

Interaction Between Insulin Sensitivity and Muscle Perfusion on Glucose Uptake in Human Skeletal Muscle

Evidence for Capillary Recruitment

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Insulin and glucose delivery (muscle perfusion) can modulate insulin-mediated glucose uptake. This study was undertaken to determine 1) to what extent insulin sensitivity modulates the effect of perfusion on glucose uptake and 2) whether this effect is achieved via capillary recruitment. We measured glucose disposal rates (GDRs) and leg muscle glucose uptake (LGU) in subjects exhibiting a wide range of insulin sensitivity, after 4 h of steady-state (SS) euglycemic hyperinsulinemia (>6,000 pmol/l) and subsequently after raising the rate of leg blood flow (LBF) 2-fold with a superimposed intrafemoral artery infusion of methacholine chloride (Mch), an endothelium-dependent vasodilator. LBF was determined by thermodilution: LGU = arteriovenous glucose difference (AVGΔ) × LBF. As a result of the 114 ± 12% increase in LBF induced by Mch, the AVGΔ decreased 32 ± 4%, and overall rates of LGU increased 40 ± 5% ($P < 0.05$). We found a positive relationship between the Mch-modulated increase in LGU and insulin sensitivity (GDR) ($r = 0.60$, $P < 0.02$), suggesting that the most insulin-sensitive subjects had the greatest enhancement of LGU in response to augmentation of muscle perfusion. In separate groups of subjects, we also examined the relationship between muscle perfusion rate and glucose extraction (AVGΔ). Perfusion was either pharmacologically enhanced with Mch or reduced by intra-arterial infusion of the nitric oxide inhibitor *N*^G-monomethyl-L-arginine during SS euglycemic hyperinsulinemia. Over the range of LBF, changes in AVGΔ were smaller than expected based on the noncapillary recruitment model of Renkin. Together, the data indicate that 1) muscle perfusion becomes more rate limiting to glucose uptake as insulin sensitivity increases and 2) insulin-mediated increments in muscle perfusion are accompanied by capillary recruitment. Thus, insulin-

stimulated glucose uptake displays both permeability- and perfusion-limited glucose exchange properties. *Diabetes* 49:768–774, 2000

Insulin is characterized by its ability to stimulate glucose uptake and does so principally in skeletal muscle (1). Insulin also displays a dose-dependent effect to increase blood flow to skeletal muscle by causing a nitric oxide (NO)-dependent vasodilation in skeletal muscle vasculature (2–5). We have previously reported that skeletal muscle glucose uptake is enhanced by insulin-mediated skeletal muscle vasodilation and that it could be modulated by pharmacologically altering the rate of skeletal muscle perfusion. Indeed, we have shown that abrogation of insulin-mediated vasodilation of human leg vasculature with the NO synthase inhibitor *N*^G-monomethyl-L-arginine (L-NMMA) (Clnalfa AG, Läufelfingen, Switzerland) could reduce muscle glucose uptake (5,6). Conversely, increasing leg skeletal muscle perfusion with the endothelium-derived vasodilator methacholine chloride (Mch) (Roche Laboratories, Nutley, NJ) resulted in enhanced glucose uptake (7).

Although changes in perfusion have the ability to modulate insulin-mediated glucose uptake, the mechanism(s) by which this occurs is not well understood. Better understanding of the underlying mechanism is an important issue, because insulin's effect to stimulate skeletal muscle perfusion is diminished in states of insulin resistance (8,9) and, thus, it is possible that diminished tissue perfusion could contribute to insulin resistance in these subjects. Microcirculatory perfusion in resting skeletal muscle is neither continuous nor uniform, but rather intermittent and heterogeneous in its distribution (10,11). Thus, capillaries perfusing skeletal muscle are not always equally perfused but rather display "on-off" or alternating perfusion. This pattern of capillary flow is, in part, controlled by precapillary arteriolar resistance and vasomotion (10). This mode of capillary perfusion is thought to make capillary exchange of oxygen and nutrients in resting muscle more energetically economical (10,11). We have previously proposed that via its effect to release NO, insulin reduces precapillary arteriolar tone and alters arteriolar vasomotion with a resultant increase and more homogeneous overall capillary perfusion termed "functional capillary recruitment" (12,13). The latter would, in turn, allow for greater capillary exchange of substrate.

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AVGΔ, arteriovenous glucose difference; FFM, fat-free mass; GDR, glucose disposal rate; HR, heart rate; LBF, leg blood flow; LGU, leg muscle glucose uptake; L-NMMA, *N*^G-monomethyl-L-arginine; MAP, mean arterial blood pressure; Mch, methacholine chloride; NO, nitric oxide; NSL, normal saline; SS, steady-state.

