

# Muscle metabolism and control of capillary blood flow: insulin and exercise

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## Abstract

The evidence that muscle metabolism is determined by available capillary surface area is examined. From newly developed methods it is clear that exercise and insulin mediate capillary recruitment as part of their actions *in vivo*. In all insulin-resistant states examined thus far, insulin-mediated capillary recruitment is impaired with little or no change to the exercise response. Control mechanisms for capillary recruitment for exercise and insulin are considered, and the failure of the microvasculature to respond to insulin is examined for possible mechanisms that might account for impaired vascular responses to insulin in insulin resistance.

## Introduction

The study of muscle metabolism by biochemists has traditionally involved the reductionist approach so that much of what is known has emerged from experiments involving isolated incubated muscles, single cells (in culture), organelles and homogenates. Unfortunately, when such knowledge gained from this approach is put back into a physiological context it is often found wanting.

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The main reason for this is the complexities of the vascular system and its unique relationship with skeletal muscle. Often the vascular system of muscle has been assumed to play a passive role delivering nutrients and hormones and removing waste products. In addition, it has been assumed that the vascular system is solely regulated by metabolic demand of the skeletal muscle. Recent evidence paints a different picture and suggests that the vasculature may play a large part in controlling muscle metabolism and contraction. Thus the muscle vasculature can respond directly to stimuli to change the pattern of microvascular perfusion, which by controlling delivery, influences muscle metabolism. Hormones such as insulin are now known to control their own, as well as substrate, delivery to muscle by vascular effects. Furthermore, there is growing evidence that insulin-resistant diabetes may originate from impaired vascular control with diminished delivery for both insulin and glucose to muscle.

The objective of this review is to examine the impact of the vasculature in controlling muscle metabolism and contraction largely through the manner in which the vascular system regulates nutrient delivery and removal.

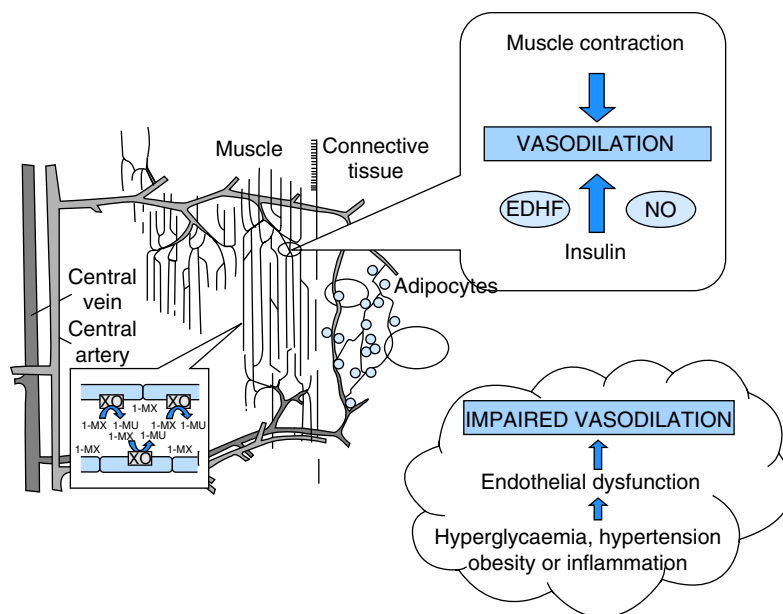
## Capillary recruitment

### Transition from rest to exercise

There is evidence that, at rest, muscle is only partly perfused with autonomic vasomotion responsible for switching flow between different regions of the microvascular network to ensure relative normoxia. Thus at any one time (i.e. if one were to take a snapshot) under basal or resting conditions a relatively small proportion of the available capillaries in muscle is perfused (albeit there is both temporal and spatial heterogeneity). With the onset of contraction (muscle work) the 'snapshot' number of perfused capillaries increases several times, ranging from a three- to fourteen-fold increase, depending on the conditions and muscle type. A direct result of the increase in perfused capillaries is the increase in nutrient delivery, providing the additional substrates required to fuel the aerobic work done by contraction.

