Free Fatty Acid Levels Modulate Microvascular Function

Relevance for Obesity-Associated Insulin Resistance, Hypertension, and Microangiopathy

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To test the hypothesis that free fatty acids (FFAs) modulate microvascular function and that this contributes to obesity-associated insulin resistance, hypertension, and microangiopathy, we examined the effects of both FFA elevation in lean women and FFA lowering in obese women on skin microvascular function. A total of 16 lean and 12 obese women underwent, respectively, Intralipid plus heparin (or saline) infusion and overnight acipimox (or placebo) treatment. We measured capillary recruitment with capillaroscopy and endothelium-(in)dependent vasodilation by iontophoresis of acetylcholine and sodium nitroprusside before and during hyperinsulinemia (40 mU · m^{-2} · min^{-1}). FFA elevation impaired capillary recruitment and acetylcholine-mediated vasodilation before (44.6 ± 16.8 vs. 56.9 ± 18.9%, $P < 0.05$; and 338 ± 131 vs. 557 ± 162%, $P < 0.01$, respectively) and during (54.0 ± 21.3 vs. 72.4 ± 25.4%, $P < 0.01$; and 264 ± 186 vs. 685 ± 199%, $P < 0.01$, respectively) hyperinsulinemia. FFA lowering improved capillary recruitment before (50.9 ± 14.6 vs. 37.4 ± 9.3%, $P < 0.01$) and during (66.8 ± 20.6 vs. 54.8 ± 15.4%, $P < 0.05$) hyperinsulinemia. Changes in FFA levels were inversely associated with changes in capillary recruitment and insulin sensitivity in lean ($r = -0.46$, $P = 0.08$; and $r = -0.56$, $P = 0.03$) and in obese ($r = -0.70$, $P = 0.02$; and $r = -0.62$, $P = 0.04$) women. Regression analyses showed that changes in capillary recruitment statistically explained ~29% of the association between changes in FFA levels and insulin sensitivity. In conclusion, FFA levels modulate microvascular function and may contribute to obesity-associated insulin resistance, hypertension, and microangiopathy. *Diabetes* 53:2873–2882, 2004

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FFA, free fatty acid; PI, phosphatidylinositol.

Obesity is an important contributor to the burden of cardiovascular disease because it increases risk not only of large artery disease (1,2) but also of disease entities that are in part caused by microangiopathy, notably retinopathy, nephropathy, and heart failure (3–5). These associations cannot be explained entirely by obesity-associated insulin resistance, hypertension, and dyslipidemia; therefore, other mechanisms must play a role (4,5).

In obesity, increased fat mass and resistance to insulin-mediated inhibition of lipolysis lead to daylong exposure to elevated free fatty acid (FFA) levels, which may contribute to cardiovascular disease via different pathways. First, through mechanisms that are not completely understood, FFA elevation impairs insulin sensitivity (6,7), increases blood pressure (8,9), and impairs large artery endothelial function (8,10). Second, in rats an acute increase of FFA levels impairs muscle capillary recruitment (11), which raises the possibility that high FFA levels can directly impair microvascular function. In turn, microvascular dysfunction in the basal state and during hyperinsulinemia has been proposed to impair glucose and insulin delivery to muscle and thus to impair insulin sensitivity (11–13). In addition, microvascular dysfunction increases peripheral vascular resistance and may thus increase blood pressure (14–16).

We hypothesize that FFA elevation impairs microvascular function in human obesity and that this may contribute to obesity-associated insulin resistance, hypertension, and microangiopathy. To test this hypothesis, we examined the effects of an acute increase of FFA levels in lean women and overnight lowering of FFA levels in obese women on both skin microvascular function—in the basal state and during hyperinsulinemia—and on insulin sensitivity and blood pressure.

RESEARCH DESIGN AND METHODS

A total of 16 lean (BMI <24 kg/m^2) and 12 obese (BMI >30 kg/m^2) women participated in this study (Table 1). All volunteers were healthy as judged from their medical history, did not have diabetes (according to 1997 American Diabetes Association criteria) (17), and were normotensive (<140/<90 mmHg), as determined by triplicate office blood pressure measurements. They all were nonsmokers and did not use any medication except oral contraceptives, which were used by five lean and seven obese women. They all used

TABLE 1
Characteristics of both study groups

	Lean women	Obese women
n	16	12
Age (years)	39.0 ± 6.7	38.8 ± 7.0
Weight (kg)	61.1 ± 8.3	111.2 ± 19.6*
BMI (kg/m^2)	21.3 ± 1.9	38.5 ± 6.6*
WHR	0.78 ± 0.06	0.92 ± 0.07*
Cholesterol (mmol/l)	5.1 ± 0.5	5.1 ± 0.8
LDL cholesterol (mmol/l)	2.8 ± 0.4	2.9 ± 0.7
HDL cholesterol (mmol/l)	2.0 ± 0.6	1.5 ± 0.3†
Triglycerides (mmol/l)	0.8 ± 0.3	1.4 ± 0.8*
24-h systolic blood pressure (mmHg)	115 ± 8	123 ± 7†
24-h diastolic blood pressure (mmHg)	72 ± 7	70 ± 6
24-h heart rate (bpm)	79 ± 11	82 ± 8

Data are means ± SD. *$P < 0.05$, †$P < 0.01$.

single-phase preparations with <50 μg ethinylestradiol. In addition, one lean and one obese woman had undergone hysterectomy. All participants gave informed consent for participation in the study. The study was undertaken with approval of the local ethics committee and was performed in accordance with the Declaration of Helsinki.

