

## Exercise-mediated angiogenesis

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Skeletal muscle is among the most plastic of tissues, remodelling to accommodate altered demands. Exercise induces a range of adaptations, notably a growth of capillaries (angiogenesis), while inactivity results in a loss of capillaries (rarefaction). As endurance activity relies on an adequate O<sub>2</sub> supply to support oxidative phosphorylation, hypoxia within working muscle may act as an angiogenic stimulus, but additional candidates include chemical factors such as metabolic by-products (e.g. acidosis) or release of signalling molecules (e.g. VEGF, NO), and mechanical factors including response to muscle contractions (strain) or increased blood flow (hyperaemia). Optimising training interventions, for performance or rehabilitation, will benefit from better understanding of the local environment controlling the pattern of capillary distribution and its consequences for tissue oxygenation.

### Addresses

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### Introduction

Capillaries are present within almost all organs, with fine structure (continuous, fenestrated, sinusoidal) differing according to functional demand. In skeletal muscle their principal role is gaseous and metabolite exchange at the blood–tissue interface. A capillary has a narrow aperture that produces resistance to blood flow through viscous drag, generating significant levels of shear stress. However, the large cumulative lumen surface area of capillaries actually provides less resistance than that of upstream arterioles. This leads to a lower linear velocity compared to that of larger vessels, which provides a sufficiently long transit time for the diffusive exchange of oxygen and nutrients (Figure 1). Despite low perfusion velocity, the small diameter combined with low compliance means

that individual capillaries may experience high levels of shear stress, which will be elevated during exercise in an intermittent manner determined by the duty cycle involved. The potential exists to mitigate effects of systemic hypoxaemia and/or local hypoxia, and contribute to improved exercise tolerance, by expanding the capillary bed and as a consequence increase surface area and increase erythrocyte transit time. Growth of capillaries is accomplished by the process of angiogenesis, which shares some similarities but many differences with other forms of vascular growth or remodelling that are often not adequately differentiated by use of loose terminology such as ‘neovascularisation’.

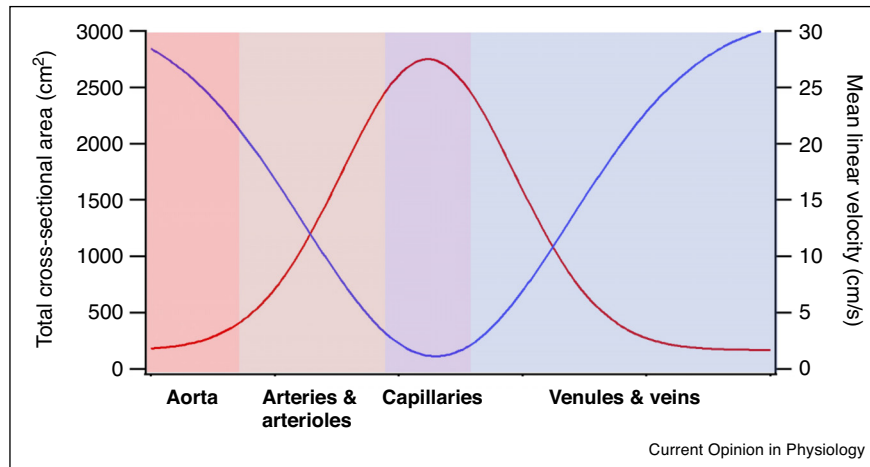
Skeletal muscle mass is an independent predictor of peak oxygen uptake (VO<sub>2</sub>) in healthy individuals and non-cachectic patients. A variety of skeletal muscle abnormalities contribute to exercise intolerance in those with cardiorespiratory limitations, that is they may be due to peripheral changes rather than to central haemodynamic dysfunction. As  $\Delta$ VO<sub>2</sub> during exercise occurs predominantly in the active muscles, due to local vasodilatation and blood shunting from the splanchnic region, this offers a viable therapeutic target. Given this relationship between skeletal muscle mass and exercise capacity, peak VO<sub>2</sub> should increase with training regimes that increase skeletal muscle bulk, hence ‘exercise as medicine’ is increasingly thought to offer a cost-effective intervention strategy. Aerobic (endurance) muscle performance relies on an adequate arterio-venous O<sub>2</sub> difference, which in turn is limited by either mitochondrial oxidative capacity or blood–tissue diffusive exchange within the microcirculation. Consequently, any supply/demand mismatch needs to have a dynamic response—acutely this may involve metabolic (functional) hyperaemia, but chronically requires growth of new vessels from the existing capillary bed (angiogenesis).

This review aims to summarise current understanding of angiogenic regulation in skeletal muscle, and identify some exciting new avenues of investigation that may help optimise microvascular performance in health and disease.

### Quantification of microvascular supply

Deriving a consensus position on what benefits accrue from exercise-induced angiogenesis, and which training regimes may be most effective, require an agreement on validated methods of quantifying size of the capillary bed. This has been a significant contributor to sometimes wide-ranging estimates of muscle capillary supply in the literature [1]. Histochemical staining has become

Figure 1



Vascular cross-sectional area (red line) and mean linear flow (blue line). Note the large cumulative surface area in the capillary bed (exchange vessels), which compensates for the slow perfusion rate to effect adequate diffusive supply of oxygen and other nutrients. Adapted from Ref. [98].

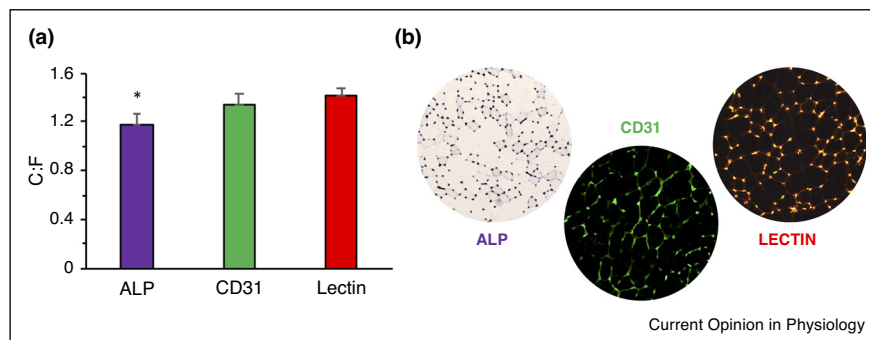
the most common method of identifying capillaries, where previously injection of dyes into the microvasculature was used [2]. Early identification of capillaries utilised a stain for alkaline phosphatase, an enzyme with high activity in the endothelium [3] but reproducibility differs among staining methods, and its validity as a marker have been questioned [1]. With the development of immunohistochemistry there are an array of antibodies available to probe for endothelial cell components, for example adhesion molecules such as CD31 (PECAM-1), and growth factor receptors such as Flk-1 and Tie-2. An increasingly common marker used to visualise capillary location are various lectins, polysaccharides that bind to the glycocalyx of capillaries, due to the relative simplicity of staining. However, alternative markers for endothelial cells generate disparate results when used to stain the same muscle sections (Figure 2a). As such, it is important

to be aware of the markers used to identify capillaries when comparing data from the literature, in particular whether they identify newly formed or mature vessels, and the varied sensitivity/specificity of markers among species in comparative studies.