### Normal action of insulin

Being able to measure changes in capillary surface area has been a significant step forward in understanding the *in vivo* vascular actions of hormones such as insulin and of exercise. To this end, a biochemical marker method using 1-MX (1-methylxanthine) has been developed for assessing capillary recruitment in muscle (Figure 1). Infused 1-MX was found to be converted stoichiometrically by rat hindlimb to 1-methylurate by xanthine oxidase, which others have shown to be largely concentrated in the capillary endothelial cells [1]. The method for measuring relative nutritive (capillary) flow was established and checked in the constant (total) flow perfused rat hindlimb system, where the proportion of total flow that is nutritive, could be varied. For example,



**Figure 1. Vascular blood flow routes in skeletal muscle**

Skeletal muscle microvascular blood flow is controlled by vasodilation at the terminal arterioles that enables perfusion of capillaries in contact with muscle myocytes (nutritive flow). Capillaries contain XO (xanthine oxidase) which can metabolize 1-MX and provide an indication of the extent of perfusion. The illustration is based upon the vascular arrangement of the rabbit tenuissimus muscle observed by Borgstrom et al. [32].

serotonin, a vasoconstrictor that reduces nutritive flow in this preparation, decreased 1-MX conversion into 1-methylurate but did so without altering either total flow or individual muscle flow rates (as determined by 15  $\mu\text{m}$  microspheres) (see review [2] and references therein). In another example, electrical stimulation of the gastrocnemius-plantaris-soleus muscle group (to simulate exercise) of the pump-perfused rat hindlimb system increased 1-MX conversion into 1-methylurate. Thus, these findings strongly suggested that 1-MX conversion is an indicator of muscle nutritive flow and changes in metabolism of 1-MX could reasonably be assumed to reflect changes in capillary recruitment. With this as background, 1-MX was infused under conditions of steady state in the anaesthetized, *ad lib.* fed rat *in vivo* and metabolism of 1-MX determined from blood samples taken across the hindlimb. It was found that insulin (at doses which increased basal plasma insulin levels 4-fold, equivalent to a maximal physiological dose) increased 1-MX metabolism approximately 80% [3] in association with increased glucose uptake in muscle of the same hindlimb. The increase in 1-MX metabolism was indicative of capillary recruitment.

Whilst the first of the two methods for assessing capillary recruitment or nutritive flow relied on the metabolism of an infused exogenous substrate, the second approach was based on imaging of the change in perfusion by CEU

(contrast enhanced ultrasound). The technique is derived from that described for the heart in which microbubbles of albumin (phospholipid microbubbles can also be used) provide the contrast medium. Essentially the technique involves simultaneously imaging and destroying all microbubbles within the ultrasound beam with the use of a high energy ultrasound pulse. The time between successive ultrasound pulses is progressively extended, allowing the tissue within the beam to be replenished with microbubbles. Eventually, the tissue will be fully replenished and further increases in time between each pulse will not alter the microbubble acoustic signal returning from the muscle. Preliminary assessment was made to ensure that the energy settings ( $> 4$  MHz) used to destroy the microbubbles did not damage the endothelium ( $< 3$  MHz). Once the images are collected a background subtraction (representing large rapidly filling vessels) is made to isolate effects on capillaries and allow calculation of the microvascular volume. Using this approach, changes in capillary blood volume in response to insulin and exercise have been assessed in skeletal muscle of the rat hindlimb *in vivo* and the CEU data have been found to correlate well with 1-MX metabolism data. Capillary blood volume increased approx. 100% during physiologic doses of insulin; with exercise slightly more of an increase. Data from these two approaches indicate that insulin mediates changes in muscle microvascular perfusion consistent with capillary recruitment. The response to administered insulin has been found in muscle of anaesthetized rats [3] and conscious humans [4] and to mixed meal and light exercise in conscious humans [5]. Furthermore, by using a number of interventions in rats, a tight link between insulin-mediated capillary recruitment and glucose uptake in the same muscle beds has begun to emerge, suggesting that capillary recruitment accounts for approximately half of the insulin-mediated muscle glucose uptake *in vivo*. Such a relationship raises the possibility that any impairment in capillary recruitment may also cause impairment in glucose uptake by muscle.

