The role of epoxyeicosatrienoic acids in the cardiovascular system

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Keywords
- cardiovascular system
- epoxyeicosatrienoic acids
- soluble epoxide hydrolase inhibitor
- vascular tone

There is increasing evidence suggesting that epoxyeicosatrienoic acids (EETs) play an important role in cardioprotective mechanisms. These include regulating vascular tone, modulating inflammatory responses, improving cardiomyocyte function and reducing ischaemic damage, resulting in attenuation of animal models of cardiovascular risk factors. This review discusses the current knowledge on the role of EETs in endothelium-dependent control of vascular tone in the healthy and in subjects with cardiovascular risk factors, and considers the pharmacological potential of targeting this pathway.

Introduction

Cardiovascular disease remains one of the greatest challenges faced by medicine today. It is responsible for approximately 3 in 10 deaths worldwide [1]. In the UK, 1 in 6 deaths in men and 1 in 10 deaths in women are attributable to cardiovascular disease, resulting in an average of 200 deaths per day [2].

The vascular system is made up of approximately 60 000 miles of different-sized blood vessels, lined by a single layer of endothelial cells [3]. The pioneering work of Furchgott in the 1980s demonstrated that the endothelium not only serves as an inert lining of blood vessels, but releases endothelium-derived relaxing factors (EDRF) [4], later identified as nitric oxide (NO). It is now known that the endothelium releases many vasodilating molecules including prostacyclin (PGI₂) [5, 6], and endothelium-derived hyperpolarizing factors (EDHF) (Figure 1) [7], and vasoconstricting molecules such as endothelin, angiotensin II and thromboxane. These regulate smooth muscle tone (Figure 2), balance anticoagulation and thrombosis, modulate immune responses, and regulate cell growth. Shear stress exerted on the vessel wall or stimulation of endothelial receptors with drugs can induce the release of endothelium-derived mediators [8]. Change in vascular tone in response to pharmacological stimulation is a reproducible ‘surrogate measure’ of overall endothelial function [9], which importantly predicts cardiovascular events in humans [10–14].

Endothelial dysfunction, characterized by an underproduction of vasodilators and an overproduction of vasoconstrictors, is a key predisposing factor to the initiation of atherosclerosis [15]. It appears early in the course of cardiovascular disease, even before the clinical manifestation of atherosclerosis or vascular disease. Traditionally, endothelial dysfunction predominantly refers to impaired NO signalling [16], but it has become evident that other endothelium-derived mediators, such as EDHF, may also be affected. EDHF (or EDHFs) describes a number of factors, including epoxyeicosatrienoic acids (EETs), hydrogen peroxide (H₂O₂) [17], potassium (K⁺) ions [18, 19] and likely other factors, depending on the vascular bed. It appears that larger conduit arteries have a greater expression of endothelial nitric oxide synthase (NOS), whereas the EDHF mediated pathway becomes more significant as vessel size reduces [20]. Indeed, resistance arteries with a diameter <400 μm are vital in modulating peripheral vascular resistance, and may be involved in the pathophysiology of hypertension. Increasing evidence suggests that EETs, in particular, exert cardio-protective effects in the smaller resistance vessels, and up-regulating the EETs signalling pathway pharmacologically may be beneficial in improving endothelial function. All drug
and molecular target nomenclature in this review conforms to the British Journal of Pharmacology's Guide to Receptors and Channels [21].

**Synthesis and metabolism of EETs**

Arachidonic acid metabolism leads to the production of two vasodilating factors, PGI₂ and EETs. EETs are the product of a number of cytochrome P450 (CYP450) enzymes. CYP2C and CYP2J produce EETs of four different isoform; 5,6-EET, 8,9-EET, 11,12-EET and 14,15-EET (Figure 3) [22]. EETs are mainly produced by CYP2C9 and CYP2J9, although CYP2C8, CYP2C19 and CYP2J2 are also involved [23, 24]. CYP2C9 mainly produces EETs in the vascular endothelial cells, and CYP2J9 is expressed in the cardiomyocytes [25], kidneys [26], pancreas [27], lung [28] and the brain [29], though, with less catalytic activity compared with the 2C family [30]. CYP4A and CYP4F families in the vascular smooth muscle cells catalyze the ω-hydroxylation of arachidonic acid to hydroxyl-eicosatetraenoic acids (HETEs), which act as vasoconstrictors in the vascular system. Although this review mainly focuses on the role of EETs metabolized from arachidonic acid, EETs can also be generated from eicosapentaenoic acid, and mediate dilatation of microvessels with comparable potency in a similar mechanism [31].

In vivo, EETs are rapidly metabolized by soluble epoxide hydrolase enzymes (sEH) to their corresponding diols, dihydroxyeicosatetraenoic acids (DHETs), with a short half-life of about 8 min [32, 33]. The C-terminal domain of the sEH enzyme is involved in the hydrolysis of EETs, whilst the N-terminal domain of sEH demonstrates lipid phosphatase activity. This is thought to limit the physiological effects of EETs, as they are generally more biologically active than DHETs [34], but in some vascular beds, such as canine coronary microcirculation [35] and murine mesenteric arteries [36], EETs and DHETs may be equipotent vasodilators. The substrate specificity for sEH is regio-isomer selective, e.g. 5,6-EET is a poor substrate for sEH [37, 38]. EETs are also metabolized by other pathways, including β-oxidation, ω-oxidation and chain elongation, particularly under sEH inhibition [39]. EETs can be incorporated into cell membrane phospholipids, through an

**Figure 1**

Mechanisms of endothelial dependent vasodilation mediated by nitric oxide, prostacyclin and endothelium derived hyperpolarizing factors. Pharmacological agonists can bind to endothelial receptors and stimulate the release of these factors in a calcium dependent manner. The vasodilating factors act on the smooth muscle and mediate vasodilation by mechanisms shown in Figure 2. R, receptor; M1 and M3, muscarinic receptors; B2, bradykinin receptor; Ca²⁺, calcium ions; NOS, nitric oxide synthase; NO, nitric oxide; GC, guanylate cyclase; cGMP, cyclic guanosine monophosphate; PGI₂, prostacyclin; AC, adenylate cyclase; cAMP, cyclic adenylic monophosphate; EDHF, endothelium derived hyperpolarising factor; EET, epoxyeicosatetraenoic acid; H₂O₂, hydrogen peroxyde; K⁺, potassium ions.
acyl-coenzyme A-dependent mechanism, and liberated through the action of phospholipase A₂ when the cell is activated [30].

**The role of EETs in regulating vascular tone**

It has long been known that derivatives of arachidonic acid regulate vascular tone [40, 41]. The hypothesis that non-cyclo-oxygenase metabolites are involved in endothelial dependent regulation of vascular tone arose from experiments showing attenuated arachidonic acid induced relaxation under CYP450 inhibition [42, 43].

There is now convincing evidence that hyperpolarizing factors released from the vascular endothelium show similar characteristics to CYP450 metabolites [44] and EETs have been identified as a hyperpolarising factor in both animal [45] and human vessels [46]. The vasodilatory effects of EETs can be region-isomer and organ selective [47]. For example, in mice mesenteric arteries, 8,9-EETs are the most potent for regulating vasorelaxation [36], whereas in rat kidneys, 8,9-EETs can be metabolized by COX enzymes to vasoconstrictor metabolites in pre-glomerular vessels [48], and in pulmonary arteries, 8,9-EETs increase pulmonary artery constriction [49].

In humans, 11,12-EETs mediate vasorelaxation in internal mammary arteries and under inhibition of NO and PGI₂ syntheses, cytochrome P450 inhibition further reduces both bradykinin and acetylcholine stimulated flow, suggesting a role for CYP450 metabolites in agonist induced vasodilatation [46]. In vivo, the role of EDHFs also varies depending on the vascular bed and the mode of stimulation. In healthy subjects, there is a greater role of EDHF in bradykinin-, but not acetylcholine-induced vasodilatation. Exercise induced vasodilatation in skeletal muscles can release EETs under NOS inhibition [50], elucidating cross-talk between the various endothelial released vasodilating factors, and this may be more significant in different cardiovascular risk groups.

The physiological role of EETs in maintaining basal tone appears to be limited. Basal flow response was
investigated in six human in vivo studies, and five reported no change in basal flow in healthy subjects [51–55], and one reported 13% and 17% reduction in response to fluconazole in 26 healthy subjects and seven patients with cardiovascular risk factors, respectively [56]. Three of these in vivo studies assessed basal tone in fewer than 10 subjects [51–53], and thus were significantly underpowered.

**Mechanisms of action in the vascular system**

A number of different pathways are involved in mediating EET-induced vasodilatation, including calcium-dependent K⁺ channels, gap junctions, endothelial NOS and transient receptor potential (TRP) channels. The precise pathway(s) involved depends on the vascular bed, and can be endothelium dependent via intermediate-conductance calcium-dependent K⁺ (IK) and small-conductance (SK) channels, TRP channels [8, 57] leading to NOS activation [36], or through a smooth muscle effect via TRP channels or a G-protein coupled receptor, and acting via large conductance (BK) channels.

Calcium-dependent K⁺ channels on endothelial and smooth muscle cells are usually activated in a calcium-dependent fashion. K⁺ efflux and hyperpolarization of the cell membrane leads to calcium channel closure on smooth muscle cells and vasorelaxation occurs as a result of reduction in intracellular calcium (Figure 2) [58].

In porcine [59] and bovine coronary arteries [60], EETs can act locally on the endothelial IK and SK channels. This interaction with calcium-dependent K⁺ channels may be through TRP channels.

TRP channels, particularly TRPV4 in the vallinoind subfamily, interact with EETs and regulate vascular tone [61, 62]. TRPV4 is a calcium permeable voltage gated channel expressed in a range of tissues including the endothelial and the smooth muscle cells. In mice, inhibition of TRPV4 with ruthenium red significantly reduces vasodilatation in CYP2C9 over-expressed arteries.
Co-inhibition of EET synthesis and TRPV4 does not have an additive inhibitory effect, suggesting that EETs act primarily through the TRPV4 pathway [63]. Under NO and PGI2 inhibition, 11,12-EETs elicit hyperpolarization in mesenteric arteries in wild type mice, but not TRPV4−/− mice, and this can be completely inhibited by blocking IK, SK and BK channels with charybotoxin, apamin and iberiotoxin, respectively [64]. Blood pressure is higher in TRPV4−/− mice, suggesting that TRPV4 may be an important regulator of vascular tone.

