Muscle microvasculature’s structural and functional specializations facilitate muscle metabolism

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Kusters YH, Barrett EJ. Muscle microvasculature’s structural and functional specializations facilitate muscle metabolism. Am J Physiol Endocrinol Metab 310: E379–E387, 2016. First published December 29, 2015; doi:10.1152/ajpendo.00443.2015.—We review the evolving findings from studies that examine the relationship between the structural and functional properties of skeletal muscle’s vasculature and muscle metabolism. Unique aspects of the organization of the muscle microvasculature are highlighted. We discuss the role of vasomotion at the microscopic level and of flowmotion at the tissue level as modulators of perfusion distribution in muscle. We then consider in some detail how insulin and exercise each modulate muscle perfusion at both the microvascular and whole tissue level. The central role of the vascular endothelial cell in modulating both perfusion and transendothelial insulin and nutrient transport is also reviewed. The relationship between muscle metabolic insulin resistance and the vascular action of insulin in muscle continues to indicate an important role for the microvasculature as a target for insulin action and that impairing insulin’s microvascular action significantly affects body glucose metabolism.

Muscle microvasculature; vasomotion; flowmotion; endothelium; insulin resistance

Muscle microvasculature is the final interface through which circulating nutrients, hormones, gases, and electrolytes must pass in journeying to and from the systemic circulation. It has evolved multiple structural and functional adaptations to flexibly and efficiently fulfill its role in optimizing muscle function. Here, we examine how the minute-to-minute metabolic activity of skeletal muscle is coupled to the function of muscle’s vasculature. We will discuss how bulk blood flow and flow distribution can regulate nutrient delivery to muscle microvasculature as well as how transendothelial transport processes can modulate the exchange of hormones and metabolites between plasma and the myocytes. We focus on acute regulatory relationships and defer discussion of chronic vascular or myocyte adaptation to environmental, nutritional, and most pathological processes.

Muscle blood flow has been measured in numerous classical limb balance and metabolic tracer studies of the acute effects of fasting, feeding, exercise, and hormonal manipulation on muscle nutrient exchange. These methods, however, treat muscle’s vasculature as a “black box.” Here, we consider muscle microvasculature’s specialized architecture and how that architecture might impact nutrient fluxes into and out of the muscle. We will discuss the role of vasomotion and flowmotion in the regulation of microvascular perfusion. We recognize that factors acting on the endothelial cell (EC), the vascular smooth muscle cell (SMC), or pericytes may affect muscle perfusion.

We will consider in more detail the effect insulin and exercise have on the endothelium and directly or indirectly on the vascular SMC to influence nutrient delivery. Beyond muscle perfusion, we will consider the transit/transport of fatty acids, glucose, and insulin across the vascular endothelium of muscle, as these processes offer another pathway whereby the vasculature may regulate myocyte metabolic function. In aggregate, this review will delineate how vascular regulation of blood flow, flow distribution, and endothelial function coordinate to optimally serve myocyte function.

Skeletal Muscle Architecture

Pioneering work by Spalteholz (105) and Krogh (63, 64) has demonstrated muscle’s highly organized vasculature. Arterioles branch off primary arteries, down to terminal arterioles, oriented perpendicular to muscle fibers, and supplying them at regular intervals (~1 mm). Each terminal arteriole supplies 15–20 capillaries running parallel to fibers, with many anastomoses, to form a rich network around muscle fibers. Each terminal arteriole with its capillaries forms the smallest unit of control for capillary perfusion (referred to as a microvascular unit) (34). Venules are arrayed as arterioles and found between two terminal arterioles. Lymph vessels originate within muscle and can ascend with arterioles or venules (see Fig. 1) (48, 103).