### **Insulin resistant states**

A number of insulin resistant states have been studied in the rat and insulin-mediated muscle capillary recruitment from 1-MX metabolism has been compared with glucose uptake in each (see review [2] and references therein). These include insulin resistance induced by the acute administration of TNF- $\alpha$  (tumour necrosis factor- $\alpha$ ) for 4 h, where a hyperinsulinaemic euglycaemic clamp was conducted over the last 2 h. In that study, it was noted that insulin-mediated capillary recruitment and limb blood flow (which occurs with this high physiological dose) were completely blocked by TNF- $\alpha$ . Total blockade of insulin's haemodynamic responses was accompanied by a 50% inhibition of muscle glucose uptake and an 18% inhibition of whole body glucose uptake. Another model involved infusion of the serotonergic agonist,  $\alpha$ -methyl serotonin, which from perfused rat hindlimb studies reduced capillary recruitment as a result of its vasoconstriction. In the study *in vivo* [6],  $\alpha$ -methyl

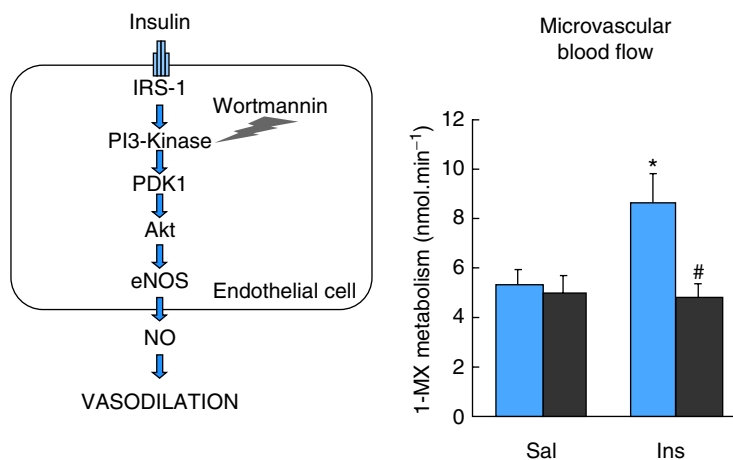
serotonin was infused shortly before and throughout an insulin clamp. Mean arterial blood pressure was increased by 25% and insulin-mediated capillary recruitment as well as limb blood flow were completely inhibited. Muscle glucose uptake was inhibited by 50% and whole body glucose uptake was inhibited by 28%. It is interesting as this study represents the first demonstration of a model of muscle insulin resistance associated with hypertension, where vasoconstriction was very likely the cause of both [6]. Two other models of fatty acid- [7] and glucosamine-induced [8] insulin resistance gave similar outcomes with complete inhibition of capillary recruitment, as measured by  $^{-1}$  MX metabolism across the hindlimb muscle, and loss of approx. 50% of muscle glucose uptake due to insulin. In addition, in the genetically obese insulin-resistant Zucker rat, hyperinsulinaemia was unable to either increase muscle capillary recruitment (by 1-MX metabolism) or glucose uptake in hindlimb muscle [9]. One recent study has compared obese and lean human subjects using ultrasound and microbubbles as the means of assessing capillary recruitment [10]. In this study, physiologic insulin administered systemically failed to increase forearm microvascular perfusion (capillary recruitment), glucose uptake or brachial artery flow in obese subjects when compared with lean controls. In total, impaired microvascular recruitment was in each case associated with a decline in skeletal muscle insulin-mediated glucose disposal and is thus suggestive of insulin resistance. This would be expected if, as discussed by Renkin et al. (references 19 and 20 in [10]), endothelial surface area is an important factor limiting delivery of substrates (and hormones) to muscle tissue.

### **Mechanisms by which capillary perfusion is controlled**

To summarize the position at this stage would be to conclude that muscle metabolism is very much dependent on the extent of microvascular perfusion. At rest, muscle is minimally perfused with vasomotion responsible for periodic redistribution so that hypoxia is avoided. However, as the metabolic demand of the muscle increases, such as in exercise, microvascular perfusion is enhanced commensurate with demand and, although the mechanism for this increased perfusion is still largely unknown, it is clearly controlled by the working myocytes themselves. A signal of myocyte or associated neuromuscular junction origin is undoubtedly responsible as only the actively contracting fibres, but not mechanically moved fibres [11], receive the benefit of increased capillary flow (recruitment). It appears that the initial rapid onset vasodilation ( $< 1$  s) is due to muscarinic receptor activation, but the prolonged vasodilation involves other mechanisms [12]. Although insulin also produces capillary recruitment, the effect is more global with large groups of muscles affected. The question is where does the signal for insulin initiate? It would seem unlikely that capillary recruitment is controlled by a mechanism similar to that used by exercise involving a signal emanating from the initially accessible myocytes that spreads outwards. Rather, the data favours the view

