

# Coronary Flow Reserve from Mouse to Man—from Mechanistic Understanding to Future Interventions

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**Abstract** Myocardial ischemia is recognized as an important mechanism increasing the risk for cardiovascular events in both symptomatic and asymptomatic patients. In addition to obstructive coronary diseases, systemic inflammation, macro- and microvascular function are additional important mechanisms contributing to the ischemic myocardium. Accumulating evidence indicates that coronary flow reserve (CFR) is a quantitative measurement of ischemia including integrated information on structure and function of the coronary artery at all levels. Not surprisingly, CFR has been shown to confer strong prognostic value for hard cardiovascular (CV) events in a number of relevant patient cohorts. Using high-resolution imaging, it is now possible to study coronary arteries from mouse to man. Therefore, CFR may be an important translational tool to risk-stratify patients and to perform both preclinical and clinical proof-of-concept studies before investing in large-scale outcome trials, thus improving the translational value for novel CV targets.

**Keywords** Coronary flow reserve · Ischemia · Cardiovascular events · Risk-stratification · Cardiac catheterization · PET · CT · MRI · Transthoracic echocardiography · Color Doppler · Translational medicine · Microvascular function · Systemic inflammation · Ischemic myocardium

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## Introduction

During the last decade, a decline in age-adjusted cardiovascular (CV) mortality has been observed [1]. Increasing public awareness as well as recent advancement in medical and surgical interventions are considered contributing factors to this positive trend. However, ischemic cardiovascular disease remains the number one killer in the westernized world and increasing prevalence of diabetes and obesity is believed to accelerate the global cardiovascular disease burden [2].

There is accumulating evidence indicating that myocardial ischemia is an important cause associated with residual CV risks among both symptomatic and asymptomatic CAD [3, 4]. Indeed, transient ischemia could also lead to sudden death due to lethal arrhythmia [5–7]. In the setting of chronic stable ischemic heart disease, angiogram-guided percutaneous coronary intervention (PCI) has not been shown to deliver superior benefits compared to optimal medical treatment, while a fractional flow reserve (FFR)-guided approach, which measures the hemodynamic significance of stenosis, has begun to demonstrate promising results (FAME II) [8].

Acute myocardial infarction (MI) is considered an atherothrombotic event, which in turn is under the influence of three major triggers according to Virchow's triad: vessel injury either through plaque rupture or endothelial erosion, hypercoagulability and finally reduced and/or disturbed flow [9, 10]. In the arterial system with normal blood flow velocity around 1 m/s, flow-stopping atherothrombosis is rare, while in the venous system with slow and occasionally stagnated flow, thrombosis may occur without atherosclerosis. In contrast to most other arteries, where flow occurs predominantly in systole, coronary perfusion is mainly a diastolic event. Also, the normal blood flow velocity in the epicardial coronary arteries is much lower than in for example: aorta and carotid arteries [11]. The unique anatomy and physiology of the coronary circulation require specialized

physiological adaptations. Understanding the biology of the coronary vascular bed may help us to risk stratify patients as well as identify novel treatment regimes to combat coronary artery disease.

### Concept of Coronary Flow Reserve

The concept of coronary flow reserve (CFR) was introduced to reflect the maximal oxygen delivery capacity during increasing demand. Since cardiomyocytes are capable of extracting oxygen with great efficiency already at rest, the only way of meeting increasing demand is to increase coronary blood flow [11, 12]. At rest, there is a significant autoregulation in the coronary vascular bed which ensures sufficient blood supply to the cardiomyocytes under a wide range of perfusion pressure. Thus, resting coronary blood flow is mainly under control of tissue metabolic demand [13]. To test the maximum flow reserve capacity, the autoregulation must be uncoupled, which will create a linear relationship between perfusion pressure and flow. Thus, CFR is usually measured during either exercise challenge or pharmacological stimuli such as dobutamine and adenosine [14].

Hemodynamically significant coronary lesions are known to cause reduced CFR [12, 14]. In the case of diffuse coronary artery atherosclerosis without angiogram-visible significant stenosis, CFR could be an objective tool to assess the hemodynamic significance, not necessarily reflected by distinct local stenosis. This has been comprehensively described by Dr. Gould et al., who pioneered the concept in humans [15]. However, impaired CFR could be due to many other pathological conditions, such as microvascular dysfunction in for example: diabetic patients [16]. Interestingly, the presence of thin cap fibroma in the coronary artery has been reported to be associated with impaired CFR, indicating an important but not completely surprising link between atherosclerotic lesion composition and downstream vascular function [17].

While FFR seems to be a fit-for-purpose tool to address hemodynamic consequences of focal lesions, CFR could be a measure of the entire coronary vascular functionality. Even though there is correlation between CFR and FFR, significant discrepancy between these two measures is evident, which may suggest that they indeed reflect distinct aspects of the coronary arteries [18]. This means that not all patients with impaired CFR would have any intervenable coronary lesions according to FFR, and that CFR will not necessarily be improved even following the best FFR-guided PCI procedure.

Even though myocardial perfusion scintigram (MPS) is considered the gold-standard for assessment of myocardial ischemia, the method relies on flow-redistribution during stress testing when a hemodynamically significant stenosis is present. In the case of balanced three vessel disease and

microvascular dysfunction, MPS results are less reliable in term of identifying the high risk patient [19]. CFR as an objective quantitative tool, will assess the hemodynamic significance of angiographic moderate or ambiguous stenosis, as well as diagnose microvascular diseases (Fig. 1) [15].