The proximity between muscle fibers and capillaries is underscored by recent findings showing capillaries embedded in grooves indenting the sarcolemma and expanding the contact area between myocytes and ECs. Moreover, muscle fiber mitochondria appear to cluster along these grooves, thereby reducing the distance oxygen and fatty acids need to diffuse.
Muscle Microvasculature and Metabolism

Nutrient supply to muscle in response to exercise or insulin will consider the relationship between muscle blood flow and supplemented with metabolite tracer measurements. Here, we humans using the limb balance method, which is at times periods of deprivation. The regulation of fuel fluxes in and out of muscle has been studied intensely in vivo in animals and storage during feeding and a major supplier of nutrients during muscle. Muscle is a major site of carbohydrate, protein, and fat triglycerides, and amino acids to and removes metabolites and Insulin.

Muscle Perfusion and Nutrient Flux With Exercise and Insulin

Perfusion delivers oxygen, free fatty acids (FFA), glucose, triglycerides, and amino acids to and removes metabolites (amino acids, lactate, carbon dioxide, and ammonia) from muscle. Muscle is a major site of carbohydrate, protein, and fat storage during feeding and a major supplier of nutrients during periods of deprivation. The regulation of fuel fluxes in and out of muscle has been studied intensely in vivo in animals and humans using the limb balance method, which is at times supplemented with metabolite tracer measurements. Here, we will consider the relationship between muscle blood flow and nutrient supply to muscle in response to exercise or insulin stimulation, which has been studied intensively. In particular, we will emphasize data from in vivo studies in humans. We will consider both intense and very modest stimulation to provide the reader with a sense of the hierarchical relationship between muscle perfusion and metabolic function.

Total limb flow can be assessed in different ways (e.g., plethysmography, dye or thermal dilution, and Doppler ultrasound). The advantages and limitations of these methods have been reviewed elsewhere (12). Although flow measurement methods have been compared with one another, none can be considered to be the gold standard to measure skeletal muscle blood flow (12, 57, 113). Of note is that each measures blood flow directed to all tissues of the limb, not just muscle. In overnight-fasted humans, resting limb blood flow averages ~2–5 ml·min⁻¹·100 ml⁻¹ of limb (84, 91, 94) in both men and women (52), using the arm or leg (33, 44, 111). Because resting skin and subcutaneous adipose blood flow (on a per 100 ml of tissue basis) are similar to that of muscle (15, 47, 71), these resting limb flow values are assumed to reflect average blood flow to muscle, i.e., the methods treat the limb as a homogenous tissue represented by muscle.

Vasomotion, the rhythmic vessel diameter oscillations first described by Jones (54) in the bat wing in 1852, is more prevalent in small arteries and arterioles than in conduit arteries (1). These oscillations modify blood flow and are one contribution to periodic flow fluctuations known as flowmotion (1, 98). In addition to vasomotion, heart rate, respiration, and autonomic factors influence flowmotion. The several components of flowmotion are distinguished by their frequencies; heart rate and respiratory contributions arise at ~1 and ~0.3 Hz, respectively, whereas low-frequency signals associated with vasomotion arise at ~0.1 Hz for myogenic activity, ~0.04 Hz for neurogenic activity, and ~0.01 Hz for endothelial activity (17, 67, 107). Vasomotion originates from an oscillator component in the SMCs and synchronizing mechanisms, which are modified by ECs (1). Upon increased metabolic needs, signals originating from the microvasculature travel upstream to larger arterioles and arteries, possibly via gap junctions in the SMCs (6), thereby influencing vessel diameter. In addition, vasomotor

