Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Physiology

Regulation of skeletal muscle blood flow during exercise

Lasse Gliemann, Camilla Vestergaard Hansen, Nicolai Rytter and Ylva Hellsten

A number of mechanisms govern the rapid and precise changes in blood flow which occur in skeletal muscle with alterations in metabolic demand. Such mechanisms include sympathetic activity, functional sympatholysis, conducted vasodilation, flow mediated dilation, and compounds which stimulate formation of endothelium derived vasodilators. Compounds identified to be of importance in vasodilation include nitric oxide, prostacyclin, potassium, and nucleotides. In this review, we briefly describe some of the basic mechanisms and present selected contemporary contributions to the field. The main focus is on basic regulation of exercise hyperemia but aspects of training, age and sex have also been included.

Address
Department of Nutrition Exercise and Sports, University of Copenhagen, Denmark

Corresponding author: Hellsten, Ylva (yhellsten@nexs.ku.dk)

Current Opinion in Physiology 2019, **10**:146–155

This review comes from a themed issue on **Exercise physiology**

Edited by **Harry B Rossiter** and **Brian Glancy**

For a complete overview see the Issue and the Editorial

Available online 8th May 2019

https://doi.org/10.1016/j.cophys.2019.05.001

Introduction

The regulation of blood flow to skeletal muscle is tightly coupled to the metabolic demand for oxygen with a change in oxygen requirement leading to a proportional change in blood flow. The precisely regulated control of blood flow serves to minimize the work of the heart, while ensuring adequate oxygen supply to the working muscle. The necessity of a precise control of blood flow to the muscle becomes evident when one considers that skeletal muscle comprises around 40% of the body mass and that muscle specific blood flow can increase almost 100-fold in going from rest to intense exercise [1]. Given the limitation in maximal cardiac output, the heart can only supply a fraction of working muscles with maximal blood flow, and during hard aerobic exercise involving larger muscle mass, vascular conductance has to be well regulated or blood pressure may fall [2].

The overall regulation of skeletal muscle blood flow is achieved through a balance between, on one hand, sympathetic vasoconstriction and circulating vasoconstrictors, and on the other hand vasodilators derived from cells in the skeletal muscle tissue, and functional sympatholysis; a compound or mechanism which reduces the vasoconstrictive effect of sympathetic activity [3]. The precise regulation of skeletal muscle blood flow is complex, involving a number of mechanisms and vasoactive compounds with close interactions (Figure 1). Although many of the separate mechanisms have been well described, our understanding of how the mechanisms interact and are integrated to allow for the required rapid, large, and precise changes in oxygen supply is limited. The integrative control of skeletal muscle blood flow thus remains one of the intriguing, yet unresolved aspects of exercise physiology.

In the present review, we focus on selected contemporary advancements in research areas related to blood flow regulation. This includes advancements in the understanding of functional sympatholysis, new findings with regard to the involvement of potassium and its interaction with other vasodilators, and new evidence for endothelial mitochondria as an important source of reactive oxygen species affecting blood vessel function. New insights into the effect of training on vasodilator control are also addressed and aspects of age and sex are included for some of the topics. We wish to emphasize that, due to the limited scope of the present review, not all new references and areas have been covered.

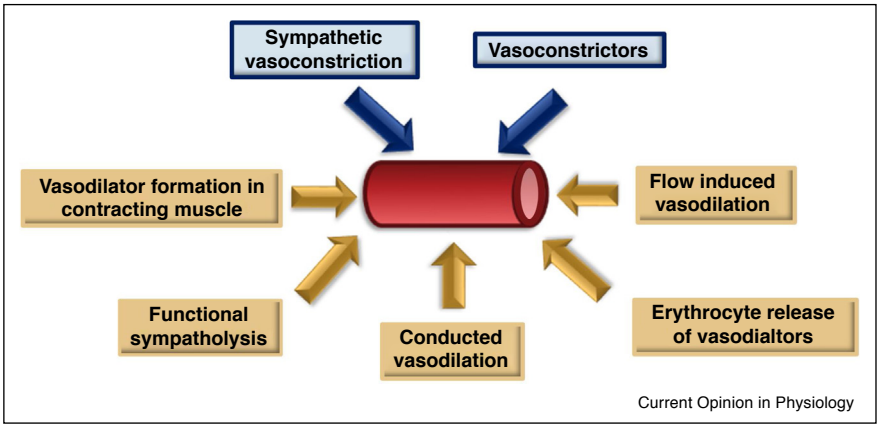
Sympathetic activity and functional sympatholysis

Sympathetic activity is increased at the onset of exercise to enhance cardiac output while maintaining blood pressure and redistributing blood flow to accommodate the enhanced oxygen demand of the working muscles. While the sympathetic outflow affects almost all vascular beds throughout the body, blood vessels within the active skeletal muscle are able to escape this sympathetic vasoconstriction, in part, by a direct reduction of the effect of noradrenaline [4], a mechanism termed functional sympatholysis [3] (Figure 2).

Mechanisms involved in functional sympatholysis

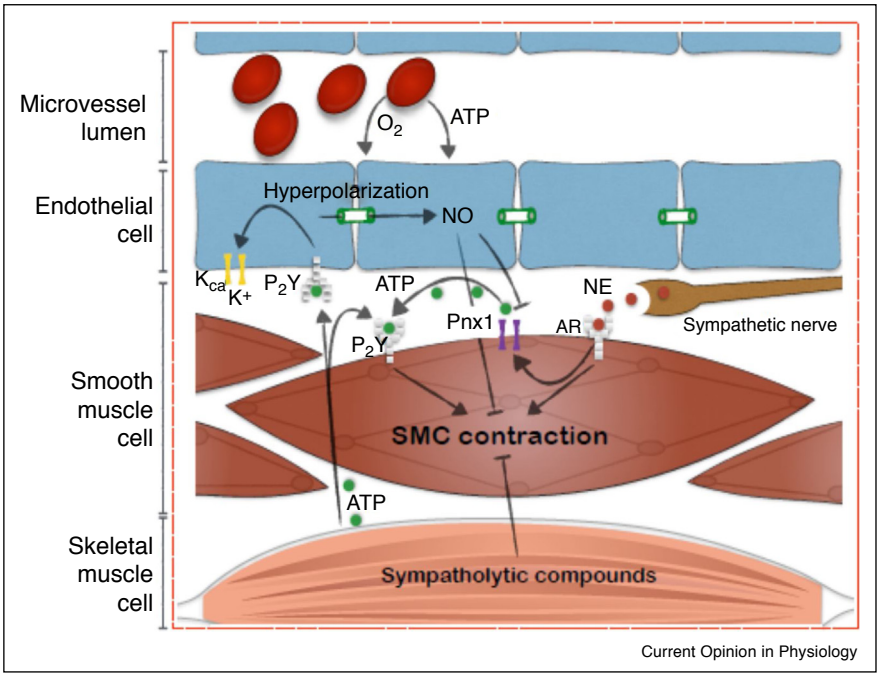
Vasoconstriction induced by stimulation of α-adrenoceptors in resting skeletal muscle is blunted during exercise in an intensity-dependent manner, but the underlying mechanisms remain unclear [4]. ATP has previously been

Figure 1



Overview over mechanisms of skeletal muscle blood flow regulation.
Blood flow is regulated by a balance between sympathetic vasoconstriction, locally formed or circulating vasoconstrictors and a number of vasodilator mechanisms. One of the unresolved questions in skeletal muscle blood flow regulation is how the different mechanisms interact and to what extent they are redundant.