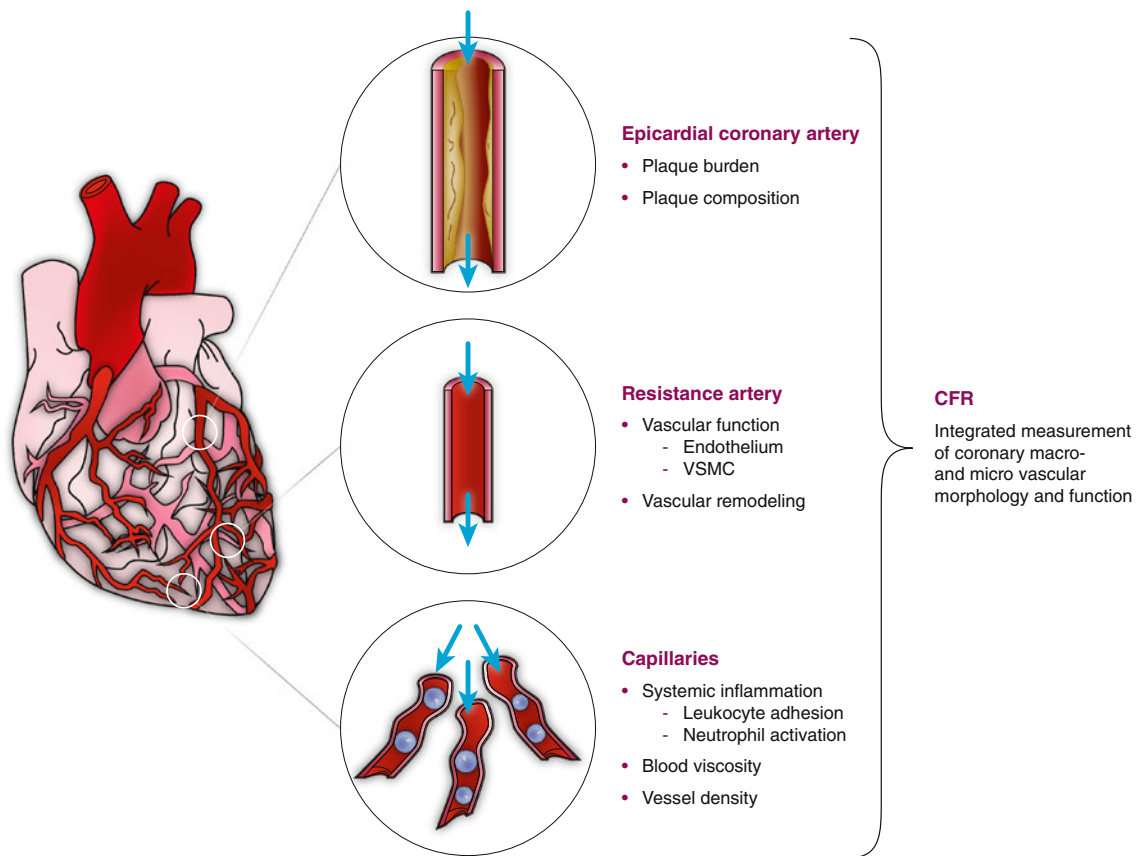
### Methods to Assess CFR in Man

Coronary flow reserve can be measured using many modalities. Thermodilution was one of the invasive techniques used to determine CFR in man [20]. Coronary flow velocity reserve (CFVR) can be measured using invasive Doppler-guide wires using, for example adenosine as a stress agent [21]. Recently, a combined FFR and CFVR catheter has been launched for simultaneous flow and pressure measurement [22].

Non-invasive techniques have been developed during the last two decades taking advantage of modern high-end imaging modalities. Positron emission tomography (PET) is now established as the gold standard for absolute coronary blood flow and flow reserve measurement. PET provides both ischemia evaluation as well as absolute flow quantification [23]. Various tracers can be used including those that do not require a full-scale cyclotron facility, for example: rubidium [24]. In combination with high-resolution computer tomography (CT), this modality can provide both structural and functional information about ischemic heart disease [25]. Even though PET is an established approach to assess CFR, radiation exposure as well as high equipment and running cost limit its wide use. Contrast CT might be a new modality for perfusion imaging. This method is also associated with radiation although the level of radiation has decreased substantially with the latest generation CT [26]. Magnetic resonance imaging is a non-radioactive valuable method, capable of assessing CFR, but is relatively costly and time-consuming [27].

Myocardial contrast echocardiography is an inexpensive and non-radioactive method for measurement of CFR. When image quality is adequate, CFVR as well as CFR can be readily measured [28]. However, for accurate and reproducible quantification of CFVR and CFR, high-quality images are required both during rest and hyperemia allowing contrast intensity measurement to derive flow velocity as well as steady-state flow signals.

Transthoracic color Doppler echocardiography (TDE-CFR) has been successfully used to measure coronary blood flow velocity (CBFV) and CFVR [29]. With proper machine setting and skilled hands, the technique is non-radioactive and feasible in every echo-lab without need of contrast agent. Using this approach, success rate is greatest in the left anterior descending (LAD) coronary artery. In addition, right posterior descending coronary artery [30] as well as circumflex artery



**Fig. 1** CFR provides an integrative assessment of coronary vascular health at all levels of the vascular tree. Accumulating evidence suggests that myocardial ischemia is the net result of pathophysiological mechanisms within the epicardial vessels, resistance arteries and the capillaries. In addition to diffuse or obstructive epicardial atherosclerosis, vascular

function and remodeling are important mechanisms contributing to overall myocardial perfusion. The microvasculature is influenced by systemic inflammation such as leukocyte adhesion and neutrophil activation with direct consequences on perfusion. Increased blood viscosity such as in hyperlipidemic patients, has also direct implications on capillary flow

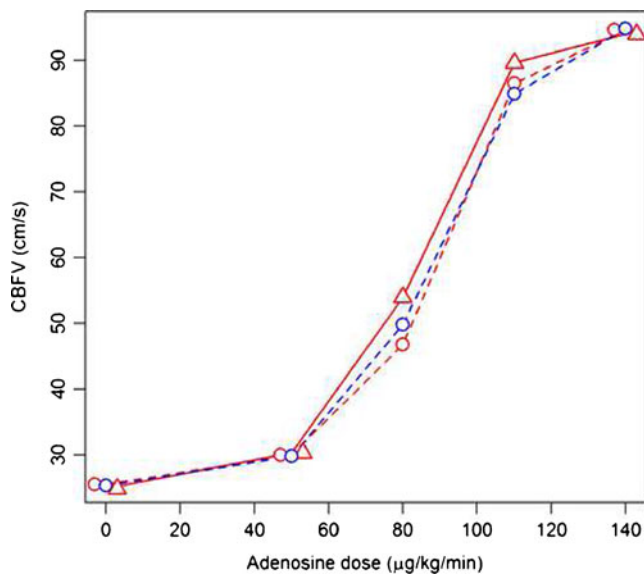
(LCX) CFBV is measurable [31]. Accuracy of these approaches has been validated previously against invasive techniques [30]. Technically, for access to the distal LAD, only a small echo-window is needed compared to for example: left ventricle ejection fraction measurement using Simpson’s rule, where a couple of cm echo-window is needed for good image quality. Thus, obesity is not an absolute hurdle for the success of this technique [32]. For real-life clinic use of this technique for diagnosis of ischemic heart disease including obstructive CAD and microvascular disease, dipyridamole-assisted TDE-CFR has been successfully applied on a routine basis in combination with wall motion analysis [33]. Using this approach, the high specificity of stress echo can be combined with the high sensitivity of CFR for diagnosis of ischemic heart disease in patients with suspect CAD.