TRPV4 agonists and 11,12-EET can activate TRPV4 channels in a cluster fashion and leverage a large calcium influx through each TRPV4 channel, leading to activation of IK and SK channels [8]. The current is then likely to spread through myoendothelial gap junctions resulting in relaxation [65–68]. When vessels are stimulated with bradykinin, other TRP channels are activated, transient receptor potential cation (TRPC) channel 3 and 6. Bradykinin-induced calcium influx can be inhibited by CYP inhibitors and EET antagonists, and enhanced by a sEH inhibitor [69]. TRP channels rapidly translocate to caveolae to modulate calcium influx in response to 11,12-EETs [69]. This process is dependent on the activation of cAMP-dependent protein kinase and may be dependent on caveolin-1 [70]. In some vascular beds, an increase in intracellular calcium stimulates endothelial NOS (Figure 4) [36, 71].

In human internal mammary arteries [46], EETs act on the BK channels expressed on smooth muscle cells, and this may be via TRPV4 channels or through a specific EET receptor. TRPV4 channels mediate the activation of BK by forming a signalling complex with ryanodine receptors and BK channels on the smooth muscle.

It appears that EET activation of BK channels is not simply by binding to an extracellular domain, but there are strict requirements for their vascular activity. In bovine coronary arteries, 14(S),15(R)-EET, but not 14(R),15(S)-EET increases BK channel activity [72], whereas 11(R),12(S)-EET is the isomer that activates the BK channel in rat renal smooth muscle cells [73]. Furthermore, tethering 14,15-EET to silica beads restricts entry into smooth muscle cells, but does not attenuate its inhibitory effect on aromatase [74]. This suggests that there is a specific EET binding site on the smooth muscle cell (Figure 4). A high affinity binding site has been characterized using radioligands in U937 monocyte membranes, where a novel radiolabelled EET agonist bound in a specific, saturable and reversible manner, resulting in the production of cAMP production with similar potency as 11,12-EET and 14,15-EET. The G-protein analogue GTPγS inhibited this binding, suggesting that EETs act via a G-protein coupled receptor [75, 76]. However, a group of 47 known receptors were screened for the ability of EET regio-isomers to displace high

Figure 4
This diagram shows the mechanisms by which EETs exert hyperpolarization effects on the endothelial cell and the smooth muscle cell. Agonist binding to a luminal receptor of the endothelial cell activates phospholipase A in a calcium dependent manner, which converts phospholipids to arachidonic acid. EETs are products of CYP450 enzyme metabolism. EETs may activate the ICa and SKCa channels via TRPV4 channels. EETs may activate BKCa and KATP channels via an EET receptor or via TRPV4 channels. R, receptor; M1 and M3, muscarinic receptors; B2, bradykinin receptor; Ca2+, calcium ions; NOS, nitric oxide synthase; NO, nitric oxide; GC, guanylate cyclase; cGMP, cyclic guanosine monophosphate; PL, phospholipids; PLA2, phospholipaseA2; AA, arachidonic acid; CYP, cytochrome P450 enzymes; K+, potassium ions; BK, large conductance calcium-dependent potassium channel; KATP, ATP sensitive potassium channel; TRP, transient receptor potential channels, RGS, G-protein coupled receptor coupled; cAMP, cyclic adenylate monophosphate
affinity radioligands, and none was identified as a receptor for EETs [77].

In bovine [78] and porcine [79] coronary smooth muscle cells, EET-mediated smooth muscle BK activation requires intracellular GTP, but not ATP, and can be blocked by a G protein inhibitor or antibodies against Ga, suggesting that a G protein is required for EETs to activate BK channels. EETs promote GTP binding to Ga in endothelial cells [80], and BK channels can be activated directly by GTP-activated Ga through a membrane-delimited action of Ga or by activation of a classic signalling cascade. In both bovine endothelial cells and U937 monocytes, EETs activate adenylyl cyclase and protein kinase A [75, 80–82], which can stimulate transmembrane of hyperpolarization through gap junctions [83]. In a similar fashion to activation of BK channels, EETs can activate ATP-sensitive K+ channels on smooth muscle cells in rats [84, 85].

**Other physiological roles of EETs**

Other than mediating vascular tone, EETs modulate calcium channels on cardiomyocytes [86, 87] and 11,12-EETs can improve recovery of cardiac contractile function following prolonged ischaemia [88]. EETs also regulate pancreatic β-cell function, where 5,6-EETs directly induce insulin secretion [89], and CYP2J is highly expressed in the cells of islets to produce a significant amount of EETs in human and rat pancreas [90].

EETs attenuate inflammatory processes, which play a key role in the pathophysiology of cardiovascular diseases [91]. Various stimuli, such as microorganisms, lipid products or hypoxia can cause vascular injury and lead to endothelial activation, a highly dynamic and complex process that intertwines endothelial dysfunction and inflammation. Leukocyte-endothelial adhesion and subsequent leucocyte transmigration across the endothelium are primary events in the vascular inflammatory process influencing the initiation of atherosclerosis and cardiovascular diseases. 11,12-EET can attenuate endothelial activation and leucocyte adhesion in induced models of inflammation by inhibiting nuclear factor-kappaB (NFκB), a central mediator of this process [92].

Increased recognition of the benefits of EETs has revealed a worrying paradox that is their broad physiological impact may potentially have deleterious effects too. EETs promote endothelial cell survival by pro-angiogenic [93] and anti-apoptotic mechanisms [94]. They contribute to vascular endothelial growth factor (VEGF) mediated stimulation of angiogenesis [95]. Whilst this may exert some protective benefits in preserving endothelial function and promoting neovascularization in ischaemic tissues [96], their potential to promote cancer metastases warrants careful consideration [97, 98]. Indeed, inhibition of CYP-derived EET synthesis increases tumour cell apoptosis, and decreases tumour growth and metastases [99].

**EET signalling in cardiovascular disease**

Dysregulated EET signalling pathways may be implicated in a number of disease states. Whilst most cardiovascular risk factors are associated with impaired EETs and induction of sEH expression, there is much crosstalk between the endothelial factors, and alteration in EET signalling may change as cardiovascular disease progresses. In the presence of stable coronary atherosclerotic disease, where there is reduced NO signalling [100], EETs may in fact be up-regulated to compensate for the overall endothelial dysfunction. The first study to quantify plasma concentrations of EETs in patients with stable coronary atherosclerosis reported that subjects with ≥50% stenosis in at least one major epicardial coronary artery had significantly higher total EETs compared with healthy controls. However, within the group of patients with coronary artery disease, obese subjects had lower plasma concentrations of total EETs [101]. This is consistent with preclinical models of obesity [102, 103], suggesting a decreased CYP450 and increased sEH expression, and the overall increased EETs in subjects with coronary artery disease may be a compensatory response to the presence of advanced cardiovascular disease. Furthermore, within the group of subjects with stable coronary atherosclerotic disease, those with comparatively higher sEH activity exhibit higher levels of inflammatory molecules, such as cellular adhesion molecules, and therefore may be predisposed to more advanced vascular inflammation [104]. Thus, sEH inhibition in these higher risk subjects may represent an effective secondary prevention strategy. Although no association between flow mediated dilatation (FMD) and plasma concentrations of EETs has been observed in subjects with coronary artery disease, bradykinin-induced changes in forearm blood flow may be more reflective of EET associated microvascular function [56, 105] and more predictive of cardiovascular outcome [10]. Interestingly, the cytochrome P450 inhibitor sulfaphenazole enhances acetylcholine-induced flow in patients with coronary artery disease and this may be due to a reduction in the generation of reactive oxygen species by CYP2C in endothelial cells, thus improving NO bioavailability [53, 106].

Diabetes and obesity are associated with reduced expression of CYP2C enzymes in mice and rat models [107–109], and increased expression of CYP4A and sEH [110, 111]. Inhibition and genetic deletion of sEH can augment pancreatic EET concentrations, and prevent hyperglycaemia in diabetic mice [112]. EDHF activity appears to be impaired in different animal models of type 1 [113] and type 2 diabetes [114, 115]. In insulin resistant
rats, chronic feeding of miconazole (CYP inhibitor) had no effect on mesenteric artery relaxation, whereas phenobarbital (CYP inducer) restored EDHF mediated relaxation [116]. Type 1 diabetic mice up-regulate the sEH mRNA and have lower concentrations of EETs in the brain, associated with worse stroke outcome [117]. Interestingly, one study reported increased EDHF mediated vasodilatation in femoral and mesenteric arteries of type 1 diabetic rats [118] and this was thought to be a compensatory mechanism for impaired NO production [119]. There are no human studies as yet which assess EET mediated endothelial function in diabetic patients.

In essential hypertension, there is certainly some alteration in EET signalling but its role in modulating human vascular function remains somewhat unclear. In animal models, an infusion of angiotensin II elevates blood pressure, and stimulates 20-HETE synthesis in renal microvessels [120], and decreases EETs by down regulating CYP450 epoxygenases, and increasing sEH activity [121]. In spontaneously hypertensive rats, sEH expression is elevated [122]. In humans, plasma concentrations of EETs are reduced in women with pregnancy-induced hypertension [123] and in subjects with renovascular hypertension [124]. This may be a result of reduced EET synthesis by CYP450 enzymes, and increased EET metabolism by sEH enzymes [124]. Another group reported no difference in basal plasma concentrations of EETs between healthy control subjects and hypertensive patients, but an impairment in induced EET release, in combination with NO and reactive oxygen species balance, and the endothelin-1 pathway contributed to endothelial dysfunction of conduit arteries (measured by flow mediated dilatation) in essential hypertensives [125]. The same group later demonstrated that inhibition of CYP450 reduced basal conduit arterial diameter only in healthy subjects, and not in essential hypertensives, but it did not change resistance arterial flow in both groups [55]. This is consistent with another study, which reported that a CYP450 inhibitor did not change basal flow within both normotensive and hypertensive patients, but conversely, it significantly blunted both acetylcholine and bradykinin induced flow only in hypertensives. The authors attributed this to EETs compensating for impaired NO activity in the hypertensive group [54]. Thus, it remains unclear whether there is true functionally important EET impairment in hypertensives, and in order to elucidate this, larger studies combining quantitative measure of plasma EETs and vascular function assessment would be required.

