Coronary vascular regulation, remodelling, and collateralization: mechanisms and clinical implications on behalf of the working group on coronary pathophysiology and microcirculation

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Introduction

Histological specimen and textbook schematics evoke static pictures of vascular networks. However, the concept of a static system is grossly misleading as vessels and their arrangement into networks exhibit a high degree of adaptation in vessel tone and vessel wall structure.1–3 These adaptive responses include the fast adjustment of vessel diameter by changes in smooth muscle tone, the slower changes of structural vessel diameter, the addition or removal of vessels by angiogenesis (sprouting/splitting), or vascular pruning (Figure 1). It is relevant to distinguish physiological adaptation, maintaining an adequate state of perfusion as well as perfusion reserve, from mal-adaptation, which may occur in the context of pathological conditions, such as a persistent increase in blood pressure. There are also differences between regulatory mechanisms in larger vessels (e.g. remodelling at the site of epicardial stenosis) and those in the microcirculation. Even within the microcirculation vascular control mechanisms are highly dependent on vessel size and type and the extent of changes in vasomotor tone and structure seem to increase with decreasing vessel size.4,5 Adaptive processes in the microcirculation are increasingly emerging as being crucial for maintenance of physiological function and for the development of relevant pathological conditions. This part of the coronary circulation, exhibiting that functional and structural plasticity requires more attention in both basic and clinical science as the basis to develop improved diagnostic and therapeutic approaches. Consequently, the present review will focus on adaptive events occurring in the coronary microcirculation which is beyond the domain of catheter investigation and intravascular imaging.

Acute changes in vascular tone maintain the balance between metabolic demand and blood supply during physiological changes in cardiac work load and compensate for pathological narrowing of epicardial feeding arteries as evidenced by the progressive exhaustion of coronary flow reserve distal to a stenosis of increasing severity.6,7 The vascular system of the heart is expanded in size and number of microvessels during growth or exercise training—while sustained reduction of physical activity leads to involution.6,7 Also, in pathological conditions, such as hypertension or hypertrophic cardiomyopathy, microvascular remodelling is observed. Narrowing of epicardial arteries will change downstream haemodynamic- and metabolic conditions and may lead to structural enlargement of arteriolar vessels and arterio-arterial anastomoses in the process of collateralization.1

The individual steps responsible for these vascular adaptations are known and many of the underlying haemodynamic, cellular, and molecular mechanisms have been described.2,8,9 However, how these mechanisms interact, especially under pathological conditions, and their responses to treatment remain incompletely understood.

The opinions expressed in this article are not necessarily those of the Editors of the European Heart Journal or of the European Society of Cardiology.

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A striking example is the limited success of attempts to use pro-angiogenic factors, such as vascular endothelial growth factor-1 (VEGF-1), to improve tissue oxygen supply in the presence of a proximal coronary artery obstruction. Apparently, an enhanced number of small distal vessels cannot simply compensate for the increased flow resistance in a proximal artery, suggesting that, here, therapeutic stimulation of collateralization is a more promising approach. Conversely, defects in coronary microvascular function cannot be fully addressed by restoring patency of epicardial vessels, suggesting that remodelling events in the microcirculation also contribute to pathophysiological events related to epicardial stenosis. These two examples highlight the importance of an integrated approach to understand coronary vascular adaptations in health and disease and to exploit these therapeutically.

In a recent study, almost 40% of patients with suspected coronary artery disease (CAD) examined by coronary angiography had normal-appearing arteries (stenosis < 20%) despite the fact that 84% of all patients had undergone prior non-invasive stress testing. The authors conclude that cardiac catheterization had a low diagnostic yield. However, a large proportion of these patients may suffer from microvascular disease and/or dysfunction. Clinically, the coronary microcirculation beyond the epicardial arteries is often considered a black box (‘if I don’t see it, I don’t believe it’), inaccessible for routine investigation, unattainable for targeted treatment. This misconception needs to change because, as outlined above, microvessels are crucial in matching perfusion to demand and methods for practical implementation to do so have been developed in recent years.

In light of such considerations, this position paper of the ESC Working Group on Coronary Pathophysiology and Microcirculation aims to present a critical and integrated view on vascular adaptations that occur at different time scales, in response to physiological and pathological challenges. These adaptations range from short-term adjustment in vessel tone to long-term vascular remodelling including changes in size, structure, and vessel number. Because of the recent improvements in physiological measurements, which guide clinical decision making, vascular remodelling mechanisms deserve closer attention. Available knowledge regarding mechanisms, stimuli, and vascular responses obtained from basic research should be used as a starting point to address (I) the clinical aspects of coronary microvascular tone, remodelling and collateralization, and (II) possible therapeutic approaches and strategies. Finally, we identify remaining open questions, thereby providing suggestions for future studies.

