Reno-protective mechanisms of epoxyeicosatrienoic acids in cardiovascular disease

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Elmarakby AA. Reno-protective mechanisms of epoxyeicosatrienoic acids in cardiovascular disease. Am J Physiol Regul Integr Comp Physiol 302: R321–R330, 2012. First published November 23, 2011; doi:10.1152/ajpregu.00606.2011. — Cardiovascular disease (CVD) is the leading cause of mortality worldwide, and it is well known that end-stage renal disease (ESRD) is a profound consequence of the progression of CVD. Present treatments only slow CVD progression to ESRD, and it is imperative that new therapeutic strategies are developed to prevent the incidence of ESRD. Because epoxyeicosatrienoic acids (EETs) have been shown to elicit reno-protective effects in hypertensive animal models, the current review will focus on addressing the reno-protective mechanisms of EETs in CVD. The cytochrome P-450 epoxygenase catalyzes the oxidation of arachidonic acid to EETs. EETs have been identified as endothelium-derived hyperpolarizing factors (EDHFs) with vasodilatory, anti-inflammatory, antihypertensive, and antiplatelet aggregation properties. EETs also have profound effects on vascular migration and proliferation and promote angiogenesis. The progression of CVD has been linked to decreased EETs levels, leading to the concept that EETs should be therapeutically targeted to prevent end-organ damage associated with CVD. However, EETs are quickly degraded by the enzyme soluble epoxide hydrolase (sEH) to their less active diols, dihydroxyeicosatrienoic acids (DHETs). As such, one way to increase EETs level is to inhibit their degradation to DHETs by using sEH inhibitors. Inhibition of sEH has been shown to effectively reduce blood pressure and organ damage in experimental models of CVD. Another approach to target EETs is to develop EET analogs with improved solubility and resistance to auto-oxidation and metabolism by sEH. For example, stable ether EET analogs dilate afferent arterioles and lower blood pressure in hypertensive rodent animal models. EET agonists also improve insulin signaling and vascular function in animal models of metabolic syndrome.

diabetes; hypertension; renal injury; hemeoxygenase; inflammation; oxidative stress

CARDIOVASCULAR DISEASE (CVD) targets the heart or blood vessels inducing a dysfunction of arteries and veins that supply oxygen to vital body organs such as the brain and the heart. In September 2011, the World Health Organization’s new report on CVD prevention and control states that CVD is the leading cause of mortality worldwide. Although a large number of cases of CVD are treatable, the incidence and progression of CVD continues to rise due to lack of adequate preventive measures. An estimated 17.3 million people died from CVD in 2008, and it is anticipated that almost 34 million people will die from CVD by 2030 (World Health Organization report, 2001). In the United States, CVD is the nation’s leading killer of both men and women among all racial and ethnic groups. About one million Americans die of CVD annually, which represents 42% of all deaths (38, 88). CVD accounts for almost 6 million hospitalizations each year and causes disability for almost 10 million Americans age ≥ 65 years. CVD costs the nation $432 billion each year, including health expenditures and lost productivity (22, 89–90). This cost is estimated to dramatically increase as the burden continues to grow as the population ages. Impaired renal function could occur as a consequence of CVD and often progress to end-stage renal disease (ESRD) (87, 91). ESRD is characterized by extensive albuminuria, increased level of inflammatory cytokines, severe decline in renal function, and elevation in blood pressure leading to increased risk of cardiovascular death (87, 91). Many factors contribute to the progression of CVD to ESRD, such as environmental pollution, daily stress, lifestyle, tobacco

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smoking, lack of physical activity, obesity, and poor nutrition (87, 120). The pathophysiology of ESRD is multifactorial; however, endothelial dysfunction and vascular inflammation develop with time and are independently associated with mortality (72, 96). Endothelial dysfunction is characterized by impaired vasomotor response, cell proliferation, platelet aggregation, and vascular permeability, which promote vascular inflammation via inducing the production of vasoconstrictor mediators, growth factors, and adhesion molecules (72, 96). Although early medical interventions and treatments of CVD are available, lack of treatment options for ESRD are the real challenge in the medical field. Accordingly, new avenues are required to halt the progression of CVD to ESRD.

