Physiologic Hyperinsulinemia Enhances Human Skeletal Muscle Perfusion by Capillary Recruitment

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Despite intensive study, the relation between insulin’s action on blood flow and glucose metabolism remains unclear. Insulin-induced changes in microvascular perfusion, independent from effects on total blood flow, could be an important variable contributing to insulin’s metabolic action. We hypothesized that modest, physiologic increments in plasma insulin concentration alter microvascular perfusion in human skeletal muscle and that these changes can be assessed using contrast-enhanced ultrasound (CEU), a validated method for quantifying flow by measurement of microvascular blood volume (MBV) and microvascular flow velocity (MFV). In the first protocol, 10 healthy, fasting adults received insulin (0.05 mU * kg−1 * min−1) via a brachial artery for 4 h under euglycemic conditions. At baseline and after insulin infusion, MBV and MFV were measured by CEU during continuous intravenous infusion of albumin microbubbles with intermittent harmonic ultrasound imaging of the forearm deep flexor muscles. In the second protocol, 17 healthy, fasting adults received a 4-h infusion of either insulin (0.1 mU * kg−1 * min−1, n = 9) or saline (n = 8) via a brachial artery. Microvascular volume was assessed in these subjects by an alternate CEU technique using an intra-arterial bolus injection of albumin microbubbles at baseline and after the 4-h infusion. With both protocols, muscle glucose uptake, plasma insulin concentration, and total blood flow to the forearm were measured at each stage. In protocol 2 subjects, tissue extraction of 1-methylxanthine (1-MX) was measured as an index of perfused capillary volume. Caffeine, which produces 1-MX as a metabolite, was administered to these subjects before the study to raise plasma 1-MX levels.

In protocol 1 subjects, insulin increased muscle glucose uptake (180%, P < 0.05) and MBV (54%, P < 0.01) and decreased MFV (−42%, P = 0.07) in the absence of significant changes in total forearm blood flow. In protocol 2 subjects, insulin increased glucose uptake (220%, P < 0.01) and microvascular volume (45%, P < 0.05) with an associated moderate increase in total forearm blood flow (P < 0.05). Using forearm 1-MX extraction, we observed a trend, though not significant, toward increasing capillary volume in the insulin-treated subjects. In conclusion, modest physiologic increments in plasma insulin concentration increased microvascular blood volume, indicating altered microvascular perfusion consistent with a mechanism of capillary recruitment. The increases in microvascular (capillary) volume (despite unchanged total blood flow) indicate that the relation between insulin’s vascular and metabolic actions cannot be fully understood using measurements of bulk blood flow alone. Diabetes 50:2682–2690, 2001

Recent studies in humans indicate that insulin enhances total limb blood flow (1,2). This increase in total flow appears dependent on the release of nitric oxide (3–5). Although multiple investigators have confirmed this vascular action of insulin (6,7), some have suggested that either prolonged exposure or supraphysiologic concentrations of insulin are required to enhance bulk muscle blood flow (2). Furthermore, although some have reported that increasing forearm or leg blood flow during hyperinsulinemia enhances glucose uptake (8), others have not seen this (9,10). As a result, the regulatory role of physiologic insulin concentrations on muscle blood flow and the relation between flow and glucose uptake remains controversial (6,11).

With few exceptions (6,12), clinical studies have used total limb blood flow to assess insulin’s vascular actions. It is inferred that these reflect changes occurring in skeletal muscle. In fact, muscle’s contribution to limb composition can vary with sex, age, and training (13), and the relative contribution of muscle to limb flow can likewise vary (14). More importantly, based on extensive observations in animal hindlimb muscle, investigators have proposed that two parallel microvascular pathways mediate muscle blood flow (15–18). Studies in the rat hindlimb under conditions of constant flow have demonstrated that vasoactive agents, including angiotensin II, epinephrine, noradrenaline, and serotoninergic compounds, simultaneously modulate metabolic activity and flow distribution in muscle (19–21) under conditions in which total blood flow is constant. These observations have led to the suggestion that microvascular flow distribution through muscle can occur via nutritive or non-nutritive pathways. This differential flow distribution may be an important regulator of nutrient and hormone delivery to muscle and thereby contribute to overall metabolic behavior.