Regardless of whether this model of insulin-induced microcirculatory modulation of substrate exchange is correct, based on known capillary/tissue glucose exchange principles, one can make some reasonable predictions. First, that perfusion would become more rate limiting for glucose uptake in situations in which tissue permeability to glucose is high (10,14). In other words, if there is little barrier for diffusion of glucose from the capillary to the intracellular space, delivery of substrate (perfusion rate) would be expected to be relatively rate limiting for overall glucose uptake. On the other hand, where tissue permeability is relatively low, one would not expect perfusion to have as great a modulating effect on glucose uptake. In the latter situation, cellular permeability of glucose would be rate limiting for overall glucose uptake. Second, under conditions of uniform capillary perfusion (no functional capillary recruitment), it would follow logically that for a given degree of cellular permeability to glucose, an increase in perfusion would be accompanied by a largely commensurate fall in glucose extraction with little or no effect on glucose uptake as proposed by Renkin (10). This would be reflected by a hyperbolic relationship between muscle perfusion rate and glucose extraction, such that as perfusion is altered, extraction would change in the opposite direction with little resultant net effect on glucose uptake. On the other hand, if, as we hypothesize, functional capillary recruitment occurs in response to insulin stimulation, the relationship between glucose extraction and perfusion would differ significantly from the predicted model. Therefore, the current study was undertaken to 1) determine the relationship between insulin sensitivity and the ability of perfusion to modulate skeletal muscle glucose uptake and 2) test the validity of the functional capillary recruitment model for glucose exchange during insulin stimulation.

RESEARCH DESIGN AND METHODS

Subjects. Characteristics of the study subjects in each protocol are given in Table 1. Subjects were characterized for body composition by dual-energy X-ray absorptiometry. All subjects were healthy and nondiabetic and displayed normal 75-g oral glucose tolerance tests. All study subjects were normotensive as determined by cuff pressure; none was ingesting medications. Studies were approved by the Indiana University Human Subjects Internal Review Board and all volunteers gave informed consent.

Diet. All subjects were admitted to the Indiana University General Clinical Research Center 2 days before study and were fed a weight-maintaining diet, the caloric content of which was distributed as 50% carbohydrate, 30% fat, and 20% protein.

Drugs. All infusates were prepared under sterile conditions on the morning of the study. Regular insulin (Humulin; Eli Lilly, Indianapolis, IN) was diluted in normal saline (NSL) to the desired concentration with added albumin. Mch was dissolved in NSL to a concentration of 25 µg/ml. L-NMMA was dissolved in NSL to a concentration of 8 mg/ml. Insulin was administered through a catheter in the antecubital vein. Mch or L-NMMA was infused directly into the femoral artery with a Harvard programmable pump model 44 (Harvard Apparatus, South Natuck, MA).

Protocol. Separate groups of subjects were studied under 2 distinct study protocols designed 1) to assess the effect of an ~2-fold increase in perfusion on skeletal muscle insulin-mediated glucose uptake (protocol 1) in subjects establishing a wide range of insulin sensitivity and 2) to establish the relationship between perfusion rate and skeletal muscle glucose extraction over a wide range of perfusion rates during insulin stimulation (protocol 2).

Methods common to both protocols are described as follows. At ~7:00 A.M., after an overnight 14-h fast, a 19-gauge catheter was inserted into the antecubital vein for infusion of substances. Subsequently, the right femoral artery and vein were cannulated. A 6 French sheath (Cordis, Miami, FL) was placed in the right femoral vein to allow the insertion of a custom-designed 5 French double lumen thermomodulation catheter (Baxter Scientific, Edwards Division, Irvine, CA) to measure leg blood flow (LBF) as previously described (1). The right femoral artery was cannulated with a 5.5 French double lumen catheter (Arrow International, Reading, PA) to allow simultaneous infusion of substances through the proximal

TABLE 1
Demographic and basal (fasting) metabolic characteristics of the study groups

	Protocol 1	Protocol 2	
		Group 1	Group 2
<i>n</i> (M/F)	16/1	12/3	7/1
Age (years)	38.1 ± 1.5	32 ± 1.7	35 ± 3
Weight (kg)	86.7 ± 4.1	66.8 ± 2.5	80.1 ± 4.7
BMI (kg/m ²)	28.3 ± 1.3	22.3 ± 0.6	27.3 ± 1.8
Fat (%)	28.5 ± 3.2	20.1 ± 2.1	26.8 ± 4.4
Glucose (mmol/l)	5.29 ± 0.11	5.12 ± 0.06	5.23 ± 0.18
MAP (mmHg)	92.0 ± 2.2	82.7 ± 1.6	91.2 ± 3.7
Insulin (pmol/l)	52.8 ± 6.0	29.4 ± 4.2	43.2 ± 5.4
Right leg muscle (kg)	9.7 ± 0.6	9.3 ± 0.5	10.2 ± 1.1
Cholesterol (mmol/l)	4.91 ± 0.44	4.67 ± 0.28	4.56 ± 0.27
Triglycerides (mmol/l)	2.05 ± 0.18	1.17 ± 0.17	1.52 ± 0.21

Data are *n* or means ± SE.

port (most caudad), and invasive blood pressure monitoring through distal port (most cephalad). Heart rate (HR) and mean arterial blood pressure (MAP) were monitored continuously via precordial leads and a pressure transducer connected to a vital signs monitor (VSM 1; Physiocontrol, Redmond, WA).

Hemodynamic measurements. All hemodynamic measurements were obtained with each subject in the supine position in a quiet temperature-controlled room and after the subject had emptied his or her bladder. Baseline measurements of LBF, MAP, and HR were obtained after allowing at least 30 min of rest after the insertion of the catheters. During graded intrafemoral artery infusion of Mch, LBF measurements were begun 2 min after the onset of each dose. LBF measurements were performed every ~30 s for a total of 10 determinations at each drug dose. Invasively determined MAP and HR were recorded with every other LBF determination. Intrafemoral artery infusions of Mch were repeated after ~200 min of euglycemic hyperinsulinemia when glucose disposal rates (GDRs) and hemodynamic parameters were in near steady-state (SS).