All individuals underwent 2 study days, which differed for lean and obese women (Fig. 1). To study the effect of an increase in FFA levels in lean women, we randomly administered either an infusion of Intralipid (200 mg/ml at a rate of 45 ml/h; Braun, Oss, the Netherlands) with heparin (10,000 units/ml at a rate of 0.2 units · kg^{-1} · min^{-1}; Akzo Nobel, Oss, the Netherlands) or an infusion of saline (0.65% NaCl; protocol A). To study the effect of a decrease in FFA levels in obese women, we administered either acipimox (a potent long-acting antilipolytic nicotinic acid analog) in doses of 250 mg (Nedios; Byk, Zwanenburg, the Netherlands) or placebo in a double-blinded, randomized manner. The drugs were administered at 11:30 P.M. the evening before the study day and at 7:30 A.M., 11:30 A.M., and 3:30 P.M. on the study day (protocol B). On the study days, participants came to the hospital at 8:00 A.M. after a 10-h fast. Weight and height were determined barefoot and without outer garments. BMI was calculated as the ratio of weight and height squared. Waist and hip circumference were measured as the horizontal circumference halfway between the lower rib margin and the anterior superior iliac spine, and as the maximal horizontal circumference over the buttocks, respectively. The waist-to-hip ratio was calculated by dividing waist circumference by hip circumference. Anthropometrical measurements were performed twice, and the mean of two measurements was calculated. Next, two polytetrafluoroethylene catheters (Venflon, Helsingborg, Sweden) were inserted, one in the right antecubital vein for blood sampling and one in a vein of the opposite arm for the infusion of fluids. Baseline microvascular measurements were started after 30 min of acclimatization in the supine position. All measurements were performed in a standardized order in a quiet and temperature-controlled room (23.4 ± 0.5°C).

Hyperinsulinemic-euglycemic clamp. Insulin sensitivity was determined with the hyperinsulinemic-euglycemic clamp method as described previously (18). Briefly, insulin (Velosulin; Novo Nordisk, Bagsvaerd, Denmark) was infused at a rate of 40 mU · m^{-2} · min^{-1}. Euglycemia (5 mmol/l) was maintained by adjusting the glucose infusion rate based on plasma glucose measurements performed at 5- to 10-min intervals. We used hypotonic saline (0.65%) as the control infusion because glucose infusion will behave like a hypotonic fluid and will increase both the intra- and extracellular fluid volume. Whole-body glucose uptake was calculated from the infusion rate during the second hour of the clamp and expressed per kilogram body weight and per unit of plasma insulin concentration (*M/I* value).

Skin microvascular measurements. Nailfold capillary studies and iontophoresis studies were performed as described previously (19). Nailfold capillaries in the dorsal skin of the third finger of the nondominant hand were visualized by a capillary microscope. A visual field of 1 mm^2 was recorded before and after 4 min of arterial occlusion with a digital cuff. Capillaries at baseline and directly after release of the cuff were counted for 15 and 30 s, respectively, from a freeze-framed reproduction of the videotape and from the running videotape when it was uncertain whether a capillary was present. Capillary density was defined as the number of erythrocyte-perfused capillaries per square millimeter of nailfold skin. This procedure was then repeated

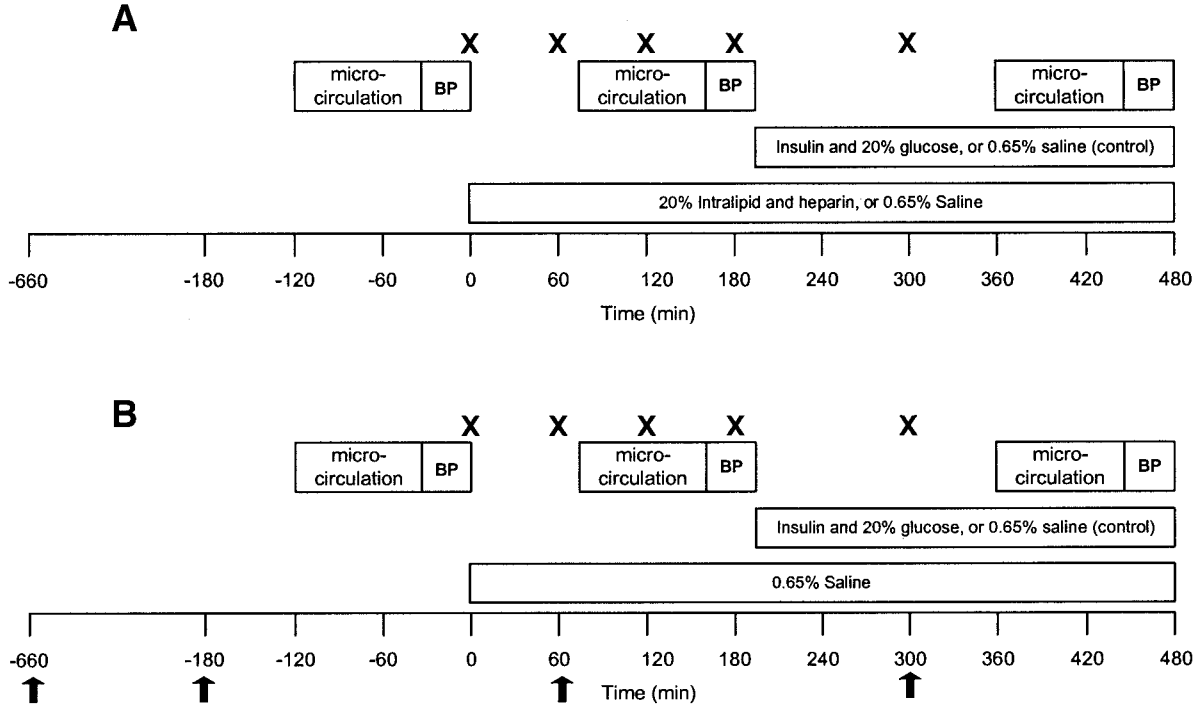

FIG. 1. Design of the study in lean (protocol A) (*A*) and obese (protocol B) (*B*) women. Microcirculation is the capillary recruitment with intravital microscopy and endothelium-(in)dependent vasodilation during iontophoresis of acetylcholine and sodium nitroprusside. For comparison between the acipimox and placebo study day, fasting insulin and FFA concentrations are the mean of four different concentrations determined at $t = 0$, 60, 120, and 180 min on the acipimox or placebo study day. Fasting glucose concentrations are the mean of two different concentrations determined at $t = 0$ and 180 min on the acipimox or placebo study day. The insulin concentration during the hyperinsulinemic-euglycemic clamp is the insulin concentration at $t = 300$ min. Arrows indicate acipimox or placebo. Bold × indicates blood sample for measurement of FFA, insulin, and glucose concentrations. BP, blood pressure and heart rate.

