Original Article

Obesity Blunts Insulin-Mediated Microvascular Recruitment in Human Forearm Muscle

Lucy H. Clerk, Michelle A. Vincent, Linda A. Jahn, Zhenqi Liu, Jonathan R. Lindner, and Eugene J. Barrett

We have previously shown that skeletal muscle capillaries are rapidly recruited by physiological doses of insulin in both humans and animals. This facilitates glucose and insulin delivery to muscle, thus augmenting glucose uptake. In obese rats, both insulin-mediated microvascular recruitment and glucose uptake are diminished; however, this action of insulin has not been studied in obese humans. Here we used contrast ultrasound to measure microvascular blood volume (MBV) (an index of microvascular recruitment) in the forearm flexor muscles of lean and obese adults before and after a 120-min euglycemic-hyperinsulinemic (1 mU · min^{-1} · kg^{-1}) clamp. We also measured brachial artery flow, fasting lipid profile, and anthropomorphic variables. Fasting plasma glucose (5.4 ± 0.1 vs. 5.1 ± 0.1 mmol/l, $P = 0.05$), insulin (79 ± 11 vs. 38 ± 6 pmol/l, $P = 0.003$), and percent body fat (44 ± 2 vs. 25 ± 2%, $P = 0.001$) were higher in the obese than the lean adults. After 2 h of insulin infusion, whole-body glucose infusion rate was significantly lower in the obese versus lean group (19.3 ± 3.2 and 37.4 ± 2.6 μmol · min^{-1} · kg^{-1} respectively, $P < 0.001$). Compared with baseline, insulin increased MBV in the lean (18.7 ± 3.3 to 25.0 ± 4.1, $P = 0.019$) but not in the obese group (20.4 ± 3.6 to 18.8 ± 3.8, NS). Insulin increased brachial artery diameter and flow in the lean but not in the obese group. We observed a significant, negative correlation between ΔMBV and BMI ($R = -0.482$, $P = 0.027$) in response to insulin. In conclusion, obesity eliminated the insulin-stimulated muscle microvascular recruitment and increased brachial artery blood flow seen in lean individuals. *Diabetes* 55:1436–1442, 2006

Obesity is increasingly prevalent and is associated with insulin resistance (1,2) and an increased risk for the development of type 2 diabetes (3) and cardiovascular diseases (4). The relationship between obesity and insulin resistance may be complex, as not all obese individuals appear to be insulin resistant. Centripetal obesity appears to be a better phenotypic marker that is associated with insulin resistance (3). Additionally, evidence from several (but not all [5]) studies indicates that obesity is associated with impaired endothelial function, as assessed by measurements of either responsiveness of resistance arterioles to infusion of cholinergic vasodilators (6) or flow-mediated conduit vessel relaxation (7). Over a decade ago, Laasko et al. (8) drew attention to a decreased sensitivity of resistance arterioles to insulin in obese, insulin resistant, but otherwise healthy individuals. Others have confirmed this observation (5,9). Impaired insulin-mediated elasticity increases of conduit vessels has also been observed in obese humans (9). More recently, using a laser Doppler and nail bed capillaroscopy, De Jongh et al. (10) have shown a diminished ability of insulin to recruit skin capillaries in obese subjects.

Our laboratory has developed and applied both a biochemical method to estimate endothelial surface exposure (11,12) and contrast-enhanced ultrasound methods (13–15) to measure microvascular blood volume (MBV) in skeletal muscle. We have reported that insulin, at physiologic concentrations, recruits skeletal muscle capillaries in rodents and humans. This effect of insulin is blocked by inhibitors of nitric oxide synthase (16) as well as by tumor necrosis factor-α (17) and free fatty acids (18). With each of these interventions, inhibition of microvascular recruitment is associated with a decline in skeletal muscle insulin-mediated glucose disposal, suggesting insulin resistance. This would be expected if, as discussed by Renkin and colleague (19,20), endothelial surface area is an important factor limiting delivery of substrates to muscle tissue. The importance of this mechanism for increasing nutrient delivery is illustrated by the marked changes in capillary recruitment observed with low-frequency muscle contraction, while substantially higher frequency stimulation is required to increase total blood flow (21). Inasmuch as skeletal muscle is the principal site of insulin-mediated glucose disposal in humans, we have examined in the current study whether in otherwise healthy obese humans, insulin resistance is associated with impaired action of insulin to recruit skeletal muscle microvasculature. MBV, microvascular flow velocity (MFV), and brachial artery velocity and diameter were measured at baseline and at the end of a 2-h 1 mU · min^{-1} · kg^{-1} euglycemic insulin clamp.

RESEARCH DESIGN AND METHODS

The study protocol was approved by the University of Virginia Human Investigation Committee. A total of 11 lean (BMI <30 kg/m^2; 8 female and 3 male) and 10 obese (BMI >30 kg/m^2; 8 female and 2 male) volunteers with no history of hypertension, diabetes, or hyperlipidemia were admitted to the General Clinical Research Center on two separate occasions (a screening and a study visit) after an overnight fast. At the screening visit blood samples were taken for cholesterol, triglycerides, insulin, and glucose measurements. Sub-

From the Division of Endocrinology and Metabolism, Department of Internal Medicine, University of Virginia Health System, Charlottesville, Virginia.

Address correspondence and reprint requests to Eugene J. Barrett, University of Virginia Health System, P.O. Box 801410, 450 Ray C. Hunt Dr., Charlottesville, VA 22908. E-mail: ejb8x@virginia.edu.

Received for publication 21 October 2005 and accepted in revised form 13 February 2006.

MBV, microvascular blood volume; MFV, microvascular flow velocity.

