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The importance of capillary distribution in supporting muscle function, building on Krogh's seminal ideas

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Abstract

Krogh's Nobel prize for insightful studies into the physiology of capillaries heralded a revolution in understanding that continues today. The view of passive conduits has been replaced by capillaries recognised as a key element in haemodynamic control, offering both a site where changes in tissue demand are sensed and a driver of integrated vascular responses. In addition, the capillary bed is known to play an important role in metabolic, hormonal and immune homeostasis. Not surprisingly, therefore, microvascular dysfunction is a hallmark of many central and peripheral diseases, leading to widespread morbidity and mortality. Consequently, there is growing interest in how best to specifically target this organ-system by means of effective angiotherapies. Underpinning a lot of our current understanding of capillary physiology has been a recognition of functional heterogeneity among different microvascular beds. In addition, there is increasing awareness of the role that spatial heterogeneity plays in determining both physiological and pathological outcomes that has led to an appreciation that quality, rather than just quantity of microvascular supply is important. This has required a re-appraisal of the methods used to determine both the extent and topology of the capillary network, with the benefit of facilitating new ways of exploring dynamic regulation of capillary supply and its potential consequences.

Key words

Capillary supply; skeletal muscle; oxygen delivery; morphometry; modelling

Dynamic variability in capillary perfusion

Building on the work of early microscopists such as Malpighi and Leeuwenhoek, August Krogh explored the organisation of capillary networks and their functional plasticity. These exchange vessels act as the diffusive interface between blood and tissue, and he reasoned that they were the most important element of the cardiovascular system. He was the first to calculate the often-quoted fact that if all the capillaries in an adult human were joined end to end the length would be twice the earth's circumference. The role of Krogh's conceptual tissue cylinder in defining our understanding of peripheral oxygen transport is mentioned in a companion article, but it is important to understand what observations lay behind its

formulation. Even at relatively low power it is possible to observe the meandering pathway of individual capillaries down the length of muscle fibres, thus increasing the surface area for exchange in the segment between arterioles and venules. His great insight on the dependence of muscle oxygen uptake and its potential regulators, such as the partial pressure of oxygen in blood (PO_2) and muscle activity, was to realise that this relationship could not be reconciled with the general opinion of the time that blood vessels were passive conduits of blood, but that perfusion was regulated in a dynamic fashion (Angleys & Østergaard 2020). His estimates of perfused capillary density in various tissues and animals showed a) a very large range of values, b) not all were perfused at any one time, c) that the number of perfused vessels increased with tissue demand, and d) that the distribution of perfused capillaries was 'always fairly regular' (Fig. 1).

We must applaud one final insight from the master of description that was ahead of its time. He realised that dynamic regulation of capillary perfusion had to involve the microvessels themselves, as arterial dilatation improved perfusion pressure and hence red-cell flux, but did little to affect the distribution of perfused capillaries. It took many decades before the mechanisms allowing feedback regulation along different elements of the vascular network, and the importance of oxygen sensing by haemoglobin saturation at the level of capillaries, provided experimental justification for his opinion (Elsworth et al. 2009, Bagher & Segal 2011, Poole et al. 2020).