Whole-body and leg glucose uptake. Whole-body glucose uptake was assessed with the euglycemic-hyperinsulinemic clamp technique. Each clamp was performed during a square wave systemic infusion of insulin at a rate of 300 mU · m⁻² · min⁻¹ (protocol 1) or 120 mU · m⁻² · min⁻¹ (protocol 2A) and 300 mU · m⁻² · min⁻¹ (protocol 2B). The serum glucose concentration was maintained at the baseline level by administering a 20% dextrose solution at a variable rate according to arterial serum glucose measurements obtained at 5-min intervals. K₂HPO₄ (~0.001–0.0038 mEq · kg⁻¹ · min⁻¹) was infused during the euglycemic-hyperinsulinemic clamps to prevent hypokalemia and hypophosphatemia. Serum potassium levels were maintained >3.5 mEq/l during all study conditions.

Rates of glucose uptake were calculated by averaging the glucose infusion rates achieved over the last 40 min of the clamp as endogenous glucose production is completely suppressed at the elevated insulin concentrations achieved (~1,680–6,000 pmol/l). Leg glucose uptake (LGU) was calculated as the product of the arteriovenous glucose difference (AVGΔ) and LBF: LGU = AVGΔ × LBF. Plasma glucose was converted to blood glucose by the following formula: blood glucose = plasma glucose × (1–0.3 × hematocrit).

Protocol 1. The study protocol was designed to determine the effect of insulin sensitivity on the augmentation of insulin-mediated glucose uptake achieved by pharmacologically induced 2-fold increment in skeletal muscle perfusion. To establish in each individual the dose of intra-arterial Mch to achieve a 2-fold increase in LBF, graded intrafemoral artery infusions of Mch at sequential doses of 2.5, 5.0, 7.5, 10.0, and 12.5 µg/min were administered in all subjects under basal conditions. The volume of Mch infusate delivered ranged from 0.1 to 0.5 ml/min. Subsequently, each subject underwent a euglycemic-hyperinsulinemic clamp for 240 min. At 240 min, each subject received an intrafemoral artery infusion of Mch, which, at baseline, was shown to increase LBF 2-fold. Thus, rates of LGU and whole-body glucose uptake were determined at baseline, during SS euglycemic hyperinsulinemia, and during superimposed intrafemoral artery infusion of Mch designed to double leg perfusion rate.

Protocol 2. To establish the relationship between muscle perfusion rate and insulin-stimulated glucose extraction by skeletal muscle over a wide range of perfusion rates, euglycemic clamps were performed in 2 separate groups of lean insulin-sensitive age- and body composition-matched subjects following a protocol similar to protocol 1. In group 1 (flow reduced, *n* = 15), the LBF rate achieved after 240 min of euglycemic hyperinsulinemia was reduced via a super-

imposed intrafemoral artery infusion of the NO synthase inhibitor L-NMMA (16 mg/min). This maneuver reduced rates of LBF to basal or below basal rates as previously reported (6). In group 2 (flow enhanced, $n = 8$), an intrafemoral artery infusion of Mch at a fixed rate, determined under basal condition, was superimposed after 240 min of euglycemic hyperinsulinemia designed to achieve a maximal increase in the rate of LBF. Groups 1 and 2 underwent euglycemic-hyperinsulinemic clamps at insulin doses of $120 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ and $300 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$, respectively, to avoid a potential bias. As result of a decrease in LBF (group 1), the $\text{AVG}\Delta$ is expected to increase. It is possible that as a result of a reduction in microcirculatory flow, higher insulin concentrations may be produced at the local tissue level and this would have the effect of further enhancing the $\text{AVG}\Delta$. Conversely, if enhancing flow (group 2) produces a lower local insulin concentration, this may have the effect of further decreasing the $\text{AVG}\Delta$. It is important to keep in mind that these 2 insulin infusion rates were maximally and equivalently effective in lean insulin-sensitive subjects (4). This is evidenced by the fact that under SS euglycemic-hyperinsulinemic conditions, the whole-body GDR is virtually identical at both insulin infusion rates, thus confirming equivalent *in vivo* insulin action under the 2 insulin infusion protocols. This probably occurred because group 2 was somewhat more insulin resistant as a result of being more adipose (% body fat 26 vs. 20, $P < 0.05$).

Analytical methods. Blood for determination of plasma insulin concentrations was collected in tubes treated with heparin. The specimens were spun, and the supernatant was removed and stored at -20°C . Insulin levels were measured using the "Coat a Count" kit (Diagnostic Products, Los Angeles, CA). Blood for serum glucose determinations was drawn, put into untreated polypropylene tubes, and centrifuged with an Eppendorf microcentrifuge (Brinkman, Westbury, NY). The glucose levels in the supernatant were determined by the glucose oxidase method with a glucose analyzer YSI 2300 (Yellow Springs, OH). Serum cholesterol and triglyceride levels were measured on an Ektachem 702 analyzer (Kodak, Rochester, NY) with an enzymatic method.

Data analysis. Results are shown as means \pm SE. LBF is expressed in liters per min (l/min). Paired and unpaired comparisons were performed using the Student's *t* test. Statistical significance was accepted at a level of $P < 0.05$. Statistics were performed on a Macintosh computer with StatView 4.5 (Abacus Concepts, Berkeley, CA) and with the general linear models procedure in the SAS statistical software package (SAS Institute, Cary, NC).