For experimental purposes, LAD TDE-CFR has been used for risk prediction as well as documentation of intervention effects. Recently, our group showed excellent inter- and intra-day variability of CBFV in a randomized double blind clinical study [34]. During this study CBFV upon increasing doses of adenosine was measured repeatedly. To

minimize environmental influences, all subjects stayed at the clinical trial unit the evening prior to the examination and received standardized meals etc. Thus, under controlled conditions, variability for CFBV measurement using this approach was satisfactorily low (Coefficient of variance for AUC for CBFV and adenosine doses <1 %) (Fig. 2).

**Determinants of CFR in Man**

To further understand biological processes and the clinical relevance of CFR, numerous studies have been performed to relate CFR to cardiovascular biomarkers. These studies revealed that CFR indeed could serve as a composite physiological marker of systemic CV risk. Besides obstructive CAD, which is an obvious determinant of CFR [12, 14], impaired CFR has been reported in patients with dilated cardiomyopathy [35], as well as patients with diabetic vascular complications [36]. Further, myocardial capillary density has also been shown to determine CFR in patients with dilated cardiomyopathy [37].



**Fig. 2** Adenosine dose versus mean coronary blood flow velocity response pre-placebo (red dashed line, circle), post-placebo (red solid line, triangle) and pre-ticagrelor (blue dashed line, circle). Red dashed and red solid lines represent thus intra-day variability, whereas red dashed and blue dashed lines represent inter-day variability (adapted from [34])

CFR is rheologically associated with blood viscosity, which in turn is dependent on lipoprotein and fibrinogen levels. In familiar hypercholesterolemic patients, CFR is related to LDL level and is improved after LDL apheresis, supporting the concept that increased blood viscosity due to extremely high LDL level can be a determinant of CFR [38].

CFR has also been shown to correlate to markers of low degree systemic inflammation, such as C-reactive protein [39] and neutrophil count [40]. Based on large-scale genetic association studies, IL-6 has recently been shown to be causally linked to CV outcome alongside LDL-regulating pathways [41]. Interestingly, a PET-CFR study conducted in twins showed that IL-6 indeed was a determinant of CFR in those asymptomatic subjects [42].

It is well-known that endothelium plays a central role in CV disease etiology and progression [43]. Even though CFR is measured using a non-endothelium dependent vasodilator, for example: adenosine, for achieving maximum hyperemic flow response, intact endothelial function is required. CFR has been shown to be associated with peripheral endothelial function both in chest pain patients and in healthy volunteers [44, 45] and direct inhibition of the nitric oxide pathway reduced hyperemic response in healthy volunteers [46].

### Prognostic Values of CFR in Man

Impaired CFR has been shown to confer great prognostic information for hard CV outcome, including all cause mortality, CV death and non-fatal MI. Independent of imaging modality used, the prognostic power of CFR appears to be consistent. In

post-MI setting, invasively assessed CFVR showed prognostic value in the “DEBATE” study. In such a setting, microvascular obstruction as well as infarct size may impact on CFVR alongside residual ischemia [47]. This concept has been extensively evaluated in patients with suspected coronary artery disease since they are undergoing ischemia testing based on clinical indication. In these patients, CFVR adds incremental prognostic values on top of wall motion analysis in terms of prediction of mortality and spontaneous CV events [33]. Interestingly, in the paper by Murthy et al., PET-CFR magnitude dependently predicted CV mortality across various levels of infarct size, ischemic burden and LV ejection fraction [48]. These data strongly supports the concept that CFR rather than just obstructive coronary artery disease contributes to CV vulnerability. In line with this, Murthy et al. showed that diabetic subjects without previous CAD but with impaired CFR had the same risk as non-diabetic patients with CAD [49]. Further, CFR showed great prognostic information in patients with dilated cardiomyopathy [50], which suggests that impaired CFR is an independent risk driving CV events in additional patient cohorts. In the context of post-infarct cardiac remodeling, impaired CFR is an independent predictor of adverse left ventricle remodeling [51]. The quantitative nature of CFR measurement is also reflected in its prognostic information, since it has also been shown that CV outcome is related to CFR in a magnitude dependent manner, that is the lower the CFR the worse the outcome and vice versa [48].

Transplant vasculopathy is a phenomenon of diffuse coronary artery disease for which traditional angiogram-based approaches cannot be used to follow the disease progression. In these patients, CFVR has been successfully used as a prognostic marker [52]. This further supports the concept that the integrative physiological assessment of the coronary functionality is of greater importance than only the luminology-based approaches.

### Effects of Established Interventions on CFR in Man

That surgical by-pass or removal of hemodynamically significant obstructive epicardial lesions can improve CFR is of course a direct consequence of these procedures and follows the purpose and definition of a successful revascularization [53]. Other established CV treatments have also been shown to improve CFR through mode of action impacting on mechanisms controlling CFR (Table 1).

#### LDL Apheresis

Blood viscosity lowering approaches, for example: LDL apheresis, induce acute improvement of CFR in familiar hypercholesterolemic patients with extraordinarily high cholesterol levels [38].

