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# 20-Hydroxyeicosatetraenoic Acid is a Potential Therapeutic Target in Cardiovascular Diseases

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**Abstract:** Arachidonic acid (AA) is metabolized by enzymes of the cytochrome P450 (CYP) 4A and CYP4F subfamilies to 20-hydroxyeicosatetraeonic acid (20-HETE), which plays an important role in the cardiovascular system. In the current work, we reviewed the formation of 20-HETE in different species by different CYPs; 20-HETE metabolism by cyclooxygenases (COXs) and different isomerases; and the current available inducers and inhibitors of 20-HETE formation in addition to its agonists and antagonists. Moreover we reviewed the negative role of 20-HETE in cardiac hypertrophy, cardiotoxicity, diabetic cardiomyopathy, and in ischemia/reperfusion (I/R) injury. Lastly, we reviewed the role of 20-HETE in different hypertension models such as the renin/angiotensin II model, Goldblatt model, spontaneously hypertensive rat model, androgen-induced model, slat- and deoxycorticosterone acetate (DOCA)-salt-induced models, and high fat diet model. 20-HETE can affect pro- and anti-hypertensive mechanisms dependent upon where, when, and by which isoform it has been produced. To the contrast to hypertension we also reviewed the role of 20-HETE in endotoxin-induced hypotension and the natriuretic effects of 20-HETE. Based on the recent studies, 20-HETE production and/or action might be a therapeutic target to protect against the initiation and progression of cardiovascular diseases.

**Keywords:** ??????????????????????????????.

## 1. INTRODUCTION

Arachidonic acid (AA), a major component of cell membranes, is currently known to be metabolized by three different pathways. The first pathway is mediated by cyclooxygenase (COX) to produce the prostaglandins (PGs). The second pathway is mediated by lipoxygenase (LOX) to produce mid chain hydroxyeicosatetraenoic acids (HETEs), lipoxins (LXs), and leukotrienes (LTs). Lastly, the third pathway is controlled by cytochrome P450s (CYPs) and divided into two different pathways, namely CYP epoxygenases and CYP ω-hydroxylases. CYP epoxygenases produce epoxyeicosatrienoic acids (EETs), while CYP ω-hydroxylases produce terminal HETE, named 20-HETE [1-2]. Metabolism of AA by CYP was first reported in 1981 [3], where CYP isoenzymes produced 20-HETE besides EETs which are further metabolized by soluble epoxide hydrolases (sEH) to their corresponding dihydroxyeicosatrienoic acids (DHETs) [4] (Fig. 1). The bioactivation of AA by CYPs was reported to be isoform and tissue-specific [5]. In this review we will focus on ω-hydroxylation product of AA, namely 20-HETE, and we will focus on the role of 20-HETE in different cardiovascular disease states.

## 2. Ω-HYDROXYLATION PRODUCT OF AA: 20-HETE

20-HETE, as an important CYP-mediated metabolite of AA, plays an important role as a second messenger in the regulation of vascular tone, renal function, cerebral blood flow and as a lung vasodilator, in addition to being considered a vascular oxygen sensor [6-8]. 20-HETE mediates the mitogenic actions of vasoactive agents and growth factors in many tissues and plays a significant role in angiogenesis [7-8]. Both mitogenic and angiogenic responses to 20-HETE were reported *in vitro* and *in vivo* [9-10]. 20-HETE plays an important role in the signal transduction processes underlying the development of pressure-dependent myogenic tone [11]. In addition, 20-HETE could bind to and activate peroxisome proliferator-activated receptor α ($PPAR_\alpha$) resulting in modulation of its target gene expression [12]. Inhibitors of 20-HETE were found to block the myogenic response of renal, cerebral, and skeletal muscle arterioles *in vitro*, autoregulation of renal and cerebral blood flow and tubuloglomerular feedback responses *in vivo*, and the vasoconstrictor response to elevations in tissue $PO_2$ both *in vivo* and *in vitro* [10, 13].

### 2.1. Formation of 20-HETE

CYP4As are generally considered the major AA ω-hydroxylases; however CYP4Fs have also been shown to catalyze the ω-hydroxylation of AA to form 20-HETE [14]. CYP1As and CYP2Es are also linked to 20-HETE formation but with a lesser degree [15]. In humans, CYP4As, CYP4Fs and CYP2U1 are responsible for 20-HETE formation [16-17], with CYP4F2 and CYP4A11 being the predominant isoforms in this process [18]. In rats, CYP4As, including CYP4A1, CYP4A2, CYP4A3 and CYP4A8 in the rat kidney, catalyze AA to produce 20-HETE [19]. CYP4F1 produces 20-HETE in abundance in the kidneys and the liver [20] besides CYP4F4 which also catalyzes the ω-hydroxylation of AA [14]. In mice, cyp4 family, including cyp4a10, cyp4a12, cyp4a14, cyp4b1, cyp4f14, cyp4f15 and cyp4f16 were found to play important role in the hydroxylation of AA [13]. In sheep, CYP2Js were found to be catalytically active towards AA forming 20-HETE as one of the main metabolites [21] (Table **1**).

### 2.2. Metabolism of 20-HETE

The range and diversity of 20-HETE activity seems to be derived from COX-dependent transformation of 20-HETE to products which affect vasomotor activity in addition to salt and water excretion [22]. COX metabolizes 20-HETE into a hydroxyl analogue of the vasoconstrictor prostaglandin (20-OH PG) $H_2$ that undergoes additional transformation by isomerases to produce the vasodilator/diuretic metabolites, 20-OH $PGE_2$ and 20-OH $PGI_2$, and the vasoconstrictor/antidiuretic metabolites, 20-OH $TXA_2$ and 20-OH $PGF_{2a}$ [3, 23-24] (Fig. **1**). 20-HETE is excreted as a glucuronide conjugate [25] as it is metabolized via glucuronidation by UGT1A1, UGT1A4, and UGT2B7 [26]. Glucuronidation may have a significant role in the modulation of 20-HETE availability for cellular processes [26].