Fig. 1. Schematic representation of muscle’s vascular architecture. Left: arterioles branch off primary arteries down to terminal arterioles; these are oriented perpendicular to muscle fibers. Top right: 2 muscle fibers and their microvascular units, with terminal arterioles (red) feeding multiple capillaries (red to blue) as the smallest unit of control and lymph vessels (green) ascending along arterioles and venules (blue). In resting muscle (top fiber), the microvasculature is intermittingly and not equally perfused. Insulin and exercise can each stimulate muscle (bottom fiber) and induce vasodilation at the level of the terminal arterioles and enhance perfusion. Bottom right: magnification of a capillary embedded in a sarcolemma “channel,” thereby expanding the contact area between myocytes and endothelial cells. Myocyte mitochondria cluster along the embedded capillary, thereby reducing the distance oxygen and nutrients need to diffuse. Illustration provided by MediCorporate bv/D & L Graphics (www.dlgraphics.nl).
responses in arterioles can be triggered by stimulation of individual capillaries, and changes in membrane potential appear to be responsible for these observations (6, 14, 104). Moreover, NO can regulate EC-to-SMC signaling at myoendothelial junctions, and reduced NO production attenuates the duration of conducted vasodilation (6, 29). These findings suggest that muscle microcirculation modulates upstream vasodilation and vasomotion.

In vivo, vasomotion may regulate microvascular flow distribution to optimize delivery of nutrients and regulate local hydraulic resistance (88). In addition to regulating blood flow distribution, vasomotion of terminal arterioles also affects lymph flow, thus influencing water transport in muscle interstitium and tissue homeostasis (103). Vasomotion, by continuously altering flow delivery to vessels over time, increases the number of ECs exposed to plasma for nutrient or hormone exchange (46) without total flow or cardiac output being changed. When perfusion demand exceeds flow motion’s compensatory capacity, blood flow control shifts upstream, enabling increases in total flow (1, 100).

Studying muscle vasomotion requires invasive direct blood vessel visualization (e.g., intravital microscopy) (13, 25, 40, 49), and it is affected by anesthetic agents and a host of experimental variables (25). Its invasive nature limits the study of vasomotion in humans. By contrast, cutaneous flow motion can be studied using laser-Doppler flowmetry (LDF) and has become popular in clinical studies (56, 81, 87, 90). To quantify flow motion, Fourier or wavelet analyses methods are used to determine the relative contribution of each frequency to the observed LDF signal (17, 25, 87). A drawback of LDF measurements is that they are affected by nearby vessels and blood pressure fluctuations, and therefore, they may not necessarily reflect only the rhythmic activities of blood vessels themselves (1, 35, 41). Several disorders influence vasomotion and flow motion; hemorrhage increases vasomotion, whereas obesity and diabetes reduce flow motion and vasomotion (1, 87). Taken together, vasomotion provides a local mechanism for vascular adaptation to altered metabolic needs, and its regulation impacts muscle perfusion.

**Exercise and Muscle Perfusion**

Myocyte and related whole body metabolic changes with acute and chronic exercise were reviewed recently (30). Oxygen and nutrient delivery to muscle during intense exercise and the accompanying blood flow changes have been studied extensively. The increased bulk blood flow during exercise arises both from local factors [e.g., potassium, adenosine, phosphate, lactate, hydrogen ion, nitric oxide (NO), and prostaglandins] released by the muscle and by neurovascular changes that relax muscle vasculature. With very intense whole body exercise, blood flow and oxygen delivery to the tissue become limiting secondary to limitations of respiratory and cardiac function (3, 83). By contrast, with intense exercise of isolated muscle groups, neither cardiorespiratory function nor hemodynamic factors limit muscle performance. Thus, blood flow to the human quadriceps can increase >50-fold during brief, intense exercise (3). Increasing the arterial oxygen content does not further increase quadriceps O2 extraction or peak work rate by either trained or untrained muscle (85). Muscle’s remarkable flow reserve can meet even extreme muscle O2 demands. In addition to oxygen, glucose and fatty acids are important fuels during exercise. Carbohydrates (both circulating glucose and muscle glycogen) are the preferred fuel for intense, brief-duration muscle exercise. With intense muscle exercise, glucose extraction can increase more than sixfold and leg blood flow 10-fold, whereas plasma glucose changes little (59). Interestingly, whereas glucose concentrations in resting muscle cytosol are extremely low, intense exercise dramatically increases glucose both in muscle interstitium (76) and within muscle cells in humans (59) and rodents (43). The >30-fold increase in leg glucose uptake seen with intense exercise indicates that both the delivery of glucose to muscle microvasculature and transcapillary glucose transfer have capacity sufficient to meet glucose metabolic needs.

