Insulin Sensitivity of Muscle Capillary Recruitment In Vivo

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We have reported that insulin exerts two vascular actions in muscle; it both increases blood flow and recruits capillaries. In parallel hyperinsulinemic-euglycemic clamp studies, we compared the insulin dose response of muscle microvascular recruitment and femoral blood flow as well as hindleg glucose uptake in fed, hooded Wistar and fasted Sprague-Dawley rats. Using insulin doses between 0 and 30 $mU^{-1} \cdot min^{-1} \cdot kg^{-1}$, we measured microvascular recruitment at 2 h by 1-methylxanthine (1-MX) metabolism or contrast-enhanced ultrasound (CEU), and muscle glucose uptake was measured by either arteriovenous differences or using 2-deoxyglucose. We also examined the time course for reversal of microvascular recruitment following cessation of a 3 $mU \cdot min^{-1} \cdot kg^{-1}$ insulin infusion. In both groups, whether measured by 1-MX metabolism or CEU, microvascular recruitment was fully activated by physiologic hyperinsulinemia and occurred at lower insulin concentrations than those that stimulated glucose uptake or hindleg total blood flow. The latter processes were insulin dose dependent throughout the entire dose range studied. Upon stopping the insulin infusion, increases in microvascular volume persisted for 15–30 min after insulin concentrations returned to basal levels. We conclude that the precapillary arterioles that regulate microvascular recruitment are more insulin sensitive than resistance arterioles that regulate total flow. *Diabetes* 53:447–453, 2004

Based in part on their observation that glucose disposal and leg blood flow displayed similar dose responses to insulin, Baron and colleagues (1,2) proposed that insulin-induced changes in blood flow were one determinant of muscle glucose uptake. Others (3,4) suggested that insulin-induced changes in flow require higher concentrations and longer exposures to insulin than those required for glucose disposal. We reported previously that insulin recruits microvascular vessels in skeletal muscle separate from any effect on total flow (5) and have suggested that insulin's microvascular action contributes to its overall effect on nutrient and hormone delivery to muscle (6). The effects of insulin on both capillary recruitment (7) and larger resistance vessels that regulate total blood flow to muscle (8) are the result of insulin-induced nitric oxide–dependent relaxation processes. We recently reported that microvascular recruitment by physiologic insulin precedes the increase in rat hindlimb total muscle blood flow by as much as 90 min (9). To further compare insulin's actions on total flow and microvascular recruitment, we examined the dose-response characteristics of these two vascular responses. In addition, to further characterize insulin's microvascular action, we examined the time course for its reversal following insulin removal. Dose-response studies were conducted on ad libitum–fed and 24-h–fasted rats using two different methods to assess microvascular recruitment, hindlimb metabolism of 1-methylxanthine (1-MX) (5) and contrast-enhanced ultrasound (CEU) (10), and total flow was measured using Doppler ultrasound.

RESEARCH DESIGN AND METHODS

For studies using 1-MX metabolism, male hooded Wistar rats weighing 230–260 g that were bred in Hobart and fed a standard rat diet and water ad libitum were used. Rats were housed at a constant temperature of 21 ± 1°C in a 12:12-h light:dark cycle. The University of Tasmania Animal Ethics Committee approved all procedures and experiments used. For studies using CEU, male Sprague-Dawley rats weighing 250–350 g were obtained from Hilltop Lab Animals (Scottdale, PA). Animals were housed at 22 ± 2°C and maintained with a 12:12-h light:dark cycle. The animals were provided with food and water ad libitum until food was removed at 5:00 P.M. on the evening before study. The University of Virginia Animal Care and Use Committee approved these experimental protocols.

Immediately before study, rats were anesthetized with sodium pentobarbital (55 mg/kg body wt i.p.) and polyethylene cannulae were inserted into the carotid artery for arterial blood sampling and measurement of arterial blood pressure (Transpac IV; Abbott Critical Care Systems, Morgan Hill, CA) and into both jugular veins for intravenous infusions. A tracheostomy was performed to facilitate spontaneous respiration during the experiment. The femoral vessels in both limbs were exposed via a small incision (~1.5 cm) in the overlaying skin. The femoral artery was carefully separated from the femoral vein and saphenous nerve, the epigastric vessels were ligated, and a flow probe (VB series 0.5 mm; Transonic Systems, Ithaca, NY) was positioned over the femoral artery of the left limb. The probe was interfaced through a flow meter to an IBM-compatible computer. Femoral artery blood flow, blood pressure, and heart rate were measured continuously using WINDAQ data acquisition software (DATAQ Instruments, Akron, OH). Anesthesia was maintained by a continuous infusion of aqueous sodium pentobarbital (0.6 mg $\cdot min^{-1} \cdot kg^{-1}$) via the carotid artery. A heat lamp positioned above the rat maintained body temperature.