that the signal arises outside the under-perfused myocytes. For example, the time course for insulin action *in vivo* shows that insulin-mediated capillary recruitment precedes activation of muscle Akt or glucose uptake by this tissue [13]. In addition, insulin dose curves conducted *in vivo* show that muscle capillary recruitment is more sensitive than muscle glucose uptake to insulin action [14]. These studies [13,14] would thus suggest that insulin acts externally to the myocytes, probably at the endothelium adjacent to smooth muscle cells constituting the terminal arterioles to achieve a dilatation into capillary units that are not at that time perfused. There is also indirect evidence that constriction of arterioles leading to nearby functional shunts occurs concomitantly.

However, the detailed mechanism by which insulin-mediated capillary recruitment occurs is unresolved. Insulin-mediated capillary recruitment is blocked by systemically infused L-NAME (*N*<sup>ω</sup>-nitro-L-arginine methyl ester), an inhibitor of NOS (nitric oxide synthase) [15]. The insulin-mediated action does appear to involve the phosphatidylinositol 3-kinase branch of the insulin signalling pathway as it is inhibited *in vivo* by wortmannin (Figure 2). This is consistent with the pathway in cultured endothelial cells that insulin uses to stimulate NO production (e.g. see [16] and [17] and references therein). However, NOS mediates numerous responses in a multitude of cell types, and global NOS inhibition *in vivo* is likely to produce complex effects. For example, systemic L-NAME administration causes elevated blood pressure, which



**Figure 2. *In vivo* effects of wortmannin in the rat hindlimb**

Wortmannin, which inhibits PI3 Kinase (phosphatidylinositol 3-kinase) activity in endothelial cells and prevents insulin mediated NO release [33], was administered to rats during either saline or insulin infusion. Muscle capillary recruitment was measured in hindlimbs of untreated (blue bars) or wortmannin treated (black bars) animals. \* Insulin treatment significantly different ( $P < 0.05$ ) from saline treatment; # wortmannin plus insulin treatment significantly different ( $P < 0.05$ ) from insulin treatment. IRS-1, insulin receptor substrate-1; PDK1, phosphoinositol-dependent protein kinase I; Akt, protein kinase B.

may be the result of central effects [18], that increase neural outputs, which are capable of inhibiting capillary recruitment. Also EDHF (endothelial-derived hyperpolarizing factor) factors, such as  $H_2O_2$ , may also be inhibited when nitric oxide synthase (NOS) is inhibited [19]. Methacholine, a NO producer, but also an EDHF producer, augments insulin-mediated capillary recruitment and muscle glucose uptake [20], and it too is inhibited by L-NAME. Furthermore, TEA (tetraethylammonium chloride), an EDHF inhibitor when used at low doses (0.5 mM) blocks insulin-mediated capillary recruitment (unpublished observation). Thus the evidence supporting NO involvement in insulin action is not conclusive even though the data overall appear to favour the notion that capillary perfusion controls muscle metabolism. Finally, there is the possibility that insulin acts to increase low frequency vasomotion, as has been reported using impaled laser Doppler flowmetry probes in human muscle [21]. An increase in vasomotion would have the net effect of extending the interval during which each capillary is perfused and reduce the interval during which blood cells are stationary in each capillary. This would translate to an increase in effective capillary surface area and is consistent with increased 1-MX metabolism and microvascular blood volume measured by contrast enhanced ultrasound. Just how insulin would act to increase low frequency vasomotion is hard to guess, but neural input is likely.

