Annu. Rev. Nutr. 1997. 17:487–99

ROLE OF BLOOD FLOW IN THE REGULATION OF MUSCLE GLUCOSE UPTAKE

Alain D. Baron
Department of Medicine, Indiana University, The Richard L. Roudebush Veterans Affairs Medical Center, Indianapolis, Indiana 46202

Michael G. Clark
Department of Biochemistry, University of Tasmania, Hobart, Tasmania, Australia 7001

KEY WORDS: glucose clamp, nitric oxide, nutritive flow, rat hindlimb perfusion, endothelium

ABSTRACT

Insulin vasodilates skeletal muscle vasculature via an endothelium-derived nitric oxide–dependent mechanism. Data suggests that insulin interacts directly with the endothelium to cause nitric oxide release. This insulin-mediated increase in muscle perfusion accounts for ~30% of insulin's overall action to stimulate muscle glucose uptake, suggesting a role for insulin and glucose delivery as a determinant of insulin action. Hindlimb perfusion experiments, where perfusion rate is fixed, suggest that changes in distribution of microcirculatory perfusion can modulate substrate uptake. The potential role of insulin to enhance flow through capillary networks that are efficient at nutrient transfer to tissue (nutritive flow) relative to non-nutritive flow is discussed.

CONTENTS

0199-9885/97/0715-0487$08.00

Introduction

It has long been recognized that perfusion of tissue to working muscle is important for adequate delivery of oxygen, fuels, and nutrients. More recently, the notion has emerged that hormone stimulation is accompanied by alterations in muscle perfusion that enhance substrate uptake. The following review addresses the role of insulin in modulating glucose delivery and uptake into skeletal muscle.

Hemodynamic Actions of Insulin

We previously reported that euglycemic hyperinsulinemia causes a dose-dependent increase in skeletal muscle blood flow in humans (5, 20, 21). In lean, insulin-sensitive subjects, insulin leads to a doubling of muscle blood flow, with half of the maximal effect occurring at the highly physiologic insulin concentration of 46 μU/ml. We showed (Figures 1*a* and *b*) that insulin's ability to vasodilate skeletal muscle vasculature is directly proportional to its ability to stimulate glucose uptake (insulin sensitivity) (21). In other words, insulin sensitivity and vasodilation are coupled such that the most insulin-sensitive subjects exhibit the greatest degree of vasodilation. On the other hand, insulin-resistant subjects—such as obese patients or those with hypertension or non-insulin–dependent diabetes mellitus—exhibit blunted vasodilatory responses to insulin (6, 20).

Mechanism of Insulin-Mediated Vasodilation

We tested whether insulin-mediated vasodilation is endothelium-derived nitric oxide (EDNO)-dependent by measuring leg blood flow (LBF), as previously described (30), in healthy volunteers during euglycemic hyperinsulinemia

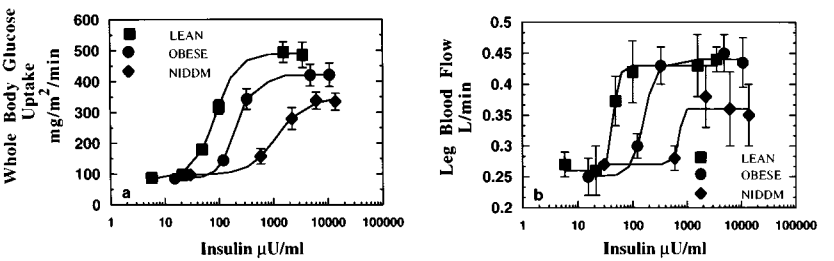


Figure 1 Rates of whole-body glucose uptake (*a*) and insulin-mediated leg blood flow (*b*) determined during euglycemic clamp studies over a wide range of steady state serum insulin concentrations in lean subjects (*closed squares*), obese non-diabetic subjects (*closed circles*), and patients with non-insulin–dependent diabetes mellitus (NIDDM; *closed diamonds*). Note the log scale on the abscissa. Reprinted with permission from the *Journal of Investigative Medicine* 44(8):406–12.