Basic concepts

Coronary vascular tone

Vascular tone, the ratio of baseline and maximal vessel diameter, is determined by the contractile state of vascular smooth muscle,
which is governed by (A) intrinsic properties of the vascular smooth muscle cells (VSMCs) itself (myogenic tone), (B) metabolic signals from adjacent tissue, (C) endothelial cells (ECs) acting as a transducer of forces exerted by the flowing blood and also as conduits for signalling along the vessel length, and (D) circulating hormones and autonomic innervation (Figure 2). Since oxygen extraction is high, already under resting conditions, increases in oxygen demand can only be met by increases in coronary blood flow (CBF). This is achieved by reduction of the substantial basal vascular tone at ‘rest’ through dilator mechanisms. Up to 80% of overall resistance in the coronary circulation resides within small arteries, arterioles and exchange vessels (resistance vessels).5,15

Myogenic responses

Vascular tone is generated in coronary resistance vessels mainly by the myogenic response to transmural distending pressure eliciting wall tension.16,17 In vitro, active smooth muscle tone increases almost linearly with transmural pressure leading to a substantial diameter reduction. Myogenic tone not only creates a vasodilator reserve but also serves to control capillary pressure preventing tissue oedema. Conversely, it contributes to reduction in flow resistance in the presence of reduced pressure, e.g. in post-stenotic areas. A key mechanism is membrane depolarization of VSMC in response to stretch due to the opening of nonspecific cation channels promoting an inward Na\(^+\) and/or Ca\(^{2+}\)-current. Mechanosensitive or stretch-activated channels may involve integrins that link the extracellular matrix to the cytoskeleton.19 Recently, members of the transient receptor potential (TRP) channel family have been suggested as initiating channels in cerebral vessels, specifically TRPC6 and TRPM4.19,20

In addition, G-protein-coupled receptors may act as mediators and/or amplifiers in myogenic responses either independent of their stimulatory agonists (AT\(_1\) receptor) or in response to their respective agonists (UTP, S1P) that are released upon stretch.21

Endothelial mechanisms

Endothelial cells importantly contribute to the modulation of vascular tone by releasing vasoactive substances and thus the evaluation of EC function in clinical settings is a relevant tool.22–24 Endothelial dilator mediators include nitric oxide (NO), prostaglandins, and a third mechanism, which is more prominent in smaller vessels and acts via smooth muscle cell hyperpolarization (endothelium-derived hyperpolarizing factor, EDHF).25–30 These mechanisms are active under resting conditions, and are enhanced in response to vasoactive agonists (including bradykinin and acetylcholine [ACh]) or mechanical stimulation.28 Nitric oxide is produced by the calcium-calmodulin regulated endothelial NO synthase (eNOS) which is additionally activated by phosphorylation.31 The latter pathway may underlie NO-mediated dilation upon mechanical stimulation of EC such as wall shear stress (‘flow-mediated dilation’).

Recently, EDHF has attracted great attention since it may compensate for the loss of NO under conditions of impaired NO bioavailability.28,29 Its exact nature is still debated and many chemical entities have been implicated, including CYP450-derived epoxyeicosatrienic acids, potassium ions, H\(_2\)O\(_2\), C-type natriuretic peptide, and H\(_2\)S.36,37 In addition, EDHF-dilation may also be elicited by endothelial hyperpolarization transferred directly from endothelial-to-smooth muscle through connexins creating myo-endothelial gap junctions.38,39 Moreover, ECs are intensely coupled through gap junctions and thus EDHF-related dilations are transferred along the vessel (Figure 2, ‘conducted responses’) creating orchestrated cellular responses40–42 that may contribute to ‘ascending dilations’ in addition to flow-mediated dilation.

Metabolic regulation

Energy production in the normal heart is primarily dependent on mitochondrial oxidative phosphorylation. In contrast to skeletal muscle, which exhibits very low metabolic requirements in the absence of exercise, the continuously beating heart consumes considerable amounts of oxygen already at ‘rest’. The heart has adapted to these conditions by maintaining a high level of oxygen extraction under these resting conditions, so that any further increase in oxygen demand can only be met by a parallel increase in myocardial perfusion.7 Thus, a typical feature of CBF adaptation to exercise is the small or even absent change of coronary venous pO\(_2\) during hyperemia.7 This suggests that (venous or capillary)
pO2 itself is not a crucial metabolic dilator while a decrease of arterial pO2 is probably one of the most powerful stimuli for coronary vasodilation. However, metabolites produced in proportion to oxidative metabolism may control CBF. Examples are carbon dioxide (CO2), which is generated in the citric acid cycle, and reactive oxygen species (ROS) formed in the respiratory chain in proportion to oxygen consumption. Increased concentrations of CO2 result in increased proton concentration, which have been proposed as a direct stimulus for coronary vasodilation. Similarly, the production of hydrogen peroxide (H2O2) may constitute a metabolic regulator as its production is directly linked to myocardial oxygen consumption.