### **Vascular dysfunction and its consequences for muscle metabolism**

It is clear that a unique intimacy exists between skeletal muscle and its vasculature. Factors such as exercise and insulin that control the capillary surface area available for nutrient and hormone delivery, as well as product removal, dictate the extent of metabolism that can be engaged by muscle. However, having said that, it is important to define the critical elements of the vasculature controlling recruitment. Because of the separation of total (limb) blood flow from metabolism by muscle at rest noted by a number of researchers, including ourselves (e.g. see review [2] and references therein), macrovascular dysfunction may prove to have little bearing on the control of muscle metabolism. Thus failure of large vessels to show vasodilatory response to cholinergic vasodilators or insulin may not necessarily have implications for muscle perfusion, metabolism and insulin's action on metabolism. This point becomes eminently visible when the total flow is manipulated by vasodilator infusion (e.g. see review [2] and references therein). Thus the focus moves to the microvasculature (Figure 1), where functional control may depend on a number of factors yet to be identified. Most consider impaired endothelial function of the microvasculature to be the key, where this may contribute to insulin resistance regardless of the presence of diabetes [22]. Thus far, causes of this dysfunction include direct effects of hyperglycaemia on the endothelial cells, indirect effects of growth factors, cytokines or vasoactive

agents produced by other cells, or the impact of components of the metabolic syndrome acting on the endothelial cells [22]. All of the mechanisms assume that insulin's action to increase capillary surface area is NO-dependent where this is a logical extension from findings where large vessel and feed artery dilatation by insulin are clearly NO-dependent (e.g. see review [2] and references therein). However, as discussed above, other factors such as EDHFs and neural input, may also be necessary for microvascular actions of insulin.

### **Hyperglycaemia**

A number of mechanisms have been proposed to explain how hyperglycaemia may interact directly with the endothelial cells and lead to vascular dysfunction. Some, if not all of these, may impact on microvascular control of muscle perfusion. Excessive glucose, even acutely following a meal, may cause a number of metabolic disturbances. Increased flux in the polyol pathway has been attributed to increased accumulation of intracellular glucose leading in turn to sorbitol accumulation, which may cause damage due to osmotic effects and reduced oxygen free radical scavenging due to NADP<sup>+</sup> depletion by aldose reductase. However, the relatively low expression of aldose reductase in endothelial cells may not be sufficient to cause significant sorbitol formation (e.g. see [22] and references therein). Increased intracellular glucose may also cause an increase in glucosamine 6-phosphate via the hexosamine pathway and this may be the mechanism by which glucosamine itself causes the loss of insulin-mediated capillary recruitment [8]. A key issue seems to be the accumulation of *N*-acetylglucosamine which by the addition to serine and threonine residues results in O-linked glycosylation. One such crucial enzyme that can be altered in this way is eNOS where O-linked glycosylation of the Akt phosphorylation site leads to decreased enzyme activity [23]. Another possible impact of hyperglycaemia is the activation of PKC (protein kinase C) via *de novo* synthesis of diacylglycerol from the excess intracellular glucose. The activation of PKC has a number of consequences particularly since activation may not necessarily only occur in the endothelial cells. For example, activation of PKC in the vascular smooth muscle cells nearby can lead to the induction of vascular endothelial growth factor which in turn causes dysregulation of endothelial cell permeability (e.g. see [22] and references therein). Activated endothelial PKC may act to decrease eNOS activity and/or increase the synthesis of endothelin-1. There may also be a PKC-mediated increase in oxidative stress by the regulation of a number of NADPH oxidases [22]. The accumulation of excessive amounts of glucose and glycolytic intermediates within the endothelial cell may also induce the overproduction of superoxide anions by uncoupled mitochondria. Some authors regard this as a key event which alone could be responsible for activation of the aldose reductase and hexosamine pathways, PKC activation, and AGE (advanced glycation end-products) formation by methylglyoxal [22].

Finally hyperglycaemia may induce non-enzymic glycosylation to form AGEs. In endothelial cells methylglyoxal is probably the main AGE formed, but AGEs either in the extracellular matrix, or formed by binding to the AGE receptors can impact on endothelial cell function [24].