Analysis of data obtained in protocol 2 was based on the equation proposed by Renkin (10) relating solute exchange (in this case, glucose), perfusion rate, and glucose concentration:

$$Jg = Ca \times Q (1 - e^{-PS/Q})$$

Where Jg = net flux of glucose from blood to tissue, Ca = arterial glucose concentration, Q = blood flow, e = base of natural logarithms, P = permeability of membranes to glucose, and S = capillary endothelial surface area exposed to blood perfusion.

In a capillary system with fixed PS (no functional capillary recruitment), a large PS relative to Q (PS/Q ratio) allows glucose clearance (Jg/Ca) to approach Q as a limit, and thus flow becomes rate limiting to glucose exchange. Conversely, as PS/Q decreases, Jg also decreases, approaching the PS as the limit for glucose exchange. Thus, in a model with a small PS -to- Q ratio, it follows that perfusion can have only a trivial modulating effect on uptake. The reason for the lack of an effect of perfusion to increase glucose uptake in a capillary system with fixed PS is related to the effect of Q on glucose extraction as discussed below.

According to Fick's principle, Jg (glucose uptake) = $Q(Ca - Cv)$. Viewed in simplistic terms, the $Ca - Cv$ ($\text{AVG}\Delta$) represents tissue glucose extraction and reflects, to a large degree, tissue permeability under conditions of fixed Q . Q or blood flow reflects the rate of delivery of glucose. The Fick equation holds that for an increase in Q , there will be a reciprocal fall in $\text{AVG}\Delta$. Thus, in a model in which no capillary recruitment occurs, one would predict that an increment in perfusion would result only in a small net increase in uptake, because the augmentation of glucose delivery is offset by the reduction in extraction, leading to a hyperbolic relationship between perfusion and extraction.

Under control of the feeding arterioles or precapillary sphincters that undergo vasomotion (14), not all capillaries are equally perfused but rather display flow heterogeneity (10,15). If insulin stimulation results in increased homogeneity of capillary perfusion (functional recruitment with increased PS/Q), one would expect a lower fall in glucose extraction in response to perfusion enhancement than predicted with a fixed PS/Q model. Moreover, increased PS/Q would result in a lower rise in glucose extraction in response to a reduction in perfusion than predicted with a fixed PS/Q model. Thus, we determined the relationship between flow and extraction based on actual data obtained in protocol 2 in which muscle perfusion was manipulated pharmacologically over a wide range of perfusion rates and compared it with the relationship as predicted by the Renkin equation. To this end, PS was determined for SS conditions (euglycemic hyperinsulinemia without flow modulation) according to the formula

$PS = -Q \ln(Cv/Ca)$, where Cv equals the venous glucose concentration. The calculated SS PS was then used to compute the predicted $\text{AVG}\Delta$ (extraction) at each measured LBF according to the equation $\text{AVG}\Delta = Ca(1 - e^{-PS/Q})$. (Applying this equation will yield identical $\text{AVG}\Delta$ for SS conditions.) Regression analysis was then performed to determine whether the observed relationship between LBF and $\text{AVG}\Delta$ differed from that predicted by the Renkin equation for fixed PS . To compare the relationship between LBF with actual and predicted $\text{AVG}\Delta$, the log-transformed data were analyzed using a linear model, $\log(\text{AV}) = \alpha + \beta_{1D1}I_1 + \dots + \beta_{1D23}I_{23} + \gamma_1 \log(\text{LBF}) + \gamma_2 \text{AVG}\Delta + \delta \log(\text{LBF}) \times \text{AVG}\Delta + \varepsilon$. The "ID" fixed effects were introduced to control for variation between subjects. The γ_1 and γ_2 terms are the main effects of the log-transformed LBF and the measured $\text{AVG}\Delta$, δ models their interaction, and ε denotes the random error term.

RESULTS

Protocol 1

SS euglycemic hyperinsulinemia. SS hyperinsulinemia was $6,396 \pm 462 \text{ pmol/l}$ ($P < 0.001$). SS glycemia was $4.96 \pm 0.06 \text{ mmol/l}$ and slightly lower than during baseline ($P < 0.05$). Rates of whole-body glucose uptake were $49.4 \pm 2.8 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$.

During SS hyperinsulinemia, the rate of LBF rose from baseline by $56 \pm 17\%$ ($P < 0.05$). The $\text{AVG}\Delta$ rose from a basal value of 0.107 ± 0.02 to $1.65 \pm 0.07 \text{ mmol/l}$ ($P < 0.001$). The SS rate of LGU was $510 \pm 70.0 \mu\text{mol} \cdot \text{leg}^{-1} \cdot \text{min}^{-1}$.

SS euglycemic hyperinsulinemia + Mch-enhanced LBF. Insulin concentrations during Mch infusion were $5,496 \pm 492 \text{ pmol/l}$ and were unchanged from euglycemic hyperinsulinemia alone. Likewise, SS euglycemia was also unaltered by Mch infusion, $4.89 \pm 0.09 \text{ mmol/l}$. Intrafemoral artery infusion of Mch caused a $114 \pm 12\%$ rise in LBF from SS hyper increase in LBF; the $\text{AVG}\Delta$ fell by $0.50 \pm 0.06 \text{ mmol/l}$ ($P < 0.01$). This represented a $32.0 \pm 3.9\%$ fall in $\text{AVG}\Delta$. As a result of Mch-induced flow enhancement, LGU rose by $40.1 \pm 5.4\%$ ($P < 0.001$). Thus, a 2-fold increment in muscle perfusion achieved with Mch, under conditions of SS euglycemic hyperinsulinemia, resulted in marked augmentation of LGU (Fig. 1).

