

Hyperinsulinemia Rapidly Increases Human Muscle Microvascular Perfusion but Fails to Increase Muscle Insulin Clearance

Evidence That a Saturable Process Mediates Muscle Insulin Uptake

Emma M. Eggleston, Linda A. Jahn, and Eugene J. Barrett

OBJECTIVE—Transport of insulin from the central circulation into muscle is rate limiting for the stimulation of glucose metabolism. By recruiting muscle microvasculature, insulin may promote its own movement into muscle interstitium. We tested whether in humans, as in the rat, insulin exerts an early action to recruit microvasculature within skeletal muscle. We further hypothesized that expansion of the microvascular volume of muscle would enhance muscle insulin clearance.

RESEARCH DESIGN AND METHODS—Microvascular volume, total blood flow, and muscle insulin and glucose uptake (forearm balance method) were measured in 14 lean, healthy volunteers before and during a 2-h hyperinsulinemic-euglycemic clamp ($1 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). Microvascular volume was measured using contrast-enhanced ultrasound.

RESULTS—Forearm muscle microvascular volume increased within 20 min of insulin infusion ($P < 0.01$), whereas an effect to increase total forearm flow was not observed until 100 min. Forearm insulin uptake increased with physiological hyperinsulinemia (15 ± 3 and $87 \pm 13 \text{ fmol} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1}$ basal vs. last 40 min of clamp, $P < 0.001$). However, the extraction fraction and clearance of insulin declined ($P = 0.02$, for each), indicating saturability of muscle insulin uptake at physiological hyperinsulinemia.

CONCLUSIONS—Skeletal muscle contributes to peripheral insulin clearance both in the basal state and with physiological hyperinsulinemia. Insulin promptly expands human muscle microvascular volume but only slowly increases blood flow. Despite increased microvascular volume available for insulin uptake, muscle insulin clearance decreases significantly. These findings are consistent with the presence of a saturable transport mechanism facilitating the transendothelial transport of insulin into human muscle. *Diabetes* 56:2958–2963, 2007

From the University of Virginia Health System, Charlottesville, Virginia.

Address correspondence and reprint requests to Emma M. Eggleston, University of Virginia Health System, P.O. Box 801410, 450 Ray C. Hunt Dr., Charlottesville, VA 22908. E-mail: ebm2n@virginia.edu.

Received for publication 18 May 2007 and accepted in revised form 23 August 2007.

Published ahead of print at <http://diabetes.diabetesjournals.org> on 24 August 2007. DOI: 10.2337/db07-0670.

CEU, contrast-enhanced ultrasound; MBV, microvascular blood volume.

© 2007 by the American Diabetes Association.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

A number of studies have demonstrated that insulin enhances skeletal muscle blood flow and suggest that changes in muscle perfusion play a role in regulating muscle glucose disposal (1,2). The potential importance of muscle perfusion for the delivery of insulin to muscle is underscored by studies demonstrating that in muscle, glucose metabolism correlates more strongly with interstitial than with plasma insulin levels (3,4) and by considerable evidence that the transendothelial transport of insulin is rate limiting for its action on muscle glucose metabolism (3,5–7). Studies using lymphatic sampling or microdialysis to quantify interstitial insulin concentrations consistently demonstrate 1) a significant time delay between increases in interstitial versus arterial insulin concentrations (3,6,8) and 2) the presence of a persistent (approximately two-fold) concentration gradient between interstitial and plasma insulin concentrations both basally (3,9,10) and during steady-state hyperinsulinemia (3,7,9,11).

The large majority of muscle endothelial surface area resides in capillaries and precapillary arterioles. However, only ~25% of skeletal muscle microvasculature is perfused at rest. Expanding the microvascular surface area perfused within muscle would proportionately increase the opportunity for insulin and glucose exchange across the skeletal muscle bed. Our laboratory has demonstrated that insulin increases capillary perfusion within muscle by recruiting microvasculature (12–14). This recruitment appears to be due to relaxation of terminal arterioles, which, like the relaxation of resistance vessels by insulin, is sensitive to inhibition of nitric oxide synthase and is diminished by insulin resistance (15,16). In the rat, insulin increases microvascular perfusion within 10–15 min. This increase temporally precedes changes in both glucose utilization and total blood flow (14) and suggests that insulin actively regulates its own delivery to the capillary endothelium. However, the time course for insulin-mediated microvascular recruitment in humans is not known.

In the current study, we used contrast-enhanced ultrasound (CEU) to ascertain whether in humans, as in the rat, insulin exerts an early action to recruit microvasculature within skeletal muscle. We further hypothesized that if insulin did expand the microvascular volume available for perfusion within skeletal muscle, enhanced muscle insulin