Figure 2



Mechanisms of sympatholysis.
During exercise, noradrenalin (NE) release from sympathetic nerve endings is increased and binds to adrenergic receptors (AR) on the smooth muscle cell leading to vasoconstriction. At the same time, sympatholytic compounds originating from the contracting skeletal muscle and the endothelial cell overrule the constrictor signal from the sympathetic nerves, so called functional sympatholysis. Emerging evidence suggests that AR activation also causes opening of the Pannexin-1 channel (Pnx1) on the smooth muscle cell whereby ATP is released and acts as an autocrine signal molecule by activating purinergic receptors (P_2Y) on the smooth muscle cell [9,11•]. Upon unloading of oxygen (O_2) the erythrocyte also releases ATP which increases the nitric oxide (NO) release from the endothelial cell. The active skeletal muscle releases NO and since NO is able to block the opening of Pnx1 this could be a mechanism of functional sympatholysis. It should however be noted that a role of NO in functional sympatholysis in humans has been questioned. It was recently suggested that functional sympatholysis is dependent on endothelial-dependent hyperpolarization via opening of calcium activated potassium channels (K_{ca}) caused by ATP binding to P_2Y whereby intracellular calcium levels are increased [6]. Endothelial hyperpolarization conducts in both directions via gap junctions and may also by conducted directly to adjacent smooth muscle cells via myoendothelial gap junctions (not shown). Recent findings in humans show that the functional sympatholytic ability is improved after a period of exercise training in different populations [74–76].

shown to have sympatholytic properties [5], and it was recently shown that arterial infusion of ATP and acetylcholine during mild exercise attenuates the vasoconstriction elicited by simultaneous phenylephrine (α_1-agonist) infusion [6]. The latter study also proposed a role for endothelial derived hyperpolarization in functional sympatholysis based on their findings. They observed that the effect of acetylcholine in attenuating the constriction to phenylephrine during mild exercise was not reproduced by infusion of a nitric oxide donor or potassium chloride and persisted during combined blockade of nitric oxide and prostaglandin formation [6]. Although there could be other underlying mechanisms, these data are the first to indicate a role of endothelial-dependent hyperpolarization in functional sympatholysis in humans, supporting previous data from experiments in animals [7].

Role of the pannexin-1 channel in sympathetically mediated vasoconstriction and sympatholysis

In rodents, NO has been shown to have sympatholytic properties [8] and recently a novel sympatholytic mechanism involving NO and the pannexin-1 channel was proposed [9]. The background for this mechanism is the finding that binding of norepinephrine to adrenergic receptors activates the pannexin-1 channel on smooth muscle cells, leading to release of ATP from the cell, which in turn binds to constrictive purinergic receptors [10]. This mechanism, which recently was supported by data in humans [11•], potentiates the constrictive effect of sympathetic activity and is potentially an important mechanism in the regulation of vascular tone (Figure 2) [12]. A sympatholytic role for NO through inhibition of this mechanism has been proposed [9] based on the finding that NO can close the pannexin-1 channel [13]. However, the role of NO in functional sympatholysis in humans has been questioned [12,11•] and this mechanism remains to be proven.

Age related alterations in functional sympatholysis

A number of studies have shown that ageing is associated with lower exercise hyperemia [14–16], although it should be mentioned that some studies contradict these findings [17,18]. At the onset of exercise, rapid vasodilation occurs at the arteriolar level to facilitate increased perfusion of the skeletal muscle tissue. In the skeletal muscle of aged mice, this rapid onset of vasodilation is impaired [19] and recently, it was shown that the ability to overcome α-adrenergic vasoconstriction differed along the arteriolar network in young versus aged mice [20]. These findings fit with observations in humans that the initial hyperemic response to exercise is lower in aged than in young men [21]. However, currently it is uncertain whether functional sympatholysis is affected by aging *per se* since some [22] but not all studies [21,23] report impaired functional sympatholysis in aged men. One explanation for the different findings could be related to the training status of the aged subjects as the subjects in the studies differed and it has been shown that very active older men have fully preserved functional sympatholysis [24]. Moreover, it has been suggested that functional sympatholysis is related to the rate of oxidative metabolism, rather than to age *per se* is [23]. Further studies are required to clarify how age versus training status affects functional sympatholysis.

Sex specific regulation of functional sympatholysis

In young women, regulation of hemodynamics via the sympathetic nervous system differs from that of men and while α-adrenergic tone dictates vascular tone in men, β-adrenergic vasodilation offsets the vasoconstrictive tone of the α-adrenergic receptors at rest [25,26••]. Moreover, there is no linear relationship between sympathetic activity and total peripheral resistance in young women [27] and the response to sympathoexcitation is lower in women compared to men [28]. While functional sympatholysis has not been compared in young men and women, it was recently shown that functional sympatholysis was more prominent in female rats compared to male rats and that this was dependent on nitric oxide [29•]. As mentioned above, there is evidence for a lack of effect of NO in sympatholysis in humans [12]; however, nitrite supplementation improves functional sympatholysis by 50% in hypertensive post-menopausal women [30], suggesting that impaired functional sympatholysis could be related to NO bioavailability in this group. Similar to what has been observed in some studies in aged men, functional sympatholysis is lower in post-menopausal women compared to young women [31]. Moreover, it was also recently reported that habitually active post-menopausal women demonstrate a better ability for functional sympatholysis compared to their untrained counterparts [32] which is in line with findings in aged men [24].

