Role of Tissue-Specific Blood Flow and Tissue Recruitment in Insulin-Mediated Glucose Uptake of Human Skeletal Muscle

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Background—Conflicting evidence exists concerning whether insulin-induced vasodilation plays a mechanistic role in the regulation of limb glucose uptake. It can be predicted that if insulin augments blood flow by causing tissue recruitment, this mechanism would enhance limb glucose uptake.

Methods and Results—Twenty healthy subjects were studied with the forearm perfusion technique in combination with the euglycemic insulin clamp technique. Ten subjects were studied at physiological insulin concentrations (≈400 pmol/L) and the other 10 at supraphysiological insulin concentrations (≈5600 pmol/L). Four additional subjects underwent a saline control study. Pulse injections of a nonmetabolizable extracellular marker (1-[3H]-L-glucose) were administered into the brachial artery, and its washout curves were measured in one ipsilateral deep forearm vein and used to estimate the extracellular volume of distribution and hence the amount of muscle tissue drained by the deep forearm vein. Both during saline infusion and at physiological levels of hyperinsulinemia we observed no changes in blood flow and/or muscle tissue drained by the deep forearm vein. However, supraphysiological hyperinsulinemia accelerated total forearm blood flow (45.0 ± 1.8 versus 36.5 ± 1.3 mL · min⁻¹ · kg⁻¹, P < 0.01) and increased the amount of muscle tissue drained by the deep forearm vein (305 ± 46 versus 229 ± 32 g, P < 0.05). The amount of tissue newly recruited by insulin was strongly correlated to the concomitant increase in tissue glucose uptake (r = 0.789, P < 0.01).

Conclusions—Acceleration of forearm blood flow mediated by supraphysiological hyperinsulinemia is accompanied by tissue recruitment, which may be a relevant determinant of forearm (muscle) glucose uptake. (Circulation. 1998;98:234-241.)

Key Words: blood flow • insulin • glucose • muscles

Skeletal muscle is the major organ responsible for the disposal of a glucose load after both intravenous and oral administration. Insulin plays a pivotal role in stimulating glucose uptake in human muscle after glucose administration and does so by activating multiple cellular steps that regulate the biochemical pathways involved in glucose disposal such as transmembrane glucose transport, glucose phosphorylation, glycogen synthesis, and glucose oxidation. Thus a widespread opinion holds that insulin sensitivity in muscle is determined by the stimulation of one or more insulin-regulated steps involved in glucose metabolism.

In recent years, this view has been challenged by studies that have demonstrated that insulin increases leg glucose uptake by simultaneously widening the arteriovenous glucose gradient and increasing leg blood flow. Furthermore, in states of insulin resistance, including obesity and type II diabetes, failure of insulin to augment leg glucose flow has been postulated to account for a sizable although variable fraction of the decrease in muscle glucose uptake.

Experimental evidence supports the view that the insulin-dependent increase in limb blood flow is nitric oxide (NO)-dependent and is secondary to a modulatory action of insulin on vascular NO synthase.

It has been proposed, therefore, that the insulin-stimulatory effect on limb glucose uptake comprises two components: (1) activation of cellular glucose metabolism, which is reflected by a widening of the arteriovenous gradient in glucose concentration, and (2) an increase in glucose supply to the cell, which is reflected by a rise in limb blood flow. Corollary to this hypothesis is that in some insulin-resistant states the defect in insulin action may result not only from some intrinsic defect in the cellular metabolic steps involved in glucose metabolism but also from a defect in glucose supply to the cell. However, a direct effect of blood flow per se in increasing limb glucose utilization has been demonstrated nicely by some investigators but challenged by others.
Glucose supply to the myocyte is a multistep process that involves the amount of glucose delivered to the tissue (ie, the product of arterial glucose concentration times the tissue-specific blood flow expressed as mL·min⁻¹·kg⁻¹ of perfused tissue), the exchanging properties of capillaries and postcapillary venules, and the diffusion processes within the interstitial fluid. The roles played by tissue-specific flow and transvascular exchange in a homogeneously perfused microvascular network were analyzed by Renkin and Crone and Levitt some decades ago. If one uses preliminary data on capillary permeability in human muscle so far reported in abstract form, beyond a critical value (≈30 mL·min⁻¹·kg⁻¹) any further increase in muscle blood flow will have a negligible effect on the amount of glucose that is transferred across the capillary wall and made available for cellular metabolism (Figure 1). As forearm blood flow at rest is typically 30 to 40 mL·min⁻¹·kg⁻¹, if insulin were to increase limb blood flow by augmenting tissue-specific flow in skeletal muscle, this phenomenon would have little impact to augment cellular and limb glucose uptake.