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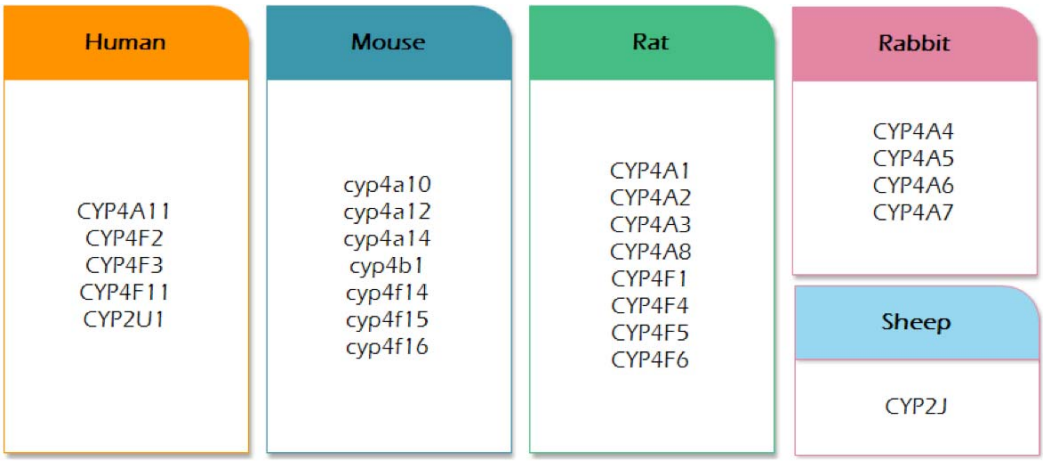
**Table 1. CYP Hydroxylases in Different Species. Adjusted from [13].**



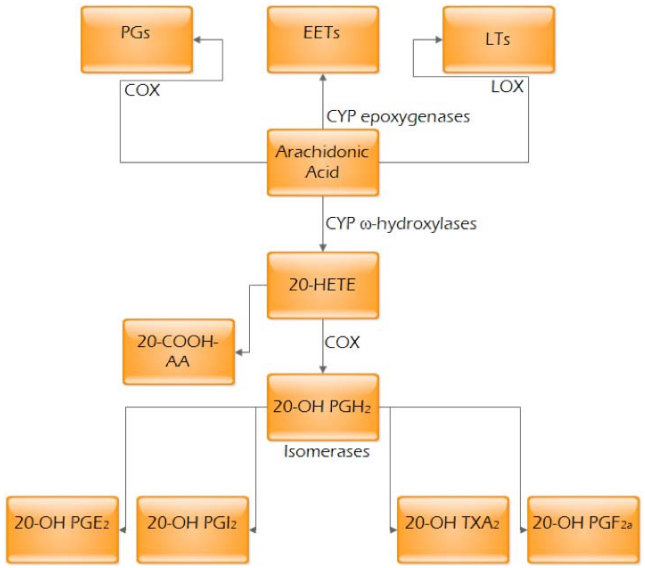




**Fig. (1).** AA metabolism by COX, LOX and CYP, with focus on 20-HETE. Adjusted from [3, 7, 23, 59].

### 2.3. Biological and Synthetic Modulators of 20-HETE Synthesis and Effects

A number of synthetic selective inhibitors that inhibit the synthesis of 20-HETE have been developed, including 17-octadecynoic acid (17-ODYA), N-methylsulfonyl-12,12-dibromododec-11-enamide (DDMS), dibromododec-11-enoic acid (DBDD), N-hydroxy-N'-(4-butyl-2methylphenyl)formamidine (HET0016), N-(3-Chloro-4-morpholin-4-yl)Phenyl-N'-hydroxyimido formamide (TS011), and acetylenic fatty acid sodium 10-undecynyl sulfate (10-SUYS) [14, 27-31]. Although HET0016 was evaluated as the first potent and selective inhibitor of 20-HETE synthase [32], it could also inhibit CYP2C9, CYP2D6, and CYP3A4 catalyzed reactions at high concentrations [33]. Also some papers reported that 17-ODYA could inhibit the formation of EETs and DHETs as well as 20-HETE [34-35]. Nonselective inhibitors of AA metabolism were also employed to inhibit 20-HETE formation such as 1-aminobenzotriazole (ABT) and cobalt (II) chloride ($CoCl_2$) [36-37]. More recently, the stable analogs 20-hydroxyeicosa-6(Z),15(Z)-dienoic acid (6,15,20-HEDE; WIT002) and 20-hydroxyeicosa-6(Z),15(Z)-dienoyl]glycine (6,15,20-HEDGE) were found to competitively antagonize the action of 20-HETE [28-30]. On the other hand, $PPAR_{\alpha}$ agonists (e.g. fibrates) or gene therapies were found to up-regulate the formation of 20-HETE. In addition, 20-HETE analogs, 20-hydroxyeicosa-5(Z),14(Z)-dienoic acid (5,14,20-HEDE; WIT003) and N-[20-hydroxyeicosa-5(Z),14(Z)-dienoyl]-glycine (5,14,20-HEDGE) were found to mimic the actions of 20-HETE [28, 30, 38-39] (Fig. **2**). Specificity of these chemicals depends on the dose/concentration used, for example high systemic HET0016 levels may inhibit not only 20-HETE synthesis, but also the ω-hydroxylation and inactivation of leukotriene B4. Moreover, high doses of 5,14-20-HEDE (mg/kg range) results in high systemic drug levels that acts not only during ischemia but also during reperfusion [40].

