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REVIEW

Functional and structural adaptations of the coronary macro- and microvasculature to regular aerobic exercise by activation of physiological, cellular, and molecular mechanisms: ESC Working Group on Coronary Pathophysiology and Microcirculation position paper

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Abstract

Regular aerobic exercise (RAEX) elicits several positive adaptations in all organs and tissues of the body, culminating in improved health and well-being. Indeed, in over half a century, many studies have shown the benefit of RAEX on cardiovascular outcome in terms of morbidity and mortality. RAEX elicits a wide range of functional and structural adaptations in the heart and its coronary circulation, all of which are to maintain optimal myocardial oxygen and nutritional supply during increased demand. Although there is no evidence suggesting that oxidative metabolism is limited by coronary blood flow (CBF) rate in the normal heart even during maximal exercise, increased CBF and capillary exchange capacities have been reported. Adaptations of coronary macro- and microvessels include outward remodelling of epicardial coronary arteries, increased coronary arteriolar size and density, and increased capillary surface area. In addition, there are adjustments in the neural and endothelial regulation of coronary macrovascular tone. Similarly, there are several adaptations at the level of microcirculation, including enhanced (such as nitric oxide mediated) smooth muscle-dependent pressure-induced myogenic constriction and upregulated endothelium-dependent/shear-stress-induced dilation, increasing the range of diameter change. Alterations in the signalling interaction between coronary vessels and cardiac metabolism have also been described. At the molecular and cellular level, ion channels are key players in the local coronary vascular adaptations to RAEX, with enhanced activation of influx of Ca^{2+} contributing to the increased myogenic tone (via voltage-gated Ca^{2+} channels) as well as the enhanced endothelium-dependent dilation (via TRPV4 channels). Finally, RAEX elicits

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a number of beneficial effects on several haemorheological variables that may further improve CBF and myocardial oxygen delivery and nutrient exchange in the microcirculation by stabilizing and extending the range and further optimizing the regulation of myocardial blood flow during exercise. These adaptations also act to prevent and/or delay the development of coronary and cardiac diseases.

Keywords Autonomic nervous system • Haemodynamic forces • Molecular signalling • Ion channels • Haemorheology

1. Introduction

Regular aerobic exercise (RAEX) elicits adaptations in all tissues and organs of the body,[1,2] that culminate in improved health and well-being.[3,4] Indeed, in over half a century, many studies have shown benefits of RAEX on cardiovascular outcome in terms of morbidity and mortality data.[3] One important effect of RAEX is that it elicits a wide range of functional and structural adaptations in the heart and its coronary circulation,[1,2] both in large and microvessels. However, the molecular and cellular mechanisms underlying these adaptations remain incompletely understood. In this article, we review the structural and functional adaptations of the coronary circulation to RAEX, with a particular focus on the cellular and molecular mechanisms underlying these adaptations.

2. Overall cardiovascular adaptations to regular exercise

During acute exercise commands from the central nervous system and exercise pressure reflexes determine the cardiovascular response.[5] At the onset of exercise, heart rate increases mainly through vagal withdrawal, with sympathetic activity starting to contribute at heart rates of 100 beats/min.[6] With exercise continuing, both metabolic and mechanical signals from active skeletal muscle groups provide feedback signals to the cardiovascular centres in the brain through afferent nervous fibres to balance oxygen delivery with metabolic demand.[7] Contracting skeletal muscle produces vasodilator metabolites which override sympathetically mediated vasoconstriction,[8,9] resulting in a decrease in local vascular resistance and increased skeletal muscle flow, while in inactive skeletal muscle and other tissues vascular resistance increases allowing the redistribution of cardiac output and maintenance or, in most cases, an increase in systemic arterial blood pressure.[1,8,10]

Repeated activation of the sympathetic system results in a reduction of sympathetic activity.[11] Numerous studies have found that the effects of regular exercise on cardiac autonomic modulation consist of lowering sympathetic activity and greater vagal modulation.[12–14] The autonomic nervous system (ANS) modulatory effects are not only observed during exercise but also immediately post-exercise, reflected in a reduction of peripheral resistance (post-exercise hypotension), more rapid heart rate recovery, and lower resting heart rate (55–60 vs. 72–74 L/min). In addition to the greater room for heart-rate (HR) increases, dilations of peripheral vessels allow to achieve a much greater cardiac output during exercise.[15] For example, one of the underlying mechanisms is the upregulated nitric oxide (NO) signalling of peripheral resistance vessels as it was shown in exercised rats that the NO releasing substance acetylcholine (ACh) elicited greater systemic blood pressure reduction in exercised than in sedentary rats.[16] Also, RAEX via activating the renal–adrenal function and increasing baroreflex sensitivity prepares the cardiovascular (CV) system for the greater workload in exercised individuals as compared to sedentary individuals (*Figure 1*).[17,18]

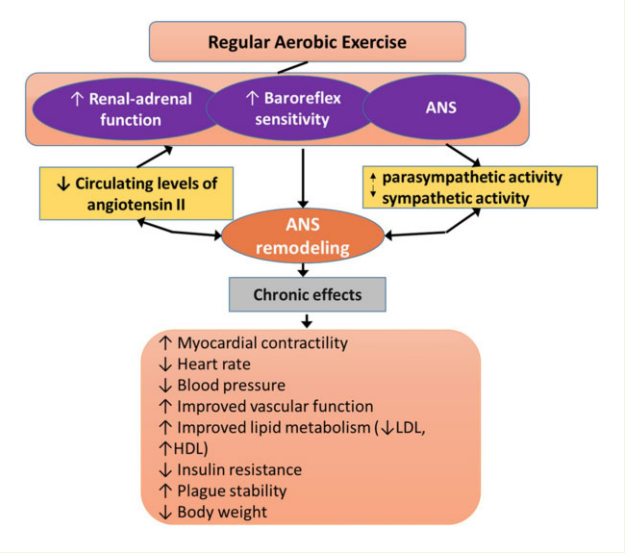

Figure 1 Overall effects of RAEX on the regulation of cardiovascular function by the autonomic nervous system (ANS). In addition to ANS changes, local adaptations, such as myocardial hypertrophy and vascular metabolic adaptations are also important that are not included in this figure. ANS, autonomic nervous system.

RAEX results in a wide range of structural and functional adaptations of the heart and coronary circulation.[1,2] For example, RAEX results in moderate concentric cardiac hypertrophy—with an increase in left ventricular end-diastolic diameter and a similar increase in left ventricular wall thickness—that enables a greater maximal stroke volume, cardiac output and hence body oxygen delivery.[1] In addition, RAEX lowers HR at rest and during submaximal exercise—through a decrease in sympathetic and an increase in parasympathetic drive—and this lower HR is accompanied by a lower myocardial oxygen demand.[1]

During exercise systemic blood pressure can reach 200 mmHg or even higher values, whereas cardiac output (blood flow) can increase from 5 to 25 L/min or more. In coronary arterial tree, these changes elicit substantial increases in perfusion pressure and four- to six-fold increase in coronary blood flow (CBF). In addition, coronary vascular wall is sensitive to changes in haemodynamic forces (pressure, shear stress) and translate these haemodynamic signals initially to functional and then structural changes, such as changes in the regulation of vasomotor tone and later remodelling of vascular wall and vascular network. Thus, these mechanisms play important roles in the adaptation of coronary arterial microvessels, which enable the coronary circulation to respond adequately to higher flow demands during exercise.

Indeed, RAEX also leads to a plethora of coronary vascular adaptations that act to maintain optimal myocardial oxygen supply in the

presence of RAEX-induced structural and functional cardiac alterations. While there is no evidence to suggest that in the normal heart CBF capacity limits aerobic metabolism even during maximal exercise, an increase in myocardial oxygen supply following RAEX could enhance maximal performance of the heart. The blood's oxygen transport capacity is typically maintained following RAEX.[1] Myocardial oxygen extraction of the left ventricular myocardium may slightly increase following RAEX,[19] but this effect is modest at best, as—even in the untrained state—oxygen extraction is already near maximal. Consequently, an increase in oxygen delivery must stem principally from an increase in blood flow. Coronary vascular adaptations in response to exercise training can be divided into functional adaptations (adaptations of vasomotor control) and structural (vascular remodelling and angiogenesis).[19] Functional adaptations include changes in neurohumoral and local vascular control mechanisms, that is, myogenic, endothelial, and metabolic control of vasomotor tone.