Insulin-mediated acceleration of limb blood flow also may result from the opening of previously closed capillaries in resting muscle, thus leading to the recruitment of previously nonperfused muscle tissue. Adding metabolically active tissue to the limb would increase limb glucose uptake and therefore would be a potentially important component of insulin action in limb tissues (Figure 1). If this mechanism were to prevail, the increase in limb blood flow would not be reflected in tissue-specific flow because it would be secondary to the addition of new areas perfused with the same tissue-specific flow. Obviously, the effect of insulin on limb blood flow could be caused by a combination of the two phenomena, that is, both increased tissue-specific flow and tissue recruitment. To the best of our knowledge, there is no experimental evidence to support either of these possibilities in human skeletal muscle.

We recently have developed a multiple tracer dilution technique for the assessment of transmembrane glucose transport in human forearm tissues. We reasoned that a detailed kinetic analysis of the extracellular marker (1-glucose or D-mannitol) injected during these studies should provide quantitative information on both muscle-specific flow and the amount of tissue accessible for glucose uptake. According to the classic kinetic theory, a nonmetabolizable extracellular marker such as 1-glucose can be used to measure the accessible extracellular (vascular plus interstitial) volume and therefore provides an index of the amount of the tissue available for metabolic exchange with the bloodstream. We therefore undertook the present investigation to assess whether an insulin-induced increase in forearm blood flow is the result of an increase in muscle-specific flow, tissue recruitment, or both.

**Methods**

**Subjects**

Twenty-four healthy young volunteers (21 men and 3 women), ranging in age from 19 to 32 years and in desirable body weight (Metropolitan Life Insurance Tables, 1983) from 92% to 115% were studied. Subjects consumed a weight-maintaining diet that contained 200 to 250 g of carbohydrate for at least 3 days before the study. Body weight was stable in all subjects for at least 3 months before the study. No subject was taking any medication, and there was no family history of diabetes mellitus. No subject participated in any heavy exercise. All subjects had a normal oral glucose tolerance test. Each subject gave informed written consent before participating in the study, which was approved by the Human Investigation Committees of the University of Texas Health Science Center at San Antonio, of the Yale University School of Medicine, and of the Verona City Hospital.

**Experimental Design**

At 8:30 AM, after a 10- to 12-hour overnight fast, subjects were admitted to the Clinical Research Center. Catheters were introduced percutaneously into the brachial artery and retrogradely into an ipsilateral deep forearm vein draining muscle. The tip of the deep forearm catheter was inserted for a distance of 2 inches from the puncture site and could not be palpated in any of the subjects. Previous studies have documented that such catheter placement is not feasible for the forearm.33 Catheter patency was maintained by a slow infusion of normal saline. To exclude blood flow from the hand, a pediatric sphygmomanometer cuff was inflated about the wrist to 100 mm Hg above the systolic pressure for 2 minutes before and during each sampling interval as well as for 2 minutes before and 10 minutes after the tracer injection. After a 60-minute basal period, either a saline control study (3 men and 1 woman) or a euglycemic insulin clamp (18 men and 2 women) was carried out for 130 minutes. Insulin was infused at the rate of 6 pmol·min⁻¹·kg⁻¹ of body weight in 10 subjects (9 men, 1 woman) (referred to as group 1 or study 1; physiological hyperinsulinemia) or at 30 pmol·min⁻¹·kg⁻¹ of body weight (6 subjects) or at 60 pmol·min⁻¹·kg⁻¹ of body weight (4 subjects) (collectively referred to as group 2 or study 2; supraphysiological hyperinsulinemia). Arterial and venous blood samples were collected at −60, −30, −15, and 0 minutes during the basal state and at 80, 100, 130 minutes during the insulin or the saline infusions, when all measurements exhibited steady-state conditions. Forearm blood flow was measured at each sampling interval from the dilution of indocyanine green dye (ICG) infused intra-arterially for 4 minutes.5,24–26 In our hands, forearm blood flow assessed in triplicate by the ICG technique has an average coefficient of variation of 9.2%.
(95% confidence intervals, 7.72% to 11.4%), as determined in 52 consecutive healthy subjects. At ~50 and 110 minutes, a bolus of an extracellular marker, [1-\textsuperscript{3}H]-L-glucose (~8 \textmu Ci), was rapidly (<2 seconds) injected into the artery, 3-O-[\textsuperscript{14}C]-methyl-D-glucose (~4 \textmu Ci) was also injected through the same syringe for a companion experiment. Frequent blood samples (every 10 to 30 seconds) were drawn from the deep vein for 10 minutes thereafter. The midpoint of the collection time (6 to 8 seconds) was recorded for each blood sample. In the computerized analysis of the washout curves (see below), each single point was considered to be the weighted average of tracer concentration within the time window corresponding to the collection time. Forearm volume was determined by water displacement. Forearm density was assumed to be 1.

