Hyperinsulinemia rapidly increases human muscle microvascular perfusion but fails to increase muscle insulin clearance: evidence that a saturable process mediates muscle insulin uptake.

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Running Title: Capillary recruitment and insulin clearance.

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Abstract

Objective: Insulin’s transport 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 muscle’s microvascular volume (MV) would enhance muscle insulin clearance.

Research Design and Methods: MV, total blood flow, and muscle insulin and glucose uptake (forearm balance method) were measured in 14 lean, healthy volunteers before and during a 2-hr hyperinsulinemic (1 mU/kg/min) euglycemic clamp. MV was measured using contrast enhanced ultrasound (CEU).

Results: Forearm muscle MV 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 physiologic hyperinsulinemia (15± 3 and 87 ± 13 fM/min/100 ml) 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 physiologic hyperinsulinemia.

Conclusion: Skeletal muscle contributes to peripheral insulin clearance both in the basal state and with physiologic hyperinsulinemia. Insulin promptly expands human muscle MV but only slowly increases blood flow. Despite increased MV 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.
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 insulin’s trans-endothelial transport 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 (~ 2-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 pre-capillary 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 arterioles 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 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**

**Study Subjects**

Studies were performed in 14 lean (BMI 23±1), 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.

**Experimental Protocol 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/hr for 60 min prior to study initiation. In the contra-lateral arm, a venous catheter was placed and an infusion of normal saline at 40 ml/hour 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 min x 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. Contrast enhanced ultrasound 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 mU/kg/min insulin infusion was started in the arm contra-lateral to the arterial and deep vein catheters, and was decreased by 0.2 mU/min/kg each min over the next 10 min until a rate of 1mU/kg/min 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 min -30 to 0 min and from 40 to 120 min. To avoid interference with the contrast enhanced ultrasound images produced by sampling from the arterial line, no arterial - venous sampling was collected from 0 to 40 min of the euglycemic clamp.

**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, N. Billerica, MA) was diluted in 60 ml of 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 approximately 30 min into the insulin clamp. After 2 min of infusion, images were obtained at a sequentially increasing pulsing interval (PI), from 1 to 20 cardiac cycles and maintained at a PI 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 3 images were acquired at each PI with a pulse mechanical index of 1.5. Images were digitized to an off-line image analysis system (Q-lab, Phillips Medical Systems). Background-subtracted acoustic intensity (AI) at each PI was measured from a region-of-interest around the deep forearm flexor muscles as described previously (16; 20). Changes in microvascular volume over time during insulin exposure were calculated from the AI expressed as mean decibels (db’s).

**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. 2-D imaging of the brachial artery was performed in the long axis approximately 10 cm proximal to the antecubital fossa. Images were triggered to the “R” wave of the cardiac cycle and the brachial artery diameter measured using on-line 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: \( Q = v \pi (d/2)^2 \), 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 two site chemiluminescent assay with a sensitivity of 12 pmol/L and an intrassay CV of 2.5, 3.3 and 7% for insulin at concentrations of ~240 pmol/l, ~60 pmol/and ~30 pmol/l, respectively (Immulite 2000 insulin assay, Diagnostic Products Corporation, Los Angeles, CA). Glucose and lactate were measured in duplicate using an YSI 2300 analyzer (Yellow Springs Instruments, Yellow Springs, Ohio). 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: balance = ([A] - [V]) x F, where [A] and [V] are arterial and venous concentrations, and F is forearm blood flow in mls/min/100 mL forearm volume for glucose and forearm plasma flow (blood flow x (1-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 as arterio-venous sampling affords the advantages of safety; specificity (using a 2-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 A-V sample pairs.

**Statistical Analysis.** Data are presented as the means ± the standard error. Initial comparisons were made via paired Student’s \( t \) test between grouped baseline (min -30 to -10) and steady state (min 80 to 120) values and by 1-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 Sigmastat 3.2 (Systat software).

**RESULTS**

**Subject characteristics.** The anthropomorphic characteristics and basal chemistries for study subjects are shown in Table 1. Data are means ± SE.

**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 ± 0.1mmol/l during the baseline period and was maintained within 4% of baseline throughout (Fig 2A). The basal forearm A-V glucose concentration
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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 vs. baseline) (Fig 2B). There was a time dependent increase in total forearm blood flow with a significant increase observed at minute 100 compared to baseline (p<0.05) (Fig 2B). Forearm glucose uptake rose from 1.2 +/- 0.14 µmol/min/100ml at baseline to an average of 6.2 ± 0.6 µmol/min/100ml during min 40 to 120 of insulin infusion (Fig 2 C). A statistically significant increase in glucose uptake was observed at all time points with insulin vs. 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 mM and 0.72 ± 0.02, respectively during the baseline period. Basal forearm lactate balance was -0.48 ±0.11 µM/min/100 ml and remained unchanged over the 120 min of insulin infusion (p=0.789).

**Insulin metabolism.** The time course for the forearm arterio-venous 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<0.01 100 and 120 min vs. baseline), (Fig 3A). Likewise, insulin clearance declined over time (p<0.05 min 120 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-state hyperinsulinemia are shown in Fig 4. Average insulin uptake increased from 15 ± 3 fM/min/100 ml under basal conditions to 87 ± 13 fM/min/100 ml during min 80-120 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 ml/min/100 ml vs. 0.30 ±0.1, p<0.05).

