Point:Counterpoint: There is/is not capillary recruitment in active skeletal muscle during exercise

POINT: THERE IS CAPILLARY RECRUITMENT IN ACTIVE SKELETAL MUSCLE DURING EXERCISE

Focus. The key issue here is whether resting skeletal muscle is fully perfused. We will make the case that it is not, and thus there exists a reserve of unperfused capillaries (capillary reserve) that are recruited to carry flow not only by muscle contraction, but also by insulin.

Our background. Our interest in this area arose from efforts to develop noninvasive methods to assess whether insulin affects muscle microvascular perfusion in vivo and whether muscle insulin resistance is at least in part due to impaired perfusion that diminishes insulin and glucose delivery. Since it is well accepted that bulk blood flow to working muscle increases, it was logical to apply our methods to compare exercise and insulin in terms of capillary recruitment.

We cite findings obtained using five different approaches that each provides evidence of capillary recruitment. Three of these are entirely noninvasive, the other two involve surgical preparation that may appreciably alter “basal” perfusion.

Exercise increases red blood cell occupancy of capillaries in muscle biopsies. Honig and colleagues (8) examined cryosections of denervated dog gracilis muscle and reported that at rest only one-third of the capillaries were perfused with erythrocytes. Muscle contraction (4/min) effectively doubled the number of capillaries containing red blood cells without increasing bulk flow. Near maximal recruitment occurred at frequencies ~8 Hz and bulk flow increased dramatically. Certainly vessel tortuosity (with a single vessel crossing a section several times) could have led to underestimation and, conversely, denervation (with resultant high basal flow rates) to overestimation of basal perfusion. Intriguingly, these observations supported those of Krogh (11) who first proposed a reserve of unperfused capillaries in muscle more than half a century earlier—a cornerstone of the work for which he was awarded the 1919 Nobel Prize.

Exercise increased red blood cell movement in exposed muscle. Intravital microscopy of surgically exposed transparent muscle of anesthetized animals has provided evidence for (6, 7, 10, 12, 16, 18) and against capillary recruitment (see accompanying “counterpoint”). The study by Lindbom (12) viewed the rabbit tenuissimus muscle using intravital microscopy and noted that one-third to one-half of all capillaries were perfused based on red blood cell movement and these were homogeneously distributed. Electrical stimulation increased perfusion of muscle and not connective tissue capillaries. The distribution of flow between muscle and connective tissue was influenced by the oxygen tension of the superfusing solution. Thus, as the Po2 increased, muscle capillary flow decreased and, conversely, when the superfusate Po2 decreased, more capillaries were perfused. The dependence of capillary flow in such preparations on Po2 was noted by others earlier (13) and confirmed recently (17). These findings both support the presence of a capillary reserve and emphasize the sensitivity of exposed muscle preparation vasculature to environmental factors.

Each of the above two methods are quite invasive, requiring general anesthesia, surgical exposure of the muscle, and, in some preparations, denervation. This makes it particularly difficult to obtain an estimate of true “basal” perfusion, which is required for any estimate of recruitment in response to exercise or other stimuli.

An increase in metabolism of the exogenous marker substrate 1-methylxanthine (1-MX). This method was developed in our laboratory (15) and measures the metabolism of 1-MX as it traverses the muscle vascular network from artery to vein. Others have shown that xanthine oxidase, which is responsible for metabolizing 1-MX in muscle, is located predominantly in the capillary endothelium (9). The method has been validated in the pump-perfused isolated rat hindlimb, where the proportion of total flow that is nutritive can be manipulated (1). With this preparation, 1-MX metabolism is proportional to the volume of nutritive flow, is increased during muscle contraction (22) and decreased when nutritive flow was decreased pharmacologically (14). Applying this method in vivo to rats, we showed that field stimulation of muscle in vivo to simulate exercise increased the metabolism of 1-MX, reinforcing the above issues (4). From a number of studies it is clear that 1-MX metabolism, and thus capillary recruitment, is not dependent on bulk blood flow (for review, see Ref. 2 and references therein). Interestingly, we showed that physiological insulin also increased 1-MX metabolism, suggesting that insulin increased microvascular perfusion (15) and, by default, indicating that prior to insulin the muscles were not fully perfused. This method does not require muscle biopsies or surgical exposure and might therefore be expected to provide a more physiological “basal” environment against which to assess the impact of recruitment.