Infusion protocols. Animals were studied using one of three protocols. The first two protocols examined the dose response of insulin action (Fig. 1). The third protocol examined the time course for the reversal of insulin-induced capillary recruitment.

Protocol 1. In fed Wistar rats, after a 60-min stabilization period, basal measurements were made and saline or insulin (1.0, 3, 10, or 30 $mU \cdot min^{-1} \cdot kg^{-1}$) infusion was begun and continued for 2 h. 1-MX was infused (0.4 mg ·

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1-MX, 1-methylanthine; 2DG, 2-deoxy-D-[2,6-^{3}H]glucose; CEU, contrast-enhanced ultrasound.

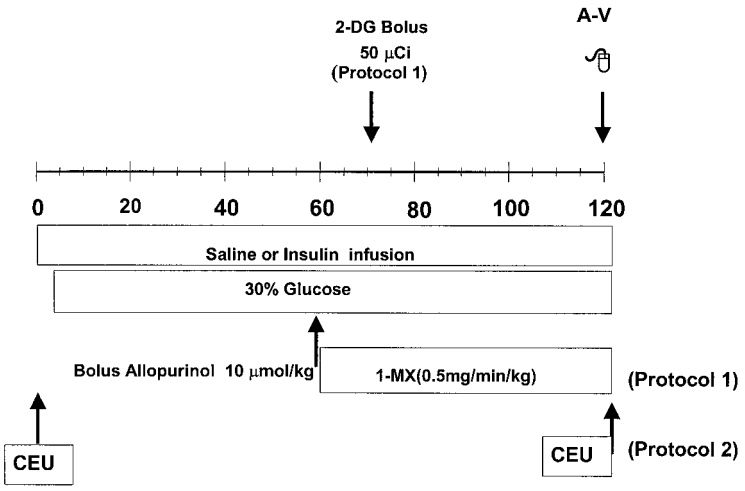


FIG. 1. Experimental protocols 1 and 2. A-V, arteriovenous.

$min^{-1} \cdot kg^{-1} \times 1$ h) for the last 60 min of the 2-h clamp, with a bolus of allopurinol (10 μmol/kg) 5 min before the infusion commenced. This allowed partial inhibition of the activity of xanthine oxidase and constant arterial concentrations of 1-MX to be maintained throughout the experiment. In these fed animals, 45 min before the completion of each experiment, a 50-μCi bolus of 2-deoxy-D-[2,6-^{3}H]glucose (2DG) (specific activity: 44.0 Ci/mmol; Amersham Life Science, Piscataway, NJ) in saline was administered. Plasma samples (20 μl) were collected at 15, 30, and 45 min to determine plasma clearance of the radioactivity. Duplicate arterial and venous samples (300 μl) were taken at the end of the experiment, immediately centrifuged, and 100 μl of the plasma mixed with 20 μl of 2 mol/l perchloric acid. The perchloric acid–treated samples were then stored at −20°C until assayed for 1-MX. At the conclusion of the experiment, the soleus, plantaris, gastrocnemius white, gastrocnemius red, extensor digitorum longus, and tibialis muscles were removed, clamp frozen in liquid nitrogen, and stored at −80°C until assayed for [^{3}H]2DG uptake.

Protocol 2. In the fasted Sprague-Dawley animals, again after a 60-min basal period during which heart rate, blood pressure, and respirations were stable, basal measurements were made and saline or insulin infusion was begun and continued for 2 h. To measure microvascular volume, perfluorocarbon gas–filled albumin-coated microbubbles (Optison; Mallinckrodt Medical, Hazelwood, MO) were infused intravenously at 120 μl/min for 2 min before and during 3 min of ultrasound data acquisition to measure microvascular volume at baseline and at 2 h of infusion of either saline or insulin at doses of 1.5, 3.0, or 10 $mU \cdot min^{-1} \cdot kg^{-1}$ for 2 h, while euglycemia was maintained using a variable rate infusion of 30% glucose.

Protocol 3. To define the time course for the cessation of the microvascular action of insulin, ad libitum–fed, hooded Wistar rats were infused with saline or insulin (3 $mU \cdot min^{-1} \cdot kg^{-1}$) was infused for 1 h while euglycemia was maintained using a variable rate infusion of 30% glucose. The insulin infusion was discontinued (euglycemia continued to be maintained) and at 0, 15, 30, or 60 min after the insulin infusions ceased, blood samples were taken to measure 1-MX metabolism. In these experiments, regardless of the cessation interval, 1-MX was infused (0.4 $mg \cdot min^{-1} \cdot kg^{-1}$) for the last 60 min with a bolus of allopurinol 10 μmol/kg, as described above.