Cytochrome P-450 Metabolites

Although cyclooxygenase (COX) and lipoxygenase metabolites of arachidonic acid are now the most widely accepted clinical targets to treat inflammation and asthma, the cytochrome P-450 (CYP) is a third class of arachidonic acid metabolites that recently emerge as a potential target for treatment of CVD (47). As shown in Fig. 1, the CYP monoxygenase pathway catalyzes the oxidation of arachidonic acid at any of the four double bonds to four regioisomeric epoxyeicosatrienoic acids (EETs) (5,6-, 8,9-, 11,12-, and 14,15-EET) by the CYP epoxygenase and/or 19- and 20-hydroxyl eicosatetraenoic acids (19- and 20-HETE) by the CYP ω-hydrolyase (34, 47, 95, 106). EETs and 20-HETE have been identified as the main metabolic products of arachidonic acid in rodent and human tissues (47, 117). The members of the CYP2C and CYP4A gene subfamilies are the most functionally relevant renal CYP epoxygenase and CYP ω-hydrolyrase, respectively (95). Arachidonic acid metabolites play a crucial role in preserving renal function and regulating blood pressure (95). Initially, pro- and antihypertensive effects were proposed for the product of CYP epoxygenase and ω-hydrolyase, respectively (95). Recently, it was shown that 20-HETE has both pro- and antihypertensive effects (95). As a prohypertensive metabolite, 20-HETE is a potent vasoconstrictor, inactivates smooth muscle calcium-sensitive potassium channels, and exacerbates the activity of other vasoconstrictor mediators such as ANG II, endothelin, and serotonin (95). In contrast, studies in salt-sensitive hypertensive animal models suggest that 20-HETE has antihypertensive and natriuretic properties (117).

EETs are synthesized in the endothelium and are considered important vasodilatory regulators of vascular tone, especially when the bioavailability of the endothelium-derived vasodilator nitric oxide is reduced (11, 56, 81). EETs are also endothelium-derived hyperpolarizing factors in a number of vascular beds, including the renal circulation (10, 57, 109). CYP epoxygenase metabolites and EETs regulate renal sodium and water excretion as indicated by increased CYP epoxygenase activity and urinary EETs excretion following excessive dietary salt intake (129–130). Besides antihypertensive properties, EETs have potent anti-inflammatory properties, as previous studies have demonstrated that EETs decrease cytokine-induced endothelial expression of leukocyte adhesion molecules (77). EETs also have thrombolytic activity, as they inhibit platelets’ adhesion to endothelial cells and increase tissue plasminogen activator expression (55, 78). EETs provide protective effects against vascular remodeling via inhibiting the proliferation of vascular smooth muscle cells (108). In endothelial cells, EETs have the opposite effect as they augment cell proliferation and migration, suggesting that EETs could improve atherogenesis and promote neovascularization in ischemic diseases (70, 116). EETs also increase endothelial cell survival and attenuate apoptosis (19–20, 68). The versatile effects of EETs make them a therapeutic target for end-organ damage associated with CVD (47), especially after recent reports suggesting that genetic variations in the CYP epoxygenase is associated with increased risk of CVD (8, 61). Additionally, the anti-inflammatory and antihypertensive properties of EETs also make then a novel strategy to halt the progression of CVD to ESRD; however, conversion of EET epoxides to their corresponding diols (DHETs) by soluble epoxide hydrolase (sEH) enzyme limits EETs availability and decreases their beneficial cardiovascular properties.

![Cytochrome P450 metabolites](image-url)
sEHs

Microsomal and soluble epoxide hydrolase are two well-known epoxide hydrolase enzymes with different subcellular localization and substrate selectivity (24, 47, 128). The microsomal epoxide hydrolase is involved in the metabolism of environmental contaminants, whereas sEH was initially discovered as a metabolizing enzyme for carcinogenic xenobiotics until it was later found that sEH also metabolizes EETs to less active DHETs (69, 73). The sEH enzyme is found in many tissues such as liver, kidney, lung, heart, and ovary and is localized in cytosol, microsomes, and peroxisomes (66, 99). The mammalian sEH is a ubiquitously expressed homodimeric enzyme consisting of two 62 kDa monomers (69, 73). Each monomer has two distinct domains, the NH2-terminal domain with phosphatase activity and the COOH-terminal domain with sEH activity (26–27, 74). There are no known selective in vivo inhibitors of the NH2-terminal domain, and current inhibitors target the COOH-terminal domain without affecting NH2-terminal domain (47). Compelling evidence in the literature using wild-type (WT) and sEH gene (Ephx2) knockout hypertensive and diabetic mice models suggest that the phosphatase domain does not contribute to blood pressure regulation or renal injury (23, 67, 101). The human Ephx2 consists of 19 exons encoding 555 amino acids and has 73% homology with mouse sEH protein sequences with 100% conservation in the catalytic residues (85). Clinically, a number of polymorphisms in Ephx2 that influence sEH enzymatic activity has been recently reported in CVD, suggesting that increased EETs bioavailability could have potential therapeutic benefits in CVD (9, 54, 58, 79, 85).