### Analytical Methods

Plasma insulin concentration was measured with a double-antibody radioimmunoassay.\textsuperscript{5,8} Plasma glucose concentration was measured in duplicate on a Beckman Glucose Analyzer II (Beckman Instruments). The concentration of ICG dye in infusate and plasma were measured spectrophotometrically at 810 nm.\textsuperscript{13} Deep vein plasma samples collected after the tracer intraarterial injection were deproteinized according to Somogyi, dried, reconstituted, and mixed with scintillation fluid (Scintiverse, Fischer). Radioactivity was quantitated in a dual-channel liquid scintillation counter with external standard correction (Packard Instruments). Known volumes of all tracer infusates were added to plasma samples obtained from the same subject before the injection of the tracers, and plasma radioactivity was determined after Somogyi precipitation as described above.

### Calculations

Whole body glucose uptake during the insulin clamps was calculated as previously reported.\textsuperscript{8,30,31} Blood glucose concentration was calculated from plasma glucose concentration according to the following formula:\textsuperscript{31}

\[
\text{Blood Glucose Concentration} = (\text{Plasma Glucose Concentration}) \times (1 - 0.3 \times \text{Hct})
\]

where Hct is the hematocrit.

Glucose uptake across the forearm (FGU) was calculated as

\[
\text{FGU} = (\text{[A]} - \text{[V]}) \times \text{FBF}
\]

where \([A]\) and \([V]\) are the arterial and venous blood glucose concentrations, respectively, and \(\text{FBF}\) is forearm blood flow.

### Measurement of Extracellular Volume and Tissue-Specific Flow

The two fundamental principles underlying our experimental approach are that [1-\textsuperscript{3}H]-L-glucose (1) traces the extracellular kinetic events, that is, distribution with blood flow and diffusion through capillary walls into the interstitial fluids, and (2) undergoes no metabolic conversions.\textsuperscript{29}

As previously reported,\textsuperscript{5,6,28–30} after the pulse injection into the brachial artery, the [1-\textsuperscript{3}H]-L-glucose washout curve in the forearm deep vein was characterized by an early peak with a prolonged, slowly decaying tail (Figure 2). This peculiar shape is due to the presence of at least 3 compartments of L-glucose molecules characterized by distinctly different transit times.\textsuperscript{28} Because the transit time is determined by the ratio of the (extracellular) volume by the rate of plasma flow,\textsuperscript{31} this characteristic of the extracellular markerwashout curves is a proof that perfusion of extracellular volume in resting human muscle is heterogeneous.

On the basis of this empirical finding, we have developed a linear flow compartmental model of the forearm system (Figure 3) with 3 parallel routes interposed between the injection site (brachial artery) and the sampling site (deep forearm vein). The characteristics of this model have been presented and discussed in detail in previous publications\textsuperscript{28,29} and will only briefly summarized herein.