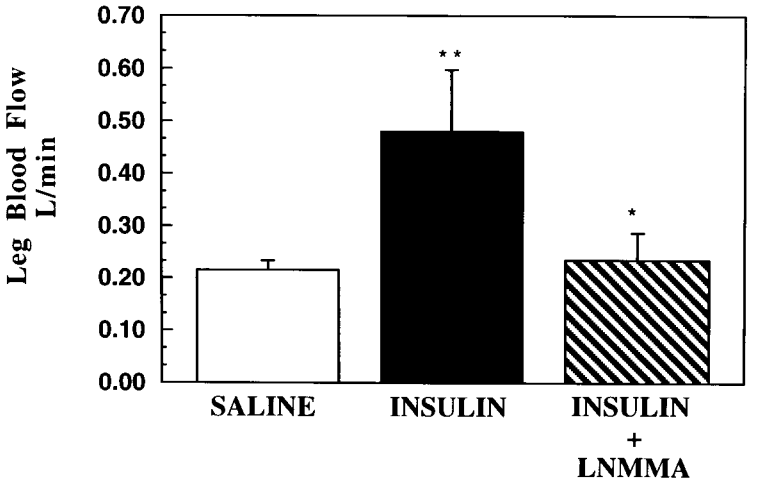


Figure 2 Effects of infusion (femoral artery) of the nitric oxide synthase inhibitor NG-monomethyl-L-arginine (L-NMMA; 16 mg/min) on leg blood flow during hyperinsulinemic (120 mU per m^2 per min) euglycemic clamp studies. *, $P < 0.05$; **, $P < 0.01$. Reprinted with permission from the *Journal of Investigative Medicine* 44(8):406–12.

(insulin levels ~220 μU/ml) alone and during a superimposed infusion of L-*n*-monomethyl arginine (L-NMMA, an inhibitor of nitric oxide synthesis) into the femoral artery. During hyperinsulinemia, LBF increased approximately twofold. With superimposed infusion of L-NMMA, LBF fell by ~50% to the basal rate (Figure 2). Neither saline nor D-NMMA infusions had any effect on LBF. Therefore, these data indicate that insulin-mediated vasodilation is largely (if not exclusively) EDNO-dependent. To verify that insulin actually causes the increased production of nitric oxide (NO), we measured the oxidative end-products NO2/NO3 (Nox) in the femoral venous effluent under each condition. Insulin caused doubling of rate of production of venous Nox, which was completely abrogated by L-NMMA. In human umbilical vein endothelial cells, insulin also causes a dose-dependent release of NO, as measured with an NO amperometric probe (34). Thus, evidence suggests that insulin stimulates the release of EDNO via direct interaction with the endothelium (Figure 3).

Skeletal Muscle Perfusion and Insulin Sensitivity

To quantitate the contribution of insulin-mediated vasodilation to insulin sensitivity, we performed hyperinsulinemic (40 and 120 mU/m^2 per min) euglycemic clamp studies, achieving insulin levels of 75 and 200 μU/ml in nondiabetic, healthy subjects. Leg glucose uptake (LGU) was measured between

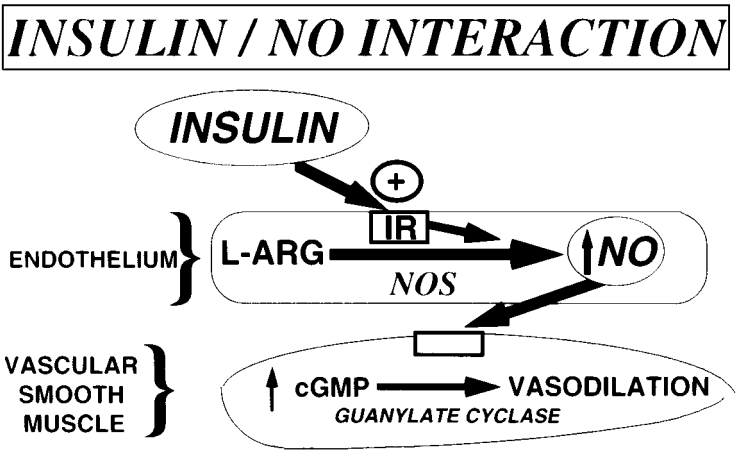


Figure 3 Schematic representation of the interaction between insulin and the endothelium during modulation of the endothelium-derived nitric oxide system. Nitric oxide (NO) is continuously synthesized and released from the endothelium in a reaction utilizing arginine as a precursor and catalyzed by nitric oxide synthase (NOS). NO diffuses to the subendothelium, where it interacts with the vascular smooth muscle cell and, through a cyclic GMP-dependent mechanism, causes smooth muscle cell relaxation and vasodilation. The endothelium-derived NO system can be probed in vivo utilizing a number of pharmacological agents. Acetylcholine or its congener, methacholine, can be utilized to probe endothelium-dependent vasodilation. Sodium nitroprusside can be used as an NO donor that acts directly at the level of the vascular smooth muscle cell, thus bypassing the endothelium. Finally, one can gauge endothelium-dependent vascular tone and vasodilation with the use of an arginine analog such as L-*n*-monomethyl arginine which is a competitive inhibitor of NOS. Our results are consistent with the hypothesis that insulin interacts with the endothelium to enhance NO production and causes vasodilation via this mechanism. IR, Insulin receptor.

