Kidney disease afflicts 33 million in the United States, and chronic kidney disease (CKD) accounts for >$60 billion in Medicare costs. Hypertension afflicts 75 million in the United States, and significant portions of those patients develop CKD and progress to end-stage renal disease. Interestingly, resistant hypertension which is defined as uncontrolled hypertension, despite 3 antihypertensive medication classes, increases the risk for cardiovascular diseases and end-stage renal disease. These recent findings in the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) highlights the fact that current treatments only slow the loss of kidney function, or have no benefit at all. New therapeutic approaches are urgently needed.

Development of drugs to increase a novel class of fatty acids, epoxyeicosatrienoic acids (EETs), represents a unique approach to treat hypertension and kidney disease. EETs are generated from the substrate arachidonic acid by cytochrome P450 (CYP) epoxygenase enzymes. There are 4 regioisomeric EETs formed: 5,6-EET; 8,9-EET; 11,12-EET; and 14,15-EET. These regioisomeric EETs are further metabolized to less active or inactive diols by the soluble epoxide hydrolase (sEH; Ephx2) enzyme. For clarity, EETs will be described as a specific regioisomeric EET. In the majority of circumstances, the primary EETs evaluated for cardiovascular and renal function have been 11,12-EET and 14,15-EET. Once formed, EETs act in an autocrine or paracrine manner to elicit biological responses. Vascular endothelial and renal epithelial cells are major sites for EET production. This localized EET generation aligns with the biological actions and contribution of EETs to cardiovascular and renal function. Prominent biological actions of EETs include their role as endothelial-derived hyperpolarizing factors and regulation of tubular sodium reabsorption by inhibiting epithelial sodium channel (ENaC) in the kidney. These actions position EETs to increase blood flow to organs, decrease peripheral vascular resistance, and enhance sodium excretion. EETs also have anti-inflammatory actions that are beneficial in cardiovascular and renal diseases.

The link between decreased EETs and hypertension, especially salt-sensitive hypertension, has been strongly established. Decreased renal epoxygenase activity and decreased renal EET levels have been associated with angiotensin-dependent hypertension, salt-sensitive hypertension, and Lyon hypertensive rats. Transgenic rats overexpressing both human renin and angiotensinogen genes (dTGR) develop hypertension and renal failure that is associated with decreased kidney epoxygenase enzymatic activity and CYP2C11 and CYP2C23 protein levels. Likewise, we have found that an inability to increase renal cortical and vascular rat CYP2C11 and CYP2C23 or mouse Cyp2c44 protein expression contributes to salt-sensitive hypertension. These CYP2C enzymes are primarily responsible for 11,12-EET and 14,15-EET formation in the rat and mouse kidneys.

Rat CYP2C23 and mouse Cyp2c44 are the predominant kidney epoxygenases which are upregulated by a high K+ (2.5%) or high Na+ (8%) salt diet. Another potential epoxygenase is the CYP2J5 protein that is abundantly expressed in the mouse kidney. However, the ability of CYP2J5 to generate EETs is questionable and Cyp2j5 mice have demonstrated that CYP2J5 seems to contribute to blood pressure control by regulating estrogen rather than EET synthesis. Genetic manipulation of CYP2C epoxygenase expression has provided additional support to the concept that CYP2C-derived EETs are essential in renal sodium handling and blood pressure regulation. Cyp2c44 mice develop hypertension when fed a high K+ or high Na+ salt diet. Similarly, Cyp4a10−/− mice have decreased renal Cyp2c44 epoxygenase activity in response to high Na+ salt and develop salt-sensitive hypertension. Differences in renal EET generation and blood pressure in response to dietary NaCl intake between the Cyp2c44−/− mice and Cyp4a10−/− mice provide additional evidence for a critical contribution for EETs in blood pressure regulation. Interestingly, Cyp4a10−/− mice have decreased urinary EET levels and an elevated blood pressure on a normal salt (0.3% NaCl) diet. Lowering dietary salt to 0.05% NaCl lowers blood pressure in Cyp4a10−/− mice. In contrast, Cyp2c44−/− mice do not have decreased urinary EET levels or elevated blood pressures on a normal salt diet. Both Cyp2c44−/− and Cyp4a10−/− mice demonstrate salt-sensitive hypertension in response to 8% NaCl feeding, which is
associated with an inability to increase renal EET generation. The fact that amiloride lowers blood pressure in Cyp2c44−/− and Cyp4a10−/− mice fed a high-salt diet suggests a significant contribution for ENaC.11,22,23