3. Structural adaptations of the coronary circulation to RAEX

3.1 Structural adaptation of large coronary vessels to RAEX

Numerous studies have shown that RAEX increases large coronary artery diameters. Increased coronary artery size has been reported in rodents, large animals, but also in humans.[19–23] These enlargements are proportional to the increase in left ventricular mass in athletes compared to healthy sedentary individuals,[22–24] and evidence suggests that this outward remodelling occurs to normalize the shear stress acting on the epicardial coronary artery wall. Haskell *et al.*[25] reported no difference in angiography measured cross-sectional areas of left main, left anterior descending, or right coronary artery between sedentary individuals vs. ultra-distance runners, under resting conditions. In contrast, the increases in coronary cross-sectional areas produced by nitroglycerin were positively correlated with aerobic exercise capacity, suggesting that the increased artery size was only apparent during nitroglycerin-induced dilation.[25] Hildick-Smith *et al.*[26] also found a larger nitroglycerin-induced dilation of the left anterior descending coronary artery in athletes compared to sedentary men, and Kozàkovà *et al.*[20] observed that a dipyridamole also produced a greater vasodilation of the left main coronary artery in athletes compared to healthy sedentary individuals. These results are consistent with the concept that RAEX stimulates outward growth of the proximal coronary arteries that is proportional to the level of cardiac hypertrophy, and that the vasodilator capacity of epicardial coronary arteries is greater after RAEX. The simultaneous changes in vascular and cardiac morphology indicate that the most important aim is to provide sufficient blood and oxygen to working cardiac muscle.

3.2 Structural adaptations of coronary microvessels to RAEX

Recent studies showed that increasing intensity of 4-week treadmill RAEX program elicited structural and functional changes in isolated coronary arterioles (~120 μm in diameter at 50 mm Hg).[27] Compared to the sedentary group arterioles isolated from RAEX-animals had reduced wall stress due to thicker walls (*Figure 2*), accompanied by increased distensibility, and a decreased elastic modulus. Reduced wall stress allows a greater range for regulation of vasomotor tone.

The coronary circulation provides ~1 mL of blood to each gram of myocardium, every minute, 24 h a day, every day of a person's life. During heavy exercise CBF can increase as much as four- to six-fold. Coronary transport reserve capacity consists of an ability to increase CBF (change in perfusion pressure and diameter) and capillary exchange (change in surface area and permeability) above resting levels.[1,28] In normal healthy subjects RAEX increases coronary oxygen transport through an increase in capillary exchange capacity in conjunction with an increase CBF capacity.[19]

Extensive research with experimental animals demonstrates that RAEX leads to cardiac hypertrophy with capillary angiogenesis commensurate with the increased cardiac mass. Thus, RAEX matches angiogenesis of capillaries to cardiac hypertrophy so that capillarization is maintained in the normal range in rats,[19,29] dogs,[30–32] and swine.[33] Evidence indicates that capillary endothelial cell division and capillary sprouting are increased in the first weeks of RAEX leading to a temporary increase in capillary densities that were no longer different from sedentary swine after 16 weeks of RAEX.[34,35] Matching of capillary growth to physiological cardiac hypertrophy is in stark contrast with the capillary rarefaction reported in pathologic forms of myocardial hypertrophy.[36] This maintenance of capillary density (capillary/muscle fiber ratio) in hypertrophied myocardium highlights the potential role of RAEX as a strategy to restore capillarity in pathological conditions. There is also evidence of increased myocardial arteriolarization (i.e. increased arteriolar density and cross-sectional area/g of cardiac muscle) following RAEX[33–35] and that RAEX increases the compliance of coronary arterioles due to changes in the collagen/elastin ratio within the coronary arteriolar wall.[37] These structural changes in the arteriolar tree may be central to increased CBF capacity after RAEX.[19]

Although the weight of evidence indicates that both coronary capillary diffusion and maximal CBF capacity are elevated by RAEX,[1,19,28] there are a few studies reporting no change in maximal CBF capacity.[33,38–44] Clinical studies of CBF capacity measured with positron emission tomography or echo-Doppler also yield a mixed view of effects of RAEX on CBF capacity with some reporting no change,[45–48] and others reporting an increase in maximal CBF.[20,21,24,26,49] Careful inspection of all these studies indicates that studies conducted in the presence of proven maximal vasodilation and controlled haemodynamic variables consistently report that following RAEX the CBF capacity is increased in swine,[35,50] dogs,[30,51] and rats.[28,29,50–55]

Studies in dogs and miniature swine have shown that RAEX also increases capillary exchange capacity.[1,19,28] Interestingly, morphometric measurements of capillarization and capillary exchange capacity in the same hearts revealed that RAEX elevated coronary capillary exchange capacity in the absence of an increase in capillary numerical density.[1,19,28,34,56] These observations suggest that RAEX produces changes in the distribution of coronary vascular resistance, likely in conjunction with a small increase in capillary diameter,[35] together resulting in an increase in effective capillary surface area in the absence of a change in capillary density.[19] These adaptations are likely responsible for the reported increase in myocardial oxygen extraction and lower coronary sinus oxygen content following exercise.[1,19,28]

In summary, coronary capillary exchange capacity and maximal CBF capacity are both increased by RAEX, acting in synergy to improve the myocardial oxygen delivery capacity and reserve. The increase in coronary transport capacity is the result of structural adaptations including enlargement of large coronary arteries and increases in arteriolar and capillary surface area, as well as functional adaptations including

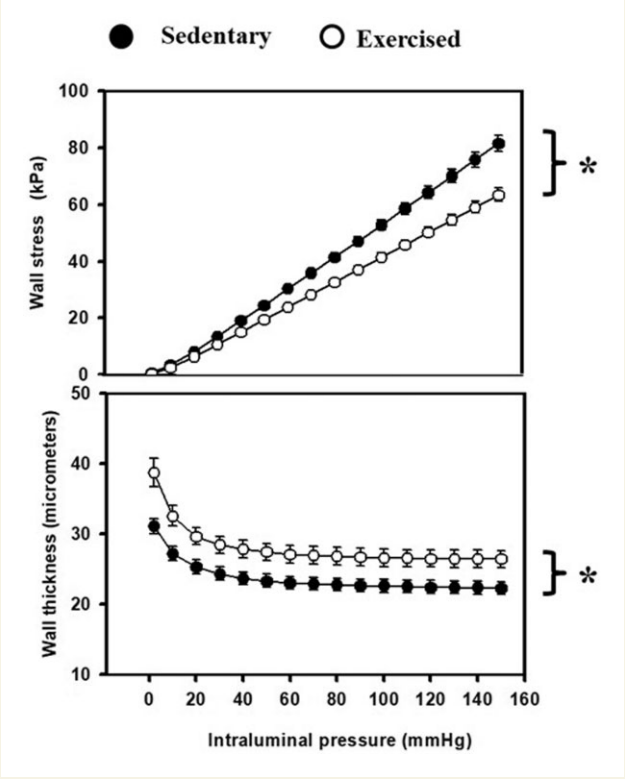

Figure 2 RAEX induces structural remodelling of rat intramural coronary arterioles resulting—among others—in reduced wall stress due to increased wall thickness (as shown in the figure), but also increased incremental distensibility, decreased incremental tangential elastic modulus. These changes allow a greater constrictor and dilator range for the coronary arterioles to operate. Mean values were used from two-way ANOVA tests with Tukey paired comparisons: *$P < 0.05$ (modified from ref.,[27] with permission of Karger).

alterations in coronary epicardial artery, resistance artery, and arteriolar vasomotor control.