200–240 min of the clamp, when near–steady state insulin action was achieved, and again during a subsequent 30-min infusion of L-NMMA, between 240–270 min. LGU was calculated by the balance technique, where

$$\text{LGU} = \text{arteriovenous glucose difference} \times \text{flow or AVG}\Delta \times \text{F}.$$

L-NMMA infusion caused a complete abrogation of the twofold rise in the insulin-induced vasodilation, returning rates of LBF back to baseline values. With the reduction in LBF, glucose extraction rose equally at both insulin doses by approximately 50% at both insulin doses, from ~28 mg/dl to 41 mg/dl; however, this increase in extraction was not sufficient to overcome the effect of the fall in perfusion, and LGU decreased by ~23% and 29% at the low and high insulin doses, respectively ($P < 0.001$; 8) (Figure 4). Thus, insulin-mediated vasodilation of skeletal muscle vasculature appears to augment the direct effect of insulin on glucose uptake.

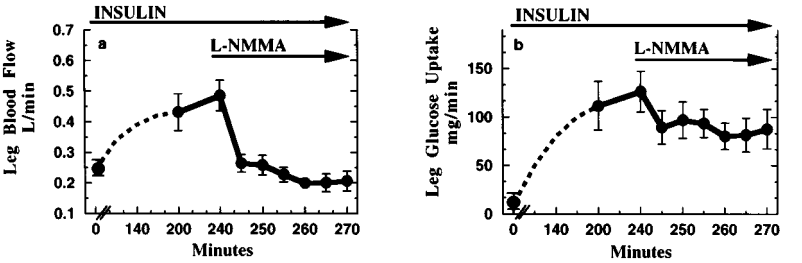


Figure 4 (*a*) Rates of blood flow in the legs of lean, insulin-sensitive subjects during a euglycemic hyperinsulinemic (200 μU/ml) clamp. Insulin causes a roughly twofold increase in blood flow over baseline during the first 240 min of insulin infusion. With superimposed intraarterial L-*n*-monomethyl arginine (L-NMMA), blood flow rapidly falls back to baseline levels during the 30 min of L-NMMA infusion. (*b*) Rates of glucose uptake in legs as a function of time during insulin infusion alone (0–240 min) and during superimposed infusion (femoral artery) of L-NMMA designed to abrogate insulin-mediated vasodilation. Inhibition of insulin-induced vasodilation results in an ~30% reduction in rates of glucose uptake in the leg. Reprinted with permission from the *Journal of Investigative Medicine* 44(8):406–12.

Given the potent effect of insulin on stimulation of tissue glucose uptake and metabolism, it is reasonable to suspect that endothelium-dependent vasodilation is coupled to metabolic need, although this proposition has been challenged (31). A number of reports have documented the ability of insulin to relax preconstricted blood vessels in vitro (33) or isolated vessel segments in vivo (15), suggesting a direct vasodilating action independent of neural or tissue metabolic signals. How is the endothelium coupled to tissue metabolic response? Insulin could act directly on the endothelium and activate nitric oxide synthase (NOS). Alternatively, insulin could stimulate glucose metabolism in the endothelium (perhaps to a similar degree as in tissues) and activate EDNO production via metabolic coupling. In either scenario, insulin resistance in tissues would be reflected in the endothelium, and insulin's ability to modulate EDNO release would be impaired to a degree commensurate with the magnitude of the insulin resistance. Alternatively, insulin-stimulated metabolism in tissues could generate a biochemical signal that could diffuse to the endothelium to cause enhanced EDNO release. These mechanisms are not mutually exclusive and, thus, may operate simultaneously in vivo.

Mechanism of Microcirculatory Glucose Exchange

To understand how an increment in skeletal muscle perfusion could cause an increase in glucose uptake, it is critical to consider glucose exchange across a capillary. A more complete discussion of this topic can be found elsewhere (4).

If glucose is freely diffusable from the capillary wall to the intracellular space and resistance to glucose diffusion is negligible, then all glucose delivered will be taken up by tissues and flow (glucose delivery) will be limiting for glucose exchange. On the other hand, if permeability of glucose through the capillary wall to the intracellular space is severely limiting, then permeability will be limiting for glucose exchange.