**Microvascular recruitment.** Fig 5 shows the time course for the change in microvascular blood volume over the first 25 min of insulin infusion. We observed a significant rise within 20 min (Fig 5; p<0.01 mean db’s vs. min 0-5 vs. and min 5-10 repeated measures ANOVA). The rise in microvascular perfusion was progressive over the 25 min and had not plateaued at min 25, with a significant increase between 20-25 min vs. 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 physiologic dose of insulin and 2) a decline in the clearance of insulin 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 physiologic 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 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 (18-20). However, divergent results across studies using differing insulin doses, duration of insulin exposure, and methods of assessing blood flow raised considerable controversy regarding the temporal sequence and physiologic significance of insulin’s effect on total blood flow (21-25). More recent studies (26-29) 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 (26), 1-methyl xanthine (1-MX) metabolism (30,31) and CEU (12-17). At physiologic 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 L-NMMA 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, physiologic hyperinsulinemia increases microvascular recruitment by 120 min (16; 17). However, the early time course of microvascular recruitment has not been defined. In the current study, microvascular blood volume increased significantly within 20 min 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 MV 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 physiologic doses of insulin, however supraphysiologic 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
Physiologic 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 pM, hyperinsulinemia 283 ± 7 pM). This suggests that within the physiologic 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 in insulin clearance is attributable to a saturable component of trans-endothelial 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 2-fold plasma-to-interstitial insulin gradient when plasma insulin concentrations are raised within the physiologic 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 trans-endothelial 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. reported that unlabeled insulin diminished the uptake of radiolabeled insulin by the canine hindlimb, indicative of a saturable process. In contrast, Steil et al reported that the plasma-to-interstitial insulin gradient diminished when the plasma insulin concentration was raised from the physiologic to the pharmacologic range. Several technical issues may have limited each of these studies. Steil pointed out that in the Sonksen study the radiolabeled insulin may not bind to the insulin receptor with the same kinetics as native insulin. Though true, this does not necessarily invalidate the observation that native insulin appeared to displace the uptake of the labeled insulin. The Steil study relied on measurements of insulin concentration in hind leg lymphatic drainage. The plasma-to-lymphatic insulin concentration gradient was measured at 2 insulin concentrations: physiologic hyperinsulinemia (~600 pM/l and supraphysiologic (~33 nM/l). At the higher concentration, the investigators observed a paradoxical increase in the rate constant for trans-endothelial 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 physiologic to pharmacologic 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 physiologic dose.
Regardless of changes in microvascular blood volume, a decrease in insulin clearance from the vasculature would not be predicted if the transport of insulin at physiologic 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 and colleagues, who used the human forearm balance technique to measure the uptake of endogenous insulin as stimulated by varying glucose infusions during hyperglycemic clamp (36; 37). However, a paucity of values at high physiologic insulin concentrations limited their regression analyses and pre-empted 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 transcapillary 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 which increases with physiologic 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. As the trans-endothelial 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.

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Table 1. Subject Characteristics $n = 14$

| Characteristic                        | Value  
|---------------------------------------|--------
| Age (years)                           | 24 ± 1 
| Gender (male/female)                  | 11/3   
| Weight (kg)                           | 72 ± 4 
| Height (cm)                           | 177 ± 3 
| Body Mass Index                       | 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 |
**Figure Legends**

**Fig 1.** Protocol for the euglycemic hyperinsulinemic (1mU/kg/min) clamp in lean healthy subjects. Continuous contrast enhanced ultrasound (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 min 40 to 120 of insulin infusion.

**Fig 2.** Glucose metabolism and brachial artery blood flow during 120 min of insulin infusion (1mU/kg/min). Arterial glucose and insulin values (A); forearm blood flow and arterial and venous glucose difference (B); glucose infusion rate and forearm glucose uptake (C). 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.

**Fig 3.** Forearm insulin metabolism at baseline and during 120 min of physiologic hyperinsulinemia (1mU/kg/min). Arterial and venous insulin difference and extraction fraction insulin (A); forearm insulin uptake and insulin clearance (B). Values are means ± SE. *p<0.01, **p<0.05 repeated measures ANOVA vs. pooled baseline. N=14.

**Fig 4.** Mean forearm insulin uptake (A); extraction fraction (B); and clearance (C) at baseline and 80-120 min of hyperinsulinemia. Values are means ± SE. P values from Student’s paired t tests. N=14.

**Fig 5.** Background subtracted forearm videointensity by time of insulin exposure. Values are means ± SE. * p<0.01 ANOVA on ranks vs. 0-5 and 5-10 min. N=13.
Figure 1

Capillary recruitment and insulin clearance

Insulin Clamp: 1 mU/kg/min

-120
-30  -20  -10  0  40  60  80  100  120 min

CEU

- Doppler US
- A-V insulin, glucose

Normal Saline

- Doppler US
- A-V insulin, glucose
Figure 2

A

Arterial glucose (mmol/l)

Arterial insulin (pmol/l)

-30 -20 -10 40 60 80 100 120

Glucose

Insulin

B

Forearm [A-V] glucose (mmol/l)

Forearm Blood Flow (mmol/min/100 ml vol)

-30 -20 -10 40 60 80 100 120

A-V glucose

Blood flow

C

GIR (µmol/kg/min)

Forearm Glucose Uptake (µmol/min/100 ml vol)

-40 -20 0 20 40 60 80 100 120 140

Glucose infusion rate

Forearm glucose uptake

Time (min)
Figure 3

A

A - V insulin
Extraction fraction insulin

[B - V] insulin (pmol/l)

-30 -20 -10 40 60 80 100 120

0.0 0.04 0.08 0.12 0.16 0.20

Extraction fraction insulin

B

Insulin clearance
Insulin uptake

Insulin clearance (mL/min)

-30 -20 -10 40 60 80 100 120

0.0 0.2 0.4 0.6 0.8

1.0

Insulin uptake (nmol/min)

-30 -20 -10 40 60 80 100 120

20 40 60 80 100 120 140 160

* * *
Figure 4
Figure 5

Capillary recruitment and insulin clearance