4. Functional adaptations of the coronary circulation to RAEX

4.1 Adaptations in autonomic control: large vs. microvessels

Although it is well established that RAEX increases parasympathetic and decrease sympathetic activity to the heart, RAEX appears to cause only minor alterations in autonomic control of CBF.[57–59] Thus, after RAEX adrenergic tone is slightly increased or maintained in coronary resistance vessels, so that during submaximal exercise there is maintained β-adrenergic vasodilation and only slightly increased or maintained α-adrenergic constriction.[19] In proximal coronary arteries, α_1-adrenergic receptor stimulation was found to be blunted by RAEX in dogs[60,61] and swine.[62] Stehno-Bittel *et al.*[63,64] showed that the decreased vasoconstrictor reactivity is due to RAEX-induced lowering of intracellular concentrations of calcium ($Ca_{[i]}$) in coronary vascular smooth muscle (VSM) cells. In contrast, RAEX does not appear to alter vasoconstrictor responses of proximal coronary arteries to prostaglandin (PG) $F_{2\alpha}$ or KCl.[61,62]

4.2 Adaptation of the endothelium: large vs. microvessels

In addition to morphological remodelling of resistance vessels, RAEX also changes the contribution of NO and PGs to the regulation of coronary vascular resistance (*Figure 3*). Thus, RAEX for 7 days, 2 h/day was reported to enhance endothelium-dependent dilation (EDD) following post-occlusion reactive hyperaemia and administration of ACh.[65] In contrast, no change was seen in EDD of proximal coronary arteries after longer RAEX programs (>10 weeks) in dogs,[61] swine,[66] or rats.[67] It appears that longer RAEX programs were accompanied by outward remodelling of the epicardial coronary arteries, resulting in a normalization of wall shear stress levels during exercise bouts, therefore endothlial-nitric-oxide-synthase (eNOS) expression returns to normal levels[68,69] and—hence EDD responses—back towards baseline levels.[61,66,68,70] RAEX increases EDD in coronary microcirculation as reflected in enhanced serotonin-induced increases CBF[60] and increased bradykinin-induced dilation in isolated coronary arterioles (64–157 μm in diameter).[71] The increased EDD is due to increased eNOS-derived NO, since eNOS expression is increased in coronary arterioles after RAEX.[69] Interestingly, RAEX produces sustained augmentation of EDD in coronary microvessels, while it increases EDD only transiently in epicardial conduit arteries. Heterogeneous effects of RAEX on gene expression along the arterial tree has also been reported in skeletal muscle and diaphragma.[72,73] These observations indicate that RAEX has heterogeneous effects in the various part of the vascular system, likely due to heterogeneity of haemodynamic forces they are exposed to.

4.3 Adaptation of the pressure-sensitive myogenic mechanism

Systemic, thus coronary blood perfusion pressure greatly increases during exercise. Thus one would expect that the pressure-sensitive myogenic response of coronary vessels is affected as well. Indeed, RAEX increases myogenic constriction in coronary arterioles[37,71] (*Figure 3*) perhaps resulting from altered calcium-dependent protein kinase C (PKC) signalling in coronary VSM cells[74] or increased calcium currents through L-type calcium channels in VSM of large arterioles.[75] The enhanced vasoconstrictor response to stretch (myogenic reactivity) is not accompanied by alterations in receptor-mediated vasoconstriction to endothelin (ET-1) or ACh, or direct stimulation of voltage-gated calcium channel activation with K^+ or by the L-type calcium channel agonist Bay K8644.[76] RAEX may also alter calcium control by sarcoplasmic reticulum and/or increase K_{Ca} and K_v channel activity in coronary VSM.[52,77,78] Studies have shown that coronary arterioles of RAEX-swine[79] and rats[27] exhibited a more powerful myogenic response, i.e. the diameter of arterioles were less in the pressure range of 60–120 mmHg.

The greater constriction in this pressure range is the result of increased smooth muscle contractility and altered endothelium-derived factors. RAEX may upregulate the calcium-dependent PKC,[19,74] increase activation of voltage-sensitive calcium channels and/or cGMP-sensitive calcium-dependent chloride channels, and voltage-gated calcium-dependent potassium channels leading to increased intracellular calcium oscillation.[80,81]

In coronary arterioles from sedentary animals an appropriate level of myogenic response is also maintained by the contribution of constrictor PGs released primarily from the endothelium, whereas in RAEX-arterioles such role for endogenous vasoconstrictor prostanoids is

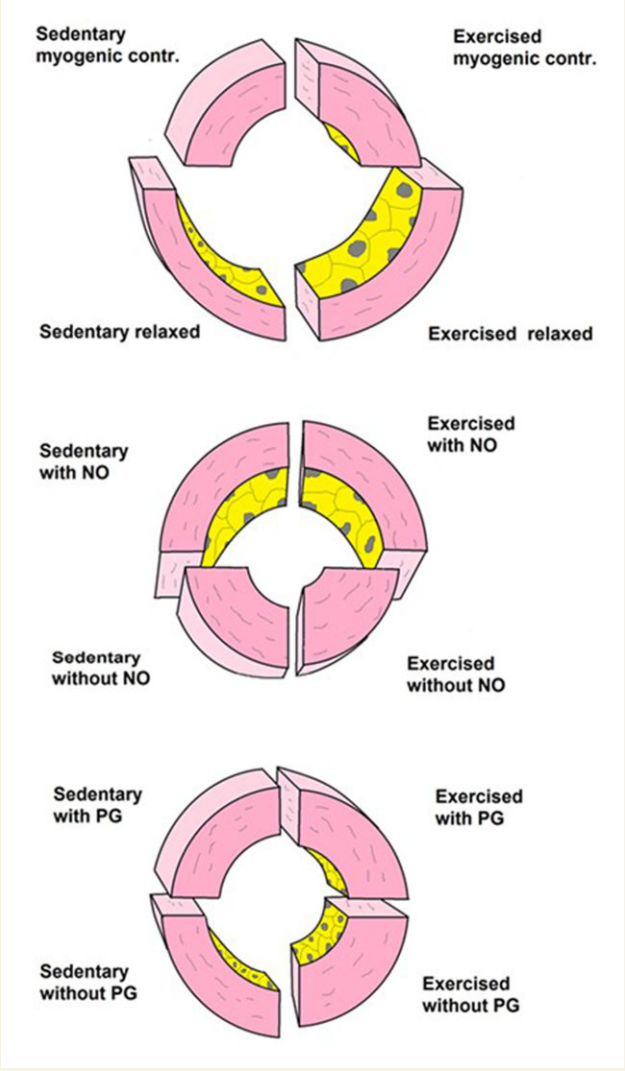

Figure 3 Illustration of morphological adaptations of small coronary arteries of rats to RAEX. Top panel: sedentary and exercised vessel in control condition at two intraluminal pressure values: upper half at 30 mmHg and lower half at 120 mmHg. RAEX elicits growth of vessels and thickening of vascular wall, resulting in reduced wall stress and increased elasticity of vessels. Middle panel illustrates the effect of NO, whereas the lower panel illustrates the effect of PG[27] (with permission of Karger). NO, nitric oxide; PG, prostaglandin.

absent. Instead, RAEX-induced augmentation of myogenic mechanism—the increased force generation to pressure—became 'built in' the smooth muscle, instead of relying on endothelial constrictor factors, especially at higher pressures.

Interestingly, NO had a greater contribution to the relaxation of exercised rat coronary arterioles at lower pressures (<60 mm Hg) compared to that of sedentary vessels. One can extrapolate this finding to the regulation of coronary circulation and propose that NO-induced opening of coronary microvessels can be important during diastole when intraluminal pressure is lower and the limited diameter effect of NO at higher pressure values protects the distal part of microcirculation from high perfusion pressure. These changes contribute to the extension of the autoregulatory range in coronary vascular tree (*Figure 4*) important to maintain CBF in face of varying pressure.

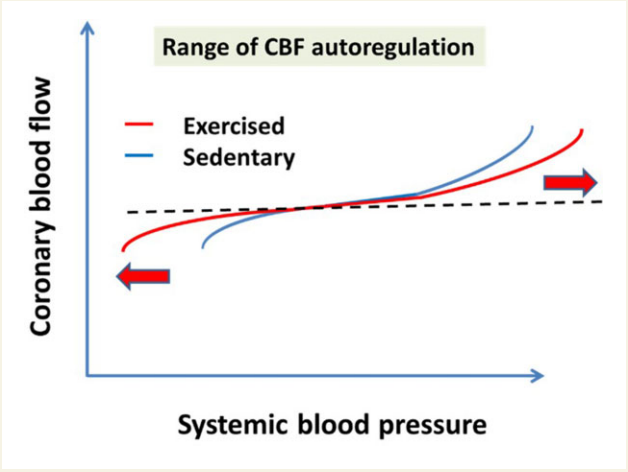

Figure 4 On the basis of experimental findings we propose that RAEX extends the range of CBF autoregulation resulting in a close to maintained CBF both at lower and higher pressures, as well. This is primarily due to the upregulated pressure-sensitive myogenic mechanism, a condition, which is then further modulated by local and remote vasomotor mechanisms. CBF, coronary blood flow.