Regression analysis. Univariate analysis revealed a continuous and positive relationship between the perfusion-modulated (Mch-induced) increase in LGU and whole-body GDR ($r = 0.60$, $P < 0.02$) (Fig. 2). Multivariate analysis including age, MAP, total cholesterol, fasting glucose, and fasting insulin levels found only GDR as a significant determinant of increments in LGU in response to changes in perfusion. These data suggest that insulin sensitivity may be a major determinant of the ability of changes in perfusion to modulate muscle glucose uptake.

Protocol 2

Subject characteristics. Characteristics of subjects undergoing protocol 2A (flow reduced, group 1) and protocol 2B (flow enhanced, group 2) are shown in Table 1. Group 2 was more adipose and exhibited higher blood pressure than group 1 but was matched for age and lipid parameters.

SS euglycemic hyperinsulinemia. After 4 h of euglycemic hyperinsulinemia, group 1 displayed significantly lower SS insulinemia ($1,261 \pm 75 \text{ pmol/l}$) compared with that of group 2 ($5,592 \pm 516 \text{ pmol/l}$) ($P < 0.05$), whereas SS glycemia was similar in both groups (4.94 ± 0.04 vs. $5.03 \pm 0.06 \text{ mmol/l}$). Despite differing insulin concentrations, rates of whole-body glucose uptake expressed per fat-free mass (FFM) were similar in groups 1 and 2 (83.8 ± 4.4 vs. $85.5 \pm 5.6 \mu\text{mol} \cdot \text{kg}^{-1} \text{ FFM} \cdot \text{min}^{-1}$ [NS]), indicating an equivalent level of insulin stimulation in both groups (Fig. 3).

During SS euglycemic hyperinsulinemia, the rate of LBF rose from baseline by $96.6 \pm 9.0\%$ to $0.45 \pm 0.05 \text{ l/min}$ in group 1 ($P < 0.01$) and by $78.2 \pm 30.2\%$ to $0.33 \pm 0.05 \text{ l/min}$ in

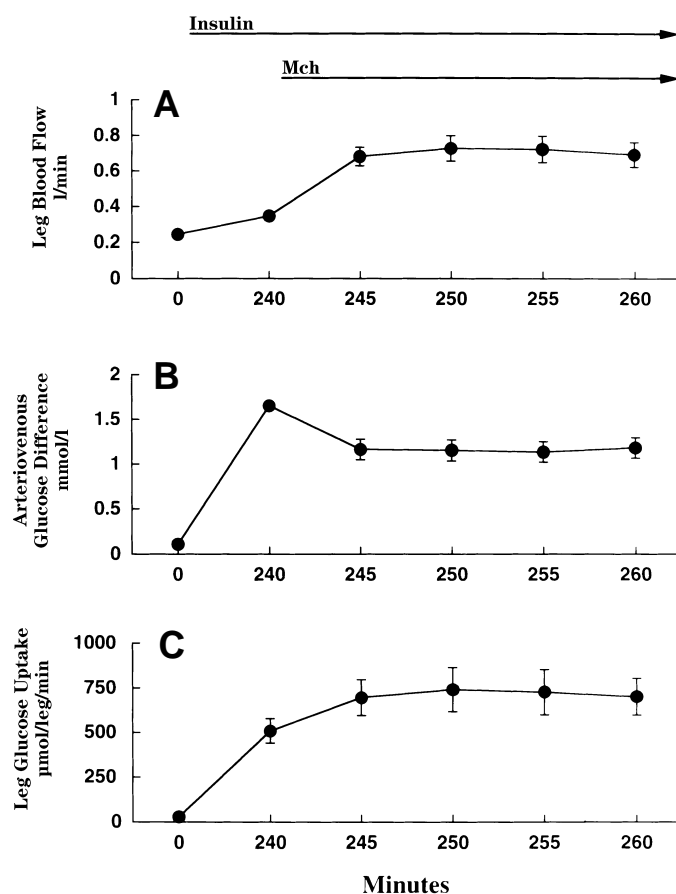


FIG. 1. Rates of LBF (A), AVG Δ (B), and LGU (C) under basal and insulin-stimulated conditions during an euglycemic-hyperinsulinemic clamp carried out for 260 min. During the last 20 min, a superimposed intrafemoral artery infusion of Mch was imposed, designed to increase LBF 2-fold over that achieved during the hyperinsulinemic-euglycemic clamp alone at the 240-min mark. Infusion of Mch led to a 2-fold increase in LBF and a fall in AVG Δ . Rates of LGU were significantly enhanced with the increase in LBF induced by Mch ($P < 0.01$).

group 2 ($P < 0.05$) ($P = \text{NS}$ group 1 vs. group 2). The AVG Δ rose from basal values of 0.11 ± 0.01 and 0.11 ± 0.03 mmol/l to 1.53 ± 0.12 and 1.65 ± 0.05 mmol/l in groups 1 and 2 (NS), respectively. Rates of insulin-stimulated LGU were 632.8 ± 99.9 vs. 471.8 ± 77.7 $\mu\text{mol} \cdot \text{leg}^{-1} \cdot \text{min}^{-1}$ in groups 1 and 2, respectively ($P = 0.29$ group 1 vs. 2).