![Figure 2. Typical washout curve of 1-[\textsuperscript{3}H]-L-glucose in the forearm deep venous plasma after an intra-arterial pulse injection at time=0 minutes.](http://circ.ahajournals.org/)

**Kinetics in compartmental models is governed by model structure and \(k\) rate constants (units: min\(^{-1}\)).** The 3 chains of the model in Figure 3 are characterized by different rates,\textsuperscript{28} that is:

\[
k_{33} > k_{31} = k_{34} = k_{35} = k_{37}
\]

Furthermore, in each route the last two rate constants are equal:\textsuperscript{29}

\[
k_{34} = k_{34} = k_{35} = k_{37}
\]

This model structure allows for three populations of L-glucose molecules appearing in the sampling compartment (deep vein) and characterized by different transit times. It has proved to be necessary and sufficient to describe the washout curves of L-glucose and is a parsimonious description of the heterogeneity of forearm perfusion.\textsuperscript{28}

The a priori uniquely identifiable parameterization of the model was fitted for each individual data set by using nonlinear least-squares parameter estimation. Measurement error was assumed additive, uncorrelated, of zero mean and with an experimentally determined variance: coefficients of variation ranged from 1% to 6% to 8% and were higher with lower counts. The conversational version (CONSAM) of the SAAM program was used in model parameter estimation.\textsuperscript{29,30}

According to the classic kinetic theory for nonmetabolizable substances,\textsuperscript{31} in a single inlet (artery)–multiple outlet (vein) system such as the forearm, the volume accessible to L-glucose in the system drained by the deep forearm vein equals the product of the deep vein plasma flow times the mean transit time of L-glucose.

The computerized analysis of the washout curves\textsuperscript{29} provides an estimate of the dose recovered in the deep vein (DR, units: dpm):\textsuperscript{29}

\[
\text{DR} = [(k_{31} + k_{34} + k_{35})(k_{32} + k_{34} + k_{35} + k_{36})] \times \text{Dose Injected}
\]

Thus plasma flow through the deep forearm vein (LPF, units: mL \times \text{min}^{-1}) can be calculated\textsuperscript{29,31} as follows:

\[
\text{LPF} = \text{DR} / \text{AUC}
\]

where AUC (units: dpm \times \text{min} \times \text{mL}^{-1}) is the area under the L-glucose washout curve extrapolated to infinite time.\textsuperscript{29,28,29} Furthermore, a measurement of the arteriovenous mean transit time (MTT\textsubscript{AV}, in the terminology of References 6 and 29; units: minutes) of L-glucose can be obtained from the following formula:

\[
\text{MTT} = (k_{31} + k_{34} + k_{35})^{-1} + (k_{34} + k_{35} + k_{36}) + 2(\Sigma k)X\]

where \(k_{31}\) and \(\Sigma k\) are defined as follows:

\[
k_{31} = k_{31} + k_{34} + k_{35} + k_{36} \quad \text{and} \quad \Sigma k = k_{31} + k_{34} + k_{35} + k_{36}
\]

Note that the symbols used in the formula for the computation of MTT in this article are different from those used in previous publications in order to allow the reader to readily recognize the computational relation between the MTT and the rate constants of the model in Figure 3.
Figure 3. Multicompartmental model used to analyze the \( L \)-glucose washout curves. \( L \)-Glucose is injected in compartment 1 (brachial artery) and its washout curve is measured in compartment 8 (deep vein). Filled arrows indicate transfer of \( L \)-glucose either to another compartment or to irreversible loss. Each transfer occurs at a rate set by the corresponding rate constant \( k \) (units: \( \text{min}^{-1} \)). The rate constant \( k_{10} \) takes into account that the forearm has a single artery but several effluent veins. Therefore, a significant amount of \( L \)-glucose is cleared through veins other than the one in which the washout curve is sampled. See text for further details.

As discussed in previous publications, both the MTT and the AUC are intrinsically model independent,\(^{28,29}\) although we used the model of Figure 3 to calculate them. The product of MTT and LPF, after subtracting the volume of the sampling compartment, is a measure of the extracellular volume in which \( L \)-glucose distributes, that is, the extracellular volume \( (V_{EC}) \in the terminology of References 6 and 29; units: milliliters) of the tissues drained by the deep forearm vein.