TABLE 2
Metabolic and hemodynamic variables before and during hyperinsulinemia in lean women: effects of FFA elevation versus saline infusion (control)

	Saline infusion			FFA elevation		
	Basal state	Saline	Saline + insulin	Basal state	FFA	FFA + insulin
Blood glucose (mmol/l)	4.3 ± 0.4	4.2 ± 0.4	4.9 ± 0.4*	4.4 ± 0.5	4.2 ± 0.4	5.0 ± 0.4*
Plasma insulin (pmol/l)	26 ± 12	23 ± 10	391 ± 75*	26 ± 10	28 ± 11†	417 ± 67*
Plasma FFA + THL (mmol/l)	0.55 ± 0.19	0.58 ± 0.22	0.01 ± 0.01*	0.52 ± 0.17	0.91 ± 0.19‡§	0.40 ± 0.13*§
M/I value ×100 (mg · kg^{-1} · min^{-1} per pmol/l)	—	—	2.17 ± 0.97	—	—	1.59 ± 0.94‖
Systolic blood pressure (mmHg)	117 ± 14	119 ± 16	117 ± 12	115 ± 10	118 ± 15	121 ± 13†
Diastolic blood pressure (mmHg)	65 ± 9	66 ± 10	63 ± 7	65 ± 8	71 ± 12	66 ± 10
Heart rate (bpm)	66 ± 11	68 ± 11	68 ± 21	67 ± 10	68 ± 11	77 ± 15*¶

Data are means ± SD. *$P < 0.01$ vs. FFA and vs. saline; †$P < 0.05$ change from basal state during FFA elevation vs. during saline infusion; ‡$P < 0.01$ vs. basal state; §$P < 0.01$ change from basal state during FFA elevation vs. during saline infusion; ‖$P < 0.05$ FFA elevation vs. saline infusion; ¶$P = 0.09$ change from basal state during FFA elevation vs. during saline infusion. THL, tetrahydrolipostatin.

using a visual field adjacent to the first visual field, and the mean of both measurements was used for analyses in the present study. Postocclusive reactive hyperemia after 4 min of arterial occlusion was used to assess functional capillary recruitment. This was calculated by dividing the increase in perfused capillary density during postocclusive reactive hyperemia by the baseline perfused capillary density. The day-to-day coefficient of variation of the mean of two measurements of capillary recruitment was 15.9 ± 8.0%, as determined in 10 healthy individuals on 2 separate days.

Endothelium-(in)dependent vasodilation of finger skin microcirculation was evaluated with laser Doppler measurements in combination with iontophoresis of acetylcholine and sodium nitroprusside, respectively. Acetylcholine (1% Miochol; Bournonville Pharma, Braine d'Alleud, Belgium) was delivered on the middle phalanx of the third finger of the nondominant hand using an anodal current, consisting of seven doses (0.1 mA for 20 s) with a 60-s interval between each dose. Sodium nitroprusside (0.01%, Nipride; Roche, Woerden, the Netherlands) was delivered on the same spot of the fourth finger using a cathodal current, consisting of nine doses (0.2 mA for 20 s) with a 90-s interval between each dose. The day-to-day coefficient of variation from baseline to the final two deliveries was 12.2 ± 9.7% for the iontophoresis of acetylcholine and 16.4 ± 8.1% for sodium nitroprusside, as determined in 10 healthy individuals on 2 separate days. Skin temperature was >30°C and monitored continuously during all skin microvascular measurements.

Blood pressure. Ambulatory monitoring (model 90207; Spacelabs, Redmond, WA) was used to obtain 24-h recordings of systolic and diastolic blood pressure, mean arterial pressure, and heart rate. The nondominant arm was used with an appropriately sized cuff. Blood pressure readings were taken at 15-min intervals from 7:00 A.M. to 10:00 P.M. and at 20-min intervals from 10:00 P.M. to 7:00 A.M. The monitor did not display results of blood pressure measurements. One of the obese women did not complete the ambulatory blood pressure measurements because of intolerance to the continuous presence of the blood pressure cuff around the upper arm. During study days, blood pressure and heart rate (Fig. 1) were determined with an oscillometric device (Press-Mate BP-8800; Colin, Komaki City, Japan). The average of three consecutive readings during each period was used for further analyses.

Analytical methods. Plasma insulin concentrations were measured by radioimmunoassay techniques (Medgenix Diagnostics, Fleurus, Belgium). Blood glucose concentrations were determined by the glucose oxidase method with a YSI2300 glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Serum total cholesterol, HDL cholesterol, and triglyceride concentrations were measured by enzymatic techniques (Hitachi 747 model 100; Roche Diagnostics, Mannheim, Germany). LDL cholesterol was calculated by the Friedewald formula. Blood samples for FFA measurements were collected in prechilled tubes. Plasma was immediately separated from cells by centrifugation and frozen (−20°C) until analysis. To inhibit in vitro lipolysis during Intralipid and heparin infusion in lean women, FFA concentrations were determined in EDTA plasma containing the lipoprotein lipase inhibitor tetrahydrolipostatin (final concentration 1 mg/l). The tetrahydrolipostatin solution was derived from a Xenical capsule, which was a generous gift from Roche (Woerden, the Netherlands). Plasma FFA concentrations were determined by an enzymatic colorimetric method (ELAN; Merck, Darmstadt, Germany).

Statistical analyses. Data are expressed as the means ± SD. Distributions of all data were tested for normality. To examine differences in microvascular function between FFA lowering and placebo in obese women, we used the mean of two microvascular measurements, i.e., the first two measurements during either FFA lowering or placebo (Fig. 1). A nonpaired Student's *t* test was used to compare differences between lean and obese women. A paired Student's *t* test was used to study effects, versus control experiments, of FFA elevation in lean women and of FFA lowering in obese women. Linear regression analysis with adjustment for age was used to investigate associations between changes in FFA levels, insulin sensitivity, and microvascular function. All analyses were performed on a personal computer using the statistical software package SPSS version 11.0.