A major cellular mechanism responsible for salt-sensitive hypertension that results from decreased renal EET levels seems to be increased ENaC activity (Figure 1).8,11,12 Actions of 11,12-EET on basolateral inwardly rectifying K+ channels and apical ENaC channels on the cortical collecting duct epithelium can explain the salt-sensitive blood pressure regulation in response to high K+ or Na+ salt diets. Hypertensive Cyp2c44−/− mice show a hyperactive ENaC and reduction in ERK1/2 and ENaC subunit phosphorylation.8,11 In regard to EET regioisomeric actions on ENaC, 11,12-EET inhibits ENaC to a greater extent than 14,15-EET and 8,9-EET had no effect on ENaC activity.11 11,12-EET can inhibit basolateral inwardly rectifying K+ channels that results in cell membrane depolarization to reduce the driving force for Na+ entry across the apical membrane.8,20 Another renal epithelial cell action attributed to 11,12-EET is stimulation of apical large-conductance Ca2+-activated K+ epithelial channels that could contribute to renal K+ secretion in response to high K+ intake.8,24,25 Interestingly, 11,12-EET is the major product of the mouse Cyp2c44 and is generated in the cortical collecting duct and increases in response to a high K+ or Na+ salt diet.11,20 The inability of Cyp2c44−/− mice to increase 11,12-EET in response to either a high Na+ or K+ diet and the lack of actions on K+ channels and ENaC in the cortical collecting duct results in salt-sensitive hypertension. Taken together, these findings clearly demonstrate a critical role for renal CYP2C enzymes in fluid and electrolyte homeostasis and blood pressure control.

**Vascular Endothelial Dysfunction**

EETs also contribute importantly to endothelial function in the pathology of hypertension and cardiovascular diseases (Figure 1).7,8 Numerous studies have shown that EETs are an endothelial-derived hyperpolarizing factor and are critical for proper regulation of blood vessels and arteries.7,8,10,26 EETs activate vascular smooth muscle cell large-conductance calcium-activated K+ channels (KCa) through a cAMP and protein kinase A–dependent mechanism.27,28 Vascular expression of epoxygenase enzymes and generation of EETs is decreased in cardiovascular diseases.7,14,18,29 Decreased renal microvessel CYP2C11, CYP2C23, and CYP2J expression in the obese Zucker rat and in rats fed a high-fat diet is thought to contribute to increased blood pressure.29 Vascular EET levels are further reduced by increased sEH expression in obese Zucker rats and this has been demonstrated to contribute to endothelial dysfunction.29 Likewise, endothelial dysfunction and inflammation are associated with decreased plasma EET levels and increased sEH activity in humans with atherosclerotic disease.30–33 Reactive oxygen species that are elevated in hypertension can also reduce EET bioavailability and vasodilation in human coronary arterioles.34,35 Thus, decreased vascular EET levels significantly contribute to the progression of cardiovascular disease and organ damage in hypertension.

**Inflammation**

Inflammation is considered a major player in hypertension and the associated progression of kidney disease. Kidney-specific elevations in T cells have also been implicated in numerous animal models of hypertension.36–38 Recent studies have implicated kidney selective increases in tumor necrosis factor-α in the development of angiotensin II–dependent hypertension and associated kidney disease.7 Likewise, a contribution for increased sEH activity and decreased EET levels has been demonstrated for the inflammation and renal injury associated with hypertension.7,14,22 On the flip side, increasing EET levels by genetic disruption of Ephx2 decreased inflammation and attenuated the progression of renal damage associated with salt-sensitive hypertension.7 Interestingly, expression of human CYP2C8 or CYP2J2 to increase mouse endothelial cell EET generation decreased blood pressure, enhanced vasodilatory responses, and decreased renal injury in angiotensin high-salt hypertension.21 These CYP2C8 and CYP2J2 transgenic mice or Ephx2−/− mice also exhibited decreased vascular nuclear factor-κB signaling and inflammation in response to endotoxin.40 This is in agreement with the increasing amount of published data that EETs decrease vascular inflammation through inhibition of phospho-IκB kinase–derived nuclear factor-κB activation.7,12,19,40 Therefore, evidence indicates that decreased EETs or increased sEH activity contribute to the vascular inflammation and pathogenesis of renal injury in hypertension and that increasing EET bioavailability can counteract disease progression.