Statistical Analysis
All data are presented as mean ± SEM. All results were compared by Student’s \( t \) test for unpaired or paired data. Correlations were sought with the use of standard formulas.\(^{41}\)

The minimum detectable difference of a variable of interest in a paired study was calculated according to the following formula:

\[
\delta = s \times (t_{a/2,n} + |b|/\sqrt{n})^{\alpha/2}
\]

where \( \delta \) is the minimum detectable fractional difference, \( s \) is the fractional coefficient of variation of the variable \( t_{a/2,n} \) and \( |b|/\sqrt{n} \) are the \( t \) values corresponding to a 90% \( (1 - \beta) \) level of significance with \( n \) degrees of freedom, and \( n \) is the number of observations.\(^{41}\)

Results

Plasma Glucose and Insulin Concentrations
Basal plasma glucose and insulin levels were similar in group 1 and in group 2 (5.1 ± 0.2 versus 5.0 ± 0.2 \( \text{mmol/L} \) and 44 ± 2.5 versus 47 ± 6 \( \text{pmol/L} \)) as well as in the saline control group (glucose: 5.27 ± 0.17 \( \text{mmol/L} \); insulin: 52.4 ± 11 \( \text{pmol/L} \)). During the insulin clamp and during the saline infusion, plasma glucose concentrations were similar in groups 1 and 2 and in the saline control group (5.1 ± 0.6, 5.2 ± 0.3 and 5.3 ± 0.2 \( \text{mmol/L} \), respectively). At the end of the saline infusion period, the plasma insulin concentration (45.2 ± 6.2 \( \text{pmol/L} \)) did not change from baseline. The steady-state plasma insulin concentrations during the insulin clamp studies were 410 ± 40 and 5640 ± 1080 \( \text{pmol/L} \), respectively (\( P < 0.01 \) versus baseline, versus the saline study, and from each other).

Forearm Glucose Uptake and Blood Flow
Forearm glucose uptake and blood flow were unchanged by the saline infusion (7.5 ± 2.9 versus 6.3 ± 2.1 \( \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \) and 39.4 ± 5.5 versus 44.1 ± 5.2 \( \text{mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \), respectively).

Whole body glucose uptake during the insulin clamp was significantly higher in group 2 than in group 1 (55.6 ± 3.8 \( \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \); \( P < 0.01 \)), whereas the increase was of borderline statistical significance in group 1 (42.3 ± 6.2 \( \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \), \( P < 0.05 \)) (Figure 4). The glucose arteriovenous gradients were also widened by hyperinsulinemia both in group 1 (0.988 ± 0.165 versus 0.155 ± 0.026 \( \text{mmol/L} \), \( P < 0.001 \)) and in group 2 (1.19 ± 0.144 versus 0.091 ± 0.02 \( \text{mmol/L} \), \( P < 0.001 \)). During the insulin clamp, therefore, forearm glucose uptake increased both in group 1 (41.2 ± 6.7 versus 5.85 ± 0.96 \( \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \), \( P < 0.01 \) versus baseline) and in group 2 (52.9 ± 5.6 versus 3.2 ± 0.6 \( \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \), \( P < 0.01 \) versus baseline); the increment over baseline was significantly greater in group 2 than in group 1 (49.8 ± 5.2 versus 35.3 ± 5.9 \( \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{kg}^{-1} \), \( P = 0.04 \) by one-tailed \( t \) test) (Figure 5).

Extracellular Volume and Muscle-Specific Blood Flow
No changes in either the extracellular volume \( (V_{EC}) \) drained by the deep forearm vein (24.2 ± 8.0 versus 22.1 ± 3.5 \( \text{mL} \) at
Insulin and Tissue Recruitment in Human Muscle

In this study, we have examined the kinetics of a radioactive extracellular marker across the deep forearm tissues (skeletal muscle) to assess whether insulin-induced changes in total forearm blood flow are secondary to changes in tissue perfusion or in the amount of tissue perfused by the bloodstream. We chose to explore two different ranges of plasma insulin concentrations, one that is physiological (group 1) and another that is supraphysiological (group 2), because, in our experience, insulin-mediated increases in forearm blood flow are readily evident only at very high insulin concentrations.5

Because our approach has not previously applied to the study of human tissues, we performed saline control studies to ascertain whether our estimates of muscle-specific blood flow and of the extracellular volume drained by the same deep forearm vein are stable and allow the investigator to detect reasonable changes in the parameters of interest. Our results show that with our technique, the average values of muscle-specific blood flow and extracellular volume are fairly reproducible and have coefficients of variation of \( \approx 20\% \). Thus the present study had a 90\% chance of detecting a minimum insulin-induced difference of \( \approx 27\% \) in muscle-specific blood flow and extracellular volume at a significance level of 0.05.