RESULTS

In lean women, FFA elevation induces acute insulin resistance and increases basal insulin levels. Metabolic measurements before the start of the infusions were comparable between the Intralipid and the saline study (Table 2). A 3-h Intralipid and heparin infusion, as compared with saline infusion, increased FFA levels (0.91 ± 0.19 vs. 0.58 ± 0.22 mmol/l, $P < 0.01$). FFA elevation reduced insulin-mediated glucose uptake (by 27%, $P < 0.05$) and increased basal insulin levels (28 ± 11 vs. 23 ± 10 pmol/l, $P < 0.05$).

In obese women, FFA lowering improves insulin sensitivity and decreases basal insulin levels. Overnight acipimox administration, as compared with placebo, lowered fasting FFA levels (0.18 ± 0.10 vs. 0.69 ± 0.21 mmol/l, $P < 0.01$) (Table 3). FFA lowering increased insulin-mediated glucose uptake (by 16%, $P = 0.06$) and decreased basal insulin levels (49 ± 17 vs. 89 ± 33 pmol/l, $P < 0.01$).

In lean women, FFA elevation impairs skin microvascular function in the basal state and during hyperinsulinemia. FFA elevation, as compared with saline infusion, impaired capillary recruitment (by 22%, $P < 0.05$) (Table 4 and Fig. 2) and microvascular vasodilatory responses to iontophoresis of acetylcholine (by 42%, $P < 0.01$). Responses to sodium nitroprusside were not significantly affected ($P = 0.2$). FFA elevation, as compared with saline infusion, increased baseline laser Doppler perfusion ($P < 0.05$).

During hyperinsulinemia, FFA elevation, as compared with saline infusion, also impaired capillary recruitment (by 25%, $P < 0.01$) and vasodilatory responses to acetylcholine (by 61%, $P < 0.01$), but it did not influence responses to sodium nitroprusside ($P = 0.6$). Thus, FFA elevation impaired capillary recruitment and microvascu-

TABLE 3
Metabolic and hemodynamic variables before and during hyperinsulinemia in obese women: effects of FFA lowering versus placebo

	Placebo		FFA lowering	
	Basal state	Insulin	Basal state	Insulin
Blood glucose (mmol/l)	4.7 ± 0.8	5.0 ± 0.2	4.7 ± 0.8	5.1 ± 0.2
Plasma insulin (pmol/l)	89 ± 33	483 ± 187*	49 ± 17†	509 ± 160*
Plasma FFA (mmol/l)	0.69 ± 0.21	0.06 ± 0.10*	0.18 ± 0.10†	0.02 ± 0.03*‡
M/I value ×100 (mg · kg^{-1} · min^{-1} per pmol/l)	—	0.52 ± 0.34	—	0.62 ± 0.28§
Systolic blood pressure (mmHg)	128 ± 8	135 ± 8‖	129 ± 14	139 ± 15*
Diastolic blood pressure (mmHg)	70 ± 9	74 ± 9	70 ± 11	76 ± 9*
Heart rate (bpm)	70 ± 6	80 ± 6*	71 ± 6	79 ± 5*

Data are means ± SD. *$P < 0.01$ vs. basal state; †$P < 0.01$ FFA lowering vs. placebo; ‡$P < 0.01$ change from basal state with FFA lowering vs. with placebo; §$P = 0.06$ FFA lowering vs. placebo; ‖$P < 0.05$ vs. basal state.

lar responses to acetylcholine both in the basal state and during hyperinsulinemia.

In obese women, FFA lowering improves skin capillary recruitment in the basal state and during hyperinsulinemia. FFA lowering, as compared with placebo, improved capillary recruitment (by 27%, $P < 0.01$) (Table 5 and Fig. 3). FFA lowering did not influence responses to acetylcholine ($P = 0.7$) or sodium nitroprusside ($P = 0.2$). FFA lowering, as compared with placebo, tended to decrease baseline laser Doppler perfusion ($P = 0.07$).

During hyperinsulinemia, FFA lowering, as compared with placebo, improved capillary recruitment (by 18%, $P < 0.05$). During hyperinsulinemia, FFA lowering did not influence responses to acetylcholine ($P = 0.4$) or sodium nitroprusside ($P = 0.5$). Thus, FFA lowering improved capillary recruitment both in the basal state and during hyperinsulinemia.

In lean and obese women, changes in capillary recruitment statistically explain part of the associations between changes in FFA levels and insulin sensitivity. Linear regression analyses demonstrated that, in lean women, increases in FFA levels were associated with decreases in capillary recruitment and insulin-mediated glucose uptake (Fig. 4). In parallel, in obese women, decreases in FFA levels were associated with increases in capillary recruitment and insulin-mediated glucose uptake. In both lean and obese women, increases in capillary recruitment were associated with increases in insulin-mediated glucose uptake. Multiple regression demonstrated, with adjustment for age, that in lean and obese women together, changes in capillary recruitment statistically explained ~29% of the association between changes in FFA levels and changes in insulin-mediated glucose uptake (β was reduced from −0.36 to −0.26).

The above results did not change materially when absolute increases in perfused capillary density were used instead of relative increases, or when absolute increases in laser Doppler perfusion during iontophoresis of acetylcholine and sodium nitroprusside were used instead of relative increases (data not shown). The use of the *M* value (glucose infusion rate during the second hour of the hyperinsulinemic-euglycemic clamp) instead of the *M/I* value (*M* value expressed per unit of plasma insulin concentration) also did not lead to different conclusions (data not shown). Also, skin temperature and insulin-mediated changes in skin temperature during microvascular measurements did not differ between FFA elevation and saline infusion in lean women and between FFA lowering and placebo treatment in obese women (data not shown).