DOI: 10.2337/db05-1373

© 2006 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

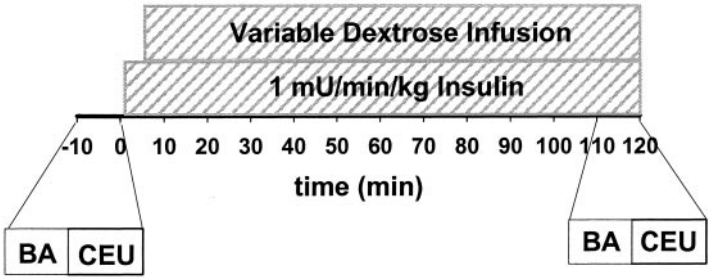

FIG. 1. Protocol for the euglycemic-hyperinsulinemic (1 mU · min^{-1} · kg^{-1}) clamp in lean and obese adults. Contrast enhanced ultrasound (CEU) measurements of the forearm and brachial artery (BA) measurements were conducted before and at the end of the 2-h insulin infusion. Venous samples for glucose estimation were taken every 5 min.

jects were excluded from the study for family history of a first-degree relative with diagnosed diabetes or if they were taking any medication known to affect either endothelial function or glucose metabolism. For the study visit, subjects were admitted to the general clinical research center the evening before the study and fasted overnight. On the morning of the study, body composition was measured using an air displacement chamber (BOD-POD; Life Management, Concorde, CA), and height, weight, hip, and waist circumferences were also measured.

Insulin clamp procedure. Catheters were placed in peripheral arm veins for blood sampling and infusions according to the protocol described in Fig. 1. After an initial rest period, baseline samples and ultrasound measurements were taken. Beginning at time 0, regular human insulin was infused intravenously at 1 mU · min^{-1} · kg^{-1}, and a variable infusion of 20% glucose was commenced at 5 min (22). Venous blood samples were taken for plasma glucose every 5 min throughout the 120-min insulin infusion. We did not heat the hand to "arterialize" venous blood, as this alters blood flow both in the heated and contralateral arms (23). Instead, we recognized that when arterial glucose is maintained at a constant level during an insulin clamp, venous glucose concentrations decline progressively with time, and the arteriovenous glucose difference increases in proportion to insulin sensitivity. We targeted for glucose to decline ~ 5 mg/dl for every 1 mg · min^{-1} · kg^{-1} increase in glucose infusion rate above the basal hepatic glucose production (estimated at 2 mg · min^{-1} · kg^{-1}). This is admittedly a rough approximation. The rationale for this was as follows: assuming that muscle constitutes ~40% of body weight, that baseline skeletal muscle muscle blood flow is ~4 ml/min per 100 g, and that muscle is responsible for 75% of the increment of glucose disposal above the basal requirements in the fasting state under euglycemic conditions, one can calculate that the arterial-venous glucose concentration difference across muscle tissue will be ~4.7 mg/dl for every 1 mg · min^{-1} · kg^{-1} of glucose infusion when arterial glucose is maintained constant. The mean glucose infusion rate in our obese subjects was 3.4 mg · min^{-1} · kg^{-1} at the end of the insulin clamp, and their plasma glucose had declined by 12.6 mg/dl (4 mg/dl more than planned). For the lean subjects with a mean glucose infusion rate of 6.7 mg · min^{-1} · kg^{-1}, the mean decline was 18 mg/dl, or ~5 mg/dl less than we had targeted. In both groups, the venous glucose (and certainly the arterial glucose) was above the range that might trigger counterregulatory responses.

Blood analysis. Plasma cholesterol, HDL and LDL cholesterol, and triglycerides were measured by the University of Virginia clinical chemistry laboratories. Plasma glucose and lactate were measured using a YSI analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was measured using an enzyme-linked immunosorbent assay with an interassay coefficient of variation of <4%. To estimate insulin sensitivity during hyperinsulinemia, we used the glucose infusion rate during the insulin clamp.

Brachial artery flow. Brachial artery measurements were made ~10 cm proximal to the antecubital fold while the patient was in the supine position using a high frequency L12-5 linear array transducer interfaced to an HDI5000 ultrasound (Philips Medical Systems, Andover, MA). Artery diameter was measured using 2-D imaging of the longitudinal artery as the distance between each inside edge of the arterial intima. Velocity was determined using pulse-wave Doppler and brachial flow calculated from the diameter and velocity measurements.

Contrast enhanced ultrasound. Contrast ultrasound measurements were performed using an HDI 5000 (Philips Medical Systems) while the patient was sitting upright. A P4-2 phased array transducer (Philips Medical Systems) was positioned with a ring stand and clamp to image the flexor muscles of the forearm in cross section. Microbubbles (Definity; Bristol-Myers Squibb, Princeton, NJ) were delivered intravenously at a rate that produced moderate opacification (below saturation level) at the longest pulsing interval used (16 s). Microbubbles were detected by power Doppler imaging using a mechanical index (1.2) capable of destroying all bubbles in the ultrasound beam. After an initial equilibration period, a pulsing interval (time) versus video-intensity curve was generated (24). The pulsing interval is the time between successive ultrasound pulses, which destroy microbubbles, and videointensity is the intensity of signal generated from the microbubbles. At a low pulsing interval (e.g., 500 ms) only microbubbles in the larger arteries and arterioles are filled before another destructive pulse is delivered, hence all of signal intensity is secondary to filling of these vessels. At a longer pulsing interval (e.g., 16 s) the microbubbles will again have filled larger arteries and arterioles but will have also entered the microvasculature. Subtracting images obtained at 500 ms from those taken at 16 s eliminates signals generated from the background tissue or larger vessels, leaving a measure of MBV. Images were recorded onto SVHS videotape, captured using Adobe Premiere software (Version 6.0) and analyzed using MCE software (University of Virginia). Pulsing interval versus video intensity curves for the forearm muscle region of interest were fitted to the exponential function: $y = A(1 - e^{-\beta t})$, where t is the pulsing interval, y is the video intensity at any given t, A is the plateau video intensity (representing MBV), and β is the MFV as described by Wei et al. (24) and validated versus radiolabeled microspheres in cardiac muscle. Microvascular flow was calculated as MBV × MFV.

