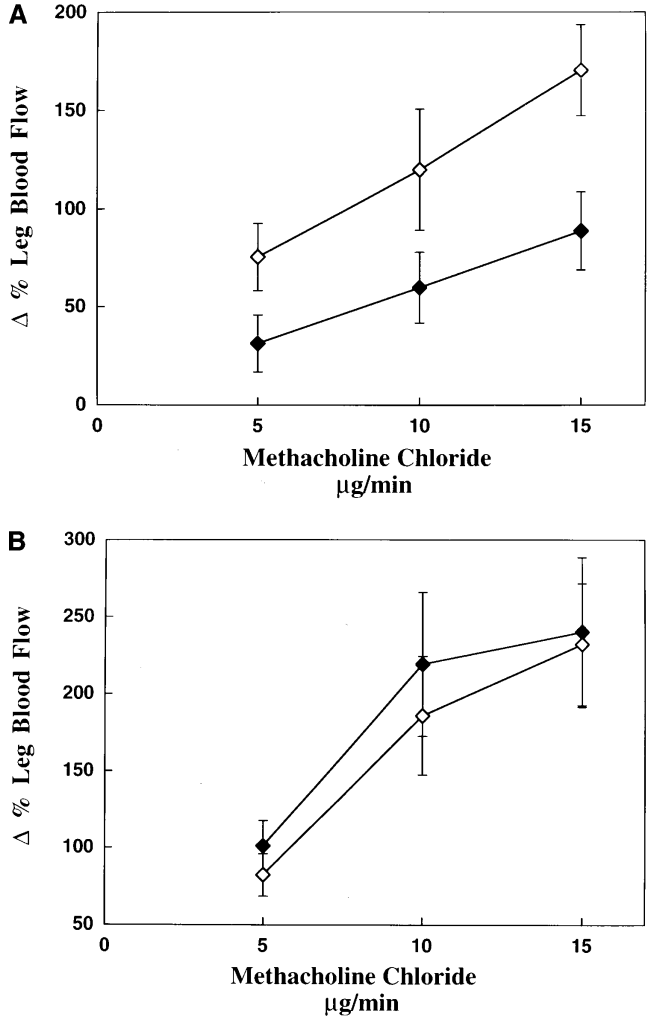

Figure 3. (*A*) LBF increments relative to baseline (Δ%LBF) in response to graded intrafemoral artery infusions of Mch during infusion of saline or SRIF (0.12 μg/kg/min). *White diamonds*, saline; *black diamonds*, SRIF. (*B*) Δ%LBF in response to graded intrafemoral artery infusions of Mch during infusion of saline or SRIF (0.12 μg/kg/min) plus replacement insulin (4 mU/min/m^2). *White diamonds*, saline; *black diamonds*, SRIF + insulin replacement.

line (Δ conductance) were on average 30% higher during saline as compared to SRIF ($P < 0.01$, ANOVA). Maximum increments in conductance above baseline in response to Mch during saline and SRIF conditions, respectively, were 4.0±0.5 and 2.9±0.4 U ($P < 0.05$, saline vs. SRIF). Changes in conductance expressed relative to baseline (%Δ to adjust for baseline differences) were higher at each Mch infusion rate during saline as compared to SRIF, respectively ($P < 0.001$ saline vs. SRIF). The magnitude of the effect of endogenously increased FFA levels with SRIF on endothelium-dependent vasodilation was comparable to the effect of exogenously elevated FFA levels in both low- and high-dose FFA groups. These data suggest that even moderate elevations of endogenous FFA cause marked impairment of endothelium-dependent vasodilation. These results demonstrate that comparable elevations of both endogenous or exogenous circulating FFA levels lead to nearly identical impairment in endothelium-dependent vasodilation.