To address whether insulin influences blood flow distribution within muscle in vivo, we have previously used 1-methylxanthine (1-MX) metabolism as a marker for

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Received for publication 19 January 2001 and accepted in revised form 15 August 2001.

1-MX, 1-methylxanthine; A-V, arterial-venous; CEU, contrast-enhanced ultrasound; HPLC, high-performance liquid chromatography; MBV, microvascular blood volume; MFV, microvascular flow velocity; PET, positron-emission tomography.
endothelial exposure to blood in vivo. In skeletal muscle, xanthine oxidase is largely restricted to capillary endothelium, and the rate of xanthine oxidase–mediated conversion of 1-MX to 1-methylurate in blood as it traverses the muscle bed is a function of capillary perfusion (22). We observed that euglycemic hyperinsulinemia enhances the extraction of 1-MX in rat skeletal muscle (23). It is of interest that changes in 1-MX extraction in these animals could be dissociated from changes in total blood flow. Thus, whereas increases in leg blood flow induced by high concentrations of insulin enhanced 1-MX disappearance rate, comparable increases in total flow induced by epinephrine did not. These findings suggest that insulin exerts a hemodynamic action to recruit additional capillaries. More recently, this finding has been confirmed using scanning-laser Doppler flow measurements in rat leg muscle in vivo (24). These observations in rats led us to attempt to examine insulin’s microvascular actions in healthy humans. Specifically, we addressed whether physiologic hyperinsulinemia acts to recruit capillaries in forearm muscle. To address this we have applied contrast-enhanced ultrasound (CEU), a technique developed to image microvascular perfusion in the myocardium (25–27), to the isolated human forearm model. This method is performed by simultaneous ultrasound imaging and intravascular administration of microbubbles that generate an acoustic signal during their microvascular transit. These microbubbles are purely intravascular and have a similar rheology to erythrocytes (28). Protocols have recently been developed that allow quantification of microvascular blood volume (MBV) and microvascular flow velocity (MFV) within these microvessels (25,26). This is accomplished using continuous infusions of microbubbles during high-power ultrasound imaging that detects and simultaneously destroys microbubbles. This is the primary approach used in the current study. Two additional approaches were used to validate results obtained with this primary approach. The first was an established indicator-dilution method of CEU in which albumin microbubbles were injected intra-arterially to serve as a flow tracer within muscle during ultrasound imaging (29). The second validation method used the extraction of 1-MX by forearm muscle after the ingestion of caffeine, which provides an endogenous source of 1-MX. Each method indicated that at physiologic concentrations, insulin increased microvascular blood volume, i.e., recruited additional capillary beds, even in the absence of significant changes in total blood flow. These findings suggest that full assessment of the relation between insulin’s vascular and metabolic actions in the forearm will require examining more than total blood flow alone, as has been the focus of investigations to date.

RESEARCH DESIGN AND METHODS

The study protocols were approved by the University of Virginia Human Investigations Committee. A total of 27 healthy, young, nonobese, adult male and female volunteers were enrolled in the study. The subjects were admitted to the General Clinical Research Center the evening before the start of the study and fasted overnight. Exclusion criteria included a history of major medical illness, including hypertension, diabetes, or dyslipidemia. None of the subjects were chronically taking medications. On the morning of the study, a catheter was placed percutaneously into the brachial artery just above the antecubital fossa in one arm for the withdrawal of arterial blood samples, the monitoring of blood pressure via fluid-filled transducer, and the infusion of insulin. A second catheter was placed retrograde in the antecubital vein of the same arm for withdrawal of deep venous blood samples, draining of forearm muscle, and venous pressure measurement. An additional deep forearm venous catheter was placed in the contralateral arm for blood sampling.