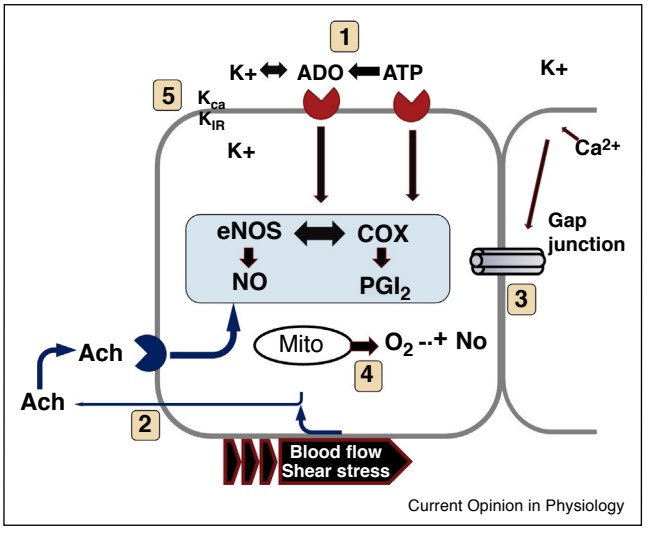
Locally formed vasoactive compounds

Endothelial cells, which line the inside of all blood vessels, are central in the formation of vasoactive compounds. A number of chemical and mechanical signals act on the surface of the endothelial cells leading to the formation of vasodilators within the cells, which diffuse to the smooth muscle cells and induce relaxation. In addition, hyperpolarization of endothelial cells, initiated via the opening of K+ channels, can be transmitted between endothelial cells via gap junctions. The hyperpolarization can then also be transmitted from endothelial cells to smooth muscle cells through myoendothelial gap junctions, leading to vasodilation [33,34]. During exercise, when blood flow to the muscle increases substantially, a number of mechanical and chemical signals of different cellular origin are thought to contribute to endothelial cell mediated vasodilation. These include 1) release of ATP from skeletal muscle cells in response to contraction; 2) release of ATP from erythrocytes in response to the off-loading of oxygen from the hemoglobin molecule; 3) blood flow mediated increase in

shear stress affecting endothelial mechanosensors; and 4) propagation of dilation along the cells of the blood vessel wall (conducted vasodilation) (Figures 1 and 3). For a more detailed review on locally formed vasoactive compounds, see Hellsten *et al.* [35].

Despite the identification of numerous putative vasodilators, no single vasodilator has been proven critical for exercise hyperemia. Studies, in which the formation of two central vasodilators, NO and prostacyclin, inhibited separately, have revealed a lack of effect on the magnitude of exercise hyperemia [36,37]. In contrast, combined inhibition of NO and prostacyclin is found to reduce leg exercise hyperemia by about 30% [38,39]. The underlying reason for these findings is most likely that vasodilators interact in a complex and redundant manner [40] whereby the impairment of one vasodilator can be compensated for by another. Such interaction may be the result of a direct chemical interaction by NO and prostacyclin [41] and NO and 11, 12 Eicosatrienoic acid [42] or be due to a functional compensation, regulated by the demand for oxygen (Figure 3).

Figure 3



Molecular mechanisms underlying endothelial cell dependent vasodilation in skeletal muscle.
Endothelial cells are influenced by a number of chemical and mechanical signals of which the majority induce vasodilation by enhancing the formation of nitric oxide and prostacyclin. **(1)** Previous observations in humans have indicated redundancy between vasodilators and recently, the first direct evidence for a complex interaction between potassium, adenosine and nitric oxide, both at the arteriolar and capillary level, has been provided [47,48••]. **(2)** Acetylcholine (Ach) binds to receptors on the endothelium leading to enhanced NO and prostacyclin formation and consequent vasodilation yet its physiological role has been questioned. Novel support for flow induced Ach formation in intact artery endothelial cells has been provided where the flow induced vasodilation was shown to be dependent on Ach and consequent NO formation [56]. **(3)** Conducted vasodilation is initiated by compounds such as Ach and by muscle contraction and occurs by propagation of endothelial hyperpolarization between endothelial cells via gap junctions. In a recent study it was demonstrated that whereas tetanic muscle contractions initiated rapid ascending vasodilation via intracellular propagation, rhythmic muscle contractions led to a slow vasodilatory effect via flow induced NO release from the feed arteries [46••]. **(4)** Enhanced presence of ROS has long been thought to be a cause of reduced NO bioavailability and endothelial dysfunction and recently, evidence has been provided for the importance of vascular cell mitochondria for ROS formation and for vascular dysfunction [62,64]. **(5)** The role of potassium in skeletal muscle blood flow regulation through its role in endothelial cell hyperpolarization and conducted vasodilation has been well established in animal models. Now also, there are studies in humans supporting involvement of potassium and endothelial hyperpolarization in exercise hyperemia [49,51•].

Potassium is important in skeletal muscle vasodilation, being responsible for hyperpolarization of endothelial and smooth muscle cells and an essential component of conducted vasodilation both in response to acetylcholine [43] and to single [44] and sustained [45] muscle contraction. An interesting recent study showed that tetanic muscle contractions initiated rapid ascending vasodilation via conduction of endothelial hyperpolarization from the arteriolar level to feed arteries whereas rhythmic muscle contractions led to a slower (10–15 s delay) vasodilatory effect via shear stress induced release of NO from the feed arteries, secondary to metabolic arteriolar vasodilation [46••]. These observations support the concept that the mechanisms of rapid vasodilation in response to the onset of muscle contraction differs from that of sustained contractions.

New insight has also been provided into the involvement of potassium in the mechanisms of redundancy between vasodilators. In a model of *in situ* blood perfused hamster cremaster muscle, it was shown that potassium inhibited arteriolar vasodilatation induced by NO and adenosine [47], and that this inhibition involved inward rectifying potassium channels (K_{IR}) and the sodium–potassium pump (Na^+/K^+-ATPase). Previous studies have demonstrated that propagated responses also occur from the level of capillaries [92,94] and in a follow-up study, Lamb *et al.* assessed the physiological role of vasodilator redundancy for vasodilation during muscle stimulation at the level of capillaries [48••]. The study identified multiple redundancy between potassium, NO and adenosine and revealed that NO and adenosine became critical for contraction-induced vasodilation only when voltage-gated potassium channels (K_V) were inhibited, [48••]. These novel findings provide further support for propagated signalling from capillaries and show that vasodilator redundancies are physiologically important and contribute to the overall regulation of exercise hyperemia (Figure 3).

Because of obvious technical limitations, conducted vasodilation via endothelial cell hyperpolarization has not been verified in human skeletal muscle *in vivo*. Nevertheless, intriguing indirect evidence for

potassium induced endothelial hyperpolarization has been provided in studies showing an involvement of potassium and K_{IR} channels in skeletal muscle blood flow at onset of and during steady state exercise [49] but also recently during hypoxia at rest [50]. Moreover, novel experiments have demonstrated that muscle contraction amplifies endothelium-dependent vasodilator signaling via K_{IR} channel activation [51•]. Collectively, these rodent and human data point at distinct regulatory mechanisms of potassium, including in interaction with other vasodilator mechanisms, and emphasize the importance of potassium in skeletal muscle blood flow regulation.

Acetylcholine potently induces local and conducted [33] vasodilation and has been used as gold standard for assessing vascular function in isolated arteries and in humans (e.g. in Refs. [52,53]). A longstanding question has, nevertheless, been whether acetylcholine is formed in the vasculature and whether it is a physiologically relevant vasodilator *in vivo*. Previous studies have provided evidence for formation of acetylcholine in cultured endothelial cells [54] and a more recent study reported acetylcholine release from isolated arterioles, but only in hypertensive animals [55]. However, in an interesting recent study, Wilson *et al.* provided convincing evidence for acetylcholine formation from mitochondria derived Acyl CoA in intact endothelial cells of mesenteric and carotid arteries in parallel with determination of a functional effect [56]. The study demonstrated a flow activated release of acetylcholine and an acetylcholine dependent shear stress induced increase in flow [56]. These findings add support for a physiological role for acetylcholine in blood flow regulation and, although attempts to prove a physiological role of acetylcholine in human exercise hyperemia using inhibitors have failed so far [57,58], this novel observation should spark interest in assessing a potential role of acetylcholine in muscle blood flow regulation.