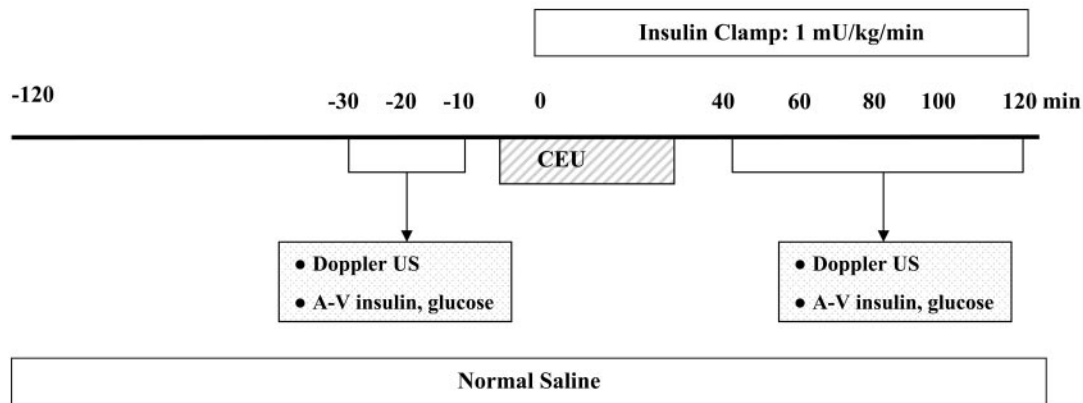


FIG. 1. Protocol for the euglycemic-hyperinsulinemic clamp ($1 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) in lean healthy subjects. Continuous CEU measurements were taken in the forearm from -5 to 30 min. Brachial artery flow and forearm glucose and insulin balance were measured at baseline and every 20 min from 40 to 120 min of insulin infusion.

clearance would be observed. To assess this, we measured skeletal muscle insulin clearance (product of forearm blood flow and arterial/venous extraction fraction) using the forearm balance method.

RESEARCH DESIGN AND METHODS

Studies were performed in 14 lean ($\text{BMI } 23 \pm 1 \text{ kg/m}^2$), healthy volunteers ages 18–35 without a family history of hypertension or diabetes and on no medications thought to affect endothelial function. All female participants had a negative pregnancy test on the evening of admission. The study protocol was approved by the University of Virginia Human Investigation Committee, and each subject gave written consent. All studies were performed in the University of Virginia General Clinical Research Center.

Screening. All subjects had an outpatient screening visit, which included a history and physical, plasma coagulation parameters, liver function tests, fasting glucose, and a lipid profile. In addition, BMI, body composition, and forearm volume (water displacement) were measured.

Admission. After an overnight 12-h fast, a brachial arterial and a retrograde median antecubital venous catheter were placed for blood sampling. Normal saline was infused at 40 ml/h for 60 min before study initiation. A venous catheter was placed in the contra-lateral arm, and an infusion of normal saline at 40 ml/h was initiated at -180 min and maintained through the duration of the study. At -30 min, paired arterial and venous samples were taken every $10 \text{ min} \times 3$ for measurement of plasma glucose, insulin, and lactate. Forearm blood flow was measured after each set of arterial and venous samples by Doppler ultrasound. CEU measurement of microvascular volume was initiated 5 min before and continued for the first 30 min of an insulin clamp as described below.

Euglycemic-hyperinsulinemic clamp. At time 0 , a primed $3 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ insulin infusion was started in the arm contra-lateral to the arterial and deep vein catheters and was decreased by $0.2 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ each minute over the next 10 min until a rate of $1 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ was reached. This rate was then maintained for the next 110 min. Arterial plasma glucose was maintained at basal levels using a variable rate 20% glucose infusion (euglycemic clamp). Whole-body glucose disposal at steady state was estimated from the glucose infusion rate required to keep arterial glucose constant. Forearm glucose uptake was determined from the arterial-venous concentration difference obtained every 10 min from -30 to 0 min and from 40 to 120 min. To avoid interference with the CEU images produced by sampling from the arterial line, no arterial-venous sampling was collected from 0 to 40 min of the euglycemic clamp (Fig. 1).

Microvascular imaging. To define the time course of insulin-mediated microvascular recruitment, microvascular blood volume (MBV) was determined using a SONOS 7500 ultrasound system (Philips Medical Systems, Bothell, WA) with harmonic imaging during the continuous infusion of microbubbles as follows. A 2.8-ml suspension of octafluoropropane gas-filled lipid microbubbles Definity (Bristol-Myers Squibb Medical Imaging, North Billerica, MA) was diluted in 60 ml normal saline and intravenously infused at a rate of 1.5 ml/min for a total of 40 min starting in the basal period and extending ~ 30 min into the insulin clamp. After 2 min of infusion, images were obtained at a sequentially increasing pulsing interval, from 1 to 20 cardiac cycles and maintained at a pulsing interval of 20 throughout the first 25 min of insulin infusion. Images were recorded from the forearm in the trans-axial

plane 5 cm distal to the antecubital fossa using a phased array transducer. At least three images were acquired at each pulsing interval with a pulse mechanical index of 1.5 . Images were digitized to an offline image analysis system (Q-lab; Philips Medical Systems). Background-subtracted acoustic intensity at each pulsing interval was measured from a region of interest around the deep forearm flexor muscles as described previously (16,17). Changes in microvascular volume over time during insulin exposure were calculated from the acoustic intensity expressed as mean decibels.

Doppler ultrasound. Brachial artery blood flow was measured at baseline and every 20 min from 40 to 120 min of the insulin clamp using the SONOS 7500 ultrasound system with a linear array transducer and a transmit frequency of 12 MHz . Two-dimensional imaging of the brachial artery was performed in the long axis $\sim 10 \text{ cm}$ proximal to the antecubital fossa. Images were triggered to the “R” wave of the cardiac cycle, and the brachial artery diameter was measured using online video calipers. Pulsed-wave Doppler sample blood volume was obtained at the same location, and the time average mean blood velocity was measured. Brachial artery mean blood flow is calculated using the following equation: $Q = v \cdot \pi(d/2)^2$, where Q is brachial blood flow, v is mean brachial artery blood flow velocity, and d is brachial artery diameter.

