

# Regulation of Muscle Microcirculation in Health and Diabetes

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Insulin increases microvascular perfusion and substrate exchange surface area in muscle, which is pivotal for hormone action and substrate exchange, by activating insulin signaling cascade in the endothelial cells to produce nitric oxide. This action of insulin is closely coupled with its metabolic action and type 2 diabetes is associated with both metabolic and microvascular insulin resistance. Muscle microvascular perfusion/volume can be assessed by 1-methylxanthine metabolism, contrast-enhanced ultrasound and positron emission tomography. In addition to insulin, several factors have been shown to recruit muscle microvasculature, including exercise or muscle contraction, mixed meals, glucagon-like peptide 1 and angiotensin II type 1 receptor (AT<sub>1</sub>R) blocker. On the other hand, factors that cause metabolic insulin resistance, such as inflammatory cytokines, free fatty acids, and selective activation of the AT<sub>1</sub>R, are capable of causing microvascular insulin resistance. Therapies targeting microvascular insulin resistance may help prevent or control diabetes and decrease the associated cardiovascular morbidity and mortality.

**Keywords:** Endothelium; Insulin; Microvasculature; Muscle; Nitric oxide; Vasoconstriction; Vasorelaxation

## INTRODUCTION

Patients with type 2 diabetes are at high risk of getting both macrovascular and microvascular complications. Though the exact underlying mechanisms remain to be elucidated, insulin resistance and endothelial dysfunction have been implicated to play a major role in their pathogenesis. Insulin, in addition to stimulating muscle glucose disposal, also plays important role in the regulation of vascular tone and tissue perfusion. We and others have shown that insulin plays important vasodilatory action on the pre-capillary arterioles in muscle. This leads to increased microvascular perfusion and capillary exchange surface area in muscle and this effect closely couples with insulin's metabolic action [1-3]. As it is in the microvasculature that the exchanges of nutrients, oxygen, and hormones between

the plasma compartment and muscle interstitium take place, insulin resistance in the microvasculature could play important role in the pathogenesis of diabetes and its complications. Here we review the regulation of muscle microcirculation in health and diabetes.

## STRUCTURE AND FUNCTION OF MUSCLE MICROVASCULATURE

Microcirculation encompasses all vessels <150 µm in diameter, including arterioles, capillaries, and venules. Its major function is to regulate muscle perfusion to ensure adequate delivery of nutrients, oxygen and hormones and to provide endothelial exchange surface area between the plasma compartment and muscle interstitium. Microvascular perfusion is determined by

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pre-capillary terminal arterioles. Their openings increase microvascular perfusion and expand endothelial exchange surface area (microvascular recruitment) and their closures lead to the opposite (microvascular decruitment) [2]. In the resting state only ~30% of the capillaries are being perfused [4].

Muscle microvascular perfusion and endothelial exchange surface area play a pivotal role in the regulation of substrate metabolism and tissue function. The rate of substrate extraction  $([V]-[A])=([I]-[A])\times(1-e^{-PS/Q})$ , where V is the venous plasma concentration, I is the interstitial concentration, A is the arterial plasma concentration, P is surface permeability, S is surface area, and Q is the plasma flow rate. Thus, a relatively small increase (decrease) in the microvascular surface area could markedly increase (decrease) substrate extraction, especially in skeletal muscle where the resting blood flow is low (3 to 5 mL/min/100 g).

## MEASUREMENT OF MUSCLE MICROVASCULAR PERFUSION

Insulin causes vasodilation and increases total muscle blood flow [5,6]. However, whether the increase in the bulk muscle blood flow contributes to insulin's metabolic effect in muscle was a subject of controversy. As microvasculature provides the necessary endothelial surface area for substrate exchange in muscle which is pivotal to muscle glucose extraction, several techniques have been developed to estimate microvascular perfusion in muscle.

