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INVITED REVIEW

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Capillary endothelial cells as coordinators of skeletal muscle blood flow during active hyperemia

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Abstract

In this invited review, we explore the burgeoning possibility of capillary endothelial cells as coordinators of skeletal muscle blood flow in response to muscle contraction. The idea that the capillary is an active vascular unit in skeletal muscle microcirculation starkly diverges from the traditional dogma that seats arterioles as the central controllers of blood flow during exercise. This review aims to incite discussion as we revisit and rethink the role of capillary endothelial cells in skeletal muscle. We discuss the potential for a mismatch in the architectural relationships between the arteriolar microvasculature and contracting motor units that would negate consistent communication between them. We review the data from the past two decades demonstrating that capillaries are ideally located architecturally to communicate with skeletal muscle fibers and are mechanistically capable of signaling upstream arterioles that control their own perfusion. We show that the orchestration of a coordinated vascular response necessary to support active skeletal muscle fibers cannot be achieved by the arterioles, but rather it is the capillaries that drive the blood flow response to muscle contraction. Thus, capillaries need to be seriously considered as critical in the coordination of skeletal muscle blood flow during active hyperemia.

KEYWORDS

active hyperemia, arteriole, blood flow, capillary, skeletal muscle

1 | INTRODUCTION

When tissues increase their metabolic demand, they require more blood flow to satisfy their increased need for substrate delivery and metabolite removal. The increase in blood flow associated with an increase in tissue metabolism is referred to as active or functional hyperemia. Although all metabolically active tissues will undergo an active hyperemic response, skeletal muscle exemplifies this phenomenon. During exercise, oxygen consumption can increase 10 to 15-fold, primarily due to the increased oxygen utilization by skeletal muscle fibers. This increase in consumption requires a compensatory increase in blood flow to match oxygen delivery to the increased oxygen demand. In skeletal muscle, blood flow has been shown to be linearly related over a wide range of metabolic demands using different indices of metabolism.[1-10] This coordination is achieved in the absence of neural connectivity,[4] indicating that the coupling of blood flow and metabolic demand occurs locally within the tissue itself. Evidence suggests that blood flow to active skeletal muscle is heterogeneously distributed within the muscle, being directed specifically to the active skeletal muscle fibers themselves.[11,12] At the microcirculatory level, the increase in oxygen delivery to a working skeletal muscle fiber is achieved by an increase in RBC flux through capillaries that supply the individual, active skeletal muscle fibers. But how are skeletal muscle fibers communicating their metabolic needs to the cells of the vasculature and how is the microvasculature coordinating the blood flow response to active skeletal muscle fibers? This review intends to

Abbreviations: ACh, acetylcholine; ADO, adenosine; ATP, adenosine triphosphate; H^+, hydrogen ion; K^+, potassium; K_{ATP}, ATP-dependent K^+ channels; NO, nitric oxide; RBC, red blood cell.

provoke critical thought regarding how skeletal muscle fibers and the vasculature communicate and the microvascular complexities of distributing blood flow to dispersed areas of the tissue. We will challenge the convention that skeletal muscle fibers communicate directly with arterioles to coordinate blood flow during muscle contraction and lay out a case supporting that capillaries are the primary level of the microvasculature that skeletal muscle communicates with to coordinate blood flow to metabolic demand. We will start by considering how skeletal muscle fibers communicate their needs to the vasculature and the challenges that skeletal muscle fiber recruitment places on the microvasculature in regard to the distribution of blood flow to active skeletal muscle fibers.

2 | THE METABOLIC HYPOTHESIS

As previously described, the linear relationship between blood flow and metabolic demand is primarily mediated at the local tissue level. The matching of blood flow and metabolic demand is hypothesized to be accomplished by chemical products of skeletal muscle metabolism, released in proportion to the tissue's metabolic rate, which diffuse out of the muscle and stimulate the vasculature. These stimuli would then initiate arteriolar vasodilation, decrease resistance, and increase flow, all in proportion to metabolic demand. Since the "metabolic hypothesis" was first proposed the hunt for the vasoactive molecules responsible for the cell-cell signaling has led to a "laundry list"[13] of potential vasodilators. Initially, the molecules investigated were products of ATP hydrolysis (H^+, ADP, and inorganic phosphate) and products of ATP resynthesis (ie, ADO, decreased O_2, increased CO_2). The list has been extended to include products related to muscle activation such as K^+ and NO and extended to include products released from other cell types such as RBCs (ATP) and endothelial cells (ie, NO, prostaglandins; for reviews 14-18). Regardless of the source of the vasodilator(s), the underlying mechanism required for communication is diffusion of the active molecule toward the vasculature to promote vasodilation. Given that capillaries cannot, themselves, control their own diameter, the assumption is that products diffuse to the arterioles causing vasodilation and an increase in capillary perfusion. This assumption would then require that arterioles controlling blood flow to capillaries supplying active skeletal muscle fibers be architecturally situated within a physiologically relevant diffusion distance to all skeletal muscle fibers within the tissue. To critically assess this assumption, we must first look at how skeletal muscle fibers are recruited to contract and consider how the vasculature relates to contracting muscle fibers.