Assays. The insulin assay used is a solid phase 2 site chemiluminescent assay with a sensitivity of 12 pmol/l and an intrassay coefficient of variation of 2.5 , 3.3 , and 7% for insulin at concentrations of ~ 240 , ~ 60 , and $\sim 30 \text{ pmol/l}$, respectively (Immulate 2000 insulin assay; Diagnostic Products, Los Angeles, CA). Glucose and lactate were measured in duplicate using an YSI 2300 analyzer (Yellow Springs Instruments, Yellow Springs, OH). Glucose measurements were made on plasma, whereas lactate was measured on whole blood. Baseline coagulation parameters, liver function tests, and fasting lipid profile were performed by standard assays in the University of Virginia Clinical Chemistries Laboratory.

Calculations. Forearm balances for glucose and insulin were calculated using the Fick principle: $\text{balance} = ([A] - [V]) \times F$, where $[A]$ and $[V]$ are arterial and venous concentrations, respectively, and F is forearm blood flow in milliliters per minute per 100 ml forearm volume for glucose and forearm plasma flow ($\text{blood flow} \times [1 - \text{hematocrit}]$) for insulin. The clearance of insulin was calculated as the product of the extraction fraction of insulin $([A] - [V])/[A]$ and forearm plasma flow per 100 ml forearm volume.

Forearm balance technique. We elected to use the forearm balance technique, rather than radiolabeled insulin, to estimate insulin uptake and clearance because arteriovenous sampling affords the advantages of safety, specificity (using a two-site chemiluminescent insulin assay for intact insulin), and the predictable binding of native, as opposed to radiolabeled, insulin with the insulin receptor. However, this method requires an assay with a low coefficient of variation (in light of the relatively small extraction of insulin across the vasculature) and multiple arterial-venous sample pairs.

Statistical analysis. Data are presented as the means \pm SE. Initial comparisons were made via paired Student’s t test between grouped baseline (-30 to -10 min) and steady-state (80 – 120 min) values and by one-way repeated-measures ANOVA with effect of time. Holm-Sidak post hoc analysis was used in the case of significance ($P < 0.05$). Statistics were calculated using Sigstatat 3.2 (Systat Software).

TABLE 1
Subject characteristics

<i>n</i>	14
Age (years)	24 ± 1
Sex (men/women)	11/3
Weight (kg)	72 ± 4
Height (cm)	177 ± 3
BMI (kg/m ²)	23 ± 1
Body fat (%)	17 ± 2
Waist (cm)	77 ± 2
Systolic blood pressure (mmHg)	121 ± 3
Diastolic blood pressure (mmHg)	69 ± 1
Total cholesterol (mmol/l)	4.2 ± 0.2
LDL cholesterol (mmol/l)	2.6 ± 0.2
HDL cholesterol (mmol/l)	1.24 ± 0.1
Triglycerides (mmol/l)	2.12 ± 0.21
Basal arterial insulin (pmol/l)	39 ± 5
Basal arterial glucose (mmol/l)	5.4 ± 0.1

Data are means ± SE.

RESULTS

The anthropomorphic characteristics and basal chemistries for study subjects are shown in Table 1.

Glucose metabolism and forearm blood flow. The intravenous insulin infusion raised arterial insulin to an average of 283 ± 7 pmol/l during the 120 min of hyperinsulinemia. Arterial glucose averaged 5.4 ± 0.1 mmol/l during the baseline period and was maintained within 4% of baseline throughout (Fig. 2A). The basal forearm arteriovenous glucose concentration difference averaged 0.2 ± 0.02 mmol/l, and this increased to 0.8 ± 0.1 mmol/l during the clamp ($P < 0.001$ at all time points versus baseline) (Fig. 2B). There was a time-dependent increase in total forearm blood flow with a significant increase observed at min 100 compared with baseline ($P < 0.05$) (Fig. 2B). Forearm glucose uptake rose from 1.2 ± 0.14 $\mu\text{mol} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1}$ at baseline to an average of 6.2 ± 0.6 $\mu\text{mol} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1}$ during min 40–120 of insulin infusion (Fig. 2C). A statistically significant increase in glucose uptake was observed at all time points with insulin versus baseline ($P < 0.001$).

The glucose infusion rate required to maintain euglycemia increased progressively over the first 40 min of the clamp and rose more slowly thereafter (Fig. 2C). Arterial and deep forearm venous lactates were 0.65 ± 0.04 and 0.72 ± 0.02 mmol/l, respectively, during the baseline period. Basal forearm lactate balance was -0.48 ± 0.11 $\mu\text{mol/l}$ per min per 100 ml and remained unchanged over the 120 min of insulin infusion ($P = 0.789$).

Insulin metabolism. The time courses for the forearm arteriovenous difference, extraction fraction, uptake, and clearance of insulin are shown in Fig. 3. In the basal state, arterial and deep forearm venous insulin concentrations averaged 39 ± 5.4 and 32 ± 5.7 pmol/l, respectively, with the arterial concentration being significantly greater ($P < 0.001$). By repeated-measures ANOVA, there was a significant increase in both the arteriovenous difference and in insulin uptake as a function of time of insulin exposure ($P < 0.01$ for each vs. baseline). The extraction fraction of insulin decreased over time ($P < 0.01$ at 100 and 120 min vs. baseline) (Fig. 3A). Likewise, insulin clearance declined over time ($P < 0.05$ at 120 min vs. baseline).

