# Meal-Related Increases in Microvascular Vasomotion Are Impaired in Obese Individuals

## A potential mechanism in the pathogenesis of obesity-related insulin resistance

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**O**besity is associated with insulin resistance, hypertension, cardiovascular disease, and type 2 diabetes (1), but the mechanisms underlying these associations are incompletely understood. This article reviews and adds original data (i.e., in the postprandial state) to the evidence for microvascular dysfunction, including impairment of insulin-stimulated microvascular perfusion as a key element in the pathogenesis of obesity-related hypertension and insulin resistance (2,3).

## MICROVASCULAR DYSFUNCTION CONTRIBUTES TO HIGH BLOOD PRESSURE AND IMPAIRED INSULIN-MEDIATED GLUCOSE UPTAKE—

The microcirculation is widely taken to encompass vessels $<150$ $\mu$m (i.e., arterioles, capillaries, and venules) (4) and has two important functions. First, arterioles regulate hydrostatic pressure and peripheral vascular resistance (4). Importantly, dysfunction of the microcirculation, with concomitant increases in vasoconstrictor tone, will increase total peripheral resistance and, other things being equal, blood pressure, as reviewed

elsewhere (2,3). Second, it regulates tissue perfusion to optimize the delivery of nutrients and removal of waste products within tissues in response to variations in demand. In this respect, insulin has been shown to play an important role (5).

Insulin redirects blood flow within the muscle microvascular bed to increase available capillary surface area, an effect referred to as "capillary recruitment" (6,7). In addition, insulin induces vasodilation of resistance vessels, resulting in an increase in total muscle blood flow (8). Whether this increase in total muscle blood flow, which occurs later in time compared with the redirection of flow to nutritive capillary beds, serves to enhance insulin-mediated glucose uptake remains controversial (9). However, it has been generally accepted that capillary recruitment is crucial for the delivery of insulin and glucose to tissue. Indeed, several studies have shown that insulin-mediated increases in capillary recruitment account for approximately half of insulin-mediated muscle glucose uptake in vivo (10–14). Insulin's effect on microvascular blood flow is, therefore, an important regulator of insulin-mediated muscle glucose uptake, the main determinant of

whole-body insulin sensitivity. Conversely, impairment of insulin-mediated increases in capillary recruitment will decrease insulin-mediated glucose uptake (5).

Insulin-induced capillary recruitment is mediated via a vasodilatory action of insulin on precapillary muscle arterioles (6,7). This action is critically dependent on insulin-stimulated production of nitric oxide (NO) in the vascular endothelium, an effect mediated by phosphatidylinositol (PI) 3-kinase, protein kinase B/Akt, and endothelial NO synthase (eNOS) (15,16). In fact, under basal conditions (i.e., at rest or in the preprandial state), only one-half to one-third of the capillary bed is perfused at any time (7,17) and these capillaries, moreover, are mainly shorter so-called nonnutritive capillaries (18). By relaxation of terminal arterioles connected to nutritive capillary networks (i.e., capillaries that perfuse sites of high glucose uptake), insulin redirects blood flow from relatively nonnutritive vessels to nutritive capillary beds, with a resultant increase in the overall number of perfused capillaries (6). In addition to this NO-dependent vasodilator effect, insulin increases the secretion of the vasoconstrictor peptide endothelin-1 (ET-1) (15,16) via the intracellular mitogen-activated protein kinase signaling pathway and the extracellular signal-regulated kinase-1/2 (ERK1/2) (19). In healthy individuals, there is a balance between the insulin-mediated NO- and ET-1–dependent effects, with the net effect being either neutral or vasodilatory.

