Free Fatty Acid Elevation Impairs Insulin-Mediated Vasodilation and Nitric Oxide Production

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The effect and time course of free fatty acid (FFA) elevation on insulin-mediated vasodilation (IMV) and the relationship of FFA elevation to changes in insulin-mediated glucose uptake was studied. Two groups of lean insulin-sensitive subjects underwent euglycemic-hyperinsulinemic (40 mU · m^{-2} · min^{-1}) clamp studies with and without superimposed FFA elevation on 2 occasions ~4 weeks apart. Groups differed only by duration of FFA elevation, either short (2–4 h, n = 12) or long (8 h, n = 7). On both occasions, rates of whole-body glucose uptake were measured, and changes in leg blood flow (LBF) and femoral vein nitric oxide nitrite plus nitrate (NOx) flux in response to the clamps were determined. Short FFA infusion did not have any significant effect on the parameters of interest. In contrast, long FFA infusion decreased rates of whole-body glucose uptake from 47.7 ± 2.8 to 32.2 ± 0.6 µmol · kg^{-1} · min^{-1} (P < 0.01), insulin-mediated increases in LBF from 66 ± 8 to 37 ± 7% (P < 0.05), and insulin-induced increases in NOx flux from 25 ± 9 to 5 ± 9% (P < 0.05). Importantly, throughout all groups, FFA-induced changes in whole-body glucose uptake correlated significantly with FFA-induced changes in insulin-mediated increases in LBF (r = 0.706, P < 0.001), which indicates coupling of metabolic and vascular effects. In a different protocol, short FFA elevation blunted the LBF response to N^G-monomethyl-L-arginine (L-NMMA), which is an inhibitor of NO synthase. LBF in response to L-NMMA decreased by 17.3 ± 2.4 and 9.0 ± 1.4% in the groups without and with FFA elevation, respectively (P < 0.05), which indicates that FFA elevation interferes with shear stress–induced NO production. Thus, impairment of shear stress–induced vasodilation and IMV by FFA elevation occurs with different time courses, and impairment of IMV occurs only if glucose metabolism is concomitantly reduced. These findings suggest that NO production in response to the different stimuli may be mediated via different signaling pathways. FFA-induced reduction in NO production may contribute to the higher incidence of hypertension and macrovascular disease in insulin-resistant patients. *Diabetes* 49:1231–1238, 2000

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ANOVA, analysis of variance; AVΔ, arteriovenous glucose differences; ΔGDR, change in whole-body glucose disposal rate; Δ%LBF, percentage of changes in leg blood flow above baseline; EDV, endothelium-dependent vasodilator; eNOS, endothelium nitric oxide synthase; FFA, free fatty acid; GDR, glucose disposal rate; HR, heart rate; IMV, insulin-mediated vasodilation; IR, insulin resistance; LBF, leg blood flow; L-NMMA, N^G-monomethyl-L-arginine; LVR, leg vascular resistance; MAP, mean arterial blood pressure; Mch, methacholine; NO, nitric oxide; NOS, nitric oxide synthase; NOx, nitrite plus nitrate; PI3K, phosphoinositol 3-kinase.

Insulin's classic action to promote glucose uptake into skeletal muscle is accompanied by increases in skeletal muscle blood flow. Indeed, numerous studies have repeatedly demonstrated that rates of whole-body glucose uptake in response to euglycemic-hyperinsulinemic clamps correlated positively and strongly with increases in skeletal muscle blood flow in the leg (1,2). In other words, subjects with low rates of insulin-stimulated glucose uptake (insulin resistance) exhibit little if any insulin-mediated vasodilation (IMV), whereas subjects with high rates of glucose uptake exhibit up to 100% increases in blood flow. Because insulin's action on glucose uptake is proportional to its vasodilatory effect in skeletal muscle, one can reasonably postulate a link between these 2 biological effects.

States of insulin resistance (IR) such as obesity and type 2 diabetes are associated with both impaired IMV and blunted vasodilation in response to the classic endothelium-dependent vasodilators (EDVs) acetylcholine (3,4) or methacholine (Mch) (5). Given that both IMV and EDV depend largely on the release of endothelium-derived nitric oxide (NO) (6), these data suggest that endothelial NO production is decreased in IR. Importantly, insulin-resistant subjects exhibit increased vascular reactivity and a higher incidence of macrovascular disease and hypertension, which may be because of the impairment in the endothelial NO system. However, the mechanism of impaired IMV or Mch response in the context of IR is unknown.

Day-long circulating free fatty acid (FFA) levels are elevated in states of IR. In turn, elevation of FFA levels for ~4–6 h induces IR (7–10) in normal insulin-sensitive volunteers. Different methods have shown that short-term (~2-h) elevation of FFA levels in healthy insulin-sensitive subjects induces endothelial dysfunction. The vasodilatory responses to both the intra-arterial infusion of Mch (11) or postischemic shear stress (12) were blunted by short-term FFA elevation. Although these results may not be directly comparable, together these findings suggest that FFA elevation impairs endothelial NO production and NO action.

Whether FFA elevation blunts in vivo insulin-mediated NO production and IMV has not been established. Vollenweider et al. (13), who studied the short-term (<3 h) effect of FFA on insulin-mediated blood flow increases in the calf, did not observe any impairment in the blood flow response to insulin. Nonetheless, decreases in insulin-mediated NO pro-

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duction and skeletal muscle vasodilation in response to FFA concomitant with impairment of insulin-mediated glucose uptake (IR) would support the idea that FFA may cause vascular dysfunction. Furthermore, similar time courses in FFA-induced impairment of IMV and insulin action (2,14) would support (but not prove) the theory that insulin's metabolic and vascular actions are coupled. A different time course in IMV impairment and induction of IR by FFA would argue against the coupling of metabolic and vascular insulin effects.

The current study was designed to examine the time course of the modulating effect of FFA elevation on IMV, insulin sensitivity, blood pressure, and NO-dependent blood flow.

RESEARCH DESIGN AND METHODS

Subjects. All study subjects were healthy, were taking no medications, had normal 75-g oral glucose tolerance tests, and had normal cuff blood pressure determinations. Studies were approved by the Indiana University Human Subjects Institutional Review Board, and all volunteers gave their informed consent.