TABLE 4
Microvascular measurements before and during hyperinsulinemia in lean women: effects of FFA elevation versus saline infusion (control)

	Saline infusion			FFA elevation		
	Basal state	Saline	Saline + insulin	Basal state	FFA	FFA + insulin
Perfused capillary density						
Baseline density (caps/mm^2)	38.2 ± 4.5	38.2 ± 5.9	38.2 ± 6.7	36.1 ± 6.2	35.9 ± 6.3	36.1 ± 7.1
Peak density (caps/mm^2)	58.1 ± 8.2	58.4 ± 5.0	63.8 ± 9.6*	55.4 ± 11.4	51.3 ± 9.9†‡	55.1 ± 10.3§‖
Percentage increase (%)	55.5 ± 21.2	56.9 ± 18.9	72.4 ± 25.1*	54.2 ± 19.3	44.6 ± 16.8†‡	54.0 ± 21.3*‖
Ach-mediated vasodilation						
Baseline perfusion (PU)	24.0 ± 7.3	25.4 ± 7.0	25.6 ± 10.0	23.1 ± 6.0	28.3 ± 11.1	34.6 ± 14.1¶
Plateau (PU)	146.0 ± 58.7	162.3 ± 54.4	199.3 ± 88.3	155.9 ± 56.1	126.5 ± 68.8‡	128.2 ± 79.5
Percentage increase (%)	516 ± 164	557 ± 162	685 ± 199*	579 ± 201	338 ± 131‖#	264 ± 186‖
SNP-mediated vasodilation						
Baseline perfusion (PU)	32.0 ± 12.3	28.8 ± 11.1	31.9 ± 16.4	30.8 ± 12.6	31.8 ± 10.9‡	34.8 ± 12.4‡
Plateau (PU)	166.6 ± 81.3	155.3 ± 56.5	160.1 ± 60.3	161.9 ± 63.1	132.1 ± 55.1	153.2 ± 52.1
Percentage increase (%)	461 ± 277	490 ± 291	450 ± 220	426 ± 334	362 ± 244	405 ± 274

Data are means ± SD. *$P < 0.01$ vs. FFA and vs. saline; †$P < 0.05$ vs. basal state; ‡$P < 0.05$ change from basal state during FFA elevation vs. during saline infusion; §$P < 0.05$ vs. FFA and vs. saline; ‖$P < 0.01$ and ¶$P = 0.09$ change from basal state during FFA elevation vs. during saline infusion; #$P < 0.01$ vs. basal state. Ach, acetylcholine; caps, number of perfused capillaries; PU, arbitrary perfusion units; SNP, sodium nitroprusside.

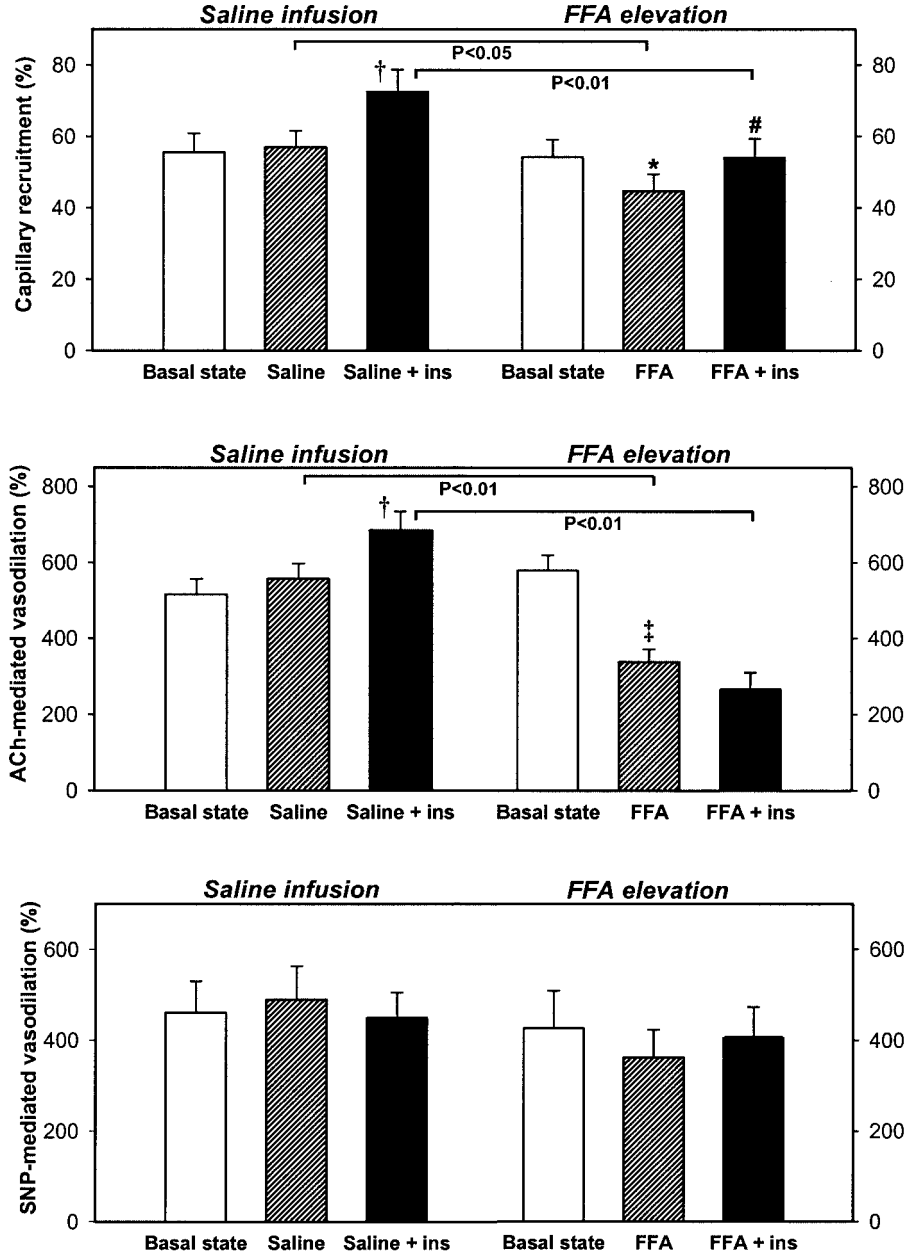

FIG. 2. Capillary recruitment, acetylcholine-mediated vasodilation, and sodium nitroprusside–mediated vasodilation before and during hyperinsulinemia in lean women: effects of FFA elevation versus saline infusion (control). FFA = Intralipid and heparin infusion. *$P < 0.05$ vs. basal state †$P < 0.01$ vs. saline alone; ‡$P < 0.01$ vs. basal state; #$P < 0.01$ vs. FFA alone. Ach, acetylcholine; ins, insulin infusion; SNP, sodium nitroprusside.