3 | SKELETAL MUSCLE FIBER RECRUITMENT

Muscle fibers are stimulated to contract by activation of motor neurons, where one alpha motor neuron, originating in the spinal cord, will innervate many skeletal muscle fibers. The nerve-to-muscle fiber ratio can vary from 1:5 to 1:2000 depending on the muscle and its function (for review, see 19). The basic functional unit for muscle fiber recruitment is the motor unit which is, by definition, the alpha motor neuron and all the fibers it innervates. Despite being a functional unit, muscle fibers associated with a motor nerve do not always reside in close proximity to each other; rather, they are dispersed throughout the skeletal muscle, intermingled with muscle fibers from other motor units. Muscle fibers within a motor unit can be found spread over up to 76% of the whole muscle cross-sectional area (for review, see 19). Force development is dictated by the number of motor units recruited and, therefore, the number of muscle fibers recruited. Motor units are recruited in order of size, from smallest to largest.[20-22] Consequently, a smaller number of muscle fibers, dispersed throughout the tissue, will be recruited first and more fibers can be recruited if more force is required. Therefore, during a submaximal contraction, the skeletal muscle itself will comprise a mix of active skeletal muscle fibers residing beside inactive muscle fibers. The task of the vasculature is to direct blood flow to this dispersed set of muscle fibers in a manner that matches their metabolic demand; thus, the question arises, how does blood flow get directed to active skeletal muscle fibers dispersed throughout the tissue?

4 | MICROVASCULAR ARCHITECTURE IN SKELETAL MUSCLE

Arteries and arterioles play critical roles in the delivery and distribution of blood flow within the tissue due to their ability to change their diameter. In contrast, capillaries have a distinct lack of vascular smooth muscle and are unable to alter their luminal diameter. The architecture of the microvascular network has been described in many skeletal muscle beds,[11,23-32] and although there are differences between these vascular beds, there are many common attributes that they share (Figure 1). There is no universally accepted strategy for the naming of branch orders of the arteriolar microvasculature, but for the ease of discussion, we will use the naming paradigm proposed by Wiedeman[33] where arterioles are classified according to their branching order beginning with a major inflow arteriole (ie, first-order arteriole, 1A) identified and subsequent branch orders being assigned higher-ordered numbers (second-order arteriole, 2A; third-order arteriole, 3A, etc.; Figure 1). Common microvascular arteriolar structure in skeletal muscle includes the arteriole that enters the muscle tissue itself and is parallel and paired with a vein. This paired arrangement of arterioles and veins continues as these vessels branch throughout the muscle tissue (1A); however, arterioles will eventually bifurcate into unpaired branch orders independent from the venous vasculature. These unpaired arterioles (2A) will branch many times, yielding multiple, smaller diameter daughter vessels, in turn, yielding multiple daughter branches (3A) until they branch into the final ramification of the arteriolar tree (4A arterioles, also called terminal arterioles, depicted in Figure 1) which gives rise to a group of capillaries. The number of arteriolar branch orders between the 1A and the capillaries will vary between muscles and between species. For our discussion here we will consider the 4A arteriole as the terminal arteriole.