Statistics. Data are presented as the means ± SE, and statistics were performed using SigmaStat (Systat Software, 2004). Within the subject comparisons between measurements made at baseline and at the end of the study were done using a paired Student's t test. Comparisons between lean and obese subjects were made using an unpaired t test. Comparison of time series measurements between lean and obese were performed by repeated-measures ANOVA.

RESULTS

The baseline anthropomorphic characteristics and basal and clamp serum chemistries of the lean and obese groups are shown in Table 1. The lean subjects weighed less, had lower BMIs, a lower percent body fat, a lower fat weight, and smaller waist and hip circumferences than the obese subjects. Fasting lipids (total cholesterol, triglycerides,

TABLE 1
Biometric parameters for 11 lean and 10 obese study subjects

	Lean	Obese	*P* value
n	11	10	
Age (years)	37 ± 3	43 ± 2	NS
Weight (kg)	74 ± 4	94 ± 4	0.001
Height (m)	1.77 ± 0.03	1.67 ± 0.04	NS
BMI (kg/m^2)	23 ± 1	34 ± 1	0.001
% body fat	25 ± 2	44 ± 2	0.001
Fat weight (kg)	19 ± 2	42 ± 3	0.001
Waist (cm)	79 ± 3	97 ± 3	0.001
Hip (cm)	99 ± 2	115 ± 3	0.001
Waist-to-hip ratio	0.8 ± 0.02	0.85 ± 0.04	NS
Systolic blood pressure (mmHg)	114 ± 3	124 ± 4	0.06
Diasystolic blood pressure (mmHg)	71 ± 2	76 ± 2	0.06
Total cholesterol (mg/dl)	176 ± 6	182 ± 7	NS
Triglycerides (mg/dl)	76 ± 6	97 ± 10	NS
LDL cholesterol (mg/dl)	109 ± 5	116 ± 7	NS
HDL cholesterol (mg/dl)	54 ± 3	50 ± 2	NS
Basal insulin (pmol/l)	38 ± 6	79 ± 11	0.003
Basal lactate (mmol/l)	0.83 ± 0.08	0.95 ± 0.29	NS
Clamp lactate (mmol/l)	1.02 ± 0.08	0.92 ± 0.07	NS

Data are means ± SE.

and LDL and HDL cholesterol) were not different between the two groups.

Fasting plasma insulin (79 ± 11 vs. 38 ± 6 pmol/l, P = 0.003) and glucose (5.4 ± 0.1 [range 5.1–5.8] vs. 5.1 ± 0.1, [4.7–5.6 mmol/l], P = 0.05) were higher in the obese compared with lean subjects.

Venous plasma insulin, plasma glucose, and the glucose infusion rate required to maintain euglycemia throughout the 120-min insulin clamp are shown in Fig. 2*A*, *B*, and *C*, respectively. Plasma insulin levels remained higher in the obese group throughout the clamp for all but the final time point. Note in Fig. 2*B* that we allowed the venous plasma glucose levels throughout the clamp to decline slowly in both the obese and lean subjects. The mean difference in venous glucose concentration between the lean and the obese subjects over the last 20 min of the insulin clamp was ~0.4 mmol/l. During the clamp the mean glucose infusion rate (Fig. 2*C*) was higher in the lean compared with the obese subjects and over the last 5 min of insulin averaged 37.4 ± 2.6 and 19.3 ± 3.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, respectively ($P < 0.001$), despite comparable or slightly higher insulin concentrations in the obese subjects. During the clamp lactate levels increased modestly in the lean subjects (Table 1).

Brachial artery diameter, velocity, and flow are shown in Fig. 3. By 120 min of insulin infusion, brachial artery diameter (Fig. 3*A*) had increased in the lean (0.33 ± 0.01 to 0.35 ± 0.01 cm, $P < 0.001$) but was unchanged in the obese patients (Fig. 3*D*; (0.31 ± 0.02 and 0.32 ± 0.02 cm, NS). Brachial artery velocity trended higher during insulin infusion in the lean subjects (Fig. 3*B*; from 9.0 ± 1.6 to 13.0 ± 2.2 cm/s, P = 0.051), and brachial artery blood flow (Fig. 3*C*) rose from 46.5 ± 8.4 to 73.7 ± 12.1 ml/min (P = 0.027). In the obese group brachial artery velocity (Fig. 3*E*) and flow (Fig. 3*F*) were unchanged by insulin infusion (12.1 ± 2.3 to 9.5 ± 1.7 cm/s and 55.8 ± 11.3 to 44.6 ± 7.5 ml/min, respectively).

As shown in Fig. 4, compared with baseline, MBV (Fig. 4*A*) significantly increased by 120 min of insulin infusion in the lean group (18.7 ± 3.3 to 25.0 ± 4.1, P = 0.019), indicating muscle microvascular recruitment. In contrast, MBV was unchanged in the obese group (Fig. 4*D*) (20.4 ± 3.6 to 18.8 ± 3.8, NS). MFV did not change between baseline and 120 min in either study group (Fig. 4*B* and *E*). MFV was significantly higher in the lean compared with the obese group (0.16 ± 0.03 vs. 0.09 ± 0.01, $P < 0.025$). The product of MBV and MFV (Fig. 4*C* and *F*) did not significantly change in either group during insulin infusion (from 2.4 ± 0.2 to 3.1 ± 0.4 in the lean and 2.0 ± 0.4 to 2.2 ± 0.6 in the obese patients).