Insulin-stimulated glucose extraction (a reasonable index of permeability) under conditions of low flow is about 30–40% (6, 20); therefore, the physiologic situation is intermediate between a flow- and permeability-limited system. If all capillaries are perfused at any one time and if glucose concentration declines in an exponential fashion from the arterial to the venous circulation, an increase in perfusion rate alone would cause a relatively minor increase in glucose concentration along the length of the capillary and, thus, would have an equally minor effect on glucose uptake (4). In this situation, one would predict an inverse linear relationship between flow and extraction (AVGΔ), where an increase in flow is accompanied by a proportionate reduction in AVGΔ and no net change in uptake. We previously demonstrated that glucose uptake under fixed insulin and glucose concentrations can be increased or decreased by methacholine chloride (endothelium-dependent vasodilator) or L-NMMA, respectively (7, 8). Thus, the continuously perfused open capillary model fails to represent actual biologic behavior and, thus, is likely to be oversimplified. Under control of the feeding arterioles, capillaries undergo intermittent perfusion (3, 4, 18) so that at any one time a proportion of capillaries are either perfused or not, and thus there is great heterogeneity of flow volume and velocity through each capillary (3, 4, 9, 26). Moreover, in response to certain stimuli (such as exercise and hypoxia), skeletal muscle exhibits great capacity for capillary recruitment, so that the number of capillaries that are perfused at any particular moment and the integrated time that capillaries remain perfused are increased (3, 4, 9, 26). In turn, because capillary recruitment and/or increased homogeneity of capillary flow leads to greater tissue exposure to glucose, it is likely accompanied by an increase in the amount of tissue participating in glucose metabolism. Another complimentary scenario is that insulin increases perfusion of predominantly nutritive vessels.

Balance of Nutritive-to-Non-Nutritive Blood Flow Within Muscle

As discussed above, there is considerable evidence that insulin acts in vivo to increase blood flow to muscle tissue and that the mechanism by which this is achieved may involve NO-dependent vasodilation. Insulin may also act to improve access for glucose and itself within muscle by increasing nutritive flow at the expense of non-nutritive flow.

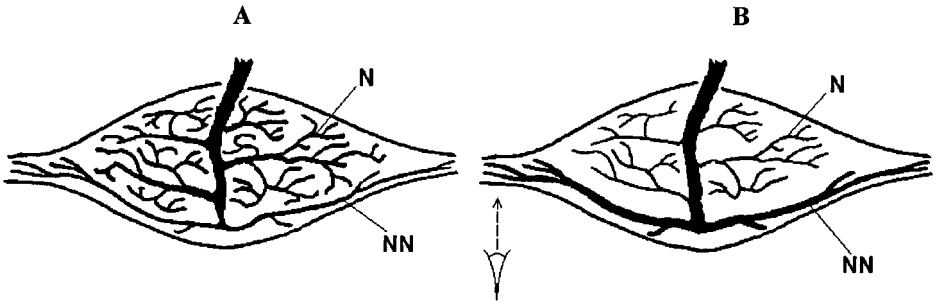
Early evidence for two circulatory systems (nutritive and non-nutritive) in, or associated with, muscle appears to date from the 1940s. Pappenheimer (23) noted disparate effects of epinephrine and stimulation of vasoconstrictor nerves in the dog gastrocnemius muscle perfused at constant pressure. While each resulted in decreased flow, oxygen uptake was either stimulated (epinephrine) or inhibited (nerve stimulation). Other workers noted disparate effects of increased flow to working muscle, where some perturbations increased flow with no change in muscle force and others did not affect flow but decreased force (29). Yet other discrepancies emerged when attempts were made to correlate flow with either clearance of intramuscular injected markers (1, 19, 32) or to extraction of markers from blood to tissue (27). However, arteriovenous shunts are not present in skeletal muscle (17, 24), and thus, an obvious anatomical explanation for high flow with low marker exchange between blood and tissue could not be invoked. An interesting proposal consistent with the existing data of the time was that non-nutritive vessels were those of the septa and tendon (2, 16). Because these were physically isolated from muscle cells but were associated with each muscle, they became prime candidates for the elusive high-flow shunt (19, 28, 29, 32).

The main recent evidence for two circulatory systems largely arises from the metabolic effects of vasoconstrictors in the rat hindlimb perfused at constant flow (10, 11 and references therein). Vasoconstrictors appear to fall into two classes. Those that increase metabolism are type A, and those that inhibit metabolism are type B. Although both A and B increase perfusion pressure in the model, they have opposing effects on oxygen uptake, as well as on lactate, glycerol, urate, and uracil efflux. Other parameters affected include insulin-mediated glucose uptake, aerobic but not anaerobic muscle contraction, and perfusate distribution volume. Type A vasoconstrictors include α_1 adrenergic agonists, angiotensins, vasopressin, vanilloids, and low-frequency nerve stimulation. Type B vasoconstrictors include serotonin, norepinephrine at high doses, high-frequency sympathetic nerve stimulation, and high-dose vanilloids. Apart from type A (stimulatory) and B (inhibitory) vasoconstrictors having the opposite metabolic effects, there is evidence that the sites on the vasculature at which they operate are biochemically distinct, with different Ca^{2+} and oxygen dependencies (14). Because of the extracellular Ca^{2+} dependency of type A vasoconstrictors, the Ca^{2+} channel blockers are selective for these. Other vasodilators (10) indiscriminately block both type A and B vasoconstrictor effects, underscoring the likelihood that the metabolic effects of both vasoconstrictor types are due to their vascular effects. As yet, a vasodilator that selectively increases metabolism, including glucose uptake, has not been identified unless it acts to dilate a type B preconstricted (metabolically inhibited) state. Since the vasodilator carbachol acts to oppose type B (serotonin)-mediated inhibition

of insulin-mediated glucose uptake in the perfused muscles (25), it is likely that the inhibition is due solely to a vascular effect that changes flow distribution within muscle (11).