### Microvascular sensors

Adaptive tissue remodelling is most likely a result of feedback regulation, so identifying the origin of proximate signals may help direct therapeutic interventions, or optimise training strategies. Key to the integrated exercise response is translation of acute reactions mediated by ergoreceptors [4], that for example, in the presence of poor capillarisation may promote the sensation of breathlessness and facilitate improved cardiac output, and translation of chronic signals into structural remodelling. Metaboreceptor stimulation by metabolite spillover is

Figure 2



(a) C:F of the mouse EDL using multiple markers for capillary location. Adapted from J Williams, PhD Thesis, University of Birmingham (2005),  $*P < 0.05$  from Lectin ( $n = 6$ ). (b) Representative histological samples. There may also be a species effect, as the discrepancy among markers in rat EDL is less pronounced (*unpublished data*). ALP; alkaline phosphatase, CD31; platelet endothelial cell adhesion molecule (PECAM-1), Lectin; biotinylated *Griffonia Simplicifolia* Lectin I.

accompanied by metabolic heat generation during muscle activity, and passive heat therapy has recently been shown to have angiogenic potential [5]. Three major candidate areas receiving a lot of attention are detection of altered tissue oxygenation, linkage with metabolic activity, and response to haemodynamic disturbance. These clearly need to invoke an appropriate signalling response on repetition (e.g. part of regular physical activity or structured exercise), as structural remodelling is energetically costly and hence endothelial responses to acute stimuli (e.g. running to catch a bus) are not readily translated into angiogenesis.

### Endothelial O<sub>2</sub> sensing

While it is clear that maximal rates of O<sub>2</sub> diffusion in muscle are limited by both the blood carrying capacity and extent of capillarisation, diffusional conductance between the microvasculature and mitochondria represents a significant limitation to maximal VO<sub>2</sub> in health and disease states [6]. The dynamic sensing of changes in local metabolic demand during exercise is therefore key to optimising aerobic capacity, and although the location has been subjected to some debate there is good evidence to suggest this is found at the level of individual capillaries, likely associated with release of ATP from erythrocytes [7]. There is clearly a need to not only provide an adequate convective supply of O<sub>2</sub> to working muscle, but also to ensure an effective distribution within a tissue of heterogeneous composition (e.g. different fibre types). Linking endothelial responses to the local environment, for example, by alteration of metabolism [8<sup>\*</sup>], to ascending dilatation within the supplying arterioles [9] provides a mechanism for such coordination. It is likely that such a feedback loop would also include stimulation of angiogenesis, providing the necessary cues to maintain optimal microvascular topology [10–12]. For example, in a recent voluntary running wheel training study on mice, exercise-induced angiogenesis was accompanied by changes in capillary fine structure and tortuosity [13]. Such a downstream response will be mediated by associated changes in the chemical and/or mechanical microenvironment surrounding capillaries.

### Endothelial chemotransduction

Intimately linked with O<sub>2</sub> sensing is the response to local disturbance in tissue oxygenation by any supply/demand mismatch, leading to pockets of relative hypoxia during muscle activity. This may invoke a range of transcriptional responses [14,15], orchestrated largely by HIF-1 $\alpha$  though requiring a more subtle regulation than implied by the usual description of a hypoxic 'switch' [16]. Investigations into release of classical pro-angiogenic signals has centred around the roles of vascular endothelial growth factor (VEGF) [17–23], as both a mitogen and vasodilator. This has expanded in recent years to include the potential involvement of exercise-induced release of endothelial microparticles and changes in microRNAs [24],

potentially modifying the response to altered levels of pro-angiogenic or angiostatic factors quantified by for example Western blot or ELISA. In addition, there is renewed interest in the role of perivascular cells in regulating adaptive angiogenesis, providing a paracrine influence of, for example, pericytes and satellite cells on the endothelium [25] offering an additional site for sensing both chemical and physical changes in the local environment and hence influencing the response to exercise. However, the role of endothelial chemotransduction in driving angiogenesis is extensively covered in the literature, largely driven by tumour angiogenesis investigations; the main players in skeletal muscle are summarised in Ref. [26] and some new angles explored in Ref. [8<sup>\*</sup>]. We shall therefore emphasise the less well reviewed concept of mechanotransduction, and identify areas of complementarity.

### Mechanotransduction-driven angiogenesis

The ability of endothelium to respond to mechanical stressors is well known, with a majority of studies emphasising the link with atheroma development and inflammation [27]. It has been noted that the signals underlying exercise-induced angiogenesis are likely multi-faceted, in both origin and effects. An impressive functional response of the microvasculature and the skeletal muscle it supports has been demonstrated across a variety of species. As little as four weeks training in humans is required to significantly increase capillary content [28–31], with similar microvascular remodelling seen after four to six weeks of voluntary wheel running or treadmill training in rodents [21,32–34,35<sup>\*</sup>]. Interestingly some changes in capillarity can be seen as early as seven days [32,33], suggesting that in these instances angiogenesis precedes the altered fibre type composition [32,36], and that microvascular changes are permissive for other adaptive responses rather than being simply reactive. However, while changes in fibre type following training usually occur very slowly, exercise-induced alterations in mitochondrial respiration are very fast, and it may be that angiogenesis is reactive to the accompanying stimuli (below).

Attempts to isolate the components largely have concentrated on elements of 1) and 2) (above), but recognition has been growing that the dynamic physical environment experienced by endothelial cells (EC) during a duty cycle (stretch and compression, elevated shear stress, increased transmural pressure) are all factors that may affect EC phenotype *in vitro*. Isolating these mechanical signals from any accompanying chemical signals *in vivo* has proved difficult, but there has been a recent surge in the translation of isolated mechanotransductive animal models [35<sup>\*</sup>,37,38] to human investigation, of particular interest is the use of passive leg exercise [39,40] and supplementation of the vasoactive  $\alpha_1$  adrenoreceptor antagonist terazosin [41<sup>\*\*</sup>,42]. Briefly, passive leg movement drives a modest increase in blood flow (hyperaemia)

and longitudinal stretch of myofibrils without the metabolic demand typically induced through active exercise [43]. Passive exercise, with no muscle activity to compound the signal, demonstrates that mechanoreceptors can drive angiogenesis *in vivo* [44]. Similarly, the well-established shear stress-induced angiogenesis model (typically through prazosin, an  $\alpha_1$  adrenoreceptor antagonist, supplemented in drinking water of rodents) has for the first time been tested in humans. Four weeks of terazosin (another vasodilator) supplementation resulted in a 12.5% increase in capillary to fibre ratio (C:F) and 24% increase in capillary density (CD) within the vastus lateralis of healthy adults. Sustained elevations in shear stress within the microcirculation induced by terazosin again represent primarily a vascular signal lacking input from surrounding muscle, and parallels the influence of functional hyperaemia on exercise-induced angiogenesis [41<sup>••</sup>]. When applied in a chronic setting, hyperaemia and the accompanying release of endogenous mediators of angiogenesis (VEGF, NO and MMP2) increase the microvascular content of skeletal muscle [40]. The associated markers of angiogenesis are also increased both within healthy adults [39] and those with peripheral arterial disease (PAD) [44]. Whether a similar translational benefit of overload-induced angiogenesis [45] could be realised is unknown.