Smoking has a synergistic effect with sEH polymorphisms coding for enhanced sEH activity and thus reduced EET signalling [126] and may initiate pulmonary vascular impairment through direct injury of endothelial cells or release of inflammatory mediators [127]. Chronic injury leads to some of the vascular impairment observed in chronic obstructive pulmonary disease (COPD), such as reduced NOS and EDHF in vitro in pulmonary vessels [128], worsening with the progression of disease [129]. A quantitative study showed 8,9-EETs are significantly reduced in the breath condensate of COPD patients [130], and one study showed no difference in bradykinin induced vasodilatation in resistance vessels between COPD patients and other healthy smokers, though not assessing the role of EETs directly [131]. This may be an interesting group to explore and target therapeutically for the vascular and anti-inflammatory effects of EETs, as a subset of COPD patients is of a systemic inflammatory phenotype [132] associated with a three-fold elevated risk of cardiovascular admissions [133]. It is estimated that approximately 30% of COPD patients die from cardiovascular disease.

In hypercholesterolaemia, EETs may be up-regulated to compensate for an impaired NO pathway. In cholesterol fed animals, EDHF is maintained, while NO is reduced [134] and only cholesterol-fed rabbits synthesize EETs in the aorta [135]. In vivo, there appears to be enhanced EDHF activity in hypercholesterolaemia where there is NO deficiency [56]. It is possible to speculate that some of the EDHF activity may be secondary to EET signalling, thus suggesting that the mechanism by which EETs act, i.e. through hyperpolarization, or via the NO signalling pathway, may be dependent on the health condition of the subject.

A summary of the in vivo studies investigating endothelial function and the EET pathway in the healthy and diseased subjects are shown in Table 1. Current evidence suggests that EET signalling may be differentially impaired in patients with cardiovascular risk factors associated with endothelial dysfunction. EETs may become up-regulated in patients with advanced coronary artery disease, suggesting that there may be a role for targeting EET impairment early to prevent disease progression.

### Genetic polymorphisms

Polymorphisms exist for both the CYP450 families involved in EET synthesis and sEH enzymes required for EET metabolism. CYP2J2 gene cloning and sequence analysis revealed a range of polymorphisms, with the commonest being the G-50 T single nucleotide polymorphism (SNP). The G-50 T SNP is in the proximal promoter of CYP2J2 gene, which regulates basal transcriptional activity. The polymorphism is found in approximately 17% Africans, 13% Asians and 10% of Caucasians and is associated with lower EET activity and an increased risk of coronary artery disease [136]. Furthermore, CYP2J2 polymorphism may be an independent risk factor for the premature onset (<45 years old) of myocardial infarction (MI) in the Chinese Han population [137], and it has a synergistic effect with smoking, increasing the risk of MI by approximately 6.7 fold compared with non-smoker
Table 1
Human in vivo studies using venous occlusion plethysmography with an intra-arterial infusion of a cytochrome P450 inhibitor (inhibit EET synthesis) to investigate EET-mediated regulation of vascular tone in basal flow and agonist induced vasodilatation

| Author          | Subjects (n)                                      | Agonists                        | Inhibitors                        | Main findings                                                                 |
|-----------------|---------------------------------------------------|---------------------------------|-----------------------------------|-------------------------------------------------------------------------------|
| Halcox et al. [51] | Healthy subjects (n = 47)                         | Bradykinin 100, 200, 400 ng min⁻¹ Acetylcholine 15, 30 µg min⁻¹ SNP 1.6, 3.2 µg min⁻¹ | Miconazole 0.0125, 0.0375, 0.125 mg min⁻¹ Aspirin 1 g intravenous LNMMA 4 µmol min⁻¹ | Miconazole did not change basal flow Miconazole did not reduce acetylcholine induced flow. |
| Passauer et al. [52] | Healthy male subjects (n = 11)                   | Bradykinin 20, 40, 80 pmol min⁻¹ | Ibuprofen 1200 mg oral Sulphaphenazole 0.02, 0.2, 2, 6 mg min⁻¹ LNMMA 4 µmol min⁻¹ | Sulphaphenazole did not change basal flow. No inhibitory effect of sulphaphenazole on bradykinin induced flow under NO inhibition. |
| Taddei et al. [54] | Healthy subjects (n = 36) Essential hypertensives (n = 32) | Acetylcholine 0.15–15 µg min⁻¹ Bradykinin 5–50 ng min⁻¹ SNP 1–4 ng min⁻¹ | LNMMA 100 µg min⁻¹ Sulphaphenazole 0.3 µg min⁻¹ | Sulphaphenazole did not change basal flow. In normotensives, sulphaphenazole was did not inhibit acetylcholine or bradykinin induced flow. In hypertensives, sulphaphenazole inhibited bradykinin induced vasodilatation more than that of acetylcholine. |
| Bellien et al. [55] | Normotensive controls (n = 14) Untreated essential hypertensive patients (n = 14) | None                            | Fluniconazole 0.4 µmol min⁻¹ LNMMA 8 µmol min⁻¹ | Fluniconazole had no effect on basal flow in both groups. In normotensives, radial artery diameter reduced by fluconazole, LNMMA, and their combination. |
| Fichtlscherer et al. [53] | Healthy subjects (n = 5) Patients with angiogram diagnosed coronary artery disease (n = 16) | Acetylcholine 20, 40 µg min⁻¹ SNP 4, 8 µg min⁻¹ | Sulphaphenazole 0.2, 2 mg min⁻¹ LNMMA 8 µmol min⁻¹ | In hypertensives, radial artery diameter was not reduced by fluconazole. Sulphaphenazole had no effect on basal flow. Sulphaphenazole significantly enhanced acetylcholine induced flow in patients. |
| Ozkor et al. [56] | Healthy subjects (n = 103) Normotensive with multiple cardiovascular risk factors (n = 71) | Bradykinin 100, 200, 400 ng min⁻¹ Acetylcholine 7.5, 15, 30 µg min⁻¹ SNP 1.6, 3.2 µg min⁻¹ | Fluniconazole 0.4 µmol min⁻¹ LNMMA 8 µmol min⁻¹ | Fluniconazole reduced basal blood flow, and addition of TEA further reduced blood flow. In healthy group, TEA inhibited bradykinin induced vasodilatation but not acetylcholine. |
| Lee et al. [150] | Healthy subjects with EPHX2 Lys55Arg and Arg287Gln polymorphisms White American (n = 198) Black American (n = 67) | Bradykinin 100, 200, 400 ng min⁻¹ Methacholine 3.2, 6.4, 12.8 µg min⁻¹ SNP 1.6, 3.2, 6.4 µg min⁻¹ | None | Reduced bradykinin induced vasodilatation in subjects with Lys55Arg (high sEH activity) in White Americans. |
wild types. In type 2 diabetes, the frequency of the CYP2J2 G-50 T polymorphism is significantly higher in younger onset diabetics (<40 years) and is associated with lower plasma EET concentration [138]. A variant of CYP4A11, which oxidizes arachidonic acid to 20-HETE is associated with hypertension [139]. CYP2C9 [140] and CYP2C19 [141] polymorphisms may be associated with hypertension in the Chinese population. Interestingly, CYP2C19 plays a key role in activating clopidogrel, and polymorphisms may determine the prognosis in young patients who are receiving clopidogrel treatment following MI [142].

Multiple reports have demonstrated an association between sEH gene polymorphisms and coronary artery disease [113, 114] and cerebrovascular disease [145–147]. The human sEH gene, EPHX2, is localized to chromosome 8p21–p12, enclosing 19 exons. A number of polymorphisms have been identified, including variants with higher (Lys55Arg) and lower (Arg287Gln) sEH activities in vitro [148]. In African American subjects selected from the Coronary Artery Risk Development in young Adults (CARDIA) study, although coding for lower sEH activity in vitro, a positive association was found between Arg287Gln and subclinical atherosclerosis defined by coronary artery plaque calcification, with no influence on blood pressure [143]. This was attributed to EETs increasing intracellular calcium concentration in vascular smooth muscle cells [149]. Another study genotyped 2065 subjects (1085 with incident coronary heart disease and 980 non-cases) selected from the Atherosclerosis Risk in Communities (ARIC) study, and reported Lys55Arg was associated with higher sEH activity in vivo, and greater risk of incident coronary heart disease in Caucasians [144]. Lys55Arg genotype is also associated with reduced vasodilator response to bradykinin in Caucasian Americans [150].

**Pharmacological target**

The cardioprotective benefits of up-regulating EET signalling have been elucidated by genetic and pharmacological modulation of this pathway. Deficiency in the sEH gene reduced EET metabolism and improved endothelial function [151], glucose homeostasis [152] and protected against experimental models of cerebral ischaemia [153]. Successful EET analogues act on a similar signalling pathway as endogenous EETs via the K+ channel, and cause vasodilator effects in bovine coronary arteries [154, 155]. In particular, one 11,12-EET analogue has the potential to reduce blood pressure in vivo in spontaneously hypertensive rats [156], but this has not progressed into humans yet. EET analogues may also exhibit some anti-inflammatory benefits in addition to anti-hypertensive effects [157, 158].

Novel sEH inhibitors developed with the aim of reducing EET metabolism have been the most progressive pharmacological agent. Older generations have weak inhibitory effectiveness and poor stability, but the newer agents are competitive, tight-binding inhibitors with nanomolar \( K_i \) values, which interact stoichiometrically with purified recombinant sEH [159]. In animal models of atherosclerosis, sEH inhibition can reduce atherosclerotic plaque lesions by up to approximately 50% in mice aortae [160, 161]. In rats with induced myocardial ischaemia and hypertension, it has the potential to reduce blood pressure [162, 163] and infarct size independent of NO [162]. In mice with induced renovascular hypertension, sEH inhibition restores the functional role of EETs in endothelium-dependent relaxation, allowing an attenuation of blood pressure, cardiac hypertrophy and prevention of coronary endothelial dysfunction [164]. Interestingly, in rats with induced malignant hypertension, the antihypertensive and renoprotective effects of sEH inhibition can be completely abolished by NO inhibition, suggesting the benefits of sEH inhibition in this condition may be dependent on the endogenous bioavailability of EETs and NO [165].