Under normal conditions, arterioles are not "open" or "closed" for any prolonged period of time. In fact, it is the rhythmic contraction and dilation of arterioles (so-called vasomotion) that regulates microvascular flow distribution so that different muscle regions are intermittently perfused (7,17). Capillary recruitment can thus be seen as a process wherein the total "open" time of capillary networks increases in conditions of

increased metabolic demand. An experimental study has demonstrated that an increase in vasomotion can result in a rise in mean blood flow of 40–60% (20,21). It seems therefore plausible that insulin-induced capillary recruitment may be mediated at least in part via effects on microvascular vasomotion. Indeed, several studies have demonstrated that insulin increases the intensity of vasomotion at the precapillary arteriolar level in human (22) and rat muscle microvasculature (14), as well as in human cutaneous microvasculature (23). Taken together, these data suggest that insulin, by altering arteriolar vasomotion and/or reducing pre-arteriolar tone, redirects blood flow from nonnutritive to nutritive capillary beds and thus increases insulin-mediated glucose uptake.

## OBESITY IS ASSOCIATED WITH MICROVASCULAR DYSFUNCTION

—Microvascular function thus appears to be crucial in the regulation of muscle glucose metabolism and peripheral vascular resistance. Obesity, in turn, has been shown to be associated with several impairments in the microvasculature, which may be an important pathway through which obesity contributes to high blood pressure and insulin resistance. Obese individuals are characterized by muscle capillary rarefaction (24) and a disturbed balance of NO and ET-1 in the muscle microcirculation (25,26). Also, obese individuals show reduced microvascular vasomotion under baseline conditions (27) and blunted responses in resistance arteries and arterioles to classic endothelium-dependent vasodilators (26,28,29). In addition, several studies have demonstrated impaired insulin-induced vasodilation and insulin-mediated increases in microvascular perfusion in skin (12,28) and skeletal muscle (30) of obese individuals. The microvascular effects of insulin are impaired because, in obesity, insulin-mediated activation of the PI 3-kinase pathway is impaired, whereas the activation of ERK1/2 and the production of ET-1 by insulin are normal. This results in an impaired vasodilator or even a net vasoconstrictor response of insulin on muscle resistance arteries and terminal arterioles (16). As a consequence, vascular resistance will increase, possibly contributing to elevations in blood pressure, as well as to extensive periods of poor delivery of insulin, glucose, and other nutrients to the muscle cells, contributing to impaired

insulin-mediated glucose disposal in muscle.

The hypothesis that microvascular dysfunction may precede and predict the development of insulin resistance and hypertension is supported by the presence of microvascular dysfunction in normoglycemic mildly overweight individuals with a genetic predisposition for type 2 diabetes (31) and improvement of endothelial function by weight loss in obese women (32). In addition, animal studies have demonstrated that diminished NO production, due to for example high-fat feeding, precedes the development of skeletal muscle insulin resistance (33), diabetes, and hypertension (34). In summary, these data support the notion that defects in microvascular function may contribute to impaired insulin-induced glucose uptake.

## MICROVASCULAR FUNCTION AND STEADY-STATE VERSUS DYNAMIC (MEAL-INDUCED) HYPERINSULINEMIA

—Most studies of the effect of insulin on microcirculatory function have been performed with the euglycemic-hyperinsulinemic clamp technique, i.e., under steady-state hyperinsulinemia (7,12–14,22,28,30,35). However, physiologically, hyperinsulinemia is usually transient and dynamic, such as after a glucose load or a meal and is then accompanied by changes in circulating concentrations of glucose, amino acids, as well as gut and pancreatic peptides, which are not replicated by the clamp technique. If insulin's effects on microcirculatory function play a physiological role in regulating insulin-mediated glucose uptake, such effects should be demonstrable not only during steady-state hyperinsulinemia, but also after a glucose load and a meal. In addition, any such

effects would be expected to be impaired in obese (insulin-resistant) individuals compared with (insulin-sensitive) healthy control subjects.

In view of these considerations, we studied, in lean and obese individuals, the effects of an oral glucose load and a liquid mixed meal on cutaneous microvascular vasomotion.