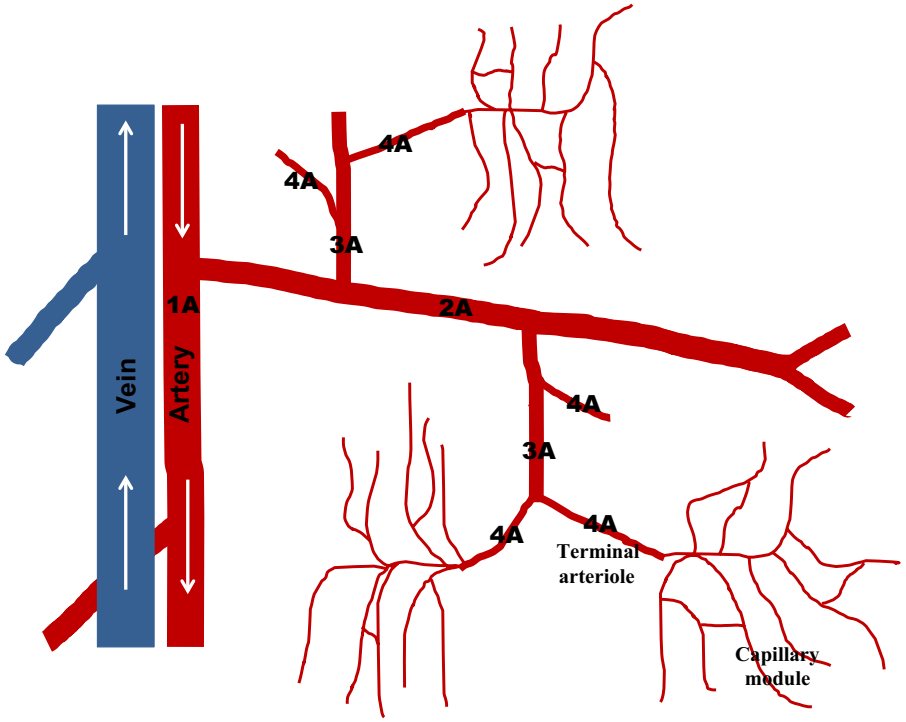

FIGURE 1 Schematic of part of the microvascular network in skeletal muscle. This schematic shows the microvascular naming paradigm, from the 1A arteriole to the capillaries, introduced by Wiedeman[33], that will be used throughout this review. The schematic is representative of the microvasculature within the hamster cremaster muscle and thus shows the TA as a 4A arteriole. The number of branch orders between the 1A and the capillaries will vary between vascular beds and between species; thus, the TA is not always a 4A arteriole. Schematic is not drawn to scale. Not all capillary modules from all 4A arterioles are shown

The 4A arteriole will give rise to a group of between 2-20 capillaries,[25,27,28,34] referred to as a capillary module,[11] a capillary unit,[25,27] or a "bundle element".[30] Capillaries are the component of the vascular network whose wall consists only of endothelial cells; they are vessels that arise from the 4A arteriole and converge into venules. Given capillaries' inability to modify their own diameter, their blood flow is dependent on the upstream arteriolar network, most immediately by the 4A arteriole from which the capillaries are derived (Figure 1). As groups of capillaries arise from a single 4A arteriole, individual capillaries within a module cannot be independently perfused; rather, all capillaries within the module will be perfused at the same time depending on the vasodilatory state of upstream arterioles.[25,32,35-37]

Capillary network organization in skeletal muscle is made up of repeated units of capillary modules, each module supplying a localized volume of skeletal muscle tissue. Lund et al.[27] described the capillary module in the hamster tibialis anterior, with repeating modules consisting of 15 capillaries. They averaged the volume of tissue supplied by the capillaries as a parallelepiped (similar to a rectangular prism) 800-900 μm long, 200 μm wide and 100 μm deep (see Figure 2). Emerson and Segal[38] describe the capillary module in the hamster retractor muscle in a similar manner with a volume of tissue supplied by each "microvascular unit" being, on average 1000 μm long, 500 μm wide, and 100 μm deep. Fraser et al.[39] describe a parallelepiped of similar dimensions in the rat extensor digitorum longus muscle. Repeating capillary units are a common feature of skeletal muscle microvasculature in different species and in different muscles.[25,28,30,34,35,39-42] Due to the conserved nature of the capillary module, it has been referred to as the fundamental building block of capillary networks.[25,38,40]

The arteriolar and capillary microvascular network has the ability to intercommunicate, such that a stimulus in one part of the network can transmit signals to distant or remote regions of the network. There is a large body of literature at the arteriolar level to show that cells of the arteriolar vasculature are connected to each other through gap junctions. Gap junctions connect the intracellular spaces between both endothelial cells, between vascular smooth muscle cells and between endothelial cells and vascular smooth muscle cells (for review, see 43,44). The signals that move from cell to cell through the gap junctions are called conducted responses (also known as transmitted or upstream responses). At the arteriolar level, ACh has been used extensively to explore the nature of conducted vasodilatory responses (for example, 45-53). Conducted responses have been characterized as the rapid movement of an electrical signal, as well as a slower calcium signal, between cells. This conducted signal produces vasodilation that can move bidirectionally in both the upstream and downstream directions from the site of stimulation, and is able to spread into upper and lower arteriolar branch orders.[46,49,52,54,55] Conducted responses are an important communication characteristic of skeletal muscle microvasculature and are necessary to distribute blood flow to individual active skeletal muscle fibers that are stimulated as part of a motor unit. Contracting individual skeletal muscle fibers will stimulate localized parts of the microvasculature scattered throughout the