Other observed benefits of sEH inhibition include amelioration of the metabolic syndrome [166], anti-inflammatory properties [167] and protection against ischaemic stroke [168, 169]. One sEH inhibitor (AR9281) improved endothelial function in mice models of diabetes, hypertension and obesity, and significantly reduced fasting plasma glucose [170]. Whilst the same compound was well tolerated in healthy subjects in a phase 1 trial, it was terminated at phase 2 due to lack of efficacy in patients with hypertension and impaired glucose tolerance (http://clinicaltrials.gov/show/NCT00847899) [171].

In rats, sEH inhibition can improve lung function, and attenuate smoking related inflammation and emphysematous changes [172]. One concern is that in the EET pathway can enhance acute hypoxic pulmonary artery vasoconstriction in mice isolated lungs, and thus possibly contribute to the development of pulmonary hypertension, but chronic treatment with sEH inhibition for 4 months did not affect muscularization of the pulmonary vasculature and exercise tolerance. It is thought that the C-terminal epoxide hydrolase of the sEH enzyme plays a lesser role in the regulation of pulmonary resistance and morphology compared with the N-terminal phosphatase [173]. Repeat dose oral administration of a potent sEH inhibitor (GSK2256294A) attenuated lung inflammation in mice exposed to cigarette smoke [174]. The authors of this review have been involved in the phase 1 clinical trial of GSK2256294 to assess its safety, tolerability and pharmacokinetics of single and repeat doses in healthy and obese smokers (http://clinicaltrials.gov/ct2/show/NCT01762774). The pharmacodynamic effects of this drug will be assessed by venous plethysmography at baseline, after acute dosing (day 1) and after chronic dosing (day 14).

Dual action compounds which act as an EET analogue and sEH inhibitor are also under development. The extent
of enzyme inhibition is dependent on the structure, and vascular relaxation has been demonstrated in bovine coronary arteries [175]. In mice with the metabolic syndrome phenotype, an EET agonist/sEH inhibitor increased vascular EET concentrations, lowered blood pressure, prevented weight gain, increased insulin sensitivity and restored acetylcholine stimulated vessel relaxation [176]. Interestingly, dual inhibition of cyclo-oxgenase 2 and soluble epoxide hydrolase may have synergistic anti-angiogenic and anti-cancer activity [177] Thus, progression of dual action agents may be a more enlightening route to unravel and balance the controversy between up-regulating EETs and their effects on cancer activity.

**Conclusion**

In the last couple of decades, the broad biological effects of EETs have gained greater recognition. The beneficial role of EETs in maintaining vascular tone, modulating inflammatory responses and mediating endothelial cell growth has propelled the development of basic and clinical pharmacological research focusing on this pathway, though this is not without some challenges considering the current lack of an identified membrane protein target for EETs. Nevertheless, the need for novel compounds to impact on the pathophysiology of cardiovascular disease remains and current research is focused on up-regulating EETs with sEH inhibitors. As impairment in EET signalling is not universal across all cardiovascular risk factors, it would be worth stratifying a group of people with the most impaired EETs to target. Theoretically, augmenting EETs with an sEH inhibitor in an ideal population should enhance their cardioprotective effects, and this may be an exciting and promising route to impact on endothelial dysfunction, a disease process thought to appear early in the development of atherosclerosis, but this is not without potential risks, and certainly warrants large scale clinical trials to demonstrate efficacy.

**Competing Interests**

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare LY had grants and fees from the Wellcome Trust Translational Medicine and Therapeutics programme in collaboration with GlaxoSmithKline, JC and IBW had support and grants from GlaxoSmithKline and KM and CM report no support from any organization for the submitted work. There are no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

**Dr Lucy Yang is funded by the Wellcome Trust TMAT programme, the Sackler fellowship, and Clare College Research Expenses Fund. Professor Ian Wilkinson and Dr Joseph Cheriyann are both funded by the Cambridge Biomedical Research Centre. Professor Ian Wilkinson and Dr Carmel McEniery are both funded by the British Heart Foundation.**

We would like to thank Ms. Naomi Morris for her help in improving the image quality of the figures.

**REFERENCES**

1. World Health Organization. Major causes of death. Available at http://www.who.int/mediacentre/factsheets/fs310/en/index2.html (last accessed 22 March 2014).
2. British Heart Foundation. Cardiovascular diseases statistics. Available at http://www.bhf.org.uk/heart-health/heart-statistics.aspx (last accessed 22 March 2014).
3. Ross MH, Pawlina W. Histology: a Text and Atlas. 6th Ed. Baltimore: Wolters Kluwer Lippincott Williams & Wilkins, 2011. Chapter 13, p. 400–30.
4. Furchgott RF, Zawadzki JW. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 1980; 288: 373–6.
5. Dusting GJ, Moncada S, Vane JR. Prostacyclin (PGX) is the endogenous metabolite responsible for relaxation of coronary arteries induced by arachindonic acid. Prostaglandins 1977; 13: 3–15.
6. Moncada S, Vane JR. The role of prostacyclin in vascular tissue. Fed Proc 1979; 38: 66–71.
7. Feletou M, Vanhoutte PM. Endothelium-dependent hyperpolarization of canine coronary smooth muscle. Br J Pharmacol 1988; 93: 515–24.
8. Sonkusare SK, Bonev AD, Liedtke J, Liedtke, W, Kotlikoff MI, Heppner TJ, Hill-Eubanks DC, Nelson MT. Elementary Ca$^{2+}$ signals through endothelial TRPV4 channels regulate vascular function. Science 2012; 336: 597–601.
9. Wilkinson IB, Webb DJ. Venous occlusion plethysmography in cardiovascular research: methodology and clinical applications. Br J Clin Pharmacol 2001; 52: 631–46.
10. Lind L, Berglund L, Larsson A, Sundstrom J. Endothelial function in resistance and conduit arteries and 5-year risk of cardiovascular disease. Circulation 2011; 123: 1545–51.
11. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR Jr, Lerman A. Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation 2000; 101: 948–54.
12. Halcox JP, Schenke WH, Zalos G, Mincemoyer R, Prasad A, Waclawiw MA, Nour KR, Quyyumi AA. Prognostic value of coronary vascular endothelial dysfunction. Circulation 2002; 106: 653–8.
13. Takase B, Hamabe A, Satumura K, Akima T, Uehata A, Matsui T, Ohsuzu F, Ishihara M, Kurita A. Comparable prognostic value of vasodilator response to acetylcholine.
in brachial and coronary arteries for predicting long-term cardiovascular events in suspected coronary artery disease. Circ J 2006; 70: 49–56.

14 Fichtlscherer S, Breuer S, Zeiher AM. Prognostic value of systemic endothelial dysfunction in patients with acute coronary syndromes: further evidence for the existence of the ‘vulnerable’ patient. Circulation 2004; 110: 1926–32.

15 Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: a marker of atherosclerotic risk. Arterioscler Thromb Vasc Biol 2003; 23: 168–75.

16 Vallance P, Collier J, Moncada S. Effects of endothelium-derived nitric oxide on peripheral arteriolar tone in man. Lancet 1989; 2: 997–1000.

17 Park Y, Capobianco S, Gao X, Falck JR, Dellsperger KC, Zhang C. Role of EDHF in type 2 diabetes-induced endothelial dysfunction. Am J Physiol Heart Circ Physiol 2008; 295: H1982–8.

18 Dawes M, Sieniawska C, Delves T, Dwivedi R, Chowienczyk PJ, Ritter JM. Barium reduces resting flow blood and inhibits potassium-induced vasodilatation in the human forearm. Circulation 2002; 105: 1323–8.

19 Edwards G, Dora KA, Gardener MJ, Garland CJ, Weston AH. K+ is an endothelium-derived hyperpolarizing factor in rat arteries. Nature 1998; 396: 269–72.

20 Shimokawa H, Yasutake H, Fujii K, Owada MK, Nakaike R, Fukumoto Y, Takayanagi T, Nagao T, Egashira K, Fujishima M, Takeshita A. The importance of the hyperpolarizing mechanism increases as the vessels size decreases in endothelium-dependent relaxations in rat mesenteric circulation. J Cardiovasc Pharmacol 1996; 28: 703–11.

21 Alexander SPH, Mathie A, Peters JA. Guide to Receptors and Channels (GRAC), 5th ed. Br J Pharmacol 2011; 164: S1–324.

22 Imig JD. Epoxides and soluble epoxide hydrolase in cardiovascular physiology. Physiol Rev 2012; 92: 101–30.

23 Wei X, Zhang D, Dou X, Niu N, Huang W, Bai J, Zhang G. Elevated 14,15-epoxyeicosatrienoic acid by increasing of cytochrome P450 2C8, 2C9 and 2 J2 and decreasing of cytochrome P450 epoxygenase: variability in expression, and cellular localization of a mouse cytochrome P-450 highly expressed in kidney. J Biol Chem 1999; 274: 17777–88.

27 Zeldin DC, Foley J, Boyle JE, Moomaw CR, Tomer KB, Parker C, Steenbergen C, Wu S. Predominant expression of an arachidonate epoxidease in islets of Langerhans cells in human and rat pancreas. Endocrinology 1997; 138: 1338–46.

28 Zeldin DC, Foley J, Ma J, Boyle JE, Pascual JM, Moomaw CR, Tomer KB, Steenbergen C, Wu S. CYP2J2 subfamily P-450 s in the lung: expression, localization, and potential functional significance. Mol Pharmacol 1996; 50: 1111–7.

29 Qu W, Bradbury JA, Tsao CC, Maronpot R, Harry GJ, Parker CE, Davis LS, Breyer MD, Waalkes MP, Falck JR, Chen J, Rosenberg RL, Zeldin DC. Cytochrome P450 CYP2J9, a new mouse arachidonic acid omega-1 hydroxylase predominantly expressed in brain. J Biol Chem 2001; 276: 25467–79.

30 Capdevila JH, Falck JR. Biochemical and molecular characteristics of the cytochrome P450 arachidonic acid monoxygenase. Prostaglandins Other Lipid Mediat 2000; 62: 271–92.

31 Zhang Y, Oltman CL, Lu T, Lee HC, Dellsperger KC, VanRollins M. EET homologs potentily dilate coronary microvessels and activate BK(Ca) channels. Am J Physiol Heart Circ Physiol 2001; 280: H2430–40.