Another compelling piece of evidence implicating a vascular effect on the control of metabolism is apparent when perfused and incubated muscle are compared. The inhibitory effect of the type B vasoconstrictor, serotonin (5-HT), on insulin-mediated 2-deoxyglucose uptake into muscles of the perfused hindlimb was not seen when muscles were removed and incubated with insulin, 5-HT, and 2-deoxyglucose. Thus, all muscles from the perfused hindlimb showed marked inhibitory effects of 5-HT, and in some muscles (e.g. extensor digitorum longes and anterior tibialis), the inhibitory effect was as great as 75%. In contrast, insulin's ability to stimulate 2-deoxyglucose uptake by isolated incubated soleus or extensor digitorum longus muscles was unaffected by 5-HT (25).

The purported presence of functional vascular shunts in the hindlimb implies that there are at least two vascular routes for flow to take: one predominantly nutritional and the other, of which shunts form part, non-nutritional (19, 28, 29, 32) (Figure 5). Thus, on the one hand, 5-HT—through its vasoconstrictor activity



	A	B
Flow in tendon vessels of biceps femoris (units of fluorescence; mean±SE, n=5)	0.39 ±0.06	2.30 ±0.23
Whole hindlimb oxygen uptake ($\mu mol.h^{-1}.g^{-1}$; mean±SE, n=5)	11.10 ±0.34	2.58 ±0.20

Figure 5 Proposed nutritive and non-nutritive flow in muscle and the observed apparent reciprocal relationship between non-nutritive flow and oxygen uptake for the rat hindlimb perfused at constant flow. Steady state flow in tendon vessels of the biceps femoris (putative non-nutritive vessels of muscle) (16) was measured using FITC dextran infusion and surface fluorometry (*arrow*). Steady state oxygen uptake was measured in the same constant flow perfusion. Nutritive vessels (N) presumably end in an extensive capillary network in intimate contact with muscle cells. Non-nutritive vessels (NN) presumably are capable of high flow and are thought to be located on septa and tendons. Vasoconstrictor infusions were 100 nM norepinephrine (enhances N flow) (*A*), or micromolar concentrations of serotonin (enhances NN flow) (*B*).

at selected sites in the rat hindlimb vasculature—controls access to the non-nutritional vessels. On the other hand, to counterbalance the selective process by 5-HT and other type B vasoconstrictors, it would appear likely that norepinephrine (NE) and type A vasoconstrictors regulate access to nutritive vessels (Figure 5).

The notion of selective flow routes was explored by comparing the effects of NE and 5-HT in the red blood cell–free, buffer-perfused hindlimb on postequilibration red blood cell efflux, vascular entrapment of fluorescein-labeled dextran, and vascular corrosion casting using methyl methacrylate (22). In addition, the presence of arteriovenous shunts (17) was assessed by monitoring passage of 12-μm microspheres. A marked transient washout of red blood cells occurred immediately following vasoconstriction by NE that was not apparent when vasoconstriction was induced by 5-HT. Assessment of perfused vascular regions with fluorescein isothiocyanate dextran during constant-flow perfusion indicated that NE recruited a new vascular space (more capillaries of the nutritive network?) that was reperfused by a second exposure to the vasoconstrictor. In contrast, 5-HT closed off a previously perfused vascular space (some of the nutritive capillary network?) that was reperfused only when the vasoconstrictor was removed. Vascular corrosion casting of the arterial tree, with 30-μm methyl methacrylate equilibrated in the absence or presence of either vasoconstrictor, showed that NE (50 nM) led to a visible increase in the number of vessels being filled without an increase in cast weight. 5-HT, in contrast, caused a marked decrease in cast weight, with visibly fewer vessels filled. Finally, very few (3%) of the infused 12-μm microspheres appeared in the venous effluent whether or not vasoconstrictors were present. Together these recent findings suggest that NE and 5-HT, in association with vasoconstriction at different sites, exert reciprocal control on the nutritive and non-nutritive flow routes in the hindlimb muscles that, in turn, may influence metabolism by increasing or decreasing access to nutrient, respectively.

