

Obesity Blunts Microvascular Recruitment in Human Forearm Muscle After a Mixed Meal

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OBJECTIVE— Ingestion of a mixed meal recruits flow to muscle capillaries and increases total forearm blood flow in healthy young lean people. We examined whether these vascular responses are blunted by obesity.

RESEARCH DESIGN AND METHODS— We fed eight middle-aged lean and eight obese overnight-fasted volunteers a liquid mixed meal (480 kcal). Plasma glucose and insulin were measured every 30 min, and brachial artery flow and muscle microvascular recruitment (contrast ultrasound) were assessed every 60 min over 2 h after the meal.

RESULTS— By 30 min, plasma glucose rose in both the lean (5.1 ± 0.1 vs. 6.7 ± 0.4 mmol/l, $P < 0.05$) and the obese groups (5.4 ± 0.2 vs. 6.7 ± 0.4 mmol/l, $P < 0.05$). Plasma insulin rose (28 ± 4 vs. 241 ± 30 pmol/l, $P < 0.05$) by 30 min in the lean group and remained elevated for 2 h. The obese group had higher fasting plasma insulin levels (65 ± 8 pmol/l, $P < 0.001$) and a greater postmeal area under the insulin-time curve ($P < 0.05$). Brachial artery flow was increased at 120 min after the meal in the lean group (38 ± 6 vs. 83 ± 16 ml/min, $P < 0.05$) but not in the obese group. Muscle microvascular blood volume rose by 120 min in the lean group (14.4 ± 2.2 vs. 24.4 ± 4.2 units, $P < 0.05$) but not in the obese group.

CONCLUSIONS— A mixed meal recruits muscle microvasculature in lean subjects, and this effect is blunted by obesity. This impaired vascular recruitment lessens the endothelial surface available and may thereby impair postprandial glucose disposal.

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Skeletal muscle is a major site for insulin-mediated glucose storage and thereby influences postprandial plasma glucose levels (1). Over the past 15 years it has become increasingly evident that insulin exerts important actions on muscle vasculature as an integral part of its action to increase glucose disposal (2–5). Within muscle the vascular endothelium is the first tissue that insulin encounters. A primary function of the endothelium is to serve as a permeability barrier that restricts transport of macromolecules such as insulin into the interstitial space. In addition to modulating delivery of insulin to muscle cells, the en-

dothelium also directly responds to insulin by increasing production of nitric oxide, a potent vasodilator, and endothelin-1, a potent vasoconstrictor, to regulate muscle blood flow (6,7).

Baron and colleagues (2,8,9) hypothesized that insulin increases leg blood flow to facilitate access of glucose and insulin to muscle, thereby increasing glucose disposal. Blocking insulin-induced increases in blood flow reduced insulin-stimulated glucose disposal by ~30%. Furthermore, this vascular action of insulin was significantly blunted in insulin-resistant obese humans (8).

These investigators used a thermolimitation method that measured only total limb blood flow and not microvascular responses. Others have measured the effect of insulin on limb blood flow using a variety of methods with divergent findings (10,11). Our laboratories have developed new methods that assess the microvascular action of insulin in muscle (3,5,12,13). We reasoned that microvascular perfusion directly mediates nutrient exchange in muscle (14). We have previously shown that insulin acts on preterminal arterioles (which control microvascular blood flow) more rapidly and at more physiologically relevant insulin concentrations than is the case for resistance arteries (which control total muscle blood flow) (3,5,12).

Using contrast-enhanced ultrasound (CEU) and the euglycemic insulin clamp method, we observed that physiological hyperinsulinemia expands the volume of microvasculature perfused in forearm muscle of healthy lean people (4,15) and that this action of insulin is markedly impaired in obese subjects (4). Indeed, we observed a negative correlation between insulin-mediated microvascular recruitment in muscle and BMI (4). Although the clamp technique provides an excellent assessment of the physiological actions of insulin, it does not mimic the changes that typically occur after the ingestion of a mixed meal. With the meal there are changes in blood glucose, amino acids, gut hormones, and parasympathetic/sympathetic tone. Few studies have reported on the effect of a meal on total limb blood flow, and these have yielded conflicting results. Although some investigators reported that the ingestion of a mixed meal (16) or an oral glucose load (17) increased total limb blood flow, others reported no effect (18). These differences may relate to techniques used to measure flow, the types of meals ingested, and differences in the population studied. However, in each of these clinical studies, investigators have measured only total limb blood flow and not microvascular responses.