32 Fulton D, Falck JR, McGiff JC, Carroll MA, Quilley J. A method for the determination of 5,6-EET using the lactone as an intermediate in the formation of the diol. J Lipid Res 1998; 39: 1713–21.

33 Fang X, Weintraub NL, McCaw RB, Hu SM, Harmon SD, Rice JB, Hamburger BD, Spector AA. Effect of soluble epoxide hydrolase inhibition on epoxyeicosatrienoic acid metabolism in human blood vessels. Am J Physiol Heart Circ Physiol 2004; 287: H2412–20.

34 Yang T, Peng R, Guo Y, Shen L, Zhao S, Xu D. The role of 14,15-dihydroxyeicosatrienoic acid levels in inflammation and its relationship to lipoproteins. Lipids Health Dis 2013; 12: 151.

35 Oltman CL, Weintraub NL, VanRollins M, Dellsperger KC. Epoxyeicosatrienoic acids and dihydroxyeicosatrienoic acids are potent vasodilators in the canine coronary microcirculation. Circ Res 1998; 83: 932–9.

36 Hercule HC, Schunck WH, Gross V, Seringer J, Leung FP, Weldon SM, da Costa Goncalves ACh, Huang Y, Luft FC, Gollasch M. Interaction between P450 eicosanoids and nitric oxide in the control of arterial tone in mice. Arterioscler Thromb Vasc Biol 2009; 29: 54–60.

37 Morisseau C, Hammock BD. Epoxide hydrolases: mechanisms, inhibitor designs, and biological roles. Annu Rev Pharmacol Toxicol 2005; 45: 311–33.

38 Newman JW, Morisseau C, Hammock BD. Epoxide hydrolases: their roles and interactions with lipid metabolism. Prog Lipid Res 2005; 44: 1–51.

39 Spector AA, Fang X, Snyder GD, Weintraub NL. Epoxyeicosatrienoic acids (EETs): metabolism and biochemical function. Prog Lipid Res 2004; 43: 55–90.

40 De Mey JG, Claes M, Vanhoutte PM. Endothelium-dependent inhibitory effects of acetylcholine, adenosine triphosphate, thrombin and arachidonic acid in the
canine femoral artery. J Pharmacol Exp Ther 1982; 222: 166–73.

41 Singer HA, Peach MJ. Endothelium-dependent relaxation of rabbit aorta. I. Relaxation stimulated by arachidonic acid. J Pharmacol Exp Ther 1983; 226: 790–5.

42 Pinto A, Abraham NG, Mullan KM. Arachidonic acid-induced endothelial-dependent relaxations of canine coronary arteries: contribution of a cytochrome P-450-dependent pathway. J Pharmacol Exp Ther 1987; 240: 856–63.

43 Singer HA, Peach MJ. Endothelium-dependent relaxation of rabbit aorta. II. Inhibition of relaxation stimulated by methacholine and A23187 with antagonists of arachidonic acid metabolism. J Pharmacol Exp Ther 1983; 226: 796–801.

44 Hecker M, Bara AT, Bausachs J, Busse R. Characterization of endothelium-derived hyperpolarizing factor as a cytochrome P450-derived arachidonic acid metabolite in mammals. J Physiol (Lond) 1994; 481: 407–14.

45 Weintraub NL, Fang X, Kaduce TL, VanRollins M, Chatterjee P, Spector AA. Potentiation of endothelium-dependent relaxation by epoxyeicosatrienoic acids. Circ Res 1997; 81: 258–67.

46 Archer SL, Gragasin FS, Wu X, Wang S, McMurtry S, Kim DH, Platonov M, Koshal A, Hashimoto K, Campbell WB, Falck JR, Michelakis ED. Endothelium-derived hyperpolarizing factor in human internal mammary artery is 11,12-epoxyeicosatrienoic acid and causes relaxation by activating smooth muscle BK(Ca) channels. Circulation 2003; 107: 769–76.

47 Quilty J, McGiff JC. Is EDHF an epoxyeicosatrienoic acid? Trends Pharmacol Sci 2000; 21: 121–4.

48 Imig JD, Navar LG, Roman RJ, Reddy KK, Falck JR. Actions of epoxygenase metabolites on the preglerular vasculature. J Am Soc Nephrol 1996; 7: 2364–70.

49 Liu Y, Zhang J, Yu L, Cao F, Rao J, Li J, Jiang C, Falck JR, Jacobs ER, Zhu D. A soluble epoxide hydrolase inhibitor—8-HUDE increases pulmonary vasoconstriction through inhibition of K(ATP) channels. Pulm Pharmacol Ther 2012; 25: 69–76.

50 Hillig T, Krustrup P, Fleming I, Osada T, Saltin B, Hellsten Y. Cytochrome P450 2C9 plays an important role in the regulation of exercise-induced skeletal muscle blood flow and oxygen uptake in humans. J Physiol (Lond) 2003; 546: 307–14.

51 Halcox JP, Narayanan S, Cramer-Joyce L, Mincemoyer R, Quyyumi AA. Characterization of endothelium-derived hyperpolarizing factor in the human forearm microcirculation. Am J Physiol Hart Circ Physiol 2001; 280: H2470–7.

52 Passauer J, Bussemaker E, Lassig G, Pistrosch F, Fauler J, Gross P, Fleming I. Baseline blood flow and bradykinin-induced vasodilator responses in the human forearm are insensitive to the cytochrome P450 2C9 (CYP2C9) inhibitor sulphinapenazole. Clin Sci (Lond) 2003; 105: 513–8.

53 Fichtlscherer S, Dimmel S, Breuer S, Busse R, Zeiher AM, Fleming I. Inhibition of cytochrome P450 2C9 improves endothelium-dependent, nitric oxide-mediated vasodilatation in patients with coronary artery disease. Circulation 2004; 109: 178–83.

54 Taddei S, Versari D, Cipriano A, Ghidoni L, Galetta F, Franzoni F, Magagna A, Virdis A, Salvetti A. Identification of a cytochrome P450 2C9-derived endothelium-derived hyperpolarizing factor in essential hypertensive patients. J Am Coll Cardiol 2006; 48: 508–15.

55 Bellien J, Remy-Jouet I, Iacob M, Blot E, Mercier A, Lucas D, Dreano Y, Gutierrez L, Donnadieu N, Thuiillez C, Jonnides R. Impaired role of epoxyeicosatrienoic acids in the regulation of basal conduit artery diameter during essential hypertension. Hypertension 2012; 60: 1415–21.

56 Ozkor MA, Murrow JR, Rahman AM, Kavtaradze N, Lin J, Manatunga A, Quyyumi AA. Endothelium-derived hyperpolarizing factor determines resting and stimulated forearm vasodilator tone in health and in disease. Circulation 2011; 123: 2244–53.

57 Vriens J, Owsianik G, Fisslthaler B, Suzuki M, Janssens A, Voets T, Morisseau C, Hammock BD, Fleming I, Busse R, Nilius B. Modulation of the Ca²⁺ permeable cation channel TRPV4 by cytochrome P450 epoxyeicosanides in vascular endothelium. Circ Res 2005; 97: 908–15.

58 Gauthier KM, Rusch NJ. Potassium channels and membrane potential in vascular endothelial and smooth muscle cells. In: EDHF 2002, ed Vanhoutte PM. London: Taylor & Francis, 2003; 1–12.

59 Fisslthaler B, Popp R, Kiss L, Potente M, Harder DR, Fleming I, Busse R. Cytochrome P450 2C is an EDHF synthase in coronary arteries. Nature 1999; 401: 493–7.

60 Campbell WB, Gebremedhin D, Pratt, PF, Harder DR. Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. Circ Res 1996; 78: 415–23.

61 Zhang DX, Mendoza SA, Bubolz AH, Mizuno A, Ge ZD, Li R, Warrtler DC, Suzuki M, Gutterman DD. Transient receptor potential vanilloid type 4-deficient mice exhibit impaired endothelium-dependent relaxation induced by acetylcholine in vitro and in vivo. Hypertension 2009; 53: 532–8.

62 Watanabe H, Vriens J, Prenen J, Droogmans G, Voets T, Nilius B. Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. Nature 2003; 424: 434–8.

63 Loot AE, Popp R, Fisslthaler B, Vriens J, Nilius B, Fleming I. Role of cytochrome P450-dependent transient receptor potential V4 activation in flow-induced vasodilatation. Cardiovasc Res 2008; 80: 445–52.

64 Earley S, Pauyo T, Drapp R, Tavares MJ, Liedtke W, Brayden JE. TRPV4-dependent dilation of peripheral resistance arteries influences arterial pressure. Am J Physiol Heart Circ Physiol 2009; 297: H1096–102.