4.4 Exercise-induced upregulation of flow/shear stress-induced vasodilator mechanism

During RAEX, in addition to pressure, blood flow and thus wall shear stress is also increased in coronary vessels. Around 1990s it was demonstrated *in vivo* that small arterioles are sensitive to changes in flow/shear stress acting on the inner surface of the endothelium and respond to this force with dilation (mechanotransduction) due to the release of various dilator factors [NO, PGs endothelium-derived-hyperpolarizing-factor (EDHF), reactive-oxygen-species (ROS)] from the endothelium.[82–84] The dilation then reduces wall shear stress back to normal levels. Thus the haemodynamic force-sensitive myogenic and flow/shear stress-dependent mechanisms are importantly involved in the regulation of the coronary microcirculation, especially because in smaller vessels these forces elicits greater diameter responses.[82] Thus it was logical to hypothesize that regular exercise by eliciting great increases in blood flow (which in the presence of constant diameter can only be achieved by increases in blood flow velocity resulting an in increase in wall shear stress) will result in an adaptation/upregulation of the 'flow sensitive' mechanism.[85] Indeed, previously we have found that a short-term exercise program augmented flow/shear stress-induced dilations of arterioles (skeletal) that are mediated by simultaneous release of NO and PGs.[86–89] Also an enhanced basal tone of exercised arterioles was found compared to sedentary vessels.[86] The upregulation of shear stress-induced dilation contributes to enlargement of the functional dilator capacity of coronary vascular tree (*Figure 5*). On the basis of studies on isolated small skeletal muscle veins one can assume that during exercise coronary venular system also dilates to increases in flow/shear stress due to release of NO and PGs[90] contributing to the increased blood supply of the cardiac muscle. The salient finding is that increases in shear stress produced by increases in perfusate flow velocity result in vasodilation of venules isolated from skeletal muscle, thereby keeping its level close to constant, which can have rheological consequences as well. An increase in shear stress results in the release of endothelium-derived

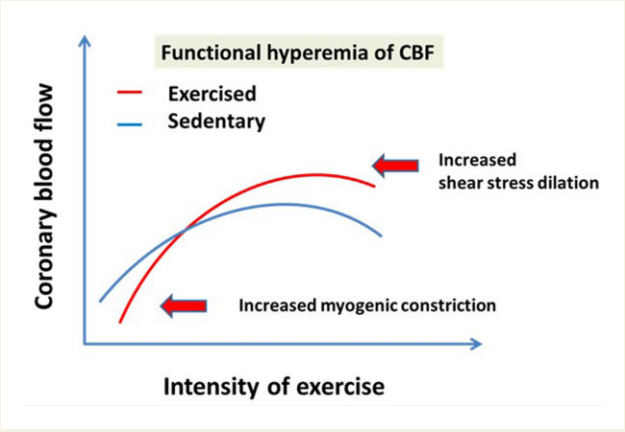

Figure 5 On the basis of experimental findings we propose that RAEX extends the maximum range of CBF resulting in exercise-induced great functional hyperaemia due to the upregulated pressure and flow/shear stress-sensitive vasomotor mechanisms, a basal tone which is then further modified by local and remote vasomotor mechanisms. CBF, coronary blood flow.

NO and PGs, whereas in the absence of these vasodilator factors, the release of an endothelium-derived vasoconstrictor factor in response to an increase in perfusate flow was also uncovered.

On the molecular level, Laughlin *et al.*[91] observed in coronary resistance vessels isolated from exercised pigs that eNOS mRNA and protein expression were increased compared to that of sedentary pigs. The increases in eNOS and Cu/Zn SOD expression were shown to be a flow-dependent phenomenon.[92] These investigators subsequently showed that RAEX-induced increases in eNOS content is not uniformly present along the coronary tree, which may be due to the type of muscle fibres they supply and/or mechanical forces exerted on their lumen.[69,91–93] In addition, Yang *et al.*[94] demonstrated that RAEX increases both endothelial and inducible NOS gene expression in rat aorta endothelial cells.

The type of exercise can be also important to activate these mechanosensitive mechanisms. For example, sudden high-intensity interval exercise results in sudden increase in intraluminal pressure and likely wall shear stress eliciting maximal stimulation of these mechanisms. For example, sudden increase in pressure leads to an increased production of ROS[95] known to play crucial role in the initiation of adaptive mechanisms leading to vascular remodelling. Also, a sudden increase in wall shear stress can elicit greater release of NO (in contrast to low-intensity exercise) via activation of platelet-endothelial adhesion molecule system.[96–98] The intensive effect of high temporal gradients shear stress could be incorporated in the design of exercise programs and may explain the pronounced efficacy of brief exercise at high intensity.

All in all, RAEX is accompanied with great increases in haemodynamic forces, which then elicit changes in the mechanosensitive vascular mechanisms, which are 'translating' physical forces to adaptive diameter changes and are balancing each other.[82,89] Depending on the prevailing levels of wall shear stress and pressure, these two mechanisms together determine the basal diameter of arterioles, which is then further modulated by metabolic and neurohormonal mechanisms. In addition, these forces use similar signalling pathways, which in a long run elicit morphological vascular remodelling such as increased wall thickness and elasticity (*Figure 2*). These adaptations then allow a greater range for the regulation of CBF to match the needs of working cardiac muscle during exercise at various pressure and flow conditions. This has also been discussed in detail in recent reviews.[19,85,99] Such remodelling of coronary arterioles is likely contributing to the optimization of CBF during exercise producing a greater range of coronary autoregulation, i.e. a greater constrictor and dilator reserve (*Figure 4*), and thereby more effective protection against coronary vascular diseases.

4.5 Adaptation of metabolic control

RAEX has been reported to increase the maximal adenosine-induced increase in CBF per gram of myocardial tissue in miniature swine and dogs.[19] At corresponding levels of left ventricular work CBF is not changed by RAEX indicating minimal RAEX-induced changes in the coupling between myocardial metabolism and CBF.[1,19,28] The interesting findings of Kuo and Chancellor[100] in the porcine subepicardial coronary arterioles (50–150 microns) extend this picture in a sense that the metabolic mediator adenosine—which is released in a threshold concentration during exercise—potentiated flow/shear stress-induced dilations of coronary arterioles by activating K_{ATP} channels in the endothelium. This mechanism could be more important when oxygen supply is not matched by demands (slight hypoxia) thus more adenosine is produced from breakdown of ATP, which metabolism is limited in this conditions.

Thus, the interplay among the local mechanisms ensures an optimal supply of blood flow during and after exercise to maintain the cardiac muscle in aerobic condition as long as possible. Interestingly, animal (porcine) experiments have shown that NO-mediated dilations are impaired distal to coronary artery narrowing (stenotic or non-stenotic), which could be reversed by exercise training. This was achieved by an additional activation of a hydrogen peroxide-mediated dilator mechanism.[101]

4.6 Adaptation of other vasomotor factors

Interestingly, there is little if any evidence for a diminished constrictor tone in coronary microvessels following RAEX. For example, vasoconstrictor responses to ET-1, ACh, voltage-gated calcium channel activator Bay K8644, or high-dose K^+ are all maintained, and myogenic tone is even enhanced, following RAEX.[19] Thus, a physiological antagonism, i.e. a decrease in constrictor mechanism is not a likely explanation for the increased vasodilator influence of NO. While there is evidence that RAEX enhances bradykinin-induced endothelium-dependent dilations in porcine coronary arterioles (64–157 micron in diameter), the observation that cytosolic Cu/Zn SOD (SOD-1) is also upregulated by RAEX indeed suggests that the increased endothelium-dependent dilator responses could be, at least in part, the result of a decreased quenching of NO by superoxide. However, the vasodilator response to sodium nitroprusside is not different between sedentary and RAEX swine, suggesting that RAEX principally increases NO production rather than increasing its half-life.[19] Consistent with this interpretation, the increased eNOS content in the coronary arterioles of exercised swine described by Laughlin *et al.*[69] may likewise account for the enhanced EDD in conscious exercised dogs found by Wang *et al.*[65]

Interestingly, RAEX does not appear to increase resistance vessel sensitivity to adenosine in isolated porcine coronary resistance vessels (which is thought to be endothelium-independent), further pointing towards the unique effects of RAEX on NO production in the coronary microvasculature. On the other hand, the coronary vasodilator sensitivity to adenosine appears to be enhanced *in vivo*.[19] An explanation for the different observations with adenosine *in vitro* vs. *in vivo* could be the

flow-mediated and endothelium-(NO)-dependent dilations of upstream small arteries and larger arterioles elicited by the increased pressure drop across the arterial network due to the adenosine-induced dilation of the very small arterioles.[19] Alternatively, the structural adaptations (i.e. increase in arteriolar densities and size) or the reduced extravascular compressive forces (due to RAEX-induced lowering of heart rate) may facilitate an increase in CBF in response to adenosine even in the absence of an increased sensitivity of the individual coronary arterioles.