EETs Signaling Mechanisms

The cellular action of EETs involves either binding to cell surface receptors and/or their intracellular uptake and direct interaction with ion channel or transcription factors. The diverse cell-signaling mechanisms of EETs suggest that EETs may have more than one receptor. Substantial data in the literature demonstrate that EETs bind to selective G protein-coupled receptors to initiate intracellular signaling pathways (47, 56, 105). However, this possibility needs further investigation as long as a putative EET receptor has yet to be clearly identified. EETs-induced vasodilatation and inhibition of the renal sodium-potassium ATPase are potential antihypertensive mechanisms (47, 80). EETs generated by endothelial cells dilate blood vessels via the activation of the G<sub>9Q</sub> protein, adenylyl cyclase, to increase cAMP, which in turn activates vascular smooth muscle cells large-conductance calcium-activated potassium channels, resulting in potassium efflux from the smooth muscle cell and subsequent membrane hyperpolarization (4, 36). There is also evidence for cAMP activation of protein kinase A (PKA) and ADP ribosylation of G<sub>alpha</sub> cellsignaling mechanisms in mediating EETs’ activation of vascular smooth muscle calcium-activated potassium channels in renal vessels (32, 48, 60). Another alternative mechanism for the EETs’ vasorelaxant response is the activation of the vanilloid (TRP) channel to increase calcium influx leading to endothelial potassium channel activation and hyperpolarization of endothelium, which could also trigger vascular smooth muscle relaxation (31, 113). Like endothelial and vascular smooth muscle cells, EETs hyperpolarize platelet cell membranes through activation of calcium-activated potassium channels (55).

EETs exert anti-inflammatory effects via inhibiting the activation of the transcription factor nuclear factor-κB (NF-κB) (23, 67, 77, 80). For example, overexpression of CYP2J2 epoxygenase in endothelial cells decreased NF-κB activation as well as treatment of endothelial cells with 11,12-EET prevented TNF-α-induced NF-κB and vascular cell adhesion molecule-1 (VCAM-1) expression (28, 77–78). EETs also mediate the activation of the anti-inflammatory nuclear receptor peroxisome proliferator-activated receptor-γ (PPARγ), which inhibits NF-κB-induced proinflammatory adhesion molecules (62).

EETs induce vascular protection via several other autocrine and paracrine effects, such as activation of endothelial cells proliferation and migration and inhibition of vascular smooth muscle cells proliferation. EETs-induced endothelial cell proliferation includes the activation of phosphotyrosil inositol-3-kinase (PI3K)/AKT pathway, mitogen-activated protein kinase (MAPK), and cAMP/PKA signaling pathways (62, 83–84). The angiogenic effects of EETs in endothelial cells involve the activation of PI3K/AKT, sphingosine kinase-1, and the Proto-oncogene, tyrosine-protein kinase, SRC-stimulated phosphorylation of STAT-3 (15, 121, 124). The cAMP/PKA cell-signaling pathway activation is involved in EET antimigratory actions in vascular smooth muscle (108). EETs have also shown to inhibit apoptosis via activation of PI3K/AKT pathway to inhibit extracellular signal-regulated kinase (ERK1/2) dephosphorylation (122).

Enhancement of EETs Availability in CVDs

Recent studies have shown a limited biological activity of EETs in CVD due to their rapid metabolism by sEH to DHETs or by fatty acid β-oxidation to 16-carbon epoxy derivatives (23, 114–115). Although the physiological properties of DHETs are not fully addressed, they have limited biological activity compared with EETs. Elevated EET levels are now being considered as a new therapeutic target for the treatment of CVD as increased EET levels protect the overall health of CVD patients from end-organ damage. There are two primary approaches to increase EETs effect in vivo. The first approach is to increase EETs survival via the inhibition of EETs degradation by sEH using sEH inhibitors. The second approach is to develop stable EETs agonists that resist auto-oxidation and metabolism by sEH enzyme. Both approaches have recently shown promising evidence in decreasing end-organ damage associated with CVD.