We have recently reported that ingestion of a mixed meal by lean young adults

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increases total forearm blood flow and increases microvascular flow (19). These vascular responses occurred at a time when plasma insulin was significantly elevated. Whether total muscle blood flow along with muscle microvascular recruitment after the ingestion of a mixed meal is normal or blunted in insulin-resistant obese subjects is unknown, and addressing this issue was the goal of the current study. We used Doppler ultrasound to study total forearm blood flow and CEU to quantify muscle microvascular responses of forearm muscle in healthy lean and otherwise healthy obese age-matched adults in response to a mixed meal. We hypothesized that both total limb blood flow and the microvascular response would be blunted by obesity.

RESEARCH DESIGN AND METHODS

The study protocol was approved by the University of Virginia Human Investigation Committee. A total of eight lean (three men and five women; BMI <25 kg/m²) and eight obese (one man and seven women; BMI >30 kg/m²) volunteers with no history of hypertension, diabetes, or hyperlipidemia were recruited and fasted overnight. Subjects were excluded from the study if they had a family history of a first-degree relative with diagnosed diabetes, had a history of smoking, or were taking any medication known to affect either endothelial function or glucose metabolism. Subjects were admitted to the General Clinical Research Center on two separate occasions. On a screening visit serum clinical chemistry values were measured by the University of Virginia clinical chemistry laboratories, and body composition was measured using an air displacement chamber (BOD POD); height, weight, and hip and waist circumferences were also measured. On the day of the study visit, overnight-fasted subjects were admitted to the General Clinical Research Center on the morning of the study. A lifestyle questionnaire was used before the study to assess level of physical activity.

Study visit

Catheters were placed in the antecubital veins of both arms. Subjects were studied in a temperature-controlled room. Subjects remained in bed 1 h before and for the duration of the study and were instructed to refrain from using their arms to minimize effects of movement (or exercise) on hemodynamic measurements. The catheter in the nondominant arm was

used for blood sampling of glucose and insulin, and the contralateral arm was used for the infusion of microbubbles. Baseline measurements of total forearm blood flow (assessed by Doppler ultrasound) and muscle microvascular blood volume (MBV) and microvascular flow velocity (MFV) (assessed by CEU) were performed as described previously (4,19). Subjects were then given a liquid meal (Boost; 480 kcal: 72 from fat, 328 from carbohydrate, and 80 from protein). A mixed meal was chosen to assess the physiological changes that occur in response to a typical meal. A liquid meal was administered to shorten the amount of time needed for digestion and absorption. The subjects drank the meal within 5 min. Plasma glucose and insulin were measured at 0, 30, 60, 90, and 120 min. Plasma glucose was measured using a YSI analyzer (YSI, Yellow Springs, OH). Plasma insulin was measured using an enzyme-linked immunosorbent assay with an interassay coefficient of variation of <4%. At 60 and again at 120 min after the mixed meal, brachial artery flow (Doppler ultrasound) and MBV and MFV (CEU) were measured.

Doppler ultrasound

For the two-dimensional and Doppler ultrasound measurements, an ultrasound system (HDI 5000; Philips Medical Systems, Bothell, WA) with a linear-array transducer was used with a transmit frequency of 12 MHz. Two-dimensional imaging of the brachial artery was performed in the long axis ~5 cm proximal to the antecubital fold. Images were triggered to the R wave of the cardiac cycle, and the brachial artery diameter was measured using on-line video calipers. A pulsed-wave Doppler sample blood volume was placed at the same location in the center of the artery and the mean blood velocity was measured using online angle correction and analysis software. Brachial artery mean blood flow was calculated from two-dimensional and Doppler ultrasound data using the equation: $Q = v\pi \cdot (d/2)^2$, where Q is brachial blood flow, v is mean brachial artery blood flow velocity, and d is brachial artery diameter.