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

Drugs. All infusates were prepared under sterile conditions on the morning of the study. Regular insulin (Humulin; Lilly, Indianapolis, IN) was diluted in normal saline to the desired concentration with added albumin. N^G-monomethyl-L-arginine (L-NMMA) (CLINALFA, Läufelfingen, Switzerland) was dissolved in normal saline to a concentration of 0.04 mol/l. Intralipid (20% fat emulsion; Pharmacia, Clayton, NC) was infused at a rate of 45 ml/h with heparin (10,000 U/ml; Elkins-Sinn, Cherry Hill, NJ) at a rate of 0.2 $U \cdot kg^{-1} \cdot min^{-1}$ to achieve elevation of systemic FFA levels. Insulin, Intralipid, and heparin were administered through a catheter into the antecubital vein. L-NMMA was administered directly into the femoral artery using a Harvard programmable pump Model 44 (Harvard Apparatus, South Natuck, MA).

Protocol. Separate groups of subjects were studied to examine the effect of FFA elevation on IMV (insulin study) or NO-dependent basal blood flow (L-NMMA study). Aspects of the protocol that are common to all studies are described below.

At ~7:00 A.M. after an overnight 14-h fast, a catheter was inserted into the antecubital vein. Subsequently, the right femoral artery and vein were cannulated. A 6 French sheath (Cordis, 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. 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 port (most caudad) and invasive blood pressure monitoring through the distal port (most cephalad). Heart rate (HR) and mean arterial blood pressure (MAP) were monitored continuously via precordial leads and a pressure transducer connected to a vital signs monitor (Spacelabs, Redmond, WA).

Hemodynamic measurements. All hemodynamic measurements were obtained with the subjects in the supine position in a quiet temperature-controlled room after the subjects had emptied their bladders. Baseline measurements of LBF, MAP, and HR were obtained after allowing at least 30 min of rest after the insertion of the catheters. LBF rates were determined by injecting 1 ml 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, Irvine, CA) that integrates the area under the thermodilution curve and displays the flow rate in liters per minute. During graded intrafemoral artery infusion of L-NMMA, LBF measurements began 2 min after the onset of the infusion. LBF measurements were performed about every 30 s for a total of ~30 determinations. Invasively determined MAP and HR were recorded with every other LBF determination.

IMV (insulin study). To study the effect of FFA elevation on IMV and insulin sensitivity, LBF and insulin sensitivity were measured in response to 4 h of euglycemic hyperinsulinemia on 2 different occasions ~4 weeks apart. The order of the studies was randomized. On 1 occasion, LBF was measured before and in response to ~4 h of euglycemic hyperinsulinemia alone. On the other occasion, LBF was determined before and after 4 h of euglycemic hyperinsulinemia with superimposed FFA elevation. To better determine the time course of FFA elevation on both IMV and insulin sensitivity, FFA levels were elevated for different time periods. FFA levels were elevated for 2, 4, or 8 h (Fig. 1). The 8-h FFA elevation included a 4-h period preceding the onset of the euglycemic-hyperinsulinemic clamp (Fig. 1). Because the results of the 2- and 4-h FFA elevation on IMV and insulin sensitivity were not different, the results were pooled and are presented together. Thus, the subjects undergoing the 2- or 4-h FFA elevations comprise the short-term FFA elevation group, and the subjects undergoing the 8-h FFA elevation comprise the long-term FFA elevation group.

To assess the effect of FFA elevation on insulin-mediated NO production in the leg, the stable oxidation products of NO (nitrite plus nitrate [NOx]) were measured in venous plasma, and venous NOx flux rates were calculated in response to euglycemic hyperinsulinemia alone and with superimposed FFA elevations according to the following: NOx flux = [NOx] × LBF.

NO-dependent vasodilation (L-NMMA study). To study the effect of FFA elevation on basal NO-dependent vascular tone, we studied the LBF response to an intrafemoral artery infusion of L-NMMA, which is an inhibitor of endothelial NO synthase (NOS), at a dose of 0.08 $mol \cdot l^{-1} \cdot min^{-1}$ for 15 min in 2 groups of subjects. The volume of L-NMMA perfusate was 2.0 ml/min. The LBF response to L-NMMA was studied during saline infusion in 1 group and after 2 h of FFA elevation in the other group.

Analytical methods. Serum total cholesterol and triglyceride levels were measured on an Ektachem 702 (Eastman-Kodak, Rochester, NY) analyzer with an enzymatic method. HDL cholesterol was measured with the Magnetic HDL kit (Reference Diagnostics, Arlington, MA), and LDL cholesterol was calculated according to the Friedewald formula. FFA was measured according to the colorimetric method described by Novak (15). Nitrite and nitrate, the stable metabolites of NO, were measured by a chemiluminescence method using a Sievers NOA 280 analyzer (Sievers, Boulder, CO). The coefficient of variation for nitrite and nitrate measurements is <5% in our laboratory. Whole-body glucose uptake rates in response to euglycemic hyperinsulinemia were obtained by infusion of D-[3-^{3}H]glucose in a primed and continuous manner (16). After 120 min of tracer equilibration and before initiation of the euglycemic-hyperinsulinemic clamp, 4 blood samples were obtained, each 10 min apart. During the clamp study, blood was obtained every 20 min to assess the contribution of hepatic glucose output to whole-body glucose disposal rates (GDRs). Blood for determination of plasma glucose specific activity was collected in sodium fluoride–treated tubes and was immediately placed on ice. The specimens were spun, and the supernatant was removed and stored at –20°C. At the time of the assay, the serum was thawed and diluted, and the proteins were precipitated with 0.6 mol/l perchloric acid. The supernatant was divided equally, evaporated to dryness, resuspended in 0.5 ml distilled water to which 10 ml liquid scintillation fluid was added (National Diagnostics, Atlanta, GA), and counted for 5 min. Samples for both study parts (with and without FFA elevation) were run together and were measured in the same assay. Body fat content was determined by dual energy X-ray absorptiometry (DXA with system software 1.2; Lunar DPX-L, Madison, WI).

Statistical analysis. Data are means ± SE. MAP is expressed in millimeters of mercury, LBF is expressed in liters per minute, and leg vascular resistance (LVR) was calculated as the MAP divided by the LBF and is expressed in arbitrary units. Changes in LBF are expressed as a percentage of change (Δ%LBF) to adjust for differences at baseline. NOx flux was calculated according to the following: NOx flux = [NOx] × LBF (in $micromoles \cdot liter^{-1} \cdot minute^{-1}$).