**Table 1** CFR and pharmacological interventions

Mode of action	Patients	Treatment	CFR before treatment	CFR after treatment	Treatment duration	Reference
Statins	No angiography-verified CAD and normal cholesterol	Atorvastatin vs placebo	2.47±0.34	3.02±0.54, <i>p</i> =0.0012 (no change in placebo)	1 h	[54]
		Fluvastatin (no placebo)	3.0±0.5	3.5±0.8, <i>p</i> <0.01	3 months	[55]
		Atorvastatin: low-dose (LD) 10 mg or moderate-dose (MD) 40 mg	LD: 2.02±0.56 MD:1.98±0.58	LD: 2.58±0.67, <i>p</i> <0.05 MD:2.96±0.73, <i>p</i> <0.05	8 months	[56]
Anti-hypertensives	Slow coronary flow	Atorvastatin (no placebo)	1.95±0.38	2.54±0.56, <i>p</i> <0.001	8 weeks	[57]
	Hypertension	Telmisartan vs nifedipine	2.4±0.4	2.9±0.4, <i>p</i> <0.01 (no change in Nifedipine)	12 weeks	[58]
Hypertension and microvascular angina pectoris	Hypertension and LVH	Enalapril (in hypertensives vs normotensive patients with hypertrophic cardiomyopathy)	2.2±0.6	3.3±1.2 ( <i>p</i> <0.001) (no change in normotensive patients)	11 to 13 months	[59]
		Perindopril (no placebo)	2.1±0.6	3.5±1.9 ( <i>P</i> =0.001)	12 months	[60]
		Lisinopril vs losartan	2.4±1	3.7±1.1, <i>p</i> =0.002 (no change in Losartan)	12 months	[61]
		Eplerenone vs hydrochlorothiazide	1.38 (1.11–1.61)	1.57 (1.36–2.14) vs hydrochlorothiazide 1.30 (1.08–1.47), <i>p</i> =0.03	6 weeks	[62]
		Perindoprilat vs placebo= saline	2.7±1.0	3.9±0.9, <i>p</i> <0.001 perindoprilat infusion	Acute infusion	[63]
Fenofibrate	Diabetes + LVH	Fenofibrate vs placebo	2.8±0.58	3.14±0.36, <i>P</i> <0.01 (no change in placebo)	6 months	[64]
Anti-inflammatory	Rheumatoid arthritis	Single dose Anakinra vs placebo (4 h) and Prednisolon (1 month)	2.39±0.6	4 h: 2.85±0.7, <i>p</i> <0.001 1 month: 3.08±0.5, <i>p</i> <0.001 (no change in placebo or Prednisolon)	4 h and 1 month	[65]
Anti-oxidant	IDC and hyperuricemia	Allopurinol (no placebo)	1.87 [1.63–2.00]	2.20 [1.87–2.49], <i>p</i> <0.001	3 months	[66]

CAD coronary artery disease, AMI acutemyocardialinfarction, IDC idiopathic dilated cardiomyopathy, LVH left ventricular hypertrophy



## Statins

Statins have shown both acute effects on CFR probably through pleiotrophic effects but also in the medium-long term, which most likely is due to their LDL-lowering anti-atherosclerotic effects.

## Anti-Hypertensives

Anti-hypertensive drugs such as angiotensin-converting enzyme blocker or receptor blockers have shown beneficial effects on CFR through their effects on endothelium as well as anti-inflammatory properties. Decreased afterload following successful blood pressure normalization will also contribute to improvement of CFR due to reduced oxygen demand.

## Anti-Inflammatory

In patients with increased systemic inflammation such as rheumatoid arthritis, blockade of IL-1 pathway using anakinra immediately improved CFR and increased peripheral endothelial function as measured by flow-mediated forearm vasodilation as well as pulse wave velocity. Compared to prednisolon, which did not show any impact on the cardiovascular system in these patients, this treatment demonstrated sustained effects on these endpoints also at one month follow-up. The beneficial CV effects were also associated with a decrease in systemic inflammatory markers.

## Translational Aspects from a Drug Discovery Perspective

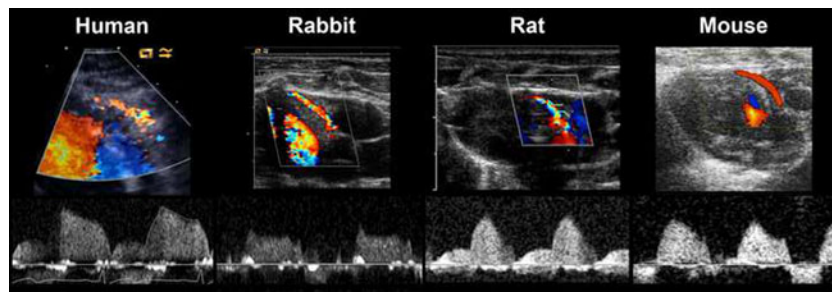
Discovery and development of novel anti-atherosclerotic drugs to reduce cardiovascular events on top of standard of care is an area of high risk. Several difficulties have to be overcome in order to succeed with delivering a positive outcome in phase III trials. Traditionally, novel targets and compounds are validated in pre-clinical models of atherosclerosis with the primary read-out being a reduction in plaque progression/size. Often such data provide the sole basis in terms of *in vivo* intervention data before investing in large clinical programs. For clinical proof-of-concept, primary end-points have focused on plaque centric endpoints such as plaque volume, intima-media-thickness or plaque composition. Although these endpoints are associated with CV disease and CV events, limited information is available on how intervening with these endpoints affects CV benefit. This is not surprising since, atherosclerotic plaques are only one component contributing to the ischemic myocardial event in humans. Many additional biological mechanisms have been shown to play a role in triggering the event including microvascular dysfunction, endothelial dysfunction, systemic inflammation, vasospasm, thrombosis and angiogenesis [67–70]. By using a more integrative approach for proof-of-

concept both pre-clinically and clinically, clinical development could be potentially de-risked.