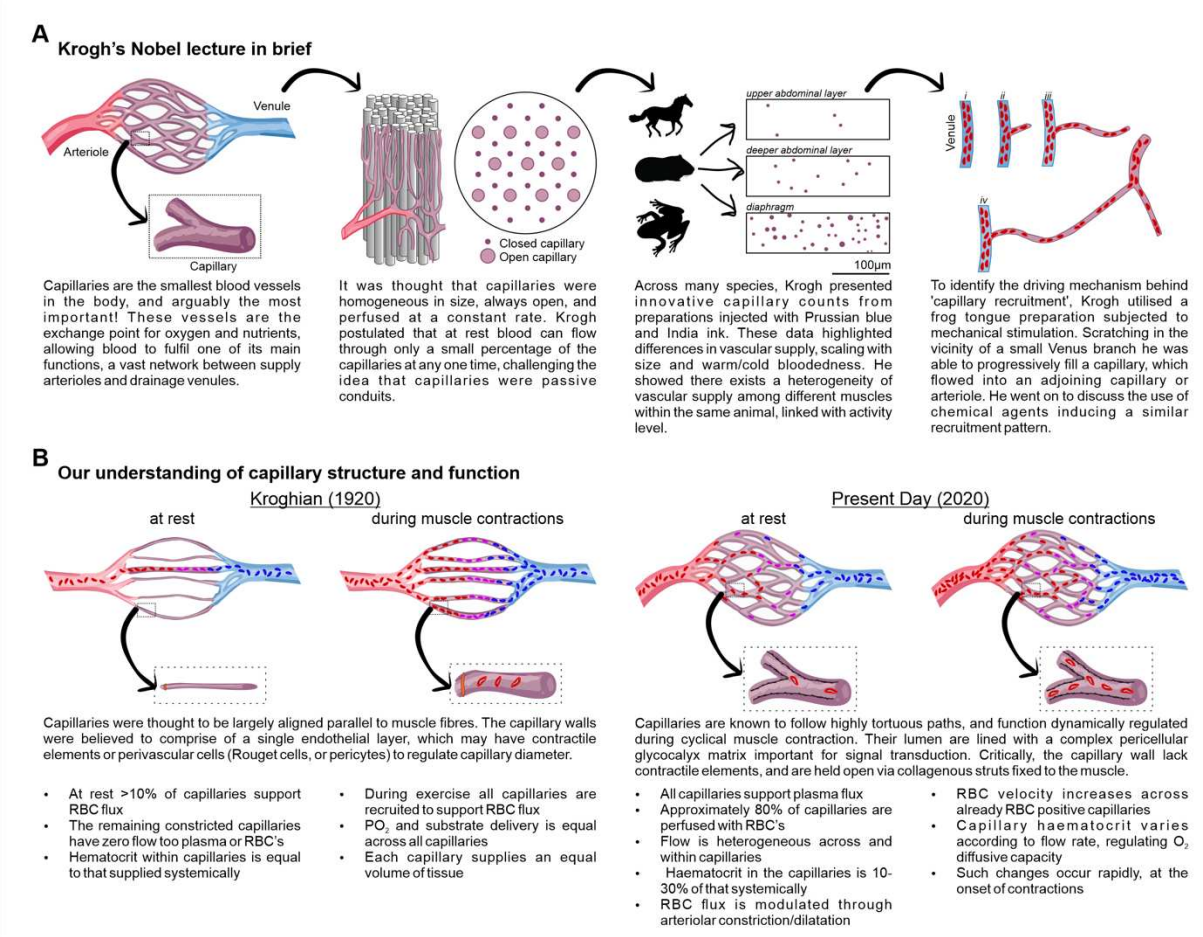

Figure 1. The legacy of Krogh in the understanding of microvascular form and function. (A) Krogh's Nobel lecture presented work across his four seminal Journal of Physiology papers. (B) Microvascular form and function as described by Krogh, and what we know today [various sources].

Informative quantification of capillary supply

Before discussing implications of capillary distribution for the efficiency of muscle oxygenation, and analytical advances that provide for more detailed exploration of capillary function than possible with Krogh's cylinder approach, we need to summarise the quantitative methods used. Any reader new to the topic may be confused about which measure of capillarity most effectively describes the microvascular content and functional consequences of changes in vascular supply during adaptive remodelling of muscle in healthy individuals, in response to varied pathologies that affect muscle function, and during ontogenetic development when muscle phenotype is adjusting to new demands and innervation patterns. New indices regularly appear in the literature that purport to offer insights not previously available. In general, while superficially attractive these often lack physiological relevance or adequate sensitivity to changes under investigation, and hence have limited utility (Egginton 1990).