Contrast-enhanced ultrasound (CEU) imaging of the muscle microvasculature. With this method an acoustic signal is obtained from intravenously infused microbubbles of inert gas as they track with erythrocytes through the vasculature. The microbubbles are smaller than red blood cells and do not change blood flow rheology. We adapted the method for use with skeletal muscle based on that described by Wei et al. (21), who validated the method to trace myocardial blood perfusion. The acoustic properties of microbubbles result in their burst when impacted by a high energy ultrasound pulse and simultaneously emitting a signal. By introducing a variable time interval between pulses, a series of images is collected and the contributions of tissue density and large, rapidly filling vessels (arteries, arterioles, veins, and venules) subtracted from the integrated video intensity in a region of interest. From this, a replenishment curve is generated that describes the refilling rate and the volume of microvasculature filled by microbubbles. This method has the advantages of being entirely noninvasive, requiring no anesthesia, and it can be used in humans performing voluntary exercise (as opposed to electrical stimulation). As a result, this method is better suited than the others mentioned above for obtaining a true measure of basal microvascular perfusion and hence to assess increments above basal.
Using this approach, changes in capillary blood volume in response to insulin (19, 23) and exercise (4) have been assessed in skeletal muscle of the rat hindlimb in vivo. The CEU data correlate well with findings obtained using the 1-MX method (4) and showed that capillary blood volume increases as much as 100% during physiological doses of insulin and 200% with field stimulation. In human forearm muscle we recently showed that mixed meal and light exercise (Fig. 1; Ref. 20) as well as insulin (3) each recruit muscle capillaries, and insulin’s effect is absent in obese insulin-resistant subjects (3).

The final method we would mention has not been used to examine the effects of exercise. However, using implanted laser Doppler probes (a minimally invasive technique), capillary recruitment in muscle as measured by an increase in intramuscular blood flow was found to increase in response to physiological hyperinsulinemia (5). This was paralleled in the same patients by physiological hyperemia was found to increase in response to physiological hyperinsulinemia assessed by contrast ultrasound. Am J Physiol Endocrinol Metab 282: E714–E720, 2002.

In summary, we find overwhelming evidence for the positive point of view that there is capillary recruitment in muscle during exercise. All of five different approaches support the notion of a capillary reserve in muscle that can be recruited not only by exercise but also insulin action.

GRANTS
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REFERENCES
COUNTERPOINT: THERE IS NOT CAPILLARY RECRUITMENT IN ACTIVE SKELETAL MUSCLE DURING EXERCISE

The notion that a substantial proportion of capillaries do not contain moving red blood cells (RBCs) in muscle at rest but are “recruited,” i.e., begin flowing with RBCs during contractions, is one basis for our present understanding of blood-muscle exchange during exercise (20, 28). This concept emanates, in part, from August Krogh, who showed that many capillaries in resting muscle did not contain India ink after high pressure perfusion (19). Despite Krogh himself recognizing that India ink particles clumped together, more likely to prevent complete perfusion (19). Despite Krogh himself recognizing that India ink particles clumped together, more likely to prevent complete perfusion of the capillary bed at rest than during exercise, these experiments, and Krogh’s O2 diffusion model based on them, are still cited by researchers invoking capillary recruitment (e.g., Refs. 22). Today capillary recruitment during exercise is accepted by many to explain important physiological phenomena, including: 1) greater blood-muscle delivery and extraction of O2, free fatty acids, and glucose and 2) reduced capillary-to-mitochondrial diffusion distances. It makes great sense that, if there were a reserve of capillaries at rest, during exercise when the muscle demands for O2 may increase up to 100-fold, all—or at least most—capillaries would contribute to meet that demand.

Why, therefore, choose to oppose the concept of capillary recruitment during exercise? In Britain, the motto of The Royal Society is “Nullius in Verba” (Take nobody’s word for it, see it for yourself). However, the majority of research papers invoking capillary recruitment have not visualized the capillary bed (e.g., Refs. 3, 7, 25). In his letter to the editor of The American Journal of Physiology, the eminent microcirculation expert, Professor Eugene Renkin (26) criticized Dr. Bentzer for entitling his paper “Capillary filtration coefficient is independent of number of perfused capillaries in skeletal muscle” (4) on the basis that “... its title is misleading. No direct measurements of [RBC-flowing] capillary number were made...” The same criticism could be leveled at almost all papers that include the words capillary recruitment in their titles. Evidence that most capillaries already sustain flow in resting muscle would preclude the possibility that recruitment of previously nonperfused capillaries occurs to any great extent during contractions.

Direct evidence for RBC flow in most capillaries in resting muscle. In resting muscle, intravital light microscopy shows that over 80% of capillaries support RBC flow, e.g., in rat spinotrapezius (14, 17, 24), diaphragm (15), and extensor digitorum longus (1), hamster cremaster and sartorius (8), cat sartorius (6), rabbit tenuissimus (30). However, animals in these experiments were anesthetized to facilitate muscle exteriorization and viewing of the capillary beds. To address this, Bailey and colleagues (2) employed minimally invasive techniques to measure blood flow (radioactive microspheres) and microvascular oxygen partial pressure in muscle in situ and neither criterion was altered by exteriorization. Moreover, the dynamic matching of increased O2 delivery and VO2 during contractions in situ was preserved in the exteriorized muscle. It is difficult to conceive how anesthesia might affect arteriolar smooth muscle function at rest (to produce a falsely high %RBC-perfused capillaries) and yet muscles increase their blood flow and VO2 at a ratio of 6:1 (11), which is precisely that seen in intact voluntary exercising animals and humans (23).