**Human Polymorphisms**

There is also evidence in humans that decreased EET levels contribute to hypertension. Human CYP2C8 and CYP2C9 are the major epoxygenases, whereas CYP2J2 has both epoxygenase and ω-1 hydroxylase activity.41 Several CYP2C8 and CYP2C9 gene variants (2C8*2, 2C8*3, 2C9*2, and 2C9*3) demonstrate reduced arachidonic acid epoxidation rates.30,41 Analysis of white and black cohorts failed to demonstrate an association between these variants and hypertension.42 However, the frequency of the CYP2C9*3 allele was lower in a subset of Chinese women with hypertension.43 A common polymorphism in the CYP2J2 gene, CYP2J2*7 allele reduces CYP2J2 transcription, reduces plasma EET levels, and has

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**Figure 1.** Cytochrome P450 epoxygenase metabolites, hypertension, and chronic kidney disease (CKD). Decreased epoxyeicosatrienoic acids (EETs) contribute to enhanced epithelial sodium channel (ENaC) activity, endothelial dysfunction, and decreased renal blood flow (RBF). These changes in kidney and vascular function contribute to hypertension and CKD.
Mediated dilation, an indicator of endothelial dysfunction that
Humans with hypertension also demonstrated decreased flow-
inhibitors has been extensively chronicled in several excellent
in hypertensive patients in the presence or absence of nitric
fluconazole, whereas it did not alter radial artery blood flow
arterial disease participants that were fed flaxseed containing
EETs to hypertension when evaluating EET bioavailability and vascular responses. Genetic variations in EPHX2 have been demonstrated to affect the magnitude of human forearm vasodilator responses. There is a reduction in the forearm vasodilator response in white Americans who have the Arg55 variant allele, which increases sEH activity and would be expected to decrease EET availability, whereas, blacks who have the Gln287 variant allele that decreases sEH activity exhibit enhanced forearm bradykinin-mediated vasodilator responses. Healthy human volunteers exhibit slightly reduced basal forearm blood flow in the presence of the CYP inhibitor fluconazole, whereas it did not alter radial artery blood flow in hypertensive patients in the presence or absence of nitric oxide inhibition. In addition, fluconazole decreased local plasma EET levels in control but not in hypertensive patients. Humans with hypertension also demonstrated decreased flow-mediated dilation, an indicator of endothelial dysfunction that was associated with a reduced EET levels. These findings demonstrate that hypertensive patients where EET levels are genetically or pharmacological manipulated have vasodilator responses that differ from those of healthy volunteers. Thus in addition to nitric oxide, EET levels contribute importantly to endothelial function in hypertensive patients.

Overall, these experimental findings in rodents and humans have generated interest in developing pharmacological means to increase EETs that could potentially lower blood pressure and protect the kidney in hypertension.