In 1963, Berne and Gerlach independently proposed that adenosine may be an important regulator of CBF in response to hypoxia and thus possibly in ischaemic tissue. Adenosine is formed by degradation of adenine nucleotides under conditions in which ATP utilization exceeds the capacity of myocardial cells to re-synthesize high-energy compounds. This results in the production of adenosine monophosphate, which is converted to adenosine by the enzyme ecto-5′-nucleotidase (CD73). Adenosine acts on specific A2 (and A1) receptors on VSMC and exerts dilator effects through the activation of the second messenger cAMP possibly involving hyperpolarization by the activation of the KATP-channel promoting K+ efflux. Since adenosine is generated also by endothelial CD73, it may be called an EDHF. In some species, adenosine-induced dilation exhibits a partially endothelium-dependent component through NO. Several findings support a relevant role of adenosine in the metabolic regulation of CBF. Indeed, its production increases in cases of imbalance between oxygen supply and demand, with the rise in interstitial concentration of adenosine paralleling the increase in CBF. However, the blockade of adenosine receptors or the enhancement of this vasodilator pathway by interfering with adenosine degradation leads only to a limited modulation of the overall dilator response in experimental settings and does not reduce the magnitude of functional hyperaemia entirely, thus suggesting that other substances may compensate for adenosine.

Autonomic nerves and circulating hormones
The contribution of the autonomic nervous system or circulating catecholamines on resting tone in the healthy coronary circulation is negligible. However, myocardial oxygen extraction was enhanced during exercise after autonomic denervation indicating insufficient CBF increase. This suggests that sympathetic activity contributes to exercise hyperaemia in a feed-forward manner likely through β-adrenoceptors. In addition, coronary vessels express α-adrenergic receptors that elicit vasoconstriction and compete with metabolic vasodilation. These are invoked during exercise as indicated by further increases of CBF in the presence of α-adrenergic blockade. Surprisingly, an adrenergic constrictor influence is still observed in ischaemic tissue and possibly aggravated in diseased states. Of other well-known vasoconstrictors, endothelin may contribute to coronary resting tone, whereas angiotensin II is less important despite the fact that coronary arterioles are in general responsive to these endogenous vasoconstrictors.

Remodelling, rarefaction, and collateralization
Signals and responses in structural vascular adaptation
It is important to understand that it is not tone alone, but rather inner vessel diameter, reflecting structure and superimposed smooth muscle tone that determines flow resistance and perfusion. Persistent changes in tone and circumferential wall tension drive structural long-term vascular adaptation (structural remodelling) leading to
long-term changes in diameter with or without alterations in wall mass (Figure 4). The responsible molecular events have been reviewed extensively, and will not be discussed in detail here. Since long-acting changes in vascular tone lead to structural vessel adaptations (see Figure 1), it is not surprising that the signals and responses in structural adaptation are very similar to those controlling vascular tone. Vessels adapt to mechanical stimuli, including fluid shear stress acting on EC (τ) and circumferential stress (or tension) acting on the wall (σ) as well as metabolic signals (Figure 4A).

To estimate the impact of structural adaptations on functional properties of vascular networks, integrations using mathematical modelling are essential (Figure 4C). Such models incorporate experimental findings and the contribution of individual mechanisms can be quantified thereby identifying potential effects of therapeutic interventions. On the basis of such models, a minimal set of biological reactions required for obtaining structures and functions comparable with the in vivo conditions was defined (solid lines in Figure 4C):

- Pressure responses result in arterio-venous asymmetry with smaller diameter, higher shear stress and larger pressure drop on the arterial side (where pressure is higher).
- Shear stress responses adjust and optimize vascular diameter to local flow thereby decreasing overall energy requirements.
- Metabolic stimuli including convection and conduction of metabolic signals support the maintenance of parallel flow pathways and avoid functional shunting.

In addition, coronary resistance and microvascular function can be affected by rarefaction, i.e. the loss of capillaries and microvessels resulting in a decrease vascular density. The process of rarefaction may be viewed as an extreme form of inward hypotrophic remodelling. Under resting conditions in the normal myocardium, the dense capillary network contributes <20% to coronary resistance (see Figure 3). This is, however, not true for situations of decreases in capillary density, e.g. in cardiac allografts or dilated cardiomyopathy. There are indications for capillary rarefaction in non-cardiac vascular beds as a surrogate of the coronary microcirculation, for example, in patients with hypertension.