SOMATOSTATIN INFUSION WITH REPLACEMENT INSULIN

FFA, glucose, and insulin levels. Basal FFA levels were comparable to the levels in the studies with exogenously elevated FFA levels (Table I). Basal FFA levels decreased significantly from 545±47 to 228±61 μmol ($P < 0.005$) in response to the systemic insulin replacement infusion. Glucose levels were 90.9±1.9 and 89.4±1.0 mg/dl ($P = 0.6$) during saline, and after the 2-h infusion of SRIF with insulin replacement, respectively. As expected, prevailing insulin levels were unchanged from baseline with SRIF + insulin replacement. Insulin levels were 9.4±1.9 μU/ml and 11.9±2.1 μU/ml at baseline saline and the systemic SRIF + insulin infusion, respectively ($P = 0.2$).

Hemodynamic data. Basal MAP was in the normal range (Table IV) and did not change during the SRIF + insulin infusion (Table II). Further analysis of the blood pressure data revealed no changes in diastolic or systolic blood pressure in response to the SRIF with insulin infusion.

LBF was 0.266±0.039 and 0.266±0.027 liter/min during saline, and after 2 h SRIF + insulin infusion, respectively ($P = 0.9$). LBF increased in a dose-dependent manner ($P < 0.001$, ANOVA) in response to the graded intrafemoral artery infusions of Mch during both saline and SRIF + insulin infusions. LBF response to Mch was nearly identical during saline and during SRIF plus insulin infusion. Maximum LBF in response to Mch was 0.854±0.130 and 0.931±0.158 liter/min ($P = 0.7$) during saline and SRIF + insulin, respectively. When LBF changes in response to Mch were expressed as percent changes above baseline to adjust for differences at baseline, Δ% LBF was nearly identical at each Mch infusion rate during saline as compared to SRIF (Fig. 3 *B*). These data suggest that impairment in endothelium-dependent vasodilation observed with SRIF infusion is likely secondary to hypoinsulinemia and its attendant rise in circulating FFA concentrations.

Control studies

TIME CONTROL STUDIES

FFA, glucose, and insulin levels. Basal FFA levels were 421± 90 μmol and comparable to the levels in the studies with exogenously elevated FFA levels (Table I). After 2 h of saline infusions, FFA levels were 525±115 μmol and not different from baseline. Glucose levels were 97.5±2.7, and declined slightly during the 2-h saline infusions to 93.3±3.5 mg/dl ($P < 0.05$). Insulin levels were 7.83±0.70 and 7.51±0.78 μU/ml at the beginning, and after a 2-h period of saline infusion (P = NS).

Hemodynamic data. Basal MAP was in the normal range (Table VI) and did not change during the 2-h saline infusion.

LBF was 0.205±0.031 and 0.213±0.031 liter/min at baseline and after 2 h saline infusion, respectively (P = NS). In response to Mch at 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 μg/min, LBF increased by 31±13, 68±20, 75±27, 130±36, 108±26, and 156±46% at baseline, and by 7±9, 45±24, 51±18, 94±32, 95±33, and 186±68% after 2 h of saline infusion, respectively. This LBF response to Mch occurred in a dose-dependent manner ($P < 0.001$, ANOVA) and was comparable under baseline conditions and after 2 h of saline infusion. Maximum LBF in response to Mch was 0.568±0.185 and 0.640±0.212 liter/min ($P = 0.6$) at baseline, and after 2 h of saline infusion, respectively. These data demonstrate that changes in endothelium-dependent vasodilation during elevations of FFA are not due to the effect of changes in endothelial responses over time.