**Blood flow measurements.** Total forearm blood flow was measured in each subject by two techniques: capacitance plethysmography (30) and brachial artery ultrasound. For the Doppler flow measurements, an ultrasound system (Sonoline Elegra; Siemens, 5500 Plum St, Andover, MA) with a linear-array transducer was used with a transmit frequency of 7.5 MHz to allow for two-dimensional (2-D) imaging of the brachial artery in the long axis. Brachial artery diameter was measured 2 cm proximal to the tip of the arterial catheter at peak systole using online video calipers. A pulsed-wave Doppler sample blood volume was placed at the same location in the center of the vessel, and the mean brachial artery blood velocity was measured using online angle correction and analysis software. Brachial artery blood flow was calculated from 2-D and Doppler ultrasound data using the equation:

\[ Q = \frac{\pi \cdot d^2}{4} \cdot \frac{Q}{H} \]

where \( Q \) is brachial blood flow, \( v \) is mean brachial artery blood velocity, and \( d \) is brachial artery diameter. In these studies, the ultrasonographonator was not blinded regarding whether the subject was receiving insulin or saline. The intra- and inter-observer coefficient of variation for the measurements of brachial artery diameter, determined from images of eight subjects, were 4.7 ± 1.7 and 5.7 ± 1.0%, respectively.

**Chemical analyses.** Plasma glucose and lactate were measured by automated analyses using glucose oxidase and lactate oxidase, respectively. Plasma insulin was measured using a double-antibody radioimmunoassay. Plasma concentrations of 1-MX, caffeine, and 1,7-dimethylxanthine were measured by high-performance liquid chromatography (HPLC) as previously described (23).

**Data analyses.** Brachial artery blood flow was used to derive forearm vascular resistance (\( R \)) (dynes s cm⁻²) by:

\[ R = \frac{1.333 (P_a - P_v)}{Q} \]

where \( P_a \) and \( P_v \) are arterial and venous pressures, respectively. Brachial blood flow (Doppler) was used for the measures of transit time and vascular resistance because it was obtained nearly simultaneously with the CEU imaging. Glucose, oxygen, and lactate balances were calculated by multiplying the arterial-venous (AV) concentration differences by forearm blood flow (plethysmographic). Calculated values for the forearm blood flow and glucose, oxygen, and lactate balances were averaged for the four samples obtained before and the four samples obtained after either saline or insulin administration. Plethysmographic forearm blood flow measurements were used in the balance calculations because these measurements were interleaved with the blood sampling, and the deep forearm catheter drains nearly exclusively forearm muscle.

**Protocols.** Beginning at 8:00 a.m. on the first day of the study, after all vascular catheters were inserted and a further 30-min stabilization period was observed, four separate arterial and bilateral deep venous blood samples were obtained every 10 min for the measurement of serum glucose, insulin, lactate, and oxygen concentrations. Blood flow to the forearm was measured by capacitance plethysmography after each blood sample and by Doppler ultrasound after the last blood sample. Arterial and venous blood pressures were then recorded. These constituted the basal measurements. The same measurement protocol was repeated during the last 40 min of either of two 4-h experimental protocols.

The two protocols differed in that two different applications of CEU methods were used to assess microvascular blood volume: 2-D intermittent harmonic imaging during continuous intravenous microbubble infusion in the first protocol and 2-D imaging during direct pulse injection of microbubbles into the brachial artery in the second protocol. In addition, in the second protocol, subjects received caffeine before the study to raise plasma concentrations of 1-MX to allow for the measurement of the extraction of this compound by the forearm vasculature (23).

**Protocol 1.** In addition to the above measurements during the basal period, in 10 subjects (8 men and 2 women, mean age 30 ± 2 years, and BMI 24 ± 1 kg/m²), forearm skeletal muscle MBV and MFV were measured using CEU with intermittent harmonic imaging (see below).