To study the effects of novel anti-atherosclerotic compounds in animals within reasonable time frames, disease progression needs to be accelerated by genetic manipulation and/or feeding high-fat high-cholesterol containing diet. No single animal model available today mimics all aspects of this complex human disease and it is important to note in particular that no model reliably and reproducibly replicates the rupture and subsequent thrombotic occlusion of coronary arteries. As discussed previously, the pathophysiological events that occur at the level of the atherosclerotic plaques have been of great interest during the past few decades and also in animal studies targets which are involved in reducing plaque progression have been in focus for potential future interventions for reducing cardiovascular events. To gain confidence in potential novel therapies for reducing CV events, it is crucial that pre-clinical studies focus on the proper vascular bed and the relevant translational endpoints. Despite the prevalent use of mouse models of atherosclerosis, few have focused on the coronary arteries due to technical difficulties with studying these small arteries. Fortunately, due to recent advances with high-resolution imaging, it is now possible to perform morphological and functional imaging studies of cardiovascular systems in living mice [71–74]. This has dramatically increased the translational value of preclinical disease models and may provide guidance for dose selection and Proof of Principle studies in humans.

Despite the high heart rate in mouse, coronary flow velocity can be measured in a reproducible way using high-resolution color Doppler ultrasound. This technique has now been applied in rodents and rabbits according to the human protocol (Fig. 3). The flow profiles in mouse left coronary artery (LCA) are strikingly human-like regarding amplitude, duration and systolic/diastolic distribution [71]. Due to its small size, little is known about coronary atherosclerosis and its functional implications in mice. By using ultrasound biomicroscopy the LCA can be visualized and the extent of atherosclerosis determined in a non-invasive, longitudinal way in living mice [75]. In a study performed in apoE/LDL receptor-deficient mice the percent wall thickness in the proximal LCA increased with age. Percent wall thickness was significantly associated with color Doppler echocardiography assessed mid- to distal coronary flow velocity ratios (Fig. 4). The extent of coronary atherosclerosis in the proximal LCA was confirmed by histological sections and correlated well with percent wall thickness and color Doppler echocardiography velocity ratios.

Our group pioneered the work to establish the TDE-CFVR approach in mice [71, 72, 74, 75] using high-resolution ultrasound. In addition to mid- to distal flow velocity ratio approach to determine LCA stenosis, CFVR can be determined in mice in a similar way as in humans using color Doppler



**Fig. 3** Representative ultrasound imaging of the LCA in human, rabbit, rat and mouse. *Upper panel:* Color Doppler is used for identification of the LCA. In the present imaging windows, the LCA is shown as *bright red-yellow streaks*. *White arrows* indicate the site of the LCA where pulsed Doppler is used to measure coronary blood flow velocity. *Lower*

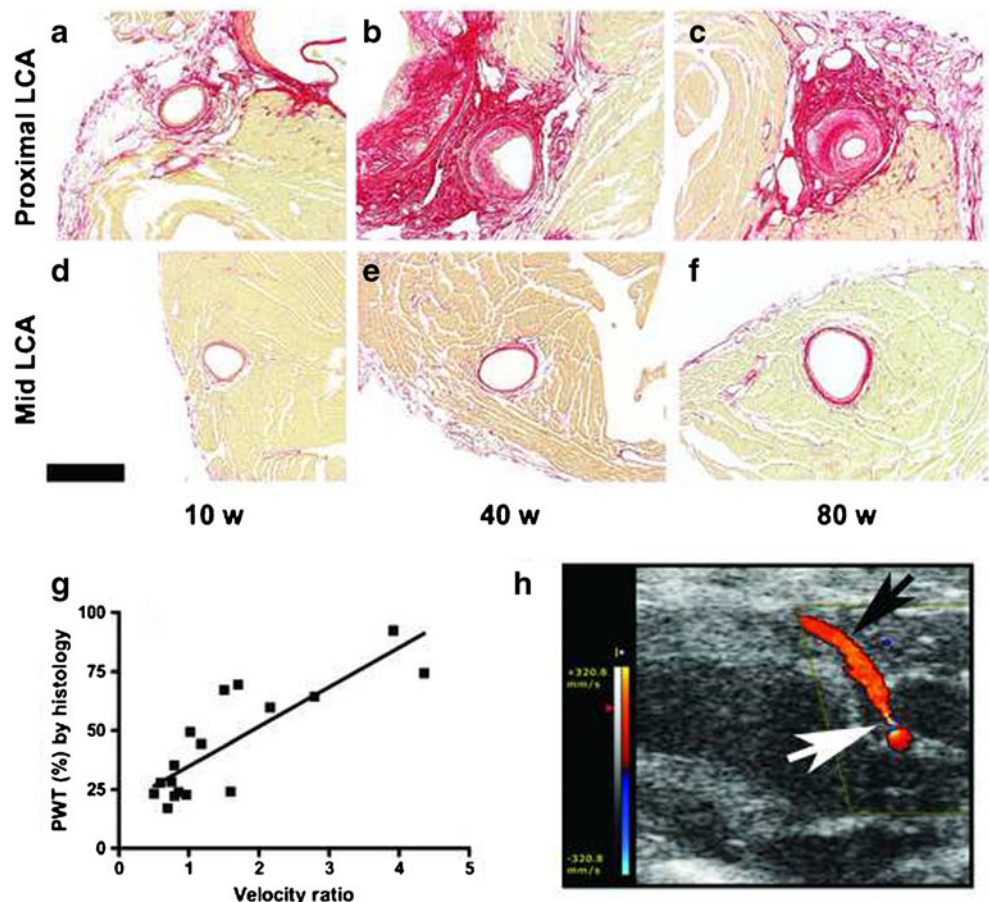
*panel:* corresponding pulsed wave Doppler signals for measurements of coronary blood flow velocity. Similar flow velocity patterns are evident between the species with minor flow signal during systole and a major flow signal during diastole. Flow velocity on *y-axis* (maximum velocity 100 cm/s in these images), time on *x-axis*