In all protocols, the total blood volume withdrawn from the animals before the final arterial and venous samples did not exceed 1.5 ml and was easily compensated by the volume of fluid infused.

CEU. Measurements of hindlimb video intensity were performed as previously reported (11). Briefly, a linear-array transducer interfaced with an ultrasound system (HDI-5000; Phillips Ultrasound, Santa Ana, CA) was positioned over the right hindlimb of the rat to image the proximal adductor muscle group (adductor magnus and semimembranosus), which comprises ~5% slow-twitch oxidative, 30% fast-twitch oxidative-glycolytic, and 65% fast-twitch glycolytic fibers (12), and secured for the duration of the experiment. Pulse inversion imaging was performed at a transmission frequency of 3.3 MHz and a mechanical index of 0.8. The acoustic focus was set at the midportion of the muscles. Gain settings were optimized and held constant, and data were recorded on 1.25-cm videotape. Perfluorocarbon gas–filled, albumin-coated microbubbles (Optison; Mallinckrodt Medical) were infused intravenously at 120 μl/min for 2 min preceding and over the 3-min duration of data acquisition. The acoustic signal that is generated from the microbubbles when exposed to ultrasound is proportional to the concentration of microbubbles within the volume of tissue being imaged. Essentially, all microbubbles within the ultrasound beam are destroyed in response to a single pulse of high-energy ultrasound, and as the time between each pulsing interval is prolonged, the beam becomes progressively replenished with microbubbles. Eventually, the beam will be fully replenished and further increases in the time between each pulsing interval will not produce a change in tissue opacification. The plateau video intensity reached at long pulsing intervals provides a measurement of microvascular blood volume.

Images were acquired at pulsing intervals from 1 to 20 s. Image analysis was performed off-line. Frames were aligned by cross-correlating several frames at each pulsing interval, and these were separately averaged and digitally subtracted from images obtained during continuous imaging, which served as background. Using images acquired with delays of 0.5 s as background allowed for the elimination of larger vessels (velocity >0.1 cm/s) that fill promptly. The background-subtracted video intensity at each pulsing interval was measured from the muscle, and pulsing interval versus video intensity data were fitted to the function, $y = A\ (1 - e^{-\beta t})$, where y is video intensity, t is the pulsing interval, A is plateau video intensity (an index of microvascular volume), and β is the rate constant, which provides a measure of flow velocity in the microvasculature.

2-Deoxyglucose uptake assay. Individual frozen muscles from the hooded Wistar rats obtained at the end of the insulin infusion were ground under liquid nitrogen and homogenized using an Ultra Turrax. Free and phosphorylated [^{3}H]2DG were separated by ion-exchange chromatography using an anion exchange resin (AG1-X8). Biodegradable Counting Scintillant-BCA (Amersham) was added to each radioactive sample and radioactivity determined using a scintillation counter (Beckman LS3801; Carlsbad, CA). From this measurement and a knowledge of plasma glucose and the time course of plasma 2DG disappearance, R_a, which reflects glucose uptake into the muscle, was calculated as previously described by others (13,14).

Analytical methods. Whole-blood glucose was measured by the glucose oxidase method. Insulin was measured by immunoassay using human insulin standards. Plasma 1-MX, allopurinol, and oxypurinol concentrations were measured by reverse-phase high-performance liquid chromatography as described previously (5,15). 1-MX metabolism was calculated from arteriovenous plasma 1-MX difference multiplied by femoral blood flow and is expressed in nanomoles per minute.

Data presentation and statistical analyses. All data are expressed as means ± SE. Mean femoral blood flow, mean heart rate, and mean arterial blood pressure were calculated from 5-s subsamples of the data, representing ~500 flow and pressure measurements every 15 min. Vascular resistance in the hindleg was calculated as mean arterial blood pressure in millimeters of mercury divided by femoral blood flow in milliliters per minute and expressed as resistance units (RUs). Hindlimb glucose uptake was calculated from arteriovenous glucose multiplied by femoral blood flow. The differences between treatment groups were examined using one-way ANOVA, repeated-measures ANOVA, or t tests as indicated in results. All tests were performed using the SigmaStat statistical program (Jandel Software, San Rafael, CA).