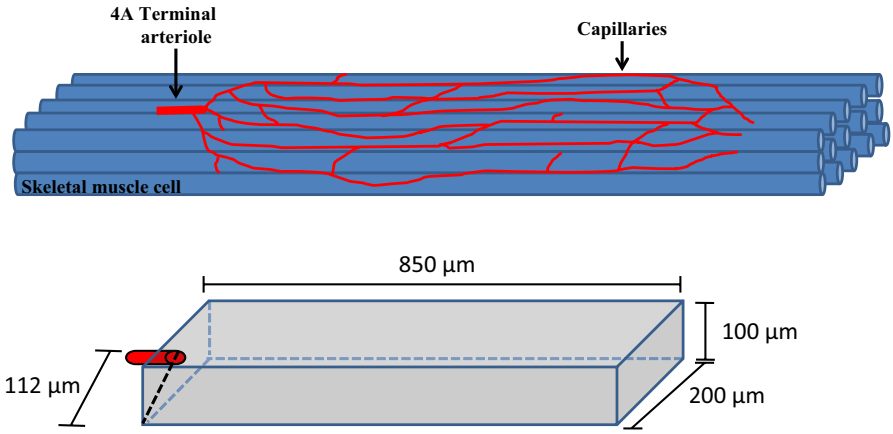

FIGURE 2 Diagram depicting the volume of skeletal muscle tissue (blue columns) supplied by a typical capillary module (in red). The dimensions of the volume of tissue supplied by a module were taken from Lund et al.,[27]. Based on these dimensions and the assumption that skeletal muscle fibers are approximately 40 µm in diameter, the volume of tissue supplied by a capillary module would be 5 muscle fibers wide by 2.5 muscle fibers deep. Assuming that the 4A arteriole branches into capillaries at the center of the border of the volume of tissue (an assumption that places the 4A arteriole as close to all skeletal muscle fibers in that volume as possible), then the furthest a skeletal muscle fiber will be from the 4A arteriole is approximately 100 µm

network. These remote parts of the vasculature must now communicate with the branching arteriolar network to coordinate the blood flow response. The important vasoactive molecules communicating between skeletal muscle and the vasculature must be able to induce a conducted response to direct blood flow to the contracting skeletal muscle fibers. While each of ADO, NO, K^+, ATP, and K_{ATP} channels have been implicated as important in the active hyperemic response (for review, see 6,14,15,17,18), K^+, ATP,[56] and the K_{ATP} channels (via K_{ATP} channel opener pinacidil)[56,57] have consistently been shown to induce conducted responses when applied to arterioles while, interestingly, NO and ADO have not.[47,48,55,58-65]

5 | VASCULAR RELATIONSHIP WITH SKELETAL MUSCLE FIBERS

The component of the microvasculature that is most intimately associated with skeletal muscle fibers is the capillaries. The number of capillaries surrounding a single muscle fiber is generally 3-6, depending on muscle fiber type and fitness or trained state of the muscle (for example, 66-74). Capillaries have a fairly conserved length across muscles and species, averaging approximately 800 µm (range: 500-1700 µm) when capillaries are defined as vessels running from 4A arteriole to the venule[27,28,34,41,42,75] and shorter lengths for those that define capillaries as a non-branching segment of vessel.[34,39,76] Generally, capillaries run parallel to skeletal muscle fibers with short cross-connections between individual capillaries[25,28,30,77-79] and can be linear in structure or more tortuous depending on muscle fiber type (for example, 30,72,80-82).

While skeletal muscle fibers can vary in length depending on the muscle, they are invariably longer than the average length of a capillary (for review see 83). This necessitates multiple capillary modules per muscle fibre. Therefore, although muscle fibers are surrounded by 3-6 capillaries along their length, these capillaries will originate from different modules and consequently different 4A arterioles that control their state of perfusion. For example, the average fiber length of the cat tenuissimus muscle is 2.5 cm,[84] and the average capillary length in this muscle is 1015 µm[41]; then, each muscle fiber will be perfused by capillaries from at least 25 modules, and each module will be associated with 25 different 4A arterioles. In hamster retractor muscle, Emerson and Segal[38] measured the capillary length within a capillary module to be 1000 µm on average and estimate that more than 50 capillary modules will be supplying a muscle fiber. Each of the 50 capillary modules will be supplied by their own 4A arteriole; therefore, an increase in metabolic rate and, consequently, the demand for blood flow will require the coordinated vasodilation of over 50 4A arterioles. Additionally, they note that the 4A arterioles supplying the muscle fiber do not come from the same part of the vascular network. Therefore, one muscle fiber will be supplied by a multitude of capillary modules along its length with their flow being controlled by equally as many 4A arterioles which arise from different parts of the arteriolar network. This begs the question: How is a metabolically active muscle fiber able to communicate with the arteriolar levels of vasculature controlling its capillary perfusion?