Therapeutic Approaches: Hypertension and Kidney Diseases

During the past decade, EET and sEH enzyme-based drugs have been developed with antihypertensive and kidney protective properties that will be particularly beneficial for hypertensive patients that develop CKD (Figure 2). Carbamate urea sEH inhibitors were developed and demonstrated to lower blood pressure and decrease renal injury in animal models of hypertension. Further development of sEH inhibitors progressed rapidly and has resulted in clinical trials for hypertension, diabetes mellitus, and more recently, chronic obstructive pulmonary disease. This development of sEH inhibitors has been extensively chronicled in several excellent review articles. More recent developments with sEH inhibitors are keeping enthusiasm for their potential use in hypertension and CKD at a high level. In a recent controlled clinical trial with peripheral arterial disease participants that were fed flaxseed containing α-linolenic acid for 6 months had decreased blood pressure. α-Linolenic acid was demonstrated in an inhibitor screening assay to decrease sEH activity and the antihypertensive effects of flaxseed feeding were associated with a decrease in plasma sEH-derived oxylipins. As for CKD, a recently published study demonstrated that Ephx2 deficiency or sEH inhibition in mice decreased renal inflammation and fibrosis associated with unilateral ureteral obstruction. The anti-inflammatory and fibroprotective effects in unilateral ureteral obstruction kidneys was via peroxisome proliferator-activated receptors activation and downregulation of nuclear factor-kB, transforming growth factor-β1/Smad3 inflammatory signaling. Another of the more recent findings is that dietary fatty acid composition can enhance the effectiveness of sEH inhibitors in cardiovascular diseases. Fish oil or ω-3 polyunsaturated fatty acid diet rich in eicosapentaenoic acid and docosahexaenoic acid coupled with sEH inhibitors lowers blood pressure and provides superior anti-inflammatory effects in angiotensin II–dependent hypertension. Eicosapentaenoic acid–derived epoxyeicosatetraenoic acids and docosahexaenoic acid–derived epoxydocosapentaenoic acids are of particular interest because these epoxygenase metabolites of ω-3 polyunsaturated fatty acid have been demonstrated to protect from coronary heart disease and atrial fibrillation. These newer findings suggest that other fatty acid epoxides could be beneficial and that sEH inhibitors still have promise for hypertension and kidney disease.