RESULTS

In rats studied using protocols 1 and 2, heart rate and blood pressure were stable and unchanged throughout the 2-h insulin clamp. Blood glucose was clamped at 4.0 mmol/l in the fasted rats and 4.3 mmol/l in the fed rats throughout the insulin infusions. The glucose infusion

TABLE 1
Steady-state values from hyperinsulinemic-euglycemic glucose clamps at various insulin infusions

Insulin infusion rate ($mU \cdot kg^{-1} \cdot min^{-1}$)	Glucose infusion rate ($mg \cdot kg^{-1} \cdot min^{-1}$)	Plasma insulin (pmol/l)
Fed		
0 (saline)	0	337.4 ± 31.4
1	2.0 ± 0.3*	356.3 ± 42.8
3	12.0 ± 0.3*	638.1 ± 84.0*
10	22.0 ± 0.9*	1,985.4 ± 230.4*
30	25.5 ± 1.2*	9,666.5 ± 659.6*
Fasted		
0 (saline)	0	270.1 ± 46.1
1.5	2.6 ± 0.7*	425.0 ± 96.9
3	7.1 ± 0.7*	521.4 ± 50.4*
10	18.4 ± 0.9*	1,679.2 ± 130.0*

Data are means ± SE. *Significantly different from saline ($P < 0.05$).

rates required to maintain euglycemia at 2 h increased progressively with insulin dose in both the fed and fasted rats (Table 1). Plasma insulin concentrations likewise rose progressively with increasing rates of insulin infusion (Table 1).

Figures 2 and 3 show the changes in femoral blood flow seen in the fed and fasted rats over the course of the 2-h insulin infusion. At very low insulin doses, there was no

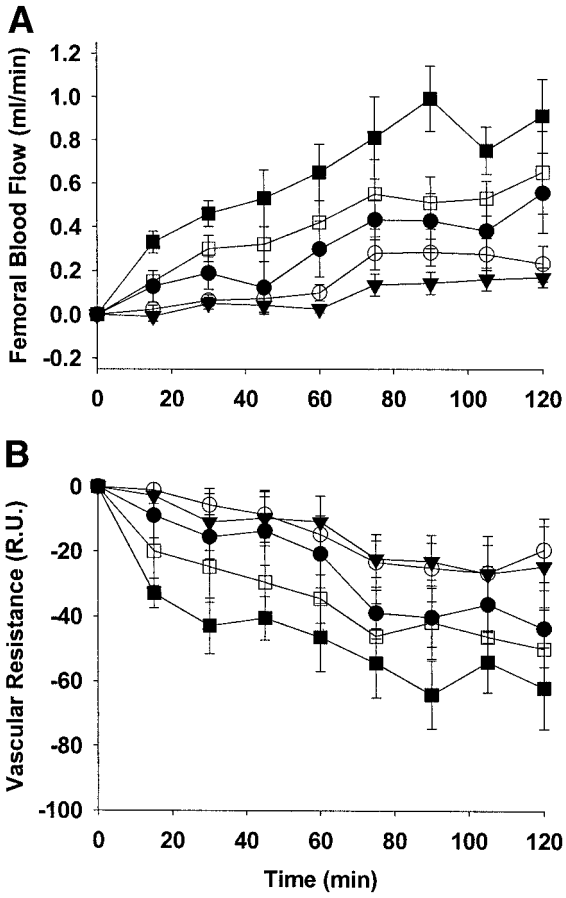


FIG. 2. Time course for the change in femoral blood flow (*A*) and vascular resistance (*B*) during saline or insulin infusions in ad libitum–fed, hooded Wistar rats. Six to eight animals were studied in each group. ▼, saline; ○, 1 mU insulin; ●, 3 mU insulin; □, 10 mU insulin; ■, 30 mU insulin.