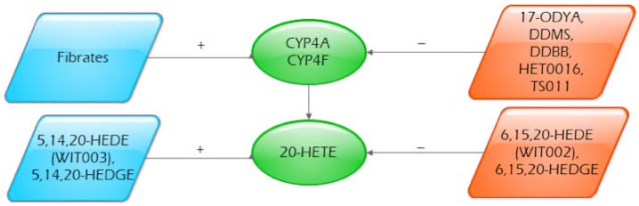



**Fig. (2).** Modulation of CYP-mediated 20-HETE synthesis and action. Adjusted from [28]

Biologically, angiotensin II (AngII), endothelin (ET) and norepinephrine stimulate 20-HETE formation in vascular smooth muscle (VSM) [10, 13, 41], whereas nitric oxide (NO) and carbon monoxide (CO) inhibited its formation [10, 13, 41-42]. NO binds to and inactivates CYP heme moiety providing a negative feedback control mechanism for this system [11]. As for CO, it desensitizes the VSM to constrictor agonists by interfering with the sensitizing influence of 20-HETE [43]. Also carbon dioxide ($CO_2$) was found to modulate CYP4As and control the levels of 20-HETE which mediate cerebrovascular reactivity in response to changes in partial pressure of $CO_2$ in the arterial blood ($PaCO_2$); $CO_2$ decreases the expression of brain CYP4As during hypercapnia while increasing its expression during hypocapnia [44]. Vasoconstriction associated with hemoglobin-based blood substitutes was supposed to be a result of 20-HETE stimulation by decreased NO production or increased $O_2$ consumption [45]. 20-HETE was also reported to be responsible for oxygen contraction in the systemic microvasculature [46]. Blockade of 20-HETE formation attenuates the vascular responses to AngII, endothelin, norepinephrine, NO, and CO [10, 13, 41]. Also CYP4As blockers were found to attenuate the vasoconstrictor response to elevations in tissue $PO_2$ [42]. Interestingly,

sesamin, the major lignan in sesame, is a 20-HETE synthesis inhibitor which could inhibit human renal and liver microsomes with selectivity towards CYP4F2 and reduced activity towards CYP4A11 [18]. A randomized controlled crossover trial on overweight men and women revealed that sesame supplementation resulted in a 28% decrease in plasma and a 32% decrease in urinary 20-HETE relative to control [18].

## 3. ROLE OF 20-HETE IN CARDIOVASCULAR SYSTEM

### 3.1. Role of 20-HETE in Cardiac Hypertrophy, Cardiotoxicity, and Diabetic Cardiomyopathy

Isoproterenol-induced cardiac hypertrophy alters AA metabolism and its associated CYP enzymes. Isoproterenol causes significant induction of CYP4A3 and significantly increases 20-HETE levels in the hypertrophied hearts suggesting an important role in the development and progression of cardiac hypertrophy [47]. Also, benzo(a)pyrene (BaP), an aryl hydrocarbon receptor (AhR) ligand, was found to induce cardiac hypertrophy which could be reversed by treatment with the ω-hydroxylase inhibitor, HET0016 [48]. Moreover, doxorubicin, a potent anti-neoplastic antibiotic, is well known for its dose-dependent cardiotoxicity which was found to be associated with high levels of 20-HETE and induced CYP ω-hydroxylases such as, CYP4A1, CYP4A3, CYP4F1, and CYP4F4 [49]. As for diabetic cardiomyopathy, 20-HETE seems to play an important role in its pathophysiology. Rats injected with streptozotocin, to induce diabetes, showed two fold increases in 20-HETE formation rate by heart microsomes when compared to control rats. Isolated perfused hearts from diabetic animals, subjected to 40 min of global ischemia followed by 30 min of reperfusion, showed three to five times lower cardiac function as compared to control hearts. However, pretreatment of the hearts with HET0016 for 30 min before ischemia/reperfusion (I/R) resulted in significant improvement in cardiac function recovery [50].

### 3.2. Role of 20-HETE in Cardiac I/R Injury

20-HETE produces detrimental effects in the heart during ischemia, and pro-inflammatory effects during reperfusion [51-52]. 20-HETE production was found to be increased during I/R, whereas inhibition of its production has been shown to reduce infarct size caused by ischemia. The molecular mechanism underlying 20-HETE action in cardiomyocytes is supposed to be through the stimulation of NADPH oxidase-derived superoxide production, which activates L-type $Ca^{2+}$ channels via a protein kinase C dependent mechanism [53-54]. A recent study investigating the direct effect of 20-HETE on cardiomyocytes revealed that 20-HETE could induce apoptosis by activation of several intrinsic apoptotic pathways which could contribute to the CYP ω-hydroxylase-dependent cardiac I/R injury [55]. In rats, 17-ODYA, DDMS or HET0016 administered 10 min prior to the 30 min of ischemia and 2 h of reperfusion significantly inhibited myocardial apoptosis [56]. In canines, myocardium subjected to 60 min of ischemia and 3 h of reperfusion was associated with higher production of 20-HETE. Inhibition of 20-HETE formation by the nonspecific CYP inhibitor, miconazole, or the specific CYP ω-hydroxylase inhibitors, 17-ODYA and DDMS resulted in profound reduction in myocardial infarct size [57-58]. The same was observed with 6,15,20-HEDE where it reduced myocardial infarct size and protected the canine heart from I/R injury [58]; whereas exogenous 20-HETE administration increased the infarct size significantly [57].

### 3.3. Role of 20-HETE in bleeding time determination and inflammation

In a murine model, subcutaneous infusion of 20-HETE shortened the tail bleeding time dramatically. Interestingly, oral administration of rofecoxib, a selective COX-2 inhibitor, for 3 months increased blood levels of 20-HETE more than 120 times which was correlated with a significantly shorter tail bleeding time. The dramatic increase in 20-HETE following rofecoxib administration was attributed to the inhibition of its metabolism and could be a partial explanation for the shortened bleeding time and adverse cardiovascular events associated with rofecoxib [59]. Interestingly, it was reported that 20-HETE exerts an inhibitory effects on COX-2 expression through PPARα mediated mechanism [60], suggesting that 20-HETE may attenuate expression of the enzymes responsible for its own metabolism. As for inflammation, intraperitoneal injection of lipopolysaccharide in Sprague Dawley (SD) rats was found to be associated with increased CYP ω-hydroxylases and 20-HETE suggesting an important role of 20-HETE in the development and progression of cardiovascular diseases by inflammation [61].