We next attempted to examine some aspects of precellular and cellular action of insulin in forearm muscle. As expected, insulin stimulated forearm glucose uptake (Figure 5), with a somewhat greater effect seen at supraphysiological (group 2) than physiological (group 1) insulin levels. Importantly, in

**Discussion**

In this study, we have examined the kinetics of a radioactive extracellular marker across the deep forearm tissues (skeletal muscle) to assess whether insulin-induced changes in total forearm blood flow are secondary to changes in tissue perfusion or in the amount of tissue perfused by the bloodstream. We chose to explore two different ranges of plasma baseline, \( P=NS \) or in the muscle-specific blood flow (42.9±16.4 versus 47.1±11.5 mL \( \cdot \) min\(^{-1} \cdot \) kg\(^{-1} \) at baseline, \( P=NS \)) were detected during the saline control studies. The within-day and within-subject coefficients of variation derived from the saline control studies were 21±11\% and 22±5\% for \( V_{EC} \) and muscle-specific blood flow, respectively.

During the insulin clamp, the MTT of L-glucose was slightly but not significantly (\( P<0.20 \) for both) prolonged both in group 1 (4.97±0.73 versus 3.92±0.29 minutes) and in group 2 (5.42±0.85 versus 3.96±0.30 minutes). Local plasma flow in the deep forearm vein was not significantly affected by hyperinsulinemia both in group 1 (7.65±1.8 versus 9.18±1.71 mL/min; \( P=0.31 \)) and in group 2 (6.82±1.13 versus 6.43±1.02 mL/min; \( P=0.69 \)). The extracellular volume drained by the deep forearm vein increased in group 2 (32.1±4.8 versus 24.1±3.3 mL, \( P<0.05 \)) but not in group 1 (33.4±6.3 versus 33.5±5.3 mL, \( P=NS \)). After extrapolation to muscle mass, in group 2 the deep forearm vein drained 229±32 g of muscle tissue at baseline and 305±46 g during hyperinsulinemia (an increase of 39.2±14.3\%, significantly different from zero at \( P<0.05 \)), whereas in group 1 the deep forearm vein drained 318±51 g at baseline and 317±59 g of muscle tissue during the clamp (\( P=NS \) (Figure 6)). Insulin-induced changes in muscle mass (or extracellular volume) drained by the deep forearm vein and in forearm glucose uptake were positively correlated (Figure 7) to each other in group 2 (\( r=0.789, P<0.007 \)) but not in group 1 (\( r=-0.39, P=NS \)).

Muscle-specific blood flow (Figure 8) decreased by 10\% to 15\% during the insulin clamp both in group 1 (43.8±6.6 versus 48.0±3.4 mL \( \cdot \) min\(^{-1} \cdot \) kg\(^{-1} \) at baseline) and in group 2 (42.3±5.9 versus 49.5±3.9 mL \( \cdot \) min\(^{-1} \cdot \) kg\(^{-1} \) at baseline), but these differences were not statistically significant.

**Figure 5.** Increment in forearm glucose uptake induced by physiological (group 1, left bar) and supraphysiological (group 2, right bar) insulin concentrations. ¶\( P=0.04 \) group 2 versus group 1 by 1-tailed \( t \) test.

**Figure 6.** Insulin-induced percent changes in the muscle mass drained by the deep forearm vein. ¶\( P<0.05 \) versus 0 by two-tailed \( t \) test.

**Figure 7.** Insulin-induced tissue recruitment (\( x \) axis) versus insulin-stimulated forearm glucose uptake over baseline (\( y \) axis) in study 2 (euglycemic clamps at supraphysiological insulin concentrations).
group 2, systemic hyperinsulinemia also caused a significant increase in total forearm blood flow (Figure 4), which was not paralleled by an increase in tissue specific blood flow (Figure 8) or in deep vein plasma flow, as assessed by the dilution curve of the extracellular marker across the forearm tissues. Thus increased muscle-specific blood flow is not part of the biological response of forearm muscle tissue to systemic hyperinsulinemia.