CEU

CEU imaging was performed as described previously (4,19). In brief, imaging of the forearm was performed in a transaxial plane 5 cm distal to be antecubital fossa, using power Doppler imaging. A suspension of microbubbles (Definity; Bristol-

Myers Squibb) was infused intravenously at a rate of 3.0 ml/min for 10 min. Once the systemic microbubble concentration reached steady state (>2 min), intermittent imaging was then performed at pulsing intervals ranging from 0.5 to 25 s, thus allowing progressively greater replenishment of microbubbles in the ultrasound beam elevation. At least three images were acquired at each pulsing interval. Images were digitized to an off-line image analysis system. Several background frames at 0.5 s were averaged and digitally subtracted from similarly averaged frames at each pulsing interval. Background-subtracted video intensity at each pulsing interval was measured from a region of interest placed around the deep forearm flexor muscles. Pulsing interval versus video intensity data were fitted to the function $y = A(1 - e^{-\beta t})$, where y is video intensity at pulsing interval t , A is plateau video intensity (MBV, an index of microvascular recruitment), and β is the rate constant that provides a measure of MFV (the rate of microvascular filling) (20).

Statistics

Data are presented as the means \pm SEM, and statistical analysis was performed using SigmaStat (Systat Software, 2004). Comparisons between lean and obese subjects were made using unpaired Student's t tests. Time-series measurements in each group were performed by two-way repeated-measures ANOVA. When data were not normally distributed, repeated-measures ANOVA on ranks was performed. When a significant difference of $P < 0.05$ was found, pairwise comparisons by a Student-Newman-Keuls test was used to assess treatment differences.

RESULTS — The baseline characteristics of the lean and obese groups are given in Table 1. The lean subjects had significantly lower body weight, fat weight, BMI, percent body fat, waist and hip circumferences, and fasting triglycerides compared with the obese subjects. HDL cholesterol was significantly higher in the lean than in the obese group. Fasting plasma glucose, total cholesterol, and LDL cholesterol did not differ between the two groups. The obese group had higher fasting plasma insulin levels ($P < 0.001$) and lower insulin sensitivity in the fasting state (estimated using the quantitative insulin sensitivity check index, a surrogate index of insulin sensitivity [21]). Although the lean group tended to

Table 1—Characteristics of lean and obese subjects

	Lean	Obese	P
Age	40.5 ± 4.1	41.8 ± 2.7	NS
Height (cm)	171 ± 3	163 ± 3	NS
Weight (kg)	65.7 ± 5.1	88.3 ± 2.8	<0.001
BMI (kg/m ²)	22.4 ± 0.9	33.7 ± 1.00	<0.001
% body fat	22.7 ± 1.5	42.8 ± 2.4	<0.001
Fat weight (kg)	15.1 ± 1.7	38.3 ± 3.1	<0.001
Lean weight (kg)	50.6 ± 4.0	50.5 ± 2.0	NS
Waist circumference (cm)	74.0 ± 3.5	96.6 ± 2.6	<0.001
Hip circumference (cm)	95.6 ± 2.0	114.4 ± 1.9	<0.001
Total cholesterol (mg/dl)	177 ± 7	185 ± 5	NS
Triglycerides (mg/dl)	62 ± 9	117 ± 12	<0.005
LDL cholesterol (mg/dl)	103 ± 9	116 ± 5	NS
HDL cholesterol (mg/dl)	64 ± 5	49 ± 3	<0.05
Plasma glucose (mmol/l)	5.1 ± 0.1	5.4 ± 0.2	NS
Plasma insulin (pmol/l)	28 ± 4	65 ± 8	<0.001
QUICKI	0.389 ± 0.011	0.336 ± 0.005	<0.001
Minimal model (× 10 ³ min · μU ⁻¹ · ml ⁻¹)	2.58 ± 0.58	1.17 ± 0.31	<0.05
Energy expenditure (kcal/day)	1,206 ± 399	849 ± 248	NS
Physical activity (h/week)	32 ± 8	26 ± 10	NS

Data are means ± SEM. QUICKI, quantitative insulin sensitivity check index.

be more active, there were no significant changes in estimated energy expenditure per day or in total physical activity hours per week (Table 1).