Endothelial mitochondria as a source of ROS formation

Although in young healthy individuals, NO availability is unlikely to be a primary factor limiting blood flow, there is good evidence that age related vascular dysfunction is dependent on attenuated NO bioavailability. Factors affecting an inadequate level of available NO include reduced NO production and excessive ROS formation in the endothelium, the latter leading to the removal of NO by a reaction with superoxide. Enzymes such as NADPH oxidase and xanthine oxidase are well documented sources of ROS in vascular cells [91,93] but it is only recently that evidence has been provided for ROS formation in their mitochondria.

Mitochondria are essential for energy production and normal cellular function but can also be a major source of ROS [59,60]. The energy expenditure in endothelial cells is much lower than in cells such as myocytes and, accordingly, mitochondrial content in endothelial cells is relatively low, around 2–5% of cell volume compared to around 36% of cell volume in cardiac myocytes [60]. Nevertheless, ROS formation via mitochondria in endothelial cells is likely to be physiologically important and contribute to endothelial dysfunction [61]. Accordingly, recent studies have shown that mitochondrial respiratory capacity of isolated skeletal muscle human feed arteries declines with age [62] and in parallel, an enhanced mitochondrial production of ROS is observed, as assessed by measurements of superoxide production by isolated arteries [63•]. Indirect proof for enhanced mitochondrial ROS production in vascular cells with aging has, furthermore, been obtained by treatment with the mitochondrial antioxidant mitoQ in rodents [63•] and of human muscle feed arteries [64]. In both of the latter studies, treatment with mitoQ also improved NO availability and vascular function [63•,64]. Moreover, chronic oral intake of mitoQ for six weeks in aged humans has been shown to reduce oxidative stress and improve vascular function [65•]. Combined, these novel data suggest that mitochondria is an important source of ROS in vascular cells and constitute a contributing factor to the reduction in NO bioavalability and consequently endothelial dysfunction in aging.

Effects of exercise training on blood flow regulation

It is well known that exercise training has a major impact on cardiovascular health and leads to improvements in blood vessel function [66]. On the functional side, aerobic exercise training can improve maximal blood flow to the muscle whereas submaximal blood flow remains unaltered or decreases after a period of training, a decrease thought to be due, at least in part, to an improved capacity for oxygen extraction [67].

Training induced improvements in blood vessel function have mainly focused on endothelial function and the ability for sympatholysis and to a lesser extent on smooth muscle cell function. Improvements in endothelial function with training has encompassed enhancements in the prostacyclin system [68] and enhanced bioavailability of NO, through upregulation of eNOS and downregulation of NADPH oxidase [69]. On the vasoconstrictor side, training reduces vasoconstriction via the endothelin-1 system [70,71] and via sympathetic activity by improving functional sympatholysis [72,73].

Effect of training on sympatholysis

Functional sympatholysis can be assessed by the ability of acute exercise to oppose the vasoconstriction induced by infusion of tyramine, a compound which induces release of noradrenalin from the nerve terminal. Recent findings in humans show that the ability for functional

sympatholysis is improved after a period of exercise training in young healthy individuals [74] and in individuals with cardiovascular disease [75,76]. Previous findings have also shown that it is preserved with lifelong physical activity in older adults [24]. The mechanisms underlying the improvements are incompletely understood, but ATP, which is a sympatholytic compound in humans [5], has been shown to be higher in lifelong physically active men than in sedentary individuals [24], and lowered with a period of immobilization concomitant with changes in the ability for functional sympatholysis [77]. In addition, a lower constrictive effect of sympathetic activity after training may also be explained by a reduction in α-adrenergic responsiveness to noradrenalin in trained muscle, as examined by infusion of tyramine at rest [75]. In rodents, the improvement in functional sympatholysis with training has been convincingly coupled to NO [72,73].

Effect of training on endothelial mechanotransduction

Shear stress is a potent activator of eNOS and believed to be central both for acute modulation of blood flow [78] and for macrovascular [79] and microvascular [80] adaptations in response to exercise training. Changes in vascular function could be related to alterations in shear stress sensing by the expression and sensitivity of mechanosensory proteins located on the endothelial membrane, thereby influencing the degree of activation of eNOS [81]. This aspect was investigated in two recent studies where in the first study, two weeks of very high glucose intake was found to lower exercise hyperemia and the flow response to passive movement, in parallel with changes in both expression and phosphorylation status of several mechanotransduction proteins, including platelet adhesion molecule 1 (PECAM-1) [82•]. In the second study the effect of training on endothelial mechanosensors and eNOS were assessed in parallel with hyperemia in response to passive leg movement in young and old individuals [83]. Overall, shear stress sensing did not appear to be greatly affected but an interesting finding was again the apparent plasticity of PECAM-1 where both aging and exercise training had a marked influence on both amount and phosphorylation state of this protein. Given the central role of shear stress for vascular function, mechanistic studies to elucidate the identity and potential role of endothelial mechanosensors *in vivo* are warranted.

The influence of physical activity on vascular aging

Aging is described to negatively influence both vascular compliance and vasodilator capacity but although aging clearly influences the cardiovascular system, much of the observed aging effects are related to years of inactivity rather than chronological age [84]. Studies comparing masters athletes and their sedentary counterparts have shown that regular physical activity through life opposes the age induced detrimental changes in vascular function both in men [85] and women (L. Gliemann, A. Tamariz Elleman, N. Rytter and Y. Hellsten, unpublished results). One molecular mechanism underlying the effects of aging on the vascular system is endothelial senescence which is associated with a reduced capacity for NO formation [86], but so far there has been little evidence for the influence of aging and physical activity on endothelial cell senescence in humans *in vivo*. This aspect was addressed by the parallel examination of vascular function and the venous endothelial cell senescence markers p53, p16, and p21 in young and old active and sedentary individuals [87]. Endothelial cell senescence and vascular dysfunction were found to be more prominent in the older sedentary group compared to the young and, importantly, also more prominent than in the older active group. Combined, these results clearly suggest that habitual physical activity oppose the cardiovascular health decline otherwise associated with age and suggest that the effect of physical activity on endothelial cell senescence may be an underlying mechanism.

A final aspect which should be mentioned with regard to training and aging is that postmenopausal women may not attain similar improvements in macrovascular or microvascular function by aerobic training as age matched men [88–90]. To what extent this blunted adaptation in women can be overcome by aspects such as earlier initiation of training after menopause or more intense training [68] should be further evaluated.