After completing these measurements in the basal period, insulin (0.05 mU · kg⁻¹ · min⁻¹) was infused into the brachial artery catheter in one forearm. Arterial plasma glucose concentrations were measured at 15-min intervals over the next 4 h. During the last 40 min, arterial and venous sampling and measurements of total blood flow (Doppler ultrasound and capacitance plethysmography) were repeated as noted above, and a second set of measurements of forearm MBV and MFV were obtained using CEU with intermittent harmonic imaging.
infused insulin in the forearm circulation. In these subjects, the higher dose of insulin infusion did not significantly enhance forearm glucose uptake in subjects given these doses of caffeine. Two factors may have contributed to this: 1) antagonism by caffeine of insulin’s metabolic effect and 2) a higher basal forearm blood flow after caffeine, resulting in local dilution of infused insulin in the forearm circulation. In these subjects, the higher dose of insulin did not provoke systemic hypoglycemia, even after 4 h of infusion. Blood sampling, measurements of forearm blood flow using capacitance plethysmography, and Doppler ultrasound were performed at baseline and at 4 h, as described under protocol 1.

**CEU with arterial bolus-injection/continuous imaging.** After the baseline and 4-h blood sampling and blood flow measurements, 2 ml of air-filled albumin microbubbles (Albunex; Mallinckrodt Medical, St. Louis, MO) were injected into the arterial catheter over 4 s using an injection pump. During injections, 2-D imaging of the forearm was performed in a transaxial plane 5 cm distal to the antecubital fossa. A transmit frequency of 5 MHz was used, and both compression and power output were set at maximal values. Gain was optimized at the beginning of the study and held constant throughout. Images were acquired at a sampling rate of 30 Hz and were recorded on 1.25-cm S-VHS videotape with a high fidelity video recorder. As in protocol 1, images obtained during CEU were transferred from videotape to an offline computer and analyzed using previously described custom-designed software. For each injection, every fourth frame was selected from just before contrast appearance until its disappearance from the forearm skeletal muscle. Selected frames were aligned using automatic cross-correlation. A region of interest was placed over the deep forearm flexor muscles, from which a background-subtracted time-intensity plot was derived for the selected frames. The time-intensity data were fitted to a γ-variate function: y = A αt where A is a scaling factor, t is time, and α is proportional to the mean transit rate of the tracer. Skeletal muscle blood volume index was then calculated by dividing brachial artery flow by a.

**Statistical analysis.** Statistical analysis was performed using RS-1 (Domain Manufacturing, Burlington, MA). Comparisons of measurements before and after insulin and between control and study arms were made using a paired Student’s t test. Comparisons of changes in study arm and control arm were made using an unpaired Student’s t test.

### RESULTS

**Protocol 1.** The mean forearm uptake of glucose and oxygen and release of lactate before and 4 h after initiation of insulin infusion are given in Table 1. Glucose uptake increased in the insulin-infused (P < 0.05) but not in the control (contralateral) arm. There was a trend toward greater lactate release in both arms, but the difference did not meet statistical significance (P = 0.08 in insulin arm). Oxygen consumption was unchanged from baseline in either arm. The modest rise in serum insulin (from 5 ± 0.6 μU/ml at baseline to 53 ± 5 μU/ml at 4 h in the insulin-infused arm) had no significant effect on the forearm (plethysmography) or brachial artery (Doppler) flow (Fig. 1).

Before and during the insulin infusion, forearm MBV and MFV were determined using intermittent harmonic imaging during intravenous infusion of microbubbles. Figure 2 illustrates an example of a pulsing interval versus videointensity curve, generated before and during insulin or saline infusion in a single individual. As the pulsing interval increased, videointensity rose to a plateau at both stages. At baseline, the plateau videointensity and rate of change in videointensity were similar in both arms. However, plateau videointensity (Fig. 2A) was greater after the insulin infusion (22.9 vs. 12.7), indicating a larger effective concentration of bubbles within the volume of interest. As the microbubble infusion rate was identical at both sampling periods, the greater videointensity during hyperinsulinemia corresponds to an increase in microvascular blood volume within the volume of interest. The rate constant of the increase in videointensity provides a measure of

### Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Insulin-infused arm</th>
<th>Contralateral arm</th>
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<tr>
<td>Glucose uptake</td>
<td></td>
<td></td>
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<tr>
<td>(μmol·min⁻¹·100 ml⁻¹)</td>
<td>1.21 ± 0.4</td>
<td>1.05 ± 0.5</td>
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<tr>
<td>Lactate release</td>
<td></td>
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</tr>
<tr>
<td>(μmol·min⁻¹·100 ml⁻¹)</td>
<td>0.38 ± 0.21</td>
<td>0.35 ± 0.28</td>
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<tr>
<td>Oxygen consumption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μmol·min⁻¹·100 ml⁻¹)</td>
<td>21.93 ± 5.01</td>
<td>21.79 ± 3.86</td>
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Data are means ± SE. *P < 0.05 vs. basal measurement.
microvascular flow velocity ($\beta$, s$^{-1}$) (25) and is lower after insulin is administered (0.18 vs. 0.38). Figure 3 shows mean values for microvascular volume and flow velocity from all subjects, obtained using the CEU data. There was a highly significant ($P < 0.01$) increase in microvascular volume after insulin infusion and a corresponding decrease in microvascular flow velocity that is of borderline statistical significance ($P < 0.07$).

Protocol 2. Table 2 indicates the mean forearm glucose and oxygen balances before and at 4 h of insulin or saline infusion for subjects in protocol 2. As in protocol 1 subjects, insulin increased glucose uptake approximately threefold and was without effect on oxygen balance. In contrast, there were no changes in the saline-treated subjects in any of these variables.

Higher insulin doses were used in protocol 2 than in protocol 1 (plasma insulin increased from 5.3 ± 0.5 μU/ml at baseline to 90.6 ± 8.1 μU/ml at 4 h), and brachial artery blood flow (measured by Doppler ultrasound) increased during infusion of insulin in protocol 2 subjects ($P < 0.01$), as shown in Fig. 4. Forearm blood flow, measured by plethysmography, was similar for all stages in these subjects. There was no significant change in blood flow during the study period in the saline-infused subjects, using either the plethysmography or Doppler methods. It is notable that basal blood flow in subjects studied in protocol 2 is greater than in subjects studied in protocol 1. In part, this may relate to the previous dosing with caffeine, as it was seen in both the insulin and saline groups.

Time-intensity curves generated during arterial injection of microbubbles in a saline- and insulin-treated subject are shown in Fig. 5. Doppler-measured brachial artery flow, rather than plethysmography flow, was used to calculate MBV from these data because of the proximity in time of the flow measurement and the microbubble injection. Skeletal muscle microvascular volume derived from the transit time of the injected microbubbles again increased significantly ($P < 0.05$) in the insulin-treated subjects (Fig. 6).

Table 3 gives the steady-state arterial and deep forearm venous concentrations of 1-MX, caffeine, and 1,7-dimethylxanthine in the insulin- and saline-treated subjects. In previous in vivo studies in rats, the circulating 1-MX concentration reached ~20 μmol/l. Because 1-MX is not available for infusion studies in humans, we relied on its generation from metabolism of caffeine. This resulted in much lower arterial concentrations of 1-MX and was likely a combined function of slow generation and rapid clearance. A small A-V difference for 1-MX was discerned, and this was slightly greater in the insulin-treated subjects, although this difference was not statistically significant.

**DISCUSSION**

The current study demonstrates that modest, physiologic increases in plasma insulin concentrations, which do not augment total forearm or brachial artery flow, increase microvascular volume. These results are consistent with an action of insulin to increase tissue perfusion by recruiting additional capillaries within skeletal muscle.

These results in human muscle complement findings of studies reporting an action of systemically infused insulin to increase capillary perfusion in rat hindlimb muscle, as...
indicated by 1-MX extraction (23) or laser Doppler (24) methods. In those studies, higher doses (per kilogram) of systemically infused insulin were used, and euglycemia was maintained using the insulin-clamp method. The current study, using local delivery of insulin, strongly suggests that insulin per se exerts effects on the microvasculature and that capillary recruitment is likely to be a local neural or humoral effect rather than a systemic response to the infused insulin. This is of interest because systemically administered insulin, even with euglycemia, can alter circulating levels of catecholamines, fatty acids, amino acids, and other factors that can secondarily affect the vasculature.