Figure 1 shows the time course of plasma glucose and insulin levels after the ingestion of the mixed meal. By 30 min after the meal, plasma glucose levels were elevated above baseline for both lean and obese groups (Fig. 1A). Over the course of the next 90 min, plasma glucose levels declined toward baseline. There were no significant differences in the time course of changes in plasma glucose between the two groups. Plasma insulin levels rose significantly by 30 min in the lean group and remained elevated for the additional 90 min of the study (Fig. 1B). The obese group had a significantly higher baseline (fasting) plasma insulin level ($P < 0.001$) and a greater postmeal area under the insulin-time curve than the lean group ($P < 0.05$, data not shown).

Brachial artery diameter, velocity, and flow are shown in Fig. 2. Baseline brachial artery diameters in the lean and obese groups were not significantly different from each other (Fig. 2A). After the meal, the brachial artery in the lean group dilated significantly ($P < 0.05$) by 60 min and remained dilated at 120 min. In the obese group, the brachial artery diameter did not change from baseline and was significantly smaller than that for the lean group at 60 min ($P < 0.05$) and was almost significant at 120 min ($P = 0.055$) after the meal (Fig. 2A). Bra-

chial artery flow velocity was markedly elevated above baseline ($P < 0.05$) in the lean group by 120 min after the meal (Fig. 2B). This effect was absent in the obese group (Fig. 2B). Brachial artery blood flow increased by 120 min after the meal in the lean group ($P < 0.05$) but not in the obese group (NS) (Fig. 2C).

Figure 3 shows the effect of the mixed meal on forearm microvascular responses. Despite hyperglycemia, muscle MBV rose significantly by 120 min in the lean group indicating muscle microvascular recruitment (Fig. 3A). In contrast, MBV was unchanged in the obese group (NS) (Fig. 3A). When the data from the female subjects were compared, we found that the lean group still showed a significant ($P = 0.02$) increase in MBV, which was absent in the obese group ($P = 0.45$). This finding suggests that it was obesity and not sex that was the distinguishing feature. The MBV increase in the lean group was accompanied by a trend for MFV to decline by 120 min ($P = 0.065$) when compared with baseline (Fig. 3B). MFV did not change in the obese group in