## 4. ROLE OF 20-HETE IN DIFFERENT MODELS OF HYPERTENSION

20-HETE displays potent and diverse biological activities, which can affect pro- and anti-hypertensive mechanisms dependent upon where, when, and by which isoform it has been produced [62]. 20-HETE is altered in genetic and experimental models of hypertension and contributes to the resetting of pressure-natriuresis and the development of different models of hypertension that will be discussed later in this review [63] (Fig. **3A** and **3B**).

### 4.1. Role of 20-HETE in the Vasculature

20-HETE, a potent vasoconstrictor produced by VSM cells, is considered a key regulator of vascular tone in the brain, kidney, heart, and splanchnic beds [10-11, 19, 27-28, 64]. 20-HETE depolarizes VSM by blocking the open-state probability of $Ca^{2+}$-activated $K^+$-channels ($K_{Ca}$) [10, 42]. Inhibition of the endogenous production of 20-HETE in renal and cerebral arterioles attenuates autoregulation of renal and cerebral blood flow *in vivo* [11]. In fact, 20-HETE plays a complex role in blood pressure regulation, where it promotes natriuresis in kidney tubules which could decrease the blood pressure, whereas in microvasculature, it has a vasopressor effect which could increase blood pressure [65]. This suggests that 20-HETE plays different roles in different models of hypertension based on its relative balance between its levels in blood vessels and renal tubules (Fig. **4**).

In the microcirculation, 20-HETE participates in the regulation of vascular tone by sensitizing the smooth muscle cells to constrictor stimuli and contributes to myogenic, mitogenic and angiogenic responses [65-66]. CYP4A inhibitors block the myogenic response of small arterioles to elevations in transmural pressure and autoregulation of renal and cerebral blood flow *in vivo* [42]. 20-HETE upregulation or augmentation could contribute to oxidative stress, endothelial dysfunction, or increased peripheral vascular resistance associated with some forms of hypertension, whereas its inhibition could protect from these adverse effects [28, 66-68]. Furthermore, 20-HETE promotes endothelial dysfunction by uncoupling endothelial NO synthase (eNOS), reducing NO bioavailability and stimulating $O_2^-$ production [65, 69-70]. eNOS uncoupling is mediated via the activation of tyrosine kinase, mitogen-activated protein kinase, and IkappaB kinase [71]. 20-HETE has been shown to induce endothelial angiotensin-converting enzyme (ACE), and thus activating the renin-angiotensin-aldosterone system [65]. 20-HETE modulates the growth of VSM cells, and its growth-inhibitory effect was reported to be mediated by transforming growth factor-beta (TGF-beta) [72]. Moreover, upregulation of the glomerular formation of TGF-beta was found to contribute to the development of proteinuria and glomerular injury through inhibition of the glomerular production of 20-HETE [73].

Injection of recombinant sense CYP4A1 cDNA in SD rats resulted in increased mean systolic pressure, whereas injection of the recombinant antisense CYP4A1 cDNA decreased mean systolic pressure with preferential activity of CYP4A1 in the kidneys at transcriptional and translational levels. This suggests that renal AA hydroxylases contributed to the formation of hypertension and


**Fig. (3).** Different animal models associated with low 20-HETE levels (**A**) and high 20-HETE levels (**B**).

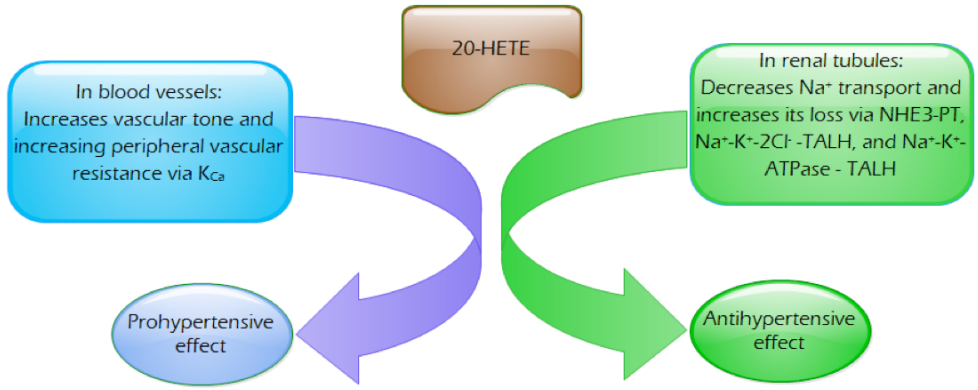



**Fig. (4).** 20-HETE role in regulation of blood pressure. sodium hydrogen exchanger-3 (NHE-3); proximal tubule (PT); sodium-potassium-2 chloride ($Na^+$-$K^+$-$2Cl^-$) cotransporter ; thick ascending limb of the loop of Henle (TAL) ; sodium potassium ATPase pump ($Na^+$-$K^+$-ATPase); $Ca^{2+}$-activated $K^+$-channels ($K_{Ca}$). Adopted from [28].

maintenance of blood pressure in normotensive rats [74]. Similarly, SD rats injected with a bolus of lentivirus construct expressing CYP4A2 suffered from increased blood pressure and proteinuria, whereas HET0016 treatment reduced blood pressure, plasma creatinine and proteinuria. Arteries from CYP4A2-transduced rats have increased levels of 20-HETE and superoxide anion as well as lower levels of eNOS and phosphorylated eNOS. In addition, these arteries displayed decreased relaxing responsiveness to acetylcholine, while HET0016 improved vascular function and increased the phosphorylated state of key proteins that regulate endothelial function, including eNOS [67]. Also intravenous injection of adenoviral vectors carrying the CYP4A2 construct to SD rats resulted in increased blood pressure and decreased relaxing responsiveness to acetylcholine in interlobular renal arteries, which was offset by treatment with DDMS, a 20-HETE synthesis inhibitor [66]. In a CYP4F2 transgenic mouse model, CYP4F2 was localized in the renal proximal tubule (PT) epithelia within the kidneys and was expressed at a high level leading to high urinary 20-HETE excretion which showed a positive correlation with the high systolic blood pressure [75]. A sex-specific effect, related to CYP4F2 polymorphisms and expression has been observed indicating that altered 20-HETE bioactivity underlay the excess of hypertension and associated vascular events observed in men [64].