### Emphasising quality, not quantity of microvascular supply

The functional relevance of any terazosin-driven angiogenic response (increase in CD) has yet to be fully examined. However, despite the associated increase in capacity for O<sub>2</sub> flux and metabolite removal, key for improved aerobic exercise performance, there is increasing evidence that highlights the importance of fine spatial control of capillary distribution needed to optimise oxygen transport [12,46,47]. Terazosin supplementation (an attempt to isolate the exercise hyperaemia response) showed no shift in distribution of the area of muscle supplied by individual vessels (or capillary domain [47]), representing the heterogeneity of capillary spacing, after four weeks (Figure 3a). This indicates a stochastic location of new vessels that does not affect the microvascular topology seen in controls, suggesting the shear stress-driven response is not responding to tissue metabolic demand. In contrast, the exercise response recruits targeted neovascularisation that is seen to reduce the supply area for individual capillaries, and hence optimise muscle oxygenation [46], to support the higher energy demand of active muscle (Figure 3b). The terazosin data are consistent with findings of prazosin in rodent models of diabetes where, despite a significant increase in microvasculature, there was little or no improvement in the functional readouts reported [35<sup>•</sup>].

The interaction of capillary spatial heterogeneity (supply) and muscle fibre type composition (demand) is becoming

a prominent topic of investigation in response to exercise and disease [18,48–50,51<sup>••</sup>,52]. Although strictly the demand is set by mitochondrial function, fibre type is used as a surrogate due to the difficulty of relating dynamic ATP production with capillary location on biopsy sections. We have recently highlighted the importance of spatial heterogeneity in skeletal muscle functional capacity within two distinct components of the rat EDL, a ‘mixed’ muscle often considered to be of homogeneous composition [50]. Within the same muscle it is possible to have varying functional characteristics and optimum working conditions, which are likely to be driven by functional demand (Figure 4). These data emphasise the need to understand the functionality of a muscle for the development of effective targeted rehabilitation and therapeutic regimes.

Until recently, the calculation of fine scale indices of capillary supply has been a laborious and undervalued technique, which may explain the lack of attention to this area of morphometric quantification. A handful of research groups have attempted to use more refined indices of capillary supply, specific to individual fibre types [49,53–55]. However, the methods used to derive these indices are inconsistent, and it is unclear how some offer appropriate representation of local capillary to fibre interactions. The recent boom in semi-automatic software packages for analysis of the phenotypic composition of skeletal muscle [56–59] has yet to allow researchers to extend this analysis and incorporate the fundamental oxygen supply component (capillary) onto muscle fibre boundary outlines, essential if one is to provide an anatomically realistic framework. However, a recent publication has made the first step in providing a means to generate valid local capillary indices, using a semi-automated data pipeline that processes immunohistochemically labelled skeletal muscle tissue, as illustrated in Figure 4 [51<sup>••</sup>].

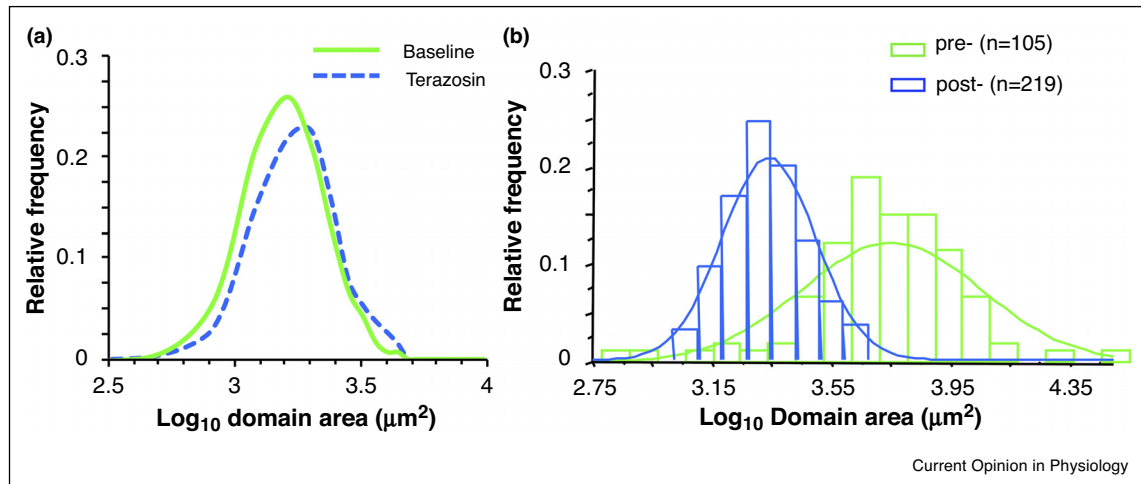
### Exercise-induced angiogenesis in disease

With national campaigns to increase levels of physical activity, and the drive for exercise as part of healthcare prescriptions, the animal data outlined above may be of particular interest for individuals with reduced exercise tolerance (e.g. chronic obstructive pulmonary disease (COPD), peripheral vascular disease (PVD) or chronic heart failure (CHF)), who could benefit from treatments that do not impose a large central cardiorespiratory or local metabolic demand, but could rectify poor microvascular supply in the periphery. The initial response in skeletal muscles may then subsequently facilitate a more active exercise program to drive central changes [35<sup>•</sup>].

Skeletal muscle inactivity [49,51<sup>••</sup>,60,61] and ageing [62,63<sup>•</sup>,64] both result in marked decreases in fibre area and microvascular supply, that correlate highly with a reduced aerobic functional capacity. Interestingly, during



Figure 3



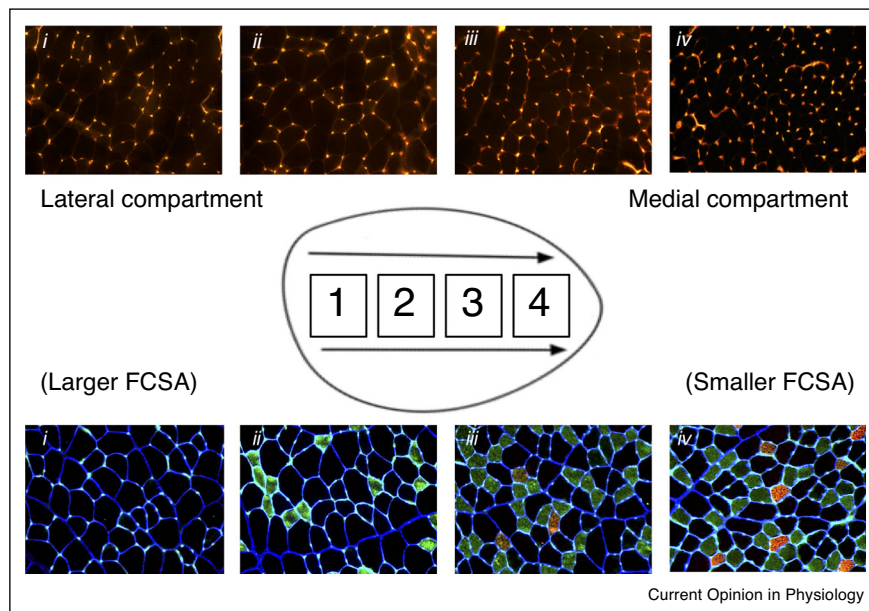
Capillary domain distributions for human angiogenic models.