Table VI. Characteristics of the Control Study Groups

	Time control	Triglyceride control
n	5	5
Male/female	4/1	5/0
Age (yr)	38.2±2.0	29.2±3.6
Body mass index (kg/m^2)	23.7±1.3	24.1±1.4
% body fat	21.1±2.4	22.0±1.6
MAP (mmHg)	83.7±3.5	87.1±1.4
Basal LBF (liter/min)	0.205±0.031	0.220±0.010
Cholesterol (mg/dl)	154±8	169±15
Triglyceride (mg/dl)	104±8	117±17
LDL-cholesterol (mg/dl)	94±7	102±14
HDL-cholesterol (mg/dl)	40±3	43±3
FFA levels (μmol)	421±90	396±45

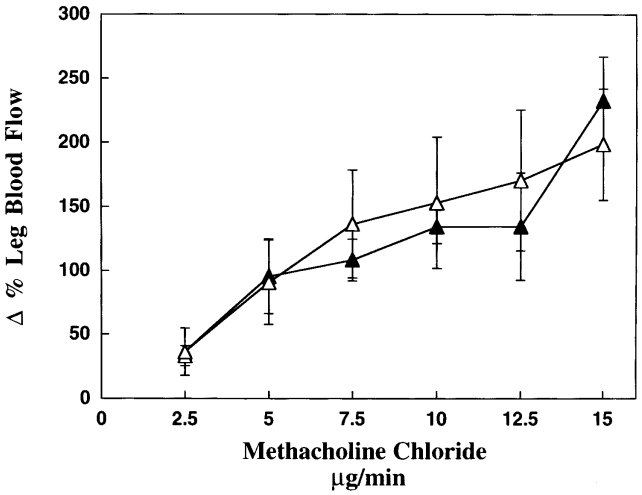

Figure 4. LBF increments relative to baseline (Δ%LBF) in response to graded intrafemoral artery infusions of Mch during infusion of saline or 20% intralipid emulsion at a rate of 45 cc/h designed to increase serum triglyceride levels approximately twofold.

Intralipid infusion without heparin (triglyceride control studies)

FFA, glucose, and insulin levels. Basal FFA levels were 396±45 μmol and comparable to the levels in the studies with exogenously elevated FFA levels (Table VI). After 2 h of Intralipid without heparin infusions, FFA levels were 527±49 μmol and not different from baseline. Triglyceride levels were 135±29 mg/dl during saline infusion, and increased to 277±41 mg/dl ($P < 0.05$) after 2 h of Intralipid infusion without heparin, resulting in a comparable twofold increase in triglyceride levels as observed during the low-dose infusion of Intralipid infusion with heparin. Glucose levels were 94.8±2.2 mg/dl, and declined slightly during the 2-h saline infusions to 90.6±1.7 mg/dl ($P = 0.058$). Insulin levels were 6.40±0.89 and 5.79±1.00 μU/ml during the infusion of saline, and after 2 h of Intralipid infusion, respectively (P = NS).

Hemodynamic data. Basal MAP was in the normal range (Table VI) and did not change during the infusion of Intralipid without heparin.

LBF was 0.215±0.010 and 0.216±0.018 liter/min at baseline (saline infusion) and after 2 h of Intralipid infusion without heparin, respectively (P = NS). LBF increased in a dose-dependent manner ($P < 0.001$, ANOVA) in response to the graded intrafemoral artery infusions of Mch during both saline and Intralipid infusion without heparin. LBF response to Mch was nearly identical under both conditions (Fig. 4). Maximum LBF in response to Mch was 0.692±0.082 and 0.728±0.125 liter/min ($P = 0.7$) during saline and Intralipid infusion without heparin, respectively. These data demonstrate that the systemic infusion of Intralipid alone causes doubling in triglyceride levels, but no impairment in endothelium-dependent vasodilation.

Correlation analysis

Our data suggest that endothelium-dependent vasodilation is modulated by prevailing FFA concentrations. To better examine the relation between FFA levels and endothelium-dependent vasodilation, we performed linear regression analysis between FFA levels and maximum percent increments in LBF (Δ%LBF) in response to Mch. Linear regression analysis demonstrated a moderately strong negative relation between FFA levels and maximum Δ%LBF which was highly significant ($r = -0.417$, $P < 0.001$). A better fit for the relation between FFA levels and maximum Δ%LBF was provided by a power function $r = 0.497$, $P < 0.001$ (Fig. 5). This result indicates that increasing FFA levels are associated with increasing impairment of endothelium-dependent vasodilation. Furthermore, the best fit model according to the power function suggests that the ability of FFA to impair endothelial function takes place up to an FFA concentration of ~ 2,000 μmol, but does not have any effect beyond that concentration.