Comparison between groups was performed by factorial analysis of variance (ANOVA). When significant differences between groups were found by ANOVA, this was followed by post hoc testing with Fisher's protected least

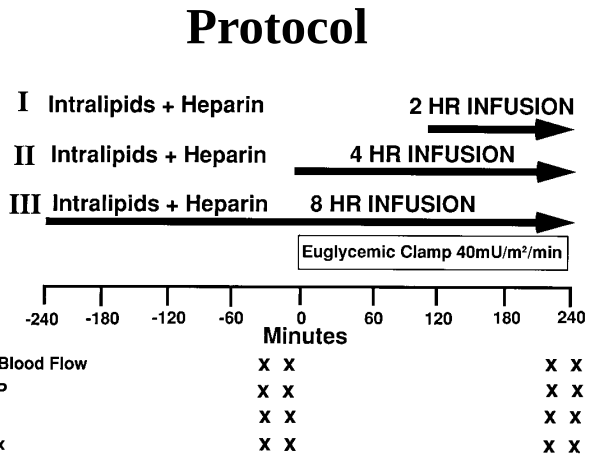

FIG. 1. Schematic description of the different periods of FFA elevation.

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TABLE 1
Demographic characteristics of the study groups (FFA elevation)

	Short term	Long term
n	12	7
Age (years)	39 ± 1	30 ± 3*
BMI	24.3 ± 2.0	24.7 ± 1.1
Body fat (%)	16.6 ± 0.9	17.6 ± 1.4
MAP (mmHg)	89.8 ± 2.2	93.9 ± 2.1
Cholesterol (mmol/dl)	4.3 ± 0.2	4.0 ± 0.2
LDL cholesterol (mmol/dl)	2.5 ± 0.2	2.2 ± 0.2
HDL cholesterol (mmol/dl)	1.3 ± 0.1	1.3 ± 0.1
Triglycerides (mg/dl)	98 ± 15	84 ± 8

Data are *n* or means ± SE. *$P < 0.05$.

significant difference. Univariate regression analysis between changes in IMV and changes in whole-body GDR (ΔGDR) caused by FFA elevation was performed to assess whether a relationship exists between metabolic and vascular changes. Statistical significance was set at $P < 0.05$. Statistical calculations were performed on a Power Macintosh computer (Cupertino, CA) with StatView IV software (Abacus Concepts, Berkeley, CA).

RESULTS

Insulin study

Insulin, glucose, and glucose metabolism. Demographic and metabolic characteristics of the study groups are shown in Table 1. Glucose and insulin levels were comparable between groups and remained stable throughout the euglycemic-hyperinsulinemic clamp studies (Tables 2 and 3). A total of 4 h of FFA elevation before the euglycemic-hyperinsulinemic clamp resulted in an increase in basal insulin levels, which indicates enhanced insulin secretion either in direct response to the FFA elevation and/or induction of IR. During the clamp studies, insulin levels increased significantly in both groups and did not differ within groups during euglycemic hyperinsulinemia with or without superimposed FFA elevation. However, insulin levels were higher ($P < 0.05$) during superimposed FFA elevation in the long-term versus the short-term FFA elevation group. As expected, hyperinsulinemia alone caused a significant decrease in FFA levels, whereas administration of Intralipid and heparin resulted in significantly increased FFA levels (Tables 2 and 3). FFA levels were comparable between short- and long-term FFA elevation groups during either steady-state euglycemic hyperinsulinemia alone or during steady-state euglycemic hyperinsulinemia with superimposed FFA elevation (Tables 2 and 3).

Arteriovenous glucose differences (AVΔ) during euglycemic hyperinsulinemia alone were similar between short- and long-term FFA groups. FFA elevation superimposed on euglycemic hyperinsulinemia caused a small but significant fall in AVΔ in both groups. The fall in AVΔ induced by FFA elevation was comparable in both groups. GDRs were not significantly attenuated by short-term FFA elevation (Fig. 2*A*). In contrast, prolonged FFA elevation significantly reduced the GDR by ~35% ($P < 0.05$) (Fig. 2*B*). Thus, long-term but not short-term FFA elevation impaired insulin-mediated glucose uptake.

TABLE 2
Metabolic and hemodynamic variables determined on 2 different occasions (Tables 2 and 3) before and during euglycemic-hyperinsulinemic clamp studies with and without superimposed FFA elevation in the 2 study groups (short-term FFA elevation)

	Saline	+FFA
n	12	12
Basal glucose (mmol/dl)	5.0 ± 0.1	5.1 ± 0.1
Clamp glucose (mmol/dl)	5.0 ± 0.2	5.1 ± 0.1
Basal insulin (pmol/l)	45.6 ± 3.6	42.6 ± 6.0
Clamp insulin (pmol/l)	389.4 ± 16.8	379.8 ± 25.2
AVΔ(mmol/dl)	1.56 ± 0.09	1.38 ± 0.17
GDR (μmol · kg^{-1} · min^{-1})	48.3 ± 2.2	44.6 ± 2.2
Basal FFA (μmol/l)	300 ± 21	324 ± 18
Clamp FFA (μmol/l)	18.0 ± 4.1	694 ± 89*
Basal LBF (l/min)	0.219 ± 0.017	0.225 ± 0.014
Clamp LBF (l/min)	0.369 ± 0.032	0.364 ± 0.040
Basal MAP (mmHg)	89.8 ± 2.2	90.1 ± 1.9
Clamp MAP (mmHg)	88.9 ± 2.0	86.5 ± 1.9
Δ%LBF	70.3 ± 10.4	67.0 ± 16.7
Basal LVR	445 ± 43	423 ± 35
Clamp LVR	250 ± 25	276 ± 34
Basal venous NOx (μmol/l)	26.3 ± 8.3	15.4 ± 2.2
Clamp venous NOx (μmol/l)	20.6 ± 8.3	10.9 ± 1.5
Basal venous NOx flux (μmol · liter^{-1} · min^{-1})	5.6 ± 1.8	3.4 ± 0.5
Clamp venous NOx flux (μmol · liter^{-1} · min^{-1})	7.4 ± 2.4	4.3 ± 0.8
Percentage of change in NOx flux	33.3 ± 9.4	23.6 ± 15.2

Data are *n* or means ± SE. *$P < 0.01$ vs. euglycemic hyperinsulinemia alone.