The type of analysis depends on aims of the study; indices of capillarity can be divided into those describing gross structure, i.e. averaged values across a muscle, and those describing local interaction between capillaries and muscle fibres (Fig. 2). The former may adequately describe the integrated responses, and with appropriate interpretation may illustrate the presence of capillary growth/rarefaction (capillary to fibre ratio, C:F), or changes in diffusive capacity of the network (capillary density, CD). Some evident influences need to be accounted for, e.g. allometric scaling, while heterogeneity of endothelial phenotype requires careful selection of markers (Corliss et al. 2019). One obvious limitation, however, is that they are unable to account for the spatial heterogeneities discussed above. Linear analyses may be used to determine the extent of clustering or variability in intercapillary distances. However, indices describing local interaction between capillaries and the muscle fibres they serve have the potential for improved descriptive power over such global approaches. These have taken varied forms, usually involving a ratiometric formulation attempting to express the extent of capillary coverage relative to fibre size (Egginton 1990). These include capillaries around a fibre/capillary contacts (CAF/CC), sharing factor (SF), capillary-fibre perimeter exchange (CFPE) index etc. Major limitations with these indices include use of integer values, individual capillaries being counted more than once, and difficulty in assessing neighbouring influences such as different fibre types or sub-adjacent capillaries. It is likely that oxygen transport to tissue, particularly the diffusion-limited component, requires a true planar analysis (i.e. assessing structural interactions in the plane of sectioning, ignoring the relatively unimportant longitudinal O_2 diffusion gradients) in order to account for such interactions (Fig. 2). Finally, there have been a number of impressive attempts to quantify 3D microvascular topology (Zeller-Plumhoff et al. 2019), but some technical challenges remain (Al-Shammari et al. 2019).