Summary and conclusion

In the past few years, a large number of interesting novel discoveries have been made in the area of skeletal muscle blood flow regulation during exercise, clearly contributing with important advancements in the field. Of note are the significant new insights attained in the area of functional sympatholysis and in vasodilator formation and interaction where the previously well-known role of potassium in propagated vasodilation, has been expanded with new evidence for a role of potassium in vasodilator redundancy and in endothelial hyperpolarization and sympatholysis in humans. Of particular interest in the area of vasodilators is also the new evidence for endothelial release of Ach and its physiological role in flow induced vasodilation.

Several recent studies have addressed multiple aspects of interactions between vasodilator mechanisms and vasodilators highlighting the importance of integration of the systems. Yet, despite the many valuable previous and novel findings, we still have an incomplete understanding of this essential area of interaction and integration of the known vasodilator mechanisms. Studies such as that of Sinkler and Segal [46••], examining aspects of fast propagated responses, flow mediated dilation and metabolically driven vasodilation in the same model with two different contraction stimuli are highly valuable in this regard.

The importance of oxygen requirement in the control of blood flow and, thus, the crucial aspect of how the demand for oxygen is sensed should be emphasized. To date we have a proposed mechanism for oxygen sensing in muscle; the attractive theory of ATP release from the red blood cell in response to oxygen desaturation, but evidence for the physiological relevance of this mechanism is lacking. One of the most groundbreaking future contributions in the area of regulation of exercise hyperemia would clearly be direct proof for how the demand for oxygen is sensed at the skeletal muscle level, whether it is through erythrocytes or through another mechanism.

Finally, the novel discoveries in the area of how blood flow regulating mechanisms are affected by aging and exercise training are important, providing insight into the essential role of physical activity to counteract the effects of age and into some of the molecular mechanisms underlying the beneficial effects of physical activity. Future studies in this area should also focus on women as most studies so far have been conducted in men and distinct sex differences are apparent.

Conflict of interest statement

Nothing declared.

Acknowledgements

The authors gratefully acknowledge research funding from The Independent Research Fund Denmark - Medical Sciences, Lundbeck Foundation and The Danish Ministry of Culture Research Fund for Sports Science.

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. Saltin B: **Exercise hyperaemia: magnitude and aspects on regulation in humans**. *J Physiol* 2007, **583**:819-823.

2. Calbet JAL, Jensen-Urstad M, van Hall G, Holmberg HC, Rosdahl H, Saltin B: **Maximal muscular vascular conductances during whole body upright exercise in humans**. *J Physiol* 2004, **558**:319-331.

3. Remensnyder JP, Mitchell JH, Sarnoff SJ: **Functional sympatholysis during muscular activity. Observations on influence of carotid sinus on oxygen uptake**. *Circul Res* 1962, **11**:370-380.

4. Saltin B, Mortensen SP: **Inefficient functional sympatholysis is an overlooked cause of malperfusion in contracting skeletal muscle**. *J Physiol* 2012, **590**:6269-6275.

5. Rosenmeier JB, Hansen J, González-Alonso J: **Circulating ATP-induced vasodilatation overrides sympathetic vasoconstrictor activity in human skeletal muscle**. *J Physiol* 2004, **558**:351-365.

6. Hearon CM, Kirby BS, Luckasen GJ, Larson DG, Dinenno FA: **Endothelium-dependent vasodilatory signalling modulates α1-adrenergic vasoconstriction in contracting skeletal muscle of humans**. *J Physiol* 2016, **594**:7435-7453.

7. Crane GJ, Gallagher N, Dora KA, Garland CJ: **Small- and intermediate-conductance calcium-activated K+ channels provide different facets of endothelium-dependent hyperpolarization in rat mesenteric artery**. *J Physiol* 2003, **553**:183-189.

8. Thomas GD, Victor RG: **Nitric oxide mediates contraction-induced attenuation of sympathetic vasoconstriction in rat skeletal muscle**. *J Physiol* 1998, **506**:817-826.

9. Nielsen MS: **Sympathetic vasoconstriction takes an unexpected pannexin detour**. *Sci Signal* 2015, **8** fs4–fs4.

10. Billaud M, Lohman AW, Straub AC, Looft-Wilson R, Johnstone SR, Araj CA, Best AK, Chekeni FB, Ravichandran KS, Penuela S, Laird DW, Isakson BE: **Pannexin1 regulates α1-adrenergic receptor-mediated vasoconstriction**. *Circul Res* 2011, **109**:80-85.

11. • Nyberg M, Piil P, Kiehn OT, Maagaard C, Jørgensen TS, Egelund J, Isakson BE, Nielsen MS, Gliemann L, Hellsten Y: **Probenecid inhibits α-adrenergic receptor-mediated vasoconstriction in the human leg vasculature**. *Hypertension* 2018, **71**:151-159.
First human evidence of the importance of the Pannexin-1 hemichannel for sympathetic vasoconstriction. Pharmacological inhibition of the Pannexin-1 channel attenuated the vasoconstrictive effect of increased noradrenalin and shows the functional involvement of the channel in regulation of vascular resistance.

12. Dinenno FA, Joyner MJ: **Blunted sympathetic vasoconstriction in contracting skeletal muscle of healthy humans: is nitric oxide obligatory?** *J Physiol* 2003, **553**:281-292.

13. Lohman AW, Weaver JL, Billaud M, Sandilos JK, Griffiths R, Straub AC, Penuela S, Leitinger N, Laird DW, Bayliss DA, Isakson BE: **S-nitrosylation inhibits pannexin 1 channel function**. *J Biol Chem* 2012, **287**:39602-39612.

14. Wahren J, Saltin B, Jorfeldt L, Pernow B: **Influence of age on the local circulatory adaptation to leg exercise**. *Scand J Clin Lab Inv* 1974, **33**:79-86.

15. Proctor DN, Shen PH, Dietz NM, Eickhoff TJ, Lawler LA, Ebersold EJ, Loeffler DL, Joyner MJ: **Reduced leg blood flow during dynamic exercise in older endurance-trained men**. *J Appl Physiol* 1998, **85**:68-75.

16. Lawrenson L, Richardson RS, Poole JG, Kim J, Brown C, Patel P: **Vascular and metabolic response to isolated small muscle mass exercise: effect of age**. *Am J Physiol Heart Circ Physiol* 2003, **285**:H1023-H1031.

17. Proctor DN, Newcomer SC, Koch DW, Le KU, MacLean DA, Leuenberger UA: **Leg blood flow during submaximal cycle ergometry is not reduced in healthy older normally active men**. *J Appl Physiol* 2003, **94**:1859-1869.

18. Rudroff T, Weissman JA, Bucci M, Seppänen M, Kaskinoro K, Heinonen I, Kalliokoski KK: **Positron emission tomography detects greater blood flow and less blood flow heterogeneity in the exercising skeletal muscles of old compared with young men during fatiguing contractions**. *J Physiol* 2014, **592**:337-349.

19. Jackson DN, Moore AW, Segal SS: **Blunting of rapid onset vasodilatation and blood flow restriction in arterioles of exercising skeletal muscle with ageing in male mice**. *J Physiol* 2010, **588**:2269-2282.