The relationship between glucose infusion rate and the change in MBV induced by insulin is shown in Fig. 5*A* (R = 0.394, P = 0.076), while Fig. 5*B* illustrates the relationship between BMI and the change in MBV between baseline and 120 min (R = −0.482, P = 0.027).

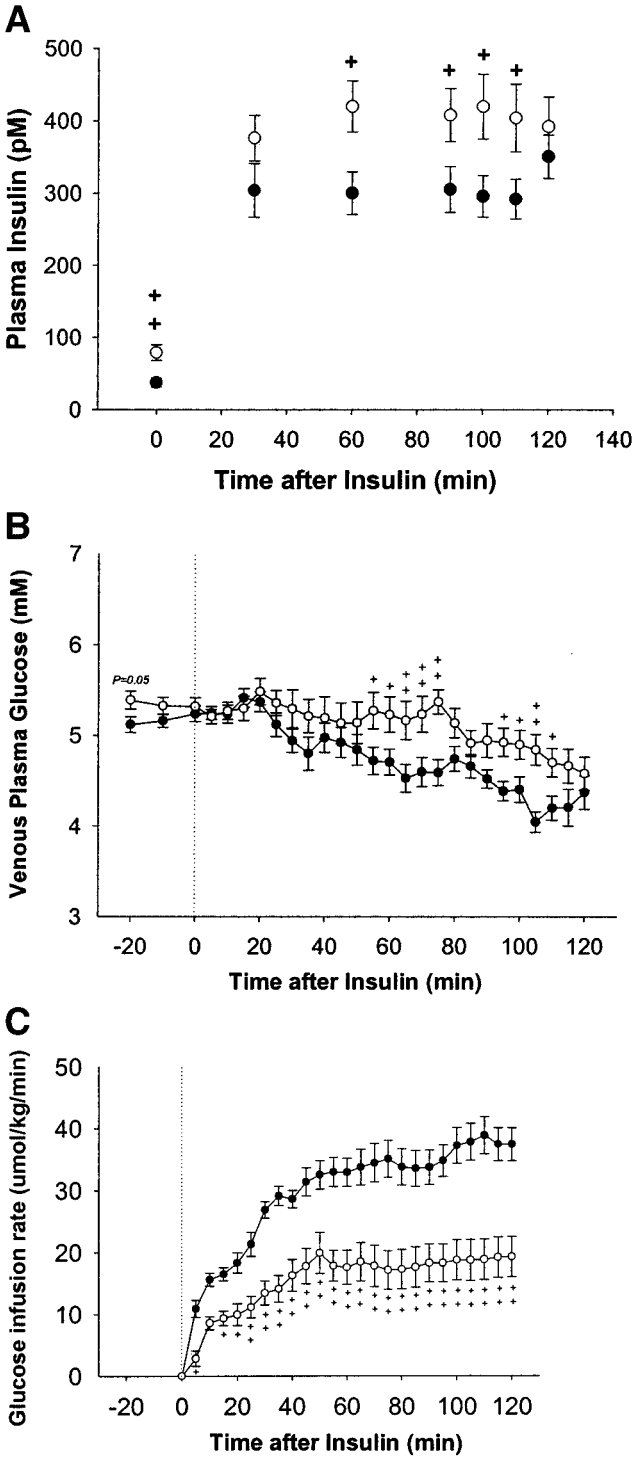

FIG. 2. Venous plasma insulin levels (*A*), glucose levels (*B*), and glucose infusion rate (*C*) at basal and throughout the 2-h insulin infusion. Values are means ± SE. ●, lean subjects; ○, obese subjects. +$P < 0.05$, ++$P < 0.01$ vs. lean subjects by repeated-measures ANOVA.

DISCUSSION

Obesity has been associated with impaired insulin-mediated leg blood flow responses in humans (8). In agreement with this, here we have shown that the increases in brachial artery diameter and flow observed in a cohort of lean individuals after insulin infusion was not seen in a group of otherwise healthy obese individuals. We had previously shown that 240 min of local insulin infusion into the brachial artery enhances forearm muscle microvascular volume in healthy young adults. In the current study, we observed that in lean healthy adults, systemically administered insulin similarly recruits microvascular vessels. The novel and potentially important finding of the current study was that forearm microvascular responses to insulin are severely blunted in otherwise healthy obese humans. Inasmuch as microvascular recruitment increases the endothelial exchange surface available for