### Metabolism of 1-methylxanthine

This technique is based on the metabolism (i.e., the extraction across a muscle bed) of 1-methylxanthine (a metabolite of caffeine) which is an exogenous substrate for xanthine oxidase. This enzyme is highly concentrated in the capillary endothelial cells [3,7] and efficiently converts 1-methylxanthine to 1-methyluric acid. Thus the changes in 1-methylxanthine metabolism can be used to estimate microvascular blood flow or volume.

### Contrast-enhanced ultrasound (CEU) imaging

CEU imaging is a noninvasive technology adapted from the myocardial contrast echocardiography technique developed by Wei et al. [8-10]. It uses microbubbles (composed of lipid shell and gas) as the contrast agent to trace the microvascular bed. The microbubbles are smaller than and have a rheology similar to the red blood cells. Thus, they can track the move-

ment of red blood cells within the muscle microvasculature. To measure skeletal muscle microvascular perfusion, intermittent imaging of the muscle of interest is performed using ultra-harmonic imaging, with the ultrasound pulses gated to an internal timer to allow progressively prolonged pulsing intervals to allow more replenishment of the microbubbles in the microcirculation. After a background subtraction to exclude the signals generated from large, rapidly filling vessels, the pulsing interval (time) versus video-intensity curve is generated and fitted to an exponential function:  $y=A(1-e^{-\beta t})$ , where y is the video intensity at a pulsing interval t, A is the plateau video intensity representing microvascular blood volume (MBV), and  $\beta$  is the rate constant reflecting the rate of rise of video intensity (i.e., microvascular flow velocity, MFV). Microvascular blood flow (MBF) is the product of MBV and MFV (i.e.,  $MBF=MBV\times MFV$ ). CEU is unique in that it provides information on three important indices of microvascular flow: MBV which reflects the volume of the microvascular bed, MFV which corresponds to the rate of red blood cells flow through this microvascular bed, and total microvascular blood flow (MBF). This technique has been successfully used in both laboratory animals and humans in combination with insulin clamp to provide dual assessment of insulin actions on both microvasculature and muscle glucose disposal [11-16].

### Positron-emission tomography (PET) imaging

PET can be used to noninvasively measure blood flow in muscle [17,18]. It uses radiowater ( $[^{15}\text{O}]\text{H}_2\text{O}$ ) as tracer and measures blood flow in tissues where there is an exchange of water molecules. It has the capacity to provide three-dimensional insight into capillary level blood flow in muscle [17]. Other than its high cost, the very low tissue uptake of the tracer increases the errors due to the contribution of arterial blood volume, statistical noise and delay of the arterial input function [17]. Nonetheless, as PET can be used to simultaneously determine glucose uptake in muscle *in vivo* by using 18F-deoxyglucose, this modality is valuable in determining the relations between muscle blood flow and glucose use. As radiowater is freely diffusible which makes it difficult to separate signals generated between larger arteries and capillaries, a change of the tracer from radiowater to labeled carbon monoxide ( $\text{C}^{15}\text{O}$ ) might provide better assessment of microvascular blood flow as  $^{15}\text{O}$  labeled carbon monoxide binds rapidly to the heme moiety of hemoglobin.

## REGULATION OF MUSCLE MICROVASCULAR PERFUSION IN HEALTH

Many physiological factors regulate muscle microvascular perfusion *in vivo*, including insulin, exercise, mixed meals, glucagon-like peptide 1 (GLP-1), and angiotensin II type 1 receptor (AT<sub>1</sub>R) blocker.