### 4.2. Role of 20-HETE in renin/AngII System-Induced Hypertension

Altered production of 20-HETE and EETs is known to play an important role in the development of AngII-dependent hypertension and associated target organ damage [76]. 20-HETE induced ACE in endothelial cells, whereas 6,15,20-HEDE prevented this effect, revealing that 20-HETE dependent vascular dysfunction and hypertension are associated with the upregulation of the renin-angiotensin system [69]. Also, in acute and chronic elevations in circulating AngII levels, there was increased formation of 20-HETE in the kidneys and peripheral vasculature potentiating vasopressor effects of AngII [77]. Inhibition of 20-HETE formation was associated with altered responsiveness of systemic and renal vascular beds to AngII without modifying their responses to noradrenaline

[76]. *In vitro*, 20-HETE was the predominant product released into renal effluent in rat isolated kidneys stimulated with AngII [78]. Similarly, incubation of AngII with isolated preglomerular microvessels resulted in the production of 20-HETE [79]. AngII reduced the diameter of renal interlobular arteries, while blocking 20-HETE synthesis by 17-ODA increased the median effective dose ($ED_{50}$) for AngII-induced constriction by a factor of 15 and diminished the maximal response by 61% [77]. *In vivo*, graded intravenous infusion of AngII increased mean arterial pressure in rats dose-dependently while acute blockade of 20-HETE with DDMS attenuated the vasopressor response to AngII [77]. Similar results were obtained with chronic intravenous infusion of AngII (50 ng/kg/min) for 5 days with or without chronic blockade of 20-HETE synthesis with intravenous infusion of DDMS. Rats infused with AngII had higher 20-HETE urinary excretion which was inhibited by DDMS [77]. SD rats that received subcutaneous infusion of AngII (120 ng/kg/min) were found to have more than 2-fold higher microvascular 20-HETE release by the third day [79].

It has been previously demonstrated that AngII increases 20-HETE via the AT receptor, whereas ET-1 increases 20-HETE through the ET receptor [69, 80]. Paradoxically, ACE inhibitors have been also shown to increase the formation of 20-HETE. As such, SD rats treated with captopril or enalapril showed increased production of 20-HETE by renal cortical microsomes and in the outer medulla as well as CYP4A protein [81]. However, the ability of ACE inhibitors to induce renal formation of 20-HETE is probably not related to the blockade of formation and/or actions of AngII [81]. In C57/B6 mice, renal tubules were found to produce low levels of 20-HETE, however administering fenofibrate (90 mg/kg/day, IP) stimulated its production by more than 2-fold with a concomitant lowering of blood pressure in AngII (1000 ng/kg/min, subcutaneous infusion) dependent hypertension model. Fenofibrate induced cyp4A proteins in the kidneys but not in the renal vasculature [82]. In uninephrectomized rats, renal vascular reactivity to AngII was found to be increased significantly. AngII was found to induce greater renal vasoconstriction in Wistar rats after 4 weeks of uninephrectomy as compared to sham rats. Of interest, indomethacin enhanced renal vasoconstriction only in sham rats, whereas HET0016 reduced renal vasoconstriction only in uninephrectomized rats suggesting a role for 20-HETE [83]. AngII inhibits $HCO^{3-}$ absorption in the perfused medullary thick ascending limb via a signaling pathway involving 20-HETE production. It is thought that this process may play a role in the ability of the kidneys to regulate sodium balance and extracellular fluid volume independently of acid base balance [84].

Ren-2 renin transgenic rats (TGRs) is an AngII-dependent hypertension model in which 20-HETE and EETs plays a permissive role in the development of hypertension and hypertension associated end-organ damage [76]. TGRs have higher kidney CYP4A protein expression, ω-hydroxylase activity, and urine 20-HETE concentration but have significantly lower CYP2C23 protein expression, renal epoxygenase activity, and urine EETs concentration [37, 85]. TGRs treated with DDMS showed that inhibition of 20-HETE formation combined with the enhanced bioavailability of EETs could attenuate the development of hypertension, cardiac hypertrophy, proteinuria, glomerular hypertrophy, and sclerosis as well as renal tubulointerstitial injury [76]. ABT and $CoCl_2$ administration to young TGRs attenuated the development of hypertension and cardiac hypertrophy, as well as preventing glomerulosclerosis. ABT and $CoCl_2$ administration in adult TGRs decreased blood pressure, cardiac hypertrophy, but did not reduce glomerulosclerosis [37]. Of interest, intrarenal inhibition of 20-HETE in TGRs increased urinary excretion of sodium without altering renal hemodynamics, while in SD, inhibition of 20-HETE and EETs synthesis decreased sodium excretion [85]. Double transgenic rats (dTGRs), overexpressing human renin and angiotensinogen genes, have lower renal epoxygenase activity compared to non-transgenic rats. Treatment of dTGRs with fenofibrate strongly induced renal CYP2C23 protein, AA epoxygenase activity, and CYP4A protein levels with an increased tubular 20-HETE production, resulting in normalization of blood pressure, albuminuria, and protection against AngII-induced renal damage [86].