TABLE 3
Metabolic and hemodynamic variables determined on 2 different occasions (Tables 2 and 3) before and during euglycemic-hyperinsulinemic clamp with and without superimposed FFA elevation in the 2 study groups (long-term FFA elevation)

	Saline	+FFA
n	7	7
Basal glucose (mmol/dl)	5.1 ± 0.1	5.2 ± 0.1
Clamp glucose (mmol/dl)	5.1 ± 0.1	5.1 ± 0.1
Basal insulin (pmol/l)	41.4 ± 4.2	64.8 ± 6.6*
Clamp insulin (pmol/l)	443.4 ± 26.4	471.6 ± 27.0
AVΔ (mmol/dl)	1.43 ± 0.12	0.98 ± 0.10*
GDR (μmol · kg^{-1} · min^{-1})	47.7 ± 2.8	32.2 ± 6.0†
Basal FFA (μmol/l)	408 ± 81	1,368 ± 98*
Clamp FFA (μmol/l) study	23.8 ± 0.8	758 ± 100†
Basal LBF (l/min)	0.213 ± 0.016	0.251 ± 0.037*
Clamp LBF (l/min)	0.357 ± 0.030	0.338 ± 0.48
Basal MAP (mmHg)	93.9 ± 2.1	102.0 ± 9.1
Clamp MAP (mmHg)	91.8 ± 3.8	101.5 ± 9.3
Δ%LBF	65.8 ± 8.1	36.6 ± 6.9*
Basal LVR	461 ± 41	465 ± 81
Clamp LVR	272 ± 28	338 ± 53
Basal venous NOx (μmol/l)	11.6 ± 0.8	12.0 ± 0.9
Clamp venous NOx (μmol/l)	8.4 ± 0.5	9.1 ± 0.9
Basal venous NOx flux (μmol · liter^{-1} · min^{-1})	2.4 ± 0.3	3.0 ± 0.4
Clamp venous NOx flux (μmol · liter^{-1} · min^{-1})	2.9 ± 0.3	3.2 ± 0.6
Percentage of change in NOx flux	24.7 ± 8.8	5.2 ± 9.1*

Data are *n* or means ± SE. *$P < 0.05$ vs. euglycemic hyperinsulinemia alone; †$P < 0.01$ vs. euglycemic hyperinsulinemia alone.

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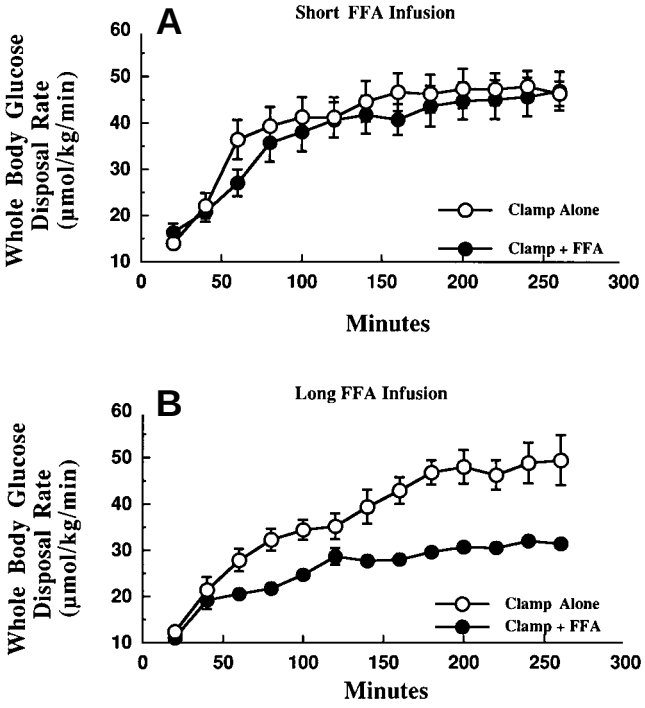

FIG. 2. Effect of short-term (*A*) and long-term elevation (*B*) of systemic FFA on whole-body GDR in response to 4 h of euglycemic hyperinsulinemia.

Hemodynamics and NOx flux. Basal MAP was comparable between groups (Tables 2 and 3) and did not change significantly in response to the clamp studies. MAP, in response to the 4 h of FFA elevation before the clamp studies, was slightly higher with saline, but the increase did not achieve statistical significance ($P = 0.09$). During euglycemic hyperinsulinemia, MAP was significantly higher with long-term FFA elevation ($P < 0.05$) than with short-term FFA elevation.

Basal LBF was comparable between groups receiving short- and long-term FFA infusions and increased by ~70% in response to euglycemic hyperinsulinemia alone in both groups ($P < 0.01$ vs. baseline). Short-term FFA elevation superimposed on euglycemic hyperinsulinemia alone did not impair IMV (Fig. 3*A*). In contrast, long-term FFA elevation superimposed on euglycemic hyperinsulinemia caused significant blunting of IMV (Fig. 3*B*).

Basal LVR was similar between groups (Table 2 and 3). LVR decreased significantly in response to euglycemic hyperinsulinemia ($P < 0.01$) alone and with superimposed FFA elevation in either group. In the short-term FFA elevation group, LVR decreased by 40 ± 4% in response to euglycemic hyperinsulinemia alone and decreased by 34 ± 6% with superimposed FFA elevation during euglycemic hyperinsulinemia (NS). In the long-term FFA elevation group, LVR decreased by 41 ± 3% in response to euglycemic hyperinsulinemia alone and decreased by 26 ± 3% with superimposed FFA elevation during euglycemic hyperinsulinemia ($P < 0.01$). Thus, long-term but not short-term FFA elevation attenuated the decrease in LVR in response to euglycemic hyperinsulinemia.

Basal venous NOx concentrations were similar between groups and decreased by ~25% after 4 h of clamp study in either group. In response to euglycemic hyperinsulinemia alone, venous NOx flux increased in both groups by ~30% ($P < 0.05$ vs. baseline). NOx flux in response to euglycemic hyperinsulinemia remained unchanged with the superimposed short-term elevation of FFA (Fig. 3). In contrast with short-term FFA elevation, long-term FFA elevation significantly ($P < 0.05$) blunted the insulin-mediated increase in NOx flux (Fig. 4). These data indicate that prolonged FFA elevation is necessary to both impair insulin-mediated glucose uptake and NO-dependent vasodilation.