Comparison of the mean baseline values for forearm skeletal muscle insulin uptake, extraction fraction, and forearm insulin clearance with the last 40 min of steady-

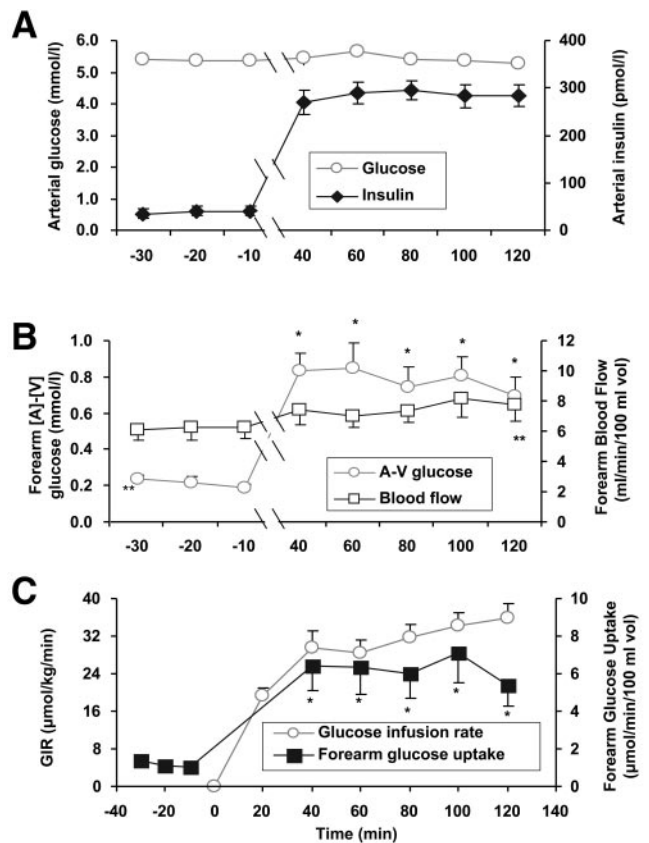


FIG. 2. Glucose metabolism and brachial artery blood flow during 120 min of insulin infusion ($1 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). A: Arterial glucose and insulin values. B: Forearm blood flow and arterial and venous glucose difference. C: Glucose infusion rate and forearm glucose uptake. Values are means ± SE. * $P < 0.001$ vs. pooled baseline by repeated-measures ANOVA with effect of time. ** $P < 0.05$ vs. pooled baseline. $n = 14$.

state hyperinsulinemia are shown in Fig. 4. Average insulin uptake increased from 15 ± 3 $\text{fmol} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1}$ under basal conditions to 87 ± 13 $\text{fmol} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1}$ during 80–120 min of insulin infusion ($P < 0.001$ paired *t* test). However, the extraction fraction of insulin across the forearm decreased significantly from basal values (0.15 ± 0.02 vs. 0.08 ± 0.01 , $P < 0.05$), and forearm insulin clearance likewise declined (0.57 ± 0.1 $\text{ml} \cdot \text{min}^{-1} \cdot 100 \text{ ml}^{-1}$ vs. 0.30 ± 0.1 , $P < 0.05$).

Microvascular recruitment. Figure 5 shows the time course for the change in MBV over the first 25 min of insulin infusion. We observed a significant rise within 20 min (Fig. 5; $P < 0.01$ mean decibels vs. 0–5 and 5–10 min repeated-measures ANOVA). The rise in microvascular perfusion was progressive over the 25 min and had not plateaued at 25 min, with a significant increase between 20–25 and 15–20 min ($P < 0.05$).

DISCUSSION

The transendothelial transport of insulin is rate limiting for insulin action in skeletal muscle (3,6). However, questions persist regarding the role that insulin plays in facilitating its own egress from the vasculature to the muscle interstitium and the mechanisms by which this transport occurs. The present study reports two findings relevant to this issue: 1) an early and significant increase in muscle microvascular perfusion in response to a physiological dose of insulin and 2) a decline in the clearance of insulin

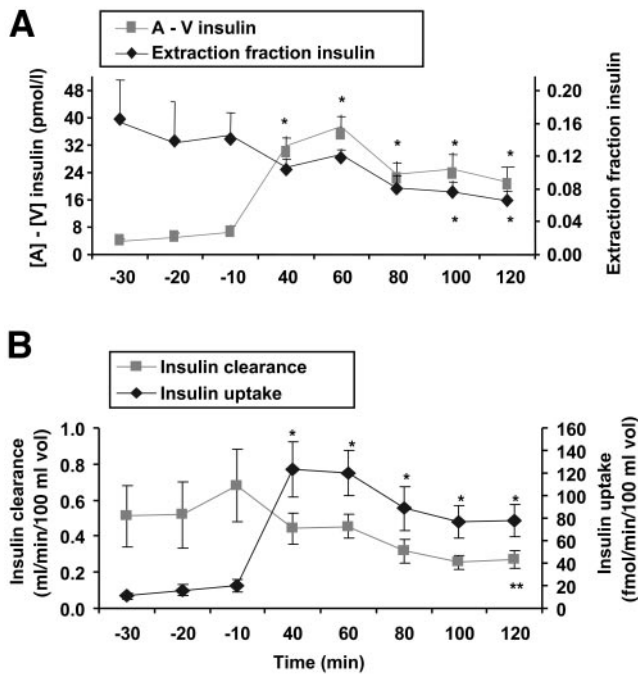


FIG. 3. Forearm insulin metabolism at baseline and during 120 min of physiological hyperinsulinemia ($1 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). *A*: Arterial and venous insulin difference and extraction fraction insulin. *B*: Forearm insulin uptake and insulin clearance. Values are means \pm SE. * $P < 0.01$, ** $P < 0.05$ repeated-measures ANOVA vs. pooled baseline. $n = 14$.