Cardiovascular Effects of sEH Inhibitors

Inhibition of sEH has been shown to provide protective effects against a variety of CVDs, such as hypertension, stroke, cardiac hypertrophy, diabetes, and atherosclerosis as well as slow the progression of end-organ damage, endothelial dysfunction, and inflammation associated with CVD (67, 80, 86, 101, 111, 126).

In hypertension, the vasodilatory effects of sEH inhibitors were tested in vitro (51, 57) followed by identifying their antihypertensive effects in vivo in experimental models of hypertension, such as spontaneously hypertensive rats (SHR), angiotensin hypertension, Ren-2 transgenic rats, and DOCA-
In heart failure, Ephx2 has been identified as a heart failure susceptible gene in SHR (16, 35). Increased EETs levels, via overexpression of CYP2J1, pharmacological inhibition of sEH, or Ephx2 gene deletion, improved postischemic left ventricular developed pressure recovery and reduced the infarct size after ischemia/reperfusion injury (5–6, 13). Similar results were obtained in mice and dogs with coronary artery ligation following reperfusion as Ephx2 gene deletion or inhibition of sEH also reduced the infarct size (39, 71). Inhibition of sEH provides antihypertrophic effects in hypertensive animal models of cardiac hypertrophy, such as in DOCA-salt hypertension, stroke-prone SHR, and ANG II-induced hypertension (3, 59, 63) and prevents the development of left ventricular hypertrophy in mice with pressure overload-induced myocardial failure (118). Ephx2 gene deletion also protected myocardium from ANG II-induced cardiac arrhythmia and pressure overload-induced heart failure (2–3). However, the clinical use of sEH inhibition or EETs analogs for treatment of heart failure could be limited by the recent observation that Ephx2 gene deletion or sEH inhibition delayed blood pressure recovery and resulted in higher mortality rate in transgenic cardiac arrest/resuscitation mice model (44).

In stroke, chronic treatment with sEH inhibitor decreased cerebral infarct size after middle cerebral artery occlusion in stroke-prone SHR via improving vascular function and protecting the neurons from cell death independent of blood pressure-lowering effects (101–102). Similarly, Ephx2 gene deletion decreased infarct size following a cerebral ischemia (101). Inhibition of sEH also exerted an angiogenic effect in stroke-prone SHR as it increased microvascular densities and cerebral artery compliance (21).

In metabolic syndrome and diabetes, CYP expression decreased and sEH increased in obese animal models (18, 115), and polymorphism of the Ephx2 gene is associated with insulin resistance in type 2 diabetic patients (79). Studies suggest that EETs are involved in glucose homeostasis and improving insulin signaling during obesity (65, 125). For example, overexpression of CYP2J3 epoxygenase improved insulin resistance in diabetic db/db mice and in fructose-induced insulin-resistant rats (119), and inhibition of sEH or Ephx2 gene deletion decreased glucose levels and improved insulin signal and sensitivity in high-fat diet-fed mice (65). Inhibition of sEH also decreased blood glucose and improved endothelial function in db/db obese mice (125). In hemeoxygenase-2 (HO-2) knockout mice, another model of obesity with decreased EETs levels, a dual-activity EET agonist/sEH inhibitor, improved phenotypic characteristics of metabolic syndrome as it reduced body weight, visceral fat, and blood glucose together with increase EETs levels (104). In the type 1 diabetic animal model, Ephx2 gene deletion or pharmacological inhibition of sEH has recently shown to reduce blood glucose in streptozotocin-induced diabetes (64).

In atherosclerosis, polymorphism in Ephx2 is also linked to incidence of atherosclerosis (58) and inhibition of sEH reduced inflammation and prevented the development of atherosclerotic plaques in apoE-knockout mouse model of atherosclerosis, suggesting that increased EETs levels may have therapeutic potential for treatment of hyperlipidemia (111, 126).