Vascular responses to light exercise (operationally, we consider ≤25% max as “light”) have been studied much less, and their link to muscle performance is less clear. However, studies of light exercise provide insight into the hierarchical regulation of muscle perfusion. With light exercise, vasomotion within the terminal arterioles appears to cease, and the number of perfused capillary increases without significant changes in total muscle blood flow (45, 46, 51). Honig et al. (46), by flash-freezing and sectioning muscle, showed that low-frequency electrical stimulation was nearly as effective as near-tetanic stimulation for recruiting hypoperfused microvasculature in canine gracilis muscle. Further increasing contractile activity increases total blood flow, as regulation shifts upstream to progressively larger arterioles (100). Factors driving microvascular perfusion changes include venous Po2, which, by enhancing venular EC NO production and erythrocyte NO release (95, 100, 106, 112), promotes dilation of nearby arterioles. Hemodynamic forces, i.e., shear stress-induced NO production, further modulate hyperemic responses (100). These are aided by prostaglandins and endothelium-derived hyperpolarizing factors to increase perfusion with exercise (83). It appears that inhibition of one system (e.g., NO synthase) can be compensated by the remaining factors, since skeletal muscle blood flow is preserved in such circumstances (18). If, however, two pathways are inhibited simultaneously, the increase in skeletal muscle blood flow is reduced (82, 83). These effectors act as sympatholytics to counteract the exercise-induced vasoconstriction of distal arterioles and feed arteries by sympathetic nerves (83).

**Insulin and Muscle Perfusion**

The effects of insulin on limb metabolism have been studied extensively using limb balance methods combined with euglycemic insulin infusion. Andres et al. (4) first reported insulin-stimulated forearm glucose uptake and noted an accompanying increase in forearm blood flow. Baron (7) and Laakso et al. (70) first reported that insulin-induced muscle glucose uptake paralleled insulin’s ability to increase total leg blood flow. In subsequent work, obesity, type 1 and type 2 diabetes, and increased plasma free fatty acid concentrations (9, 69, 70, 109) each impaired insulin-stimulated limb blood flow and glucose uptake in parallel. These studies suggested that 1) insulin stimulates limb blood flow in both humans and animals; 2) during a high-dose insulin clamp, increased limb flow correlates with muscle glucose uptake; 3) acute and chronic metabolic insulin resistance is associated with im-
paired insulin-stimulated increases of limb blood flow; and 4) insulin enhances limb blood flow by increasing nitric oxide production (70, 78, 108, 109). This led investigators to suggest that impaired arteriolar relaxation in response to insulin contributes significantly to metabolic dysfunction in insulin-resistant muscle.

Insulin’s vasodilatory effects arise from its binding to the insulin receptor on ECs. This stimulates the phosphatidylinositol 3-kinase/Akt pathway to activate endothelial NO synthase (eNOS) (53, 124) and induces NO-mediated vasodilation (20). Insulin also stimulates the MAPK pathway and enhances production of the vasoconstrictor endothelin-1 (78, 86, 108). In health, insulin’s vasodilatory effect dominates. With endothelial dysfunction, as occurs with obesity and type 2 diabetes, the PI3K pathway appears to be selectively inhibited by low-grade inflammation, FFA, oxidative stress, and reduced perivascular adiponectin release (55, 61, 75, 79, 123). In these circumstances, a reduced vasodilatory response or even paradoxical vasoconstriction can be seen with hyperinsulinemia (23, 55).