Taken together, these findings indicate the complexity of adaptations, and while perhaps not all mechanisms are revealed, the beneficial effects of RAEX on coronary circulation—even in diseased conditions—are unquestionable.

Thus as summarized in *Figure 6*, RAEX-induced adaptations in the coronary circulation—epicardial and microcirculation—include both structural (angiogenesis and vascular remodelling) and functional (alterations in control of vascular resistance) adaptations.[19,52,53,102] Adaptations in the regulation of coronary vascular resistance are elicited by changes in neurohumoral, cardiac metabolic, and local vasomotor mechanisms and their interplay. All these beneficial modifications play important roles in the RAEX-induced increase in myocardial oxygen (and nutrition) delivery and extraction during and following exercise activities.[19]

4.7 Interactions among blood, coronary vessels and cardiac tissue

For the sake of simplicity it is customary to treat events taking place in blood vessels and cardiac muscle separately; however, they are—together with other tissues in the heart—form a 'continuum', which allow significant interactions among them. *In vivo*, there are no 'hard' borders among tissues. Molecular factors or even cells are trafficking between the myocardium, smooth muscle cells, endothelium, and blood by a variety of mechanisms, including diffusion, passive or active transport across membranes, tight junctions, etc. Many details need to be considered, but we would like to highlight this important aspect, with one particular signalling mechanism outlined in *Figure 7*.

Because myocardial oxygen extraction is close to maximal, already in resting conditions, CBF must greatly increase to supply the myocardium with sufficient oxygen during exercise. Nevertheless, there is evidence that RAEX produces a small further increase in oxygen extraction capacity, likely as a result of an increase in capillary exchange capacity.[19,103] Sudden increases in blood flow/wall shear stress—especially during acceleration type exercise—induces high temporal gradients,[98] which greatly stimulate eNOS to release large amounts of NO, which then elicit great dilations. In addition, vascular, thus endothelial deformation during each cardiac cycle can lead to NO release—as shown previously[104]—contributing to coronary dilations. All these mechanisms despite continuously increased cardiac load, delay the development of hypoxia and development of anaerobic, lactic acid metabolism.

Although many of the signalling roles of NO have been appreciated (such as controlling vascular tone, platelet aggregation, adhesion, and vascular growth, etc.) two should be emphasized here, as they have a major impact on both coronary and cardiac function. First, about two decade ago Kobzik *et al.*[105] have shown that eNOS is expressed in the capillary wall, which was later confirmed in the heart by Hintze *et al.*[106–108] Since in the capillary wall consists exclusively of endothelial cells and is devoid of smooth muscle, it was suggested that NO may serve an additional role in this part of the microcirculation. Indeed NO as a highly diffusible molecule can reach the mitochondria underneath the cell membranes of cardiac muscle fibres and compete for the oxygen binding site at cytochrome A3.[105]

Early studies of Kayar and Banchero[109] showed that groups of mitochondria are located very close to the wall of capillaries further

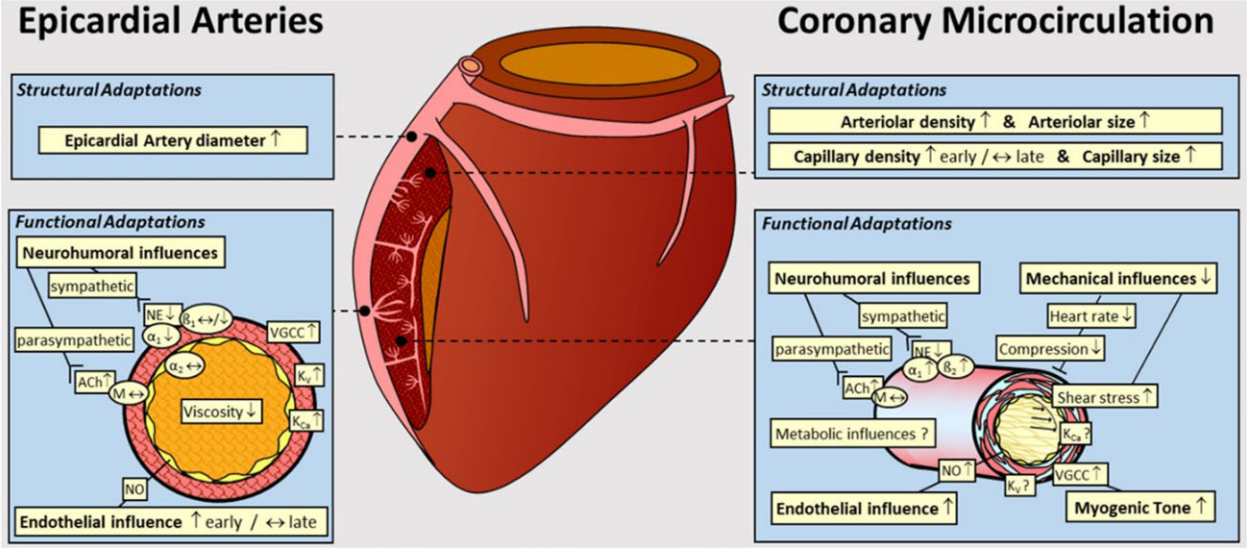

Figure 6 Graph summarizing the structural and functional coronary adaptations to RAEX in normal subjects. The coronary circulation has been divided into Epicardial Arteries and the Coronary Microcirculation, showing the structural and functional adaptations detailed for each of these coronary vascular compartments. α1, α1-adrenergic receptor; ß1, ß1-adrenergic receptor; ß2, ß2-adrenergic receptor; ACh, acetylcholine; KCa, Ca^{2+}-dependent K channel; Kv, voltage-dependent K channel; M, muscarinic receptor; NE, norepinephrine; NO, nitric oxide; VGCC, voltage-gated Ca^{2+} channel. Modified from ref.[19] with permission of the American Physiological Society.

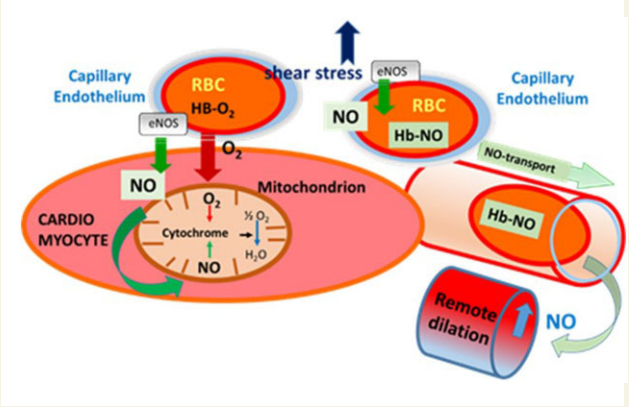

Figure 7 Schematic illustration of the effects of eNOS-derived NO released from capillary endothelium in response to increases in flow/wall shear stress, during exercise on mitochondrial function and remote vascular dilation. The highly diffusible NO can reach and then bind to cytochromes in the mitochondria of cardiac muscle, but also can be carried away by binding to the haemoglobin in RBC and later released eliciting dilation of remote microvessels, thereby increasing oxygen supply. eNOS, endothlial-nitric-oxide-synthase; NO, nitric oxide; RBC, red-blood-cell.

supporting this concept that physiologic levels of NO can exert a tonic control of cellular respiration and metabolism[108] by regulating tissue oxygen consumption through reversible inhibition of cytochrome c oxidase in the mitochondrial respiratory chain as well as substrate selection.[108] Second, Stamler *et al.*[110] and later Allen *et al.*[111] showed that NO binds to haemoglobin in the erythrocytes to form the more stable Hb-NO, from which NO can be released under conditions of low oxygen, eliciting vasodilation and increasing blood flow in remote areas or back flow to proximally occluded network segments to prevent/reduced tissue hypoxia. This inter-tissue signalling among blood, coronary vessels, and the myocardium likely optimizes cardiac performance thereby contributing to the cardiovascular adaptations to exercise.