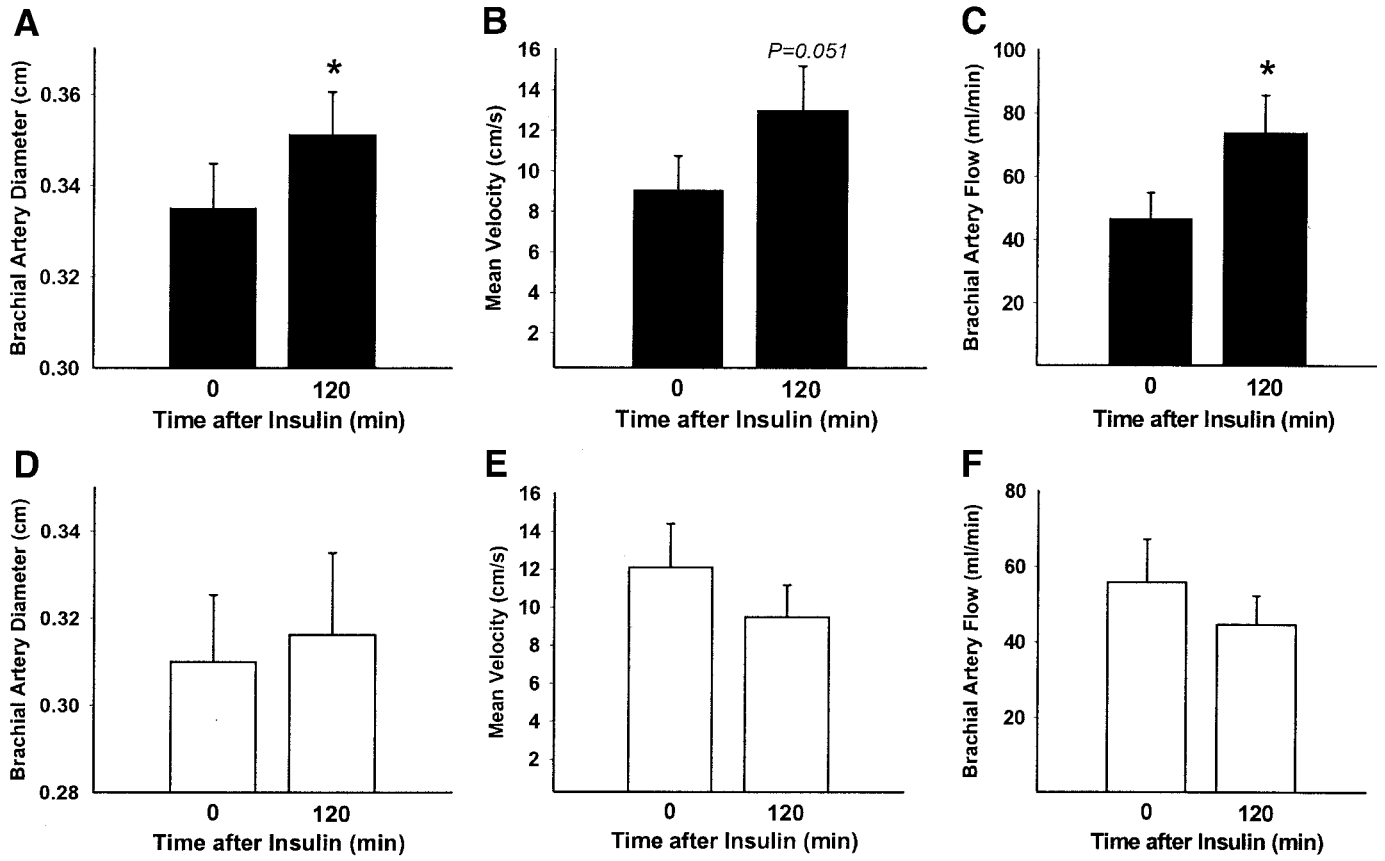

FIG. 3. Brachial artery diameter (*A* and *D*), velocity (*B* and *E*), and flow (*C* and *F*) before and at the end of a 2-h insulin infusion. Values are means ± SE. ■, lean subjects; □, obese subjects. **P* < 0.05 for 0 vs. 120 min of insulin by paired *t* test.

glucose and insulin to enter skeletal muscle (25), we would anticipate that the blunted recruitment seen in these obese individuals contributes at least in part to the diminished insulin-mediated glucose disposal seen under insulin clamp conditions. The absence of microvascular response in obese humans seen here is reminiscent of the absent microvascular response we observed in the obese Zucker rat (26).

We have previously pointed out that in rat skeletal muscle the actions of insulin on resistance vessels (which modulate total limb blood flow) and precapillary arterioles (which regulate microvascular recruitment) can be dissociated both by their differing time course (27) (microvascular recruitment precedes increases of total blood flow) as well as by their differing sensitivity to insulin (microvascular recruitment being more sensitive than total flow) (13). We did not attempt in the current study to address either concentration responses or the time course for insulin action. In part, this relates to limitations upon the amount of microbubbles that can be infused in humans. Typically this allows for only two (or in some cases three) sets of images to be obtained in a single study. However, in the current study, despite low doses of insulin and a study duration of only 2 h, we observed changes both in total blood flow as well as in microvascular recruitment in the control subjects. As a result, we cannot ascertain whether in humans the same differences in time course and sensitivity for insulin's actions on precapillary arterioles and resistance vessels prevail as in the rat.

It is important to recognize that the concentrations of insulin achieved during the clamp procedure are well within those seen physiologically during the course of an average meal. We recognize that within the literature there is controversy with regard to whether or not insulin at physiologic concentrations increases total blood flow in healthy individuals (28). In a previous study from our laboratory, local infusion of insulin into the brachial artery did not significantly increase forearm total blood flow even after 4 h of infusion; however, microvascular recruitment was observed (15). In the current study, with a systemic insulin infusion we observed both increases in total flow and microvascular recruitment. Whether the differences in route of insulin delivery contributed to this difference is uncertain. However, systemically administered insulin is known to increase plasma catecholamines (29,30) and cardiac output.