A major question concerns the anatomical nature of the non-nutritive vessels of the hindlimb. Failure of microspheres to pass to the venous side suggests that these high-flow vessels that have little or no capacity for nutrient exchange to muscle [e.g. type B vasoconstrictors have no effect on aerobic muscle contraction (13)] end in capillaries. A key issue is whether these non-nutritive vessels are associated with muscle or are in nonmuscle tissue of the hindlimb (e.g. skin, bone, etc). This has been answered by using fluorescent microspheres to measure perfusate flow in each tissue. Two conditions were assessed in the rat hindlimb perfused at constant flow: basal, when nutritive and non-nutritive flow occur; and type B (5-HT) vasoconstricted, when non-nutritive flow predominates and metabolism/contractile performance is markedly inhibited. Values for microsphere content as a percentage of the total infused were

determined. Leg muscle contained 40 ± 5% under basal conditions, and the content was unchanged as a result of 5-HT–mediated vasoconstriction. In addition, 5-HT did not significantly alter the distribution of microspheres in skin, white adipose tissue, abdominal tissue, or tissue surrounding the spine. Only bone showed a significant decrease, from ~9% to 5%. Because tissues are excised for the assessment of microsphere entrapment, a failure to find changes in distribution of the microspheres from muscle to other tissues, or between muscles, implies that the non-nutritive vessels are associated with each muscle. A clue to the anatomical identity of the non-nutritive vessels of muscle may reside in the observations of Grant & Payling Wright (16), who proposed that non-nutritive vessels of the rat hindlimb could be visualized on the tibial tendon of the biceps femoris. Accordingly, flow in these vessels has now been studied during hindlimb perfusion using surface fluorometry of infused fluorescein-5-isothiocyanate (FITC)-dextran (M_r 150,000). Apparent flow in the putative non-nutritive vessels was greatest when metabolism for the whole hindlimb was lowest (Figure 5). However, it remains to be resolved whether flow in connective tissue vessels of muscle septa and tendons overall could be high enough to account for the observed inhibition of metabolism with type B vasoconstrictors.

Another approach to assessing relative nutritive:non-nutritive flow has been to quantify the metabolism of an infused substrate targeted for a capillary endothelial enzyme. The principal assumption is that the extent of the nutritive capillary network greatly exceeds that of the non-nutritive system. A candidate substrate is 1-methylxanthine (1-MX), which appears to be metabolized exclusively to 1-methylurate (1-MU) (at least in rat hindlimb) by xanthine oxidase and is nonvasoactive at the concentrations required (i.e. 20–25 μM). Infusion of 23 μM 1-MX establishes a steady state value for 1-MU/1-MX of ~1.15 (Figure 6). When 5-HT is infused and total arterial flow is kept constant, there is a decrease in the ratio to ~0.70, coinciding with both the rise in perfusion pressure and the decrease in metabolism (VO_2). This would appear to be consistent with 5-HT–mediated repartitioning of flow from nutritive to non-nutritive vessels.

The 1-MX method has been applied in vivo in rats (12). Anesthetized rats were treated with either saline or α-methyl serotonin (a serotonin 5-HT_2 agonist and putative type B vassoconstrictor in vivo), alone or in combination with 10 mU of insulin per min per kg under euglycemic clamp conditions. Mean arterial blood pressure, heart rate, and femoral blood flow were monitored continuously. Hindlimb blood flow distribution was assessed by arterial-venous femoral blood sampling to determine the metabolism of 1-MX. The serotonin agonist alone increased blood pressure and heart rate but did not change femoral blood flow. However, consistent with perfused hindlimb data (Figure 6), 1-MX disappearance decreased by 46%, indicating less capillary (nutritive?) flow.

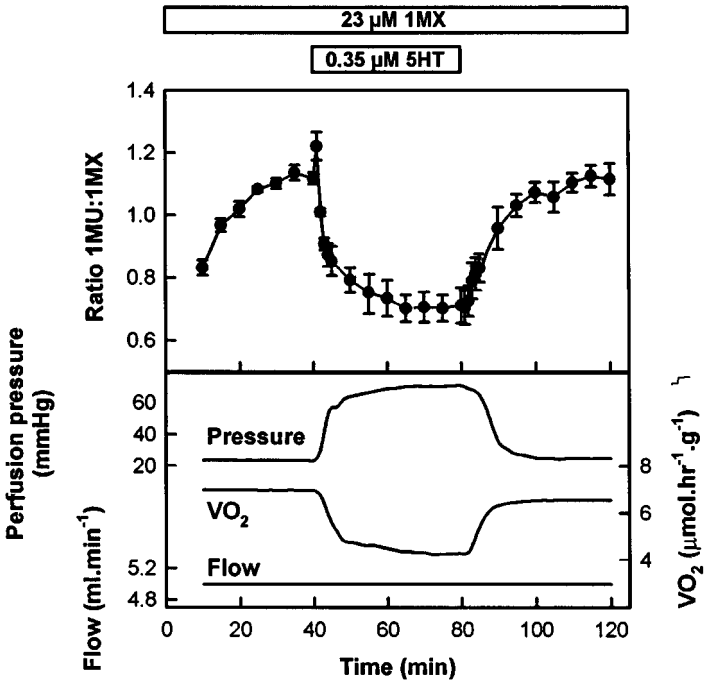


Figure 6 Effect of serotonin on the metabolism of 1-methylxanthine (1MX) to 1-methylurate (1MU) in the rat hindlimb perfused at constant flow. Values for 1MU:1MX are means plus or minus the standard error ($n = 5$). Representative traces of pressure, VO_2, and flow are shown.