4.8 Adaptations in ion channel function in the vascular wall

In addition to molecular mediators, ion channels are also involved in the regulation of coronary resistance and in their adaptations to RAEX as indicated partly in *Figure 6*. However, the role of these channels in the adaptation to RAEX deserves a more detailed analysis.

4.8.1 Ion channels in the microcirculation

Opening and closing of ion channels, in vascular cells particularly K^+ channels, modulate the electrical potential across the plasma membrane (membrane potential) which impacts on the activity of (other) voltage-gated ion channels thereby feeding back to membrane potential or altering cytosolic ion concentrations.[112] The latter effect is specifically important for Ca^{2+} because cytosolic Ca^{2+} concentrations are very low. In smooth muscle cells, Ca^{2+} activates the contractile machinery and thereby significantly determines arteriolar tone. Moreover, Ca^{2+} is also an important activator of specific K^+ channels (Ca^{2+}-dependent K^+ channels), which are expressed mainly in endothelial cells, and in this setting localized Ca^{2+} increases are sufficient to induce opening of such channels. As the opening of K^+ channels drives the membrane potential from the physiological range of -45 to -30 mV towards the K^+ equilibrium potential of about -90 mV (hyperpolarization) it prevents the opening of voltage-gated Ca^{2+} channels (VGCC) in smooth muscle cells and thereby promotes arteriolar dilation.[113] The activation of endothelial K^+ channels likewise induces hyperpolarization which initiates a similarly directed membrane potential change in smooth muscle either through gap junctional-mediated coupling or by the transfer of a factor that induces the hyperpolarization in these adjacent cells (endothelium-derived hyperpolarization, EDH).[114,115]

The most important modulators are K^+ channels and they consist in vascular cells of four (or more) classes, namely voltage-gated (K_V), Ca^{2+}-dependent (K_{Ca}), inwardly rectifying (KIR), and ATP-dependent (KATP). K^+ channels have been implicated in functional dilation[116,117] (specifically in the coronary vascular bed $K_V1.5$ and $K_V1.3$)[118,119] and are therefore also likely targets of exercise training. The focus in this section will therefore be on K^+ channels and the main effector in smooth muscle, i.e. VGCC.

4.8.2 Ion channels in smooth muscle cells affected by RAEX

An enhanced vascular tone is frequently observed in exercised animals[19] most likely due to a selectively exaggerated myogenic tone (see above) specifically in resistance vessels.[76,79] Myogenic responses are well known to rely on depolarization and subsequent opening of VGCC[120] and, in fact, smooth muscle cells isolated from coronary vessels of different sizes of exercise-trained animals exhibited two-fold Ca^{2+} current densities through L-type VGCC.[75] Whether this phenomenon is due to enhanced channel expression or related to a phosphorylation of the channel with enhanced conductance remains to be determined. Concomitantly, constrictions upon non-selective K^+ channel blockade were enhanced in such vessels indicating increased negative feedback through K^+ channels thereby limiting further constrictions. However, the enhanced K^+ channel activity was not observed in isolated smooth muscle cells suggesting that the density of K^+ channels and their conductance remained unchanged.[121] Similar results were obtained in a follow-up study for overall K^+ currents or currents through K_{Ca} channels ($K_{Ca}1.1$) in isolated smooth muscle cells from exercised animals.[122]

This suggests that these channels are only more active in intact pressurized vessels and pressure (and stretch) may then provide the appropriate stimulus which is either a stronger depolarization (K_V channels)[120] or subsequent enhanced Ca^{2+} influx (K_{Ca} channels).[123,124] Despite enhanced Ca^{2+} currents and higher rates of cytosolic Ca^{2+} increases the net accumulation of free Ca^{2+} was not increased suggesting a compensatory Ca^{2+} extrusion mechanism (distinct from SERCA pump and sodium-calcium exchange) which prevents overshooting Ca^{2+} levels in vessels of exercised animals.[77] Taken together, modulation of ion channel function, mainly VGCC, in smooth muscle supports enhanced arteriolar tone in the resting state in exercise-trained animals.[125]

4.8.3 Ion channels in endothelial cells potentially targeted by regular exercise

Exercise-induced flow increases may increase shear stress acting on endothelial cells[85,126] which is a major factor contributing to augmented NO-mediated responses in the coronary circulation as discussed above. It is also well established that endothelial K^+ channels are important players in EDD, particularly in arterioles.[115,127] Although some K^+ channels are likewise activated in a flow-dependent manner, little is known about exercise-induced changes of the function of these endothelial K^+ channels in coronary arterioles. Only rarely EDH-type dilations are

investigated although a beneficial effect of regular exercise was reported more than 20 years ago.[128] In the endothelium two types of K_{Ca} channels ($K_{Ca3.1}$/IK_{Ca} and $K_{Ca2.3}$/SK_{Ca}) and the inwardly rectifying K^+ channel (KIR) are functionally dominant.[129,130] Specifically, K_{Ca} are activators of the EDH-type dilation.[127,131,132] SK_{Ca} channels are also activated by shear stress[132] and support active hyperaemia[133] (*Figure 8*).

Endothelial Ca^{2+} increases induced by agonists or wall shear stress may not only enhance NO production but also activate K_{Ca} channels. In this setting, the transient receptor potential channel TRPV4[134] is reported to contribute by enabling Ca^{2+} influx[135,136] generating localized Ca^{2+} increases that activate K_{Ca} channels[137,138] or release mitochondrial reactive oxygen species to mediate flow/shear stress-induced dilations in coronary arterioles.[139] In endothelial cells *in vitro*, TRPV4 channels are redistributed upon mechanical stimulation[140] which suggests that exercise may also have an impact on these signalling cascades in coronary endothelium (*Figure 8*).

Future work will possibly elucidate these pathways further and hopefully define a more precise picture of the phenomenon which is nowadays briefly summarized as an enhancement of EDD, specifically pronounced in cardiac diseased conditions[141] or during physiologic ageing.[142,143]

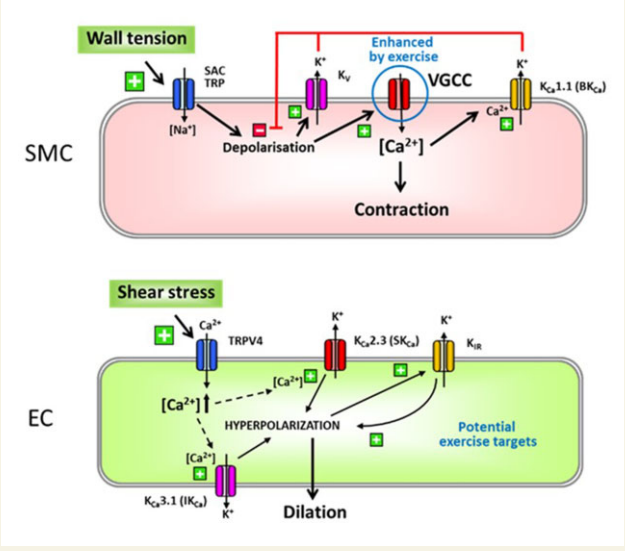

Figure 8 Regular exercise may affect multiple ion channels in SMC (upper panel) or EC (bottom panel). However, experimental data have shown up to now only enhanced Ca^{2+} currents through VGCC (encircled in blue) in SMC after RAEX. This is either due to changes in conductance upon channel phosphorylation or due to an upregulation at the protein level. VGCC are involved in myogenic responses initiated by a depolarization through activation of SAC or TRP channels. The increased activity of K^+ channels (BK_{Ca} and K_V) found in exercised animals is most likely attributable to the initial enhanced VGCC activity because they act in negative feedback manner limiting depolarization. The ion channels involved in shear stress-induced endothelium-dependent responses are shown for EC. TRPV4 channels enable Ca^{2+} influx leading to activation of SK_{Ca} and IK_{Ca} and subsequent EDH-type dilation, while endothelial K_{IR} acts as an amplifier of hyperpolarizing signals. All channels may be functionally modulated by RAEX. However, up to date this was not verified at the molecular level although RAEX enhances EDDs including EDH-type dilation. For further details see text. EC, endothelial cells; SAC, stretch-activated channels; SMC, smooth muscle cells; TRP, transient receptor potential channels; VGCC, voltage-gated Ca^{2+} channel.