from the vasculature despite the increase in microvascular perfusion. Taken together, these findings suggest that insulin facilitates its own delivery to muscle by increasing the microvascular surface available for uptake and that at physiological insulin concentrations, insulin transport occurs via a saturable process.

The time course of insulin-mediated microvascular recruitment has not previously been studied in humans. Here, we observed a significant increase within 20 min of giving insulin. This rise in MBV preceded by 80 min a small, but significant, increase in total forearm blood flow.

Baron and colleagues (17–19) provided the first detailed studies of skeletal muscle blood flow regulation by insulin. In a series of experiments, they found that insulin increased both total blood flow and glucose uptake by human leg muscle and that both effects were blunted by obesity, diabetes, and hypertension. However, divergent results across studies using differing insulin doses, dura-

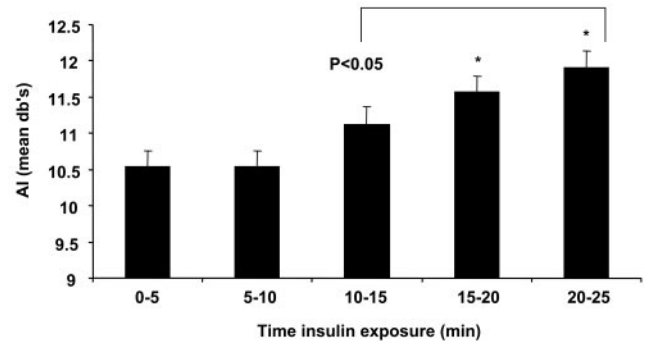


FIG. 5. Background-subtracted forearm video intensity by time of insulin exposure. Values are means \pm SE. * $P < 0.01$ ANOVA on ranks vs. 0–5 and 5–10 min. $n = 13$.

tion of insulin exposure, and methods of assessing blood flow raised considerable controversy regarding the temporal sequence and physiological significance of the effect of insulin on total blood flow (20–24). More recent studies (25–28) have focused on the regulation of tissue perfusion at the microvascular level by capillary recruitment. Expanding the microvascular bed by recruiting previously underperfused capillaries would predictably increase the exchange surface available, even in the absence of changes in total limb blood flow.

The hypothesis that insulin recruits capillaries in muscle to facilitate its own delivery to target tissues by increasing the available endothelial surface has been examined using a variety of methods including laser Doppler (25), 1-methyl xanthine metabolism (29,30), and CEU (12–16,31). At physiological doses, the effect of insulin to increase microvascular perfusion occurs earlier than changes in total flow or glucose uptake (14,16). Moreover, inhibiting this increase with N^G -monomethyl-L-arginine reduces muscle glucose uptake by as much as 40% (16). These data suggest that microvascular perfusion is the primary flow mechanism by which insulin delivery to skeletal muscle is enhanced, and this increased delivery in turn augments glucose uptake in skeletal muscle.

In rodents, insulin recruits skeletal muscle microvasculature sooner (10 vs. 90 min) and at lower concentrations than changes in total blood flow (14). In humans, physiological hyperinsulinemia increases microvascular recruitment by 120 min (16,31). However, the early time course of microvascular recruitment has not been defined. In the current study, MBV increased significantly within 20 min

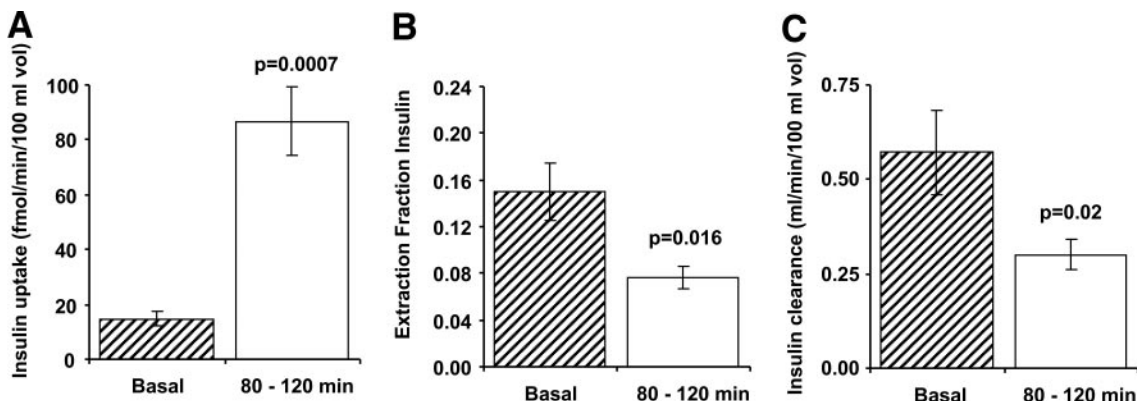


FIG. 4. Mean forearm insulin uptake (*A*), extraction fraction (*B*), and clearance (*C*) at baseline and 80–120 min of hyperinsulinemia. Values are means \pm SE. P values from Student's paired t tests. $n = 14$.

of insulin infusion. Restrictions on the cumulative dose of microbubbles allowed in humans limited the total time of continuous microvascular imaging we were able to perform; however, the continued increase in microvascular volume at 25 min suggests that the recruitment process continued beyond the imaging period.