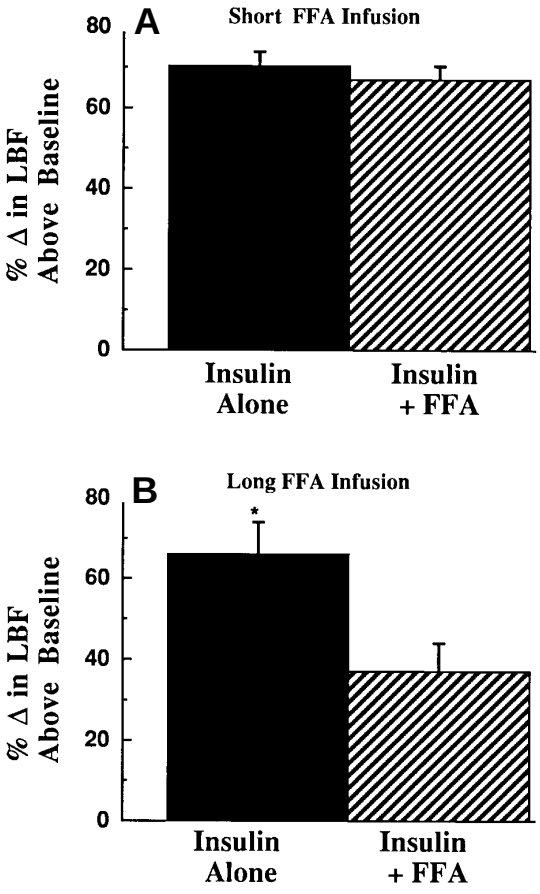

FIG. 3. Effect of short-term (*A*) and long-term elevation (*B*) of systemic FFA on Δ%LBF in response to 4 h of euglycemic hyperinsulinemia. *$P < 0.05$.

Univariate analysis between FFA-induced ΔGDR and FFA-induced changes in IMV (ΔLBF) demonstrated a significant

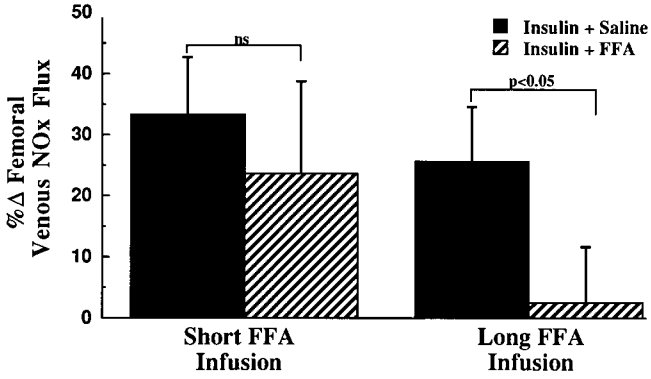

FIG. 4. Effect of short- and long-term elevation of systemic FFA on increases above baseline in venous NOx flux (Δ% femoral venous NOx flux) in response to 4 h of euglycemic hyperinsulinemia.

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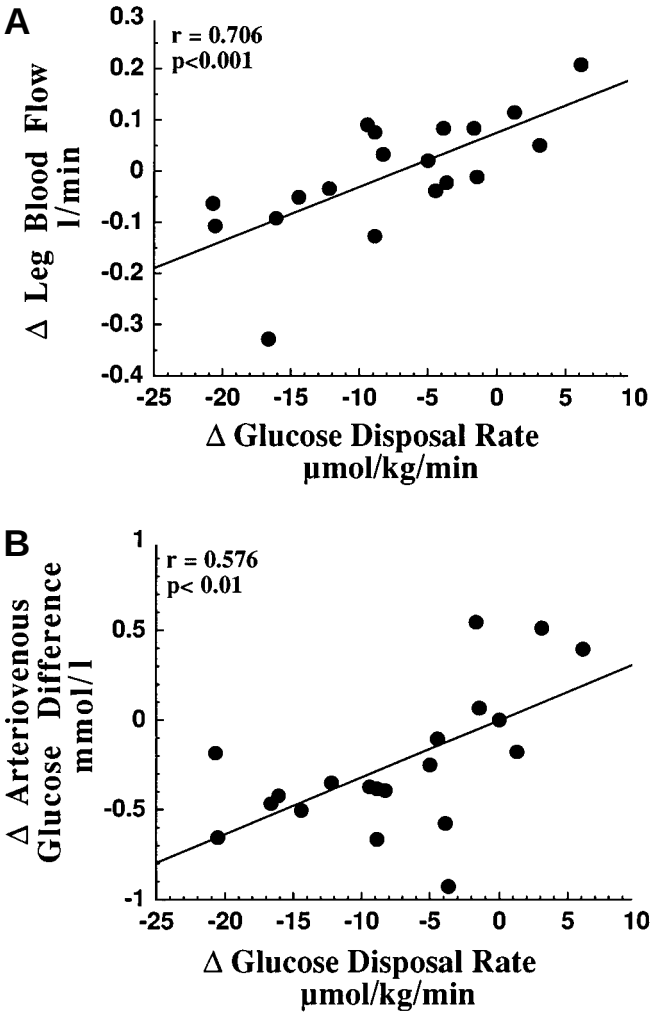

FIG. 5. ***A*****: Relationship between ΔLBF and ΔGDR caused by elevation of circulating FFA levels.** ***B*****: Relationship between AVΔ and ΔGDR caused by elevation of circulating FFA levels.**

and strongly positive relationship (r = 0.706, P < 0.001) between these variables (Fig. 5*A*). This finding suggests coupling between insulin's metabolic and vascular effects. Univariate analysis between FFA-induced ΔGDR and AVΔ (Fig. 5*B*) also correlated significantly (r = 0.576, P < 0.01).

Multivariate analysis with ΔGDR as the dependent variable and ΔLBF and AVΔ induced by FFA elevation as independent variables revealed that only ΔLBF remained significantly correlated with the ΔGDR (P < 0.005), whereas AVΔ was no longer a significant determinant (P = 0.062). These associations suggest that the effect of elevated FFA to impair insulin-mediated increases in blood flow may be a major determinant of FFA-induced IR (reduction of GDR).

L-NMMA study. Demographic and metabolic characteristics are shown in Table 4. No differences were evident in age, body fat content, blood pressure, or lipid levels.

Basal MAP was comparable between both groups (Table 4). After 2 h of FFA elevation, MAP was 88.4 ± 2.1 mmHg, which was not significantly different from baseline (P = 0.26). In response to L-NMMA, MAP rose by 4.6 ± 0.5 and 5.3 ± 1.5 mmHg in the control and FFA groups, respectively (P < 0.01 vs. basal for both groups). The magnitude of MAP increases in response to L-NMMA was similar in both groups.