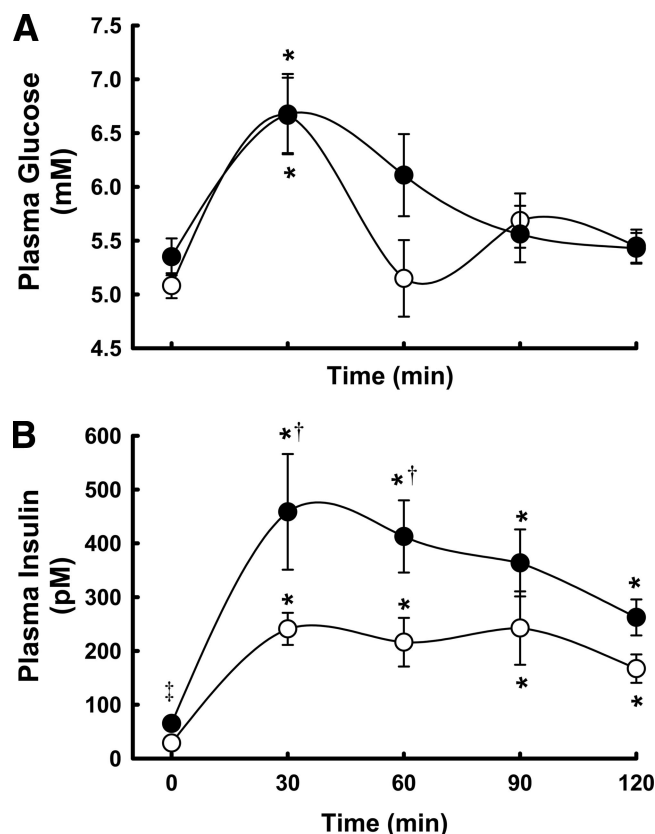


Figure 1—Time courses of plasma glucose and insulin concentrations after the ingestion of a mixed meal in lean (○, n = 8) and obese (●, n = 8) volunteers. Measurements were made every 30 min for 2 h. Data are means ± SEM. * $P < 0.05$ vs. 0 min; † $P < 0.05$ vs. lean; two-way repeated-measures ANOVA and post hoc Student-Newman-Keuls test. ‡ $P < 0.001$ vs. lean; Student's unpaired t test.

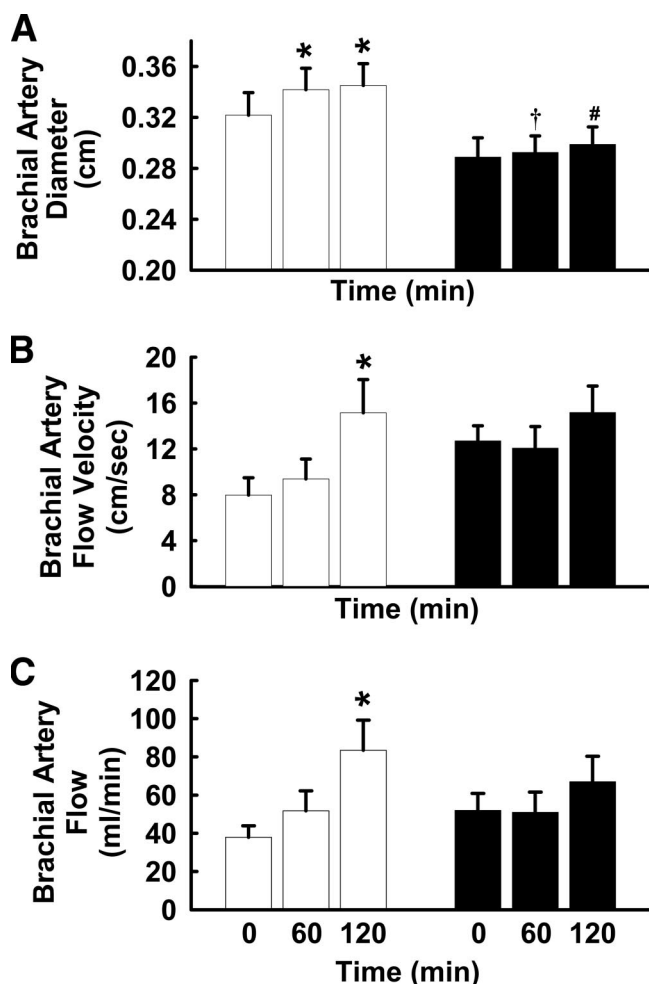


Figure 2—Time courses of brachial artery diameter, brachial artery flow velocity, and total brachial artery blood flow measured by Doppler ultrasound after the ingestion of a mixed meal in lean (\square , $n = 8$) and obese (\blacksquare , $n = 8$) volunteers. Data are means \pm SEM. * $P < 0.05$ vs. 0 min; † $P < 0.05$ vs. lean; # $P = 0.055$ vs. lean; two-way repeated-measures ANOVA and post hoc Student-Newman-Keuls test.

response to the meal challenge (Fig. 3B). The product of MBV and MFV (muscle microvascular perfusion) did not significantly change in response to the mixed meal in either group (Fig. 3C).