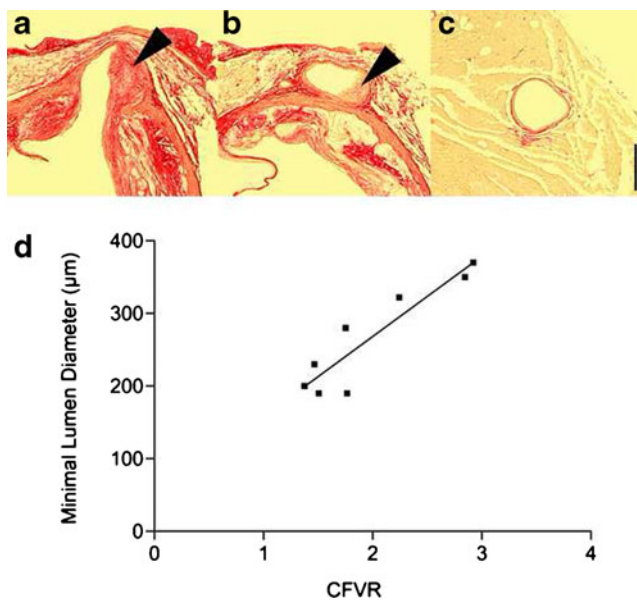
echocardiography. Hyperemia can be induced using adenosine at 160  $\mu\text{g}/\text{kg}/\text{ml}$  which is similar to the dose used in humans. This dose was shown to induce hyperemia without any apparent effects on systemic hemodynamics. Hypoxia is an alternate non-invasive means of inducing a similar increase in CFVR. In LDLR-deficient mice with coronary atherosclerosis, a correlation could be found between the extent of lumen narrowing and CFVR (Fig. 5) [72]. Although a correlation between coronary atherosclerosis and CFVR was demonstrated in mice it has also been shown that inflammation could impact on

CFVR, for example: CFVR is reduced following experimental coxsackie virus-induced myocarditis [73].

Although similar protocols are used to study CFVR in mice and humans, caution should be taken when comparing the absolute magnitude of changes since anesthesia is necessary in animal experiments and this has been shown to influence CFR in rodents [76]. Indeed, the most commonly used anesthetic agent isoflurane has been to shown to increase CFBV per se [77]. To assess coronary artery function in mice, CFVR is a good surrogate for CFR. In humans, a

**Fig. 4** Plaque growth with increasing age in apoE/LDL double knock-out mice in the proximal (a,b,c) and mid-LCA (d,e,f) and the correlation between coronary artery percentage wall thickness as measured with histology and proximal to mid flow velocity ratio in the double knock-out mice of different age (g). *Scale bar*=300  $\mu\text{m}$ . A typical color Doppler image (h) showing site of proximal stenosis (*white arrow*) and stenosis-free segment in the more distal parts of the LCA (*black arrow*). Adapted from [75] with permission





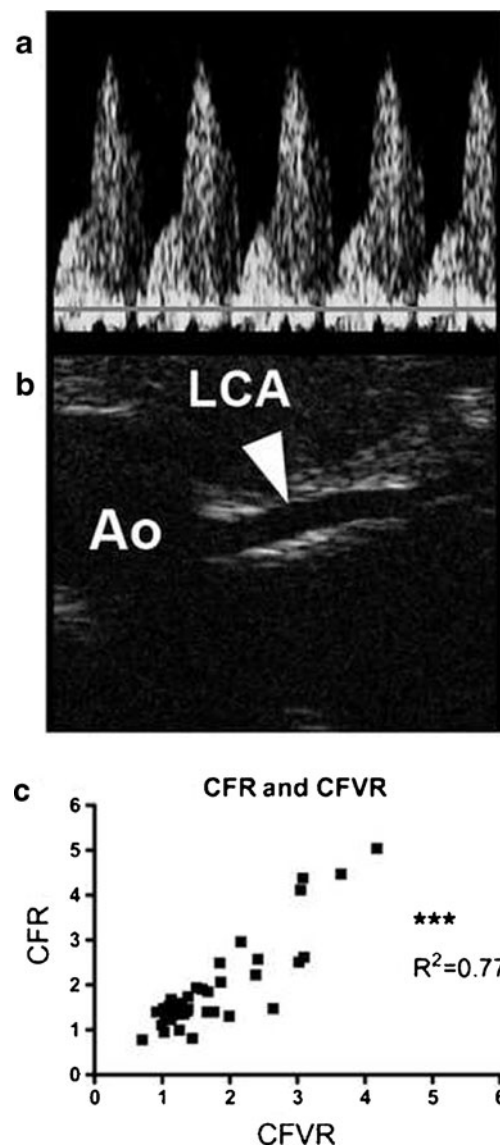
**Fig. 5** Evidence of coronary artery atherosclerosis in the proximal segment (a, b) but not the mid-segment (c) of the left coronary artery in low-density lipoprotein receptor gene-deficient mice. A correlation was evident between minimal lumen diameter and CFVR (d) as imaged with high-resolution ultrasound. *Arrowheads* indicate coronary lesions. *Scale bar*=200 µm. Adapted from [72] with permission

good correlation between CFVR and CFR has been demonstrated [78]. By measuring LCA lumen diameter and flow velocity simultaneously in mice, a good correlation between CFVR and CFR was evident. However, due to the larger intra- and inter-observer variability in CFR measures, CFVR is a more robust marker of coronary vascular health in mice [74] (Fig. 6).

### Effects of Interventions on CFR in Rodents

The TDE-CFR approach has also been developed and applied in other species, for example: rat, to demonstrate effects of various interventions. We showed that spontaneous hypertensive rats improved their CFVR following voluntary endurance training through increased vascular anti-oxidant capacity [79]. Improvement of CFVR was also associated with improved vascular compliance and acetylcholine-mediated vasodilation in peripheral vascular beds [80] (Fig. 7).