In these same subjects, brachial artery flow (Doppler ultrasound) increased by an average of 17% at 100 min of hyperinsulinemia. This is a time course typically observed with physiological doses of insulin; however, supraphysiological doses of insulin have been reported to increase femoral artery blood flow as early as 30 min (32).

Echo interference from microbubbles precluded the measurement of total forearm blood flow during the 30 min of continuous CEU and therefore direct comparison of the time course of these two actions of insulin. Thus, the current data suggest but do not prove that in humans, the microvascular effect of physiological hyperinsulinemia precedes its effect on total blood flow.

If the passage of insulin from the vasculature to the interstitium occurs via passive diffusion alone, one would expect an increase in insulin clearance from the vasculature with increased microvascular surface area. This did not occur. Rather, the clearance of insulin decreased by 47% between baseline and 120 min of hyperinsulinemia. We would emphasize that this decline in muscle insulin clearance was observed at insulin concentrations well within the range seen physiologically (basal, 39 ± 5 pmol/l; hyperinsulinemia, 283 ± 7 pmol/l). This suggests that within the physiological range, the uptake of insulin by human skeletal muscle is a saturable process. Because this measurement was made using simple arterial-venous concentration differences and estimates of muscle blood flow, it does not directly inform as to whether this decline of insulin clearance is attributable to a saturable component of transendothelial insulin transport into skeletal muscle or to a saturable uptake of insulin by the muscle cells themselves. Several considerations would favor the former explanation. Among these, the observation (made by several laboratories) that in muscle there is a persistent approximate twofold plasma-to-interstitial insulin gradient when plasma insulin concentrations are raised within the physiological range, as in the current study. In addition, we and others have reported that insulin is transported across endothelial cell monolayers via a receptor-mediated pathway (33,34). These latter observations are consistent with the potential for a saturable process being involved in transendothelial insulin transport *in vivo*.

It is of interest to consider this evidence that insulin exits the vasculature via a saturable process in the light of previous studies addressing this question. Two canine studies reached somewhat opposite conclusions (9,35). Consistent with the current findings, Sonksen et al. (35) reported that unlabeled insulin diminished the uptake of radiolabeled insulin by the canine hindlimb, indicative of a saturable process. In contrast, Steil et al. (9) reported that the plasma-to-interstitial insulin gradient diminished when the plasma insulin concentration was raised from the physiological to the pharmacological range. Several technical issues may have limited each of these studies. Steil et al. pointed out that in the Sonksen et al. study, the radiolabeled insulin may not bind to the insulin receptor with the same kinetics as native insulin. Although true, this does not necessarily invalidate the observation that native insulin appeared to displace the uptake of the labeled insulin. The Steil et al. study relied on measurements of

insulin concentration in hind leg lymphatic drainage. The plasma-to-lymphatic insulin concentration gradient was measured at two insulin concentrations: physiological hyperinsulinemia (~ 600 pmol \cdot l⁻¹ \cdot l⁻¹) and supraphysiological (~ 33 nmol \cdot l⁻¹ \cdot l⁻¹). At the higher concentration, the investigators observed a paradoxical increase in the rate constant for transendothelial insulin transport as derived from a kinetic model. The investigators suggest that this increase in insulin transport may be due to an increased endothelial surface area attributed to capillary recruitment induced by the insulin increment from high physiological to pharmacological doses. However, capillary recruitment was not measured. We have measured the dose response of recruitment to insulin (13), and this suggests that most, if not all, of the recruitment would have already occurred at the insulin concentration achieved at the physiological dose.

Regardless of changes in MBV, a decrease in insulin clearance from the vasculature would not be predicted if the transport of insulin at physiological doses occurs via passive diffusion alone. If the uptake of insulin is linearly related to arterial insulin concentrations, as would be the case with passive diffusion, then the clearance of insulin should remain constant with increasing insulin concentrations. A linear association between insulin uptake and increasing arterial insulin concentrations was suggested by early work by Kalant et al. (36,37), who used the human forearm balance technique to measure the uptake of endogenous insulin as stimulated by varying glucose infusions during hyperglycemic clamp. However, a paucity of values at high physiological insulin concentrations limited their regression analyses and preempted any firm conclusions regarding the relationship between arterial insulin concentration and forearm insulin uptake.

Obese, insulin-resistant subjects display impaired insulin-mediated increases in microvascular perfusion (16). However, whether insulin resistance decreases skeletal muscle insulin clearance, as has been shown for hepatic insulin clearance (38,39), is currently unknown.

We caution that the current study examines microvascular perfusion and insulin flux in forearm skeletal muscle, which is made up predominantly of fast-twitch fibers. It is possible that larger weight-bearing muscles, which have a higher proportion of slow-twitch fibers, may behave differently. The capillary density of distinct muscle groups may also differentially affect microvascular transport of insulin as muscle capillary density has been correlated with transcappillary substrate transport (40), blood flow (41), and insulin sensitivity (42). These questions remain to be determined experimentally.