## SUBJECTS AND METHODS

### Subjects
A total of 18 lean and 13 obese Caucasian individuals participated (Table 1). Participants were healthy, as judged by medical history; nondiabetic, as defined by an oral glucose tolerance test; normotensive (<135/<85 mmHg), as determined by ambulatory 24-h blood pressure monitoring (SpaceLabs 90207, Redmond, WA); and nonsmokers. Participants did not use any medication except oral contraceptives (three lean and two obese women). All participants gave informed consent for participation in the study. The study was undertaken with the approval of the local ethics committee and performed in accordance with the Declaration of Helsinki.

### Study design
All subjects were allocated to three interventions in random order, i.e., a glucose drink (75 g glucose in 250 mL water [similar to an oral glucose tolerance test]), a 495-kcal liquid mixed meal (volume: 330 mL, 60% carbohydrates, 25% proteins, 15% fat), or a control drink (300 mL tap water). Investigators were blinded with regard to the type of drink ingested. The interval between each of the interventions was 1 week.

All measurements were conducted in a temperature-controlled room ($T = 23.4 \pm 0.5°C$) at 8:00 A.M., after a 10-h

**Table 1—Characteristics of both study groups**

|  | Lean individuals | Obese individuals |
|---|---|---|
| *n* (men/women) | 18 (6/12) | 13 (5/8) |
| Age (years) | 37.3 ± 12.9 | 35.9 ± 14.9 |
| Weight (kg) | 68.9 ± 10.3 | 100.3 ± 16.3* |
| BMI (kg/m$^2$) | 22.5 ± 1.7 | 34.0 ± 3.5* |
| Waist-to-hip ratio | 0.80 ± 0.08 | 0.90 ± 0.09* |
| Systolic blood pressure daytime, 24-h ABPM (mmHg) | 122 ± 7 | 119 ± 9 |
| Diastolic blood pressure daytime, 24-h ABPM (mmHg) | 77 ± 5 | 73 ± 5† |
| Fasting plasma glucose (mmol/L) | 5.0 ± 0.6 | 5.1 ± 0.4 |
| Fasting insulin (mU/L) | 3.6 ± 1.0 | 10.4 ± 6.0* |
| HOMA2-IR | 0.46 ± 0.13 | 1.35 ± 0.75* |

Data are means ± SD or *n*. ABPM, ambulatory 24-h blood pressure monitoring. *$P < 0.01$. †$P < 0.05$ obese vs. healthy subjects.

fast, with the subjects in the supine position. Subjects were asked to refrain from drinking alcohol for a period of 24 h before each study day and to perform no strenuous exercise for a period of 48 h before each study day. Baseline measurements were obtained after allowing 30 min of rest and acclimatization after the insertion of one intravenous catheter (Venflon; Viggo, Gotenborg, Sweden).

### Laser Doppler flowmetry

To perform vasomotion analyses, skin blood flow (in arbitrary perfusion units) was measured with a laser Doppler probe (PF 457; Perimed, Stockholm, Sweden) at the dorsal side of the wrist of the dominant arm. Signals were recorded for 30 min, both at baseline and 30 min after intake of the glucose drink, the mixed-meal drink, or water. Fast-Fourier transform analysis was performed by means of Perisoft dedicated software (PSW version 2.50; Perimed) to determine the contribution of the five frequency components to the variability of the laser Doppler signal (i.e., endothelial, 0.01–0.02 Hz; neurogenic, 0.02–0.06 Hz; myogenic, 0.06–0.15 Hz; respiratory, 0.15–0.40 Hz; and heart beat, 0.40–1.60 Hz).

### Plasma insulin and glucose

Plasma insulin concentrations were measured by radioimmunoassay techniques (AutoDELFIA; PerkinElmer, Waltham, MA).

Blood glucose concentrations were determined with a glucose analyzer YSI2300 (Yellow Springs Instruments, Yellow Springs, OH). Insulin resistance was estimated by the calculation of the homeostasis model assessment–insulin resistance (HOMA2-IR) index (36).

### Statistical analysis

Data are expressed as means ± SD. A paired Student $t$ test was used to study effects of the different drinks, on glucose and insulin levels, within each group. The independent Student $t$ test was used to ascertain differences between the lean and obese groups.