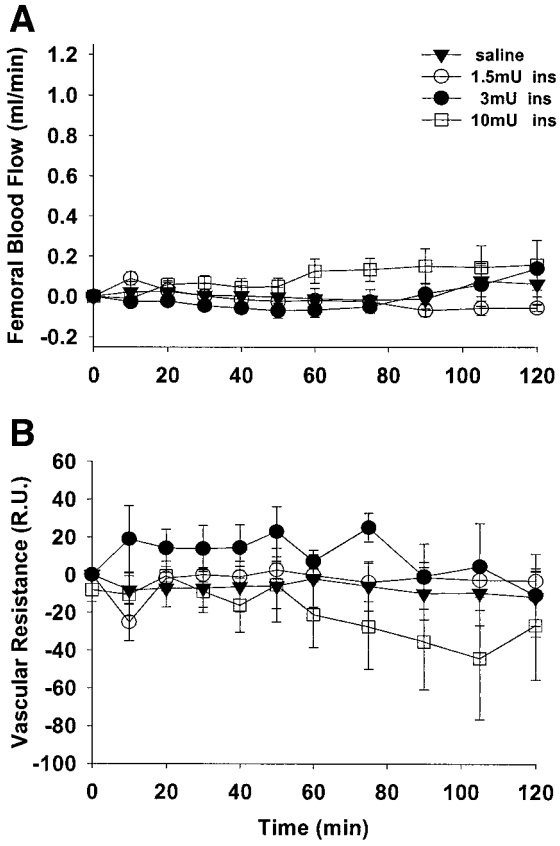


FIG. 3. Time course for the change in femoral blood flow (*A*) and vascular resistance (*B*) during saline or insulin infusions in overnight-fasted Sprague-Dawley rats. Six to eight animals were studied in each group.

significant change in blood flow throughout the 2-h insulin infusion in either group. As the insulin dose was increased, femoral flow rose more markedly and changes were seen sooner after starting the insulin infusion. These changes were more marked in the fed, hooded Wistar rats. Inasmuch as heart rate and blood pressure did not change in either protocol 1 or 2, the observed increase in flow could be attributed to changes in hindlimb vascular resistance. Figures 2 and 3 illustrate the changes in vascular resistance seen over time in the rats fed ad libitum and the rats fasted overnight, respectively. Vascular resistance did not differ between saline and either 1- or 1.5-$mU \cdot min^{-1} \cdot kg^{-1}$ insulin infusions in either the fed or fasted rats, respectively. With the 3.0-$mU \cdot min^{-1} \cdot kg^{-1}$ insulin infusion, femoral blood flow began to increase at the end of the first hour and rose further during the second hour in the fed animal and tended to increase between 1–2 h in the fasted rats, though this was not statistically significant. With insulin doses of 10 $mU \cdot min^{-1} \cdot kg^{-1}$ or greater, vascular resistance declined significantly in both groups and the response occurred earlier as the insulin dose was further increased in the fed rats.

Glucose uptake by rat hindlimb rose with increasing insulin doses in both the fasted and fed rats (Fig. 4). In both groups, the lowest dose of insulin failed to increase glucose uptake, but with doses ≥3 $mU \cdot min^{-1} \cdot kg^{-1}$, glucose uptake rose significantly in both groups. In the fed animals, where glucose disposal was also measured using 2DG uptake, a similar pattern was seen for individual

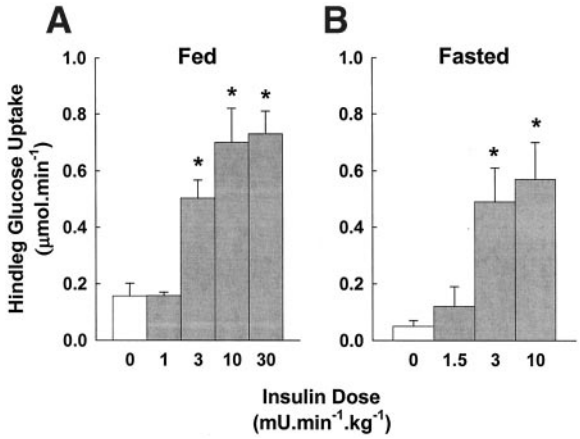


FIG. 4. The rate of hindlimb glucose uptake at the end of the 2 h of insulin infusion (arteriovenous glucose concentration times blood flow) in ad libitum–fed (*A*) and overnight-fasted (*B*) rats.

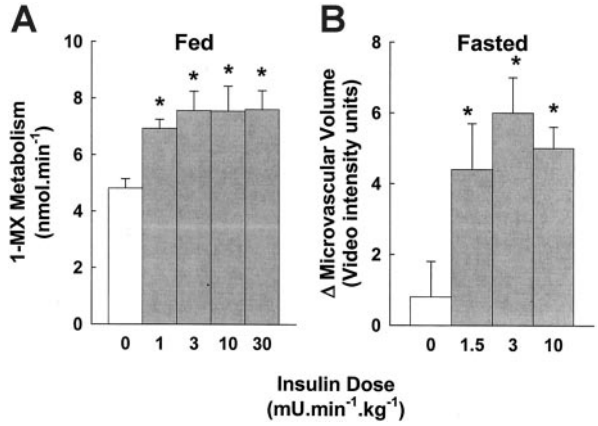
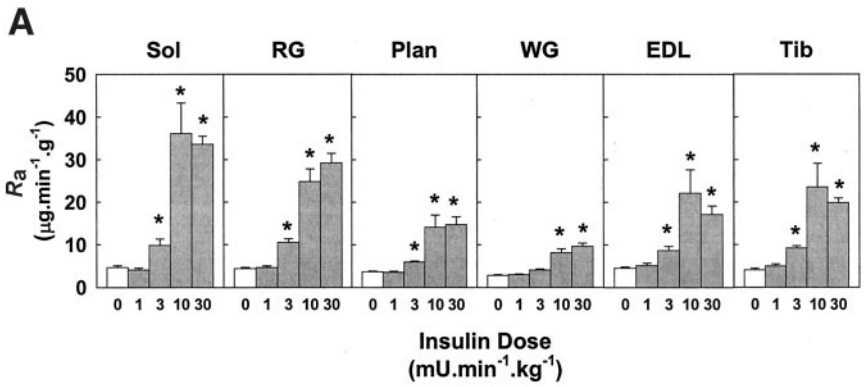