### **Cytokines and vasoactive agent effects**

The most prominent of the cytokines that has been reported to have deleterious effects on endothelial NO production is TNF- $\alpha$ . This cytokine when incubated with cultured endothelial cells inhibits insulin signalling by activating an opposing protein kinase system [16,17]. Acute administration of TNF- $\alpha$  into rats *in vivo* completely blocks insulin's haemodynamic effects [25] which very likely involves endothelial NO production in the microvasculature, causing a state of acute insulin resistance resulting in diminished insulin-mediated muscle glucose uptake [25]. Indeed there is some evidence in experimental animals that blockade or lowering of TNF- $\alpha$  ameliorates the insulin resistance [26]. Another important potential antagonist of capillary recruitment is angiotensin II, which impacts on NO bioavailability by increasing vascular NAD(P)H oxidases, superoxide production and NO scavenging [27]. There are also other aspects of endothelial dysfunction that may be independent of NO production. One example is endothelin-1, which is produced predominantly by the endothelium [28], and is elevated in the plasma of diabetes. When over produced, endothelin-1 may cause the redistribution of flow, reducing the nutritive component and increasing functional shunting [29]. Such a scenario could lead to decreased insulin delivery and insulin resistance.

### **Impact of components of the metabolic syndrome**

The endothelium can control the initiation of atherosclerosis and its progression. Contributing issues may include elevated plasma cholesterol, elevated fatty acids and triglycerides, smoking and diabetes. Each of these decrease endothelial NO bioavailability, whether through decreased production or through increased degradation. One mechanism is through free radical attack, in particular via the increased production of ROS (reactive oxygen species). Increased plasma levels of low-density lipoprotein-cholesterol increases the production of ROS as well as decreasing the level of NOS, the enzyme responsible for NO production. Increased production of ROS quenches NO also reducing its concentration. Elevated non-esterified fatty acids are another component of the metabolic syndrome that bears on NO production. Fatty acids may do so by acting to inhibit the insulin signalling process, preventing insulin-mediated activation of Akt and thus the phosphorylation and activation of eNOS [16,17].

### **Comparing insulin- and exercise-mediated capillary recruitment**

In all of the models of insulin resistance studied thus far there appears to be corroborative data from cellular studies to account for the basis by

which the insulin resistance has occurred. In cultured endothelial cells acutely administered TNF- $\alpha$  blocked insulin-mediated NO production (see commentaries [16,17] and references therein). Non-esterified fatty acids may act similarly to TNF- $\alpha$  to cause insulin resistance through loss of the vascular action of insulin and loss of NO production at the endothelium [16,17]. The elevated TNF- $\alpha$  and nonesterified fatty acids in plasma of the obese Zucker rat may account for the impairment of capillary recruitment by insulin in this model. It is important to note, however, that acutely administered TNF- $\alpha$  does not inhibit exercise-mediated capillary recruitment [30] and the insulin resistant obese Zucker rat, which exhibits a complete loss of insulin-mediated capillary recruitment and has elevated plasma levels of TNF- $\alpha$ , responds normally in terms of exercise-mediated capillary recruitment [31]. This suggests that the loss of capillary recruitment, as measured by 1-MX metabolism, in obesity may result from impaired insulin signalling or endothelial vasodilator mechanisms and these defects do not exert significant effects on the response to exercise. Notwithstanding the impaired response to insulin in obesity there are perfusion limitations during exercise *in vivo* that reduce exercise capacity. This is dealt with elsewhere in this series.

## Conclusions

Insulin, by enhancing total blood flow and capillary recruitment in muscle, enhances delivery of itself and glucose for optimal glucose metabolism within the muscle myocyte. The process insulin uses is independent of skeletal muscle metabolism and probably involves the endothelial cell. Muscle contraction, which has similar microvascular actions, uses processes that are dependent on skeletal muscle metabolism and thus differ from insulin. Further elucidation of the mechanisms and interactions between contraction- and insulin-mediated capillary recruitment is important to provide insight into how insulin resistance can be overcome.

## Summary

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- *Total flow reaching the muscle may not necessarily affect the extent of capillary recruitment.*
- *Newly developed methods demonstrate that exercise and insulin each mediate capillary recruitment as part of their actions in vivo.*
- *In all insulin resistant states examined thus far the action of insulin to recruit capillary flow is impaired; exercise induced recruitment seems to be less affected.*
- *Control mechanisms for capillary recruitment are different for contraction and insulin.*