20. Sinkler SY, Fernando CA, Segal SS: **Differential α-adrenergic modulation of rapid onset vasodilatation along resistance networks of skeletal muscle in old versus young mice**. *J Physiol* 2016, **594**:6987-7004.

21. Piil P, Jørgensen TS, Egelund J, Rytter N, Gliemann L, Bangsbo J, Hellsten Y, Nyberg M: **Effects of aging and exercise training on leg hemodynamics and oxidative metabolism in the transition from rest to steady-state exercise: role of cGMP signaling**. *Am J Physiol Regul Integr Comp Physiol* 2018, **315**:R274-R283.

22. Dinenno FA, Masuki S, Joyner MJ: **Impaired modulation of sympathetic alpha-adrenergic vasoconstriction in contracting forearm muscle of ageing men**. *J Physiol* 2005, **567**:311-321.

23. Wray DW, Nishiyama SK, Richardson RS: **Role of α_1-adrenergic vasoconstriction in the regulation of skeletal muscle blood flow with advancing age**. *Am J Physiol Heart Circ Physiol* 2009, **296**:H497-H504.

24. Mortensen SP, Nyberg M, Winding K, Saltin B: **Lifelong physical activity preserves functional sympatholysis and purinergic signalling in the ageing human leg**. *J Physiol* 2012, **590**:6227-6236.

25. Kneale BJ, Chowienczyk PJ, Brett SE, Coltart DJ, Ritter JM: **Gender differences in sensitivity to adrenergic agonists of forearm resistance vasculature**. *J Am Coll Cardiol* 2000, **36**:1233-1238.

26. •• Hart EC, Charkoudian N, Wallin BG, Curry TB, Eisenach J, Joyner MJ: **Sex and ageing differences in resting arterial pressure regulation: the role of the β-adrenergic receptors**. *J Physiol* 2011, **589**:5285-5297.

The findings of Hart *et al.* are very important in that they show a distinct sex difference in resting arterial blood pressure regulation of men and women. Their data demonstrate that the beta-adrenergic receptors offset alpha adrenergic constriction in young women but not in young men.

27. Hart EC, Joyner MJ, Wallin BG, Charkoudian N: **Sex, ageing and resting blood pressure: gaining insights from the integrated balance of neural and haemodynamic factors**. *J Physiol* 2012, **590**:2069-2079.

28. Hogarth AJ, Mackintosh AF, Mary DA: **Gender-related differences in the sympathetic vasoconstrictor drive of normal subjects**. *Clin Sci* 2007, **112**:353-361.

29. • Just TP, Delorey DS: **Sex differences in sympathetic vasoconstrictor responsiveness and sympatholysis**. *J Appl Physiol* 2017, **123**:128-135.

This study shows that functional sympatholysis is more prominent in female rats compared to male rats and furthermore provides evidence for that the underlying mechanism is dependent on nitric oxide.

30. Caldwell JT, Sutterfield SL, Post HK, Craig JC, Baumfalk DR, Copp SW, Ade CJ: **Impact of acute dietary nitrate supplementation during exercise in hypertensive women**. *Med Sci Sports Exerc* 2018, **51**:1014-1021 Epub ahead of print.

31. Kruse NT, Hughes WE, Ueda K, Hanada S, Feider AJ, Iwamoto E, Bock JM, Casey DP: **Impaired modulation of postjunctional α1 - but not α2-adrenergic vasoconstriction in contracting forearm muscle of postmenopausal women**. *J Physiol* 2018, **596**:2507-2519.

32. Kruse NT, Hughes WE, Hanada S, Ueda K, Bock JM, Iwamoto E, Casey DP: **Evidence of a greater functional sympatholysis in habitually aerobic trained postmenopausal women**. *J Appl Physiol* 2018, **124**:583-591.

33. Segal SS, Duling BR: **Flow control among microvessels coordinated by intercellular conduction**. *Science* 1986, **234**:868-870.

34. Little TL, Xia J, Duling BR: **Dye tracers define differential endothelial and smooth muscle coupling patterns within the arteriolar wall**. *Circul Res* 1995, **76**:498-504.

35. Hellsten Y, Nyberg M, Jensen LG, Mortensen SP: **Vasodilator interactions in skeletal muscle blood flow regulation**. *J Physiol* 2012, **590**:6297-6305.

36. Shoemaker JK, Naylor HL, Pozeg ZI, Hughson RL: **Failure of prostaglandins to modulate the time course of blood flow during dynamic forearm exercise in humans**. *J Appl Physiol* 1996, **81**:1516-1521.

37. Rådegran G, Saltin B: **Nitric oxide in the regulation of vasomotor tone in human skeletal muscle**. *Am J Physiol* 1999, **276**:H1951-H1960.

38. Boushel R, Langberg H, Gemmer C, Olesen J, Crameri R, Scheede C, Sander M, Kjaer M: **Combined inhibition of nitric oxide and prostaglandins reduces human skeletal muscle blood flow during exercise**. *J Physiol* 2002, **543**:691-698.

39. Mortensen SP, Gonzalez-Alonso J, Damsgaard R, Saltin B, Hellsten Y: **Inhibition of nitric oxide and prostaglandins, but not endothelial-derived hyperpolarizing factors, reduces blood flow and aerobic energy turnover in the exercising human leg**. *J Physiol* 2007, **581**:853-861.

40. Murrant CL, Sarelius IH: **Local control of blood flow during active hyperaemia: what kinds of integration are important?** *J Physiol* 2015, **593**:4699-4711.

41. Nicholson WT, Vaa B, Hesse C, Eisenach JH, Joyner MJ: **Aging is associated with reduced prostacyclin-mediated dilation in the human forearm**. *Hypertension* 2009, **53**:973-978.

42. Bauersachs J, Popp R, Hecker M, Sauer E, Fleming I, Busse R: **Nitric oxide attenuates the release of endothelium-derived hyperpolarizing factor**. *Circulation* 1996, **94**:3341-3347.

43. Yuill KH, Yarova P, Kemp-Harper BK, Garland CJ, Dora KA: **A novel role for HNO in local and spreading vasodilatation in rat mesenteric resistance arteries**. *Antioxid Redox Signal* 2011, **14**:1625-1635.

44. Armstrong ML, Dua AK, Murrant CL: **Potassium initiates vasodilatation induced by a single skeletal muscle contraction in hamster cremaster muscle**. *J Physiol* 2007, **581**:841-852.

45. Milkau M, Köhler R, de Wit C: **Crucial importance of the endothelial K+ channel SK3 and connexin40 in arteriolar dilations during skeletal muscle contraction**. *FASEB J* 2010, **24**:3572-3579.

46. •• Sinkler SY, Segal SS: **Rapid versus slow ascending vasodilatation: intercellular conduction versus flow-mediated signalling with tetanic versus rhythmic muscle contractions**. *J Physiol* 2017, **595**:7149-7165.

By comparing the effect of tetanic and rhythmic muscle contraction the authors were able to show that, whereas rapid onset vasodilation occurs via conduction of endothelial hyperpolarization, rhythmic muscle contractions led to a delayed vasodilatory effect via shear stress induced release of NO from the feed arteries, secondary to metabolic arteriolar vasodilation.