DISCUSSION

We investigated whether acute FFA elevation in lean women impairs, and whether overnight FFA lowering in obese women improves, microvascular function in the basal state and during physiological hyperinsulinemia. We report four novel findings: *1*) in lean women, acute elevation of FFA levels impairs microvascular function (capillary recruitment and acetylcholine-induced vasodilation) both in the basal state and during hyperinsulinemia, *2*) in obese women, overnight lowering of FFA levels improves capillary recruitment both in the basal state and during hyperinsulinemia, *3*) in lean and obese women, changes in FFA levels are inversely associated with changes in capillary recruitment and insulin-mediated glucose uptake, and *4*) in lean and obese women, changes in microvascular function can statistically explain part of the association between changes in FFA levels and changes in insulin-mediated glucose uptake. These findings are consistent with a role for FFA-induced microvascular dysfunction in the development of obesity-related insulin resistance, hypertension, and microangiopathy.

Recently, it has been suggested that one of insulin's physiological functions may be to switch blood flow from nonnutritive to nutritive capillary beds, thereby enhancing access of glucose and insulin to muscle tissue independently of changes in total flow (12). Indeed, both human and rat studies suggest a functional coupling between insulin-induced effects on muscle microvascular perfusion and glucose uptake (12,20–22). Obese Zucker rats are characterized by both impaired insulin-induced glucose uptake and impaired capillary recruitment in the basal state and during hyperinsulinemia (23), and we recently demonstrated similar impairments in human obesity (24). In addition, acute FFA elevation in lean rats concomitantly impairs effects of insulin on capillary recruitment and glucose uptake (11). We now show, in healthy women, that

TABLE 5
Microvascular measurements before and during hyperinsulinemia in obese women: effects of FFA lowering versus placebo

	Placebo		FFA lowering	
	Basal state	Insulin	Basal state	Insulin
Perfused capillary density				
Baseline density (caps/mm^2)	38.6 ± 5.7	37.4 ± 5.2	38.1 ± 6.0	37.9 ± 5.2
Peak density (caps/mm^2)	52.5 ± 7.7	56.6 ± 5.0	56.2 ± 5.6	61.6 ± 5.0*†
Percentage increase (%)	37.4 ± 9.3	54.8 ± 15.4*	50.9 ± 14.6‡	66.8 ± 20.6*†
Ach-mediated vasodilation				
Baseline skin perfusion (PU)	25.6 ± 8.5	26.3 ± 12.4	23.6 ± 7.0	22.3 ± 10.8
Plateau (PU)	106.4 ± 40.9	99.5 ± 44.0	102.1 ± 36.3	90.1 ± 52.4
Percentage increase (%)	345.4 ± 158.8	302.3 ± 157.2	363.9 ± 198.2	342.5 ± 260.3
SNP-mediated vasodilation				
Baseline skin perfusion (PU)	26.8 ± 9.4	25.7 ± 8.1	20.7 ± 5.8§	23.2 ± 7.6
Plateau (PU)	132.1 ± 57.3	99.0 ± 32.5	99.9 ± 48.3	96.4 ± 44.0
Percentage increase (%)	470.9 ± 301.4	411.0 ± 182.6	413.2 ± 252.7	362.3 ± 294.5

Data are means ± SD. *$P < 0.01$ vs. basal state, †$P < 0.05$, ‡$P < 0.01$, §$P = 0.07$ FFA lowering vs. placebo. Ach, acetylcholine; caps, number of perfused capillaries; PU, arbitrary perfusion units; SNP, sodium nitroprusside.

insulin's effects on microvascular function and glucose uptake are impaired when FFA levels are increased (in lean women) and enhanced when FFA levels are lowered (in obese women), i.e., insulin's microvascular and metabolic effects may be coupled during changes in exposure to FFAs. Notably, ~29% of the effects of FFA elevation or lowering on insulin-induced glucose uptake could be explained statistically by changes in microvascular function. Taken together, these (11,12,20–23) and the present findings support the concept that insulin-induced effects on microvascular function may affect glucose uptake. In addition, these findings suggest, but do not prove, a causal relationship between FFA-mediated effects on microvascular perfusion and insulin-induced glucose uptake. A direct effect of FFAs to inhibit glucose transport/phosphorylation may be important because previous studies have demonstrated a time difference in FFA-mediated effects on insulin-induced increases in whole-body glucose uptake (160 min) and intramuscular glucose-6-phosphate concentrations (<60 min) (25–27). On the other hand, capillary recruitment is an early insulin effect (<10 min) that precedes both activation of insulin-signaling pathways and increases in glucose disposal in rat muscle (28), an effect that is impaired during Intralipid infusion (11). Therefore, capillary recruitment may play a role in the early insulin-induced increase in intramuscular glucose concentration, and it would be of interest to study the time course of FFA effects on capillary recruitment and *M* values in future studies.

In the present study, effects of FFA elevation on endogenous glucose production were not measured, and therefore the *M* value may not solely reflect insulin-stimulated peripheral glucose uptake. Previous studies have demonstrated that FFA elevation had either no effect on (7,29) or impaired the insulin-induced decrease in (30,31) endogenous glucose production. However, during FFA elevation in healthy women, at most 30% of glucose appearance can be explained by impaired insulin-induced suppression of endogenous glucose production (31), indicating that the *M* value still partially reflects insulin-stimulated peripheral glucose uptake.

It might be argued that substances derived from glucose metabolism contribute to insulin-induced effects on microvascular function. However, recent studies have demonstrated that effects of insulin on microvascular function precede muscle glucose uptake in time (28) and that these occur at lower insulin concentrations (32). In addition, local application of insulin causes microvascular vasodilation without influencing the systemic glucose metabolism (22). Taken together, these studies support the idea that insulin-induced effects on microvasculature are, at least in part, independent of effects on glucose metabolism.

A mechanism inducing simultaneous effects on microvascular function and glucose uptake may lie in pathways of insulin signaling that are shared between skeletal muscle and vascular endothelial cells. Specifically, phosphatidylinositol (PI) 3-kinase activity is necessary for insulin-induced effects on both glucose transport in skeletal muscle and nitric oxide production in vascular endothelium (11,33,34). Indeed, FFA elevation blunts insulin-induced PI 3-kinase activation in human muscle (33). In obese Zucker rats, insulin-induced PI 3-kinase activation is reduced in endothelial cells and isolated arterioles (34). Concomitant impairment, by FFAs, of insulin-induced increases in PI 3-kinase activity in skeletal muscle and endothelial cells should therefore be considered as an alternative explanation for our observations. This possibility requires further investigation.