It is worth noting that we had modified the usual insulin clamp procedure in the current study. We did not use the "hot hand" method to arterialize venous blood. As noted in RESEARCH DESIGN AND METHODS, heating the hand per se increases blood flow both to the heated and the contralateral limbs. As blood flow was one of our critical end point measures, this had to be avoided. We might have simply done arterial sampling; however, we opted instead to use venous blood and allow a slow decline over the course of the insulin infusion anticipating the increasing arterial-venous glucose gradient consequent to insulin-mediated glucose disposal in both the lean and obese individuals. As shown in Fig. 2, to compensate for this and avoid hyperglycemia we allowed the venous plasma glucose concentration to decline by ~1 mmol/l in the lean and 0.6 mmol/l in the obese individuals. We recognize that attempting to

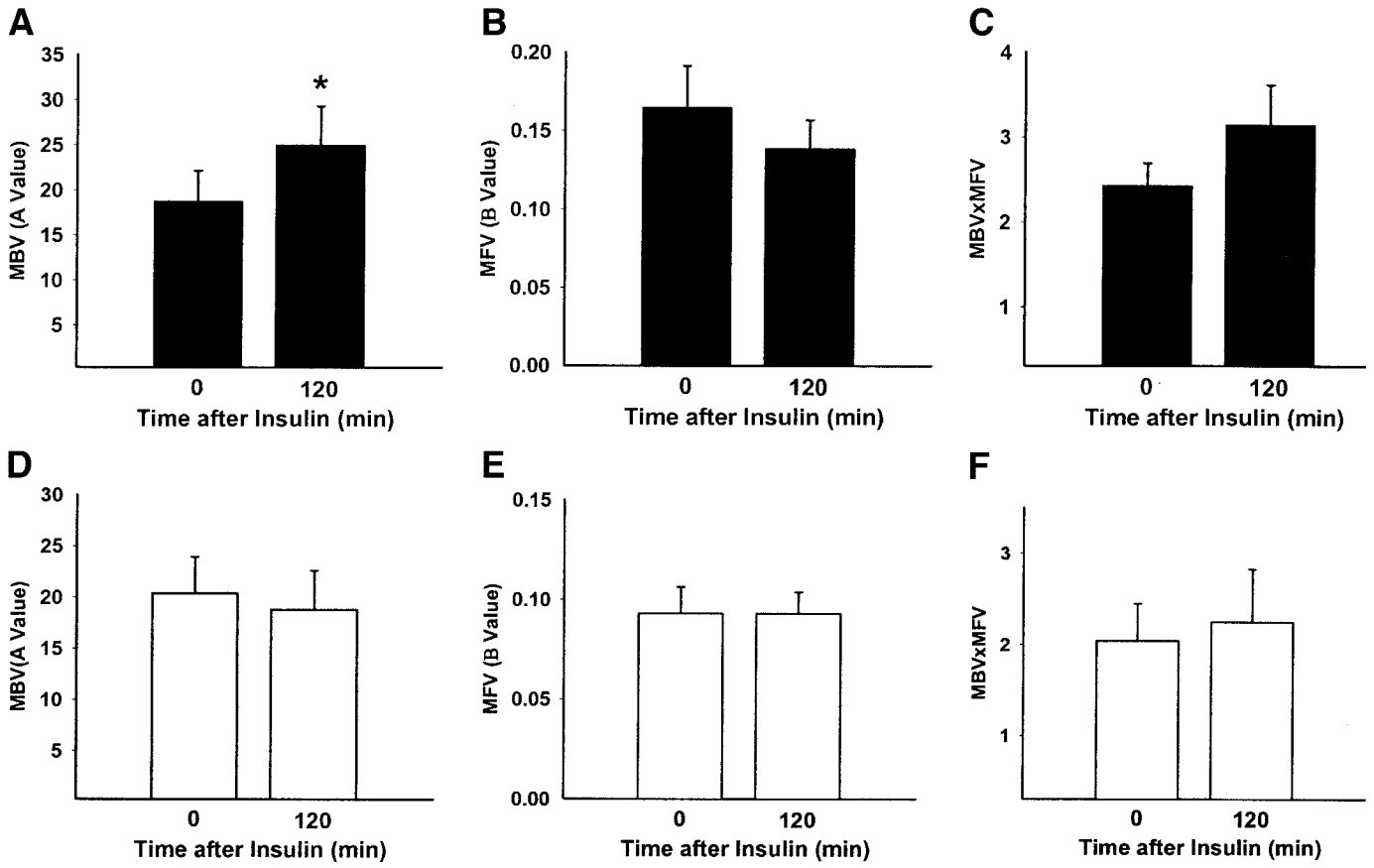

FIG. 4. Forearm MBV (*A* and *D*), MFV (*B* and *E*), and the product of MBV and MFV (*C* and *F*) before and at the end of the insulin infusion. Values are means ± SE. ■, lean subjects; □, obese subjects. **P* < 0.05 for 0 vs. 120 min of insulin by paired *t* test.

clamp plasma glucose without arterial sampling or heating of the extremity likely diminishes the precision of our clamp procedure. More particularly there is the possibility that the insulin-sensitive leaner subjects may have had a modest elevation in plasma glucose during the clamp procedure. The plasma insulin concentrations in the lean subjects, however, did not demonstrate any time-dependent rise, as might be expected if there were significant hyperglycemia, and the plasma insulin concentrations were similar or slightly lower than in the obese subjects. Likewise the change in plasma insulin concentration was similar in the two groups. We note that the lower venous plasma glucose concentrations obtained in the lean individuals would not be expected to trigger counterregulatory hormone release.

Our results reported here with regard to microvascular recruitment in skeletal muscle appear to be in good accord with those responses observed in skin capillaries (10). That obesity is associated with impaired microvascular recruitment in skeletal muscle is particularly important inasmuch as this appears to be the major site of insulin-mediated glucose disposal (at least under insulin clamp conditions) (31). Renkin and colleague (19,20) have pointed out that increasing total flow through an organ has little impact upon tissue substrate uptake unless there is a significant arterial-venous gradient. In contrast, expanding the capillary exchange surface can increase the delivery of nutrients or hormones to muscle in direct proportion to the surface area available for diffusion as predicted by Fick's law: $Js = P \times A(C_p - C_i)$, where Js is flux, P is permeability, A is area, and C_p and C_i are the plasma and interstitial concentrations, respectively (32). Thus, in the current study, the 34% increase in microvascular volume seen with the insulin infusion could be expected to directly enhance insulin and glucose delivery to the muscle. The impairment in insulin-mediated microvascular recruitment seen in the obese individuals might consequently contribute significantly to the overall impaired glucose disposal. Interestingly, in studies in the Zucker rat, we have observed that microvascular recruitment in response to insulin is totally blocked, and glucose disposal in the obese Zucker rat is severely impaired. However, microvascular recruitment in response to exercise is preserved in the Zucker rat, and glucose uptake with exercise in the obese Zucker rat is similar to that seen in the control rat (33). Whether microvascular recruitment in response to exercise might help to overcome some of the insulin resistance observed in human obesity remains to be investigated.