The Wilcoxon signed-rank test for paired data was used to study differences in vasomotion within each group between fasting and postprandial values. The Mann-Whitney $U$ test was used to compare effects of the interventions on vasomotion between the lean and obese group. A two-tailed $P$ value of $< 0.05$ was considered significant. All analyses were performed using the statistical software package SPSS version 15.0.

**RESULTS**—Subject characteristics are shown in Table 1. Compared with lean individuals, obese individuals had higher weight, BMI, waist-to-hip ratio, and fasting insulin levels and were more insulin resistant (higher HOMA2-IR). In addition, 24-h diastolic blood pressure was lower in obese individuals.

### Effects of the glucose and mixed-meal drink on plasma insulin and glucose levels

Glucose and insulin levels before and after ingestion of the different drinks in lean and obese subjects are shown in Fig. 1.

After ingestion of the glucose drink, peak plasma glucose levels were higher compared with the mixed-meal drink in both lean ($P < 0.05$) and obese ($P < 0.001$) individuals. Obese individuals, compared with lean individuals, had higher glucose levels after both glucose ingestion and the mixed meal ($P < 0.001$ and $P < 0.05$, respectively) (Fig. 1A). In lean individuals, but not obese individuals, plasma insulin levels rose to higher levels after ingestion of the mixed-meal drink compared with the glucose drink ($P < 0.01$). In addition, peak insulin levels after both the glucose and the mixed-meal drink reached higher levels in obese compared with lean individuals ($P < 0.05$ and $P = 0.07$, respectively) (Fig. 1B). Water intake did not alter plasma glucose or insulin levels in both groups.

### Effects of the glucose and mixed-meal drink on microvascular vasomotion

At baseline, microvascular vasomotion was not different between lean and obese individuals (baseline energy density [mean of three study days] for lean vs. obese individuals for each of the five frequency bands and the total spectrum:
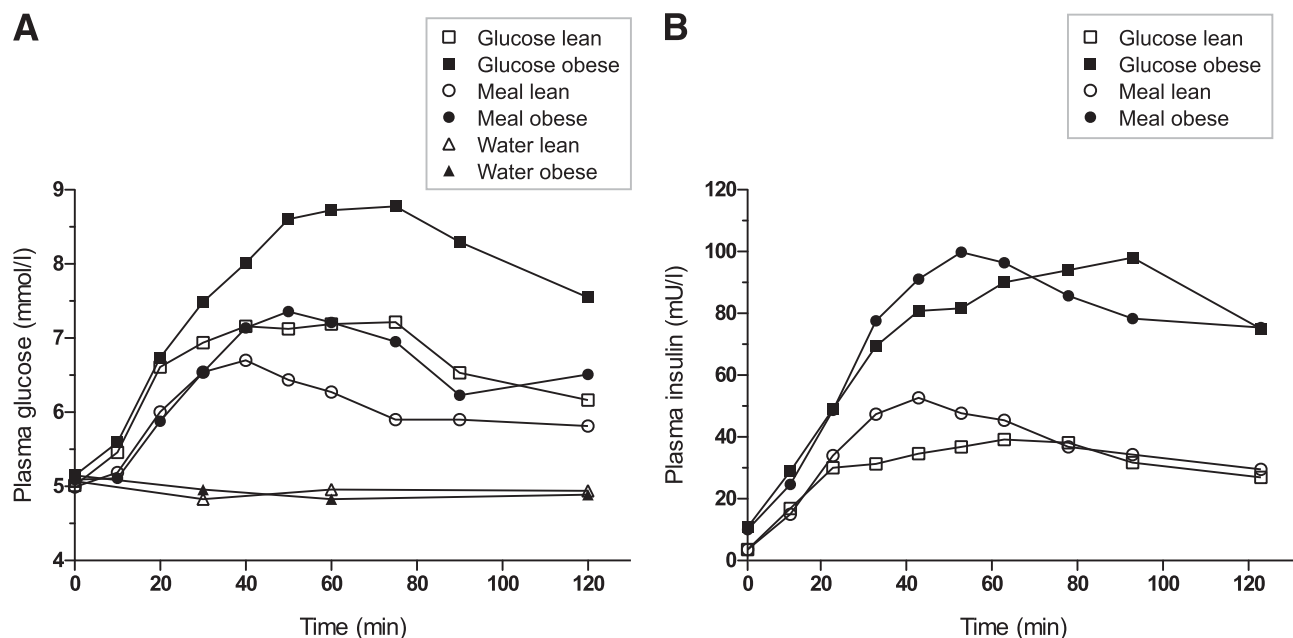