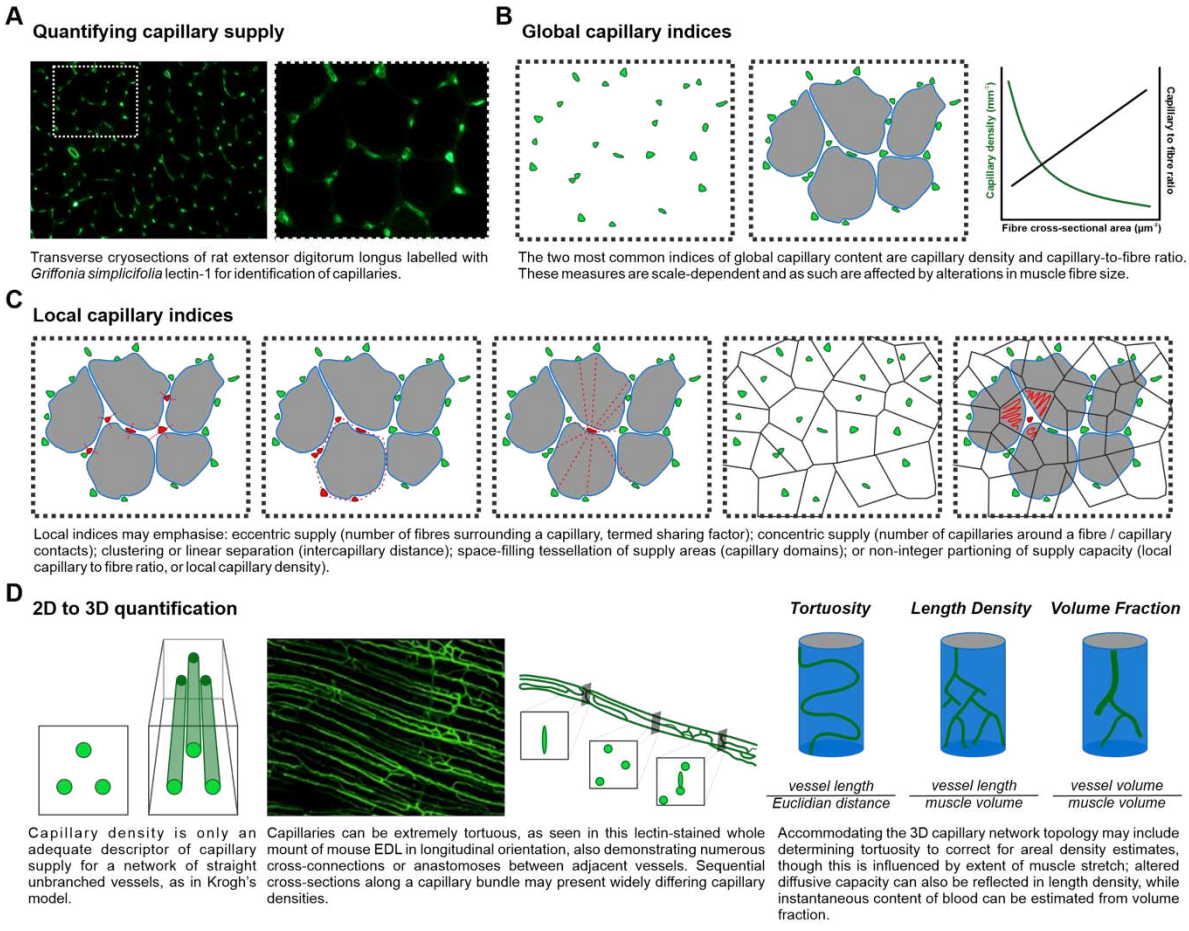

Figure 2. Developments in quantifying microvascular supply to improve descriptive power. There are numerous indices of capillarity described in the literature that aim to improve the descriptive power over the simple counts made by early histologists. Depending on the question posed, their varied analytical limitations may be acceptable (or ignored).

Implications of variable capillary distribution

The Krogh-Erlang cylinder describing radial oxygen efflux from a capillary has been widely used, and in some areas still finds utility as evidenced by regular updates and appearance in recent primary research articles (Poole et al. 2013). Indeed, an independent derivation of the process of oxygen diffusion from vascular elements, leading to the generation of a Krogh cylinder-type solution, provided an adequate explanation for oxygenation in submerged plant stems (Beckett & Armstrong 1992). However, there are up to 20 assumptions underlying this idealised, theoretical approach; many are evidently inappropriate under a range of biologically relevant conditions (Kreuzer 1982).

The key limitation of most modelling studies (whether using the Kroghian approach or not) is representation of the capillary network as a regular pattern of supply points, serving tissue with a homogeneous demand. Both these principles are evidently gross simplifications, but accommodating such spatial heterogeneity has taken a great deal of development in the analyses currently available (Al-Shammari et al. 2019). An additional

complication is the temporal heterogeneity in flow patterns observed within the microcirculation, which requires variation in distribution of both functional and structural elements to be integrated.

The construction of Krogh cylinders was based on an assumption of a perfect spatial symmetry of capillaries (Fig. 3A). This geometrical simplicity permitted an elegant and mathematically tractable description of oxygen diffusion (Krogh-Erlang formula). However, capillary distribution is variable and can be highly asymmetric (Hoofd 1985), in addition to the impossibility of close-packing cylinders to account for O_2 delivery to all areas of tissue. Equivalent Krogh cylinders are sometimes used in an attempt to account for this apparent variability. Nonetheless, the radial symmetry of Krogh cylinders still undermines the effort to capture the influence of capillary variability (Fig. 3). These observations suggest that the numerical value of mean diffusion area is not an adequate description for potential microvascular contribution to muscle performance.