Detrimental effects of FFA elevation on microvascular function may play a role in the development not only of insulin resistance but also of hypertension and microangiopathy, all of which contribute to increased cardiovascular risk. FFAs increase systolic blood pressure, as measured by invasive blood pressure measurements (8). Impaired microvascular dysfunction has been shown to increase peripheral vascular resistance and antedate the development of hypertension, indicating a role for microvascular dysfunction in the development of hypertension (14–16). In addition, microvascular changes may directly contribute to the development of microangiopathy.

Although muscle is considered the main peripheral site of insulin-induced glucose uptake and vasodilatation, comparable metabolic (35–37) and vascular (22,38–40) effects of systemic insulin infusion can be demonstrated in skin. In rats, during hyperinsulemia, skin and muscle glucose uptake accounted for the majority (26 and 59%, respec-

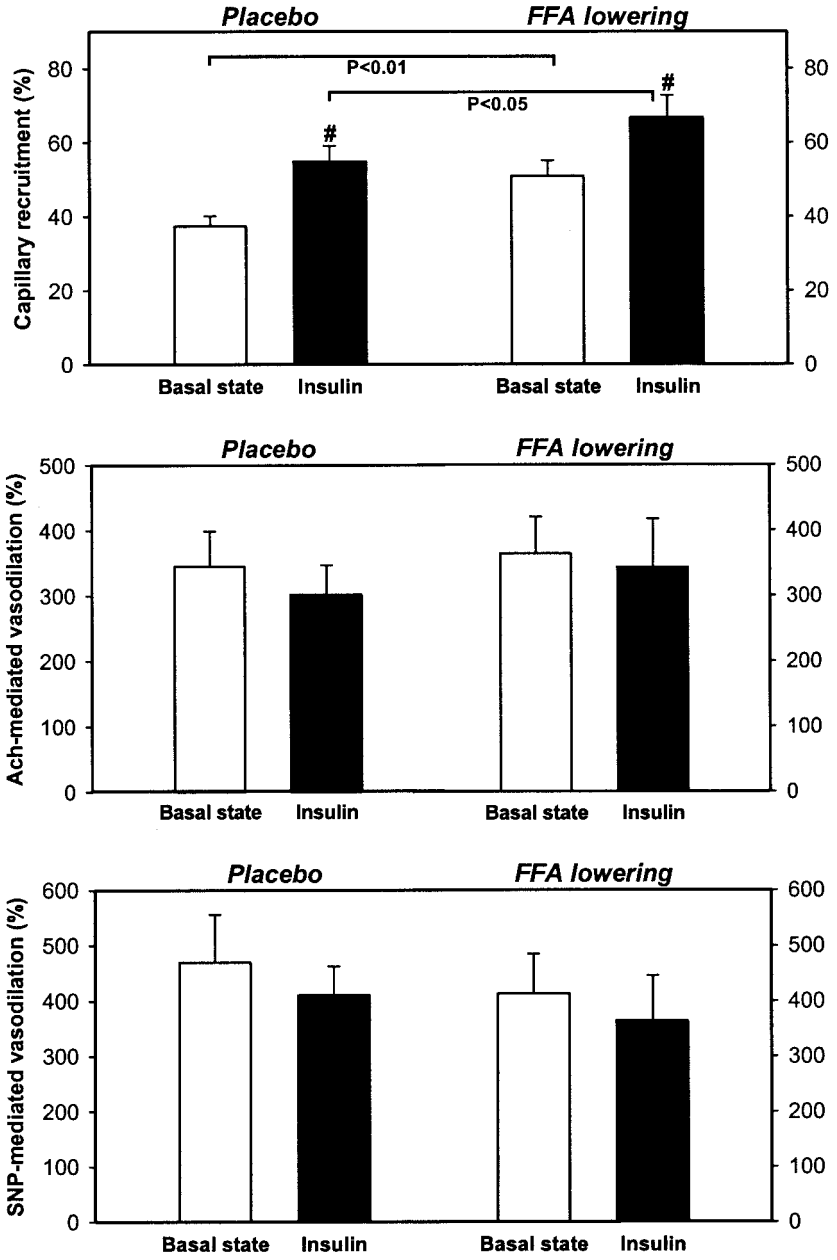

FIG. 3. Capillary recruitment, acetylcholine-mediated vasodilation, and sodium nitroprusside–mediated vasodilation before and during hyperinsulinemia in obese women: effects of FFA lowering versus placebo. #$P < 0.01$ vs. basal state. Ach, acetylcholine; SNP, sodium nitroprusside.

tively) of whole-body glucose uptake. In humans, glucose disposal in skin is very difficult to measure and therefore is rarely assessed. Recently, we have demonstrated insulin-induced recruitment of capillaries in skin (22) that is comparable to muscle (12,20). In addition, skin microvascular vasodilator capacity is associated with insulin's metabolic (19,41–43) and vascular (41) actions in muscle. Therefore, it appears reasonable to investigate skin to learn more about the insulin-induced effects on microvascular perfusion. Nevertheless, it should be stated that findings concerning skin microcirculation should be extrapolated to muscle with caution.

FFA elevation impaired both capillary recruitment and endothelium-dependent vasodilation, whereas FFA lowering improved capillary recruitment but not endothelium-dependent vasodilation (Fig. 3). The explanation for this discrepancy is not entirely clear. The stimulus used in capillary recruitment (i.e., peak reactive hyperemia after arterial occlusion) differs from that used in microvascular endothelium-dependent vasodilation (i.e., acetylcholine), and FFA lowering may preferentially influence the microvascular response to peak reactive hyperemia. In addition, FFA lowering may affect characteristics of the capillary network that contribute to capillary perfusion (44) but not to endothelium-dependent vasodilation.