47. Lamb IR, Murrant CL: **Potassium inhibits nitric oxide and adenosine arteriolar vasodilatation via K(IR) and Na(+)/K(+) ATPase: implications for redundancy in active hyperaemia**. *J Physiol* 2015, **593**:5111-5126.

48. •• Lamb IR, Novielli NM, Murrant CL: **Capillary response to skeletal muscle contraction: evidence that redundancy between vasodilators is physiologically relevant during active hyperaemia**. *J Physiol* 2018, **596**:1357-1372.

This study provides important further proof for contraction induced vasodilator responses from the capillary level and novel evidence for a physiological role for vasodilator interactions/redundancy at the capillary level.

49. Crecelius AR, Luckasen GJ, Larson DG, Dinenno FA: **KIR channel activation contributes to onset and steady-state exercise hyperemia in humans**. *Am J Physiol Heart Circ Physiol* 2014, **307**: H782-H791.

50. Racine ML, Crecelius AR, Luckasen GJ, Larson DG, Dinenno FA: **Inhibition of Na^+/K^+-ATPase and KIR channels abolishes hypoxic hyperaemia in resting but not contracting skeletal muscle of humans**. *J Physiol* 2018, **596**:3371-3389.

51. • Hearon CM, Richards JC, Racine ML, Luckasen GJ, Larson DG, Dinenno FA: **Amplification of endothelium-dependent vasodilatation in contracting human skeletal muscle: role of KIR channels**. *J Physiol* 2019, **597**:1321-1335.

The data in this study provide evidence in humans for endothelial hyperpolarization in exercise hyperemia and show that endothelial hyperpolarization influences acetylcholine mediated vasodilation during exercise.

52. Oltman CL, Parker JL, Adams HR, Laughlin MH: **Effects of exercise training on vasomotor reactivity of porcine coronary arteries**. *Am J Physiol* 1992, **263**:H372-H382.

53. Martin CM, Beltran-Del-Rio A, Albrecht A, Lorenz RR, Joyner MJ: **Local cholinergic mechanisms mediate nitric oxide-dependent flow-induced vasorelaxation in vitro**. *Am J Physiol* 1996, **270**:H442-H446.

54. Milner P, Kirkpatrick KA, Ralevic V, Toothill V, Pearson J, Burnstock G: **Endothelial cells cultured from human umbilical vein release ATP, substance P and acetylcholine in response to increased flow**. *Proc Biol Sci* 1990, **241**:245-248.

55. Zou Q, Leung SWS, Vanhoutte PM: **Transient receptor potential channel opening releases endogenous acetylcholine, which contributes to endothelium-dependent relaxation induced by mild hypothermia in spontaneously hypertensive rat but not Wistar-Kyoto rat arteries**. *J Pharmacol Exp Ther* 2015, **354**:121-130.

56. Wilson C, Lee MD, McCarron JG: **Acetylcholine released by endothelial cells facilitates flow-mediated dilatation**. *J Physiol* 2016, **594**:7267-7307.

57. Shoemaker JK, Halliwill JR, Hughson RL, Joyner MJ: **Contributions of acetylcholine and nitric oxide to forearm blood flow at exercise onset and recovery**. *Am J Physiol* 1997, **273**:H2388-H2395.

58. Hellsten Y, Krustrup P, Iaia FM, Secher NH, Bangsbo J: **Partial neuromuscular blockade in humans enhances muscle blood flow during exercise independently of muscle oxygen uptake and acetylcholine receptor blockade**. *Am J Physiol Regul Integr Comp Physiol* 2009, **296**:R1106-R1112.

59. Potente M, Carmeliet P: **The link between angiogenesis and endothelial metabolism**. *Annu Rev Physiol* 2017, **79**:43-66.

60. Caja S, Enríquez JA: **Mitochondria in endothelial cells: sensors and integrators of environmental cues**. *Redox Biol* 2017, **12**:821-827.

61. Ito A, Shiroto T, Godo S, Saito H, Tanaka S, Ikumi Y, Kajitani S, Satoh K, Shimokawa H: **Important roles of endothelial caveolin-1 in endothelium-dependent hyperpolarization and ischemic angiogenesis in mice**. *Am J Physiol Heart Circ Physiol* 2019, **316**: H900-H910.

62. Park S-Y, Kwon OS, Andtbacka RHI, Hyngstrom JR, Reese V, Murphy MP, Richardson RS: **Age-related endothelial dysfunction in human skeletal muscle feed arteries: the role of free radicals derived from mitochondria in the vasculature**. *Acta Physiol (Oxf)* 2018, **222**:e12893.

63. • Gioscia-Ryan RA, LaRocca TJ, Sindler AL, Zigler MC, Murphy MP, Seals DR: **Mitochondria-targeted antioxidant (MitoQ) ameliorates age-related arterial endothelial dysfunction in mice**. *J Physiol* 2014, **592**:2549-2561.
The study provides some of the first evidence for ROS formation in mitochondria of vascular cells of isolated mice arteries and shows that mitochondrial ROS reduces NO bioavailability and may be one of the underlying causes of endothelial dysfunction.

64. Park SH, Kwon OS, Park SY, Weavil JC, Andtbacka RHI, Hyngstrom JR, Reese V, Richardson RS: **Vascular mitochondrial respiratory function: the impact of advancing age**. *Am J Physiol Heart Circ Physiol* 2018, **315**:H1660-H1669.

65. • Rossman MJ, Santos-Parker JR, Steward CAC, Bispham NZ, Cuevas LM, Rosenberg HL, Woodward KA, Chonchol M, Gioscia-Ryan RA, Murphy MP, Seals DR: **Chronic supplementation with a mitochondrial antioxidant (MitoQ) improves vascular function in healthy older adults**. *Hypertension* 2018, **71**:1056-1063.
The study provides evidence in humans for a role for mitochondria derived ROS in vascular dysfunction in older adults. The study treated older adults with endothelial dysfunction with the mitochondria specific antioxidant MitoQ for six weeks and found a 42% imprvement in flow mediated dilation.

66. Hellsten Y, Nyberg M: **Cardiovascular adaptations to exercise training**. *Compr Physiol* 2015, **6**:1-32.

67. Mortensen SP, Egginton S, Madsen M, Hansen JB, Munch GDW, Iepsen UW, Akerstrom T, Pedersen BK, Hellsten Y: **Alpha adrenergic receptor blockade increases capillarization and fractional O_2 extraction and lowers blood flow in contracting human skeletal muscle**. *Acta Physiol (Oxf)* 2017, **221**:32-43.

68. Nyberg M, Egelund J, Mandrup CM, Nielsen MB, Mogensen AS, Stallknecht B, Bangsbo J, Hellsten Y: **Early postmenopausal phase is associated with reduced prostacyclin-induced vasodilation that is reversed by exercise training: the copenhagen women study**. *Hypertension* 2016, **68**:1011-1020.