In summary, using classical arterial-venous sampling, we observe a significant extraction of endogenous insulin by skeletal muscle in the basal state that increases with physiological hyperinsulinemia in lean healthy subjects. We also observe that hyperinsulinemia promptly expands muscle microvascular volume and more slowly increases muscle blood flow. Despite this, muscle insulin clearance decreases significantly. These findings are consistent with the presence of a saturable transport mechanism facilitating the movement of insulin from the vasculature to its target tissue. Because the transendothelial transport of insulin plays a central and rate-limiting role in overall insulin action in muscle, further clarification of the mechanisms by which this transport occurs is of particular clinical relevance.

ACKNOWLEDGMENTS

E.J.B. has received National Institutes of Health (NIH) Grants DK-057878, DK-073759, and DK-063609. The University of Virginia General Clinical Research Center has received NIH Grant RR-00847.

We thank Dr. S. Rattigan for thoughtful suggestions for the manuscript.

REFERENCES

- Baron AD, Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G: Insulin-mediated vasodilation contributes to both insulin sensitivity and responsiveness in lean humans. *J Clin Invest* 96:786–792, 1995
- Vincent MA, Rattigan S, Clark MG, Barrett EJ: Inhibition of nitric oxide synthase prevents insulin-mediated capillary recruitment and glucose uptake in skeletal muscle in vivo (Abstract). *Diabetes* 50 (Suppl. 1):A334, 2001
- Yang YJ, Hope ID, Ader M, Bergman RN: Insulin transport across capillaries is rate limiting for insulin action in dogs. *J Clin Invest* 84:1620–1628, 1989
- Castillo C, Bogardus C, Bergman R, Thuillez P, Lillioja S: Interstitial insulin concentrations determine glucose uptake rates but not insulin resistance in lean and obese men. *J Clin Invest* 93:10–16, 1994
- Sherwin RS, Kramer KJ, Tobin JD, Insel PA, Liljenquist JE, Berman M, Andres R: A model of the kinetics of insulin in man. *J Clin Invest* 53:1481–1492, 1974
- Miles PD, Levisetti M, Reichart D, Khourshed M, Moossa AR, Olefsky JM: Kinetics of insulin action in vivo: identification of rate-limiting steps. *Diabetes* 44:947–953, 1995
- Poulin RA, Steil GM, Moore DM, Ader M, Bergman RN: Dynamics of glucose production and uptake are more closely related to insulin in hindlimb lymph than in thoracic duct lymph. *Diabetes* 43:180–190, 1994
- Sjostrand M, Gudbjornsdottir S, Holmang A, Lonn L, Strindberg L, Lonnroth P: Delayed transcapillary transport of insulin to muscle interstitial fluid in obese subjects. *Diabetes* 51:2742–2748, 2002
- Steil GM, Ader M, Moore DM, Rebrin K, Bergman RN: Transendothelial insulin transport is not saturable in vivo: no evidence for a receptor-mediated process. *J Clin Invest* 97:1497–1503, 1996
- Herkner H, Klein N, Joukhar C, Lackner E, Langenberger H, Frossard M, Bieglmayer C, Wagner O, Roden M, Muller M: Transcapillary insulin transfer in human skeletal muscle. *Eur J Clin Invest* 33:141–146, 2003
- Sjostrand M, Holmang A, Lonnroth P: Measurement of interstitial insulin in human muscle. *Am J Physiol* 276:E151–E154, 1999
- Coggins MP, Lindner J, Rattigan S, Fasy E, Jahn L, Kaul S, Barrett EJ: Physiologic hyperinsulinemia enhances human skeletal muscle perfusion by capillary recruitment. *Diabetes* 50:2682–2690, 2001
- Zhang L, Vincent MA, Richards SM, Clerk LH, Rattigan S, Clark MG, Barrett EJ: Insulin sensitivity of muscle capillary recruitment in vivo. *Diabetes* 53:447–453, 2004
- Vincent MA, Clerk LH, Lindner JR, Klibanov AL, Clark MG, Rattigan S, Barrett EJ: Microvascular recruitment is an early insulin effect that regulates skeletal muscle glucose uptake in vivo. *Diabetes* 53:1418–1423, 2004
- Vincent MA, Barrett EJ, Lindner JR, Clark MG, Rattigan S: Inhibiting NOS blocks microvascular recruitment and blunts muscle glucose uptake in response to insulin. *Am J Physiol Endocrinol Metab* 285:E123–E129, 2003
- Clerk LH, Vincent MA, Jahn LA, Liu Z, Lindner JR, Barrett EJ: Obesity blunts insulin-mediated microvascular recruitment in human forearm muscle. *Diabetes* 55:1436–1442, 2006
- Laasko M, Edelman SV, Brechtel G, Baron AD: Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man. *J Clin Invest* 85:1844–1852, 1990
- Baron AD, Laasko M, Brechtel G, Edelman SV: Mechanism of insulin resistance in insulin-dependent diabetes mellitus: a major role for reduced skeletal muscle blood flow. *J Clin Endocrinol Metab* 73:637–643, 1991