Our findings suggest that impaired microvascular function observed in obese individuals is mediated, at least in part, through elevated FFA levels. FFA elevation in lean women impaired microvascular function to a level comparable to that in obese women, and, conversely, FFA lowering in obese women improved microvascular function to a level comparable to that in lean women. The fact that obesity-related impairment of microvascular function in our previous study (24) could not be (statistically) explained by differences in fasting plasma FFAs does not exclude a role for FFA dynamics in modulating microvas-

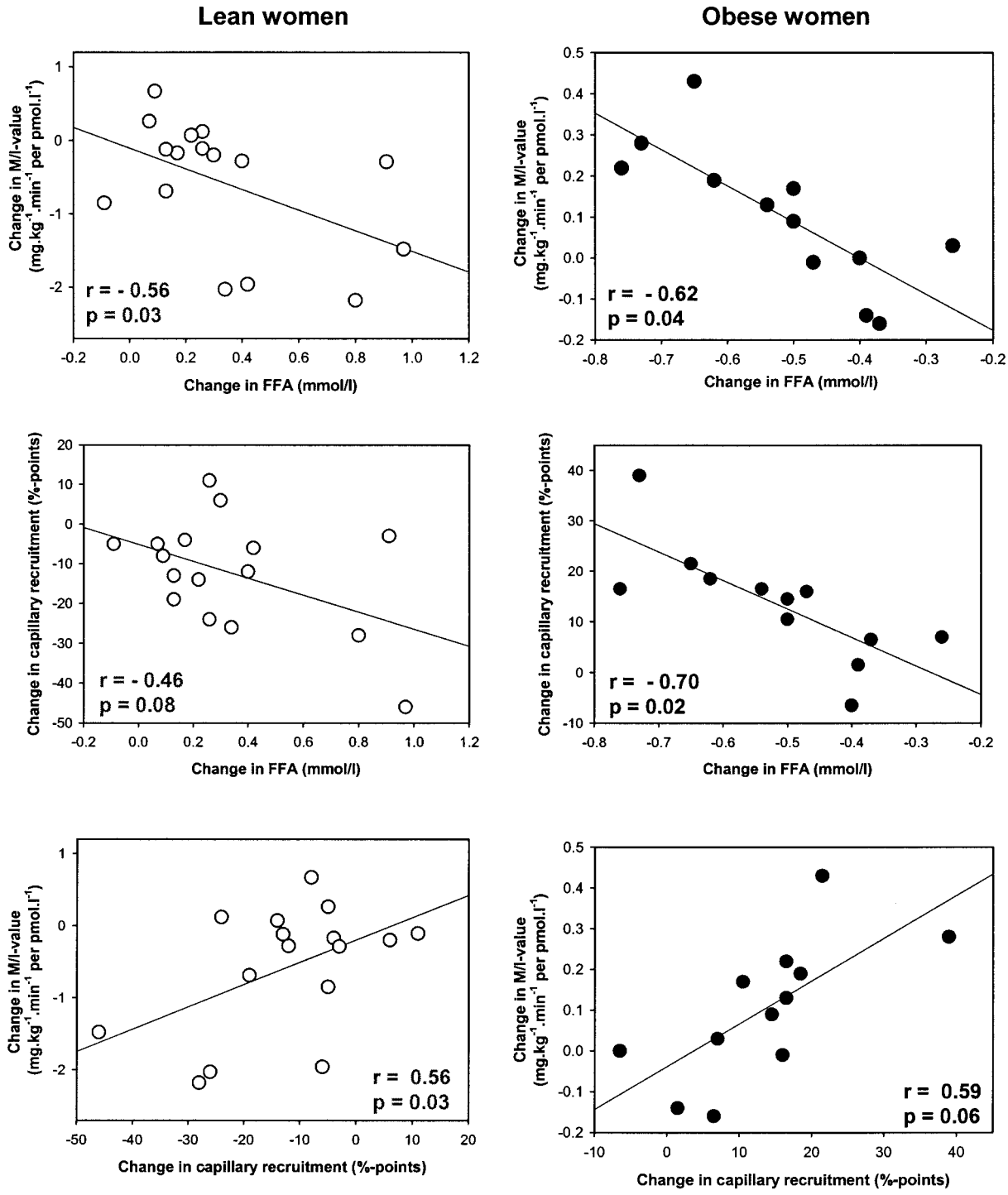

FIG. 4. Associations among changes in FFA levels, capillary recruitment, and insulin-mediated glucose uptake between FFA elevation and saline infusion in lean women (○) and between FFA lowering and placebo in obese women (●). In lean women, change in FFA levels and change in capillary recruitment are the differences in these variables before the start of the insulin or saline infusion (t = 180 min) (Fig. 1). In obese women, change in FFA levels is the difference in the mean of FFA levels at t = 0, 60, 120, and 180 min, and change in capillary recruitment is the mean of the first two measurements during either FFA lowering or placebo (Fig. 1). Change in insulin-mediated glucose uptake is the difference between both study days in *M/I* values as calculated from the glucose infusion rate during the second hour of the clamp. Correlation coefficients are adjusted for age.

cular function. In addition, one could speculate that not plasma FFAs themselves, but instead intracellular accumulation of substances derived from FFAs, such as triglycerides or acetylcoenzyme A, determine effects of FFAs on microvascular function. These intracellular lipids have also been suggested to play a role in FFA-induced insulin resistance because intracellular lipids (45,46) are associated with impaired insulin-induced glucose uptake. However, it remains to be established whether accumulation of intracellular lipids also occurs in the microvasculature, and whether this influences microvascular function.

In conclusion, acute FFA elevation in lean women

impairs, and overnight FFA lowering in obese women improves, microvascular function. This suggests that elevated FFA levels in obesity contribute to microvascular dysfunction and consequently may play a role in the development of obesity-related insulin resistance, hypertension, and microangiopathy. A functional coupling between insulin's stimulatory effects on microvascular function and glucose uptake is supported by concomitant changes in both effects during FFA elevation and lowering. Furthermore, FFA-induced changes in capillary recruitment can statistically explain part of FFA-induced changes in insulin-mediated glucose uptake. These insights underline the importance of microvascular dysfunction as a partial explanation for FFA-induced insulin resistance in obesity. Our data suggest that strategies to lower FFA concentrations in obesity may improve microvascular function and thereby contribute to prevention of insulin resistance, hypertension, and microangiopathy. Studies to test these hypotheses are warranted.

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