(a) Percutaneous needle biopsies from *m. vastus lateralis* following supplementation with terazosin for four weeks (modified from Mortensen *et al.* [41]). (b) Percutaneous needle biopsies from the lateral quadriceps muscle, pre-isokinetic and post-isokinetic training at 60% maximum voluntary contraction using a Cybex Orthotron KT2 (Egginton and Jakeman, unpublished data). Note: both interventions produced significant increases in C: F, but the capillary domain area distributions differ greatly.

healthy aging there appears to be local feedback that coordinates capillary rarefaction in order to preserve appropriate supply to the individual fibre requirements [63]. However, there are a number of pathologies that are accompanied by impaired peripheral microvascular supply that may not maintain this relationship; including

COPD [65], CHF [66–68], diabetes [30,35,48,69,70], PVD [44] and spinal cord injury (SCI) [71]. This supply – demand mismatch is an obvious candidate driving development of the observed exercise intolerance, and hence exercise-induced angiogenesis would appear to be a worthwhile therapeutic goal.

Figure 4



Structural and functional heterogeneity within the EDL across four sample regions. The heterogeneous oxygen supply capacity of the muscle is demonstrated by density of rhodamine labelled capillaries (lectin histology top row, i–iv). The increased oxidative supply medially across the muscle is matched by an increasing oxidative demand, in the form of greater oxidative fibre content (immunohistology bottom row, i–iv) (Type I red, Type IIa green, Type IIb black).

Surprisingly, the microvascular component of SCI is a largely ignored pathology in relation to trauma, and as a potential driver of functional recovery. In chronically injured patients there is a well-maintained hyperaemic response to functional electrical stimulation [72], which points to the microvasculature as the limiting interface leading to impaired fatigue resistance [71]. Following eight weeks of locomotor training combined with epidural stimulation it is possible to significantly blunt the microvascular rarefaction seen in skeletal muscle innervated caudal to the lesion [71]. There is also evidence that passive limb movement [73] and functional electrical stimulation [72] promote muscle hyperaemia, and applied early enough following trauma these may be able to alleviate some of the capillary rarefaction observed. The parallels with exercise-induced angiogenesis are clear, but it remains to be tested whether the signals induced are the same.

Given the established role of skeletal muscle microcirculation in mediating glucose homeostasis exercise-induced angiogenesis is an attractive, non-pharmacological intervention for the burgeoning diabetic population. Again, any exercise-intolerance may be alleviated by invoking shear-stress induced angiogenesis to improve insulin sensitivity [74], with the hope of subsequently encouraging more traditional exercise therapy. The increasing prevalence of diabetes and CHF as comorbidities poses a complex therapeutic niche [75,76]. As standalone pathologies, both diabetes [30,35\*,48,69,70,77,78] and CHF [66–68,79–81] present with modest microvascular rarefaction, and those suffering with both are likely to have more pronounced rarefaction. To further complicate matters the metabolic response to these isolated pathologies is mixed, with evidence that diabetes may preserve [82] or increase oxidative demand of muscle, while CHF tends to present with a reduced oxidative capacity in locomotor muscles [83,84]. Diabetes [30,35\*,69,78,85,86] and CHF [81,84] induced capillary rarefaction are both attenuated with increased activity, with concomitant increases in oxidative demand of skeletal muscles [86,87], although the functional benefits of these structural and enzymatic changes are still to be determined. When considering the effects of exercise as a holistic response, differences in phenotype among tissues may have important consequences for targeted angiotherapy, for example, endothelium in the central and peripheral nervous systems are different from skeletal muscle, hence addressing diabetic exercise intolerance may need to distinguish between poor oxygenation and neuropathy [88,89].

Difficulty in unpicking the proximal causes and subsequent aetiology of many diseases mean that tailoring the most efficient exercise regime represents a significant challenge. It is possible to increase microvascular content using conventional endurance exercise such as cycling

and running [29,30,78,85,90], but often these modalities are poorly tolerated. However, it is also possible to modify microvascular composition and muscle metabolism through moderate intensity interval training (MIIT) [53,90,91], high intensity interval training (HIIT) [14,91,92] or resistance training [15,31,55,93], offering the potential for disease-specific and patient-specific interventions. It remains a fine balance between optimising any exercise intervention to avoid overloading the central (cardiorespiratory) systems, while inducing peripheral adaptations to modify the metabolic status (demand) and/or the microvascular composition (supply) sufficiently to impact exercise tolerance. The use of pharmacologically induced rise in shear stress and/or the passive movement to avoid these central challenges are likely to be beneficial, particularly in the early stages of rehabilitation.

## Conclusions and future directions

The realisation that even previously assumed ‘homogeneous’ muscles actually contain functionally and structurally distinct regions demands a reappraisal of both the analytical approach when handling biopsies, and interpretation of resulting data describing the outcomes of different training regimes. This will allow improved understanding of the local factors that drive tissue remodelling, and consequently help deliver more effective therapeutic exercise interventions that offer the equivalent of ‘personalised medicine’ for individual conditions. There is a wealth of recent research to suggest that exercise adaptation differs greatly among individuals who receive the same activity stimulus. This may be underpinned by differential molecular responses [94–96], but surprisingly is not accounted for by genetic variation [97]. One unexplored avenue that may complement such studies is how vicarious events and lifestyle influence the microvascular distribution in skeletal muscle, and the importance of spatial heterogeneity in utilising the intrinsic capacity for oxygen transport to tissue that ultimately may define an individual’s endurance exercise performance.

## Conflict of interest statement

Nothing declared.

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## References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Egginton S: *Morphometric Analysis of Tissue Capillary Supply, Vertebrate Gas Exchange*. Springer; 1990:73–141.