Insulin at high physiological concentrations relaxes resistance arterioles and increases limb blood flow in humans. In addition, there is convincing evidence that this response is blunted in insulin-resistant individuals with obesity (68) and type 1 (8) or type 2 diabetes (10). This effect of insulin on blood flow is most evident when insulin is infused continuously at a rate of 3 mU·kg⁻¹·min⁻¹ to achieve marked steady-state hyperinsulinemia for ≥2 h. It has been more difficult to reproduce this effect infusing insulin at a lower rate or for shorter duration (121). This has led to questioning of the physiological importance of this response. In addition, in insulin-resistant subjects, pharmacologically increasing blood flow often does not improve insulin’s metabolic action measured as limb glucose uptake (89). However, in the latter circumstance, it remains likely that while perfusion is improved insulin resistance in the myocyte persists, and short-term correction of perfusion is insufficient to overcome that metabolic defect.

Over the last 15 years, research on insulin’s action on muscle perfusion has added focus on the microvasculature. There, analogous to the effect of low-frequency stimulation reported by Honig et al. (45, 46), relaxation of terminal arterioles could recruit hypoperfused capillaries and expand the EC surface available for nutrient exchange (11) without changing total flow. However, there were no noninvasive methods available to quantify the microvascular volume perfused in intact muscle in animals or humans. In 1997, a technique was introduced to estimate the endothelial surface available for nutrient exchange based on the single-pass conversion of 1-methylxanthine (1-MX) to 1-methylurate (1-MU) by xanthine oxidase, which is present on capillary endothelium. Increased endothelial exposure to substrate (i.e., capillary recruitment) would increase 1-MX to 1-MU conversion (92, 93). Later, contrast-enhanced ultrasound (CEUS) was used to investigate microvascular perfused volume regulation (24, 28). Using either method, we found that, from the start of an euglycemic insulin clamp, microvascular blood volume increases within 15–30 min, and this microvascular change precedes and correlates with the rise in forearm glucose uptake (31, 32, 116). Moreover, chronic (obesity) and acutely induced (lipid infusion) insulin resistance are associated with an impaired microvascular response to insulin or meal ingestion (23, 50, 60, 74). Using CEUS (51, 115), we also confirmed the original finding by Honig et al. (45) that light exercise increased microvascular perfused volume ~3-fold with minimal increases in total limb blood flow (see Fig. 2). However, with more intense exercise, both microvascular blood volume and total limb blood flow increase (51, 101, 115). Interestingly, cardiac muscle behaves similarly to skeletal muscle during hyperinsulinemia (73) and feeding (99), and its increases in microvascular blood volume are blunted by insulin resistance as well (72).

Insulin is not the only hormone that can “recruit” muscle microvasculature. Both glucagon-like peptide-1 (GLP-1) (21, 110) and adiponectin (125), like insulin, increase microvascular volume, albeit each acting by different biochemical pathways. By contrast, epinephrine can increase muscle blood flow without increasing perfused microvascular volume (22). The responses to physiological hyperinsulinemia and low-intensity exercise suggest a hierarchical response by muscle vasculature whereby microvascular units respond to these stimuli, whereas with more intense stimulation (high physiological insulin concentrations or heavy exercise) more proximal arterial elements also respond, leading to the flow increases seen in those settings. Whether this graded response involves antidiromic signals through the vascular network or again is a response to multiple locally generated metabolic signals is not known.

![Fig. 2. Top: contrast-enhanced ultrasound images of rat thigh muscle as a function of time after a high-energy ultrasound pulse with the muscle at rest or given a 1-Hz electrical stimulation. Bottom: greater plateau video intensity (red) corresponding to a larger number of microbubbles in the imaged volume of the stimulated muscle.](http://ajpendo.physiology.org/doi/10.1152/ajpendo.00443.2015)
Nutrient and Hormone Transfer Across the Endothelium

Here we very briefly review evolving evidence that the vascular endothelium in tissues like muscle and adipose may actively mediate the transendothelial transport (TET) of substrates (e.g., glucose or FFA) and hormones, including insulin. As would be expected, such a role for the endothelium is of consequence principally in tissues with a continuous endothelium, like muscle and adipose, but less consequential in liver and other splanchnic tissues (2).