Local perfusion in the heart is highly heterogeneous with flow distribution being stable over hours. Similar heterogeneity is reported for local oxygen consumption and metabolic activity and, interestingly, the latter correlates with local flow. The heterogeneous perfusion is a corollary of the structural heterogeneity of the terminal vasculature and the heterogeneity of metabolism may result from adaptation of tissue function to oxygen availability, which is partially limited by conduction and convection of signals along the vessel (Figure 4). It is unknown to date, to which degree microvascular heterogeneity contributes to pathological conditions and how microvascular heterogeneity is influenced by changes in upstream coronary function.

**Gender differences**

Gender differences in coronary regulation have been addressed in a number of original publications, and have recently been reviewed by the working group. Intracoronary infusion of testosterone may induce coronary artery dilation and increase CBF in men, which contrasts with the concept that testosterone is harmful to the male cardiovascular system. Similarly, there are gaps in our knowledge regarding the effects of oestrogen, and the concept that ischaemic heart disease rates in women rise remarkably after menopause, due to lower levels of oestrogen, is still open to debate. It was reported from the ONTARGET and TRANSCEND trial that, after controlling for confounders, the sex difference in cardiovascular risk showed no clear dependence on age and there was no indication that the natural menopause is associated with an increased risk for ischaemic heart disease. The Nurses’ Health Study investigators show that 4-year treatment with oestrogen did not deliver a protective effect for those women with a lower baseline risk for coronary heart disease. It is beyond the scope of this review to address all gender-related aspects, instead we focus on concepts that are relevant to vascular remodelling.

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**Figure 4**

Mechanisms and models of vascular remodelling. Left: Signals for vascular adaptation include wall shear stress at the endothelial surface (τ), circumferential wall stress (σ), and metabolic signals. Metabolic signals may be elicited by low oxygen availability and act as vasodilators and stimulate vascular growth, or they could be vasoconstricting mediators produced at high oxygen partial pressures but in decreasing amounts with decreasing pO2. Metabolic substances are convected downstream, but elicit also a signal that is conducted upstream within the vessel wall. Middle: Vascular responses elicited by these stimuli comprise changes of diameter and wall mass. Right: An integrated model which connects the local conditions (pressure, flow, and metabolic state) with derived stimuli (τ, σ, and metabolic stimuli) and the vascular changes in vessel diameter or wall mass. Lines indicate biological reactions (solid) and physical relations (dashed).
not reduce risk of CHD events. Controversies are even greater when looking at the multiple effects of oestrogens on the vascular bed ranging from coagulation to inflammation. Importantly, it should be noted that virtually all studies using oral oestrogen preparations, which represents only one aspect of gender differences, may turn out to have little relevance towards explaining the gender difference in regulation of vascular structure and function and development of ischaemic heart disease.

Vascular biology of collateralization
Collaterals are inter-arterial connections that provide blood flow to a myocardial territory with an obstruction in the original supply vessel. Collaterals result from pre-existing small arterial interconnections that enlarge to functionally relevant conduction vessels and provide a bypass and alternative blood supply. Coronary collateralization exists latently even in the normal heart, but becomes predominant in patients with coronary occlusion. Although well-developed coronary collaterals exert a protective effect against myocardial ischaemia and necrosis, their presence does not imply viability of the subtended myocardium.

In the human heart, the main mechanism of collateralization is arteriogenesis. Small collateral arteries remodel and expand in response to wall shear stress exerted onto EC by enhanced flow along collaterals. This, in turn, is caused by increased pressure gradients between pre- and post-occlusive vessels. The increased flow augments NO production through endothelial NO-synthase, followed by reprograming the endothelial gene machinery for migration (i.e. tissue factor dependent) and secretion of angiogenic factors (VEGF and others), which induce monocyte chemotactic protein-1 synthesis in EC and VSMC. Consequently, monocytes and T-cells are attracted and activated into the adventitial space (peripheral vessels) or are attached to the endothelium (coronaries). The proteases produced by these cells digest the extracellular matrix, providing space for new cells. Vascular smooth muscle cells in the media switch from contractile to proliferative phenotype. Actin polymerization and maturation are initiated by secretion of proteins like actin-binding Rho-activating (ABRA) protein, coflin, and thymosin β-4. The transcription factors AP-1, egr-1, carp, ERK-1, and ERK-2 play decisive roles in this complex process, which can result in 2- to 20-fold increases in vascular diameter.

Clinical implications
Clinical investigation of the coronary microcirculation
There is still no technique that allows direct visualization of coronary microcirculation in vivo in humans. However, both non-invasive techniques such as positron emission tomography (PET) and catheter-based approaches can be used to obtain information regarding microcirculatory function. Positron emission tomography permits the absolute quantification of myocardial perfusion, and is employed to investigate the function of the microvasculature and to monitor the effect of treatment on coronary microvascular dysfunction. A recent meta-analysis by Gould et al. has confirmed the ability of this technique to detect coronary microvascular dysfunction in subjects with CAD risk factors, in cardiomyopathies and in transplant vasculopathy.