### 4.3. Role of 20-HETE in Goldblatt Hypertensive Rats

Goldblatt hypertensive rats have two models, two kidneys and one constriction model (2K1C), and one kidney and one constriction model (1K1C). In the 2K1C model, hypertension is maintained by a continuously activated renin-angiotensin system because pressure diuresis of the contralateral normal kidney prevents hypervolemia. To the contrast, volume retention by the single stenotic kidney of the 1K1C animal shuts off renin secretion, providing a model of low-renin, volume-dependent hypertension [87-88]. In 2K1C, bioavailability and urinary excretion of 20-HETE were increased while renal blood flow was decreased [89-90]. Of interest, intrarenal inhibition of 20-HETE by DDMS decreased sodium excretion in sham-operated rats but elicited increases in renal hemodynamics and sodium excretion in 2K1C rats [90]. Also, HET0016 increased renal blood flow but didn't provide blood pressure-lowering effects in this model most likely due to its anti-natriuretic effect [89]. As for 1K1C, CYP4A/20-HETE system contributes to arteriolar constriction in response to elevated $PO_2$ in the established stage of 1K1C renovascular hypertension [88].

### 4.4. Role of 20-HETE in Spontaneously Hypertensive Rats (SHR)

CYP content and activities are increased in the kidneys of SHRs as compared with those of normotensive control rats [91-93]. CYP hydroxylase activities were significantly higher in SHRs, whereas epoxygenase activity demonstrated no differences between SHRs and Wistar Kyoto (WKY) rats at different ages [92]. Increased expression of the CYP4A genes and 20-HETE production in the kidneys was considered an early event in the development of hypertension in SHRs [62, 94-97]. Some reports mentioned that deficiency of vascular CYP2E1-derived products, 19(R)-HETE and 18(R)-HETE, could participate in the amplification of 20-HETE sensitizing actions [98]. 20-HETE contributes to the elevated renal vascular tone in adult SHRs due to its action in renal vasculature rather than in renal cortical tissue [34]. Also, 20-HETE formation was found to be higher in the PT of SHR than WKY [99]. *In vitro*, examination of juxtamedullary nephron microvessels revealed that basal internal diameters of arcuate and interlobular arteries and proximal and distal afferent arterioles of the SHRs were 5-29% smaller than WKY rats. On the other hand, addition of the CYP inhibitor ketoconazole or 20-HETE synthesis inhibitor 17-ODYA increased the diameters of the preglomerular vasculature in this model [34].

Renal cortical CYP4A1 protein levels were higher in SHR compared to SD and WKY rats indicating that it might contribute to the increased cortical and proximal production of 20-HETE seen in 7 weeks old SHR [62]. CYP4A1 cDNA treatment increased mean systolic pressure in SD rats while anti-CYP4A1 treatment decreased mean systolic pressure in SHR 5 weeks after the injection and up to 24 weeks. CYP4A overexpression in sense-treated animals and inhibition in antisense-treated animals were preferential in the kidneys [19]. CYP4A1 antisense oligonucleotide reduced CYP4A-immunoreactive proteins along with 20-HETE synthesis in mesenteric arterial vessels [95]. Mesenteric arteries from these rats also exhibited decreased sensitivity to the constrictor action of phenylephrine, in addition to attenuation of myogenic constrictor responses to increased transmural pressure [95]. Also, CYP4A2 level in SHR was found to be much higher than that of normotensive rat [100]. CYP4A3 and CYP4A8 expressions were reported to be critical to the early changes in eicosanoid formation and renal function in the young SHR [96]. In addition to CYP4As, CYP4Fs produce 20-HETE; with CYP4F1 being the most critical

isoform of these isoenzymes in the SHR kidney [94]. Interestingly, cerebral production of 20-HETE increased whereas renal tubular production decreased in postmenopausal SHR, suggesting that 20-HETE contributes to the elevation of postmenopausal blood pressure. Administering ABT for 7 days, reduced blood pressure in postmenopausal rats but had no effect in young females, also acute intravenous infusion of HET0016 over 3 h reduced blood pressure in this model [36]. Moreover, drinking saline instead of water for 2 weeks decreased 20-HETE renal production by 45% in SHR and 22 % in Brown Norway rats, however mean arterial pressure rose in SHR and fell in Brown Norway rats after sodium intake was elevated [93].

Single dose of 10-SUYS caused an acute reduction in mean arterial blood pressure in 8 weeks old SHR which was associated with a decrease in urinary 20-HETE formation *in vivo* and attenuation of the vasoconstrictor response of renal interlobar arteries to AngII *in vitro* [27]. Similarly, single injection of ABT to 7 weeks old SHRs caused an acute reduction in mean arterial pressure which was associated with 70-85% inhibition of 20-HETE formation in the cortex and outer medulla [101]. ABT administration to SHR chronically treated with N-nitro-L-arginine methyl ester (L-NAME), NO synthesis inhibitor to further elevate mean arterial blood pressure, resulted in significant attenuation in blood pressure elevation. ABT significantly decreased L-NAME-mediated increase in urine volume and protein suggesting that inhibition of 20-HETE synthesis in hypertensive individuals with endothelial dysfunction and chronic NO deficiency could attenuate development of high blood pressure and end-organ damage [102]. DDMS was found to decrease sensitivity of arteries to phenylephrine, whereas 20-HETE reverses the desensitizing effect of DDMS with lower concentration in SHR (0.1 micromol/L) than WKY (10 micromol/L) [98].

SHR fed borage oil-enriched diet have lower systolic blood pressure due to enhanced synthesis of the vasodilatory EETs and decreased formation of the vasoconstrictive 20-HETE [103]. Of interest, inhibition of sEH in 8 to 12 weeks old female SHR was associated with profound decreases in renal 20-HETE, however it did not reduce blood pressure [104]. Single injection of cobalt protoporphyrin (50 mg/kg), an inducer of heme oxygenase-1 (HO-1), in 7-week-old SHR significantly increased HO-1 protein expression in the renal cortex and outer medulla with a 65% decrease in renal 20-HETE levels, with a concomitant decrease in blood pressure, suggesting an important role for HO-1 activity in the regulation of 20-HETE levels, urine volume, electrolyte excretion, and blood pressure [105]. Similarly, administration of heme arginate (15 mg/kg for 4 d), a potent inducer of HO-1, to 7 weeks old SHR resulted in a marked decrease in blood pressure with increased HO-1 activity in both hepatic and renal microsomes and decreased ω-hydroxylases activity in kidneys [91].