65 Popp R, Brandes RP, Ott G, Busse R, Fleming I. Dynamic modulation of interendothelial gap junctional communication by 11,12-epoxyeicosatrienoic acid. Circ Res 2002; 90: 800–6.
Inceoglu B, Schmelzer KR, Morisseau C, Jinks SL, Chen Y, Falck JR, Manthati VL, Jat JL, Campbell WB. 20-
Yang W, Tuniki VR, Anjaiah S, Falck JR, Hillard CJ, Campbell S, Snyder GD, Krisna UM, Falck JR, Spector AA. Evidence for a
Imig JD, Navar LG, Roman RJ, Reddy KK, Falck JR. Role of caveolar compartmentation in endothelium-
derived hyperpolarizing factor-mediated relaxation: Ca2+ signals and gap junction function are regulated by
caveolin in endothelial cells. Circulation 2008; 117: 1065–74.
Kohler R, Heyken WT, Heinau P, Schubert R, Si H, Kacik M, Busch C, Grgic I, Maier T, Hoyer J. Evidence for a functional role of endothelial transient receptor potential V4 in shear stress-induced vasodilatation. Arterioscler Thromb Vasc Biol 2006; 26: 1495–502.
Campbell WB, Holmes BB, Falck JR, Capdevila JH, Gauthier KM. Adenoviral expression of cytochrome P450
epoxygenase in coronary smooth muscle cells: Regulation of potassium channels by endogenous 14(S),15(R)-EET. Am J Physiol 2006; 290: H64–71.
Snyder GD, Krisna UM, Falck JR, Spector AA. Evidence for a membrane site of action for 14,15-EET on expression of
aromatase in vascular smooth muscle. Am J Physiol 2002; 283: H1936–42.
Yang W, Tuniki VR, Anjaiah S, Falck JR, Hillard CJ, Campbell WB. Characterization of epoxyeicosatrienoic acid binding site in U937 membranes using a novel radiolabeled agonist, 20-125i-14,15-epoxyeicosa-8(Z)-enoic acid. J Pharmacol Exp Ther 2008; 324: 1019–27.
Inceoglu B, Schmelzer KR, Morisseau C, Jinks SL, Hammock BD. Soluble epoxide hydrolase inhibition reveals novel biological functions of epoxyeicosatrienoic acids (EETs). Prostaglandins Other Lipid Mediat 2007; 82: 42–9.
Li PL, Campbell WB. Epoxyeicosatrienoic acids activate K+ channels in coronary smooth muscle through a guanine nucleotide binding protein. Circ Res 1997; 80: 877–84.
Hayabuchi Y, Nakaya Y, Matsuoka S, Kuroda Y. Endothelium-derived hyperpolarizing factor activates Ca2+ –activated K+ channels in porcine coronary artery smooth muscle cells. J Cardiovasc Pharmacol 1998; 32: 642–9.
Node K, Ruan XL, Dai J, Yang SX, Graham L, Zeldin DC, Liao JK. Activation of Goa mediates induction of tissue-type plasminogen activator gene transcription by epoxyeicosatrienoic acids. J Biol Chem 2001; 276: 15983–9.
Wong PYK, Lai PS, Shen SY, Belosludtsev YY, Falck JR. Post-receptor signal transduction and regulation of 14(R), 15(S)-epoxyeicosatrienoic acid (14,15-EET) binding in U-937 cells. J Lipid Mediat Cell 1997; 16: 155–69.
Wong PYK, Lai PS, Falck JR. Mechanism and signal transduction of 14(R), 15(S)-epoxyeicosatrienoic acid (14,15-EET) binding in guinea pig monocytes. Prostaglandins Other Lipid Mediat 2000; 62: 321–33.
Griffith TM, Chaytor AT, Edwards DH. Permissive role of cAMP in the mediation of relaxations initiated by endothelial hyperpolarization. In: EDHF 2002, ed Vanhoutte PM. London: Taylor & Francis, 2003; 211–22.
Li PL, Chen CL, Bortell R, Campbell WB. 11,12-
Epoxyeicosatrienoic acid stimulates endogenous mono-
ADP-ribosylation in bovine coronary arterial smooth muscle. Circ Res 1999; 85: 349–56.
Ye D, Zhou W, Lee HC. Activation of rat mesenterial arterial KATP channels by 11,12-epoxyeicosatrienoic acid. Am J Physiol 2005; 288: H358–64.
Xiao YF, Huang L, Morgan JP. Cytochrome P450: a novel system modulating Ca2+ channels and contraction in mammalian heart cells. J Physiol 1998; 508: 777–92.
Chen J, Capdevila JH, Zeldin DC, Rosenberg RL. Inhibition of cardiac L-type calcium channels by epoxyeicosatrienoic acids. Mol Pharmacol 1999; 55: 288–95.
Wu S, Chen W, Murphy E, Gabel S, Tomer KB, Foley J, Steenberg C, Falck JR, Moomaw CR, Zeldin DC. Molecular cloning, expression, and functional significance of a cytochrome P450 highly expressed in rat heart myocytes. J Biol Chem 1997; 272: 12551–9.
Falck JR, Manna S, Moltz J, Chacos N, Capdevila J. Epoxyeicosatrienoic acids stimulate glucagon and insulin release from isolated rat pancreatic islets. Biochem Biophys Res Commun 1983; 114: 743–9.
Zeldin DC, Foley J, Boyle JE, Moomaw CR, Tomer KB, Parker C, Steenberg C, Wu S. Predominant expression of an arachidonate epoxygenase in islets of Langerhans cells in human and rat pancreas. Endocrinology 1997; 138: 1338–46.
Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002; 105: 1135–43.
P450 epoxygenase-derived eicosanoids. Science 1999; 285: 1276–9.

93 Fleming I. Epoxycosatrienoic acids, cell signaling and angiogenesis. Prostaglandins Other Lipid Mediat 2007; 82: 60–7.

94 Dhanasekaran A, Gruenloh SK, Buonaccorsi JN, Zhang R, Gross GJ, Falcon JR, Patel PK, Jacobs ER, Medhora M. Multiple antiapoptotic targets of the PI3K/Akt survival pathway are activated by epoxycosatrienoic acids to protect cardiomyocytes from hypoxia/anoxia. Am J Physiol Heart Circ Physiol 2008; 294: H724–35.

95 Yang S, Wei S, Pozi A, Capdevila JH. The arachidonic acid epoxygenase is a component of the signaling mechanisms responsible for VEGF-stimulated angiogenesis. Arch Biochem Biophys 2009; 489: 82–91.

96 Dhanasekaran A, Al-Saghir R, Lopez B, Zhu D, Guttermann DD, Jacobs ER, Medhora M. Protective effects of epoxycosatrienoic acids on human endothelial cells from the pulmonary and coronary vasculature. Am J Physiol Heart Circ Physiol 2006; 291: H517–31.

97 Panigraphy D, Green ER, Pozi A, Wang DW, Zeldin DC. EET signaling in cancer. Cancer Metastasis Rev 2011; 30: 525–40.

98 Jiang JG, Ning YG, Chen C, Ma D, Liu ZJ, Yang S, Zhou J, Xiao X, Zhang XA, Edin ML, Card JW, Wang J, Zeldin DC, Wang DW. Cytochrome p450 epoxygenase promotes human cancer metastasis. Cancer Res 2007; 67: 6665–74.

99 Chen C, Li G, Liao W, Wu J, Liu L, Ma D, Zhou J, Elbekai RH, Edin ML, Zeldin DC, Wang DW. Selective inhibitors of CYP2J2 related to terfenadine exhibit strong activity against human cancers in vitro and in vivo. J Pharmacol Exp Ther 2009; 329: 908–18.

100 Schachinger V, Zeiher AM. Atherosclerosis-associated endothelial dysfunction. Z Kardiol 2000; 89: 70–4.

101 Theken KN, Schuck RN, Edin ML, Tran B, Ellis K, Bass A, Lih FB, Tomer KB, Poloyac SM, Wu MC, Hindler AL, Zeldin DC, Stouffer GA, Lee CR. Evaluation of cytochrome P450-derived eicosanoids in humans with stable atherosclerotic cardiovascular disease. Atherosclerosis 2012; 222: 530–6.

102 Zhao X, Dey A, Romanko OP, Stepp DW, Wang MH, Zhou Y, Jin L, Pollock JS, Webb RC, Imig JD. Decreased epoxygenase and increased epoxide hydrolase expression in the mesenteric artery of obese Zucker rats. Am J Physiol Regul Integr Comp Physiol 2005; 288: R188–96.

103 Theken KN, Deng Y, Schuck RN, Oni-Orisan A, Miller TM, Kannon MA, Poloyac SM, Lee CR. Enalapril reverses high fat diet-induced alterations in cytochrome P450-mediated eicosanoid metabolism. Am J Physiol Endocrinol Metab 2012; 302: E500–9.

104 Schuck RN, Theken KN, Edin ML, Caughhey M, Bass A, Ellis K, Tran B, Steele S, Simmons BP, Lih FB, Tomer KB, Wu MC, Hindler AL, Stouffer GA, Zeldin DC, Lee CR. Cytochrome P450-derived eicosanoids and vascular dysfunction in coronary artery disease patients. Atherosclerosis 2013; 227: 442–8.

105 Rahman AM, Murrow JR, Ozkor MA, Kavtaradze N, Lin J, Staerck CD, Hooper WC, Manatunga A, Hayek S, Quyumi AA. Endothelium-derived hyperpolarizing factor mediates bradykinin-stimulated tissue plasminogen activator release in humans. J Vasc Res 2014; 51: 200–8.

106 Fleming I, Michaelis UR, Bredenpotter D, Fisslthaler B, Dehghani F, Brandes RP, Busse R. Endothelium-derived hyperpolarizing factor synthase (Cytochrome P450 2C9) is a functionally significant source of reactive oxygen species in coronary arteries. Circ Res 2001; 19: 44–51.

107 Pass GI, Becker W, Kluge R, Linnartz K, Plum L, Giesen K, Joost HG. Effect of hyperinsulinemia and type 2 diabetes-like hyperglycemia on expression of hepatic cytochrome P450 and glutathione s-transferase isomers in a New Zealand obese-derived mouse backcross population. J Pharmacol Exp Ther 2002; 302: 442–50.

108 Zhao X, Dey A, Romanko OP, Stepp DW, Wang MH, Zhou Y, Jin L, Pollock JS, Webb RC, Imig JD. Decreased epoxygenase and increased epoxide hydrolase expression in the mesenteric artery of obese Zucker rats. Am J Physiol 2005; 288: R188–96.

109 Lam JL, Jiang Y, Zhang T, Zhang EY, Smith BJ. Expression and functional analysis of hepatic cytochromes P450, nuclear receptors, and membrane transporters in 10- and 25-week-old db/db mice. Drug Metab Dispos 2010; 38: 2252–8.

110 Enriquez A, Leclercq I, Farell GC, Robertson G. Altered expression of hepatic CYP2E1 and CYP4A in obese, diabetic ob/ob mice, and fa/fa Zucker rats. Biochem Biophys Res Commun 1999; 255: 300–6.

111 Kroetz DL, Yook P, Costet P, Bianchi P, Pineau T. Peroxisome proliferator-activated receptor alpha controls the hepatic CYP4A induction adaptive response to starvation and diabetes. J Biol Chem 1998; 273: 31581–9.

112 Luo P, Chang HH, Zhou Y, Zhang S, Hwang SH, Morisseau C, Wang CY, Inscho EW, Hammock BD, Wang MH. Inhibition or deletion of soluble epoxide hydrolase prevents hyperglycemia, promotes insulin secretion, and reduces islet apoptosis. J Pharmacol Exp Ther 2010; 334: 430–8.