2. Krogh A: **The number and distribution of capillaries in muscles with calculations of the oxygen pressure head necessary for supplying the tissue.** *J Physiol* 1919, **52**:409-415.
3. Klingmuller G: **Demonstration of alkaline phosphatase in the capillaries.** *Hautarzt* 1958, **9**:84-88.
4. Scott AC, Francis DP, Davies LC, Ponikowski P, Coats AJS, Piepoli MF: **Contribution of skeletal muscle 'ergoreceptors' in the human leg to respiratory control in chronic heart failure.** *J Physiol* 2000, **529**:863-870.
5. Hesketh K, Shepherd SO, Strauss JA, Low DA, Cooper RG, Wagenmakers AJM, Cocks M: **Passive heat therapy in sedentary humans increases skeletal muscle Capillarisation and eNOS content but not mitochondrial density or GLUT4 content.** *Am J Physiol Heart Circ Physiol* 2019 <http://dx.doi.org/10.1152/ajpheart.00816.2018>.
6. Wagner PD: **Diffusive resistance to O<sub>2</sub> transport in muscle.** *Acta Physiol Scand* 2000, **168**:609-614.
7. Ellsworth ML, Ellis CG, Sprague RS: **Role of erythrocyte-released ATP in the regulation of microvascular oxygen supply in skeletal muscle.** *Acta Physiol* 2016, **216**:265-276.
8. Eelen G, Zeeuw Pd, Treps L, Harjes U, Wong BW, Carmeliet P: **Endothelial cell metabolism.** *Physiol Rev* 2018, **98**:3-58.  
This review provides essential reading for those who consider the endothelium to be a passive conduit for substrate delivery. This tissue is of huge importance in regulating muscle metabolism, as both a delivery interface and a modifier of numerous biomolecules. The concept is developed that in addition, the metabolism of endothelial cells is itself plastic, which offers exciting new possibilities for therapeutic manipulation of the microvascular supply in health and disease.
9. Behringer EJ, Segal SS: **Spreading the signal for vasodilatation: implications for skeletal muscle blood flow control and the effects of ageing.** *J Physiol* 2012, **590**:6277-6284.
10. Al-Shammari AA, Gaffney EA, Egginton S: **Modelling capillary oxygen supply capacity in mixed muscles: capillary domains revisited.** *J Theor Biol* 2014, **356**:47-61.
11. Deveci D, Marshall JM, Egginton S: **Relationship between capillary angiogenesis, fiber type, and fiber size in chronic systemic hypoxia.** *Am J Physiol Heart Circulatory Physiol* 2001, **281**:H241-H252.
12. Egginton S, Gaffney E: **Tissue capillary supply—it's quality not quantity that counts!** *Exp Physiol* 2010, **95**:971-979.
13. Baum O, Sollberger C, Raaflaub A, Odriozola A, Spohr G, Frese S, Tschanz SA: **Increased capillary tortuosity and pericapillary basement membrane thinning in skeletal muscle of mice undergoing running wheel training.** *J Exp Biol* 2018, **221**:jeb171819.
14. Miyamoto-Mikami E, Tsuji K, Horii N, Hasegawa N, Fujie S, Homma T, Uchida M, Hamaoka T, Kanehisa H, Tabata I, Iemitsu M: **Gene expression profile of muscle adaptation to high-intensity intermittent exercise training in young men.** *Sci Rep* 2018, **8**:16811.
15. Kon M, Ohiwa N, Honda A, Matsubayashi T, Ikeda T, Akimoto T, Suzuki Y, Hirano Y, Russell AP: **Effects of systemic hypoxia on human muscular adaptations to resistance exercise training.** *Physiol Rep* 2014, **2**:e12033.
16. Lindholm ME, Rundqvist H: **Skeletal muscle hypoxia-inducible factor-1 and exercise.** *Exp Physiol* 2016, **101**:28-32.
17. Taylor CW, Ingham SA, Hunt JE, Martin NR, Pringle JS, Ferguson RA: **Exercise duration-matched interval and continuous sprint cycling induce similar increases in AMPK phosphorylation, PGC-1 $\alpha$  and VEGF mRNA expression in trained individuals.** *Eur J Appl Physiol* 2016, **116**:1445-1454.
18. Gomes JLP, Fernandes T, Soci UPR, Silveira AC, Barretti DLM, Negrão CE, Oliveira EM: **Obesity downregulates microRNA-126 inducing capillary rarefaction in skeletal muscle: Effects of aerobic exercise training.** *Oxid Med Cell Longevity* 2017, **2017**.
19. Ishiuchi Y, Sato H, Tsujimura K, Kawaguchi H, Matsuwaki T, Yamanouchi K, Nishihara M, Nedachi T: **Skeletal muscle cell contraction reduces a novel myokine, chemokine (CXC motif) ligand 10 (CXCL10): potential roles in exercise-regulated angiogenesis.** *Biosci Biotechnol Biochem* 2018, **82**:97-105.
20. O'Carroll L, Wardrop B, Murphy RP, Ross MD, Harrison M: **Circulating angiogenic cell response to sprint interval and continuous exercise.** *Eur J Appl Physiol* 2019, **119**:743-752.
21. Herrera NA, Jesus I, Dionísio EJ, Dionísio TJ, Santos CF, Amaral SL: **Exercise training prevents dexamethasone-induced rarefaction.** *J Cardiovasc Pharmacol* 2017, **70**:194-201.
22. Bellafiore M, Battaglia G, Bianco A, Palma A: **Expression pattern of angiogenic factors in healthy heart in response to physical exercise intensity.** *Front Physiol* 2019, **10**.
23. Strömberg A, Rullman E, Jansson E, Gustafsson T: **Exercise-induced upregulation of endothelial adhesion molecules in human skeletal muscle and number of circulating cells with remodeling properties.** *J Appl Physiol* 2017, **122**:1145-1154.
24. Improta Caria AC, Nonaka CKV, Pereira CS, Soares MBP, Macambira SG, Souza BSdF: **Exercise training-induced changes in MicroRNAs: beneficial regulatory effects in hypertension, type 2 diabetes, and obesity.** *Int J Mol Sci* 2018, **19**:3608.
25. Nagahisa H, Miyata H: **Influence of hypoxic stimulation on angiogenesis and satellite cells in mouse skeletal muscle.** *PLoS One* 2018, **13**:e0207040.
26. Egginton S: **Invited review: activity-induced angiogenesis.** *Pflügers Archiv* 2009, **457**:963-977.
27. Chatterjee S: **Endothelial mechanotransduction, redox signaling and the regulation of vascular inflammatory pathways.** *Front Physiol* 2018, **9**.
28. Hoier B, Nordborg N, Andersen S, Jensen L, Nybo L, Bangsbo J, Hellsten Y: **Pro- and anti-angiogenic factors in human skeletal muscle in response to acute exercise and training.** *J Physiol* 2012, **590**:595-606.
29. Baum O, Gübeli J, Frese S, Torchetti E, Malik C, Odriozola A, Graber F, Hoppeler H, Tschanz SA: **Angiogenesis-related ultrastructural changes to capillaries in human skeletal muscle in response to endurance exercise.** *J Appl Physiol* 2015, **119**:1118-1126.
30. Walton RG, Finlin BS, Mula J, Long DE, Zhu B, Fry CS, Westgate PM, Lee JD, Bennett T, Kern PA, Peterson CA: **Insulin-resistant subjects have normal angiogenic response to aerobic exercise training in skeletal muscle, but not in adipose tissue.** *Physiol Rep* 2015, **3**:e12415.
31. Holloway TM, Snijders T, van Kranenburg J, van Loon LJC, Verdijk LB: **Temporal response of angiogenesis and hypertrophy to resistance training in young men.** *Med Sci Sports Exerc* 2018, **50**:36-45.
32. Waters RE, Rotevatn S, Li P, Annex BH, Yan Z: **Voluntary running induces fiber type-specific angiogenesis in mouse skeletal muscle.** *Am J Physiol Cell Physiol* 2004, **287**:C1342-C1348.
33. Gute D, Laughlin MH, Amann JF: **Regional changes in capillary supply in skeletal muscle of interval-sprint and low-intensity, endurance-trained rats.** *Microcirculation* 1994, **1**:183-193.
34. Degens H, Veerkamp JH, van Moerkerk HT, Turek Z, Hoofd LJ, Binkhorst RA: **Metabolic capacity, fibre type area and capillarization of rat plantaris muscle. Effects of age, overload and training and relationship with fatigue resistance.** *Int J Biochem* 1993, **25**:1141-1148.
35. Dunford EC, Leclair E, Aiken J, Mandel ER, Haas TL, Birot O, Riddell MC: **The effects of voluntary exercise and prazosin on capillary rarefaction and metabolism in streptozotocin-induced diabetic male rats.** *J Appl Physiol* 2017, **122**:492-502.  
The authors highlight a key finding that diabetes-induced rarefaction of skeletal muscle is recoverable with exercise. Treating diabetic rats with prazosin alleviates capillary rarefaction, and the combination of both exercise and prazosin has an additive benefit. Unlike a previous report on healthy rats prazosin alone was insufficient to improve glycaemic control, while exercise alone and in combination did so.
36. Andersen P, Henriksson J: **Capillary supply of the quadriceps femoris muscle of man: adaptive response to exercise.** *J Physiol* 1977, **270**:677.