5. Effects of exercise on 'haemorheological' resistance

From the Hagen–Poiseuille equation it is clear that viscosity also an important factor determining resistance to flow. Thus although it is not specific to coronary circulation, changes in the properties of blood in response to RAEX can greatly affect the blood delivery function of coronary vascular tree as well. At present, however, there are no means to specifically determine rheological changes only in the coronary lumens, yet they can be very important. Because in smaller vessels blood behaves as a non-Newtonian fluid, its viscosity varies with haemodynamic conditions and shear rate. Blood becomes less viscous at high shear rates, which occur with increased flow (velocity), such as during exercise or in peak-systole. Contrarily, blood viscosity increases when shear rate goes down as with increased vessel diameters or with low flow, such as downstream from an obstruction or during diastole.[144] One of the most important determinant of plasma viscosity is fibrinogen concentration, a haemostatic protein, which level could be affected by exercise. Changes in the balance between haemorheological and haemostatic determinants can lead to thrombotic complications.[145] Exercise affects haemorheology and haemostasis but results are equivocal, likely due to confounding factors, such as age, exercise intensity and duration, or exercise conditions of the individual.[146]

Acute exercise is associated with a rise in haematocrit, decreased red blood cell deformability, increased red blood cell aggregation, and increased fibrinogen level and therefore with high plasma and whole blood viscosity. These haemorheological changes result in reduced capillary circulation—acting against haemodynamic changes—and thus can diminish tissue perfusion. In contrast, RAEX has a beneficial effect on all of these haemorheological parameters (called 'haemorheological fitness') reducing cardiovascular risk and improving capillary blood flow even in patients with ischaemic heart disease (*Figure 9*).

A large number of studies in healthy humans, considering different RAEX programs, show a direct association between intensity of aerobic physical activity (endurance and resistance exercise) and transient prothrombotic states,[147–151] including increased platelet counts, higher levels of coagulation factors (i.e. FVIII) and/or reduced fibrinolytic factors and stimulated coagulation activity mainly through activation of the intrinsic-pathway resulting in increased levels of fibrinopeptide A and D-dimers, as markers of fibrin formation.[151,152] Current evidence supports that exercise intensity rather than duration is the main factor modulating the haemostatic parameters. Regular exercise returns the initial activation of pro-thrombotic variables to baseline[153,154] preventing thrombosis. It is of note that acute exercise (in sedentary individuals) can induce hyperviscosity (mostly due to haemoconcentration and alterations of red blood cell properties), whereas regular exercise improves all haemorheological parameters, thus improving haemorheological fitness (*Figure 9*).

6. Sex, age, and cold environment modulate cardiovascular responses to exercise

6.1 Influence of sex

Acute exercise increases sympathetic tone, which results in positive chronotropic and inotropic responses, thereby increasing myocardial oxygen demand. High levels of sympathetic drive also stimulate the

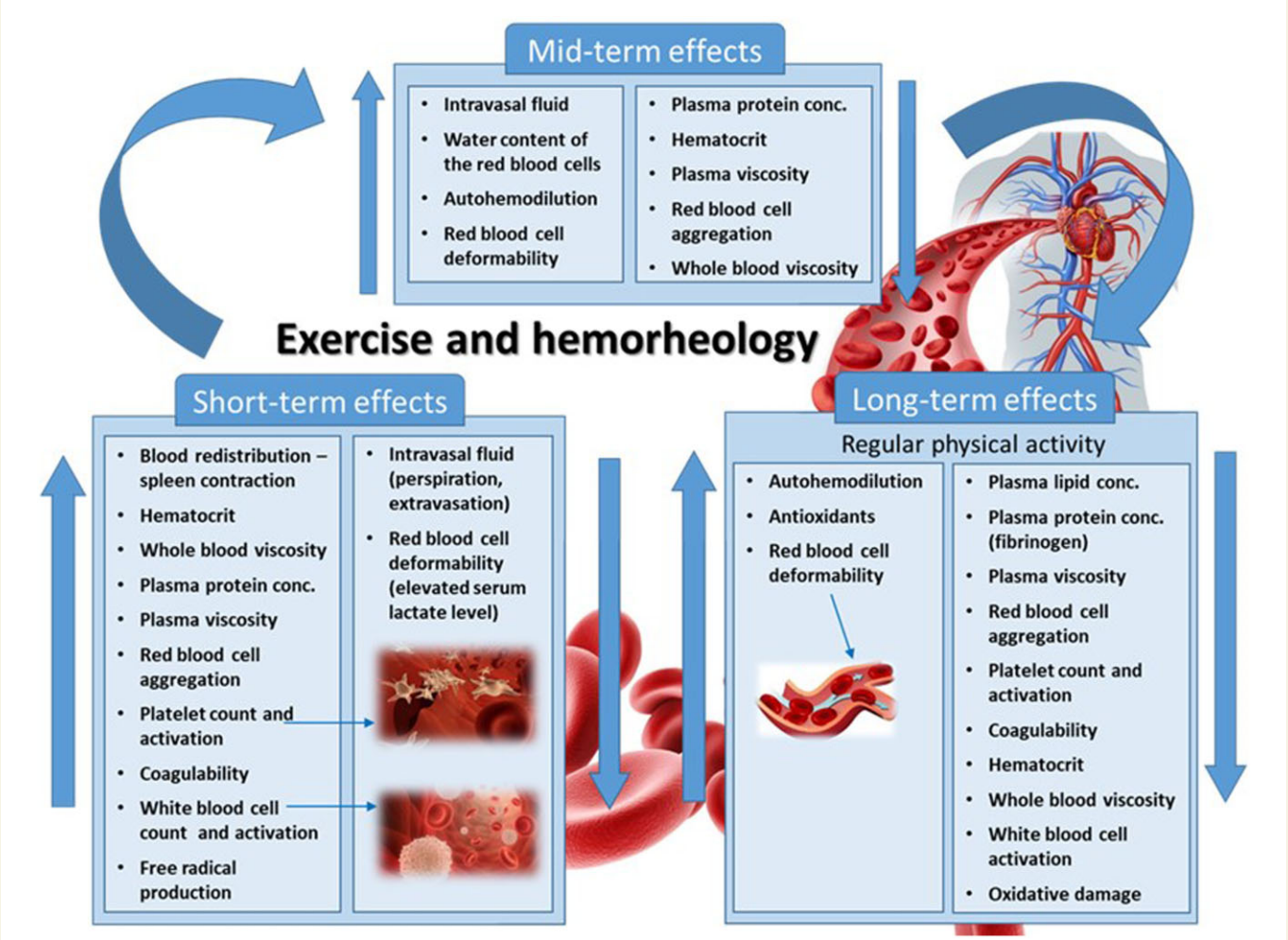

Figure 9 Illustration of short-, medium-, and long-term effects of RAEX on the rheological behaviours of constituents and cellular elements blood achieving rheological fitness further supporting the blood to pass the extremely complex microvascular network of coronary circulation.

endothelial cells to produce vasodilator molecules, such as NO. Several studies have shown sex differences with respect to autonomic nervous control of cardiac function in response to RAEX. Despite higher heart rates, women exhibit lower sympathetic activity, greater vagal modulation, and reactivation following maximal exercise, while men display elevated resting sympathetic tone.[155–157] Sex differences in cardiac autonomic modulation are largely described in premenopausal women as compared to age-matched men.[158–161] Oestrogens have been shown to prevent parasympathetic/sympathetic imbalance and improve baroreflex sensitivity.[162] Sex-specific differences are mitigated progressively after the menopause and the sex-related difference of heart rate variability (HRV) also decreases with ageing.[161] Additionally, oestrogen may improve vasomotor tone though the vasodilator effect of β-adrenergic activation, which also activates NO mechanism and lowers blood pressure.[162,163] It was also found that the presence of oestrogen via an increased NO release and antioxidant activity result in a greater flow-dependent dilation.[164–166] Recently, after 12 weeks of swimming exercise program Török *et al.*[167] found many similar adaptation in rat coronary vessels isolated from both sexes compared to that of sedentary controls, but they also found differences related to sex, such an increased spontaneous and TxA_2 agonist-induced tone in coronaries of exercised females and a more effective endothelium-dependent relaxation in coronaries of exercised males compared to that of sedentary groups.[167] At the moment, the clinical consequences of these experimental findings are not yet clear and highlight the existence of still somewhat conflicting findings in sex-related adaptation of coronary vascular tree and other vascular territories.