FIG. 6. *A*: The rate of 1-MX disappearance across the rat hindlimb at the end of the 2-h infusion of saline or insulin at each dose to ad libitum–fed, hooded Wistar rats. *B*: The changes in microvascular volume (CEU) seen between baseline and 2 h in overnight-fasted Sprague-Dawley rats. n = 6–8 rats in each group. *Significant differences between insulin and the saline-infused animals ($P < 0.05$).

muscles as well as for the aggregate pool of muscle tissues (Fig. 5).

Figure 6 shows the observed changes in microvascular recruitment (microvascular blood volume) in hindlimb muscle measured by 1-MX in the fed rats and by CEU in the fasted animals as a function of insulin dosage. The two methods gave similar outcomes, regardless of whether the animals were fasted or fed. In the fed animals given even the lowest dose of insulin (1 mU · min^{-1} · kg^{-1}), hindlimb 1-MX metabolism increased. There was a further small rise in 1-MX hindlimb metabolism as the insulin dose increased to 3 mU · min^{-1} · kg^{-1}, but no further increase despite much greater increases in total flow in these animals at high insulin doses (Fig. 2). In fasted rats, video intensity began to rise even with the lowest dose of insulin infused, although this increment was not statistically significant (P = 0.067). However, doses of 3 mU · min^{-1} · kg^{-1}



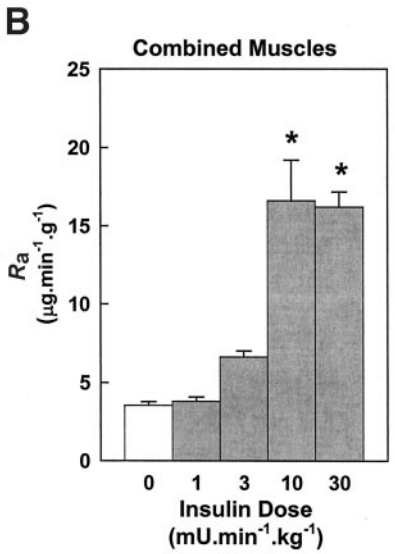


FIG. 5. *A*: The rate of uptake in soleus (Sol), red gastrocnemius (RG), plantaris (Plan), white gastrocnemius (WG), extensor digitorum longus (EDL), and tibialis anterior (Tib) muscles with increasing concentrations of insulin as indicated. *B*: The average increments in insulin-stimulated glucose uptake by these same muscles as a pooled sample. Data are means ± SE for n = 6–9 rats. *Significantly different from saline ($P < 0.05$).

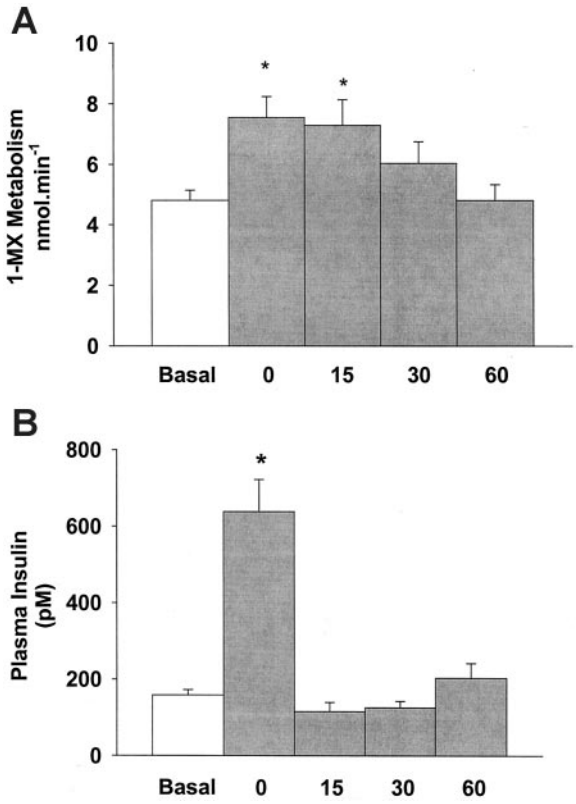


FIG. 7. Plasma insulin concentration and 1-MX metabolism after stopping the 1-h, 3-mU · min^{-1} · kg^{-1} insulin infusion. Data are means ± SE for basal (n = 7–8 animals in each group). *Significantly different from basal values ($P < 0.05$).

appeared to fully recruit microvascular volume, and higher doses did not further stimulate it.