Intracoronary measurements of flow velocity and pressure enable functional assessment of the coronary physiology (Supplementary material online, Table S1). Coronary flow reserve (CFR) assessed by different means (non-invasively, thermodilution, and Doppler-flow) is widely used but cannot distinguish between epicardial or microvascular resistance, and is dependent on haemodynamic indices partially due to its normalization to resting conditions. Combining distal coronary pressure measurements with flow parameters (transit time in thermodilution or Doppler guidewire-derived velocity) during hyperaemia circumvents these problems. The derived indices (thermodilution transit time: index of microvascular resistance [IMR]; flow velocity: hyperaemic microvascular resistance [HMR]) provide valuable information on minimal structural resistance of the coronary microcirculation in its fully vasodilated state. Moreover, such additional measures as well as measures of ‘no-reflow’ are useful predictors for long-term outcome implying that microvascular resistance determines disease progression and prognosis.

Coronary microvascular dysfunction
The endothelium exerts important functions at the crossroad of blood flow and tissue oxygen demand (see above). However, endothelial dysfunction, characterized clinically by impaired epicardial dilation or even constriction in response to the endothelium-dependent vasodilator ACh, is common in patients with CAD or ischaemic heart disease and predicts acute cardiovascular events. Even subjects without clinical signs of ischaemic heart disease but with traditional risk factors (smoking, hypercholesterolaemia, hypertension, obesity, and diabetes) or with chronic inflammatory vessel disease exhibit endothelial dysfunction which is thus one of the first clinically detectable alterations in the development of atherosclerosis. Moreover, it has been proposed that a hyperconstriction or spasm of coronary microvessels may cause coronary microvascular angina. Crea and coworkers suggested a clinical classification of coronary microvascular dysfunction according to the following clinical conditions: (i) In the absence of myocardial diseases and obstructive CAD, (ii) associated with myocardial diseases, (iii) associated with obstructive CAD, and (iv) iatrogenic.

It is clear that assessment of endothelial function, e.g. by testing flow-mediated dilation, is clinically important. Endothelial dysfunction is characterized by increased oxidative stress and production of ROS with gradual loss of endothelial NO availability and/or increased production of vasoconstrictors. ‘Endothelial dysfunction’ is not only an impairment of dilator function but also involves a switch from a quiescent to an activated state promoting inflammatory responses, chemokine and adhesion molecule expression and consecutive interaction with platelets and leukocytes. and will contribute to structural changes as described below (see Figure 1).

Coronary microvascular dysfunction is also initiated by classical cardiovascular risk factors which also maintain a low-grade inflammation. Additionally, chronic systemic inflammation is associated with coronary microvascular dysfunction that is possibly mediated through C-reactive protein (CRP) which were related to coronary flow reserve impairment in patients with a chest pain syndrome without risk factors for CAD and angiographically
normal epicardial arteries. Further, coronary microvascular dysfunction in non-obstructive CAD was associated with higher serum hs-CRP and increased plaque vulnerability. Interestingly, microvascular resistance was recently found to be increased in patients with syndrome X and correlated positively with exercise treadmill testing. These observations suggest that mechanical obstruction, structural vascular changes, and/or attenuated smooth muscle cell dilator efficacy are indeed contributing factors in these conditions in coronary microvascular dysfunction.

Coronary microvessels react to changes in mechanical forces due to upstream epicardial coronary artery stenosis. This not only affects vascular tone regulation but also initiates structural remodelling in the downstream microvascular bed. Such remodelling (e.g. arteriolar narrowing, inward remodelling) blunts dilator capacity, regulatory capability, and increases microvascular resistance and may thus be a substrate of microvascular dysfunction. Its potential reversibility upon elimination of the epicardial stenosis or pharmacotherapy has important implications for the progression of the disease and appropriateness of revascularization. However, arteriolar thickening is also reported for settings without epicardial coronary obstruction, e.g. in arterial hypertension and non-obstructive cardiac diseases, such as left ventricular hypertrophy, cardiomyopathies, and heart transplantation.

### Coronary collateral circulation

Coronary angiography allows only assessment of vessels with diameters > 100 μm, and visualization markedly depends on the pressure gradient exerted upon the coronary vasculature. Thus, a large part of the collateral network remains unexplored. These recruitable collaterals in the microcirculation serve an important therapeutic role, protecting against myocardial ischaemia and left ventricular dysfunction during brief coronary occlusion. Only one-third of patients with angina symptoms have a well-developed collateral network with sufficient capacity to prevent myocardial ischaemia during coronary occlusion. Nevertheless, a large proportion of patients without significant lesions exhibit high ‘pressure-derived collateral flow index’ (CFIp), suggesting that innate factors are contributing to collateral development. This is in line with a wide distribution of CFIp, in brief subtotal coronary occlusion. A meta-analysis of 12 studies enrolling 6529 participants with CAD showed that the lower mortality risk with a high degree of coronary collateralization was consistent in patients with both stable disease and acute coronary syndromes. Patients with high collateralization (defined as collaterals visible in coronary angiography, Rentrop scores 1–3, or collateral flow index, CFIp greater than or equal to 0.25) had a 36% reduced all-cause mortality risk compared with patients with low collateralization (no collaterals visible, Rentrop score 0, or CFIp less than 0.25; P = 0.012). Thus, the exploration of possible therapeutic approaches to foster collateralization is of greatest clinical impact.