In conclusion, the current study provides the first evidence for impaired muscle microvascular action of insulin in human obesity. Inasmuch as insulin's microvascular actions appear important to enhancing the delivery of insulin and glucose to skeletal muscle, the impaired responses to insulin seen in the obese subjects may contribute to the impaired metabolic response to insulin vis-à-vis glucose disposal. Beyond that, our current findings, taken together with those previously published by others showing that obesity impairs insulin's action to increase the elasticity of conduit (9) and to dilate resistance vessels (8), suggest a generalized endothelial dysfunction throughout the arterial-arteriolar vascular tree in human obesity. The relationship of this to the endothelial dysfunction seen in

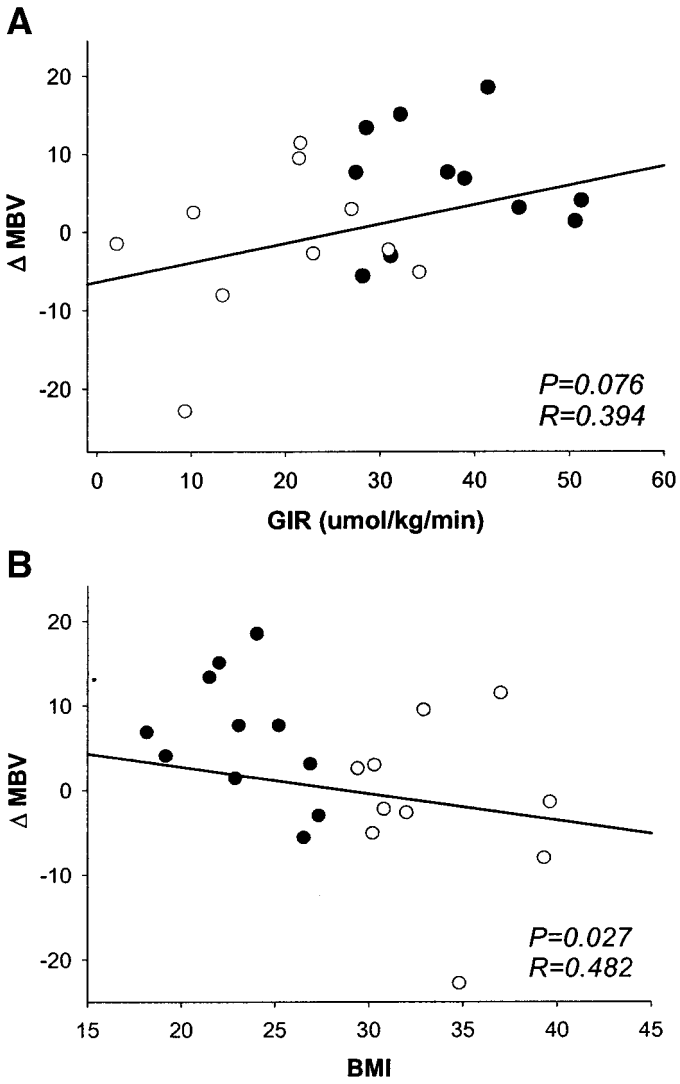

FIG. 5. Regression analysis of final glucose infusion rate (μmol · min^{-1} · kg^{-1}) versus change in MBV (*A*) and BMI versus change in MBV (*B*) after 120-min insulin infusion. Values are means ± SE. ●, lean subject; ○, obese subjects.

response to shear force or cholinergic agents and the increased cardiovascular risk of obesity remains to be delineated.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Grants DK-38578, DK-54058, and DK063609 (to E.J.B.) and RR00847 (to the University of Virginia General Clinical Research Center).