Another valid concern about intravital microscopy is that there are non-RBC containing capillaries at rest that cannot be seen because of their translucency. However, neither observation of contracting (17) nor vasodilated (16) muscles revealed a significant number of such vessels (see also Refs. 8, 14). The technical requirements necessary to observe capillaries within living muscle restrict the procedure to a limited selection of animal muscles so that one question to ask is: How representative of other muscles in the animals’ body and in humans are these? In anesthetized and conscious animals, Snyder et al. (29) used systemic indicator injections and demonstrated that essentially all capillaries in each muscle examined (vastus lateralis, diaphragm, soleus) were perfused within 3–7 s.

Indirect evidence for RBC flow in most capillaries in resting human muscle. Noninvasive near-infrared spectroscopy (NIRS) measures muscle hemoglobin concentration ([Hb]). If there were significant recruitment of previously non-RBC containing capillaries during exercise, say from 20 to 90%, [Hb] would be expected to increase several-fold. However, the rest-to-exercise [Hb] increase is less than one-fold (e.g., 10) and can be accounted for by increased capillary hematocrit (18). Thus, as in animal muscles, there is little room for substantial capillary recruitment in human muscle.

Against the evidence for capillary recruitment during exercise. The literature that purports to demonstrate capillary recruitment deserves to be evaluated on its own merits, but the following must be considered as possible explanations for reports of many non-RBC flowing capillaries in resting muscle. 1) Capillaries are fragile structures, subject to damage by blunt trauma, surgery, and/or manipulations such as stretching (24). 2) Po2 within resting muscle is normally very low and raising this will cause arteriolar constriction and capillary flow stoppage (22). 3) Anesthetized preparations are often hypovolemic and hypotensive, which provokes reflex vasoconstriction.

In addition, misinterpretation of histological techniques has supported the notion of capillary recruitment. The conclusion that a RBC in the muscle capillary cross-section indicates RBC flow, whereas its absence supports no flow is erroneous (e.g., Ref. 13). In resting animal muscle observed in vivo, RBC movement in flowing capillaries varies over time, appearing either continuous or stop-start (9). Muscle contractions result...
in more continuously flowing vessels with higher RBC velocities (5). Hargreaves et al. (12) used thioflavine S (a plasma marker) to show that, since all capillaries were perfused, during contractions the increase in muscle blood flow (microspheres) could be accounted for by the increased velocity rather than capillary recruitment. Reduced flow heterogeneity and hence augmented capillary hematocrit from rest to exercise decreases the length of inter-RBC plasma gaps and increases the probability that an RBC will appear in cross section.

**Is the concept that most capillaries support RBC flow in resting muscle mathematically possible?** In a typical 70-kg human with 31.5 kg of muscle (45% body mass), resting muscle blood flow is estimated as ~1 l/min (or 5.4 x 10^12 RBCs/min; Refs. 20, 28). Accepting a mean value for capillary density and length of 300/mm^2 and 1,000 μm, respectively, if 80% of the 8.9 x 10^9 capillaries support RBC flow at rest, as in the rat (14, 17, 24), this would be ~12 RBCs per capillary per second—very close to the 15–20 RBCs per capillary/s actually measured in rat muscle (17). Whereas such calculations are certainly not proof that most skeletal muscle capillaries have RBC flow in humans at rest, they support that it is feasible.

**Why is it crucial that we question the dogma of capillary recruitment?** If most capillaries support RBC flow at rest and are not recruited at exercise onset, increased substrate delivery must occur within already flowing capillaries. Accordingly, is the recruitment of more surface area along the length of already flowing capillaries, rather than de novo flow in previously stagnant capillaries, key to increased blood-myocyte exchange? Diabetes (21), heart failure (27), and chronic ischemia (5) decrease the proportion of RBC-perfused capillaries in resting muscle. If we do not recognize that most capillaries may support RBC flow at rest in healthy muscle, our ability to appreciate the mechanisms for impaired blood-muscle exchange, which may be pathognomonic to these and other diseases, is crippled.

**Nullus in verba!**

The online version of this article contains supplemental data showing intravital microscopy recordings demonstrating RBC flow in almost all capillaries in healthy resting spinotrapezius muscle (first video, Refs. 24, 27) and diaphragm (third video, Ref. 15). The second video demonstrates the effects of chronic heart failure (CHF; left coronary artery ligation, Ref. 27) on capillary perfusion in spinotrapezius muscle. Note, in the CHF condition, the presence of stopped RBCs in central capillaries and other capillaries that have intermittent RBC flow and/or very low/sporadic RBC flux. Adherence to the misconception that many capillaries do not flow in healthy resting muscle (i.e., capillary recruitment notion) would confound identification of the effects of this disease on capillary hemodynamics and therefore O2 delivery and substrate exchange.