### Therapeutic approaches and strategies

#### Pharmacotherapy

The most important modifier of microvascular and endothelial dysfunction is aerobic exercise (see below) in conjunction with other life-style changes (e.g. smoking cessation, weight loss). Nevertheless, different interventions have been shown to restore endothelium-dependent vasodilation at least partially. These include folic acid, statins, and angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. Also, an improvement of endothelial function after prolonged application of endothelin receptor antagonists was demonstrated, but this awaits confirmation in larger clinical trials. Other targets, that are currently studied experimentally, include endothelial K⁺-channels (Ca²⁺-dependent K⁺-channels) which upon activation promote EDHF-type dilations and possibly NO release, and modulation of eNOS coupling. For acute myocardial infarction with no obstructive coronary atherosclerosis, diagnosis, and therapeutic treatments have been detailed in a recent review.

A central target of pharmacotherapy of the cardiac microcirculation is a ‘normalization’ of altered microvascular vascular structure (‘re-remodelling’) thus reducing the resistance of the coronary microvascular bed. Studies with anti-hypertensive drugs suggest a rather different potency of different classes of drugs with respect to re-remodelling. While β-blockers seem to have a very limited effect with respect to re-remodelling and diuretics are only a little more potent, re-remodelling is sensitive for vasodilators (e.g. calcium antagonists, ACE-blockers, and AT1-receptor antagonists) and may even be independent from the reduction in blood pressure. The preferential potency of vasodilators seems to be obvious considering the stimulus (enhanced tone) to initiate remodelling of microvessels in hypertension. In contrast, a successful approach to reverse arteriolar obliteration and capillary rarefaction is currently not available.

In many disease conditions (diabetes mellitus, hypertension, atherosclerosis, and hyper-homocysteaemia), however, the availability of NO is reduced. The physiological stimulus for endothelial release of NO is wall shear stress and its phasic changes. Thus, it was logical to explore the potential of phosphodiesterase inhibitors to reduce coronary resistance by elevating cGMP levels. At this time, however, their clinical use is limited. Interestingly, there is also a class of β-blockers, which release NO. Statins are likely improving coronary circulation by their long-term effects on vascular structure and function which seem to independent from their LDL lowering effects. Statins increase NO bioavailability, reduce oxidative stress, and inflammation, all of which are favourable to maintain and improve coronary microcirculation.

A relevant area to consider is the interference with vasoconstrictor mechanisms in coronary microcirculation disorders. Vasoconstrictor mediators include endothelin, thromboxane A₂ and other COX1, COX2, and Cytochrome P450-derived metabolites. Also, the balance between the constrictor α and dilator β receptors may be tilted towards α receptors in diseased conditions, representing another potential target for intervention. For an in-depth review of pharmacotherapeutical strategies in coronary microvascular dysfunction, the reader is referred to two excellent recent reviews.

#### Exercise training

Exercise training is perhaps the most physiological and best approach to exploit the adaptive capacity of the coronary vascular bed and to evoke a number of functional and structural changes (Figure 5). Exercise training produces long-term adaptations in the coronary microvasculature, including angiogenesis with increased...
arteriolar and (transiently) capillary densities and structural diameter increase. Together, these adaptations act to enhance peak CBF rates to maintain or even slightly increase maximal myocardial blood flow per gram of myocardium, and enlarge capillary exchange capacity despite the exercise-induced cardiac hypertrophy. Improved coronary capillary blood flow distribution may result from structural changes in the coronary tree as well as from alterations in tone regulation in resistance arteries. The latter includes increased myogenic changes in the coronary tree as well as from alterations in tone regulation in resistance arteries. The latter includes increased myogenic changes in the coronary tree as well as from alterations in tone regulation in resistance arteries.6 The latter includes increased myogenic changes in the coronary tree as well as from alterations in tone regulation in resistance arteries.6 The latter includes increased myogenic changes in the coronary tree as well as from alterations in tone regulation in resistance arteries.6