CONCLUSIONS—The present study demonstrated that the ingestion of a mixed meal significantly increased total muscle blood flow and MBV of lean, middle-aged volunteers and that both of these vascular responses are blunted by obesity. The meal-induced increase in MBV in the lean volunteers reported here is similar to that reported previously in young, lean adults (19). In that study, MBV increased even earlier (60 min) than reported in the current study (120 min). The somewhat slower response noted here may represent an age-related phenomenon; however, this has not been tested directly. The most

striking finding of the current study is the absence of any increase in MBV in the obese individuals.

We have noted previously that obesity appears to prevent microvascular recruitment in response to euglycemic hyperinsulinemia (4). In that study, plasma insulin levels were increased to high physiological levels, and total forearm blood flow did not change in either lean or obese subjects (4). In the current study, the ingestion of a mixed meal increased total limb blood flow in lean but not in obese subjects. The increase in blood flow noted in the lean subjects serves to emphasize the difference in stimulus of insulin alone under physiological euglycemic conditions.

Scognamiglio et al. (22) demonstrated that the ingestion of a mixed

meal increased myocardial microvascular perfusion in healthy control subjects. However, myocardial microvascular perfusion actually diminished in the patients with type 2 diabetes. In the current study, we also found an increase in microvascular perfusion in skeletal muscle in response to a mixed meal in lean subjects, and this effect was blunted in the obese subjects. We did not observe a net decline in microvascular perfusion as reported by Scognamiglio et al. The lack of microvascular response in the obese subjects in response to the meal may simply reflect resistance to the vascular action of insulin as was indicated by the insulin clamp studies in obese subjects (4). However, we cannot discount the possibility that obesity altered microvascular responses to other hormonal, nutritional, cardiac, and neural factors that change in the postprandial state. The lack of a significant change in MBV in the obese group is unlikely because of increased muscle adiposity quenching the microbubble signal. We have shown (P. St-Pierre, M.A.K., S.M. Richards, and S. Rattigan, unpublished observations) that high fat-fed, insulin-resistant rats with increased tissue adiposity have a microbubble dose-response curve similar to that in healthy lean rats. Thus, the effects seen herein are due to changes in microvascular perfusion and not to changes in signal characteristics of the microbubble in vivo.

Expanding MBV enhances the surface area of the vascular endothelium, which affords nutrients and hormones, such as insulin, increased access for exchange with the muscle interstitium. For insulin, this entry into the muscle interstitium appears to be rate limiting for overall insulin action within skeletal muscle (23,24). In this manner insulin, which signals to the body that feeding has occurred, may exert a feed-forward signal to enhance muscle blood flow and thereby increase both its own access to the myocyte as well as that of other nutrients such as glucose and amino acids within the meal.

In the current study it remains possible that the failure of insulin to exert a vascular action within the skeletal muscle of obese subjects contributed to their significantly raised postprandial insulin levels. If these increases in insulin and glucose levels occur chronically, they have the potential to increase hepatic VLDL production and raise plasma triglyceride concentrations (25), as occurs in insulin-resistant obese subjects (Table 1).