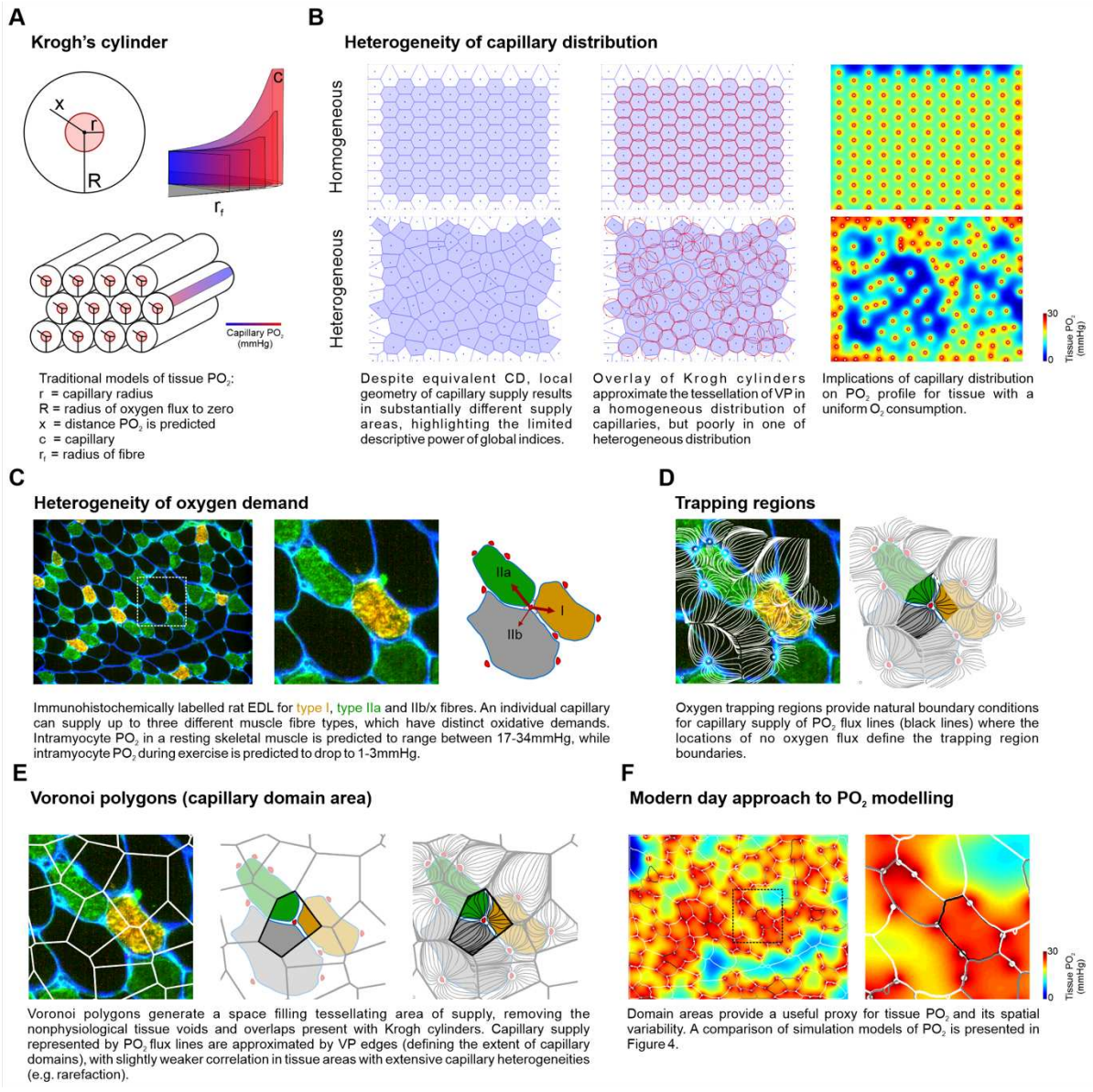

Figure 3. Microvascular distribution and implications of tissue PO_2. (A) A tubular supply area around the capillary, where R denotes the Krogh cylinder radius, r the capillary radius and x the distance from the capillary of which the equation predicts PO_2. (B) Stacking supply cylinders as described by Krogh provides a modest approximation of capillary supply area for tissue with a homogeneous distribution of capillaries, though there still exists inevitable overlapping supply regions or anoxic voids, emphasised by more physiologically appropriate heterogeneous distributions. (C) Intramuscular distribution of PO_2 is influenced by the spatial heterogeneity of supply (capillary distribution) and demand (fibre type heterogeneity). (D) A computationally intensive estimation of O_2 flux lines produces boundaries (oxygen trapping regions) that align with those of the more tractable capillary domains, to a first approximation. (E) Capillary domains offer a space-filling alternative to Krogh cylinders, that allows the effect of heterogeneous distributions to be quantified. (F) Using this approach, we can readily visualise the predicted effects of *in vivo*, or *in silico*, experimental interventions on muscle PO_2. Content based on the authors' studies.