The limitations of pro-arteriogenic therapy led to the search for key factors in patients that may explain the aforementioned innate variability of development of collaterals.142 Unfortunately, arteriogenesis in humans is largely unexplored as collaterals are not accessible for biopsy and their post-mortem identification is cumbersome. However, circulating mononuclear cells may aggravate atherosclerosis in mice, which constitutes a major drawback of pro-arteriogenic therapy.143 In a murine hind limb model of arteriogenesis, an anti-arteriogenic approach was indeed verified.143 In total coronary occlusion, a gene-polymorphism related to galactin-2 expression was pivotal in facilitating the acceptance of this approach.143 The combined use of FFR and CFR has underscored the role of microvascular dysfunction in ischaemic heart disease. However, adoption of FFR in clinical practice remains low in Europe and the USA and is virtually non-existing in most ‘emergent countries’ in which the number of PCI procedures is increasing exponentially. Therefore, the stenosis-centred concept of myocardial ischaemia has to be replaced by an integrative approach also including coronary microvascular dysfunction, structural remodelling, rarefaction, and collateralization.

Nevertheless, there are many open questions that need to be addressed in future studies as a basis of such an integrated approach: With respect to coronary microvascular dysfunction, it remains to be established whether endothelial dysfunction is a key mechanism in all types of coronary microvascular dysfunction. We also need to better understand a possible change in vasomotor regulatory mechanisms, ranging from NO to other endothelial dilators such as prostaglandins or EDHF with age and/or disease. We need to establish the occurrence and impact of arteriolar obliteration and capillary

### Evaluation of pro-arteriogenic therapies in clinical studies: a bedside-to-bench approach

Several growth factors were originally identified in experimental models of collateral artery development and subsequently tested in patient trials. Administration of growth factor (GM-CSF, GSF) is easy (subcutaneously) and was tested in patients with stable CAD.141 However, the clinical study using GM-CSF was prematurely halted because of development of unstable angina in some patients. In fact, stimulation of mononuclear cells may aggravate atherosclerosis in mice, which constitutes a major drawback of pro-arteriogenic therapy using growth factors. Nevertheless, GSF enhanced CFI, which suggests stimulation of coronary arteriogenesis (Figure 6).

The limitations of pro-arteriogenic therapy led to the search for key factors in patients that may explain the aforementioned innate variability of development of collaterals.142 Unfortunately, arteriogenesis in humans is largely unexplored as collaterals are not accessible for biopsy and their post-mortem identification is cumbersome. However, circulating monocytes, the key players in arteriogenesis, are easily obtainable and transcriptome analysis of monocytes provided 244 differentially regulated genes between good and bad arteriogenic responders.143 In non-complete obstruction, genes related to interferon-β, a type I interferon, were up-regulated in poor arteriogenic responders suggesting a hampering effect on arteriogenesis. In a murine hind limb model of arteriogenesis, an anti-arteriogenic effect of applied interferon-β was indeed verified.143 In total coronary occlusion, a gene-polymorphism related to galactin-2 expression was demonstrated in all monocyte cell subtypes. Again, poor responders were characterized by overexpression of galactin-2 suggesting that inhibition of interferon-β or galactin-2 expression may stimulate arteriogenesis.143 Thus, direct comparison of monocyte expression patterns of patients with either poor or well-developed collateral circulation may lead to the identification of new targets for stimulation of arteriogenesis.

### Cell strategies to stimulate angiogenesis/vasculogenesis and vascular remodelling

Neo-vascularization in the adult does not rely solely on angiogenesis (mature EC sprout from pre-existing vessels, migrate, and proliferate to form new vessels), but also on vasculogenesis, i.e. new vessels formed from circulating or tissue-resident endothelial stem or progenitor cells which proliferate to de novo EC.144 Cell-based therapy has the potential to supply stem/progenitor cells and multiple angiogenic and trophic factors to the region of ischaemia to stimulate therapeutic neo-vascularization. A variety of stem cells (SCs) have been used to enhance cardiac vascular repair, although many past and ongoing clinical trials exploit predominantly adult autologous bone marrow cells. Due to ease of harvest and abundance, interest has recently grown in the developmental plasticity and therapeutic potential of SCs isolated from adipose tissue (ADSC). One of the most interesting characteristic of ADSC is their potential to stimulate angiogenesis, reduce apoptosis, and exert anti-inflammatory effects suggesting an active role of ADSC in revascularization of ischaemic damaged tissues. Most of these effects are believed to be mediated via paracrine activity.146 Further away of clinical applications, but holding promise for the future, are induced pluripotent stem cells. Starting from the pluripotency of embryonic SCs, a new type of SC has been created by reprogramming somatic cells.

However, cell therapy clinical trials, to date, have not really shown the expected beneficial results.147,148 Therefore, finding ways to stimulate or even induce structural angioadaptation, collateralization, angiogenic and vasculogenesis using various SCs therapies is the challenge of the future.