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## References

1. Jarasch, E.D., Bruder, G. & Heid, H.W. (1986) Significance of xanthine oxidase in capillary endothelial cells. *Acta Physiol. Scand. Suppl.* **548**, 39–46
2. Clark, M.G., Wallis, M.G., Barrett, E.J., Vincent, M.A., Richards, S.M., Clerk, L.H. & Rattigan, S. (2003) Blood flow and muscle metabolism: a focus on insulin action. *Am. J. Physiol. Endocrinol. Metab.* **284**, E241–E258
3. Rattigan, S., Clark, M.G. & Barrett, E.J. (1997) Hemodynamic actions of insulin in rat skeletal muscle: evidence for capillary recruitment. *Diabetes* **46**, 1381–1388
4. Coggins, M., Lindner, J., Rattigan, S., Jahn, L., Fasy, E., Kaul, S. & Barrett, E. (2001) Physiologic hyperinsulinemia enhances human skeletal muscle perfusion by capillary recruitment. *Diabetes* **50**, 2682–2690
5. Vincent, M.A., Clerk, L.H., Lindner, J.R., Price, W.J., Jahn, L.A., Leong-Poi, H. & Barrett, E.J. (2006) Mixed meal and light exercise each recruit muscle capillaries in healthy humans. *Am. J. Physiol. Endocrinol. Metab.* **290**, E1191–E1197
6. Rattigan, S., Clark, M.G. & Barrett, E.J. (1999) Acute vasoconstriction-induced insulin resistance in rat muscle *in vivo*. *Diabetes* **48**, 564–569
7. Clerk, L.H., Rattigan, S. & Clark, M.G. (2002) Lipid infusion impairs physiologic insulin-mediated capillary recruitment and muscle glucose uptake *in vivo*. *Diabetes* **51**, 1138–1145
8. Wallis, M.G., Smith, M.E., Kolka, C.M., Zhang, L., Richards, S.M., Rattigan, S. & Clark, M.G. (2005) Acute glucosamine-induced insulin resistance in muscle *in vivo* is associated with impaired capillary recruitment. *Diabetologia* **48**, 2131–2139
9. Wallis, M.G., Wheatley, C.M., Rattigan, S., Barrett, E.J., Clark, A.D. & Clark, M.G. (2002) Insulin-mediated hemodynamic changes are impaired in muscle of Zucker obese rats. *Diabetes* **51**, 3492–3498
10. Clerk, L.H., Vincent, M.A., Jahn, L.A., Liu, Z., Lindner, J.R. & Barrett, E.J. (2006) Obesity blunts insulin-mediated microvascular recruitment in human forearm muscle. *Diabetes* **55**, 1436–1442
11. Sarelius, I.H., Cohen, K.D. & Murrant, C.L. (2000) Role for capillaries in coupling blood flow with metabolism. *Clin. Exp. Pharmacol. Physiol.* **27**, 826–829
12. VanTeeffelen, J.W. & Segal, S.S. (2006) Rapid dilation of arterioles with single contraction of hamster skeletal muscle. *Am. J. Physiol. Heart Circ. Physiol.* **290**, H119–H127
13. Vincent, M.A., Clerk, L.H., Lindner, J.R., Klibanov, A.L., Clark, M.G., Rattigan, S. & Barrett, E.J. (2004) Microvascular recruitment is an early insulin effect that regulates skeletal muscle glucose uptake *in vivo*. *Diabetes* **53**, 1418–1423
14. Zhang, L., Vincent, M.A., Richards, S.M., Clerk, L.H., Rattigan, S., Clark, M.G. & Barrett, E.J. (2004) Insulin sensitivity of muscle capillary recruitment *in vivo*. *Diabetes* **53**, 447–453
15. Vincent, M.A., Barrett, E.J., Lindner, J.R., Clark, M.G. & Rattigan, S. (2003) Inhibiting NOS blocks microvascular recruitment and blunts muscle glucose uptake in response to insulin. *Am. J. Physiol. Endocrinol. Metab.* **285**, E123–E129
16. Kim, F., Tysseling, K.A., Rice, J., Pham, M., Haji, L., Gallis, B.M., Baas, A.S., Paramsothy, P., Giachelli, C.M., Corson, M.A. & Raines, E.W. (2005) Free fatty acid impairment of nitric oxide production in endothelial cells is mediated by IKK $\beta$ . *Arterioscler. Thromb. Vasc. Biol.* **25**, 989–994