SS euglycemic hyperinsulinemia + modulation of LBF. SS insulin concentrations were unchanged during infusion of either L-NMMA (group 1) or Mch (group 2), $1,344 \pm 58.8$ and $6,534 \pm 540$ pmol/l, respectively ($P = \text{NS}$ vs. SS). Likewise,

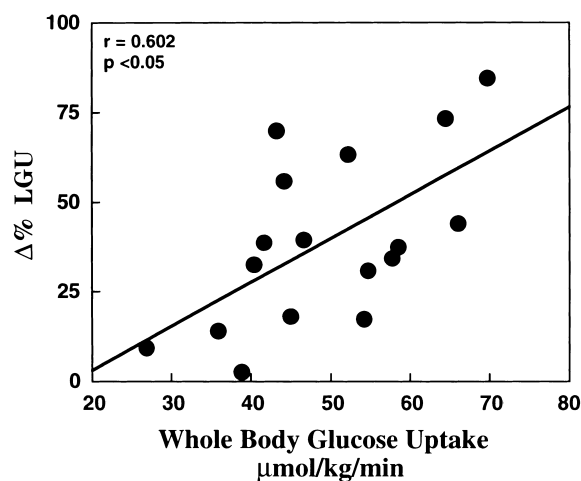


FIG. 2. Relationship between the rate of whole-body glucose uptake (insulin sensitivity) and the relative increment in LGU induced by the enhancement of LBF by Mch. The most insulin-sensitive subjects displayed the greatest perfusion-mediated increase in LGU.

SS euglycemia was also unaltered by either L-NMMA or Mch infusion (Fig. 4). Compared with SS, intrafemoral artery infusion of L-NMMA caused a $50.1 \pm 3.4\%$ reduction in LBF and infusion of Mch caused a $105.3 \pm 22.4\%$ rise in LBF ($P < 0.01$) vs. SS in both groups (Table 2). In response to the reduction in LBF, the AVG Δ in group 1 rose 56.6% to 2.38 ± 0.11 mmol/l ($P < 0.01$) (Table 2). In response to the 105.2% increase in LBF in group 2, the AVG Δ fell 25.7 \pm 5.8% to 1.24 ± 0.13 mmol/l ($P < 0.01$) (Table 2). As a result of L-NMMA-induced flow reduction, LGU in group 1 fell by $21.5 \pm 4.5\%$ to 472 ± 75 $\mu\text{mol} \cdot \text{leg}^{-1} \cdot \text{min}^{-1}$ ($P < 0.05$) (Table 2). In response to Mch-induced flow enhancement, LGU rose by $49.4 \pm 8.4\%$ to 655 ± 108.8 $\mu\text{mol} \cdot \text{leg}^{-1} \cdot \text{min}^{-1}$ ($P < 0.05$) (Table 2).

Regression analysis. The plotted relationships between LBF and glucose extraction (AVG Δ) based on actual and predicted (with fixed PS/Q) data are shown in Fig. 5. Regression analysis reveals the curves to be different ($P < 0.001$). Indeed, the actual AVG Δ was greater than predicted when LBF was increased, and smaller than predicted when LBF was decreased, suggesting that PS changes considerably under insulin-stimulated conditions.

DISCUSSION

Although changes in muscle perfusion are able to modulate insulin-mediated glucose uptake (6,7,16–18), the biophysical events taking place at the level of exchange vessels which permit such modulation are not well understood. This study was

TABLE 2

LBF, AVG Δ , and LGU during SS euglycemic insulinemia and after manipulation of blood flow by superimposed intrafemoral artery infusion of L-NMMA (protocol 2A) or Mch (protocol 2B)

	Protocol 2A		Protocol 2B	
	SS	L-NMMA	SS	Mch
LBF (l/min)	0.45 ± 0.05	$0.23 \pm 0.03^*$	0.33 ± 0.05	$0.66 \pm 0.11^*$
AVG Δ (mmol/l)	1.53 ± 0.12	$2.38 \pm 0.11^*$	1.65 ± 0.06	$1.24 \pm 0.13^*$
LGU ($\mu\text{mol} \cdot \text{leg}^{-1} \cdot \text{min}^{-1}$)	633 ± 99.9	$472 \pm 75.0^\dagger$	472 ± 7.8	$655 \pm 108.8^\dagger$

* $P < 0.01$ vs. SS; $^\dagger P < 0.05$ vs. SS.

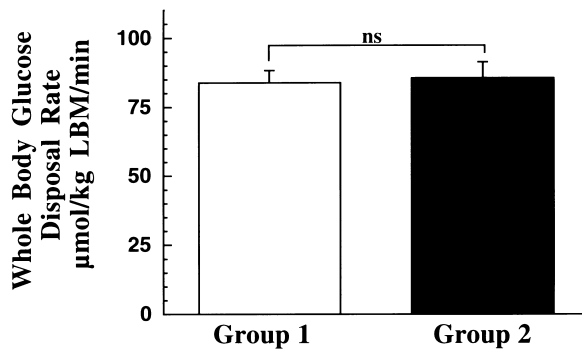


FIG. 3. Rates of whole-body glucose disposal expressed in $\mu\text{mol} \cdot \text{kg}^{-1}$ lean body mass $\cdot \text{min}^{-1}$ in group 1 (L-NMMA infused) and group 2 (Mch infused).

undertaken 1) to determine the relationship between insulin sensitivity and the ability of perfusion to modulate skeletal muscle glucose uptake (flow-limited glucose exchange) and 2) to test the functional validity of the capillary recruitment model for glucose exchange during insulin stimulation.