Under normal physiology, insulin upon binding to and activation of its receptors on endothelial cell membrane actively regulates muscle microvascular perfusion. We and others have repeatedly demonstrated a vasodilatory effect of physiological concentrations of insulin in the muscle microcirculation in both humans and laboratory animals [15,19,20]. Insulin exerts its vaso-regulatory actions mainly through two signaling pathways. It activates the phosphatidylinositol 3-kinase (PI-3 kinase)/protein kinase B (PKB or Akt) pathway which leads to the phosphorylation and activation of the endothelial nitric oxide (NO) synthase (eNOS) and NO production [21]. NO is a potent vasodilator and causes vasodilation of the peripheral resistance arteries and the pre-capillary terminal arterioles, leading to increased total blood flow [22] and muscle microvascular perfusion [11,12,15,19,23]. The effect on microvascular perfusion, or recruitment, occurs rapidly (within 5 to 10 minutes) [12], and is NO-dependent as inhibition of NO production prevents insulin-mediated microvascular recruitment [11,12]. On the other hand, activation of the insulin receptors also phosphorylates and activates the mitogen-activated protein kinase (MAPK) pathway which results in the production of a potent vasoconstrictor endothelin-1 [24,25]. Thus, insulin fine-tunes pre-capillary arteriole tone and muscle perfusion via balancing its signals through these two signaling pathways.

Increased muscle microvascular perfusion not only facilitates nutrient exchanges between the plasma compartment and muscle interstitium, it also increases muscle delivery of insulin. For insulin to exert its metabolic actions it has to be first delivered to tissue interstitium. Insulin has been shown to regulate its own delivery to muscle interstitium by dilating resistance vessels to increase total blood flow, relaxing pre-capillary arterioles to recruit microvasculature and increase exchange surface area (microvascular recruitment), and trans-endothelial transport of insulin from the plasma compartment to interstitium [2]. Insulin-mediated microvascular recruitment clearly precedes insulin-stimulated glucose uptake in skeletal muscle [12] and blockade of insulin's microvascular action with NOS inhibitor decreases insulin-stimulated glucose disposal

by ~40% [11,12]. This is thus far the strongest evidence linking insulin's microvascular action to its metabolic action in muscle.

Muscle contraction is probably the most potent inducer of muscle microvascular recruitment. Even light exercise, such as gentle hand grip (at 25% of maximal strength) [26], or electric stimulation at low frequency that does not increase conduit artery blood flow (0.1-Hz contraction) [27,28], significantly increases muscle microvascular volume. This low frequency muscle contraction also significantly increases muscle uptake of insulin, suggesting that the microvascular response to muscle contraction may in part explain enhanced insulin action in exercising muscle. Though the underlying signalling pathways remain to be studied, NO does not appear to be involved in this process as co-infusion of L-NAME, an NOS inhibitor, does not appear to blunt muscle contraction-induced microvascular recruitment [28].

AT<sub>1</sub>R blockers have been widely used clinically to treat patients with hypertension and numerous clinical trials have confirmed that they are able to decrease cardiovascular morbidity and mortality and new onset of diabetes in patients with various cardiovascular conditions. AT<sub>1</sub>R blockade leads to unopposed action of endogenous angiotensin II on the G-protein-coupled type 2 receptors (AT<sub>2</sub>R) (through G<sub>iα</sub>), resulting in increased phosphotyrosine phosphatase activity, inhibition of the p44/42 MAPK, and vasodilation via the bradykinin-NO-cGMP signaling cascade. AT<sub>2</sub>R antagonist PD123319, bradykinin-B<sub>2</sub> receptor antagonist icatibant, and NOS inhibition all independently blocks AT<sub>2</sub>R-mediated vasodilatory action [29]. Strong *in vivo* evidence has confirmed the vasodilatory role of AT<sub>2</sub>R in both resistance microvessels as well as in large capacitance vessels including rat uterine artery, mesenteric arterial segments, coronary arterioles, and thoracic aorta [29,30]. We have recently shown that AT<sub>1</sub>R blockade with losartan potently recruits muscle microvasculature and increases muscle use of glucose while specific antagonism of the AT<sub>2</sub>R with PD123319 decreases basal microvascular blood volume by ~80% which is associated with significantly decreased muscle glucose extraction and decreased muscle insulin delivery and action [14,31]. Thus, selective AT<sub>1</sub>R blockade may improve insulin sensitivity via muscle microvascular recruitment in addition to its inhibitory effect on AT<sub>1</sub>R-mediated oxidative stress.