Quantifying O_2 flux

Radial symmetry of oxygen efflux may be a reasonable approximation in close proximity of the capillary wall but interaction with neighbouring capillaries may influence its validity (Titcombe 2000), suggesting that a natural boundary condition must depend on the distribution of nearby microvessels. Incorporating the influence of adjacent/sub-adjacent capillaries can be naturally achieved by assuming that the supply area between two neighbours is bisected by a line of symmetry. Connecting these supply bisectors leads to a natural polygonal tessellation (Voronoi polygons or VPs) of the tissue plane (Fig. 3E), thereby forming a natural polygonal extension to Krogh's cylinder, sometimes referred to as a capillary domain (Hoofd et al. 1985; Egginton & Ross 1992). Importantly, this construct captures both the immediate tissue area and the influence of neighbouring vessels, and automatically characterises the variability in spatial distribution of capillaries (Al-Shammari et al. 2014). Indeed, recent results indicate that equivalent Krogh cylinders largely overestimate tissue PO_2 (Fig. 4). As an extension, VPs are less sensitive to such variabilities (Al-Shammari et al. 2012) given their close approximation to the boundary of physiological supply areas (oxygen trapping regions). Hence, VPs allow sensible boundary conditions to tissue PO_2 while also retaining some geometric simplicity. But more generally, oxygen trapping regions provide a modern version of Krogh cylinder and VPs (Al-Shammari et al. 2014b), and are ultimately the most robust to capillary variability, particularly in rarefied capillary networks (e.g. diabetes).

In addition to spatial heterogeneity of microvascular O_2 supply, spatial variability is also observed in tissue demand (Fig. 3C). In skeletal muscle, a capillary can be surrounded by a range of fibre types (I, IIa, IIb/x), which have distinct oxidative demands as well as myoglobin and lipid content. This implies oxygen demand is spatially variable nearby individual capillaries, suggesting capillary supply of oxygen is additionally influenced by the variability of the surrounding tissue demand (Zeller-Plumhoff et al. 2019) and permeability (Al-Shammari et al. 2014a). Such influence is automatically captured by Voronoi polygons through their aforementioned boundary conditions. Indeed, these boundaries allow for a natural overlap between nearby muscle fibres and the Voronoi polygon of the 'central'

capillary. Hence, just as Krogh and Erlang were able to give spatial estimates of PO_2 around a central capillary, with VPs one can do the same for every individual capillary while also accounting for the influence of nearby fibre types and capillaries. Moreover, such estimates can be further improved by considering oxygen trapping regions (Al-Shammari et al. 2014a, 2019). Finally, using the capillary domain approach offers an objective way of calculating non-integer indices of local capillary supply, which are more sensitive to physiological or pathological changes in capillarisation than more traditional indices (Egginton 1990, Egginton & Turek 1990).