TABLE 4
Demographic and metabolic characteristics of the study groups undergoing intrafemoral artery infusion of L-NMMA under basal conditions without (saline) or with (FFA) superimposed elevation of FFA

	Saline + L-NMMA	FFA + L-NMMA
n	19	9
Age (years)	31.6 ± 1.4	33.2 ± 2.3
BMI	25.1 ± 0.8	23.2 ± 0.4
Body fat (%)	21.9 ± 1.4	18.0 ± 1.5
Glucose (mmol/dl)	5.1 ± 0.1	5.2 ± 0.1
MAP (mmHg)	87.8 ± 1.8	84.7 ± 3.0
Cholesterol (mmol/dl)	4.37 ± 0.26	4.68 ± 0.34
Triglycerides (mg/dl)	121.3 ± 15	90.0 ± 6
FFA (µmol/l)	400 ± 150	1,473 ± 247*

Data are *n* or means ± SE. *P < 0.05 vs. saline + L-NMMA.

LBF before the intrafemoral artery infusion of L-NMMA was 0.226 ± 0.021 l/min in the control group. LBF before administration of Intralipid and heparin was 0.208 ± 0.012 l/min, which increased to 0.261 ± 0.013 l/min (P < 0.05) in response to the 2 h of FFA elevation. LBF before L-NMMA infusion was not different between the control and the FFA groups (NS). In response to L-NMMA, LBF decreased to 0.183 ± 0.015 and 0.237 ± 0.013 l/min in the control and FFA groups, respectively (P < 0.01 vs. baseline for both groups, P < 0.05 for the control vs. FFA group), which represents a 17.3 ± 2.4 and 9.0 ± 1.4% decrease in LBF (Fig. 6), respectively (P < 0.05 for the control vs. FFA group). Thus, FFA elevation blunted the LBF response to L-NMMA, which indicates that short-term FFA elevation reduces NO-dependent vascular tone.

The increase in MAP and the decrease in LBF in response to L-NMMA resulted in an increase in LVR in both groups (P < 0.01 vs. basal). The increases in LVR mirrored the reduction in LBF. LVR increased in response to L-NMMA by 29.4 ± 3.9 and 16.7 ± 2.7 U in the control and FFA groups, respectively (P < 0.05, control vs. FFA). Taken together, these data indicate that short-term elevation of FFA reduces basal NO release.

DISCUSSION

Obesity and type 2 diabetes, which are both insulin-resistant states, are characterized by impaired vascular function,

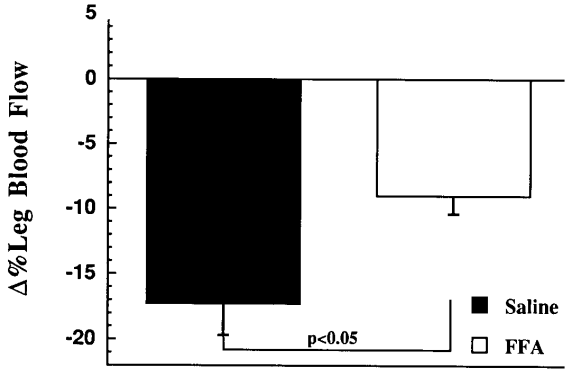

FIG. 6. The Δ%LBF in response to an intrafemoral artery infusion of L-NMMA (16 mg/min) under conditions of basal circulating FFA levels (saline) and with 2- to 3-fold elevation of FFA levels.

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including decreased vasodilation in response to insulin and EDVs such as acetylcholine (3,4,17), Mch (5), and bradykinin (18). The relationship between metabolic and vascular abnormalities in insulin-resistant states such as obesity and type 2 diabetes is not well understood. Elevated FFA levels are a characteristic feature of insulin-resistant states. Regarding vascular effects, we have previously shown that an acute 2-h elevation of circulating FFA concentration attenuates the response to the EDV Mch but not sodium nitroprusside (an endothelium-independent vasodilator), which suggests FFA-induced impairment of the release of endothelium-derived NO. Because IMV is mediated in large part by NO (19,20) and possibly to a lesser extent via a direct effect on vascular smooth muscle (21), one can logically postulate a causal link between elevated FFA levels and vascular dysfunction observed in the insulin-resistant states of obesity and type 2 diabetes. Importantly, this vascular dysfunction may explain at least in part the increased incidence of macrovascular disease and hypertension observed in these patients.

The current study was undertaken to better define the effect of elevated FFA on IMV, insulin sensitivity, and basal NO-dependent LBF. The results of our study demonstrate that prolonged ~8-h (but not ≤4-h) elevation of systemic FFA levels induced impairment of IMV in normotensive lean insulin-sensitive subjects. Impairment of IMV by FFA was observed concomitantly and commensurately with induction of resistance to insulin-mediated glucose uptake. The results further demonstrate that short-term elevation of FFA decreased basal NO-dependent LBF by ~50%. These results suggest that short-term elevation of FFA is sufficient to cause significant impairment of shear stress (basal NO-dependent blood flow) and Mch-induced vasodilation (11), whereas longer exposure to FFA elevation with induction of IR is required to cause impaired IMV.

In our study, circulating FFA levels were elevated 2- to 3-fold from baseline in lean insulin-sensitive subjects and achieved concentrations observed in insulin-resistant obese and type 2 diabetic subjects. Because we had previously demonstrated that short-term elevation of FFA was sufficient to cause impaired EDV in response to Mch chloride (11), we also expected decreases in IMV to occur after 2 h. Surprisingly, elevation of FFA levels for periods >4 h were required to blunt IMV. Because FFA levels during hyperinsulinemia were comparable between the groups, these data indicate that the duration of FFA elevation is an important factor in the induction of endothelial dysfunction in response to insulin.

The impairment in IMV occurred under conditions of slightly increased basal LBF rates. Although we cannot completely exclude the possibility that the observed effect of FFA elevation to blunt IMV may be explained at least in part by the elevated basal LBF, this is not very likely. We know from previous studies (20) that the LBF response to insulin does not represent the total vasodilator capacity. During euglycemic hyperinsulinemia, LBF increases in response to Mch even in insulin-resistant obese and type 2 diabetic subjects who do not exhibit normal IMV. In addition, we found a strong relationship (r = 0.706) between FFA-induced decreases in IMV and decreases in insulin-mediated glucose uptake, which indicates that we observed a true effect of FFA elevation to impair IMV.