69. Cocks M, Shaw CS, Shepherd SO, Fisher JP, Ranasinghe AM, Barker TA, Tipton KD, Wagenmakers AJM: **Sprint interval and endurance training are equally effective in increasing muscle microvascular density and eNOS content in sedentary males**. *J Physiol* 2013, **591**:641-656.

70. Van Guilder GP, Westby CM, Greiner JJ, Stauffer BL, DeSouza CA: **Endothelin-1 vasoconstrictor tone increases with age in healthy men but can be reduced by regular aerobic exercise**. *Hypertension* 2007, **50**:403-409.

71. Nyberg M, Mortensen SP, Hellsten Y: **Physical activity opposes the age-related increase in skeletal muscle and plasma endothelin-1 levels and normalizes plasma endothelin-1 levels in individuals with essential hypertension**. *Acta Physiol (Oxf)* 2013, **207**:524-535.

72. Jendzjowsky NG, DeLorey DS: **Role of neuronal nitric oxide in the inhibition of sympathetic vasoconstriction in resting and contracting skeletal muscle of healthy rats**. *J Appl Physiol* 2013, **115**:97-106.

73. Mizuno M, Iwamoto GA, Vongpatanasin W, Mitchell JH, Smith SA: **Exercise training improves functional sympatholysis in spontaneously hypertensive rats through a nitric oxide-dependent mechanism**. *Am J Physiol Heart Circ Physiol* 2014, **307**:H242-H251.

74. Piil P, Jørgensen TS, Egelund J, Gliemann L, Hellsten Y, Nyberg M: **Effect of high-intensity exercise training on functional sympatholysis in young and older habitually active men**. *Transl Sports Med* 2018, **1**:37-45.

75. Mortensen SP, Nyberg M, Gliemann L, Thaning P, Saltin B, Hellsten Y: **Exercise training modulates functional sympatholysis and α-adrenergic vasoconstrictor responsiveness in hypertensive and normotensive individuals**. *J Physiol* 2014, **592**:3063-3073.

76. Munch GW, Iepsen UW, Ryrsø CK, Rosenmeier JB, Pedersen BK, Mortensen SP: **Effect of 6 wk of high-intensity one-legged cycling on functional sympatholysis and ATP signaling in patients with heart failure**. *Am J Physiol Heart Circ Physiol* 2018, **314**:H616-H626.

77. Mortensen SP, Mørkeberg J, Thaning P, Hellsten Y, Saltin B: **Two weeks of muscle immobilization impairs functional sympatholysis but increases exercise hyperemia and the vasodilatory responsiveness to infused ATP**. *Am J Physiol Heart Circ Physiol* 2012, **302**:H2074-H2082.

78. Pohl U, Lamontagne D: **Impaired tissue perfusion after inhibition of endothelium-derived nitric oxide**. *Basic Res Cardiol* 1991, **86(Suppl. 2)**:97-105.

79. Carter HH, Dawson EA, Birk GK, Spence AL, Naylor LH, Cable NT, Thijssen DHJ, Green DJ: **Effect of SR manipulation on conduit artery dilation in humans**. *Hypertension* 2013, **61**:143-150.

80. Zhou A, Egginton S, Hudlicka O, Brown MD: **Internal division of capillaries in rat skeletal muscle in response to chronic vasodilator treatment with alpha1-antagonist prazosin**. *Cell Tissue Res* 1998, **293**:293-303.

81. Tzima E, Irani-Tehrani M, Kiosses WB, Dejana E, Schultz DA, Engelhardt B, Cao G, DeLisser H, Schwartz MA: **A mechanosensory complex that mediates the endothelial cell response to fluid shear stress**. *Nature* 2005, **437**:426-431.

82. • Gliemann L, Rytter N, Lindskrog M, Slingsby MHL, Akerstrom T, Sylow L, Richter EA, Hellsten Y: **Endothelial mechanotransduction proteins and vascular function are altered by dietary sucrose supplementation in healthy young male subjects**. *J Physiol* 2017, **595**:5557-5571.
This study shows for the first time that a period of high sugar intake influences mechanotransduction proteins and exercise hyperemia and thus indicates a detrimental role of glucose on vascular function.

83. Gliemann L, Rytter N, Pill P, Nilton J, Lind T, Nyberg M, Cocks M, Hellsten Y: **The endothelial mechanotransduction protein platelet endothelial cell adhesion molecule-1 is influenced by aging and exercise training in human skeletal muscle**. *Front Physiol* 2018, **9**:1807.

84. Seals DR: **Edward F. Adolph Distinguished Lecture: the remarkable anti-aging effects of aerobic exercise on systemic arteries**. *J Appl Physiol* 2014, **117**:425-439.

85. Nyberg M, Blackwell JR, Damsgaard R, Jones AM, Hellsten Y, Mortensen SP: **Lifelong physical activity prevents an age-related reduction in arterial and skeletal muscle nitric oxide bioavailability in humans**. *J Physiol* 2012, **590**:5361-5370.

86. Matsushita H, Chang E, Glassford AJ, Cooke JP, Chiu CP, Tsao PS: **eNOS activity is reduced in senescent human endothelial cells: preservation by hTERT immortalization**. *Circul Res* 2001, **89**:793-798.

87. Rossman MJ, Kaplon RE, Hill SD, McNamara MN, Santos-Parker JR, Pierce GL, Seals DR, Donato AJ: **Endothelial cell senescence with aging in healthy humans: prevention by habitual exercise and relation to vascular endothelial function**. *Am J Physiol Heart Circ Physiol* 2017, **313**:H890-H895.

88. Pierce GL, Eskurza I, Walker AE, Fay TN, Seals DR: **Sex-specific effects of habitual aerobic exercise on brachial artery flow-mediated dilation in middle-aged and older adults**. *Clin Sci* 2011, **120**:13-23.

89. Moreau KL, Stauffer BL, Kohrt WM, Seals DR: **Essential role of estrogen for improvements in vascular endothelial function with endurance exercise in postmenopausal women**. *J Clin Endocrinol Metab* 2013, **98**:4507-4515.

90. Santos-Parker JR, Santos-Parker KS, McQueen MB, Martens CR, Seals DR: **Habitual aerobic exercise and circulating proteomic patterns in healthy adults: relation to indicators of healthspan**. *J Appl Physiol* 2018, **125**:1646-1659.

91. Cai H, Harrison DG: **Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress**. *Circulation Research* 2000, **87**:840-844.

92. Cohen KD, Sarelius IH: **Muscle contraction under capillaries in hamster muscle induces arteriolar dilatation via KATP channels and nitric oxide**. *J Physiol* 2002, **539**:547-555.

93. de Zeeuw P, Wong BW, Carmeliet P: **Metabolic adaptations in diabetic endothelial cells**. *Circ J* 2015, **79**:934-941.

94. Mitchell D, Yu J, Tyml K: **Comparable effects of arteriolar and capillary stimuli on blood flow in rat skeletal muscle**. *Microvasc Res* 1997, **53**:22-32.