REFERENCES

1. Rabinowitz D, Zierler KL: Forearm metabolism in obesity and its response to intra-arterial insulin: characterization of insulin resistance and evidence for adaptive hyperinsulinism. *J Clin Invest* 41:2173–2181, 1962
2. Everson SA, Goldberg DE, Helmrich SP, Lakka TA, Lynch JW, Kaplan GA, Salonen JT: Weight gain and the risk of developing insulin resistance syndrome. *Diabetes Care* 21:1637–1643, 1998
3. Stern MP: Diabetes and cardiovascular disease: the "common soil" hypothesis. *Diabetes* 44:369–374, 1995
4. Calle EE, Thun MJ, Petrelli JM, Rodriguez C, Heath CW Jr: Body-mass index and mortality in a prospective cohort of U.S. adults [see comments]. *N Engl J Med* 341:1097–1105, 1999
5. Tack CJJ, Ong MKE, Lutterman JA, Smits P: Insulin-induced vasodilatation and endothelial function in obesity/insulin resistance: effects of troglitazone. *Diabetologia* 41:569–576, 1998
6. 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
7. Hashimoto M, Akishita M, Eto M, Kozaki K, Ako J, Sugimoto N, Yoshizumi M, Toba K, Ouchi Y: The impairment of flow-mediated vasodilatation in obese men with visceral fat accumulation. *Int J Obes Relat Metab Disord* 22:477–484, 1998
8. Laasko M, Edelman SV, Brechtel G, Baron AD: Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. *J Clin Invest* 85:1844–1852, 1990
9. Westerbacka J, Vehkavaara S, Bergholm R, Wilkinson I, Cockcroft J, Yki-Jarvinen H: Marked resistance of the ability of insulin to decrease arterial stiffness characterizes human obesity. *Diabetes* 48:821–827, 1999
10. de Jongh RT, Serne EH, RG IJ, de Vries G, Stehouwer CD: Impaired microvascular function in obesity: implications for obesity-associated microangiopathy, hypertension, and insulin resistance. *Circulation* 109: 2529–2535, 2004
11. Rattigan S, Clark MG, Barrett EJ: Hemodynamic actions of insulin in rat skeletal muscle: evidence for capillary recruitment. *Diabetes* 46:1381–1388, 1997
12. Rattigan S, Clark MG, Barrett EJ: Acute insulin resistance in rat skeletal muscle in vivo induced by vasoconstriction. *Diabetes* 48:564–569, 1999
13. Zhang L, Vincent MA, Richards SM, Clerk LH, Rattigan S, Clark MG, Barrett EJ: Insulin sensitivity of muscle capillary recruitment in vivo. *Diabetes* 53:447–453, 2004
14. Vincent MA, Clerk LH, Lindner JR, Klibanov AL, Clark MG, Rattigan S, Barrett EJ: Microvascular recruitment is an early insulin effect that regulates skeletal muscle glucose uptake in vivo. *Diabetes* 53:1418–1423, 2004
15. Coggins MP, Lindner J, Rattigan S, Fasy E, Jahn L, Kaul S, Barrett EJ: Physiologic hyperinsulinemia enhances human skeletal muscle perfusion by capillary recruitment. *Diabetes* 50:2682–2690, 2001
16. Vincent MA, Barrett EJ, Lindner JR, Clark MG, Rattigan S: Inhibiting NOS blocks microvascular recruitment and blunts muscle glucose uptake in response to insulin. *Am J Physiol Endocrinol Metab* 285:E123–E129, 2003
17. Youd JM, Rattigan S, Clark MG: Acute impairment of insulin-mediated capillary recruitment and glucose uptake in rat skeletal muscle in vivo by TNF-α. *Diabetes* 49:1904–1909, 2000
18. Clerk LH, Rattigan S, Clark MG: Lipid infusion impairs physiologic insulin-mediated capillary recruitment and muscle glucose uptake in vivo. *Diabetes* 51:1138–1145, 2002
19. Renkin EM: B. W. Zweifach Award lecture: Regulation of the microcirculation. *Microvasc Res* 30:251–263, 1985
20. Renkin EM, Crone C: Microcirculation and capillary exchange. In *Comprehensive Human Physiology.* 1st ed. Greger R, Windhorst U, Eds. Berlin-Heidelberg, Springer-Verlag, 1996, p. 1965–1979
21. Honig CR, Odoroff CL, Frierson JL: Active and passive capillary control in red muscle at rest and in exercise. *Am J Physiol* 243:H196–H206, 1982
22. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique, a method for quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979
23. Astrup A, Simonsen L, Bulow J, Christensen NJ: Measurement of forearm oxygen consumption: role of heating the contralateral hand. *Am J Physiol* 255:E572–E578, 1988
24. Wei K, Jayaweera AR, Firoozan S, Linka A, Skyba DM, Kaul S: Quantification of myocardial blood flow with ultrasound-induced destruction of microbubbles administered as a constant venous infusion. *Circulation* 97:473–483, 1998
25. Clerk LH, Vincent MA, Lindner JR, Clark MG, Rattigan S, Barrett EJ: The vasodilatory actions of insulin on resistance and terminal arterioles and their impact on muscle glucose uptake. *Diabete Metab Res Rev* 20:3–12, 2004
26. Wallis MG, Wheatley CM, Rattigan S, Barrett EJ, Clark AD, Clark MG: Insulin-mediated hemodynamic changes are impaired in muscle of Zucker obese rats. *Diabetes* 51:3492–3498, 2002
27. Vincent MA, Dawson D, Clark AD, Lindner JR, Rattigan S, Clark MG, Barrett EJ: Skeletal muscle microvascular recruitment by physiological hyperinsulinemia precedes increases in total blood flow. *Diabetes* 51:42–48, 2002
28. Yki-Jarvinen H, Utriainen T: Insulin-induced vasodilatation: physiology or pharmacology? *Diabetologia* 41:369–379, 1998
29. Vollenweider P, Tappy L, Randin D, Schneiter P, Jequier E, Nicod P, Scherrer U: Differential effects of hyperinsulinemia and carbohydrate metabolism on sympathetic nerve activity and muscle blood flow in humans. *J Clin Invest* 92:147–154, 1993

30. Tack CJJ, Lenders JWM, Willemsen JJ, Vandruten JAM, Thien T, Lutterman JA, Smits P: Insulin stimulates epinephrine release under euglycemic conditions in humans. *Metab Clin Exp* 47:243–249, 1998
31. DeFronzo RA, Ferrannini E, Hendler R, Felig P, Wahren J: Regulation of splanchnic and peripheral glucose uptake by insulin and hyperglycemia in man. *Diabetes* 32:35–45, 1983
32. Bonadonna RC, Saccomani MP, Delprato S, Bonora E, Defronzo RA, Cobelli C: Role of tissue-specific blood flow and tissue recruitment in insulin-mediated glucose uptake of human skeletal muscle. *Circulation* 98:234–241, 1998
33. Wheatley CM, Rattigan S, Richards SM, Barrett EJ, Clark MG: Skeletal muscle contraction stimulates capillary recruitment and glucose uptake in insulin-resistant obese Zucker rats. *Am J Physiol Endocrinol Metab* 287:E804–E809, 2004