As mentioned previously, products of skeletal muscle contraction must use diffusion to communicate with the vasculature and increase capillary flow. Given that capillary blood flow is controlled by proximal arterioles, it is logical to conclude that skeletal muscle fibers must be communicating directly with arterioles to cause vasodilation to increase capillary perfusion. Yet, the increase in blood flow to contracting muscle occurs within 1-second after the initiation of muscle contraction,[5,85-91] and the immediate vasodilation in response has been attributable to a diffusible factor.[92,93] Thus, if diffusion is the method of transport of the communicating molecule

and the response occurs within 1 second, then, using the diffusion equation:

$$t = \frac{L^2}{2D}$$

(where t=time in seconds, L=length (or distance) of the diffusing molecule to travel in cm, and D=the diffusion coefficient for small molecules which have been estimated to be 10^{-5} cm^2/s.) We calculate the distance between the skeletal muscle and the important stimulated component of the vasculature as 40 μm. Thus, if contracting skeletal muscle fibers are communicating with arterioles, then skeletal muscle fibers must be within 40 μm of the each of the arterioles that controls blood flow to the capillaries feeding the active skeletal muscle.

Although the relationship between arterioles and skeletal muscle is not well defined, it has been noted that the branching of arterioles does not consistently follow the arrangements of skeletal muscle fibers regardless of the muscle fiber orientation.[38] Further, using measurements of the volume of skeletal muscle tissue that a capillary module supplies, we calculate that skeletal muscle fibers can, indeed, be further than 40 μm away from arterioles (Figure 2). For example, Lund et al.,[27] estimated that the volume of the parallelepiped of tissue supplied by a capillary module is 800-900 μm long, 200 μm wide, and 100 μm high. Assuming that a skeletal muscle fiber is 40 μm in diameter,[34] the volume of tissue supplied by a capillary module would encompass a rectangular volume of muscle fibers 5 muscle fibers wide and 2.5 muscle fibers deep and includes approximately 12.5 muscle fibers. If you assume that the arteriole is entering this volume of tissue centrally, at one end, then skeletal muscle fibers may be as far as 100 μm away from the modules 4A arteriole (Figure 2), a distance over which it would take a small molecule 10 s to diffuse. Using measurements from Emerson and Segal,[38] the volume of tissue supplied by each capillary "microvascular unit" is 1000 μm long, 500 μm wide, and 100 μm deep. In this case, skeletal muscle fibers may be as far as 270 μm away from the modules 4A arteriole, a distance over which it would take a small molecule 20 s to diffuse. Fraser et al.[39] mapped out the volume of tissue that seven capillary units in the rat extensor digitorum longus muscle supplied and, using the above calculations and assumptions, skeletal muscle fibers could be between 68.2 and 169.5 μm from the modules 4A arteriole. These three studies demonstrate the variability in the distances between skeletal muscle fibers and 4A arterioles, highlighting the lack of a consistent architectural relationship between the two. Given this lack of consistent architecture, it is unlikely that skeletal muscle fibers are consistently within 40 μm of all of the 4A arterioles controlling the capillaries along its entire length.

Potential variability in the distance between arterioles and skeletal muscle fibers presents further complications regarding the concentration of communicating molecule released by the source (i.e. skeletal muscle cells). For example, Figure 2 shows that in the volume of tissue supplied by a capillary module, some muscle fibers can be adjacent to the 4A arteriole and some muscle fibers can be up to 100 μm away. During muscle contraction, if the concentration of K^+ at the 4A arteriole was increased by 1 mmol/L to promote vasodilation, and the diffusion distance was 100 μm, then the initial concentration at the source (skeletal muscle) must be 59 mmol/L. The change in K^+ during contraction have been reported as high as 4-5 mmol/L (for example, see 94-97), necessitating an initial source concentration of 236 mmol/L from the skeletal muscle fiber 100 μm away. These non-physiological concentrations of K^+ demonstrate that the architectural relationship between skeletal muscle fibers and 4A arterioles does not support the possibility that they are communicating directly. Further, this calculation raises the architectural issue regarding the ability to produce vasodilation of a predictable magnitude: With the assumption of large effluxes of K^+ during contraction, the skeletal muscle fibers directly adjacent to 4A arterioles will have a significantly shorter diffusion distance (ie, 10 μm), and consequently, the K^+ concentration at the 4A arteriole will be substantially higher and the vascular response will be different. In fact the difference between arteriolar concentrations of K^+ of 10 mmol/L and 50 mmol/L is vasodilation and vasoconstriction, respectively.[98-100] The fundamental problem presented is that if the amount of vasodilator released by each contracting muscle fiber is constant, then the 4A arteriolar response will vary depending on the diffusion distance from the contracting muscle fiber. Any variability in the vasodilatory response due to variable diffusion distances will be problematic with regard to matching blood flow to metabolic rate. Therefore, the assumption that arterioles are the primary site of stimulation during active hyperemia necessitates a level of architectural complexity that is not supported by the literature to date.