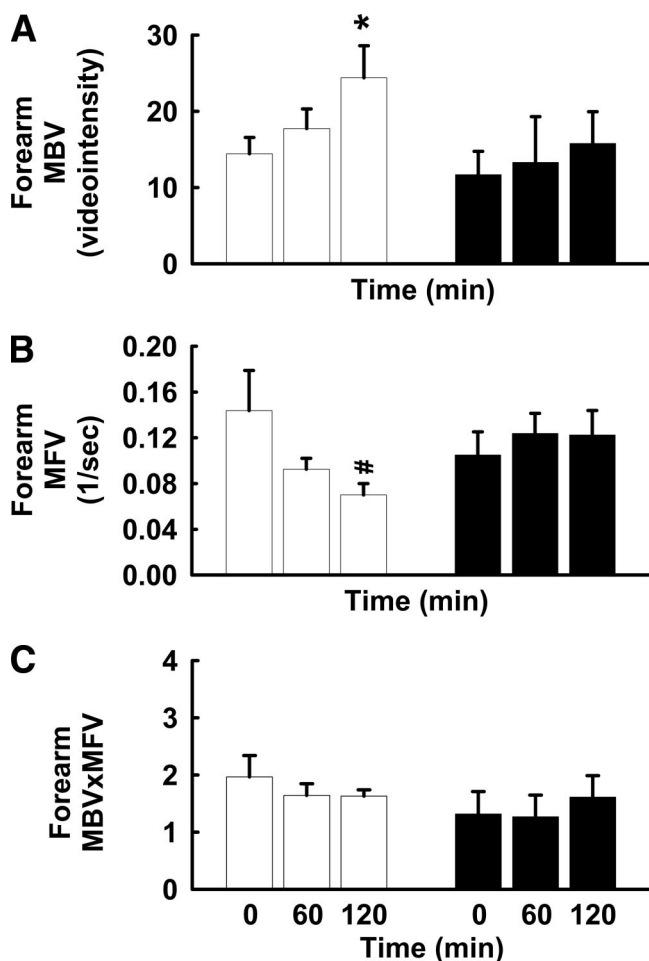


Figure 3—Time course of forearm MBV, MFV, and MBV × MFV measured by contrast-enhanced ultrasound after the ingestion of a mixed meal in lean (□, n = 8) and obese (■, n = 8) volunteers. Data are means ± SEM. *P < 0.05 vs. 0 min; #P = 0.065 vs. 0 min; repeated-measures ANOVA on ranks and post hoc Student-Newman-Keuls test.

A curious aspect of the current study was the observed increase in forearm blood flow in the lean subjects when muscle microvascular blood flow (MBV × MFV) was not altered. Several factors may account for this effect. First, total forearm blood flow is measured 5–10 min before the measurement of microvascular flow (these measurements cannot be done simultaneously because of interference of microbubbles with Doppler recording). Second, the microvascular blood flow measurements are an integrated value of flow occurring over ~5 min of data acquisition, whereas Doppler flow measurements are made more acutely over the course of 10–30 heartbeats. Third, forearm blood flow includes flow to subcutaneous adipose tissue, muscle, bone, and the hand, whereas the microvascular flow assessment is restricted to blood flow within muscle. A fourth possibility

is that microvascular blood flow within muscle is distributed between nutritive and non-nutritive flow routes, with insulin increasing nutritive flow (3).

In summary, the findings reported here suggest a physiological role for microvascular recruitment provoked by meal ingestion in the process of delivery of nutrients and hormones to skeletal muscle. Failure of this microvascular recruitment, particularly in obese individuals, may be one factor that contributes to postprandial hyperinsulinemia associated with insulin resistance/metabolic syndrome.

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No potential conflicts of interest relevant to this article were reported.

References

- DeFronzo RA, Ferrannini E, Sato Y, Felig P, Wahren J. Synergistic interaction between exercise and insulin on peripheral glucose uptake. *J Clin Invest* 1981;68:1468–1474
- Baron AD. Hemodynamic actions of insulin. *Am J Physiol* 1994;267:E187–E202
- Rattigan S, Clark MG, Barrett EJ. Hemodynamic actions of insulin in rat skeletal muscle: evidence for capillary recruitment. *Diabetes* 1997;46:1381–1388
- Clerk LH, Vincent MA, Jahn LA, Liu Z, Lindner JR, Barrett EJ. Obesity blunts insulin-mediated microvascular recruitment in human forearm muscle. *Diabetes* 2006;55:1436–1442
- 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* 2002;51:42–48
- Montagnani M, Chen H, Barr VA, Quon MJ. Insulin-stimulated activation of eNOS is independent of Ca²⁺ but requires phosphorylation by Akt at Ser¹¹⁷⁹. *J Biol Chem* 2001;276:30392–30398
- Cardillo C, Nambi SS, Kilcoyne CM, Choucair WK, Katz A, Quon MJ, Panza JA. Insulin stimulates both endothelin and nitric oxide activity in the human forearm. *Circulation* 1999;100:820–825
- Laakso M, Edelman SV, Brechtel G, Baron AD. Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man: a novel mechanism for insulin resistance. *J Clin Invest* 1990;85:1844–1852
- Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD. Insulin-mediated skeletal muscle vasodilation is nitric oxide dependent: a novel action of insulin to increase nitric oxide release. *J Clin Invest* 1994;94:1172–1179
- Utriainen T, Malmstrom R, Makimattila S, Yki-Jarvinen H. Methodological aspects, dose-response characteristics and causes of interindividual variation in insulin stimulation of limb blood flow in normal subjects. *Diabetologia* 1995;38:555–564
- Scherrer U, Randin D, Vollenweider P, Vollenweider L, Nicod P. Nitric oxide release accounts for insulin's vascular effects in humans. *J Clin Invest* 1994;94:2511–2515
- 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* 2004;53:1418–1423
- Dawson D, Vincent MA, Barrett EJ, Kaul S, Clark A, Leong-Poi H, Lindner JR. Vas-