6.2 Influence of age

Ageing is associated with sympathetic dysregulation and a decrease in heart rate variability (HRV) as well as endothelial dysfunction, which are associated with abnormal regulation of CBF.[168] Elderly healthy individuals have a reduced exercise tolerance and a decreased left ventricular inotropic reserve related to increased afterload, physical deconditioning, and impaired autonomic regulation resulting—in part—from 'beta-adrenergic desensitization'.[161,169,170] Beta-adrenergic receptor density reduction may lower intracellular Ca^{2+} transients with subsequent impaired inotropic and chronotropic responses to adrenergic stimuli.[161,170–172] A recent study in old individuals showed that 5-year leisure-time activity

and walking were associated with higher global HRV and vagal-related indexes indicative of improved circadian fluctuations, which suggests that RAEX may counterbalance the age-related decline in cardiac autonomic control.[173] According to the above-mentioned hypothesis, increased vagal tone appears to induce an anti-inflammatory milieu: HRV is inversely related to the production of many inflammatory markers such as interleukin-6, C-reactive protein, and fibrinogen in healthy individuals as well as in older adults especially in cardiovascular disease.[174,175] In sum, these observations suggest that preserved vagal tone is crucial to cardiovascular health in ageing and RAEX can potentially modulate cardiac ageing phenotypes.

6.3 Influence of ambient temperature

A cold environment can have an effect on the adaptation of cardiovascular system to regular exercise by its impact on the parasympathetic/sympathetic balance. Exposure to cold leads to various parasympathetic/sympathetic responses such as an increase in heart rate probably due to parasympathetic withdrawal including, an increase in systemic vascular resistance, systolic and diastolic blood pressure.[163,176–179] Initially cold exposure increases sympathetic activity, which is blunted, whereas parasympathetic activity is enhanced after cold acclimation. In sum, one can assert that cold adaptation lowers sympathetic activation and causes a shift towards increased parasympathetic activity,[180] with the consequent changes on CBF.

More importantly an underlying cardiovascular disease (CAD) may modify the relationship between exercise and acute or prolonged cold exposure. Cold exposure reduces myocardial oxygen supply in CAD, which may lead to more severe ischaemia. Exercise in cold augments cardiac workload in patients with CAD more than when it is performed in regular temperature/condition. At the same time, exercise may reduce myocardial perfusion, due to endothelial dysfunction or flow-limiting stenosis, leading to early ischaemia, angina, impaired performance, and release of pro-arrhythmic substrates.[18,163,179] Furthermore, in the setting of CAD, autoregulation may lead to uneven distribution of microvascular resistance and thus flow among different coronary territories. In case of increased myocardial oxygen demand/supply, such as cold exposure the accentuation of this pathophysiological condition can lead to haemodynamic interactions between a collateral receiving and collateral supplying vascular bed in the form of coronary steal, defined as a drop of coronary flow below resting levels in the affected area. The phenomenon of coronary steal is clinically relevant due to a prevalence of 10–20% in non-obstructive CAD.[181–184]

Another interesting issue of relevance is the exercise in cold water. It has been hypothesized that submersion in cold water and the release of breath holding promotes an 'autonomic conflict' due to activation of two opposing reflexes 'cold shock reflex' and the 'diving reflex'.[185] The 'cold shock reflex' driven by cutaneous cold thermoreceptors induces the activation of a sympathetically driven tachycardia, hyperventilation, peripheral vasoconstriction, and hypertension. The 'diving reflex' driven by activation of facial trigeminal receptors during facial immersion promotes an acute bradycardia mediated by the vagus and an expiratory apnoea, which in turn leads to arterial hypoxaemia and hypercapnia producing further vasoconstriction.[185] This 'autonomic conflict' may be responsible for sudden alteration in coronary circulation, arrhythmias, particularly at release of breath holding, which increases vagal tone that varies with respiration.[179,185] Such disarrangement in the ANS can lead to dysregulation of cardiac and coronary function especially in extreme cold, CAD, myocardial hypertrophy, and channelopathies.[185,186] Further studies are needed, however, to uncover the underlying coronary vasomotor mechanisms.

7. Important areas for future research

There are insufficient number of studies elucidating the RAEX-induced differences in the adaptation of human coronary circulation in both sexes, as a function of age and temperatures (heat and cold) in sedentary people, athletes, and elite athletes or after retirement of elite athletes.[187] All of which has great impact not only on the life of individuals but also on the 'fitness' of societies. Moreover for personalized prescription of exercise as medicine necessitate the delineation of these differences and the uncovering of the underlying mechanisms.

8. Clinical perspective and concluding remarks

There is ample evidence that RAEX elicits beneficial cardiovascular adaptations in healthy subjects as well as in individuals affected by cardiovascular diseases—including heart failure and ischaemic heart disease—and their comorbidities—including hypertension, obesity, type II diabetes, hypercholesterolaemia, and chronic kidney disease.[4,161,171] RAEX—likely, in part, due to haemodynamic force stimulation—induces anti-atherogenic adaptations in endothelial function and vascular structure, regardless of traditional and novel risk factors cardiovascular-disease (CVD) risk factors. For the same reasons and due to intermittent hypoxia RAEX may improve angiogenesis of coronary capillaries and myocardial arteriolarization, thus promoting coronary collateral circulation growth, which in turn may improve CBF, increase ischaemic threshold, limit infarct size and provide protection against myocardial ischaemia–reperfusion injury. Furthermore, the mechanisms activated by RAEX by releasing NO, PGs and hyperpolarization factors may reduce prothrombotic risk, by improving haemorheological parameters. Another benefit of regular exercise is that it promotes anti-inflammatory milieu mediated by myokines.

Taken together, there is ample evidence that RAEX, such as running, cycling, swimming, or walking is associated with improved functional capacity, better quality of life, and prolonged survival in health and disease.[4] RAEX also improves mental health, e.g. it promotes positive self-esteem and mitigates depression, effects that may also contribute to the beneficial effects of RAEX on the pathogenesis and progression of cardiovascular disease.[188] For example, these adaptations can be useful in the treatment of early hypertension highlighting the potential mechanism(s) by which exercise can be used as an antihypertensive medicine. Also, greater range for the regulation of coronary function would extend the range for aerobic metabolism, delaying lactate production, and contribute to the benefit of RAEX in the setting of coronary artery disease. Regular exercise-induced coronary dilations are primarily due to activation of factors intrinsic to the vascular wall by the changes in haemodynamic forces and cardiac metabolism, all of which interact with each other at several levels. Finally, it should be noted that the mechanisms described in this review provide mechanistic underpinning for the recommendations regarding the frequency, intensity, time, and type and mode of exercise regimen in cardiovascular diseases, an approach summarized by the 2020 ESC Guidelines on sports cardiology and exercise in patients with cardiovascular diseases,[187,189] This Guidelines are giving substantial help to make decisions regarding the above-mentioned aspects, although most of them are based on Level of Evidence C, necessitating further research.

In conclusion, in this review we summarized the physiological, cellular, ion channel, and molecular mechanisms as well as haemorheological

mechanisms activated by RAEX eliciting adaptations of the coronary circulation and some of the human and clinical aspects. All these adaptations serve to provide a greater range for the regulation of vascular and haemorheological resistance, allowing an enhanced blood supply and exchange capacity during the increased workload of heart during aerobic exercise. These adaptations enable an increased oxygen supply to the heart to match the needs of increased cardiac workload during exercise. Future experimental studies should reveal so far unknown molecular mechanisms activated by different types of exercise regimens, in both sexes, which also contribute to the coronary vascular adaptations in health and disease conditions promoting thereby the specific, personalized use of various exercise regimens in prevention and rehabilitation of cardiovascular disease as described in the 2020 ESC Guidelines on sports cardiology and physical activity in patients with CVD: implications for practice, so we can base on mechanisms the statement: exercise is medicine.[187]

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