### 4.5. Role of 20-HETE in Androgen-Induced Hypertension

Men have a higher susceptibility to develop hypertension than age-matched premenopausal women indicating that androgen plays an important role in blood pressure regulation. Interestingly, postmenopausal women and women with polycystic ovary syndrome, both of which have increased endogenous androgen production, have elevated risks of hypertension [65, 70]. 5 alpha-dihydrotestosterone (DHT) treatment was found to increase blood pressure in both male and female rats [70]. In male rats renal interlobar arteries, the ratio between 20-HETE and EETs levels was 2-fold higher than female rats, however DHT treatment significantly increased this ratio by 85% and 230% in male and female rats, respectively, besides eliminating the difference in the ratio between both genders [70]. This data suggests that 20-HETE mediates the hypertension in rodents treated with androgen [65]. DHT increased blood pressure and 20-HETE production in the renal microvessels, but had no effect on urinary sodium excretion [106]. In addition, it increased the sensitivity of renal interlobar arteries to vasoconstriction induced via phenylephrine and decreased acetylcholine-induced vasorelaxation. Furthermore, 6,15,20-HEDE attenuated the effects associated with DHT in the renal interlobar arteries [107]. In SD rats treated with DHT, renal vascular 20-HETE levels increased by day 2 of treatment, whereas blood pressure elevation reached significance by day 3 [107]. Androgen effects were found to be associated with up-regulation in the kidney levels of CYP4A8, the primary enzyme responsible for 20-HETE synthesis in the renal vasculature, as well as down-regulating CYP2C23, the major epoxygenase in the kidney, resulting in increased vascular tone [70, 108-109]. Moreover, dissected renal microvessels, the target tissue for most of the pro-hypertensive actions of 20-HETE, showed an androgen-dependent up-regulation of vascular CYP4A8 mRNA and a 4-fold increase in 20-HETE synthase activity [108].

Treatment with DHT induced both 20-HETE production and cyp4a12a expression more than 4-fold in male C57BL/6 mice. cyp4a12a is the predominant 20-HETE synthase in the mouse kidney, and its expression determines the sex and strain specific differences in 20-HETE generation and may explain sex and strain differences in the susceptibility to hypertension and target organ damage. Female mice showed low AA hydroxylase activities and very low cyp4a12a mRNA and protein levels, but high cyp4a10 and cyp4a14 expression [110]. It was found that cyp4F2 transgenic mice treated with DHT suffered from elevated systolic blood pressure which was associated with strong induction of renal cyp4F2, renal AA ω-hydroxylation and urinary 20-HETE excretion, and decreased EETs levels. 20-HETE/EETs ratio in the urine and kidney homogenate were significantly elevated contributing to the androgen aggravated hypertension; however HET0016 was able to completely eliminate these androgen-induced effects [111]. Interestingly, androgen plays an important role in hypertension development in SHR model. Male SHR have plasma free-testosterone levels twice as high as male WKY rats in addition to higher renal production of 20-HETE. Castration in addition to treatment with the androgen receptor antagonist, flutamide, reduced blood pressure, renal production of 20-HETE and CYP4A protein levels in both strains, however castrated SHR had significantly lower 20-HETE renal production than castrated WKY [109]. 20-HETE may play a role in adrenocorticotrophic hormone (ACTH)-induced but not dexamethasone-induced hypertension. As such, it was found that HET0016 prevents and reverses ACTH-induced but not dexamethasone-induced hypertension. Interestingly, ACTH, but not dexamethasone, increased renal microsomal 20-HETE formation [112].

### 4.6. Role of 20-HETE in Deoxycorticosterone Acetate (DOCA)-Salt, Salt-Dependent and Dahl Salt-Sensitive (Dahl S) Models of Hypertension

Wild-type mice treated with DOCA-salt had higher mean arterial pressure and higher cumulative sodium balance, but lower renal 20-HETE production than did vehicle-treated mice. However, treatment of DOCA-salt mice with clofibrate (500 mg/kg/day in corn oil) attenuated the increase in mean arterial pressure and cumulative sodium balance while increasing 20-HETE production and renal tubular cyp4a expression [113]. Similarly, fenofibrate (500mg/kg/day) significantly reduced blood pressure and sodium retention during DOCA-salt hypertension by inducing cyp4A, increasing 20-HETE production, and attenuating renal expression of COX-2 [114]. DOCA-salt treatment was found to increase excretion of ET-1 with associated 4-fold increase in 20-HETE excretion, whereas $CoCl_2$ lowered the increase in their levels by 58% and 72%, respectively [115]. $CoCl_2$ also was found to prevent the effects associated with DOCA-salt in uninephrectomized rats such as hypertension, organ hypertrophy, and renal injury [115].

20-HETE is known to produce vasoconstricting effect on blood vessels, whereas it inhibits the renal tubular sodium transport resulting in an important role in salt excretion regulation [116-117]. In

human, salt-sensitivity of blood pressure in essential hypertension may result from impairment of a natriuretic mechanism dependent on 20-HETE [116]. In rats, 20-HETE excretion was elevated when the rats were switched from low to high salt diet, also 20-HETE formation in kidney homogenates was elevated by 30% and epoxygenase activity doubled when rats were switched to high salt diet [118]. It was found that animals that are unable to increase renal 20-HETE formation do not excrete sodium and are prone to hypertension [117]. Adult rats given saline showed increased renal expression of CYP4A and CYP2C, while excreting more 20-HETE and remained normotensive. In contrast, young rats given saline showed no induction, and even reduced CYP4A and CYP2C, decreased urinary 20-HETE excretion, and retained sodium resulting in increased blood pressure. Clofibrate increased renal 20-HETE excretion and prevented sodium retention while lowered blood pressure in saline-treated young rats [117].