Figure 7 shows the time course for the reversal of microvascular recruitment in fed rats following cessation of a 1-h hyperinsulinemic-euglycemic clamp at 3 mU · min^{-1} · kg^{-1} (protocol 3). Plasma insulin decreased within 15 min to basal, but 1-MX metabolism remained activated until 30 min after insulin infusion had stopped (Fig. 7*B*). Euglycemia was maintained following cessation of insulin infusion, but the glucose infusion could be discontinued within 30 min. The plasma 1-MX concentration remained stable throughout this time.

DISCUSSION

Two important findings emerge from this study. First, the current dose-response findings provide further support for insulin having two discrete actions on the arterial vasculature in skeletal muscle. One action involves dilation of resistance arterioles that regulate total blood flow to the hindlimb. This action is analogous to that described by multiple authors in human leg and arm muscle, where total flow has been measured by a variety of methods. As reported in the introduction, while there is virtually uniform acknowledgment of this action of insulin to enhance total flow (16–18), there is considerable divergence of the findings among investigators with regard to the dose relationship between insulin and increases in blood flow and as a result, the physiologic importance of this action of insulin to stimulate glucose disposal (4).

We have a previously observed that in both the rat hindlimb (5) and human forearm (19), insulin exerts a second action to recruit microvascular elements (presumably capillaries) and that this action can be separated from the actions of insulin on resistance arterioles. This second vascular action presumably occurs further down the arterial tree at terminal arterioles that regulate the distribution of flow to individual fibers. The current dose responses seen in both fed and fasted rats further clarifies that this action of insulin can be separated from the actions of insulin on total blood flow. In both fed and fasted animals using two different strains of rodents, microvascular recruitment required less insulin than enhancement of total flow. Ad libitum feeding (and the insulin secretion that would accompany this) did not appear to affect the response to intravenously infused insulin. However, because of the time required for surgical preparation of the animals, several hours had elapsed since rats had access to food, and the last time feeding had occurred during the overnight period was not monitored. As there were no differences in the basal insulin concentrations between the two groups of rats, the last feeding may have been 6 or 8 h earlier.

The current observations in rats are consistent with observations we have made previously in the human forearm, where raising insulin concentration from fasting levels to ~60 μU/ml increased forearm microvascular volume without affecting total forearm flow (19). In the current study, both the 1-MX and CEU measurements were made after 2 h of steady-state saline or insulin infusion. We previously observed in the rat hindlimb that insulin at doses of 3 mU · min^{-1} · kg^{-1} increases microvascular volume within 30 min. In both those and the current studies with either fed or overnight-fasted animals, there was no increase in total blood flow at this insulin dose during the first 1 h of the infusion (11).

The observation that the effect of insulin on microvascular recruitment saturates at insulin infusion rates between 1 and 3 mU · min^{-1} · kg^{-1}, whereas total flow continues to increase with increments of insulin infusion rates between 3 and 10 mU · min^{-1} · kg^{-1} and increases further between 10 and 30 mU · min^{-1} · kg^{-1}, suggests that microvascular insulin sensitivity is quite distinct from that of resistance vessels. This apparent difference in insulin sensitivity cannot be attributed to differences in measurement sensitivity for total flow and capillary recruitment. We estimate that we would have >80% power to detect a 30% change in hindlimb blood flow by studying six animals ($\alpha < 0.05$). The change in microvascular recruitment was ~50% at the lowest insulin dose used.

Although we observed that capillary recruitment is also more sensitive than glucose disposal to small increments in plasma insulin, some caution must be used in this comparison because considerable data indicate that there is a concentration gradient for insulin between the vascular and interstitial compartments within muscle, and the insulin concentration in the interstitium may be only one-half that of the plasma (20,21). In addition, the arteriovenous glucose measurement is not a very sensitive measure of glucose metabolism. However, measurement of glucose uptake by 2DG is very sensitive, and flow does not enter the calculation. We are confident that even small

(30–50%) increases in glucose uptake would be detectable with this method.