**Figure 1**—A: *Time course of mean plasma glucose levels in lean and obese individuals after the glucose drink, the mixed-meal drink, and the water drink.* B: *Time course of mean plasma insulin levels in lean and obese individuals after the glucose and the mixed-meal drink.*

0.62 ± 0.35 vs. 0.49 ± 0.15, P = 0.49; 0.45 ± 0.18 vs. 0.41 ± 0.13, P = 0.60; 0.23 ± 0.11 vs. 0.23 ± 0.21, P = 0.41; 0.09 ± 0.04 vs. 0.07 ± 0.04, P = 0.16; 0.07 ± 0.03 vs. 0.06 ± 0.03, P = 0.23; and 1.46 ± 0.67 vs. 1.25 ± 0.45 Hz, P = 0.44, respectively).

Microvascular flow (perfusion units) did not change during any of the experiments (data not shown). Water intake did not alter the energy density of any of the five frequency components in either group (data not shown).

In lean individuals, the glucose drink increased the energy density of the frequencies between 0.01 and 0.02 Hz (Fig. 2A). Intake of the liquid mixed meal increased the energy density of all five frequency bands and the total energy density of the entire spectrum (Fig. 2B). In obese individuals, neither the glucose (Fig. 2C) nor the mixed-meal drink (Fig. 2D) had any effect on the energy density of the five frequency bands or the total energy density of the entire spectrum.

After the mixed meal, but not after glucose, obese individuals had lower increases in the energy density of the frequency bands between 0.01–0.02, 0.02–0.06, and 0.15–0.40 Hz, as well as in the total energy density of the entire spectrum compared with lean individuals (−0.03 ± 0.27 vs. 0.20 ± 0.21, P < 0.01; 0.05 ± 0.19 vs. 0.17 ± 0.21, P < 0.05; 0.00 ± 0.04 vs. 0.07 ± 0.09, P < 0.05; and 0.00 ± 0.60 vs. 0.57 ± 0.61 Hz, P < 0.01, respectively).