In the current study, we have attempted to examine blood flow distribution using several techniques. There is no gold standard for examining flow distribution within human skeletal muscle or the number of capillaries that are being perfused at any one time. Histological techniques can identify capillary density (33,34) but offer no information on the fundamental parameters of blood flow, which are the volume of perfused microvessels and the velocity of flow within them. In careful studies of the effects of exercise on capillary perfusion in dog muscle, Honig et al. (35) demonstrated capillary recruitment by quantifying the number of erythrocyte-containing capillaries in flash-frozen tissue samples. This exacting method is too invasive to apply to clinical studies. The microbubbles used with CEU have similar rheology to erythrocytes (28), and as with red cells, they are confined to the vascular compartment; hence, they should report reliably only on this volume that constitutes a very small percentage (<5%) of total muscle volume. Albumin microbubbles resonate when exposed to ultrasound energy in order to generate an acoustic signal that is directly related to the microbubble concentration in tissue, resulting in opacification of tissue. At the transmit frequencies and acoustic powers used in protocol 1, microbubbles have been shown to undergo destruction within the ultrasound sector (26), resulting in an enhanced signal. This signal can best be detected above tissue scatter by receiving it at a harmonic of the transmit frequency (36). We have used microbubbles as intravascular tracers using two distinct approaches. All applications of CEU share the notable benefit of clinical ultrasound’s excellent spatial resolution (1–2 mm) compared with other imaging modalities, such as positron-emission tomography (PET) (12) or single photon emission–computed tomography.

In protocol 1, harmonic imaging is performed during continuous infusion of microbubbles, and ultrasound is delivered in intermittent pulses. The variable time delay imposed between ultrasound pulses allows variable filling of the volume of interest by the continuously infused bubbles. With the repeated cycles of bubble destruction, filling of the microvasculature is imaged with this intravascular tracer. The plateau videointensity (A, eq. 3), which is proportional to the volume of the microvasculature occupied by bubbles, increased significantly after infusion of insulin. The steady-state plasma insulin concentration achieved in these subjects (53 ± 5 μU/mL) is well within the normal physiological range seen in healthy, postprandial humans. It bears emphasis that vessels larger than small arterioles, capillaries, and venules are not imaged with this method. Flow through these large vessels is more rapid (e.g., cm/s). As a result, in large vessels, microbubble flow through the ~0.5-cm thick volume of interest is swept by the ultrasound beam and is essentially complete even at the shortest pulse-delay intervals. As a result, the signal from these larger vessels is included in the background images and contributes little, if any, to the background-subtracted videointensity time course that is observed (Fig. 2).

The second microbubble method (protocol 2) uses a more traditional indicator-dilution approach, whereby the signal intensity and mean transit time for a bolus injection of tracer (in this case albumin microbubbles) are used to derive a distribution volume. This is similar to approaches

**TABLE 2**

Glucose uptake and oxygen consumption: protocol 2

<table>
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<th>Variable</th>
<th>Insulin infusion</th>
<th>Saline infusion</th>
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<tr>
<td></td>
<td>Basal</td>
<td>4 h</td>
</tr>
<tr>
<td>Glucose uptake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μmol·min⁻¹·100 ml⁻¹)</td>
<td>0.99 ± 0.14</td>
<td>3.16 ± 0.51*</td>
</tr>
<tr>
<td>Oxygen consumption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μmol·min⁻¹·100 ml⁻¹)</td>
<td>18.9 ± 4.0</td>
<td>20.6 ± 3.6</td>
</tr>
</tbody>
</table>

Data are means ± SE. *P < 0.01 compared with basal measurement.
taken using conventional radionuclide tracer methods (37,38). It is noteworthy that the ultrasound in protocol 2 is delivered at a higher frequency (5 MHz), and bubbles are therefore not destroyed by the ultrasound. The time-intensity curve then displays the relative concentrations of microbubbles that enter and wash through the vasculature. Finally, it is helpful that these methods are relatively noninvasive, although the studies in protocol 2 in which the mean transit of microbubbles is measured require arterial catheterization to deliver microbubbles locally. Furthermore, unlike techniques based on PET imaging, no isotopes are involved and the hardware required is greatly simplified and widely available, and for the local infusion (protocol 2), fewer assumptions are required regarding the nature of the tracer input function.