Discussion

In the current study we have tested the hypothesis that acute elevations of circulating FFA in the range observed in insulin-resistant patients can cause endothelial dysfunction in lean insulin-sensitive subjects. The study reveals the following new findings: (*a*) acute three to ninefold elevations in circulating FFA concentrations from exogenous or endogenous sources cause endothelial dysfunction, and have no effect on endothelium-independent vasodilation; (*b*) the basal circulating insulin

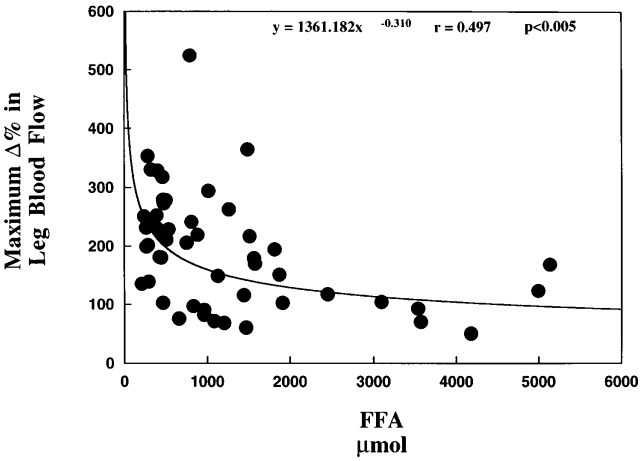

Figure 5. Relation between prevailing serum FFA levels and maximum increments in LBF relative to baseline (Δ%LBF) in response to the endothelium dependent vasodilator methacholine chloride (Mch).

concentration modulates endothelial function via its ability to control lipolysis and prevailing FFA levels; and (*c*) acute FFA-induced endothelial dysfunction is associated with a modest rise in systolic arterial pressure.

The syndrome of insulin resistance and central obesity is associated with a three to fourfold elevation in risk of myocardial infarction, and is characterized by a cluster of cardiovascular risk factors including modest elevations of blood pressure, serum triglycerides, small dense LDLs and FFAs, and reductions in levels of HDLs (11). We have recently reported endothelial dysfunction as a novel cardiovascular risk factor associated with insulin resistance/obesity (4). It is interesting to note that the magnitude of the impairment in endothelial function that we reported in obese insulin-resistant subjects was similar to what we have found in lean subjects infused with Intralipid and heparin in the current study. The findings suggest that the endothelial dysfunction observed in obese insulin-resistant subjects may be mediated, at least in part, via the elevated circulating FFA levels that these patients exhibit. Thus, because of their effect to cause endothelial dysfunction, elevated serum levels of FFAs may contribute significantly to the increased risk of macrovascular disease in insulin-resistant patients.

It is noteworthy that in our previous report, as in the present study, we could not find any relationship between the fasting FFA concentration and the degree of endothelial dysfunction (4). Because fasting FFA levels exhibit great variability, however, it is not surprising that such a relationship was not found. Indeed, while FFAs are well known to cause insulin resistance to insulin's effect to promote glucose uptake, a relationship between fasting FFA levels and insulin sensitivity has not been established. On the other hand we found a significant inverse correlation between the prevailing FFA level during the various infusion protocols and maximal endothelium-dependent vasodilation. Interestingly, the effect of FFA elevations to impair endothelium-dependent vasodilation was found to occur over the range of FFA levels observed in clinical states, while higher levels had no further effect. It is important to note that FFA elevations cause insulin resistance in a dose- and time-dependent fashion (12). While the effect of time of FFA exposure was not examined in the current study, it is possible that the FFA effect to cause endothelial dysfunction might have been more pronounced with exposure periods longer than 2 h.