The most important finding of this study is that in parallel with the increase in total forearm blood flow detected at supraphysiological insulin concentrations (Figure 4), the volume of distribution of L-glucose was increased by \( \approx 39\% \) (Figure 6). This result could be anticipated from the behavior of the MTT of L-glucose, if one considers that the L-glucose volume of distribution equals the product of flow by the mean transit time of L-glucose. If the insulin-induced increase in forearm blood flow, documented in group 2 by the ICG dilution technique (Figure 4), had taken place in the same amount of tissue perfused at baseline, the MTT of L-glucose should have fallen proportionally to the rise in tissue perfusion. Because the MTT of L-glucose did not fall but actually somewhat increased, the inescapable conclusion is that the volume of distribution of L-glucose within the forearm must have increased during supraphysiological hyperinsulinemia. Importantly, this same conclusion and the quantitation of this increase in L-glucose space above baseline observed in this study was \( \approx 40\% \); (2) the increase in blood volume (and vascular space) found by Raitakari et al. at pharmacological insulin levels was only \( \approx 3 \text{ mL/kg of muscle tissue} \), whereas we observed an increase in muscle extracellular volume of \( \approx 24 \text{ mL/kg} \). From these observations, it follows that from 70% to 80% of the phenomenon we report herein was subtended by an expansion in interstitial volume.

The expansion in the interstitial volume caused by insulin could be secondary either to a swelling of the interstitial volume itself, to new access of the extracellular marker to previously inaccessible interstitial volume, or both. The former mechanism might be mediated by the increased transcapillary escape rate of albumin that takes place during hyperinsulinemia, which in turn would bring about a shift of water into the interstitial space from both the vascular and the intracellular spaces. During an insulin clamp, \( \approx 6 \text{ g of albumin leaves the intravascular pool} \). Even if one assumes that all of this albumin is sequestered within muscle interstitial volume (\( \approx 2.7 \text{ L in a 70 kg man} \)), this would lead to an increase of \( \approx 30 \text{ to 35 } \mu\text{mol of albumin per liter of interstitial fluid, ie, a rise of } <0.1\% \text{ in osmolality} \). Hence this explanation is untenable on quantitative grounds. Thus we strongly favor the interpretation that the primary mechanism underlying the increase in L-glucose space documented in our study is the recruitment of previously inaccessible interstitial volume.

The choice of \(^3\text{H-L-glucose} \) (molecular mass of 182 Da) limits our conclusions to molecules with similar molecular mass, such as D-glucose (molecular mass=180 Da). The effects of insulin on the interstitial volume herein reported may not be applicable to smaller molecules than L-glucose, such as water and oxygen.

If new interstitial space was made accessible to L-glucose by insulin, previously inaccessible cellular surface (and space) also must have been made accessible to cell permeant molecules such as D-glucose. Stated otherwise, our data strongly suggest that supraphysiological hyperinsulinemia caused tissue recruitment. Such an event would be expected to lead to increased forearm glucose uptake (Figure 1). Indeed, we found a strong correlation between the increase in extracellular volume (muscle mass) and the increase in forearm glucose uptake, which, however, was of quantitative bearing only beyond \( \approx 40 \mu\text{mol/min} \cdot \text{kg}^{-1} \) of forearm glucose uptake (Figure 7), thereby suggesting that two distinct, although possibly related, insulin-induced phenomena occurred in the forearm: (1) stimulation of cellular glucose metabolism and (2) recruitment of metabolically active tissue. Both are of apparent quantitative importance, although, as suggested by the correlation shown in Figure 7, stimulation of cellular glucose metabolism appears to play a predominant role. A further corollary to our findings is that because the intercept (ie, the zero recruitment point) of the graph relating forearm glucose uptake to recruitment (Figure 7) is almost identical to the forearm glucose uptake at physiological hyperinsulinemia (41 \mu\text{mol/min} \cdot \text{kg}^{-1}), the greater stimulatory effect of supraphysiological hyperinsulinemia on glucose uptake may be accounted for largely by tissue recruitment.