We consider first the endothelium’s role in FFA transport, as FFAs are a major muscle fuel. It has long been known that lipoprotein lipase (LPL) is secreted by skeletal myocytes and adipocytes but resides on the luminal surface of the vascular EC in muscle and fat, where it cleaves fatty acid monomers from triglycerides bound to circulating lipoproteins (97). This process can be regulated by insulin, which increases LPL expression. Recently, it was found that the EC possesses a receptor for LPL [glycosylphosphatidylinositol-anchored high-density lipoprotein binding protein 1 (GPIHBP1)] (26) and that when LPL binds to GPIHBP1 a vesicular transport system mediates LPL’s TET to the luminal membrane, where it encounters circulating triglycerides (27). Mutations in either GPIHBP1 or LPL that interfere with this pathway can cause severe hypertriglyceridemia in humans (122).

In addition to this pathway for LPL transport that provides triglyceride-derived fatty acids to the EC, the EC possesses membrane-associated fatty acid transport proteins (FATPs) as well as fatty acid-binding proteins (FABPs). The former take up FFA derived either from triglyceride hydrolysis or circulating as FFA associated with albumin or other plasma proteins. FATP3 and FATP4 are expressed by ECs along with the scavenger receptor CD36. Each appears to be involved in movement of fatty acids across the EC luminal plasma membrane. Expression of both FATP3 and FATP4 is increased by VEGF secreted by myocytes and adipocytes acting via VEGFR1. Muscle expression of VEGFβ is increased by the transcriptional coactivator PGC-1α, which is a central player in the regulation of mitochondrial oxidative metabolism within the myocyte. In the context of increasing muscle oxidative capacity, muscle signaling to adjacent ECs to enhance FFA uptake may be part of an adaptive response. Expression of CD36 by ECs is upregulated by peroxisome proliferator-activated receptor-γ, which enhances fat mass and adipogenesis. Interestingly, inhibition of VEGFβ in diabetic animal models decreases muscle ectopic fat and improves glucose metabolism (42). By contrast, VEGFA knockout in muscle increases susceptibility to high-fat diet induced insulin resistance, which is due at least in part to decreased microvascular perfusion (16). The transport of FFA across the ECs is presumably bidirectional, particularly in adipose tissue, although this has not been clarified in detail.

In contrast to the complex regulatory system involved in the movement of FFA across the EC, glucose transport from the vascular lumen into and across the endothelium is mediated by the constitutive activity of the GLUT1 transporter. The system has a high capacity in the absence of exogenous insulin, as indicated by the marked increases in glucose transport that occur with exercise (see above). The GLUT1 transporter is expressed in both vascular smooth muscle and endothelial cells. In the former, the transporter activity is downregulated by high ambient glucose concentrations (58). This adaptive response is absent in aortic ECs. The function of this transporter is important to the metabolic activity of the EC, which receives a significant fraction of its energy from glycolytic ATP production (77). The specific transport capacity in brain microvascular ECs has been studied extensively, as ECs perform a critical function in assuring adequate glucose delivery to the central nervous system. Cardiac muscle microvascular glucose transport capacity has been investigated and utilizes the same high-capacity GLUT1 transporter reported for other endothelia (77). To our knowledge, GLUT1 transporter function specific to skeletal muscle microvasculature has not been studied. As noted previously, the observation that muscle interstitial and myocyte glucose concentrations rise during intense exercise suggests that glucose transport through the vascular endothelium is not limiting even under conditions of high substrate demand. Indeed, the high capacity of the system may in part be responsible for damage that occurs to the microvascular endothelium as a result of hyperglycemia in the diabetic state (19). The EC does not appear to defend itself from high levels of intracellular glucose, which via several pathways can generate reactive oxygen species (19, 37). When this is unchecked, as occurs in persistent hyperglycemic states, vascular injury occurs.