37. Egginton S, Hussain A, Hall-Jones J, Chaudhry B, Syeda F, Glen KE: **Shear stress-induced angiogenesis in mouse muscle is independent of the vasodilator mechanism and quickly reversible.** *Acta Physiol* 2016, **218**:153-166.
38. Hotta K, Behnke BJ, Arjmandi B, Ghosh P, Chen B, Brooks R, Maraj JJ, Elam ML, Maher P, Kurien D *et al.*: **Daily muscle stretching enhances blood flow, endothelial function, capillarity, vascular volume and connectivity in aged skeletal muscle.** *J Physiol* 2018, **596**:1903-1917.
39. Hoier B, Rufener N, Bojsen-Møller J, Bangsbo J, Hellsten Y: **The effect of passive movement training on angiogenic factors and capillary growth in human skeletal muscle.** *J Physiol* 2010, **588**:3833-3845.
40. Hellsten Y, Rufener N, Nielsen JJ, Hoier B, Krstrup P, Bangsbo J: **Passive leg movement enhances interstitial VEGF protein, endothelial cell proliferation, and eNOS mRNA content in human skeletal muscle.** *Am J Physiol Regul Integr Comp Physiol* 2008, **294**:R975-R982.
41. Mortensen SP, Egginton S, Madsen M, Hansen JB, Munch GDW, Iepsen UW, Åkerström T, Pedersen BK, Hellsten Y: **Alpha adrenergic receptor blockade increases capillarization and fractional O<sub>2</sub> extraction and lowers blood flow in contracting human skeletal muscle.** *Acta Physiol* 2017, **221**:32-43.
- This elegant study describes the acute and chronic effects of vasodilator (terazosin) supplementation. Having supplemented participants for four weeks, muscle histology demonstrated a significant angiogenesis, in addition to an altered haemodynamic response to an acute bout of exercise. This is the first direct comparison with animal models demonstrating mechanotransduction-initiated angiogenesis in humans.
42. Nyberg M, Piil P, Kiehn OT, Maagaard C, Jørgensen TS, Egelund J, Isakson BE, Nielsen MS, Gliemann L, Hellsten Y: **Probenecid inhibits  $\alpha$ -adrenergic receptor-mediated vasoconstriction in the human leg vasculature.** *Hypertension* 2018, **71**:151-159.
43. Hoier B, Hellsten Y: **Exercise-induced capillary growth in human skeletal muscle and the dynamics of VEGF.** *Microcirculation* 2014, **21**:301-314.
44. Hoier B, Walker M, Passos M, Walker PJ, Green A, Bangsbo J, Askew CD, Hellsten Y: **Angiogenic response to passive movement and active exercise in individuals with peripheral arterial disease.** *J Appl Physiol* 2013, **115**:1777-1787.
45. Deveci D, Egginton S: **Muscle ischaemia in rats may be relieved by overload-induced angiogenesis.** *Exp Physiol* 2002, **87**:479-488.
46. Al-Shammari A, Gaffney E, Egginton S: **Modelling oxygen capillary supply to striated muscle tissues.** *Adv Appl Math* 2014:13-21. Springer.
47. Al-Shammari A, Gaffney E, Egginton S: **Re-evaluating the use of voronoi tessellations in the assessment of oxygen supply from capillaries in muscle.** *Bull Math Biol* 2012, **74**:2204-2231.
48. Kondo H, Fujino H, Murakami S, Tanaka M, Kanazashi M, Nagatomo F, Ishihara A, Roy RR: **Low-intensity running exercise enhances the capillary volume and pro-angiogenic factors in the soleus muscle of type 2 diabetic rats.** *Muscle Nerve* 2015, **51**:391-399.
49. Dirks ML, Wall BT, van de Valk B, Holloway TM, Holloway GP, Chabowski A, Goossens GH, van Loon LJC: **One week of bed rest leads to substantial muscle atrophy and induces whole-body insulin resistance in the absence of skeletal muscle lipid accumulation.** *Diabetes* 2016, **65**:2862.
50. Kissane RWP, Egginton S, Askew GN: **Regional variation in the mechanical properties and fibre-type composition of the rat extensor digitorum longus muscle.** *Exp Physiol* 2018, **103**:111-124.
51. Al-Shammari AA, Kissane RWP, Holbek S, Mackey AL, Andersen TR, Gaffney EA, Kjaer M, Egginton S: **Integrated method for quantitative morphology and oxygen transport modeling in striated muscle.** *J Appl Physiol* 2019, **126**:544-557.
- This innovative methodological paper describes the generation of a high throughput data pipeline that allows characterisation of the structural composition of skeletal muscle, with the potential of calculating local capillary indices and illustrating the consequences of changes using oxygen tension modelling. The software is free to download as a runtime version, with instructive user manuals provided.