17. Kim, J.A., Koh, K.K. & Quon, M.J. (2005) The union of vascular and metabolic actions of insulin in sickness and in health. *Arterioscler. Thromb. Vasc. Biol.* **25**, 889–891
18. Shankar, R., Zhu, J.S., Ladd, B., Henry, D., Shen, H.Q. & Baron, A.D. (1998) Central nervous system nitric oxide synthase activity regulates insulin secretion and insulin action. *J. Clin. Invest.* **102**, 1403–1412
19. Shimokawa, H. & Morikawa, K. (2005) Hydrogen peroxide is an endothelium-derived hyperpolarizing factor in animals and humans. *J. Mol. Cell. Cardiol.* **39**, 725–732
20. Mahajan, H., Richards, S.M., Rattigan, S. & Clark, M.G. (2004) Local methacholine but not bradykinin potentiates insulin-mediated glucose uptake in muscle *in vivo* by augmenting capillary recruitment. *Diabetologia* **47**, 2226–2234
21. De Jongh, R.T., Clark, A.D., Ijzerman, R.G., Serne, E.H., De Vries, G. & Stehouwer, C.D. (2004) Physiological hyperinsulinaemia increases intramuscular microvascular reactive hyperaemia and vasomotion in healthy volunteers. *Diabetologia* **47**, 978–986
22. Schalkwijk, C.G. & Stehouwer, C.D. (2005) Vascular complications in diabetes mellitus: the role of endothelial dysfunction. *Clin. Sci.* **109**, 143–159
23. Musicki, B., Kramer, M.F., Becker, R.E. & Burnett, A.L. (2005) Inactivation of phosphorylated endothelial nitric oxide synthase (Ser-1177) by O-GlcNAc in diabetes-associated erectile dysfunction. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 11870–11875
24. Stern, D.M., Yan, S.D., Yan, S.F. & Schmidt, A.M. (2002) Receptor for advanced glycation end-products (RAGE) and the complications of diabetes. *Ageing Res. Rev.* **1**, 1–15
25. Youd, J.M., Rattigan, S. & Clark, M.G. (2000) Acute impairment of insulin-mediated capillary recruitment and glucose uptake in rat skeletal muscle *in vivo* by TNF- $\alpha$ . *Diabetes* **49**, 1904–1909
26. Borst, S.E. & Bagby, G.J. (2002) Neutralization of tumor necrosis factor reverses age-induced impairment of insulin responsiveness in skeletal muscle of Sprague–Dawley rats. *Metabolism* **51**, 1061–1064
27. Cheng, Z.J., Vapaatalo, H. & Mervaala, E. (2005) Angiotensin II and vascular inflammation. *Med. Sci. Monit.* **11**, RA194–RA205
28. Luscher, T.F. & Barton, M. (2000) Endothelins and endothelin receptor antagonists: therapeutic considerations for a novel class of cardiovascular drugs. *Circulation* **102**, 2434–2440
29. Kolka, C.M., Rattigan, S., Richards, S. & Clark, M.G. (2005) Metabolic and vascular actions of endothelin-1 are inhibited by insulin-mediated vasodilation in perfused rat hindlimb muscle. *Br. J. Pharmacol.* **145**, 992–1000
30. Zhang, L., Wheatley, C.M., Richards, S.M., Barrett, E.J., Clark, M.G. & Rattigan, S. (2003) TNF- $\alpha$  acutely inhibits vascular effects of physiological but not high insulin or contraction. *Am. J. Physiol. Endocrinol. Metab.* **285**, E654–E660
31. Wheatley, C.M., Rattigan, S., Richards, S.M., Barrett, E.J. & Clark, M.G. (2004) Skeletal muscle contraction stimulates capillary recruitment and glucose uptake in insulin-resistant obese Zucker rats. *Am. J. Physiol. Endocrinol. Metab.* **287**, E804–E809
32. Borgstrom, P., Lindbom, L., Arfors, K.E. & Intaglietta, M. (1988)  $\beta$ -adrenergic control of resistance in individual vessels in rabbit tenuissimus muscle. *Am. J. Physiol.* **254**, H631–H635
33. Montagnani, M., Chen, H., Barr, V.A. & Quon, M.J. (2001) Insulin-stimulated activation of eNOS is independent of  $Ca^{2+}$  but requires phosphorylation by Akt at Ser(1179). *J. Biol. Chem.* **276**, 30392–30398