The EC in tissues with a continuous endothelium also constitutes a barrier to insulin movement into the tissue. Indeed, estimates of muscle interstitial insulin concentrations using either lymphatic sampling (120) or microdialysis (102) suggest that insulin TET could be rate limiting for insulin’s action on muscle. This led us to examine the cellular pathway of insulin TET. Most ECs express insulin, IGF-I, and the insulin/IGF-I hybrid receptor (62). In aortic ECs and adipocytes (36), the insulin receptor associates with a specialized lipid raft domain, i.e., caveolae. Caveolae can be internalized and mediate TET of a variety of proteins (e.g., albumin in pulmonary microvascular ECs) (80). In a series of experiments, we found that caveolae are required for insulin uptake by and transport across aortic ECs (118). In these ECs, preserved insulin signaling through phosphatidylinositol 3-kinase to Akt and eNOS is necessary for insulin TET (119). Interestingly, inflammatory cytokines or several days of high-fat diet diminish EC insulin transport (119). If muscle microvascular ECs behave similarly, then impaired EC insulin signaling and TET could contribute to the impaired muscle insulin action of insulin resistance. As we had seen for microvascular recruitment, for insulin TET, NO appears to be a critical regulatory factor. When phosphatidylinositol 3-kinase or NO synthase is inhibited, insulin transport declines. Giving sodium nitroprusside (3–30 μM) can restore insulin uptake (117) in aortic ECs. Surprisingly, an EC-specific insulin receptor knockout mouse (with Cre-recombinase driven by Tie-2) did not display any metabolic phenotype (114). However, more recently, another EC-specific insulin receptor knockout (with Cre-recombinase driven by a vascular endothelial-cadherin promoter) displayed glucose intolerance and delayed insulin action on insulin target tissues (Konishi M, Sakaguchi M, Cai W, Rask-Madsen C, and Kahn CR, unpublished observations). Likewise, the EC-specific IRS-2 knockout mouse is glucose intolerant and has impaired insulin-induced microvascular perfusion and diminished muscle insulin delivery (66). These genetic models...
underscore the role of EC insulin signaling to whole body metabolic functioning.

Recent work using adipose microvascular ECs has suggested a similar vesicle-mediated insulin TET process, and initial studies implicate clathrin-coated vesicles, not caveolae (5). However, there is a lack of data clarifying whether insulin regulates that transport or whether a transendothelial insulin concentration gradient is present in adipose tissue and whether insulin TET limits insulin action in adipose tissue (96).

In summary, a hierarchical, graded control of muscle perfusion arises from cooperative interactions among the factors released locally by myocytes, by SMCs, or by ECs along with systemically and neurally delivered signals. Very modest contractile activity or physiological hyperinsulinemia promptly increases microvascular perfusion volume even in the absence of changes in limb blood flow. In both cases, EC exchange surface for nutrient or hormone delivery expands without requiring increased cardiac work. Greater contractile activity or hyperinsulinemia triggers signals that may ascend the vascular network and provoke relaxation of progressively larger vessels, increasing total flow to the expanded microvascular network. Beyond insulin and exercise, other humoral factors (e.g., GLP-1, angiotensin II, and adiponectin) similarly enhance muscle microvascular perfusion. However, the role of these factors in normal muscle physiology is still being unravelled. Evolving evidence indicates that (at least for insulin and triglycerides/FFA), beyond altered perfusion, the microvasculature, particularly the EC, actively regulates the delivery of insulin and FFA to the muscle via regulation of specialized transport mechanisms. Thus, the EC of muscle vasculature is increasingly recognized as a key regulator of normal muscle physiology and metabolic health. Much remains to be learned regarding the normal regulation of the integrated functioning of the skeletal muscle myocytes and its vasculature and how this regulation is altered by disease or dysfunction.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Y. H. K. and E. J. B. conception and design of research; Y. H. K. and E. J. B. analyzed data; Y. H. K. interpreted results of experiments; Y. H. K. and E. J. B. prepared figures; Y. H. K. and E. J. B. drafted manuscript; Y. H. K. and E. J. B. approved final version of manuscript.

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