To address the first issue, we studied a group of healthy subjects who exhibited a wide range of insulin sensitivity. We reasoned that the more insulin-resistant subjects would exhibit reduced cellular permeability to glucose. This notion is supported by the observation that glucose uptake is reduced in these subjects largely due to a defect or defects in glucose transport (19–23), thus reducing facilitative diffusion of glucose. We further reasoned that if cellular permeability to glucose were indeed greater in the more insulin-sensitive subjects, that perfusion would be more rate limiting to glucose uptake in these subjects than in insulin-resistant subjects. To formally test this idea, in each subject we imposed an equivalent increase in muscle perfusion during SS insulin stimulation and measured the resultant increment in glucose uptake. We found that a 2-fold increase in leg perfusion resulted in an ~40% increase in LGU in the group as a whole, with the greatest increases seen in the most insulin-sensitive subjects (Fig. 1). Indeed, we found a rather strong correlation between the level of insulin sensitivity (whole-body GDR) and the magnitude of the rise in LGU engendered by the increment in leg perfusion. Thus, our findings are consonant with a model of tissue glucose exchange as proposed by Renkin (10,11) in which perfusion becomes more rate limiting to glucose uptake as cellular permeability to glucose increases.

These findings have clinical implications. Indeed, insulin-resistant states, such as obesity and type 2 diabetes, characterized by defects in glucose transport (20–23), would not be expected to exhibit perfusion-limited glucose exchange and, thus, strategies to improve perfusion would not be expected to significantly improve insulin sensitivity in patients in these states. Conversely, in insulin-sensitive subjects or in previously insulin-resistant subjects in whom insulin action has been ameliorated, perfusion would be predicted to become relatively more rate limiting. Therefore, it follows logically that complete normalization of insulin action requires a physiologic vascular response to insulin. Stated differently, perfusion appears to contribute more to overall insulin sensitivity than to insulin resistance, at least in insulin-resistant subjects who exhibit permeability-limited glucose exchange, such as obese or type 2 diabetic subjects. Conversely, in subjects in whom

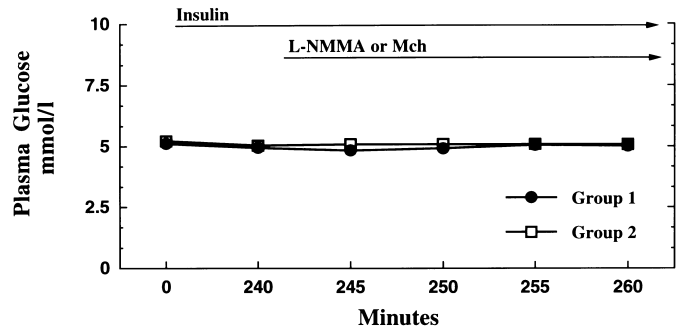


FIG. 4. Plasma glucose concentrations over the period of euglycemic hyperinsulinemia with and without LBF modulation with L-NMMA or Mch in groups 1 and 2, respectively.

glucose exchange is less permeability limited, a defect in perfusion has the most potential for causing insulin resistance. Potential clinical states displaying insulin resistance on the basis of defective perfusion are essential hypertension (24,25) and type 1 diabetes (26). Both of these clinical states are associated with altered microcirculatory flow and abnormal vascular responses to insulin. Although reports are conflicting (27,28), we have previously suggested that decreased insulin sensitivity in these groups may be contributed to by perfusion defects rather than solely by defects in glucose extraction (24,26). However, because perfusion and extraction are inversely related, it is difficult to ascertain the relative contributions of perfusion or extraction to overall glucose uptake under insulin-stimulated conditions.

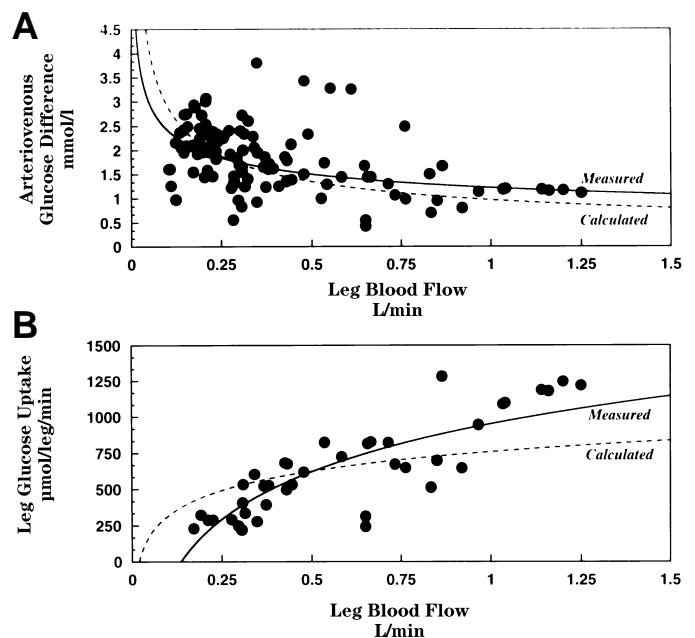


FIG. 5. Femoral AVGD (A) and rates of LGU (B) as a function of the rate of LBF established during euglycemic hyperinsulinemia alone. The rate of LBF was enhanced or reduced by intra-arterial infusion of Mch or L-NMMA, respectively. Actual AVGD or LGU (—) was compared with calculated values (---) based on the assumption that enhancement or reduction of blood flow is not accompanied by changes in capillary exchange surface area and permeability, fixed P/S (see text for details). Measured and calculated curves differ significantly ($P < 0.001$) (see data analysis), which suggests that insulin-mediated changes in perfusion are associated with capillary recruitment.