The mechanism for FFA-induced endothelial dysfunction is unresolved by the current data. Insulin-induced vasodilation is nitric oxide–dependent (2) and impaired in insulin-resistant states (4), suggesting that the endothelial nitric oxide system is dysregulated in insulin-resistant patients. If FFAs are instrumental in causing endothelial dysfunction in obese/insulin-resistant subjects, it follows logically that FFAs are highly likely to cause endothelial dysfunction via an effect on the nitric oxide system. Support for this formulation comes from data indicating that oleic acid causes reduction of nitric oxide synthase activity in cultured endothelial cells (5). Whether FFAs also cause endothelial dysfunction via other mechanisms remains to be established.

Infusion of Intralipid and heparin is highly efficacious in raising prevailing FFA levels. This approach, however, may not qualitatively reflect endogenous composition of circulating FFAs. To circumvent this problem, we examined endothelial function during an infusion of somatostatin designed to cause hypoinsulinemia, and thus allow lipolytic rates to rise with resultant increase in endogenous circulating FFAs. We were able to match circulating FFA levels using both exogenous lipid infusion (low-dose intralipid) and somatostatin protocols. Interestingly, both approaches achieved similar degrees of endothelial dysfunction, suggesting that FFA elevations of both exogenous and endogenous sources cause marked endothelial dysfunction. Moreover, because basal insulin replacement during somatostatin infusion was able to reestablish normal (albeit subbasal) FFA levels and restore normal endothelial function, it follows logically that basal insulin action to suppress lipolysis indirectly modulates endothelial function. Insulin-resistant subjects have resistance to insulin's action to suppress lipolysis, and thus have elevated FFAs on that basis. In contrast, insulin-dependent diabetics may exhibit relative or absolute insulin deficiency, and thus may exhibit intermittent FFA elevations via that mechanism. Based on the current data, regardless of the cause, it is reasonable to suspect that elevations of circulating FFA concentrations, whether day long (NIDDM) or intermittent (insulin-dependent diabetes mellitus) may result in endothelial dysfunction which over time is likely to increase greatly the risk of macrovascular disease.

Important control studies for time-dependent changes of endothelial function and potential effects associated with intralipid infusion per se were also performed. Control saline infusions revealed no effect of time on endothelial function over a 2-h period. Similarly, Intralipid infusion without heparin resulted in a twofold rise in serum triglyceride concentrations with no effect on endothelial function. Because infusion of heparin alone is associated with elevations of serum FFAs, a study to control for the effect of heparin on endothelial function was not performed. Because somatostatin infusion which was not accompanied by heparin infusion caused both a rise in serum FFA levels and endothelial dysfunction, however, it is unlikely that heparin per se caused the observed endothelial dysfunction.

It is interesting to note that low-dose FFA infusions caused modest but significant rises in systolic blood pressure, but only the higher prevailing FFA concentrations were accompanied by a small but significant rise in mean blood pressure. It is probable that the higher FFA concentrations raised blood pressure further by (*a*) causing more profound endothelial dysfunction in skeletal muscle vasculature, (*b*) causing endothelial dysfunction in other vascular beds, and/or (*c*) triggering other pressor forces such as activation of the sympathetic nervous system (13), or the release of endothelium-derived vasoconstrictors. Consistent with this formulation, Stepniakowski et al. (14) have shown that elevated circulating FFA levels can increase the sensitivity of the vasculature to pressor agents such as norepinephrine.

In summary and conclusion, these studies indicate for the first time that elevation of circulating FFA concentrations in normal humans causes endothelial dysfunction, which is similar to that observed in obese insulin-resistant individuals with and without NIDDM. Given the central vasoprotective role of the endothelium (15), the data would indicate that circulating FFA concentrations may play an important role in the pathogenesis of macrovascular disease in states of insulin-resistance associated with FFA elevations. Moreover, FFAs may add to the cardiovascular risk of insulin-resistant humans by its effect to increase systolic arterial blood pressure. Although the mechanism of FFA-induced endothelial dysfunction remains to be defined, the data would predict that clinical strategies to

reduce prevailing FFA concentrations would have beneficial cardioprotective effects.