- Laasko M, Edelman SV, Brechtel G, Baron AD: Impaired insulin-mediated skeletal muscle blood flow in patients with NIDDM. *Diabetes* 41:1076–1083, 1992
- Natali A, Buzzigoli G, Taddei S, Santoro D, Cerri M, Pedrinelli P, Ferrannini E: Effects of insulin on hemodynamics and metabolism in human forearm. *Diabetes* 39:490–500, 1990
- Yki-Jarvinen H, Young AA, Lamkin C, Foley JE: Kinetics of glucose disposal in whole body and across the forearm in man. *J Clin Invest* 79:1713–1719, 1987
- Utriainen T, Malmstrom R, Makimattila S, Yki-Jarvinen H: Methodological aspects, dose-response characteristics and causes of interindividual variation in insulin stimulation of limb blood flow in normal subjects. *Diabetologia* 38:555–564, 1995
- Yki-Jarvinen H, Utriainen T: Insulin-induced vasodilatation: physiology or pharmacology? *Diabetologia* 41:369–379, 1998
- Clark MG, Wallis MG, Barrett EJ, Vincent MA, Richards SM, Clerk LH, Rattigan S: Blood flow and muscle metabolism: a focus on insulin action. *Am J Physiol Endocrinol Metab* 284:E241–E258, 2003
- Clark ADH, Barrett EJ, Rattigan S, Wallis MG, Clark MG: Insulin stimulates laser Doppler signal by rat muscle in vivo consistent with nutritive flow recruitment. *Clin Sci* 100:283–290, 2001
- Clerk LH, Vincent MA, Lindner JR, Clark MG, Rattigan S, Barrett EJ: The vasodilatory actions of insulin on resistance and terminal arterioles and their impact on muscle glucose uptake. *Diabetes Metab Res Rev* 20:3–12, 2004
- Gudbjornsdottir S, Sjostrand M, Strindberg L, Wasahren J, Lonnroth P: Direct measurements of the permeability surface area for insulin and glucose in human skeletal muscle. *J Clin Endocrinol Metab* 88:4559–4564, 2003
- Gudbjornsdottir S, Sjostrand M, Strindberg L, Lonnroth P: Decreased muscle capillary permeability surface area in type 2 diabetic subjects. *J Clin Endocrinol Metab* 90:1078–1082, 2005
- Rattigan S, Clark MG, Barrett EJ: Hemodynamic actions of insulin in rat skeletal muscle: evidence for capillary recruitment. *Diabetes* 46:1381–1388, 1997
- Clerk LH, Rattigan S, Clark MG: Lipid infusion impairs physiologic insulin-mediated capillary recruitment and muscle glucose uptake in vivo. *Diabetes* 51:1138–1145, 2002
- Vincent MA, Clerk LH, Lindner JR, Price WJ, Jahn LA, Leong-Poi H, Barrett EJ: Mixed meal and light exercise each recruit muscle capillaries in healthy humans. *Am J Physiol Endocrinol Metab* 290:E1191–E1197, 2006
- Dela F, Stallknecht B, Biering-Sorenson F: An intact central nervous system is not necessary for insulin-mediated increases in leg blood flow in humans. *Pflugers Arch Eur J Physiol* 441:241–250, 2000
- Wang H, Liu Z, Li G, Barrett EJ: The vascular endothelial cell mediates insulin transport into skeletal muscle. *Am J Physiol Endocrinol Metab* 291:E323–E332, 2006
- King GL, Johnson SM: Receptor-mediated transport of insulin across endothelial cells. *Science* 227:1583–1586, 1985
- Sonksen PH, McCormick JR, Egdahl RH, Soeldner JS: Distribution and binding of insulin in the dog hindlimb. *Am J Physiol* 221:1672–1680, 1971
- Kalant N, Leibovici T, Rohan I, Ozaki S: Interrelationships of glucose and insulin uptake by muscle of normal and diabetic man: evidence of a difference in metabolism of endogenous and exogenous insulin. *Diabetologia* 16:365–372, 1979
- Kalant N, Leibovici T, Rohan I, McNeil K: Effect of exercise on glucose and insulin utilization in the forearm. *Metabolism* 27:333–340, 1978
- Polonsky KS, Given BD, Hirsch L, Shapiro ET, Tillil H, Beebe C, Galloway JA, Frank BH, Karrison T, Van Cauter E: Quantitative study of insulin secretion and clearance in normal and obese subjects. *J Clin Invest* 81:435–441, 1988
- Jones CN, Pei D, Staris P, Polonsky KS, Chen YD, Reaven GM: Alterations in the glucose-stimulated insulin secretory dose-response curve and in insulin clearance in nondiabetic insulin-resistant individuals. *J Clin Endocrinol Metab* 82:1834–1838, 1997
- Vock R, Weibel ER, Hoppeler H, Ordway G, Weber JM, Taylor CR: Design of the oxygen and substrate pathways. V. Structural basis of vascular substrate supply to muscle cells. *J Exp Biol* 199:1675–1688, 1996
- Hedman A, Andersson P-E, Reneland R, Lithell HO: Insulin-mediated changes in leg blood flow are coupled to capillary density in skeletal muscle in healthy 70-year-old men. *Metab Clin Exp* 50:1078–1082, 2001
- Lillioja S, Young AA, Culter CL, Ivy JL, Abbott WG, Zawadzki JK, Yki-Jarvinen H, Christin L, Secomb TW, Bogardus C: Skeletal muscle capillary density and fiber type are possible determinants of in vivo insulin resistance in man. *J Clin Invest* 80:415–424, 1987