To test the validity of the functional capillary recruitment model for insulin-stimulated glucose exchange, we measured the change in glucose extraction as a function of a change in muscle perfusion. As proposed by Renkin (10,11), the ability of changes in perfusion to modulate glucose exchange is limited if under conditions of fixed permeability (P), capillary recruitment (S) does not occur. The experimental data discussed above indicate that, in fact, changes in perfusion are able to markedly modulate glucose uptake. Indeed, even in the face of near-maximal insulin stimulation, enhancement of muscle perfusion resulted in a 50% increase in LGU in insulin-sensitive subjects. Therefore, the data would suggest that Mch enhancement of leg perfusion under insulin-stimulated conditions is accompanied by capillary recruitment. To more formally and quantitatively ascertain insulin-mediated capillary recruitment, we established the relationship between leg glucose extraction and leg perfusion during equivalent insulin stimulation in 2 separate groups of subjects. Muscle perfusion was manipulated pharmacologically during SS insulin stimulation by performing intra-arterial infusions of the NO synthase inhibitor L-NMMA to reduce blood flow to basal rates (group 1) and the muscarinic agonist, Mch, to increase blood flow rates (group 2) above those achieved with insulin stimulation alone. Thus, the relationship between insulin-stimulated glucose extraction and perfusion was measured over a wide range of perfusion rates. This experimentally established relationship was compared with one predicted on the basis of the Renkin equation reflecting a noncapillary recruitment model. As can be observed in Fig. 5, the measured and predicted curves are statistically different, thus supporting an effect of insulin to alter the P/S ratio, further suggesting an increase in capillary exchange surface area or capillary recruitment. Indeed, extraction falls to a lesser extent than predicted (based on a noncapillary recruitment model) as perfusion is increased, and extraction augments less than predicted as perfusion rate is reduced. Furthermore, our data suggest that NO inhibition induces insulin resistance, at least in part, by causing capillary de-recruitment or by preventing insulin-mediated capillary recruitment. Finally, it is important to recognize that observed and predicted differences in glucose extraction persist over a wide range of perfusion rates, and thus differences in extraction cannot be secondary to any errors in the measurement of blood flow rates. Given that the measurement error of glucose extraction is negligible, the differences between predicted and observed are all the more robust.

It is important to point out that not all studies in humans have found an effect of insulin to enhance perfusion (29–31), and not all have been able to show an effect of pharmacological modulation of perfusion on glucose uptake (32,33). The reasons for this are likely multiple and include technical, methodological, biological, and pharmacological issues (12,34,35). Certainly, one's ability to precisely and sensitively measure changes in flow and extraction is critical. One issue that deserves consideration is that different muscle groups are likely to exhibit varying capacities for capillary recruitment. Compared with the leg musculature, arm muscles fatigue more rapidly and exhibit greater oxygen requirements for a given work load (36). Leg muscle vasculature must routinely adapt to a remarkably wide range of workloads from rest (blood flow 0.20 l/min) to moderately high intensity (10 l/min), thus displaying up to a 50-fold change in perfusion rate,

which can be sustained for hours (37). This suggests that compared with arm muscle, leg muscle is more adapted to accommodating a greater range of work loads and thus, it is reasonable to assume that leg muscle vasculature is appropriately adapted to this task. Therefore, it is logical to suggest that differences in limb vascular architecture and/or dynamics may account for some of the divergent findings, i.e., that capillary recruitment is likely to be more extensive in leg than in arm musculature and, thus, more difficult to demonstrate in the latter. It is noteworthy that our data are nevertheless consonant with those of Bonadonna and Bonora (38) obtained in human forearm utilizing a multiple-tracer approach showing an insulin-induced increase in volume of distribution suggesting capillary recruitment. Moreover, our data are consistent with those of Rattigan et al. (39) obtained in the isolated rat hind limb perfusion model in which insulin was able to modulate the clearance of 1-methylxanthine by the endothelial-based enzyme xanthine oxidase independent of perfusion rate, strongly suggesting capillary recruitment.

In summary, our data indicate, for the first time in humans, that muscle perfusion becomes more rate limiting to glucose uptake as insulin sensitivity increases and, thus, muscle perfusion appears to be a more potent determinant of muscle glucose uptake in insulin-sensitive than in insulin-resistant subjects. Moreover, we have also provided data strongly supporting the notion that insulin stimulation is associated with a change in pattern of microcirculatory perfusion consistent with capillary recruitment, which allows for changes in perfusion to modulate glucose uptake. The latter action has the net effect to amplify a given insulin concentration's ability to stimulate glucose uptake. Thus, defects in the microcirculatory effects of insulin have the potential to contribute to in vivo insulin resistance in certain pathophysiologic states. Finally, it is important to consider that insulin-mediated capillary recruitment may also play an important role in the clearance of other substrates, such as triglyceride-rich particles.

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