Significant recent advancements in the development of robust EET analogs that mimic the actions of endogenous EETs position them as a potential therapeutic for renal and cardiovascular diseases. First generation EET analogs were methyl esters and sulfonimide substitutions of the carboxylic
acids, which obviated esterification and resisted β-oxidation.59
The next generation of EET analogs removed the 1,4-diene
responsible for autooxidation and replaced the labile epoxide
with biososteres that resist metabolism (Figure 2).59,60 Studies
of the second generation of EET analogs assessing vascular
inflammation and dilation resulted in the following structural
requirements: an acidic carboxyl group, Δ8-olefin bond,
20-carbon chain length, and a cis epoxide.59,60
EET analogs have substantial promise for the treatment of
kidney and cardiovascular diseases. One such EET analog that
has been successfully used in vivo in rodents is the aspartic
amide of 11-nonyloxy-undec-8(Z)-enoic acid, NUDSA.61,62
NUDSA has been found to decrease blood pressure, improve
metabolic status in metabolic syndrome, and provide cardiolipase
in ischemic injury.63–65 Overall, the effects of NUDSA are linked to its ability to reduce inflammation
and cell death, supporting the notion that EET analogs could
be beneficial in renal pathologies. In support of this notion,
oral active EET analogs, EET-A and EET-B, were found to
protect the kidneys from cisplatin-induced nephrotoxicity.64
Attenuated nephrotoxicity correlated with reduced inflamma-
tion, oxidative stress, and decreased apoptosis through a reduction in Bcl-2 protein-mediated proapoptotic sig-
naling, reduced renal capase-12 expression, and reduced renal
caspase-3 activity.64 EET-A and EET-B have been shown to
decrease blood pressure and prevent hypertensive renal injury
dramatically.26,65 EET-A lowers blood pressure in angiotensin-
dependent hypertension and in Cyp2c44−/− mice with salt-sen-
sitive hypertension.66 Additional findings demonstrated that
EET-A inhibits ENaC activity in cultured cortical collecting
duct cells and reduced kidney expression of ENaC subunits
in angiotensin II hypertension.67 Interestingly, kidney protec-
tion in Dahl SS rats independent of blood pressure lowering
was demonstrated after 2 weeks of EET-B treatment. EET-B
decreased renal injury by reducing oxidative stress, endoplas-
mic reticulum stress, and macrophage infiltration.65 There are 2
potential explanations for the lack of blood pressure by EET-B
in the Dahl SS rats. First, EET-B does not inhibit ENaC in the
same manner as EET-A.65 Although EET-B treated Dahl SS
rats had decreased macrophage infiltration, EET-B failed to
lower kidney T-cell levels, which is known to be a major con-
tributor to the elevated blood pressure in this animal model of
salt-sensitive hypertension.67,68,69 Taken together, these diverse
biological actions and development of oral EET analogs dem-
onstrate their therapeutic potential for hypertension and CKD.
Perspectives
It is now established that a reduction in EETs can contribute to
hypertension and the associated renal injury and that approaches
to increase EETs have therapeutic potential. As with every ther-
apapeutic approach, there is always a downside that is of concern.
In the case of EETs, that concern has been their angiogenic and
tumorigenic actions.49,60,67 Although initial studies demonstrated
that EETs or sEH inhibition enhanced angiogenesis, tumorigen-
esis, and resulted in metastasis; recent studies have shown that
sEH inhibition or Ephx2 gene deficiency inhibits inflammatory
bowel tumor development and supports the notion that EETs
can inhibit cancer by blocking inflammation.68,69 Interestingly,
dual inhibition of cyclooxygenase-2 and sEH synergistically
inhibits primary tumor growth and metastasis by suppressing
tumor angiogenesis.70 EET analogs also failed to increase
cultured tumor cell proliferation and did not interfere with the
ability of cisplatin to kill tumor cells.64 Although these findings
do not eliminate the concern for unwanted tumorigenesis with
EET-based therapies, this concern seems to be considerably less
than originally thought.
Other considerations for blood pressure regulation and
hypertension are differences in sEH and EET levels between
males and females and central nervous system effects. Cerebral
vascular sEH expression is higher in male mice and females have increased EET-mediated protection from isch-
emic injury when compared with males.71,72 Furthermore, sEH
inhibition abolishes sex-specific differences in endothelial cell
survival and ischemic brain injury.71,72 Brain sEH inhibition
via intracerebroventricular delivery of AUDA (12-[(tricy-
clo[3.3.1.13,7]dec-1-ylamino)carbonyl]amino)-dodecanoic
acid) increases blood pressure and heart rate in spontaneously
hypertensive rats.73 In contrast, neuronal-specific expression
of sEH to increase activity 3-fold failed to increase arterial
blood pressure in mice.74 Sex differences have also been found
with regard to blood pressure regulation. Basal blood pressure
in Ephx2−/− mice was lower in males but not in females when
compared with wild-type mice.75 This decrease basal blood
pressure in male Ephx2−/− mice has not been observed when
other colonies on various genetic backgrounds were gener-
ated.76–78 More recently, renal vascular EET levels were higher
in female spontaneously hypertensive rats compared with
that in males.79 In this study, 10-day treatment with the sEH
inhibitor AUDA increased EET levels but did not lower blood
pressure in either male or female spontaneously hypertensive
rats.77 This finding is consistent with previous studies that
have found variable effects of sEH inhibition on blood pres-
sure in the spontaneously hypertensive rats.79 These experi-
mental findings highlight the need to consider brain actions of
EETS and sex-specific actions of EETS when evaluating sEH
inhibitors and EET analogs for hypertension and CKD.
The further development of EET analogs will be greatly
enhanced if protein targets and receptors for EETs can be
identified. Although the identity of EET-binding sites/recep-
tors remain elusive, EETs activate renal and coronary vas-
cular smooth muscle cell K_{Ca} channels through G protein
(Gqα)–dependent mechanism.8,10,26,27,76,79 Other investigations
provide evidence that cAMP and protein kinase A are key
signaling molecules required for K_{Ca} channel activation.26–28
Likewise, endothelial cell action of 11,12-EET are protein
kinase A–dependent and require the Gs protein.80 There are
also differences in potency and activity when comparing
11,12-EET and 14,15-EET in various vascular tissues.74,75
11,12-EET is more potent than 14,15-EET in renal arterioles,
whereas rat mesenteric resistance arteries respond similarly to
11,12-EET and 14,15-EET.7 In addition, mesenteric resistance
to flow-induced dilation was inhibited by the 14,15-EET
antagonist, 14,15-DHE5ZE, but unchanged by the 11,12-EET
antagonist and 11,12,20-THESZE.81 These findings suggest
unique biological activities and the potential for multiple vas-
cular EET-binding sites/receptors.
Recent studies on the contribution of EETs to inflammation,
kidney function, and blood pressure regulation in hypertension
have shed light on their potential as a target for therapeutic intervention. Thus, there is a bright future for sEH inhibitors and EET analogs as novel therapies to treat hypertension and stop the progression of CKD to renal failure effectively.

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**Disclosures**

Dr Imig has patents and patent applications that cover the composition of matter for epoxyeicosatetraenoic acid analogs.

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