**Reno-Protective Effects of EETs in CVD**

Inhibition of sEH has been shown to protect kidney from end-organ damage in ANG II hypertensive rats, as it decreased glomerular and tubular collagen expression and vascular hypertrophy and reduced albuminuria (50, 131). Inhibition of sEH also attenuated afferent arteriolar diameter responses to ANG II in isolated kidneys from chronic ANG II hypertensive rats (131). Moreover, urinary albumin excretion was decreased and macrophage infiltration was reduced by sEH inhibitor treatment in ANG II-salt sensitive hypertension (50). Inhibition of sEH also increased urinary salt and water excretion and decreased renal vascular resistance in ANG II-infused mice (51). The reno-protective effect of sEH inhibition was quite obvious either during the progression of hypertension or after hypertension was established (49–51, 131). In Goldblatt two-kidney, one-clip (2K1C) hypertensive rats, inhibition of sEH decreased blood pressure and sodium excretion together with improved renal blood flow and glomerular filtration rate (GFR) (107, 114). Inhibition of sEH also provides renal protection in stroke-prone SHR, as it decreased blood pressure, renal arteriolar hypertrophy, and proteinuria (59). Ephx2 gene deletion or pharmacological inhibition of sEH also decreased blood pressure and attenuated renal inflammation and glomerular injury in DOCA-salt hypertension (67). Inhibition of sEH could exacerbate the beneficial effects of 20-HETE inhibition, as this combination slowed the progression of hypertension, and protect kidney from hypertensive induced end-organ damage in Ren-2 transgenic rats (12). In an animal model of metabolic syndrome, increased EETs levels improved abnormal renal hemodynamics and hypertension as inhibition of sEH lowered mean arterial pressure, renal vascular resistance, and GFR and increased renal blood flow in high-fat diet-fed rats (43). Inhibition of sEH also provides renal protection in ANG II-induced hypertensive diabetic Goto-Kakizaki rats as it decreased albuminuria and glomerular and tubular injury together with reduced macrophage infiltration and MCP-1 excretion (80). Recent data from the laboratory demonstrate that renal blood flow was high in Ephx2 knockout mice compared with control WT mice. Induction of diabetes with streptozotocin decreased renal blood flow in WT mice, and this effect was reduced with either Ephx2 gene deletion or pharmacological inhibition of sEH (Fig. 2), suggesting that increased EETs levels via Ephx2 gene deletion or sEH inhibition could prevent the impairment in renal function during diabetes. Ephx2 gene deletion or sEH inhibition also reduced renal injury and inflammation in streptozotocin-induced diabetic mice (23). In addition to their protective effects against chronic progressive kidney disease, sEH inhibitors have been shown to provide kidney protection against cisplatin-induced acute renal injury as inhibition of sEH decreased blood urea nitrogen levels and reduced tubular damage induced by cisplatin (82).

Similarly to the effect of sEH inhibition, stable EETs analogs also provide renal protection in CVD. Analogs of 8,9-EET improve glomerular dysfunction as 8–9 EET analogs attenuated focal and segmental glomerulosclerosis permeability fac-
tor-induced increased in glomerular albumin permeability (100). 11,12 EETs analogs also mediate afferent arteriolar relaxation utilizing the phospho-protein phosphatase 2A to activate the large-conductance calcium-activated potassium channels (45). Similarly, 11,12-EET agonists exerted vasodilatory and anti-inflammatory effects via the inhibition of TNF-α induced VCAM-1 expression (28). In vivo, the potential protective effects of EETs agonists against end-organ damage in CVD have been recently explored in animal models of hypertension and metabolic syndrome (46, 104). EETs agonists dilated afferent arterioles and lowered blood pressure in SHR (46). A dual-activity EET agonist/sEH inhibitor has recently been tested in HO-2 knockout mice, a model of metabolic syndrome phenotype and endothelial dysfunction (104). Recent data from Dr. John Imig’s laboratory (unpublished data) demonstrated that EET agonist also decreased blood pressure and reduced renal injury and inflammation in ANG II-induced hypertensive rats. Overall, EETs ability to improve renal vascular function and decrease renal injury could provide a promising role of sEH inhibitors in treatment of acute and chronic kidney disease.

**Reno-Protective Mechanisms of EETs in CVDs**

The potential reno-protective mechanisms of EETs are summarized in Fig. 3 and as follows.

**EET’s hypotensive and hypoglycemic effects.** EETs improve renal function and protect the kidney from renal inflammation and injury associated with the progression of CVD as they increase renal blood flow, GFR, and sodium excretion and decrease renal vascular resistance (47, 105–106). Previous studies suggest that the antihypertensive effect of EETs mediates their reno-protective effects as inhibition of sEH lowered blood pressure together with reduced renal injury in hypertensive and metabolic syndrome animal models (43, 50, 131). However, the antihypertensive effect of sEH inhibitors is not the sole reno-protective mechanism as inhibition of sEH maintained renal protection in hypertensive diabetic Goto-Kakizaki independent on blood pressure-lowering effects (80). Moreover, the Ephx2 deletion demonstrated variable results on blood pressure in hypertensive and diabetic mice models (23, 67, 103, 127), although these mice exhibited reduced renal injury when diabetes was experimentally induced, despite blood pressure-lowering effects (23).