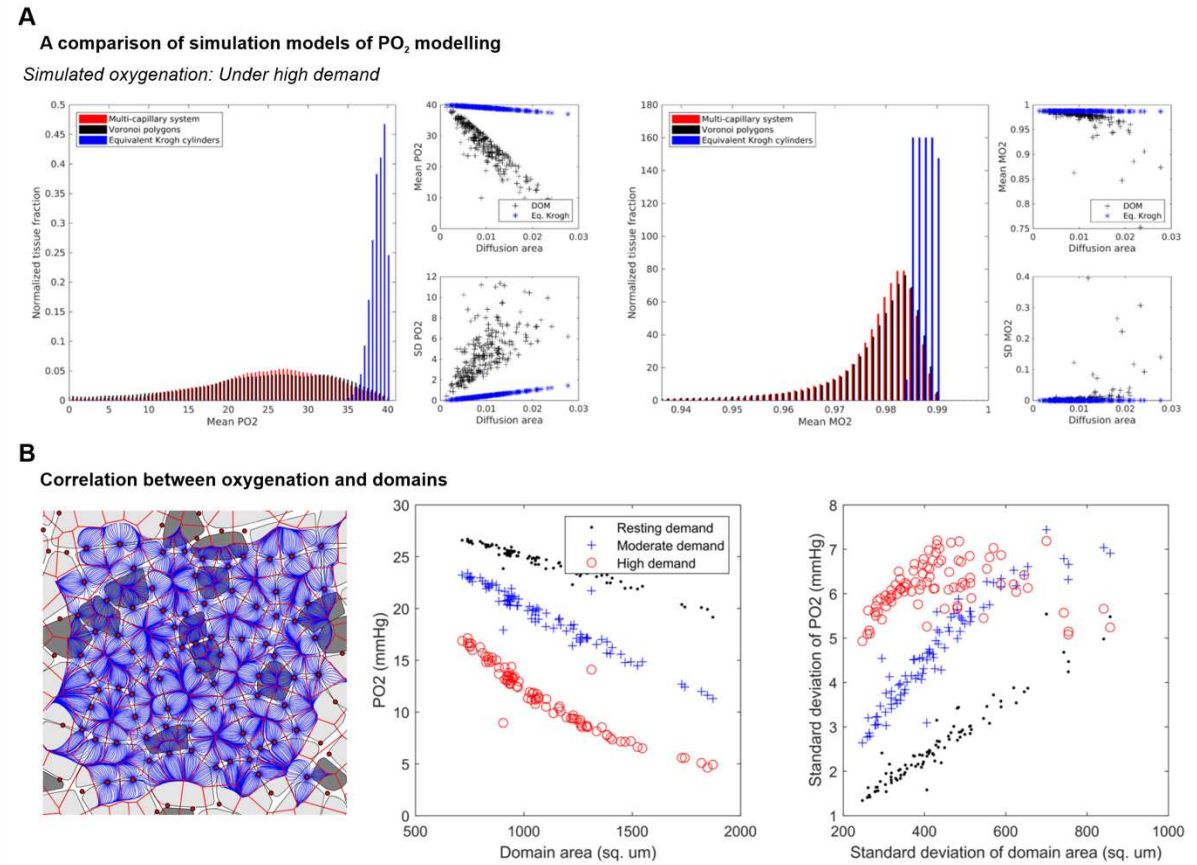

Figure 4. A comparison between models simulating muscle PO_2. (A) Krogh cylinders are based on highly symmetrical supply and only account for the numerical value of the area of supply, leading to symmetrical and peaked distribution of PO_2 (blue). In contrast, modelling PO_2 based on capillary domains (a realistic generalization of Krogh cylinder, black) accounts for the capillary diffusion/supply area, local capillary interaction and the spatial arrangement of capillaries. Although simple in their geometrical construction, these domains allow simulation models to capture the intricate details of the spatial distribution of PO_2 as predicted in multi-capillary model models (red). This is a direct result of their very good approximation of the 'natural' boundary conditions of capillary supply. (B) Capillary domains (red lines) overlaid on a digitised image of a skeletal muscle section (grey outlines), containing fast (light grey fill) and slow (dark grey fill) fibres. Using values of muscle metabolism under varying conditions of activity demonstrates an inverse relationship between the amount of tissue supplied by an individual capillary (domain area) and

resultant O_2 tension, with the tight association breaking down only in the largest supply areas at highest demand. Content based on the authors' studies.

Functional consequences of heterogeneity

It can be shown mathematically that any degree of asymmetry in location of a capillary supplying a given volume of tissue will disturb oxygen flux, irrespective of varied extraction within that domain, but the implications for individual capillary dropout in the integrated supply has until recently be difficult to prove (Egginton & Gaffney 2010). This is largely due to the fact that where capillary rarefaction occurs naturally, e.g. with diabetes or hypertension, the attendant comorbidities make it difficult to ascertain the proximal cause of any tissue dysfunction. So, in addition to interest in resolving this issue from a better mechanistic understanding of fundamental physiological principles, there are many practical reasons for pursuing this line of enquiry. An example would be in the case of heart failure, where both cardiac and skeletal muscle show impaired function (Fig. 5), and exercise intolerance is a hallmark of the condition. However, the extent to which this is due to microvascular as opposed to macrovascular insufficiency is a matter of current debate.