113 Makino A, Ohuchi K, Kamata K. Mechanisms underlying the attenuation of endothelium-dependent vasodilatation in the mesenteric arterial bed of the streptozotocin-induced diabetic rat. Br J Pharmacol 2000; 130: 549–56.

114 Wigg SJ, Tare M, Tonta MA, O’Brien RC, Meredith IT, Parkinson HC. Comparison of effects of diabetes mellitus on an EDHF-dependent and an EDHF-independent artery. Am J Physiol Heart Circ Physiol 2001; 281: H232–40.

115 Miller AW, Katakam PV, Ujhelyi MR. Impaired endothelium-mediated coronary arteries from insulin-resistant rats. J Vasc Res 1999; 36: 385–92.

116 Katakam, PVG, Hoenig M, Ujhelyi MR, Miller AW. Cytochrome P450 activity and endothelial dysfunction in insulin resistance. J Vasc Res 2000; 37: 426–34.

117 Jouihan SA, Zuloaga KL, Zhang W, Shangraw RE, Krasnow SM, Marks DL, Alkayed NJ. Role of soluble epoxide hydrolase in exacerbation of stroke by streptozotocin-
induced type 1 diabetes mellitus. J Cereb Blood Flow Metab 2013; 33: 1650–6.

118 Shi Y, Ku DD, Man RY, Vanhoutte PM. Augmented endothelium-derived hyperpolarizing factor-mediated relaxations attenuate endothelial dysfunction in femoral and mesenteric, but not in carotid arteries from type 1 diabetic rats. J Pharmacol Exp Ther 2006; 318: 276–81.

119 Makimattila S, Liu ML, Vakkilainen J, Schlenzka A, Lahdenpera S, Syvanne M, Mantysaari M, Summanen P, Bergholm R, Taskinen MR, Yki-Jarvinen H. Impaired endothelium-dependent vasodilation in type 2 diabetes. Diabetes Care 1999; 22: 973–81.

120 Croft KD, McGiff JC, Sanchez-Mendoza A, Carroll MA. Angiotensin II releases 20-HETE from rat renal microvessels. Am J Physiol Renal Physiol 2000; 279: F544–51.

121 Ai D, Fu Y, Guo D, Tanaka H, Wang N, Tang C, Hammock BD, Shyy JY, Zhu Y. Angiotensin II up-regulates soluble epoxide hydrolase in vascular endothelium in vitro and in vivo. Proc Natl Acad Sci U S A 2007; 104: 9018–23.

122 Yu Z, Xu F, Huse LM, Morisseau C, Draper AJ, Newman JW, Parker C, Graham L, Engler MM, Hammock BD, Zeldin DC, Kroetz DL. Soluble epoxide hydrolase regulates hydrolysis of vasoactive epoxyeicosatrienoic acids. Circ Res 2000; 24: 992–8.

123 Catella F, Lawson JA, Fitzgerald DJ, FitzGerald GA. Endogenous biosynthesis of arachidonic acid metabolites in humans: increased formation in pregnancy-induced hypertension. Proc Natl Acad Sci USA 1990; 87: 5893–7.

124 Minuz P, Jiang H, Fava C, Turolo L, Tacconelli S, Ricci M, Patrignani P, Morganti A, Lechi A, McGiff JC. Altered release of cytochrome p450 metabolites of arachidonic acid in renovascular disease. Hypertension 2008; 51: 1379–85.

125 Bellien J, Iacob M, Remy-Jouett J, Lucas D, Monteil C, Gutierrez L, Vendeville C, Dreano Y, Mercier A, Thulillez C, Joannides R. Epoxyeicosatrienoic acids contribute with altered nitric oxide and endothelin-1 pathways to conduit artery endothelial dysfunction in essential hypertension. Circulation 2012; 125: 1266–75.

126 Wei Q, Doris PA, Pollizotto MV, Boerwinkle E, Jacobs DR Jr, Siscovick DS, Fornage M. Sequence variation in the soluble epoxide hydrolase gene and subclinical coronary atherosclerosis: interaction with cigarette smoking. Atherosclerosis 2007; 190: 26–34.

127 Yang Q, Underwood MJ, Hsin MKY, Liu XC, He GW. Dysfunction of pulmonary vascular endothelium in chronic obstructive pulmonary disease: basic considerations for future drug development. Curr Drug Metab 2008; 9: 661–7.

128 Barbera, JA, Peinado VI, Santos S, Ramirez J, Roca J, Rodriguez-Roisin R. Reduced expression of endothelial nitric oxide synthase in pulmonary arteries of smokers. Am J Respir Crit Care Med 2001; 164: 709–13.

129 Yang Q, Shigemura N, Underwood MU, Hsin M, Xue HM, Huang Y, He GW, Yu CM. NO and EDHF pathways in pulmonary arteries and veins are impaired in COPD patients. Vascul Pharmacol 2012; 57: 113–8.

130 Fritscher LG, Post M, Rodrigues MT, Silverman F, Balter M, Chapman KR, Zamel N. Profile of eicosanoids in breath condensate in asthma and COPD. J Breath Res 2012; 6: 026001.

131 Maclay JD, McAllister DA, Mills NL, Paterson FP, Ludlam CA, Drost EM, Newby DE, Macnee W. Vascular dysfunction in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2009; 180: 513–20.

132 Sabit R, Bolton CE, Edwards PH, Pettit RJ, Evans WD, McEniery CM, Wilkinson IB, Cockcroft JR, Shale DJ. Arterial stiffness and osteoporosis in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2007; 175: 1259–65.

133 Garcia-Aymerich J, Serra Pons I, Mannino DM, Maas AK, Miller DP, Davis KJ. Lung function impairment, COPD hospitalisations and subsequent mortality. Thorax 2011; 66: 585–90.

134 Ashraf MZ, Reddy MK, Hussain ME, Podrez EA, Fahim M. Contribution of EDHF and EDHF to restoration of endothelial function following dietary restrictions in hypercholesterolemic rats. Indian J Exp Biol 2007; 45: 505–14.

135 Pfister SL, Falck JR, Campbell WB. Enhanced synthesis of epoxyeicosatrienoic acids by cholesterol-fed rabbit aorta. Am J Physiol 1991; 261: H843–52.

136 Spiecker M, Darius H, Hankeln T, Soufi M, Sattler AM, Schaefer JR, Node K, Borgel J, Mugge A, Lindpaintner K, Huesing A, Maisch B, Zeldin DC, Liao JK. Risk of coronary artery disease associated with polymorphism of the cytochrome P450 epoxygenase CYP2J2. Circulation 2004; 110: 2132–6.

137 Liu PY, Li YH, Chao TH, Wu HL, Lin LJ, Tsai LM, Chen JH. Synergistic effect of cytochrome P450 epoxygenase CYP2J2*7 polymorphism with smoking on the onset of premature myocardial infarction. Atherosclerosis 2006; 195: 199–206.

138 Wang CP, Hung WC, Yu TH, Chiu CA, Lu LF, Chung FM, Hung CH, Shin SJ, Chen HJ, Lee YJ. Genetic variation in the G-50 T polymorphism of the cytochrome P450 epoxygenase CYP2J2 gene and the risk of younger onset type 2 diabetes among Chinese population: potential interaction with body mass index and family history. Exp Clin Endocrinol Diabetes 2010; 118: 346–52.

139 Gainer JV, Bellamine A, Dawson EP, Womble KE, Grant SW, Wang Y, Cupples LA, Guo CY, Demissie S, O’Donnell CJ, Brown NJ, Waterman MR, Capdevila JH. Functional variant of CYP4A11 20-hydroxyeicosatetraenoic acid synthase is associated with essential hypertension. Circulation 2005; 111: 63–9.

140 Yu BN, Luo CH, Wang D, Wang A, Li Z, Zhang W, Mo W, Zhou HH. CYP2C9 allele variants in Chinese hypertension patients and healthy controls. Clin Chim Acta 2004; 348: 57–61.

141 Ma Y, Ni W, Zhu W, Xiong Y, Deng X. Association of genetic polymorphisms of CYP 2C19 with hypertension in a Chinese Han population. Blood Press 2011; 20: 166–70.

142 Collet JP, Hulot JS, Pena A, Villard E, Esteve JB, Silvain J, Payot L, Brugier D, Cayla G, Beygui F, Bensimon G, Funck-
Brentano C, Montalescot G. Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. Lancet 2009; 373: 309–17.

143 Fornage M, Boerwinkle E, Doris P, Jacobs D, Liu K, Wong ND. Polymorphism of the soluble epoxide hydrolase is associated with coronary artery calcification in African-American subjects. The Coronary Artery Risk Development in Young Adults (CARDIA) study. Circulation 2004; 109: 335–9.

144 Lee CR, North KE, Bray MS, Fornage M, Seubert JM, Newman JW, Hammock BD, Couper DJ, Heiss G, Zeldin DC. Genetic variation in soluble epoxide hydrolase (EPHX2) and risk of coronary heart disease: The Atherosclerosis Risk in Communities (ARIC) study. Hum Mol Genet 2006; 15: 1640–9.

145 Gschwendtner A, Ripke S, Freiling B, Lichtner P, Muller-Myszkowski B, Wichmann NE, Meitinger T, Dichgans M. Genetic variation in soluble epoxide hydrolase (EPHX2) is associated with an increased risk of ischemic stroke in white Europeans. Stroke 2008; 39: 1593–6.

146 Fava C, Montagnera M, Danese E, Almgreen P, Hedblad B, Engstrom G, Berglund G, Minuz P, Melander O. Homozygosity for the EPHX2 K55R polymorphism increases the long-term risk of ischemic stroke in men: a study in Swedes. Pharmacogenet Genomics 2010; 20: 94–103.

147 Fornage M, Lee CR, Doris PA, Bray MS, Heiss G, Zeldin DC, Boerwinkle E. The soluble epoxide hydrolase gene harbors sequence variation associated with susceptibility to and protection from incident ischemic stroke. Hum Mol Genet 2005; 14: 2829–37.

148 Przybyla-Zawislak BD, Srivastava PK, Vazquez-Matias J, Mohrenweiser HW, Maxwell JE, Hammock BD, Bradbury JA, Enayetallah AE, Zeldin DC, Grant DF. Polymorphisms in human soluble epoxide hydrolase. Mol Pharmacol 2003; 64: 482–90.