The question arises as to whether our findings imply that under normal circumstances areas of muscle tissue are permanently unperfused. This is not necessarily the case. The pulse injection technique “sees” as perfused areas only those that are open during the time that the tracer leaves the syringe...
(<2 seconds) and reaches the capillaries. Because blood flow velocity in the brachial artery typically is 10 to 20 cm/s, the entire time during which the tracer can physically gain access to the capillaries should not be >3 seconds. If vasomotion (rhythmic opening and closing) of precapillary sphincters takes place within muscle tissue, then the tracer will gain access to only that fraction of tissue that is perfused during those 3 seconds, although the nonperfused tissue also will be perfused shortly thereafter. Studies in rabbit muscle have shown that terminal arterioles constrict up to the point of functional closure (diameter of ≈2 μm) and relax at a fundamental frequency of ≈18 cycles per minute.44 Furthermore, in rats, perfusion of muscle capillaries by a fluorescent dye is critically dependent on the duration of the injection, with only 36% to 55% of the capillaries accessible to the dye when the injection lasts 1 to 2 seconds.45

We propose that resting muscle is perfused by blood flow in an intermittent fashion and that during the time of nonperfusion, anaerobic metabolism (glycogenolysis, anaerobic glycolysis, and lactate production) prevails, whereas when the muscle is perfused, aerobic metabolism (plasma glucose and free fatty acid uptake/oxidation) predominates. At any single time point, blood flow and glucose uptake depend on the fraction of tissue that is in the aerobic mode. Insulin, by prolonging the open time of the precapillary sphincters and/or changing the frequency of their vasomotion, causes an increase in bulk limb flow and, at any given moment, shifts a higher percentage of muscle tissue toward the aerobic metabolism and glucose uptake. Consistent with, although not a proof of, this hypothesis is our recent finding in human skeletal muscle that hyperinsulinemia increases regional blood flow proportionally more in the areas that at baseline are characterized by low flow.46

Such a scenario, although fascinating, needs to be experimentally proved in human beings. Thus we cannot rule out the alternative albeit unlikely implication of our data that under normal circumstances, there are areas in resting muscle that are permanently unperfused and are recruited only when exercise or hyperinsulinemia stimulate muscle metabolism.

The results herein reported may help to explain some of the discrepancies found in the relation between flow- and insulin-mediated glucose uptake.21–24 Insulin causes vasodilation through an NO synthase–dependent mechanism and recruits muscle tissue, thereby amplifying its stimulatory effect on cell glucose metabolism. In several studies intraarterial infusion of different vasodilators was used to test the hypothesis that vasodilation during hyperinsulinemia is a quantitatively important determinant of muscle glucose uptake in vivo in human beings, but the results have been contradictory.21–24 However, not all vasodilators are expected to increase insulin-mediated glucose metabolism to the extent that their vasodilatory effect is not coupled to tissue recruitment. Intraarterial infusions of various vasodilators, including meta-choline,21 adenosine,22 and bradykinin,24 have been used to increase muscle blood flow during hyperinsulinemia. However, it is unknown whether these substances cause muscle tissue recruitment in vivo in human beings, and it is unclear whether they are of help in dissecting out the relative roles of vascular versus cellular events in the stimulatory effects of insulin on glucose metabolism in human skeletal muscle.

We observed insulin-induced increases in blood flow and tissue recruitment only at insulin concentrations beyond the physiological levels. Our experimental design had a 90% chance of detecting changes in forearm flow and tissue recruitment of ≈12% and ≈27%, respectively, at a significance level of 0.05. Smaller changes induced by physiological insulin levels in group 1 would be likely to be missed. Therefore the relation of our findings to normal physiology remains unclear. However, in several laboratories,10,47,48 insulin-induced increases in leg blood flow have been observed even at physiological insulin levels. Thus it is possible that what we have documented in forearm tissues at supraphysiological insulin levels already is occurring in leg tissues at physiological insulin concentrations. Because leg tissues harbor ≈40% of total body muscle, the quantitative impact of our findings on whole body glucose homeostasis would be much greater than expected on the basis of our forearm (≈4% of body muscle) studies. Additional studies in human leg tissues are needed to resolve this issue.

In summary, the evidence reported in this study sheds new light on the relation between vascular and metabolic effects of insulin. Evidence has been provided by some investigators that insulin can increase limb blood flow in human beings. However, these studies did not examine whether vasodilation was or was not accompanied by tissue recruitment. Our results document that supraphysiological hyperinsulinemia increases limb blood flow and that this is associated with tissue recruitment. This finding offers a potential mechanistic determinant of limb (and whole body) insulin sensitivity in human beings.

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