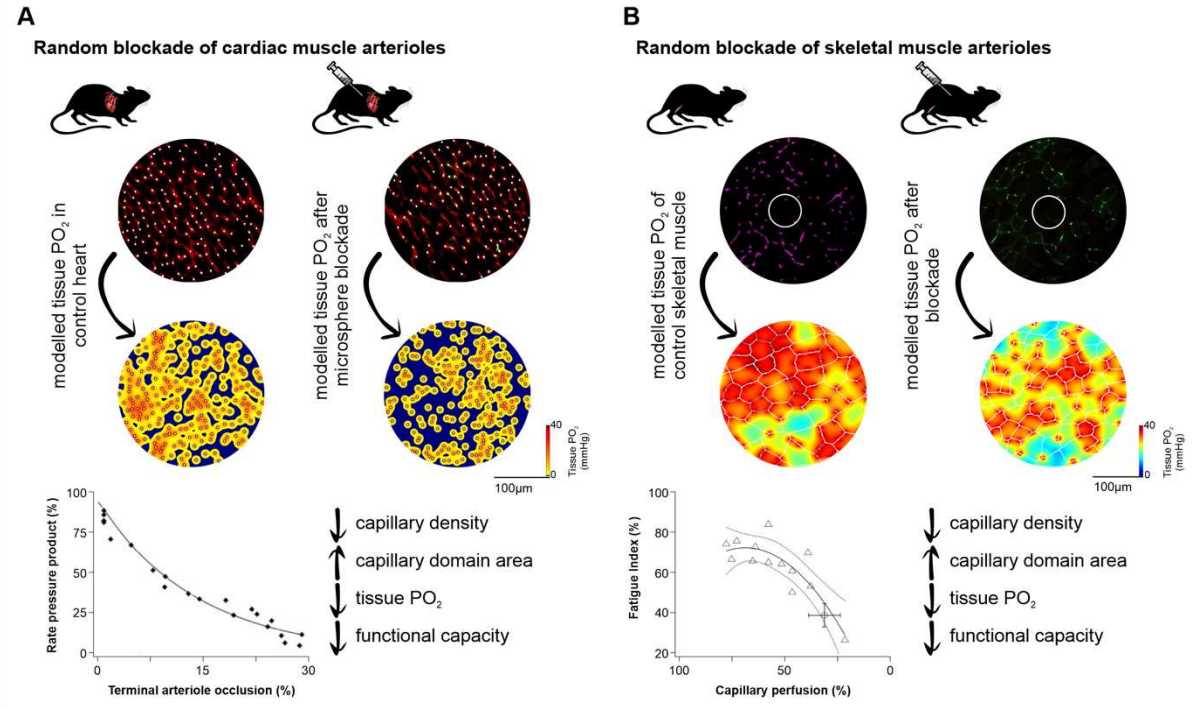

Figure 5. Functional capillary rarefaction leads to reduced cardiac and skeletal muscle performance. Recent work by Hauton *et al*. (2015) (A) and Tickle *et al*. (2020) (B) demonstrate the profound impact of random arteriole blockade (and subsequent lack of downstream capillary perfusion) on muscle function. Through injection of microspheres into the circulation, dose-response curves show the deterioration in cardiac and skeletal muscle function with decreasing capillary perfusion, accompanied by a progressive increase in area of muscle expected to develop hypoxia.

Conclusions

We consider here the influence of fine scale heterogeneity of capillary supply for peripheral O_2 supply, but of course other levels of organisation may have a significant influence on functional outcomes, e.g. the relative influence of perfusion-metabolism heterogeneity in skeletal muscle in response to differing physiological challenges (Piiper, 2000). Such considerations may help to optimise targetted angiotherapies, by e.g. focussing on macrovascular (perfusion) or microvascular (diffusion) impediment to muscle performance.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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