During the clamp, α-methyl serotonin also decreased the whole-body glucose infusion rate by 43%, further suggesting of an acute state of insulin resistance at the whole-body level. Insulin alone increased femoral blood flow (62%), hindleg glucose uptake, and 1-MX disappearance (54%), suggesting that insulin may act to favor nutritive flow at the expense of non-nutritive flow. These findings emphasize the importance of blood flow distribution within skeletal muscle and its control by vasomodulators, including insulin.

Summary and Conclusion

We presented evidence that insulin acts at the level of the endothelium to regulate skeletal muscle perfusion. The vasodilatory effect of insulin can amplify the direct stimulatory effect of insulin on glucose uptake. The mechanism is complex and likely involves capillary recruitment and redistribution of flow through nutritive vessels. Insulin may specifically enhance nutritive vessel perfusion so that changes in total muscle flow may not reveal the vascular effect

of insulin on glucose metabolism. Conversely, pathologic states associated with maldistribution or lack of enhancement of perfusion in response to insulin may be associated with insulin resistance. Clearly, more work is required to better understand the relationship between muscle glucose metabolism and microcirculatory perfusion.

ACKNOWLEDGMENTS

We wish to thank Drs. H. O. Steinberg and S. Rattigan for their helpful suggestions, and Ginger Brechtel-Hook and Joyce Ballard for their help in preparing the manuscript. This work was supported in part by grants DK42469, M01-RR750, and DK20542 from the National Institutes of Health, a Veterans Affairs Merit Review Award, a Grant-in-Aid A3392 from the American Heart Association, and grants from the National Health and Medical Research Council of Australia and Diabetes Australia.

Literature Cited

1. Barlow TE, Haigh AL, Walder DN. 1958. A search for arteriovenous anastomoses in skeletal muscle. *J. Physiol. London* 143:80P
2. Barlow TE, Haigh AL, Walder DN. 1961. Evidence for two vascular pathways in skeletal muscle. *Clin. Sci.* 20:367–85
3. Baron AD. 1993. Cardiovascular actions of insulin in humans. Implications for insulin sensitivity and vascular tone. In *Clinical Endocrinology and Metabolism*, ed. E Ferrannini, pp. 961–86. London: Bailliere Tindal
4. Baron AD. 1994. Hemodynamic actions of insulin. *Am. J. Physiol.* 267:E187–202
5. Baron AD, Brechtel G. 1993. Insulin differentially regulates systemic and skeletal muscle vascular resistance. *Am. J. Physiol.* 265:E61–67
6. Baron AD, Brechtel-Hook G, Johnson A, Hardin D. 1993. Skeletal muscle blood flow. A possible link between insulin resistance and blood pressure. *Hypertension* 21:129–35
7. Baron AD, Steinberg H, Brechtel G, Johnson A. 1994. Skeletal muscle blood flow independently modulates insulin-mediated glucose uptake. *Am. J. Physiol.* 266:E248–53
8. Baron AD, Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G. 1995. Insulin-mediated skeletal muscle vasodilation contributes to both insulin sensitivity and responsiveness in lean humans. *J. Clin. Invest.* 96:786–92
9. Caro GG, Pedley TJ, Schroter RC, Seed WA. 1978. *The Mechanisms of the Circulation*, pp. 418–22. New York: Oxford Univ. Press
10. Clark MG, Colquhoun EQ, Dora KA, Rattigan S, Eldershaw TPD, et al. 1994. Resting muscle: a source of thermogenesis controlled by vasomodulators. In *Temperature Regulation: Advances in Pharmacological Sciences*, ed. AS Milton, pp. 315–20. Basel: Birkhauser
11. Clark MG, Colquoun EQ, Rattigan S, Dora KA, Eldershaw TPD, et al. 1995. Vascular and endocrine control of muscle metabolism. *Am. J. Physiol.* 268(E31): 797–812
12. Clark MG, Rattigan S, Barrett EJ. 1996. Vasoconstrictor-mediated blood flow redistribution and acute insulin resistance in rat skeletal muscle in vivo. *Diabetologia* 39(Suppl. 1):A175
13. Dora KA, Rattigan S, Colquhoun EQ, Clark MG. 1994. Aerobic muscle contraction impaired by serotonin-mediated vasoconstriction. *J. Appl. Physiol.* 77:277–84
14. Dora KA, Richards SM, Rattigan S, Colquhoun EQ, Clark MG. 1992. Serotonin