52. Bowen TS, Herz C, Rolim NPL, Berre A-MO, Halle M, Kricke A, Linke A, da Silva GJ, Wisloff U, Adams V: **Effects of endurance training on detrimental structural, cellular, and functional alterations in skeletal muscles of heart failure with preserved ejection fraction.** *J Cardiac Fail* 2018, **24**:603-613.
53. Tan R, Nederveen JP, Gillen JB, Joanisse S, Parise G, Tarnopolsky MA, Gibala MJ: **Skeletal muscle fiber-type-specific changes in markers of capillary and mitochondrial content after low-volume interval training in overweight women.** *Physiol Rep* 2018, **6**:e13597.
54. Nederveen JP, Joanisse S, Snijders T, Ivankovic V, Baker SK, Phillips SM, Parise G: **Skeletal muscle satellite cells are located at a closer proximity to capillaries in healthy young compared with older men.** *J Cachexia Sarcopenia Muscle* 2016, **7**:547-554.
55. Snijders T, Nederveen JP, Joanisse S, Leenders M, Verdijk LB, van Loon LJC, Parise G: **Muscle fibre capillarization is a critical factor in muscle fibre hypertrophy during resistance exercise training in older men.** *J Cachexia Sarcopenia Muscle* 2017, **8**:267-276.
56. Smith LR, Barton ER: **SMASH – semi-automatic muscle analysis using segmentation of histology: a MATLAB application.** *Skeletal Muscle* 2014, **4**:21.
57. Wen Y, Murach KA, Vechetti IJ Jr, Fry CS, Vickery C, Peterson CA, McCarthy JJ, Campbell KS: **MyoVision: software for automated high-content analysis of skeletal muscle immunohistochemistry.** *J Appl Physiol* 2018, **124**:40-51.
58. Mayeuf-Louchart A, Hardy D, Thorel Q, Roux P, Gueniot L, Briand D, Mazerand A, Bouglé A, Shorte SL, Staels B *et al.*: **A high-content analysis method to study skeletal muscle with a new Fiji tool.** *Skeletal Muscle* 2018, **8**:25.
59. Desgeorges T, Liot S, Lyon S, Bouvière J, Kemmel A, Trignol A, Rousseau D, Chapuis B, Gondin J, Mounier R *et al.*: **Open-CSAM, a new tool for semi-automated analysis of myofiber cross-sectional area in regenerating adult skeletal muscle.** *Skeletal Muscle* 2019, **9**:2.
60. Coker RH, Williams RH, Wolfe RR, Hays NP, Evans WJ: **Bed rest promotes reductions in walking speed, functional parameters, and aerobic fitness in older, healthy adults.** *J Gerontol Ser A* 2014, **70**:91-96.
61. Arentson-Lantz EJ, English KL, Paddon-Jones D, Fry CS: **Fourteen days of bed rest induces a decline in satellite cell content and robust atrophy of skeletal muscle fibers in middle-aged adults.** *J Appl Physiol* 2016, **120**:965-975.
62. Suetta C, Frandsen U, Mackey AL, Jensen L, Hvid LG, Bayer ML, Petersson SJ, Schröder HD, Andersen JL, Aagaard P *et al.*: **Ageing is associated with diminished muscle re-growth and myogenic precursor cell expansion early after immobility-induced atrophy in human skeletal muscle.** *J Physiol* 2013, **591**:3789-3804.
63. Barnouin Y, McPhee JS, Butler-Browne G, Bosutti A, De Vito G, Jones DA, Narici M, Behin A, Hogrel J-Y, Degens H: **Coupling between skeletal muscle fiber size and capillarization is maintained during healthy aging.** *J Cachexia Sarcopenia Muscle* 2017, **8**:647-659.
- This study utilises a number of histochemical staining techniques to quantify changes in local capillary supply and oxidative capacity of skeletal muscle in young and old adults. The main finding is that oxidative capacity of skeletal muscle is surprisingly well maintained, and that muscle atrophy with aging is accompanied by targeted capillary rarefaction that maintains the relationship between supply and demand.
64. Huang C-C, Wang T, Tung Y-T, Lin W-T: **Effect of exercise training on skeletal muscle SIRT1 and PGC-1 $\alpha$  expression levels in rats of different age.** *Int J Med Sci* 2016, **13**:260.
65. Gouzi F, Maury J, Bughin F, Blaquièrre M, Ayoub B, Mercier J, Perez-Martin A, Pomiès P, Hayot M: **Impaired training-induced adaptation of blood pressure in COPD patients: implication of the muscle capillary bed.** *Int J Chron Obstruct Pulmon Dis* 2016, **11**:2349-2357.
66. Bowen TS, Brauer D, Rolim Natale PL, Bækkerud Fredrik H, Kricke A, Ornbostad Berre AM, Fischer T, Linke A, da Silva Gustavo J, Wisloff U, Adams V: **Exercise training reveals inflexibility of the diaphragm in an animal model of patients**