Acknowledgments

The authors wish to thank Joyce Ballard for her expert and invaluable help in preparing the manuscript, and Kerry Holloway for technical assistance.

This work was supported by grants DK42469, MO1-RR750-19, and DK20452 from the National Institutes of Health, a Veterans Affairs Merit Review Award, and a Grant-In-Aid from the American Heart Association.

References

1. Laakso, M., S.V. Edelman, G. Brechtel, and A.D. Baron. 1990. Decreased effect of insulin to stimulate skeletal muscle blood flow in obese men. *J. Clin. Invest.* 85:1844–1852.

2. Steinberg, H.O., G. Brechtel, A. Johnson, N. Fineberg, and A.D. Baron. 1994. Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. *J. Clin. Invest.* 94:1172–1179.

3. Laakso, M., S.V. Edelman, G. Brechtel, and A.D. Baron. 1992. Impaired insulin-mediated skeletal muscle blood flow in patients with NIDDM. *Diabetes.* 41:1076–1083.

4. Steinberg, H.O., H. Chaker, R. Leaming, A. Johnson, G. Brechtel, and A.D. Baron. 1996. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. *J. Clin. Invest.* 97: 2601–2610.

5. Davda, R.K., K.T. Stepniakowski, G. Lu, M.E. Ullian, T.L. Goodfriend, and B.M. Egan. 1995. Oleic acid inhibits endothelial nitric oxide synthase by a protein kinase C-independent mechanism. *Hypertension.* 26:764–770.

6. Bruce, R., I. Godsland, C. Walton, D. Crook, and V. Wynn. 1994. Associations between insulin sensitivity, and free fatty acid and triglyceride metabolism independent of uncomplicated obesity. *Metabolism.* 43:1275–1281.

7. Swislocki, A.L.M., Y.-D.I. Chen, A. Goley, M.-O. Chang, and G.M. Reaven. 1987. Insulin suppression of plasma-free fatty acid concentration in normal individuals and patients with type 2 (non-insulin-dependent) diabetes. *Diabetologia.* 30:622–626.

8. Baron, A.D., G. Brechtel, P. Wallace, and S.V. Edelman. 1988. Rates and tissue sites of non-insulin and insulin-mediated glucose uptake in humans. *Am. J. Physiol.* 255:E769–E774.

9. Baron, A.D., O.G. Kolterman, J. Bell, L.J. Mandarino, and J.M. Olefsky. 1985. Rates of non-insulin-mediated glucose uptake are elevated in type II diabetic subjects. *J. Clin. Invest.* 76:1782–1788.

10. Novak, M. 1965. Colorimetric ultramicro method for the determination of free fatty acids. *J. Lipid Res.* 6:431–433.

11. Reaven, G.M. 1994. Syndrome X: 6 years later. *J. Intern. Med.* 236 (Suppl. 736): 13–22.

12. Bonadonna, R.C., K. Zych, C. Boni, E. Ferrannini, and R.A. DeFronzo. 1989. Time dependence of the interaction between lipid and glucose in humans. *Am. J. Physiol.* 257:E49–E56.

13. Grekin, R.J., A.P. Vollmer, and R.S. Sider. 1995. Pressor effects of portal venous oleate infusion. A proposed mechanism for obesity hypertension. *Hypertension.* 26:193–198.

14. Stepniakowski, K.T., T.L. Goodfriend, and B.M. Egan. 1995. Fatty acids enhance vascular alpha-adrenergic sensitivity. *Hypertension.* 25 (4):774–778.

15. Lloyd-Jones, D.M., and K.D. Bloch. 1996. The vascular biology of nitric oxide and its role in atherogenesis. *Ann. Rev. Med.* 47:365–375.