The above argument assumes that the diffusing vasodilators are coming from skeletal muscle fibers; however, there is the potential that vasodilators originate from other cellular sources such as endothelial cells and RBCs. These sources will encounter the same spatial and architectural complexities in the relationship between skeletal muscle fiber stimulation, the cellular source of the vasodilator, and the 4A arteriole as mentioned above. For example, the hypothesis that RBCs release ATP and produce vasodilation[14] would still require diffusion of ATP to the 4A arteriole that is connected to the capillaries that feed the contracting skeletal muscle fiber. This hypothesis would then require an architectural relationship between ATP release and the 4A arterioles. RBCs release ATP when compressed, during lowered PO_2 conditions, and under conditions of a lower pH,[14] all conditions found at the level of the capillary, not necessarily the 4A arteriole. Regardless of the source of the vasodilator, if the assumption is that arterioles are the primary site for stimulation and diffusion is the communication mechanism, then there are spatial and architectural complexities that will need to be addressed to facilitate the diffusion of a vasodilator to the correct 4A arteriole.

The above argument also assumes that the 4A arteriole is the critical microvascular level that skeletal muscle fibers communicate with; however, we can consider other arteriolar levels upstream (ie, 3A arterioles).[32] Once stimulated, such arterioles could induce conducted responses and signal downstream vasodilation toward 4A arteriole which would result in an increase in capillary perfusion. In actuality, this is unlikely for two reasons: (i) There are fewer blood vessels that comprise the ascending vascular tree,[23,101] thereby decreasing the likelihood of having all skeletal muscle fibers within a physiologically

relevant diffusion distance of 3A and 2A arterioles, and (ii) the direct stimulation of 3A or 2A arterioles has no mechanism to coordinate a conducted response to create a specific pathway of decreased vascular resistance to perfuse an individual 4A arteriole, as opposed to dilation of multiple 4A arterioles. For example, if a 3A arteriole were stimulated to vasodilate and induce a conducted response downstream, how would this response be directed to a specific 4A arteriole? How is the 3A arteriole able to recognize which skeletal muscle fiber is contracting in order to vasodilate a specific 4A arteriole to increase its capillary module perfusion? If the 3A arteriole conducts its vasodilation to all branching 4A arterioles, then there would be a large region of the muscle perfused where there were no contracting skeletal muscle fibers and blood flow would no longer be matched to skeletal muscle metabolism. Therefore, stimulating arterioles other than the 4A arteriole present mechanistic problems in being able to match skeletal muscle activity with perfusion.

Thus, arterioles fail, both architecturally and mechanistically, to provide a means in which blood flow can be specifically recruited to active skeletal muscle fibers. Capillaries, however, do not fail in these regards. Dietrich and Tyml[102] were the first to recognize the potential importance of capillaries in the control of blood flow. Since then, there has been mounting evidence that underscores that capillaries should be investigated as central coordinators of blood flow during exercise. Architecturally, capillaries are intimately related to each skeletal muscle fiber, with many capillary endothelial cells residing immediately beside each skeletal muscle fiber, and along the entire length of the skeletal muscle fiber with little variability in distance between skeletal muscle fibers and capillary endothelial cells. Thus, capillary endothelial cells are ideally positioned to be exposed to products of skeletal muscle contractions. Further, there is an architectural relationship between the capillaries and the upstream arteriole that controls their perfusion. But in order for capillaries to be the critical communicating medium between skeletal muscle fibers and 4A arterioles, capillaries must be mechanistically capable of being stimulated by products of skeletal muscle contraction and, further, be able to communicate this signal to cause upstream arterioles to vasodilate. Therefore, this raises the question: Can capillaries be stimulated by products of skeletal muscle contraction and can they signal the upstream 4A arterioles that control their perfusion?