Statins have become a standard therapy for CV patients and any novel therapy will need to demonstrate significant benefit on top of statins. Thus, back translation of statin effects to animal models is of fundamental importance for drug discovery and development. In addition to their cholesterol lowering effects, statins have been shown to retard the progression of atherosclerosis [81], improve endothelial function [82], reduce systemic inflammatory markers and reduce cardiovascular mortality [82–84]. The response to statins in animal models

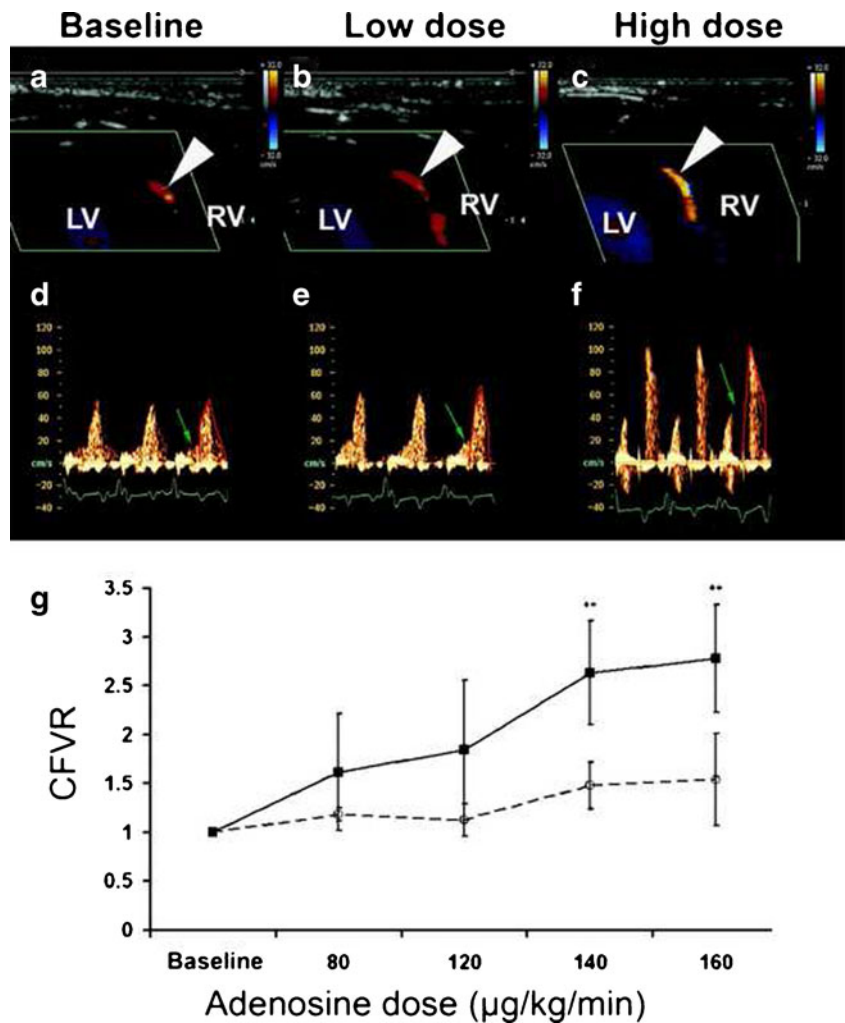


**Fig. 6** CFR can be calculated from measurements of flow velocity measurements with pulsed Doppler (a) (flow velocity on the y-axis and time on the x-axis) and diameter measurements in B-MODE images (total image width 3 mm) of the proximal to mid-segment (b) of the left coronary artery in vivo. *Arrowhead* indicate site where the LCA diameter was measured. There was a good correlation between CFVR and CFR (c). *Ao*=Aorta, *LCA*=left coronary artery. Adapted from [74] with permission

varies with species, strain, gender, diet and protocol used. Although statins reduce plasma cholesterol and plaque area in some murine models, the exact mechanisms behind the cholesterol lowering effects are largely unknown. Statins have shown to reduce atherosclerosis in the apoE\*3Leiden by changing lipoprotein profiles and total lipid levels [85, 86] in plasma. However, the so called “pleiotropic effects” may also play a significant role in this model [87]. In apoE- and LDLr-deficient mouse strains, statins have shown variable results on plasma cholesterol levels and plaque progression. ApoE-deficient mice on a mixed C57BL/6 and 129SvJ (Taconic



**Fig. 7** Ultrasound imaging of the LCA in rat and improvement in CFVR with exercise. *Upper panel (a–c)*: Color Doppler images from baseline (a), low dose (b), towards high dose of adenosine (c) with corresponding pulsed wave Doppler images (*mid-panel:d–f*). CFVR and adenosine dose–response curves for voluntarily exercising spontaneous hypertensive rats (solid line) vs non-exercising controls (*dashed line*) (g). As evident, voluntary exercising rats had higher CFVR compared with controls after 6 weeks.  $**p < 0.01$ . Adapted from [79] with permission



Denmark) background on a high-fat diet (21 % pork lard and 0.15 % cholesterol) responded to rosuvastatin in a human like manner. Plasma Cholesterol levels were reduced on average by 20 % during the 16-week study period [88]. In addition, brachiocephalic plaque area and the inflammatory serum biomarker serum amyloid-A (SAA) were significantly reduced at the end of the study suggesting additional effects similar to those observed in humans. Assessing coronary artery flow, calculated from UBM measured coronary diameter and color Doppler echocardiography flow velocity revealed an increase in hyperemic blood flow at the end of the study, again in line with findings in humans. Generating data in this animal model responding to statin therapy in a human-like way will improve our confidence in novel targets and facilitate future translational drug intervention studies in mice.

Grönros et al. used the TDE-CFVR approach to document effects of a novel arginase inhibitor, which may act by restoring normal nitric oxide balance in the vascular endothelium. In a rat model of type 2 diabetes, microvascular dysfunction as measured by CFVR was demonstrated relative to control rats.

Further, the arginase inhibitor succeeded to improve CFVR only in diabetic rats and not in control rats [89].

### CFR as a Translational Tool for Target Validation

We and others have shown that CFR in vivo is reduced in late stage mouse atherosclerosis mouse models [72, 90], but also in the absence of coronary atherosclerosis such as in models of diabetes [91], blood pressure overload induced heart failure [92] and myocarditis [73]. CFR can thus be measured in various disease models in vivo for exploration of disease mechanisms and might be used to validate novel drug targets.

5-Lipoxygenase (5-LO), five lipoxygenase activating protein (FLAP) and its products leukotrienes B<sub>4</sub> (LTB<sub>4</sub>) and cysteinyl leukotrienes (cys LTs) have been linked to cardiovascular disease [93]. The ALOX5AP (FLAP) haplotype HapA has been associated with risk of MI [94] and levels of 5-LO, FLAP and LTA<sub>4</sub>H mRNA in human carotid plaques have been correlated with the occurrence of plaque instability

[95]. In a phase II study, the 5-LO inhibitor, VIA-2291 reduced coronary plaque volume and new formation of rapidly growing coronary lesions in ACS patients following 6-months treatment [96]. These findings are in great agreement with many preclinical studies performed in animal models of atherosclerosis [97, 98].