Second, although plasma insulin levels and muscle glucose uptake (data not shown) return to basal levels within 15 min of stopping the insulin infusion, 1-MX metabolism required a further 15 min to reach basal. This suggests that the signaling mechanisms involved in capillary recruitment differ from those involved in glucose uptake. There is evidence that capillary recruitment (7) and total blood flow (8) are each nitric oxide dependent and that nitric oxide production in endothelial cells involves a phosphorylation cascade from the insulin receptor via insulin receptor substrate-1, phosphatidylinositol 3-kinase, and Akt to endothelial nitric oxide synthase (22). Thus, although sharing some elements of the insulin-signaling cascade with glucose transport, the slow reversal of microvascular recruitment may reflect lower phosphatase activity at one or more steps in the insulin-signaling cascade. This could also increase the sensitivity of capillary recruitment to activation by insulin. Further work will be needed to resolve this issue.

A greater sensitivity to insulin and a slower reversal from activation for capillary recruitment may each confer an advantage for insulin (21) and glucose (23) delivery to muscle by ensuring that optimal muscle glucose storage occurs after meals. Capillary recruitment generally affords a greater available surface area for nutrient exchange with the muscle cells for glucose and nutrient uptake. To have it activated early (9) and by lower insulin concentrations would facilitate delivery of glucose and insulin to the myocytes. A slow reversal following the decline of the peak of plasma insulin at the end of the absorptive state could allow washout of insulin from muscle for clearance by liver and kidney. In this manner, anabolic processes stimulated by insulin in the myocytes would be more readily reversed. This might limit late hypoglycemia.

Even with the different methodological approaches for determining capillary recruitment, it is apparent that this is stimulated at insulin concentrations that are lower than those that appeared to be required to stimulate glucose uptake by the hindleg or 2DG uptake into muscles. The observation that video intensity and hindlimb 1-MX metabolism are fully stimulated using insulin infusions of 3 mU $\cdot$ min^{-1} $\cdot$ kg^{-1} while increasing insulin dosage beyond 3 mU $\cdot$ min^{-1} $\cdot$ kg^{-1} progressively increases femoral flow and glucose uptake supports the hypothesis that steps in insulin action beyond any effect on capillary recruitment are important determinants of muscle glucose uptake. Indeed, at very high insulin concentrations, when the extraction ratio for glucose across a muscle bed approaches 50%, it would be predicted on theoretical grounds (24) and is observed experimentally (8) that total blood flow would be a determinant of glucose uptake. However, at more physiologic insulin concentrations, capillary recruitment is likely a necessary primary response for insulin-stimulated glucose uptake. Impairment of this process, as occurs in the genetically obese Zucker rat, has a marked effect on muscle glucose uptake (25). It is also probable that fatty acid uptake by muscle is also impaired when capillary recruitment is impaired (26).

In other studies, we have observed that inhibition of microvascular recruitment, either directly by vasoactive agents like α-methyl serotonin (15) or the nitric oxide inhibitor L-NAME (7) or with tumor necrosis factor-α (27) or free fatty acids (28) blunts insulin-stimulated glucose disposal in muscle. The latter two agents almost certainly affect glucose disposal at sites beyond any action of insulin in the vasculature. The actions of L-NAME and α-methyl serotonin are more strongly suggestive of a role for a vascular action of insulin in glucose disposal. Indeed, in studies (7) using low doses of insulin and early measurements, when insulin has not affected resistance vessels and total flow is unchanged, blocking microvascular recruitment by L-NAME diminishes glucose disposal significantly. Taken together with the current observations, this would suggest that microvascular recruitment is necessary for the full expression of the action of insulin to stimulate glucose disposal but in itself is not sufficient to enhance muscle glucose uptake.

It is relevant to note that because measurements of limb blood flow and capillary recruitment require the animals to be motionless, the experiments were conducted under pentobarbital anesthesia. Pentobarbital at the level used in the current study does not significantly affect heart rate or mean arterial blood pressure, but muscle blood flow and glucose uptake, particularly by muscles rich in red-type fibers, are decreased (14). However, neither basal nor insulin-stimulated glucose uptake by white fiber–type muscles is affected by pentobarbital anesthesia (14).

In summary, the current studies demonstrate that insulin recruits microvasculature within skeletal muscle at concentrations lower than those required to enhance total muscle blood flow and lower than those needed to stimulate glucose disposal. Recruitment of microvasculature alone does not seem to be a sufficient stimulus to enhance the glucose disposal but is necessary to obtain the optimal metabolic response to insulin in muscle. Persistence of microvascular recruitment after insulin withdrawal may facilitate the delivery of nutrients to muscle for a brief time after the postprandial rise in insulin has subsided.

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