6 | CAPILLARY STIMULATION BY PRODUCTS OF SKELETAL MUSCLE CONTRACTION

In skeletal muscle of both frog and rodent animal models, capillary endothelial cells have been shown to be stimulated by many different vasoactive molecules (ACh,[103-105] bradykinin,[103,106] norephinepherine,[103-107] phenylephrine[106]) and, importantly, molecules that may be relevant to muscle contraction (NECA (ADO analogue),[104,106] K^+,[104,105] and H^+[104]) with the result of this stimulation being a change in RBC flux through the stimulated capillary module. Critically, the physiological relevance of capillary stimulation has been demonstrated by showing that capillaries can be stimulated by contracting skeletal muscle fibers[11] resulting in an increased RBC flux through the stimulated capillaries. This increase in RBC flux is ultimately the product of vasodilation of the associated upstream 4A arteriole controlling blood flow to the stimulated capillary module. Upstream 4A arteriolar vasodilation has been observed directly when stimulating the capillaries with ACh,[108] ADO,[109] NO,[109] and opening K_{ATP} channels (with pinacidil)[57,109] and with skeletal muscle fiber contraction.[11,108,109] In total, these observations have clearly established that capillary endothelial cells can be stimulated by substances relevant to skeletal muscle contraction and, further, show that capillary endothelial cell stimulation can induce conducted responses to promote upstream arteriolar vasodilation.

As mentioned previously, capillary endothelial cells are interconnected through gap junctions. While capillary initiation of downstream conducted responses into venules has not been explored, functional data show that upstream conducted responses initiated by stimulating capillaries can be blocked if gap junction uncouplers are placed in the signal transmission pathway between the capillary stimulation site and the arteriolar observation site. Gap junction uncouplers have been shown to block 4A arteriole vasodilation in response to capillary stimulation via muscle contraction[11,108] and ACh[108] as well as block the increase in RBC flux that accompanies capillary stimulation with muscle contraction.[108] Capillary endothelial cells have been shown to be electrically connected to the arteriolar vasculature by locally stimulating arterioles and observing a conducted hyperpolarization that changed the membrane potential in the downstream connected capillaries.[46,110] In the same experiments, application of depolarizing concentrations of K^+ directly on the capillaries caused depolarization. Therefore, capillary endothelial cells can be stimulated by muscle contraction and products relevant to muscle contraction, and they have the gap junction connectivity required to transmit signals upstream to remote parts of the arteriolar vasculature. But capillaries also appear to have a unique ability to conduct responses upstream in an ordered, directional manner, very different from the bidirectional, regional spread of vasodilation that results from stimulating arterioles.

7 | CAPILLARIES STIMULATE SPECIFIC ORDERED OPENING OF UPSTREAM ARTERIOLES

To direct blood flow to specific capillaries that are supplying an active skeletal muscle fiber, the upstream path of vasodilation must be very ordered. To increase flow in a specific capillary module, the upstream 4A arteriole must vasodilate. To increase blood flow further, the parent 3A arteriole must vasodilate, and for further increases in blood flow, the supplying 2A arteriole must vasodilate. This creates a path of least resistance to the specific capillary module being perfused. Importantly, once a 4A arteriole vasodilates and this vasodilatory signal spreads upstream into the parent 3A arteriole, the vasodilation should not spread into all 4A arterioles branching from that 3A arteriole. As briefly mentioned above, the vasodilation of all branching

4A arterioles would increase perfusion of all capillary modules associated with the 3A arteriole. This would produce perfusion of larger regions of the skeletal muscle including perfusion of capillary modules supplying inactive muscle fibers, resulting in an over-perfusion of the region and a stark mismatch between blood flow and metabolic demand. Therefore, the spread of vasodilation must be very ordered and directional upstream so as not to over perfuse inactive regions of the muscle.