**CONCLUSIONS**—This study, in lean and (insulin-resistant) obese individuals, had three main findings. First, in the basal preprandial state, there were no significant differences in microvascular vasomotion between lean and obese individuals. Second, ingestion of a glucose drink and a liquid mixed-meal drink induced an increase in microvascular vasomotion in lean individuals. Third, the increase in microvascular vasomotion after a meal was impaired in obesity. In addition, the lack of meal-induced stimulation of microvascular
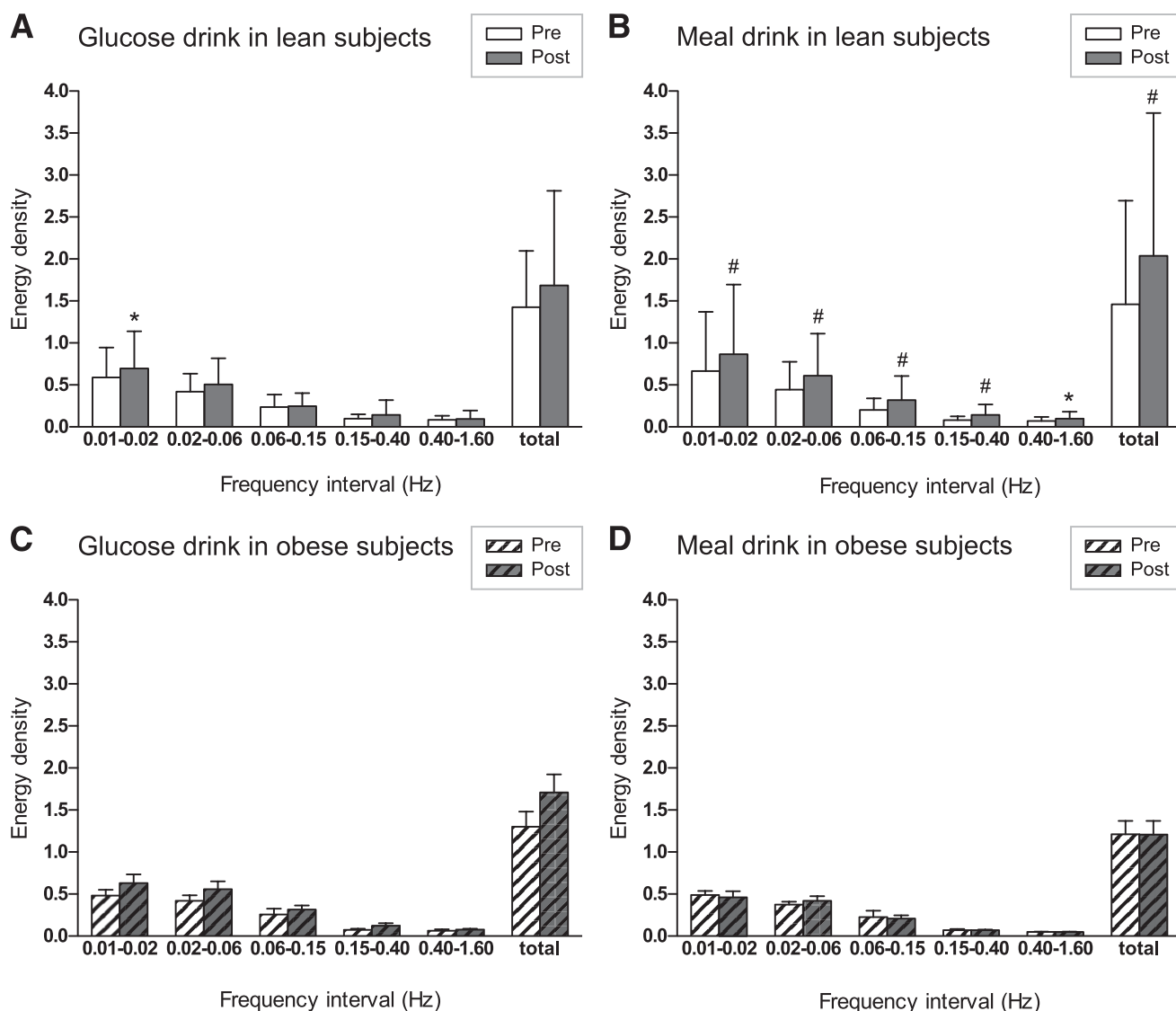


**Figure 2**—*Microvascular vasomotion before and after the glucose drink* (A) *and the mixed-meal drink* (B) *in lean subjects. Microvascular vasomotion before and after the glucose drink* (C) *and the mixed-meal drink* (D) *in obese subjects. Bars present means ± SD. #P < 0.01, *P < 0.05 vs. before intake.*

vasomotion in obese individuals was associated with elevated plasma glucose levels (e.g., attenuated insulin-stimulated glucose disposal) after meal feeding in these subjects. These findings are consistent with a role for microvascular dysfunction, specifically, impaired vasomotion, in the development of obesity-related insulin resistance.