- with obesity-driven heart failure with a preserved ejection fraction. *J Am Heart Assoc* 2017, **6**:e006416.**
67. Schaufelberger M, Eriksson BO, Grimby G, Held P, Swedberg K: **Skeletal muscle fiber composition and capillarization in patients with chronic heart failure: Relation to exercise capacity and central hemodynamics.** *J Cardiac Fail* 1995, **1**:267-272.
  68. Nusz DJ, White DC, Dai Q, Phippen AM, Thompson MA, Walton GB, Parsa CJ, Koch WJ, Annex BH: **Vascular rarefaction in peripheral skeletal muscle after experimental heart failure.** *Am J Physiol Heart Circ Physiol* 2003, **285**:H1554-H1562.
  69. Machado MV, Martins RL, Borges J, Antunes BR, Estado V, Vieira AB, Tibiriçá E: **Exercise training reverses structural microvascular rarefaction and improves endothelium-dependent microvascular reactivity in rats with diabetes.** *Metab Syndr Relat Disord* 2016, **14**:298-304.
  70. Aiken J, Mandel ER, Riddell MC, Birot O: **Hyperglycaemia correlates with skeletal muscle capillary regression and is associated with alterations in the murine double minute-2/ forkhead box O1/thrombospondin-1 pathway in type 1 diabetic BioBreeding rats.** *Diabetes Vasc Dis Res* 2018, **16**:28-37.
  71. Kissane RWP, Wright O, Al'Joboori YD, Marczak P, Ichiyama RM, Egginton S: **Effects of treadmill training on microvascular remodeling in the rat after spinal cord injury.** *Muscle Nerve* 2019, **59**:370-379.
  72. Olive JL, Slade JM, Dudley GA, McCully KK: **Blood flow and muscle fatigue in SCI individuals during electrical stimulation.** *J Appl Physiol* 2003, **94**:701-708.
  73. Burns KJ, Pollock BS, Stavres J, Kilbane M, Brochetti A, McDaniel J: **Passive limb movement intervals results in repeated hyperemic responses in those with paraplegia.** *Spinal Cord* 2018, **56**:940-948.
  74. Akerstrom T, Laub L, Vedel K, Brand CL, Pedersen BK, Lindqvist AK, Wojtaszewski JFP, Hellsten Y: **Increased skeletal muscle capillarization enhances insulin sensitivity.** *Am J Physiol Endocrinol Metab* 2014, **307**:E1105-E1116.
  75. Lehrke M, Marx N: **Diabetes mellitus and heart failure.** *Am J Cardiol* 2017, **120**:S37-S47.
  76. Kristensen SL, Preiss D, Jhund PS, Squire I, Cardoso JS, Merkely B, Martinez F, Starling RC, Desai AS, Lefkowitz MP: **Risk related to pre-diabetes mellitus and diabetes mellitus in heart failure with reduced ejection fraction: insights from prospective comparison of ARNI with ACEI to determine impact on global mortality and morbidity in heart failure trial.** *Circ Heart Fail* 2016, **9**:e002560.
  77. Groen BBL, Hamer HM, Sniijders T, van Kranenburg J, Frijns D, Vink H, Loon LJC: **Skeletal muscle capillary density and microvascular function are compromised with aging and type 2 diabetes.** *J Appl Physiol* 2014, **116**:998-1005.
  78. Machado MV, Vieira AB, da Conceição FG, Nascimento AR, da Nóbrega ACL, Tibiriçá E: **Exercise training dose differentially alters muscle and heart capillary density and metabolic functions in an obese rat with metabolic syndrome.** *Exp Physiol* 2017, **102**:1716-1728.
  79. Wadowski PP, Hülsman M, Schörghenhofer C, Lang IM, Wurm R, Gremmel T, Koppensteiner R, Steinlechner B, Schwameis M, Jilka B: **Sublingual functional capillary rarefaction in chronic heart failure.** *Eur J Clin Invest* 2018, **48**:e12869.
  80. Sun G, Liu F, Xiu C: **High thoracic sympathetic block improves coronary microcirculation disturbance in rats with chronic heart failure.** *Microvasc Res* 2019, **122**:94-100.
  81. Ranjbar K, Ardakanizade M, Nazem F: **Endurance training induces fiber type-specific revascularization in hindlimb skeletal muscles of rats with chronic heart failure.** *Iran J Basic Med Sci* 2017, **20**:90-98.
  82. Gorres-Martens BK, Field TJ, Schmidt ER, Munger KA: **Exercise prevents HFD- and OVX-induced type 2 diabetes risk factors by decreasing fat storage and improving fuel utilization.** *Physiol Rep* 2018, **6**:e13783.
  83. Bowen TS, Rolim NPL, Fischer T, Bækkerud FH, Medeiros A, Werner S, Brønstad E, Rognmo O, Mangner N, Linke A *et al.*: **Heart failure with preserved ejection fraction induces molecular, mitochondrial, histological, and functional alterations in rat respiratory and limb skeletal muscle.** *Eur J Heart Fail* 2015, **17**:263-272.
  84. Esposito F, Mathieu-Costello O, Wagner PD, Richardson RS: **Acute and chronic exercise in patients with heart failure with reduced ejection fraction: evidence of structural and functional plasticity and intact angiogenic signalling in skeletal muscle.** *J Physiol* 2018, **596**:5149-5161.
  85. Mortensen SP, Winding KM, Iepsen UW, Munch GW, Marcussen N, Hellsten Y, Pedersen BK, Baum O: **The effect of two exercise modalities on skeletal muscle capillary ultrastructure in individuals with type 2 diabetes.** *Scand J Med Sci Sports* 2019, **29**:360-368.
  86. Schmederer Z, Rolim N, Bowen TS, Linke A, Wisloff U, Adams V: **Endothelial function is disturbed in a hypertensive diabetic animal model of HFpEF: moderate continuous vs. high intensity interval training.** *Int J Cardiol* 2018, **273**:147-154.
  87. Sharma NM, Rabeler B, Zheng H, Raichlin E, Patel KP: **Exercise training attenuates upregulation of p47phox and p67phox in hearts of diabetic rats.** *Oxid Med Cell Longevity* 2016, **2016**:11.
  88. Morland C, Andersson KA, Haugen ØP, Hadzic A, Kleppa L, Gille A, Rinholm JE, Palibrk V, Diget EH, Kennedy LH *et al.*: **Exercise induces cerebral VEGF and angiogenesis via the lactate receptor HCAR1.** *Nat Commun* 2017, **8**:15557.
  89. Vanlandewijck M, He L, Mäe MA, Andrae J, Ando K, Del Gaudio F, Nahar K, Lebouvier T, Laviña B, Gouveia L *et al.*: **A molecular atlas of cell types and zonation in the brain vasculature.** *Nature* 2018, **554**:475.
  90. Cocks M, Shaw CS, Shepherd SO, Fisher JP, Ranasinghe A, Barker TA, Wagenmakers AJM: **Sprint interval and moderate-intensity continuous training have equal benefits on aerobic capacity, insulin sensitivity, muscle capillarisation and endothelial eNOS/NAD(P)H oxidase protein ratio in obese men.** *J Physiol* 2016, **594**:2307-2321.
  91. Hoier B, Passos M, Bangsbo J, Hellsten Y: **Intense intermittent exercise provides weak stimulus for vascular endothelial growth factor secretion and capillary growth in skeletal muscle.** *Exp Physiol* 2013, **98**:585-597.
  92. Cochran AJ, Percival ME, Tricarico S, Little JP, Cermak N, Gillen JB, Tarnopolsky MA, Gibala MJ: **Intermittent and continuous high-intensity exercise training induce similar acute but different chronic muscle adaptations.** *Exp Physiol* 2014, **99**:782-791.
  93. Ferguson RA, Hunt JE, Lewis MP, Martin NR, Player DJ, Stangier C, Taylor CW, Turner MC: **The acute angiogenic signalling response to low-load resistance exercise with blood flow restriction.** *Eur J Sport Sci* 2018, **18**:397-406.
  94. Keller P, Vollaard N, Babraj J, Ball D, Sewell D, Timmons J: **Using systems biology to define the essential biological networks responsible for adaptation to endurance exercise training.** *Biochem Soc Trans* 2007, **35**:1306-1309.
  95. Timmons JA, Knudsen S, Rankinen T, Koch LG, Sarzynski M, Jensen T, Keller P, Scheele C, Vollaard NB, Nielsen S: **Using molecular classification to predict gains in maximal aerobic capacity following endurance exercise training in humans.** *J Appl Physiol* 2010, **108**:1487-1496.
  96. Keller P, Vollaard NB, Gustafsson T, Gallagher IJ, Sundberg CJ, Rankinen T, Britton SL, Bouchard C, Koch LG, Timmons JA: **A transcriptional map of the impact of endurance exercise training on skeletal muscle phenotype.** *J Appl Physiol* 2011, **110**:46-59.
  97. Bouchard C, Rankinen T: **Individual differences in response to regular physical activity.** *Med Sci Sports Exerc* 2001, **33**(Suppl. 6):S446-S451 discussion S452-3.
  98. Widmaier EP, Raff H, Strang KT: *Vander's Human Physiology: the Mechanisms of Body Function.* McGraw-Hill Education; 2006.