Similarly, Ephx2 deletion or sEH inhibitors have also been shown to reduce blood glucose and enhance glucose utilization via increased insulin release, reduced β-cell apoptosis, and decreased insulin resistance in obese and diabetic mice (64–65). The hypoglycemic effect of sEH inhibition also could be considered a potential reno-protective mechanism against diabetic-induced renal injury; however, Ephx2 gene deletion or pharmacological inhibition of sEH failed to lower blood glucose in streptozotocin-induced diabetic mice, although it provided renal protection against diabetic-induced renal injury (23). Collectively, the hypotensive and hypoglycemic effects of sEH inhibition could not completely explain the reno-protective effects of EETs in CVD and additional reno-protective mechanisms of EETs in CVD are yet to be explored.

**EETs anti-inflammatory effects.** It well known that inflammatory cytokines are key components in end-organ damage associated with CVD (7). The relationship between inflammatory cytokines and CYP epoxygenase has been recently investigated. Cytokines decreased CYP epoxygenase expression and its vasodilatory effects, whereas blocking inflammatory cytokines, such as inhibition of TNF-α or chemokine receptor 2b blockade, increased renal CYP epoxygenase expression and decreased renal injury and inflammation in ANG II-salt-sensitive hypertension (24–25, 33–34). Accordingly,
increase of EETs’ level via the inhibition of sEH could provide renal protection against end-organ damage in CVD via EETs anti-inflammatory effects. EETs have been shown to exert anti-inflammatory effects via the inhibition of NF-κB inflammatory signaling activation (23, 67, 77, 80). For example, inhibition of sEH reduced the production cytokines and pro-inflammatory mediators in LPS-induced inflammation in mice with improved survival rates (98). Inhibition of sEH reduced macrophage infiltration in ANG II-salt sensitive hypertensive rats and in hypertensive diabetic Goto-Kakizaki rats (80, 131).

Pharmacological inhibition of sEH or Ephx2 gene deletion inhibited renal NF-κB activation and decreased MCP-1 excretion in DOCA-salt hypertensive mice (67). We have recently shown that Ephx2 gene deletion also inhibited renal inflammation via inhibition of phospho-IκB kinase-induced NF-κB signaling activation in streptozotocin-induced diabetic mice (23). Inhibition of sEH exerted analgesic effects and reduced COX-2 expression in lipopolysaccharide-induced hyperalgesia (97).

Additionally, inhibition of sEH also reduced the number of renal apoptotic/necrotic cell in right kidney from WT mice subjected to 45-min ischemia followed by 4-h perfusion as well as in the left contralateral control (Fig. 4). Accordingly, the anti-inflammatory and antiapoptotic effects of EETs could be a potential reno-protective mechanism against CVD-induced renal injury.

**EETs-PPAR interaction.** Peroxisome proliferator-activated receptor-α (PPAR-α) activator is involved in regulating fatty acid metabolism and attenuates vascular smooth muscle cell proliferation (40, 92). PPAR-α agonists have been shown to reduce renal injury, oxidative stress, and inflammation during CVD with or without blood pressure-lowering effects (37, 42–43). EETs have been shown to activate PPAR-α (30, 75), and inhibition of sEH also activates PPAR-α and inhibits cyclin D1 (29, 76). Accordingly, the ability of sEH inhibitors to activate PPAR-α could be beneficial in protecting kidney from end-organ damage associated with patients with hyperlipidemia, diabetes, and hypertension. EETs also increase PPAR-γ transcription activity where PPAR-γ agonists are insulin sensitizer and are key elements in inhibiting NF-κB-induced inflammation (62). Present commercially available PPAR-γ agonists for treatment of diabetes have an unwanted fluid-retaining effect, which is detrimental to patients with CVD, especially those with heart failure. Accordingly, using sEH inhibitor with or without PPAR-γ agonist could provide a new avenue for treatment of cardiometabolic syndrome and protect kidney from ESRD, because EETs diuretic and natriuretic effects could lessen the fluid-retaining properties of PPAR-γ agonist.