Mixed meal is another potent muscle microvascular recruitment inducer [15,26,32]. It appears that its effect is stronger than insulin alone as raising plasma insulin concentrations to levels that are similar to those achieved with mixed meal in-

gestion by systemic insulin infusion recruits less microvasculature than mixed meals [15]. This is not surprising as mixed meals induce more neural and hormonal changes *in vivo* in addition to inducing insulin secretion. One of the major changes is the secretion of GLP-1 which causes glucose-dependent insulin secretion. However, ample evidence has confirmed that GLP-1 has many extra-pancreatic effects including a vasodilatory action on the vasculature. It has also been shown to stimulate glucose uptake in both cardiac muscle and skeletal muscle independent of insulin. We have recently shown that GLP-1 potently recruits muscle microvasculature in rats which is associated with increased muscle insulin delivery and action. These effects are NO-dependent as inhibition of NO production completely abolishes GLP-1-induced microvascular recruitment and insulin delivery in muscle [33].

## MICROVASCULAR INSULIN RESISTANCE IN DIABETES AND OBESITY

Insulin resistance is present in skeletal muscle microcirculation in patients with or animal models of obesity and/or diabetes. Basal muscle microvascular volume is decreased in obese Zucker rats (an animal model of metabolic syndrome) and Zucker diabetic fatty rats (an animal model of type 2 diabetes), which is associated with impaired insulin-mediated glucose disposal and microvascular recruitment [34,35]. In humans, moderate insulin resistance as seen in people with simple obesity blunts insulin- or mixed meal-induced muscle microvascular recruitment [20,32]. It appears that insulin resistance only selectively occurs in the PI3-kinase/Akt/eNOS pathway and insulin signaling through the MAPK pathway remains intact or even enhanced [21,36,37], leading to decreased NO availability and same or enhanced endothelin-1 production, tilting the vasomotion balance between endothelin-1 and NO production and resulting in vasoconstriction [25,38]. This may contribute to the pathogenesis of hypertension and organ dysfunction in patients with diabetes and insulin resistance.

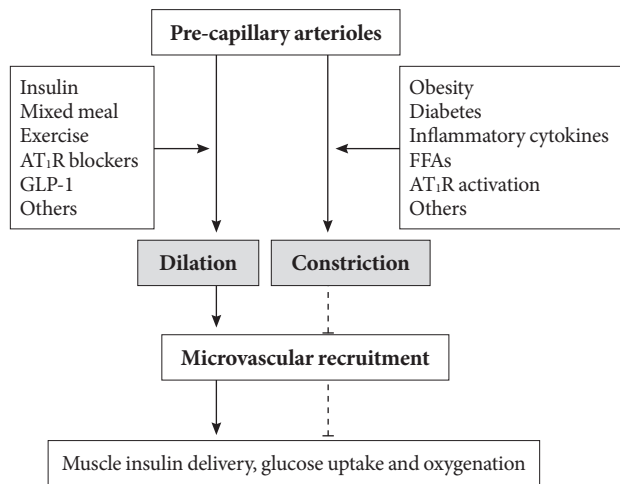
Though the mechanisms underlying muscle microvascular insulin resistance remain to be elucidated, ample evidence has confirmed the critical roles of inflammatory cytokines, free fatty acids (FFAs) and the activation of vascular renin-angiotensin system (RAS) in its pathogenesis. Each of them has been shown to blunt insulin-mediated microvascular recruitment in muscle. This is not surprising as each of them causes oxidative stress, inflammation, and insulin resistance. However, un-

derlying pathways vary.