To further explore the potential role of the 5-LO pathway in low degree subclinical inflammation and coronary vascular function, we carefully investigated CV function in 5-LOX-deficient mice (AstraZeneca Transgenic Centre, Mölndal, Sweden). We hypothesized that these mice could be protected from deleterious effects of lipopolysaccharide (LPS) challenge in a low-grade inflammatory, sub-acute setting.

Adenosine-induced CFVR, cardiac function and morphology were measured before and one week after LPS-challenge (2 mg/kg) in 5-LOX<sup>-/-</sup> and in back-crossed littermate C57BL/6 wild-type mice. Several cytokines and ex vivo aortic endothelial function were measured one week after LPS challenge to avoid acute sepsis-like condition. Cardiac function (not shown) and CFVR did not differ between the groups before the LPS challenge. However, one week after LPS challenge, CFVR was decreased in WT mice ( $p < 0.05$ ) while no significant change was evident in 5-LOX<sup>-/-</sup> mice (Fig. 8). In vivo cardiac measurements were similar after LPS (not shown). Ex vivo aortic acetylcholine-mediated relaxation capacity in phenylephrine pre-contracted segments of the thoracic aorta was impaired in WT mice compared to 5-LOX<sup>-/-</sup> mice post LPS ( $p < 0.01$ ). Levels of IL-10 were higher in the plasma of 5-LO<sup>-/-</sup> mice compared to WT mice post LPS ( $p < 0.05$ ).

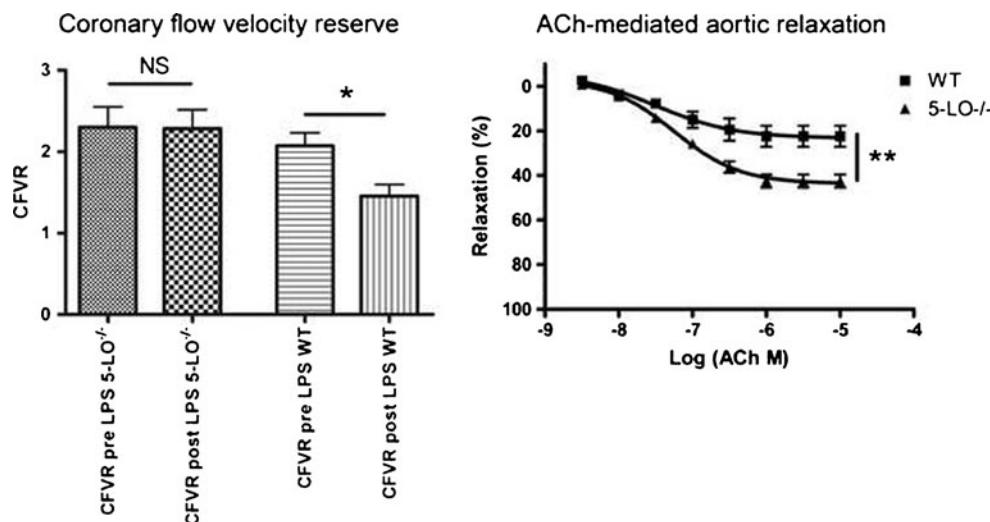
Higher doses of LPS might induce septic shock with acute effects such as myocardial contractile dysfunction, drop in systemic blood pressure, vasoplegia, tissue hypoperfusion, microvessel injury and multiple organ injury [99], whilst low or moderate doses of LPS have been shown to induce endothelial dysfunction in experimental settings [100]. Our data seems to be consistent with the previous findings that 5-

LOX-deficient mice maintain blood pressure control better than control mice [101] and rats treated with a 5-LO inhibitor (Zileuton) showed reduced organ failure [99] following severe LPS endotoxemia. In addition to mediating inflammation [93, 102], direct vasoactive actions of the LTs have been frequently reported in both coronary [103] and aortic [101] arteries of several species. Thus, 5-LO metabolites seem to have both direct and indirect vasomodulating properties, especially in abnormal conditions, as observed in atherosclerotic arteries of humans [103]. Taken together, in addition to its anti-atherosclerotic effects, 5-LO pathway inhibition may also improve coronary vascular function in patients with systemic low-grade inflammation.

### Concluding Remarks

In summary, accumulating evidence indicates that CFR may serve as a composite marker for coronary macro- and microvascular status, as well as systemic risk profile. Quantitative measurement of CFR provides us with an accurate prognostic tool to risk stratify patients as well as to follow disease progression and potential effects of intervention. Mechanistically, CFR is closely associated with many major pathoetiological factors driving residual risk in CV patients, such as epicardial vessel atherosclerosis burden and composition, left ventricle hypertrophy, vascular rarefaction, dyslipidemia, subclinical inflammation, endothelial dysfunction, etc. Establishment of the translational imaging techniques opens up exciting possibilities for deepened mechanistic understanding of this important pathophysiological phenomenon. Accumulated knowledge will hopefully accelerate development of novel therapeutic approaches targeting unexplored pathways to improve CFR and thereby further reduce CV mortality and morbidity. A translational approach and comprehensive understanding of the biology may potentially de-risk clinical development programs of

**Fig. 8** CFVR in the LCA in vivo before (pre) and after (post) LPS challenge (a) and ex vivo aortic relaxation capacity with increasing doses of acetylcholin (ACh) (b) in 5-LO KO mice after LPS challenge. \* $p < 0.05$ , \*\* $p < 0.01$



novel therapies. In addition to treatment correcting dyslipidemia and emerging drugs to reduce systemic inflammation, recent scientific progress within regenerative medicine may also in the not too distant future contribute to step-change therapies in terms of for example: regeneration of cardiomyocytes and coronary microvessels, and thereby restore a functional myocardium with well-dimensioned vascular bed.

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**Conflict of Interest** All authors are employers of AstraZeneca R&D but have no conflicts to disclose in relation to this paper.

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