and norepinephrine vasoconstriction in rat hindlimb have different oxygen requirements. *Am. J. Physiol.* 262:H698–703
15. Feldman RD, Bierbrier GS. 1993. Insulin mediated vasodilation: impairment with increased blood pressure and body mass. *Lancet* 342:707–9
16. Grant RT, Payling Wright H. 1970. Anatomical basis for non-nutritive circulation in skeletal muscle exemplified by blood vessels of rat biceps femoris tendon. *J. Anat.* 106:125–33
17. Hammersen F. 1970. The terminal vascular bed in skeletal muscle with special regard to the problem of shunts. In *Capillary Permeability: The Transfer of Molecules and Ions Between Capillary Blood and Tissue*, ed. C Crone, NA Lassen, pp. 351–65. Copenhagen: Munksgaard
18. Honig CR, Odoroff CL, Frierson JL. 1982. Active and passive capillary control in red muscle at rest and exercise. *Am. J. Physiol.* 243:H196–206
19. Hyman C, Rosell S, Rosen A, Sonnenschen RR, Uvnas B. 1959. Effects of alterations of total muscular blood flow on local tissue clearance of radio-iodide in the cat. *Acta Physiol. Scand.* 46:358–74
20. Laakso M, Edelman SV, Brechtel G, Baron AD. 1990. Decreased effect of insulin to stimulate skeletal muscle blood flow in obese man: a novel mechanism for insulin resistance. *J. Clin. Invest.* 85:1844–52
21. Laakso M, Edelman SV, Brechtel G, Baron AD. 1992. Impaired insulin mediated skeletal muscle blood flow in patients with NIDDM. *Diabetes* 41:1076–83
22. Newman JMB, Dora KA, Rattigan S, Edwards SJ, Colquhoun EQ, Clark MG. 1996. Norepinephrine and serotonin vasoconstriction in rat hindlimb control different vascular flow routes. *Am. J. Physiol. Endocrinol. Metab.* 270:E689–99
23. Pappenheimer JR. 1941. Vasoconstriction nerves and oxygen consumption in the isolated perfused hindlimb muscles of the dog. *J. Physiol. London* 99:182–200
24. Piiper J, Rosell S. 1961. Attempt to demonstrate large arteriovenous shunts in skeletal muscle during stimulation of sympathetic vasodilator nerves. *Acta Physiol. Scand.* 53:214–17
25. Rattigan S, Dora KA, Colquhoun EQ, Clark MG. 1993. Serotonin-mediated acute insulin-resistance in the perfused rat hindlimb but not in incubated muscle: a role for the vascular system. *Life Sci.* 53:1545–55
26. Renkin EM. 1979. Control of microcirculation and blood-tissue exchange. In *Handbook of Physiology—The Cardiovascular System IV*, ed. RM Berne, N Sperelakis, SE Geiger, 4:627–87. Baltimore: Williams & Wilkins
27. Renkin EM, Rosell S. 1962. Effects of different types of vasodilator mechanisms on vascular tonus and on transcapillary exchange of diffusible material in skeletal muscle. *Acta Physiol. Scand.* 54:241–51
28. Rosell S, Uvnas B. 1960. Vasomotor control of oxygen consumption in skeletal muscle. *Acta Physiol. Scand.* 50(Suppl. 175):129–30
29. Sonnenschein RR, Hirvonen L. 1961. Effects of vasoactive drugs on blood flow and work performance in skeletal muscle. *Biochem. Pharmacol.* 8:166
30. Steinberg HO, Brechtel G, Johnson A, Fineberg N, Baron AD. 1994. Insulin mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. *J. Clin. Invest.* 94:1172–79
31. Vollenweinder P, Tappy L, Randin D, Schneiter P, Jequier E, et al. 1993. Differential effects of hyperinsulinemia and carbohydrate metabolism on sympathetic nerve activity and muscle blood flow in humans. *J. Clin. Invest.* 92:147–54
32. Walder DN. 1955. The relationship between blood flow, capillary surface area, and sodium clearance in muscle. *Clin. Sci.* 14:303–15
33. Yagi S, Takata S, Kiyokawa H, Yamamoto M, Noto Y, et al. 1988. Effects of insulin on vasoconstrictive responses to norepinephrine and angiotensin II in rabbit femoral artery and vein. *Diabetes* 37:1064–67
34. Zeng G, Quon MJ. 1996. Insulin-stimulated production of nitric oxide is inhibited by wortmannin. Direct measurement in vascular endothelial cells. *J. Clin. Invest.* 98(4):894–98