- cular recruitment in skeletal muscle during exercise and hyperinsulinemia assessed by contrast ultrasound. *Am J Physiol Endocrinol Metab* 2002;282:E714–E720
14. Renkin EM, Renkin EM, Michel CC, Geiger SR. Control of microcirculation and blood-tissue exchange. In *Handbook of Physiology. Section 2: The Cardiovascular System*. Vol. IV. Renkin E, Michel CC, Eds. Bethesda, MD, American Physiological Society, 1984, p. 627–687
 15. Coggins M, Lindner J, Rattigan S, Jahn L, Fasy E, Kaul S, Barrett E. Physiologic hyperinsulinemia enhances human skeletal muscle perfusion by capillary recruitment. *Diabetes* 2001;50:2682–2690
 16. Hoost U, Kelbaek H, Rasmussen H, Court P, Christensen NJ, Pedersen-Bjergaard U, Lorenzen T. Haemodynamic effects of eating: the role of meal composition. *Clin Sci* 1996;90:269–276
 17. Baron AD, Laakso M, Brechtel G, Hoit B, Watt C, Edelman SV. Reduced postprandial skeletal muscle blood flow contributes to glucose intolerance in human obesity. *J Clin Endocrinol Metab* 1990;70:1525–1533
 18. Hernandez MA, Jensen MD. Contribution of blood flow to leg glucose uptake during a mixed meal. *Diabetes* 1995;44:1165–1169
 19. Vincent MA, Clerk LH, Lindner JR, Price WJ, Jahn LA, Leong-Poi H, Barrett EJ. Mixed meal and light exercise each recruit muscle capillaries in healthy humans. *Am J Physiol Endocrinol Metab* 2006;290:E1191–E1197
 20. 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* 1998;97:473–483
 21. Quon MJ. QUICKI is a useful and accurate index of insulin sensitivity. *J Clin Endocrinol Metab* 2002;87:949–951
 22. Scognamiglio R, Negut C, De Kreutzenberg SV, Tiengo A, Avogaro A. Postprandial myocardial perfusion in healthy subjects and in type 2 diabetic patients. *Circulation* 2005;112:179–184
 23. Chiu JD, Richey JM, Harrison LN, Zuniga E, Kolka CM, Kirkman E, Ellmerer M, Bergman RN. Direct administration of insulin into skeletal muscle reveals that the transport of insulin across the capillary endothelium limits the time course of insulin to activate glucose disposal. *Diabetes* 2008;57:828–835
 24. Richey JM, Ader M, Moore D, Bergman RN. Angiotensin II induces insulin resistance independent of changes in interstitial insulin. *Am J Physiol* 1999;277:E920–E926
 25. Park J, Lemieux S, Lewis GF, Kuksis A, Steiner G. Chronic exogenous insulin and chronic carbohydrate supplementation increase de novo VLDL triglyceride fatty acid production in rats. *J Lipid Res* 1997;38:2529–2536