149 Fang X, Weintraub NL, Stoll LL, Spector AA. Epoxycosatrienoic acids increase intracellular calcium concentration in vascular smooth muscle cells. Hypertension 1999; 34: 1242–6.

150 Lee CR, Pretorius M, Schuck RN, Burch LH, Bartlett J, Williams SM, Zeldin DC, Brown NJ. Genetic variation in soluble epoxide hydrolase (EPHX2) is associated with forearm vasodilator responses in humans. Hypertension 2011; 57: 116–U309.

151 Elmarakby AA, Faulkner J, Al-Shabrawey M, Wang MH, Maddipati KR, Imig JD. Deletion of soluble epoxide hydrolase gene improves renal endothelial function and reduces renal inflammation and injury in streptozotocin-induced type 1 diabetes. Am J Physiol Regul Integr Comp Physiol 2011; 301: R1307–17.

152 Luria A, Bettaieb A, Xi Y, Shieh GJ, Liu HC, Inoue H, Tsai HJ, Imig JD, Haj FG, Hammock BD. Soluble epoxide hydrolase deficiency alters pancreatic islet size and improves glucose homeostasis in a model of insulin resistance. Proc Natl Acad Sci U S A 2011; 108: 9038–43.

153 Zhang W, Otsuka T, Sugo N, Ardeshiri A, Alhadid YK, Liiff JJ, DeBarber AE, Koop DR, Alkayed NJ. Soluble epoxide hydrolase gene deletion is protective against experimental cerebral ischemia. Stroke 2008; 39: 2073–8.

154 Yang W, Holmes BB, Gopal VR, Kishore RV, Sangras B, Yi XY, Falck JR, Campbell WB. Characterization of 14,15-epoxyeicosatrienoyl-sulfonamides as 14,15-epoxyeicosatrienoic acid agonists: use for the studies of metabolism and ligand binding. J Pharmacol Exp Ther 2007; 321: 1023–31.

155 Dimitropoulou C, West L, Field MB, White RE, Reddy LM, Falck JR, Imig JD. Protein phosphatase 2A and Ca2+ activated K+ channels contribute to 11,12-epoxyeicosatrienoic acid analog mediated mesenteric arterial relaxation. Prostaglandins Other Lipid Mediat 2007; 83: 50–61.

156 Elmarakby AA, Falck JR, Imig JD. Ether epoxyeicosatrienoic acid (EET) analogs lower blood pressure when administered in-vivo to the spontaneously hypertensive rats. FASEB J 2008; 22(meeting abstract supplement): 479.44.

157 Imig JD, Elmarakby A, Nithipatikom K, Wei S, Capdevila JH, Tuniki VR, Sangras B, Anjaiah S, Manthath VL, Sudarshan Reddy D, Falck JR. Development of epoxyeicosatrienoic acid analogs with in vivo anti-hypertensive actions. Front Physiol 2010; 3: 157.

158 Hye Khan M, Pavlov TS, Chistain SV, Neckar J, Staruschenko A, Gauthier KM, Capdevila JH, Falck JR, Campbell WB, Imig JD. Epoxyeicosatrienoic acid analogs with in vivo anti-hypertensive actions. Front Physiol 2010; 3: 157.

159 Bellien J, Joannides R, Richard V, Thuillez C. Modulation of cytochrome-derived epoxyeicosatrienoic acids pathway: A promising pharmacological approach to prevent endothelial dysfunction in cardiovascular diseases? Pharmacol Ther 2011; 131: 1–17.

160 Ulu A, Davis BB, Tsai HJ, Kim IH, Morisseau C, Inceoglu B, Fiehn O, Hammock BD, Weiss RH. Soluble epoxide hydrolase inhibitors reduce the development of atherosclerosis in apolipoprotein E-knockout mouse model. J Cardiovasc Pharmacol 2008; 52: 314–23.

161 Wang YX, Cassis LA. Angiogensin II-induced aortic aneurysms. In: A Handbook of Mouse Models for Cardiovascular Diseases, ed Xu Q. Chichester England: Life and Medical Sciences, John Wiley & Sons; 2006: 125–36.

162 Neckârf J, Kopkan L, Husková Z, Kolar F, Papousek F, Kramer HJ, Hwang SH, Hammock BD, Imig JD, Maly J, Netuka I, Ostadal B, Cervenka L. Inhibition of soluble epoxide hydrolase by cis-4-(3-adamantan-1-ylureido) cyclohexyl-oxy]benzoic acid exhibits antihypertensive and cardioprotective actions in transgenic rats with angiotensin II-dependent hypertension. Clin Sci (Lond) 2012; 122: 513–25.

163 Kopkan L, Husková Z, Sporková A, Varcanova S, Honetschlagerova Z, Hwang SH, Tsai HJ, Hammock BD, Imig JD, Kramer HJ, Burgelova M, Vojitiskova A, Kujal P, Verneeven Z, Cervenka L. Soluble epoxide hydrolase inhibition exhibits antihypertensive actions.
independently of nitric oxide in mice with renovascular hypertension. Kidney Blood Press Res. 2012; 35: 595–607.

164 Gao J, Bellien J, Gomez E, Henry JP, Daubreaux B, Bounoure F, Skiba M, Thuillez C, Richard V. Soluble epoxide hydrolase inhibition prevents coronary endothelial dysfunction in mice with renovascular hypertension. J Hypertens 2011; 29: 1128–35.

165 Honetschlagerova Z, Kitada K, Huskova Z, Sporkova A, Kopkan L, Burgelova M, Varcabova S, Nishiyama A, Hwang SH, Hammock BD, Imig JD, Kramer HJ, Kujal P, Verneerova Z, Cervenka L. Antihypertensive and renoprotective actions of soluble epoxide hydrolase inhibition in ANG II-dependent malignant hypertension are abolished by pretreatment with L-NAME. J Hypertens 2013; 31: 321–32.

166 Iyer A, Kauter K, Alam MA, Hwang SH, Morisseau C, Hammock BD, Brown L. Pharmacological inhibition of soluble epoxide hydrolase ameliorates diet-induced metabolic syndrome in rats. Exp Diabetes Res 2012; 2012: 758614.

167 Sanders WG, Morisseau C, Hammock BD, Cheung AK, Terry CM. Soluble epoxide hydrolase expression in a porcine model of arteriovenous graft stenosis and anti-inflammatory effects of a soluble epoxide hydrolase inhibitor. Am J Physiol Cell Physiol 2012; 303: C278–90.

168 Simpkins AN, Rudic RD, Schreihofer DA, Roy S, Manhiani M, Tsai HJ, Hammock BD, Imig JD. Soluble epoxide inhibition is protective against cerebral ischemia via vascular and neural protection. Am J Pathol 2009; 174: 2086–95.

169 Zhang W, Koerner IP, Noppens R, Grafe M, Tsai HJ, Morisseau C, Luria A, Hammock BD, Falck JR, Alkayed NJ. Soluble epoxide hydrolase: a novel therapeutic target in stroke. J Cereb Blood Flow Metab 2007; 27: 1931–40.

170 Zhang LN, Vincellette J, Chen D, Gless RD, Anandan SK, Rubanyi GM, Webb HK, MacIntyre DE, Wang YX. Inhibition of soluble epoxide hydrolase attenuates endothelial dysfunction in animal models of diabetes, obesity and hypertension. Eur J Pharmacol 2011; 654: 68–74.

171 Chen D, Whitcomb R, MacIntyre E, Tran V, Do ZN, Sabry J, Patel DV, Anandan SK, Gless R, Webb HK. Pharmacokinetics and pharmacodynamics of AR9281, an inhibitor of soluble epoxide hydrolase in single- and multiple-dose studies in healthy human subjects. J Clin Pharmacol 2012; 52: 319–28.

172 Wang L, Yang J, Guo L, Uyeminami D, Dong H, Hammock BD, Pinkerton KE. Use of a soluble epoxide hydrolase inhibitor in smoke-induced chronic obstructive pulmonary disease. Am J Respir Cell Mol Biol 2012; 46: 614–22.

173 Keseru B, Barbosa-Sicard E, Schermuly RT, Tanaka H, Hammock BD, Weissmann N, Fisslthaler B, Fleming I. Hypoxia-induced pulmonary hypertension: comparison of soluble epoxide hydrolase deletion vs inhibition. Cardiovasc Res 2010; 85: 232–40.

174 Podolin PL, Bolognese BJ, Foley JF, Long E 3rd, Peck B, Umbrecht S, Zhang X, Zhu P, Schwartz B, Xie W, Quinn C, Qi H, Sweitzer S, Chen S, Galop M, Ding Y, Belyanskaya SL, Israel DI, Morgan BA, Behm DJ, Marino JP Jr, Kurafi E, Barnette MS, Mayer RJ, Booth-Genthe CL, Callahan GF. In vitro and in vivo characterization of a novel soluble epoxide hydrolase inhibitor. Prostaglandins Other Lipid Mediat 2013; 104–105: 25–31.

175 Falck JR, Kodela R, Manne R, Atcha KR, Puli N, Dubasi N, Manthati VL, Capdevila JH, Yi XY, Goldman DH, Morisseau C, Hammock BD, Campbell WB. 14,15-Epoxyeicosicosa-5,8,11-trienoic acid (14,15-EET) surrogates containing epoxyeicosatrienoic acid (14,15-EET) surrogates in ex vivo and in vivo characterization of a novel soluble epoxide hydrolase inhibitor. Prostaglandins Other Lipid Mediat 2013; 104–105: 25–31.

176 Sodhi K, Inoue K, Gotlinger KH, Canestrao M, Vanella L, Kim DH, Manthati VL, Koduru SR, Falck JR, Schwartzman ML, Abraham NG. Epoxyeicosatrienoic acid agonist rescues the metabolic syndrome phenotype of HO-2-null mice. J Pharmacol Exp Ther 2009; 331: 906–16.

177 Zhang G, Panigrahy D, Hwang SH, Yang J, Mahakan LM, Wettersten HI, Liu JY, Wang Y, Ingham ES, Tam S, Kieran MW, Weiss RH, Ferrara KW, Hammock BD. Dual inhibition of cyclooxygenase-2 and soluble epoxide hydrolase synergistically suppresses primary tumor growth and metastasis. Proc Natl Acad Sci USA 2014; 111: 11127–32.