The time required for FFA elevation to impair basal NO-dependent LBF was ~2 h, which was similar to the time required to blunt the response to the EDV Mch. In contrast, >2-fold longer exposure to elevated FFA was needed to impair IMV, which depends in large part on the release of NO. One must consider that NO release in response to Mch accounts for only ~50% of the vasodilation (6), which indicates that other mediators such as the endothelium-dependent hyperpolarizing factor or cyclo-oxygenase products may contribute to the increases in blood flow. Therefore, impairment of non–NO-dependent vasodilator mechanisms by FFA may be 1 possible explanation for the time course differences in response to FFA. On the other hand, if FFA caused decreased NO release during Mch stimulation, then concomitant intact IMV would suggest that short-term FFA elevation does not interfere with NO synthesis but may interfere with shear stress and muscarinic receptor signaling related to NOS activation. This is consistent with our data showing that short-term FFA elevation causes decreased NO-dependent basal (shear stress–induced) flow. Regardless of the molecular mechanism by which FFA elevation inhibits Mch-induced vasodilation, it does not involve a rate-limiting step of NO generation because insulin at 2 and 4 h of FFA elevation is able to induce vasodilation via augmented NO production. In contrast with the effect of FFA on Mch-induced vasodilation, the appearance of impaired IMV (which is entirely NO dependent) required prolonged FFA exposure and occurred concomitantly with the induction of IR. This suggests that FFAs induce IR simultaneously in skeletal muscle, fat, and endothelium. In the skeletal muscle, the defect is reflected as reductions in glucose transport, whereas in the vascular endothelium, it is reflected as reduced NO release.

The mechanisms by which FFA elevation impairs basal NO-dependent blood flow or the response to Mch chloride and postischemic hyperemia is not well characterized. FFA elevation may cause vascular endothelial dysfunction either indirectly via increased release of vasoconstrictor substances such as endothelin-1 as proposed by Piatti et al. (22) and/or through a direct effect of its own. Elevated FFA may induce formation of oxygen radicals, which could quench NO and thus result in decreased NO action at the level of the vascular smooth muscle. This explanation, however, is less likely because we have shown previously (11) that short-term FFA elevation did not impair the blood flow response to the endothelium-independent vasodilator sodium nitroprusside and more importantly does not impair IMV, which is in large part NO dependent. Because sodium nitroprusside acts as a donor of NO, this indicates similar NO action under conditions of normal and elevated FFA levels.

Davda et al. (23) and Gupta et al. (24) demonstrated in endothelial cell cultures that FFA elevation inhibits NO production by decreasing endothelial NOS activity because endothelium NOS (eNOS) protein content was not altered by FFA elevation. Although some have proposed that the inhibition of NOS is mediated via a protein kinase C–dependent mechanism (25–27), other studies have yielded conflicting results (23). FFA has been shown to interfere with insulin signaling in skeletal muscle by reducing the activity of phosphoinositol 3-kinase (PI3K) (10). Recently, IR has been shown to be associated with reduced insulin-stimulated PI3K activation of vascular cells in vivo and in vitro as well as a reduction in the serine/threonine kinase Akt, which is downstream of PI3K (28). Adding to this line of reasoning, recent reports have identified Akt as the most distal signaling molecule for

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activation of the eNOS (29,30). Taken together, this set of findings suggests that insulin-signaling pathways may be shared in skeletal muscle, fat, and endothelium as proposed by Zeng and Quon (31) and that insulin signal transduction abnormalities associated with IR in classical target tissues are also reflected in impaired NOS activation at the level of the endothelium. Specifically, FFA may impair PI3K both in skeletal muscle and in endothelium, thus causing impaired insulin-mediated glucose uptake and vasodilation, respectively. This proposal is a parsimonious explanation for the ubiquitousness of endothelial dysfunction observed in insulin-resistant states and as such provides a mechanism for the increased incidence of hypertension and macrovascular disease observed in these patients.

Regardless of the validity of the above mechanism, the findings of our study indicate that insulin's vascular and metabolic actions are linked. This notion is supported by the significant relationship between changes in IMV and ΔGDR. The idea that coupling exists between insulin's vascular and metabolic actions is also underscored by the observation that IMV is impaired under conditions of IR as diverse as hypertension (32,33), polycystic ovary syndrome (34), obesity (18), or type 2 diabetes (5). Our findings contrast with those of Vollenweider et al. (13) who measured the effect of FFA elevation on IMV in the calf. The reason for the different findings is not clear. One possible explanation is that the increase in calf blood flow observed in their study was actually secondary to FFA (rather than insulin), which is consistent with our current study and our previous report (11).

In summary, we have shown that FFA elevation impairs basal and insulin-mediated NO production. Importantly, the FFA-induced decrease in IMV was only observed when rates of insulin-mediated glucose uptake were concomitantly decreased, which indicates that metabolic coupling exists between insulin's effect to dilate skeletal muscle vasculature and its effect to promote glucose uptake. This impairment of NO production in response to insulin may contribute to the IR observed in obesity and type 2 diabetes. More speculatively, the fall in basal NO production in response to FFA elevation may contribute to the incidence of hypertension in insulin-resistant states such as obesity and type 2 diabetes. Elucidation of the mechanisms responsible for the decrease in NO production in response to elevated FFA levels may help to design interventions to enhance NO production in obesity or type 2 diabetes. Given the antiatherogenic effects of NO (35,36), enhanced endothelial NO production may lower the high rates of cardiovascular morbidity and mortality in these high-risk populations.