The videointensity measured cannot be converted to conventional measures of volume in volume units (e.g., ml/100 ml muscle) in a straightforward manner.

However, the changes in videointensity seen before and after intervention should accurately reflect changes in MBV. It is of interest that the response to insulin seen using 1-MX in protocol 2 bears resemblance to those obtained in vivo in studies of rat hindlimb skeletal muscle. In those infusion studies, 1-MX was itself intravenously infused, and insulin increased conversion of 1-MX to 1-methylurate. Those findings suggested that insulin increased the accessibility of 1-MX to the endothelial xanthine oxidase. In the current study, the concentrations of 1-MX achieved in arterial blood by caffeine loading of these subjects are only 2–5% of those achieved in previously reported rat studies. Adapting the HPLC analytical method allowed detection of these low concentrations. There was a detectable A-V difference for 1-MX at basal, and this tended to increase with insulin, although the latter change was not statistically significant. In addition, other metabolites of caffeine (e.g., 1,7-dimethylxanthine) compete with 1-MX for xanthine oxidase (S.R., unpublished observations) and may thereby diminish the sensitivity of the method. These undesirable effects of caffeine suggest that future studies that use chemical extraction of xanthine oxidase substrates to study capillary recruitment in humans will require a different approach. There is precedence for infusion of 1-MX in clinical settings, although this is not currently available in the U.S. Nevertheless, the 1-MX extraction data obtained in the current study are at least consistent with and lend support to data obtained with the intrabrachial microbubble infusion in protocol 2 and are consistent with the effect of insulin to enhance the microvascular volume that was shown using the intermittent harmonic imaging CEU in protocol 1. Taken together, these three separate approaches appear to provide concordant evidence of a microvascular action of insulin to enhance capillary recruitment.

The ongoing controversy involving the hypothesis that insulin exerts a physiologically significant vascular action in muscle may need to be reconsidered. Before the current study, nearly all studies have used total blood flow as a measure of insulin’s vascular action. Our findings suggest that this will under-represent a significant vascular effect of insulin. Baron et al. (39) initially demonstrated an action of modest physiological concentrations of insulin to increase
total human leg blood flow during an insulin clamp. They used a thermodilution method to measure femoral blood flow with a custom-designed thermodilution catheter. Controversy arose when other investigators, using different methods to measure blood flow, including dye dilution, plethysmography, and even PET imaging, did not consistently reproduce this finding (6,40–42). In part, methods used to measure blood flow likely contributed to the differing results. Yet, even when similar thermodilution methods were used, findings supporting (43) or denying (11) a defect in insulin-induced flow enhancement have been reported. Additional factors, including insulin dose, duration of insulin infusion, and whether local or systemic insulin infusions were used, also likely contributed (2). The demonstration that insulin increases nitric oxide production in skeletal muscle lends support to the suggestion that insulin regulates muscle blood flow (4,42,44). However, even granting that higher insulin doses or longer infusion times enhance muscle total blood flow, there is little or no agreement currently as to whether these flow changes impact on glucose metabolism by the muscle. Thus, although blocking insulin’s action to stimulate leg blood flow (using the nitric oxide synthase inhibitor L-NMMA) diminished glucose uptake, enhancing muscle blood flow using bradykinin (10), adenosine (45), or IGF-I (46) did not enhance glucose uptake in normal subjects or overcome insulin resistance. In light of current results, the question arises as to whether each of these vasodilators (nitric oxide, adenosine, bradykinin, etc.), which can increase total blood flow, may in fact have differing effects with regard to capillary perfusion and perhaps with regard to muscle metabolic activity.