Stimulating a capillary elicits an ordered opening of upstream arterioles. Using muscle contraction under capillaries as a stimulus, Berg et al.[11] showed that contraction initiated a conducted response in capillaries that vasodilated the upstream, associated 4A arteriole, and the upstream parent 3A arteriole, but vasodilation did not spread into all 4A arterioles that arose from the 3A arteriole. This ordered opening of terminal microvascular arterioles decreased resistance specifically to the stimulated capillary module and not to all capillary modules stemming from the 3A arteriole. At higher contraction frequencies, when metabolic rate of the skeletal muscle fibers was higher and more flow was required, vasodilation spread further upstream into the 2A arteriole (Figure 3). This ordered opening was further explored by Twynstra et al.[57] using micropipette application of pinacidil (a K_{ATP} channel opener). When pinacidil was applied to capillaries in a module, vasodilation was evoked in the upstream 4A arteriole and spread to the upstream 3A arteriole, in a similar manner as muscle contraction. Vasodilation did not spread from the 3A arteriole to other 4A arterioles branching from it. Further, the spread of vasodilation into the 2A arteriole occurred only in the upstream direction from the 3A branch point, not downstream of the 3A branch point (Figure 3B). Therefore, stimulating capillaries changed resistance in a specific pathway that would lead to a decrease in resistance and, presumably, an increase in flow to the module containing the stimulated capillaries specifically. When pinacidil was applied to the 2A arteriole directly, vasodilation spread in the upstream and downstream direction as well as into upstream 3A and 4A arterioles and downstream 3A and 4A arterioles (Figure 3A). Clearly, stimulating the 2A arteriole directly will not cause a coordinated change in flow to specific capillaries but would result in a regional increase in blood flow.

8 | PERSPECTIVES ON CAPILLARIES AS COORDINATORS OF ACTIVE HYPEREMIA

Given that capillaries are ideally located architecturally with skeletal muscle fibers and capillaries are mechanistically equipped to respond to products of skeletal muscle contraction, we propose that capillaries are the level of the vasculature that active skeletal muscle fibers communicate with and, further, that capillaries coordinate the blood flow response to contracting skeletal muscle. We raise doubts over the ability of arterioles alone to coordinate the blood flow response to exercise. Arterioles lack the architecturally consistent relationship with each skeletal muscle fiber required to couple contraction of each skeletal muscle fiber with the vasodilation required to increase blood flow. Further, arteriolar communication with the vascular network appears to lack the specificity of directionality of the conducted response required to direct blood flow to stimulated capillary modules. Capillaries, however, are ideally located in close proximity to every skeletal muscle fiber to be exposed to products from skeletal muscle by diffusion. In addition, capillaries are mechanistically equipped to be stimulated by metabolic products of skeletal muscle contraction and capable of sending signals up the arteriolar tree in an ordered, directional manner that would direct a localized increase in blood flow to the capillary module that contains the stimulated capillary and the contracting skeletal muscle fiber. Thus, skeletal muscle fibers control their own perfusion by using capillaries as the communication medium to the arteriolar network. Both the conserved architectural proximity between skeletal muscle fibers and capillaries, and the capillaries' ability to increase their own perfusion implicates capillaries as the critically important vascular component in the coordination of skeletal muscle blood flow during active hyperemia. This idea needs to be further explored experimentally and could fundamentally shift the thinking on blood flow control in response to skeletal muscle contraction.

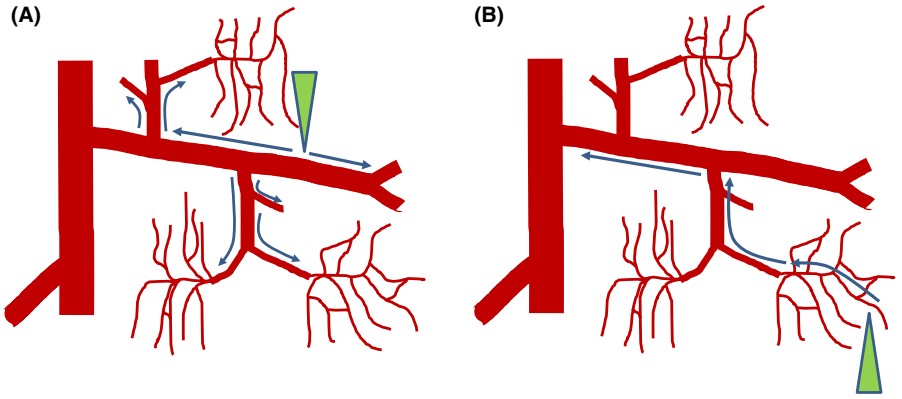

FIGURE 3 Schematic of the direction of upstream vasodilation produced by stimulating (A) the 2A arteriole directly and (B) the capillaries from a single capillary module directly. (A) Stimulation of the 2A arteriole directly would induce conducted responses that would spread in the upstream and downstream directions, into upstream and downstream 3A and 4A arterioles which would result in an increased in regional perfusion of multiple capillary modules. (B) Stimulation of capillaries within a capillary module will result in an ordered opening of upstream arterioles that would lead more specifically to an increased perfusion of the stimulated capillary module

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