**EETs-HO relationship.** HO is the primary pathway for heme metabolism-generating biliverdin, iron, and CO and biliverdin, which is further metabolized to bilirubin (1, 110). Two isoenzymes of HO are well known: HO-1 and HO-2. HO-1 is induced in response to many pathophysiological changes in CVD, such as hypoxia, oxidative stress, ischemia, and increased cytokines, whereas HO-2 is the constitutive isoenzyme that accounts for most HO activity in normal healthy state (1, 110). Induction of HO-1 provides protective effects via the dissipation of the pro-oxidant heme and the generation of the antioxidant, anti-inflammatory and antiapoptotic metabolites (1, 110). It is well known that HO-1 induction could protect kidney from end-organ damage in CVD via antioxidant and inflammatory effects, as induction of HO-1 have been shown to inhibit NADPH-derived oxidative stress and inflammation; however, the use of HO-1 inducers are limited due to their potential toxic effects (1, 17, 110). EETs and HO share overlapping anti-inflammatory properties, and the possible link between them has been initially established in vitro. Activity and expression of HO-1 increased in cultured endothelial cells treated with 11,12-EET. 11,12-EETs vasorelaxant properties in mesenteric vessel was also shown to be mediated by increased HO activity (93–94). EETs’ agonist or sEH inhibition inhibited adipogenesis and decreased levels of inflammatory cytokines together with increased HO-1 expression in mesenchymal stem cell-derived adipocytes proliferation and differentiation and inhibition of HO activity reversed EET-induced inhibition of adipogenesis (52, 112). In vivo, Sodhi et al. (104) demonstrated that a dual-activity EET agonist/sEH inhibitor increased renal EETs levels and renal HO-1 expression, and these changes were associated with a reduction inflammatory cytokines levels and restoration of relaxation of aortic rings to acetylcholine together with improved metabolic syndrome phenotype in obese HO-2 knockout mice. We have recently shown that renal HO-1 expression and activity increased in diabetic Ephx2 knockout mice with no change in HO-2 expression, and these changes were associated with decreased renal oxidative stress, inflammation, and damage compared with diabetic WT mice (23). Furthermore, inhibition of HO activity negated the reno-protective effects of Ephx2 gene deletion or sEH inhibition during diabetes as it increased inflammatory and renal injury markers (23). Overall, increased EETs levels induce HO-1 in CVD, which in turn protects the kidney from end-organ damage via the reduction in oxidative stress and the inhibition of NF-κB induced inflammation.

Perspectives and Significance

sEH inhibitors or EET agonists have potential therapeutic use to slow the progression of ESRD associated with CVD especially after a sEH inhibitor has recently completed phase
IIA clinical trial in hypertensive patients with impaired glucose tolerance (14). Because the inhibition of sEH has been shown to reduce COX-2 expression (97), sEH inhibitors could be used in combination with traditional nonsteroidal anti-inflammatory drugs or selective COX-2 inhibitors to synergize their anti-inflammatory properties in CVD and reduced their cardiotoxic side effects. Another potential approach to halt the progression of ESRD is to investigate the effect of combined administration of EET agonist and sEH inhibitor on the progression of ESRD in CVD. A dual EET agonist and sEH inhibitor improved endothelial function and metabolic syndrome phenotype and reduced blood pressure in obese HO-2 knockout mice (104). Gross et al. (39) recently showed that sEH inhibitor or exogenous EET exert cardioprotection in canine ischemia/reperfusion, and combined administration of both resulted in a synergistic effect. Accordingly, future clinical studies should determine whether combined administration of EET agonist and sEH inhibitors will provide better reno-protective effects than either treatment alone. Moreover, the potential use of 20-HETE sEH inhibitors will provide better reno-protective effects than either treatment alone. Moreover, the potential use of 20-HETE sEH inhibitors may provide favorable synergistic effects against ESRD in CVD, especially after recently published data demonstrated that combined inhibition of 20-HETE formation and sEH inhibition attenuated the development of hypertension and protected the kidney from hypertension-induced injury in Ren-2 transgenic rats (12).

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AUTHOR CONTRIBUTIONS
A.A.E. conception and design of research; A.A.E. analyzed data; A.A.E. prepared figures; A.A.E. drafted manuscript; A.A.E. edited and revised manuscript; A.A.E. approved final version of manuscript.

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