**ACKNOWLEDGMENTS**

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**REFERENCES**


REBUTTAL FROM DRs. POOLE, BROWN, HUDDLICA

We congratulate David Poole and his associates for a clear and thoughtful argument (6). The accompanying intravital microscopy video of the spinotrapezius muscle is equally instructive where over 80% of the capillaries are supporting RBC flow at rest.

However, the data are only as good as the techniques used to acquire them, and the authors themselves (6) acknowledge some of the problems associated with exposing a muscle such as the spinotrapezius for viewing under a microscope. These problems, which could alter the extent of capillary recruitment, include the effect of manipulations required to expose the muscle; the question of whether the thin muscles that can be exposed are representative of the cylindrical load-bearing muscles; the effects of anesthesia; and the superfusion PO2. Since the weight of argument against exercise-mediated capillary recruitment is based on studies using intravital microscopy it is essential that the findings from this system can be extrapolated to in vivo. Not least among the problems is the superfusion PO2 in skeletal muscle. Am J Physiol Heart Circ Physiol 291: H2439–H2445, 2006.


REBUTTAL FROM DRs. CLARK, RATTIGAN, BARRETT, AND VINCENT

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REFERENCES


REBUTTAL FROM DRs. POOLE, BROWN, HUDDLICA

My learned colleagues are to be congratulated for considering different techniques (1–5 below) to assess changes in the proportion of flowing capillaries in muscle. However, the resultant data must be judged in light of limitations inherent in those techniques.

It is also important to define precisely what we are talking about. Professor Clark and colleagues’ (1) opening statement that “The key issue here is whether resting skeletal muscle is fully perfused” is clearly erroneous. “Perfusion,” (from the Latin perfusus), infers nothing about the distribution of blood, which, along with the veracity of presumptions made to assess that distribution, is what is at issue here.
Technique 1: Red blood cell occupancy in cryosections. PRESUMPTIONS. An RBC in capillary cross-section indicates capillary was flowing (4).

CHALLENGES. 1) Cannot discriminate stopped versus moving RBCs. 2) Low capillary hematocrit means large inter-RBC (plasma) spaces.

CONCLUSION. Apparent increase in capillaries “recruited” might simply reflect elevated hematocrit during hyperemia (6, 7).

Technique 2: direct intravital microscopy observation. Reports of a majority of nonperfused capillaries in resting tenuissimus muscles (8) are opposed by others (10).

Technique 3: increased metabolism of 1-methyl xanthine by xanthine oxidase. PRESUMPTIONS. Xanthine oxidase (XO) found predominantly in capillary endothelial cells and increased 1-methyl xanthine (1-MX) metabolism occurs: 1) in direct proportion to the number of RBC-perfused capillaries and 2) is unaffected by capillary hemodynamics.

CHALLENGES. 1) XO is found in plasma (9) and smooth muscle (3), 2) increased 1-MX metabolism results from increased delivery to low-flow capillaries.

Technique 4: contrast-enhanced ultrasound. PRESUMPTIONS. Microbubbles are distributed the same as RBCs.

CHALLENGE. If true (doubtful), concentration in already-flowing capillaries would increase two- (or more) fold in hyperemia without obligatory capillary “recruitment” (5–7).

Technique 5: laser Doppler flowmetry shows “... an increase in intramuscular hyperemia ...”. PRESUMPTION. Hyperemia parallels capillary recruitment.

CHALLENGE. Presumption is baseless: hyperemia reflects primarily increased RBC flux in already flowing capillaries (2, 5, 6).

The famous Indian/Chinese/African legend “The Blind Men and the Elephant,” popularized by John Godfrey Saxe in 1878, bears some similarity to my opponents’ approach to this debate. When several blind men seek to discover what an elephant is like, each man only touches one part and argues as follows:

<table>
<thead>
<tr>
<th>Part Touched</th>
<th>Conclusion “Elephant is like...”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Side</td>
<td>wall</td>
</tr>
<tr>
<td>Tusk</td>
<td>spear</td>
</tr>
<tr>
<td>Trunk</td>
<td>snake</td>
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<tr>
<td>Knee</td>
<td>tree</td>
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<tr>
<td>Ear</td>
<td>fan</td>
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<tr>
<td>Tail</td>
<td>rope</td>
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Hence, although all were partly right, all were wrong. This 2,000 year-old missive argues for a better integration of current techniques and knowledge if we are to understand muscle capillary exchange.

Nullius in Verba!

REFERENCES