Our finding that, in the basal state, the energy density within each of the five frequency bands and that of the total spectrum was, although numerically smaller in obese than in lean individuals, not significantly different between these groups contrasts with findings of de Jongh et al. (27), who demonstrated significantly lower energy densities within the frequency intervals of 0.01–0.02 and 0.02–0.06 Hz and reduced energy density of the total frequency spectrum in obese compared with lean women. The explanation for this discrepancy is not entirely clear, but may be related to the fact that the individuals in the current study were less obese than in the previous study (27) and to the fact that we studied a smaller group of obese individuals (i.e., we had less power to detect small differences).

There is convincing evidence that insulin-induced changes in skeletal muscle microvascular blood flow distribution play an important role in regulating muscle glucose disposal (5). Herein, we propose a significant role for insulin-induced increases in microvascular vasomotion. Indeed, several studies have demonstrated that insulin increases the intensity of vasomotion at the precapillary arteriolar level in human (22) and rat muscle microvasculature (14), as well as in human cutaneous microvasculature (23). However, until now, these effects of insulin on microvascular vasomotion have only been measured during an insulin clamp (14,22) or local application of insulin to the surface of the skin (23) and not postprandially. In the current study, we are the first to demonstrate that ingestion of glucose or a mixed meal indeed stimulates microvascular vasomotion in healthy lean individuals. Likely, this effect may be secondary to meal-induced hyperinsulinemia, as indicated by the larger increase in both insulin levels and microvascular vasomotion after the mixed-meal drink compared with the glucose drink in lean individuals. However, given the complex neuroendocrine responses to feeding and the effects of different meal components thereupon (i.e., fat, carbohydrates, proteins) (37), we cannot exclude

other factors contributing to the postprandial changes in microvascular vasomotion.

Another key finding in this study was that the increase in microvascular vasomotion with meal ingestion was impaired in insulin-resistant obese individuals. In addition, this lack of meal-induced stimulation of microvascular vasomotion paralleled blunted insulin-stimulated glucose disposal (i.e., elevated plasma glucose levels) after meal feeding in obese individuals. This link between meal-induced microvascular vasomotion and insulin-stimulated glucose uptake is consistent with a role for insulin-stimulated microvascular vasomotion in insulin-mediated glucose uptake in daily life and consequently with a role for microvascular dysfunction in the development of insulin resistance in obesity.

A possible limitation of the current study is that although muscle is the main peripheral site of insulin-induced microvascular function and insulin-mediated glucose uptake, we studied skin and not muscle microvascular vasomotion. However, there is substantial evidence that skin cutaneous microcirculation is representative of microcirculation in general (e.g., skeletal muscle). This is underscored by several studies demonstrating that cutaneous microvascular function mirrors generalized systemic microvascular function (38). With regard to insulin infusion, several studies demonstrated comparable metabolic (39) and vascular effects (12,13) of insulin in muscle and skin. In addition, skin microvascular vasodilator capacity is associated with both vascular and metabolic (40) actions of insulin in skeletal muscle. The latter is clearly demonstrated in a study in which acutely increasing or decreasing skin nutritive flow has been shown to result in parallel changes in skeletal muscle insulin-mediated glucose uptake (12).

## Possible pathophysiological mechanisms for obesity-associated microvascular dysfunction

The pathophysiological mechanisms behind the relationship between obesity and microvascular dysfunction are probably multifactorial. Adipose tissue and in particular visceral adipose tissue cells secrete a variety of hormones and cytokines, such as free fatty acids (FFAs), tumor necrosis factor (TNF)-$\alpha$, interleukin-6, adiponectin, leptin, and angiotensinogen (1). Some of these adipokines have been shown to affect microvascular function. For example,