### Outlook and open questions

Revisiting the concepts and areas of knowledge on regulation and remodelling in the coronary microcirculation has an enormous importance for some of the most contemporary issues in diagnosis and treatment of ischaemic heart disease. The benefit of coronary revascularization in stable CAD, boosted by the development of percutaneous techniques, has been recently challenged, highlighting the importance of properly identifying ischaemia-generating stenoses as targets of PCI. The development of fractional flow reserve (FFR), a pressure-derived index of stenosis severity, and the solid evidence on the superiority of physiology-based decisions over coronary angiography, has been pivotal in facilitating the acceptance of this approach. The combined use of FFR and CFR has underscored the role of microvascular dysfunction in ischaemic heart disease. However, adoption of FFR in clinical practice remains low in Europe and the USA and is virtually non-existing in most ‘emergent countries’ in which the number of PCI procedures is increasing exponentially. Therefore, the stenosis-centred concept of myocardial ischaemia has to be replaced by an integrative approach also including coronary microvascular dysfunction, structural remodelling, rarefaction, and collateralization.

Nevertheless, there are many open questions that need to be addressed in future studies as a basis of such an integrated approach: With respect to coronary microvascular dysfunction, it remains to be established whether endothelial dysfunction is a key mechanism in all types of coronary microvascular dysfunction. We also need to understand a possible change in vasomotor regulatory mechanisms, ranging from NO to other endothelial dilators such as prostaglandins or EDHF with age and/or disease. We need to establish the occurrence and impact of arteriolar obliteration and capillary
Figure 5  Structural and functional coronary microcirculatory adaptations to chronic exercise training in normal subjects. Exercise produces a number of functional adaptations, of which the most important are improvement of endothelial function and reduction in heart rate to reduce average extravascular compressive forces. Exercise also produces a number of structural adaptations (arteriolar densities and diameters) that serve to maintain maximal myocardial blood flow in the face of cardiac hypertrophy. Ach, acetylcholine; M, muscarinic receptor; NE, norepinephrine; $\alpha_1$, $\beta_1$, $\beta_2$: adrenergic receptors; $K_v$, voltage-dependent $K^+$ channel; $K_{Ca}$, $Ca^{2+}$-dependent $K^+$ channel. Adapted from Laughlin et al. with permission of the American Physiological Society.
rarefaction on patient prognosis. With respect to microvascular dysfunction distal to a coronary artery stenosis, we only have an incomplete understanding of its molecular mechanisms. Furthermore, we do not know what its relevance in the human heart is and to what extent it can be reversed by relieving a proximal stenosis and re-establishing physiologic flow. Other important questions are whether results from non-cardiac (e.g., skeletal muscle) vessels can be extrapolated to the coronary circulation, and whether simple and non-invasive methods can be developed to identify coronary endothelial dysfunction and predict prognosis in patients.

With respect to collateral circulation, we need to identify the exact molecular mechanisms underlying structural adaptation and collateral formation in the coronary vascular bed which can guide therapeutic developments in coronary and peripheral artery disease. Furthermore, we need to address the question whether there is a significant role for EPC in arteriogenesis, and whether these cells are affected by the systemic effects of the disease process and local endothelial dysfunction. In case EPC and SCs are effective, approaches to boost their function and/or an efficient delivery platform for therapeutic approaches targeting the coronary microcirculation are required. Another important question is how intracoronary parameters, available to assess collateral flow, and non-invasive diagnostic techniques (MRI, PET) allow to quantify modulation of arteriogenesis, how they can be validated and routinely applied. We also need to identify which therapeutic strategies apart from exercise training can induce efficient structural collateralization and angiogenesis. Finally, we need to determine suitable endpoints for therapeutic interventions. Answering these questions will be critical for the advancement of treatment of patients with CAD.

Conclusions

Atherosclerotic disease of the epicardial coronary arteries has been accepted as the principal cause of angina pectoris for more than two centuries, and sudden thrombotic occlusion of an epicardial coronary artery has been well established as the cause of acute myocardial infarction for >100 years. However, in recent years it has been demonstrated that functional vascular alterations such as endothelial dysfunction may preclude development of overt atherosclerotic disease of the epicardial arteries and contribute to myocardial ischaemia. Furthermore, it must be considered that epicardial arteries are only one segment of the coronary circulation. The importance of the coronary microcirculation in the maintenance of appropriate myocardial perfusion is well established and evidence accrued over the past 20 years has proven that abnormalities in the function and structure of the coronary microcirculation occur in many clinical conditions and can contribute to the pathogenesis of myocardial ischaemia (metabolic syndrome, diabetes, hypertension, etc.). Both experimental studies for the evaluation of different mechanisms involved in coronary microvascular dysfunction and prospective clinical studies that evaluate the effect of interventions restoring these microvascular abnormalities on patient prognosis are mandatory.

Supplementary material

Supplementary material is available at European Heart Journal online.

Acknowledgements


References

Coronary vascular regulation