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REFERENCES

1. Laakso M, Edelman SV, Brechtel G, Baron AD: Decreased effect of insulin to stimulate skeletal muscle blood flow in obese men. *J Clin Invest* 85:1844–1852, 1990
2. Baron AD, Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G: Insulin-mediated skeletal muscle vasodilation contributes to both insulin sensitivity and responsiveness in lean humans. *J Clin Invest* 96:786–792, 1995
3. Hogikyan RV, Galecki AT, Pitt B, Halter JB, Greene DA, Supiano MA: Specific impairment of endothelium-dependent vasodilation in subjects with type 2 diabetes independent of obesity. *J Clin Endocrinol Metab* 83:1946–1952, 1998
4. McVeigh GE, Brennan GM, Johnston GD, McDermott BJ, McGrath LT, Andrews JW, Hayes JR: Impaired endothelium dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 35:771–776, 1992
5. Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD: Obesity/insulin resistance is associated with endothelial dysfunction: implications for the syndrome of insulin resistance. *J Clin Invest* 97:2601–2610, 1996
6. Vallance P, Collier J, Moncada S: Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. *Lancet* 2:997–1000, 1989
7. Boden G, Chen X, Ruiz J, White JV, Rossetti L: Mechanisms of fatty acid-induced inhibition of glucose uptake. *J Clin Invest* 93:2438–2446, 1994
8. Boden G: Fatty acids and insulin resistance. *Diabetes Care* 19:394–395, 1996
9. Bonadonna RC, Zych K, Boni C, Ferrannini E, DeFronzo RA: Time dependence of the interaction between lipid and glucose in humans. *Am J Physiol* 257:E49–E56, 1989
10. Dresner A, Laurent D, Marcucci M, Griffin ME, Dufour S, Cline GW, Slezak LA, Andersen DK, Hundal RS, Rothman DL, Petersen KF, Shulman GI: Effects of free fatty acids on glucose transport and IRS-1-associated phosphatidylinositol 3-kinase activity. *J Clin Invest* 103:253–259, 1999
11. Steinberg HO, Tarshoby M, Monestel R, Hook G, Cronin J, Johnson A, Bayazeed B, Baron AD: Elevated circulating free fatty acid levels impair endothelium-dependent vasodilation. *J Clin Invest* 100:1230–1239, 1997
12. Lundman P, Erickson M, Schenck-Gustavson K, Karpe K, Tornvall P: Transient triglyceridemia decreases vascular reactivity in young, healthy men without factors for coronary heart disease. *Circulation* 96:3266–3268, 1997
13. Vollenweider L, Tappy L, Owlya R, Jequier E, Nicod P, Scherrer U: Insulin-induced sympathetic activation and vasodilation in skeletal muscle. *Diabetes* 44:641–645, 1995
14. Baron AD, Steinberg H, Brechtel G, Johnson A: Skeletal muscle blood flow independently modulates insulin-mediated glucose uptake. *Am J Physiol* 266:E248–E253, 1994
15. Novak M: Colorimetric ultramicro method for the determination of free fatty acids. *J Lipid Res* 6:431–433, 1965
16. Finegood DT, Bergman RN, Vranic M: Estimation of endogenous glucose production during hyperinsulinemic euglycemic glucose clamps: comparison of unlabeled and labeled exogenous glucose infusates. *Diabetes* 36:914–924, 1987
17. Makimattila S, Liu ML, Vakkilainen J, Schlenzka A, Lahdenpera S, Syvanne M, Mantysaari M, Summanen P, Bergholm R, Taskinen MR, Yki-Jarvinen H: Impaired endothelium-dependent vasodilation in type 2 diabetes: relation to LDL size, oxidized LDL, and antioxidants. *Diabetes Care* 22:973–981, 1999
18. Laine H, Yki-Jarvinen H, Kirvela O, Tolvanen T, Raitakari M, Solin O, Haaparanta M, Knuuti J, Nuutila P: Insulin resistance of glucose uptake in skeletal muscle cannot be ameliorated by enhancing endothelium-dependent blood flow in obesity. *J Clin Invest* 101:1156–1162, 1998
19. Scherrer U, Randin D, Vollenweider P, Vollenweider L, Nicod P: Nitric oxide release accounts for insulin's vascular effects in humans. *J Clin Invest* 94:2511–2515, 1994
20. Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD: Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent. *J Clin Invest* 94:1172–1179, 1994
21. Zemel MB, Johnson BA, Ambrozy SA: Insulin stimulated vascular relaxation: the role of Ca^{++}-ATPase. *Am J Hypertens* 5:637–641, 1992
22. Piatti PM, Monti LD, Conti M, Baruffaldi L, Galli L, Phan CV, Guazzini B, Pontiroli AE, Pozza G: Hypertriglyceridemia and hyperinsulinemia are potent inducers of endothelin-1 release in humans. *Diabetes* 45:316–321, 1996
23. Davda RK, Stepniakowski KT, Lu G, Ullian ME, Goodfriend TL, Egan BM: Oleic acid inhibits endothelial nitric oxide synthase by a protein kinase C-independent mechanism. *Hypertension* 26:764–770, 1995
24. Gupta MP, Steinberg HO, Hart CM: H_2O_2 causes endothelial barrier dysfunction without disrupting the arginine-nitric oxide pathway. *Am J Physiol* 274:L508–L516, 1998
25. Davda RK, Chandler LJ, Guzman NJ: Protein kinase C modulates receptor-independent activation of endothelial nitric oxide synthase. *Eur J Pharmacol* 266:237–244, 1994
26. Morrison KJ, Pollock D: Impairment of relaxations to acetylcholine and nitric oxide by a phorbol ester in rat isolated aorta. *Br J Pharmacol* 101:432–436, 1990

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27. Murakami K, Chan SY, Routtenberg A: Protein kinase C activation by *cis*-fatty acid in the absence of Ca^{2+} and phospholipids. *J Biol Chem* 261:15424–15429, 1986
28. Jiang ZY, Lin YW, Clemont A, Feener EP, Hein KD, Igarashi M, Yamauchi T, White MF, King GL: Characterization of selective resistance to insulin signaling in the vasculature of obese Zucker (*fa*/*fa*) rats. *J Clin Invest* 104:447–457, 1999
29. Fulton D, Gratton JP, McCabe TJ, Fontana J, Fujio Y, Walsh K, Franke TF, Papapetropoulos A, Sessa WC: Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. *Nature* 399:597–601, 1999
30. Dimmeler S, Fleming I, Fissthaler B, Hermann C, Busse R, Zeiher AM: Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. *Nature* 399:601–605, 1999
31. Zeng G, Quon MJ: Insulin stimulated production of nitric oxide is inhibited by Wortmannin: direct measurement in vascular endothelial cells. *J Clin Invest* 98:894–898, 1996
32. Laine H, Knuuti MJ, Ruotsalainen U, Utriainen T, Oikonen V, Raitakari M, Luotolahti M, Kirvela O, Vicini P, Cobelli C, Nuutila P, Yki-Jarvinen H: Preserved relative dispersion but blunted stimulation of mean flow, absolute dispersion, and blood volume by insulin in skeletal muscle of patients with essential hypertension. *Circulation* 97:2146–2153, 1998
33. Panza JA, Quyyumi A, Brush JE, Epstein SE: Abnormal endothelium dependent vascular relaxation in patients with essential hypertension. *N Engl J Med* 323:22–27, 1990
34. Paradisi G, Steinberg HO, Shepard M, Baron AD: Polycystic syndrome is associated with endothelial dysfunction (Abstract). *Diabetes* 47 (Suppl. 1): 309A, 1998
35. Lloyd-Jones DM, Bloch KD: The vascular biology of nitric oxide and its role in atherogenesis. *Annu Rev Med* 47:365–375, 1996
36. Cannon RO III: Role of nitric oxide in cardiovascular disease: focus on the endothelium. *Clin Chem* 44:1809–1819, 1998

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