Diabetes and obesity are associated with a chronic elevation of inflammatory cytokines in tissue and in plasma. Among them tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) has been extensively studied and plays critical role in the pathogenesis of insulin resistance in skeletal muscle, adipose tissue, liver and vasculature. It also causes microvascular insulin resistance, as evidenced by its inhibitory effect on insulin-induced microvascular recruitment and glucose disposal in rats [39], possibly via p-38 MAPK-mediated insulin resistance through the PI3-kinase pathway [40].

Diabetes is associated with an elevation of plasma FFAs which blunts insulin-mediated vasodilation and NO production in humans, and insulin- or mixed meal-induced muscle microvascular recruitment in rats and humans [15,23,41,42]. It appears that FFAs contribute to microvascular insulin resistance via the NF- $\kappa$ B pathway as pre-treatment with salsalate, an anti-inflammatory agent that inhibits NF- $\kappa$ B, markedly improves insulin's microvascular action in muscle during systemic lipid infusion [16]. This observation is consistent with *in vitro* experiments using cultured endothelial cells demonstrating that palmitate inhibits insulin-mediated phosphorylation of eNOS and NO production via increasing IKK $\beta$  activity [36, 43].

Diabetes up-regulates the activity of RAS in the cardiovascular system [44-46]. Angiotensin II, a terminal product of RAS, acts on the AT<sub>1</sub>R which cross-talks with the insulin signaling cascades to cause insulin resistance, including an antagonistic effect on insulin-mediated vasodilation, likely via AT<sub>1</sub>R-mediated NAD(P)H oxidase activation and increased production of superoxide and reduced NO bioavailability [44, 47-50]. The AT<sub>1</sub>R mediates the vast majority of the cardiovascular, renal, and adrenal actions of angiotensin II, resulting in arterial vasoconstriction, aldosterone secretion, and renal sodium reabsorption. We have recently reported that the AT<sub>1</sub>R also exerts potent tonic effect on muscle microvasculature. Systemic administration of AT<sub>1</sub>R blocker losartan acutely recruits muscle microvasculature and increases muscle glucose extraction, suggesting a tonic restriction of the AT<sub>1</sub>R activity on muscle microvascular perfusion and substrate delivery [31]. On the other hand, selective activation of the AT<sub>1</sub>R (in the presence of specific AT<sub>2</sub>R blockade) by endogenous angiotensin II drastically reduces muscle microvascular perfusion and insulin delivery, resulting in reduced insulin-stimulated muscle glucose disposal [14,31]. Whether this effect contributes to



**Fig. 1.** Schematic diagram illustrating the regulation of microvasculature in muscle. AT<sub>1</sub>R, angiotensin II type 1 receptor; GLP-1, glucagon-like peptide 1; FFAs, free fatty acids.

decreased cardiovascular morbidity and mortality and new onset of diabetes in patients on chronic treatment of AT<sub>1</sub>R blockers remain to be examined. Treatment of insulin resistant Zucker diabetic fatty rats for 15 weeks with an angiotensin-converting enzyme (ACE) inhibitor quinapril did restore insulin's microvascular action [34].

## CONCLUSION

Muscle microvasculature plays a pivotal role in the regulation of nutrient metabolism by providing the necessary exchange surface area between the plasma compartment and muscle interstitium (Fig. 1). Insulin causes vasodilation in the insulin sensitive state to recruit muscle microvasculature. Several other factors, including exercise, mixed meal, GLP-1, and AT<sub>1</sub>R blockers, also recruit muscle microvasculature which may contribute to increased insulin delivery to and action in muscle. Microvascular insulin resistance and dysfunction are closely related with metabolic insulin resistance in diabetes. Major factors that have been shown to cause metabolic insulin resistance, including inflammatory cytokines, FFAs, and AT<sub>1</sub>R activation, have all been shown to cause microvascular insulin resistance. Thus, muscle microvasculature could be a therapeutic target for diabetes prevention and control.

## CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was re-

ported.

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