elevation of FFAs has been demonstrated to impair endothelium-dependent vasodilation (41,42), at least in part through decreased NO production (41). Also, both FFAs and TNF-$\alpha$ directly inhibit insulin-mediated capillary recruitment and insulin-mediated glucose disposal in rat skeletal muscle (10,11,43). In addition, in lean individuals, FFA elevation impairs insulin-mediated stimulation of microvascular flow (12,13,41), and these impairments correlate closely with inhibition of insulin-mediated muscle glucose uptake (12,13). Conversely, lowering of FFAs in obese humans has an opposite effect (12). Also, circulating TNF-$\alpha$ levels are associated with impaired skin capillary recruitment (44), and weight loss resulted in a significant amelioration of endothelial function that closely correlated with a reduction in circulating TNF-$\alpha$ (32). Thus, FFAs and TNF-$\alpha$ have been shown to contribute to microvascular insulin resistance. This effect may be due to inhibiting effects on the insulin-signaling cascade. Both FFA and TNF-$\alpha$ elevation have been demonstrated to blunt insulin-induced PI 3-kinase activation directly or via the production of reactive oxygen species (45–49). Also, both FFAs and TNF-$\alpha$ upregulate the expression of ET-1 in human endothelial cells (35,50,51), whereas TNF-$\alpha$ downregulates the expression of eNOS (52). Thus, circulating FFAs and TNF-$\alpha$ are likely candidates to link adipose tissue with defects in microvascular function, at least in part by influencing insulin signaling and thereby the vascular effects of insulin. In addition, locally produced adipokines from perivascular fat depots may directly inhibit vasodilatory pathways distal in the arteriole and thereby cause loss of blood flow in the nutritive capillary network supplied by this arteriole (53,54). Nevertheless, whether postprandial changes in FFAs and TNF-$\alpha$ concentrations are involved in the impairment of meal-induced vasomotion in obesity cannot be derived from the current data, and further studies in this area are needed.

Another potential mechanism between adipose tissue and the microvasculature is the renin-angiotensin system (RAS). Obese individuals are characterized by increased activity of the RAS (55). Multiple mechanisms may contribute to RAS-induced vascular insulin resistance. Angiotensin II (AngII) was demonstrated to blunt insulin-induced PI 3-kinase activation (56) directly or via stimulated reactive oxygen species generation (57). In addition, AngII was shown to activate the

ERK1/2 pathway and consequently stimulates ET-1 production. Support for a role of AngII in insulin-mediated microvascular dysfunction is provided by a study in the Zucker obese diabetic rat, in which chronic ACE inhibition improved insulin-mediated capillary recruitment and insulin-mediated glucose disposal (58). Therefore, we hypothesize that AngII may play an important role in compromising microvascular function and thus provide another potential link between obesity and obesity-related microvascular dysfunction. Whether systemic or local RAS activity can be regulated by nutritional factors is not known. Nevertheless, several studies have demonstrated that acute hyperinsulinemia (i.e., an insulin clamp) increases plasma renin and AngII levels and upregulates angiotensinogen mRNA expression in adipose tissue (59). This increase in systemic RAS activity with insulin is likely mediated via sympathetic activation (60), which has been shown to be more pronounced in obese compared with lean individuals (60).

In summary, we are the first to report that ingestion of a meal increases microvascular vasomotion in healthy lean individuals. This increased vasomotion is likely to play an important role in skeletal muscle blood flow distribution and, therefore, the regulation of insulin and glucose delivery to skeletal muscle. In addition, we demonstrated that the increase in microvascular vasomotion with meal ingestion was impaired in obese individuals. This lack of meal-induced stimulation of microvascular vasomotion paralleled blunted insulin-stimulated glucose disposal after meal feeding in these subjects, which again is consistent with a role for insulin-stimulated microvascular vasomotion in insulin-mediated glucose uptake in the postprandial state. This article has further reviewed the evidence for microvascular dysfunction as a key element in the development of obesity-related hypertension and insulin resistance and consequently in the development of CVD and type 2 diabetes. We propose that, in obesity, microvascular dysfunctions may be the result of alterations in endocrine and vasocrine (1,53) signaling, in which adipokines and the RAS play a prominent role. Additional studies are required to fully understand the precise mechanisms that link obesity to insulin-mediated microvascular dysfunction. Such studies are an important step toward development of strategies in the prevention and treatment of obesity-associated hypertension and insulin resistance.

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