Using a very different approach—PET scanning after inhalation of labeled CO—Raitakari et al. (47) measured leg muscle blood volume before and during systemic insulin infusion (5 mU · kg⁻¹ · min⁻¹) in healthy volunteers and later in type 1 diabetic patients (12) and hypertensive subjects (48). The supraphysiologic insulin levels resulted in large increases in total leg blood flow and small (∼10%) but significant increases in blood volume. In those studies, volume was measured with static PET images that were integrated over a 4-min acquisition period. This method has the potential disadvantage of being strongly influenced by blood in larger vessels, which may not show the same responses as the microvasculature to vasoactive agents. Furthermore, systemic infusion of high doses of insulin causes secondary changes in systemic and local release of catecholamines, which may themselves induce changes in both blood flow and volume (49,50).

In a subsequent study, Bonadonna et al. (51) used a radiotracer dilution method and estimated mean transit time, forearm plasma flow, and the extracellular volume to which L-glucose was distributed. Capillary recruitment is not assessed directly by this method, as the vascular volume constitutes only ∼10% of extracellular space. It is of interest, however, that these investigators concluded that pharmacologic doses of insulin both increased flow and significantly expanded the extracellular tissue space drained by deep forearm veins. The authors pointed out that if muscle tissue is homogenously perfused, one would not predict a major effect of increased blood flow on transcapillary glucose exchange in human skeletal muscle, with a basal flow of 30–40 ml · min⁻¹ · 100 g⁻¹. However, if the microvascular system is nonhomogeneous and insulin opens previously unperfused vessels, then the potential for improving nutrient exchange, as well as exposure of the tissue to insulin itself, would be considerable.

The finding that a hormone (in this case insulin) can alter tissue perfusion in the absence of changes in total blood flow is in accordance with an extensive body of data in the isolated constant flow–perfused rat hindlimb. In that preparation, vasoactive hormones (norepinephrine, serotonin, and angiotensin II) can direct blood flow distribution within skeletal muscle under constant flow conditions, and this flow redistribution is paralleled by changes in nutrient exchange. This has led to the concept of nutritive and non-nutritive flow pathways being available in skeletal muscle (21). We have recently observed that during an insulin clamp, infusion of α-methylserotonin decreases muscle 1-MX uptake and concomitantly decreases insulin-stimulated glucose uptake (52). α-Methylserotonin does not affect glucose uptake in isolated muscle preparations, suggesting that the effects seen in vivo may be secondary to the vascular actions of the hormone.

It is of interest that insulin has also been recently demonstrated to have an effect on the elastic properties of very large blood vessels. Westerbacka and colleagues (53,54) observed that insulin, at physiologically relevant concentrations and exposure times, enhanced the distensibility of aortic tissues in humans. Presumably, this action is very distinct from insulin’s action on the microvasculature reported here.

In summary, using several distinct methods, we have observed that physiologic concentrations of locally infused insulin enhance microvascular perfusion in human skeletal muscle. This occurs at insulin concentrations that do not affect total forearm blood flow or brachial artery flow or systemic glucose concentrations. This suggests a
specific action of insulin to influence tissue perfusion in the absence of overall increases in blood flow. Whether these changes in muscle perfusion relate in any necessary manner to insulin’s action to stimulate glucose uptake is not known. However, as local muscle perfusion can potentially affect delivery of insulin and glucose as well as other nutrients, it will be important to further assess the physiologic relation between capillary recruitment and metabolic action in healthy individuals and those in states of impaired insulin action.

ACKNOWLEDGMENTS
This work was supported by Grants USPHS DK-54058, DK-38578, K08 HL-03810, and RR-00847 to the University of Virginia General Clinical Research Center and by a grant-in-aid from the American Heart Association Atlantic coast affiliate. M.C. was supported by a Medical Student Fellowship from the American Diabetes Association.

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