It was also demonstrated that chronic blockade of 20-HETE formation promotes the development of salt-sensitive hypertension in rats. In this regard, blocking 20-HETE formation using ABT reduced blood pressure in rats fed a low salt (0.4% NaCl) diet, but blood pressure rose by 20 mm Hg after these rats were switched to a high salt (8% NaCl) diet for 10 days [118]. Similarly, HET0016 had no effect on blood pressure in rats fed a low salt diet; however blood pressure was elevated by 18 mm Hg after feeding the rats with high salt diet [118]. Chronic treatment with ABT and HET0016 inhibited the renal formation of 20-HETE by approximately 90% and decreased its excretion by 68% to 85% [118]. In Lewis rats that were fed high salt diet, it was found that inhibiting renal 20-HETE formation produced hypertension [119-120]. Uninephrectomized Lewis rats that were fed high salt diet and received chronic direct infusion of 17-ODYA (400 pmol/min) into the outer medulla of the left kidney, showed reduced formation of 20-HETE by 70% in the outer medulla while having no effect in renal cortex. The lower medullary levels of 20-HETE was associated with increased mean arterial blood pressure after 5 days of infusion [120].

High sodium intake results in increased NO synthase (NOS) activity and higher production of the vasodilator NO which could modulate the formation of CYP metabolites by binding to heme moiety resulting in inhibition and limitation of CYP impact on blood pressure. However, this NO-mediated protection disappears after prolonged sodium intake, where high sodium intake seems also to be responsible for increased activity of CYP enzymes enhancing the synthesis of 20-HETE in the arteries of skeletal muscles leading to an increase in total peripheral resistance and mean arterial blood pressure [121]. However, long-lasting high sodium intake lowers NO bioavailability and promotes systemic and intrarenal vasoconstrictor activity of 20-HETE [121]. In addition to NO, ET-B receptor activation has relationship with the maintenance of CYP4A protein expression in the kidneys of rats fed a high-salt diet [122]. ET-B receptor blockade in male and female SD rats caused salt-dependent hypertension which could be reduced by chronic clofibrate treatment. Clofibrate by itself increases renal cortical and medullary CYP4A protein and 20-HETE synthesis in the kidneys of rats on high, whereas it had no effect on mean arterial blood pressure under normal salt conditions [122]. Interestingly, 20-HETE was found to modulate the natriuretic response to furosemide, whereas impaired natriuresis in salt-sensitive subjects involves a mechanism that alters the 20-HETE response to furosemide and is linked to salt-sensitive blood pressure [123-124].

Dahl S rats require a higher renal perfusion pressure to excrete the same amount of sodium and water as normotensive rats due to elevated $Cl^-$ transport in the thick ascending limb of the loop of Henle (TAL) [119]. Perfusion of the loop of Henle of Dahl S rats with exogenous 20-HETE normalized the elevated loop $Cl^-$ transport [119, 125]. ω-Hydroxylases activities were lower in 3-5 weeks old Dahl S than Dahl salt-resistant rats [126]. Deficiency in 20-HETE formation in the outer medulla of the kidney combined with the overexpression of renal outer medullary $K^+$ channel (ROMK) and sodium-potassium-2 chloride (Na+-K+-2Cl-) co-transporter in the thick ascending limb may predispose Dahl S rats fed a high-salt diet to $Na^+$ retention and hypertension [116, 119-120, 125, 127-129]. Diminished production of 20-HETE in the outer medulla was a result of 3-fold reduction in the level of CYP4A2 protein [129], however, induction of renal CYP4A activity with clofibrate prevented the development of hypertension in this model [119, 130]. Similarly, fenofibrate (95 mg/kg/day for 7 days) was found to prevent the development of hypertension and reduced subsequent glomerular injury in Dahl S rats due to the increased production of renal 20-HETE with resulting natriuresis [130]. Transfer of chromosome 5 from the Brown Norway rat or Lewis rat onto the Dahl S genetic background increases the renal expression of CYP4A protein and the production of 20-HETE which contributes to the pressure-natriuresis resulting in antihypertensive and renoprotective effects [131-133]. Superoxide was reported to inhibit the synthesis of 20-HETE by renal cortical microsomes and enhance breakdown of 20-HETE to a more polar product, suggesting that superoxide has a role in Dahl S hypertension. Addition of Tempol, as an antioxidant therapy, (1 mmol/L) to the drinking water of Dahl S rats fed with high-salt diet reduced mean arterial blood pressure and severity of renal damage while increased 20-HETE excretion and creatinine clearance. Interestingly, HET0016 (10 mg/kg/day) blunted the antihypertensive and renoprotective effects of Tempol [134].

### 4.7. Role of 20-HETE in High-Fat Diet-Induced Model of Hypertension

High fat diet caused sodium retention, hypertension and increased renal plasma flow, and glomerular filtration rate (GFR) but no significant change was observed in renal vascular resistance [135]. These changes were associated with reduction in renal CYP4A1 and CYP4A8 expression, increased alpha-subunit of the epithelial sodium channel, beta-subunit of the epithelial sodium channel, sodium hydrogen exchanger-3 (NHE-3), and ROMK expression in renal tubules. Clofibrate (240 mg/kg/day) induced CYP4A expression in PT and in TAL but not in renal microvessels, resulting in attenuation of cumulative sodium balance and decreased blood pressure and GFR [135]. Similarly, SD rats fed high fat diet for 10 weeks revealed downregulation in CYP4A/CYP2C23, and decreased renal ω-hydroxylases and epoxygenases activity in cortex, medulla, and papilla, with no significant changes in the renal microvessels [136].

### 4.8. Effect of Polymorphism on 20-HETE Production and Development of Hypertension

Several population-based studies have reported ethnic differences in the prevalence of hypertension, with the highest prevalence in African populations [137]. Interestingly, polymorphisms of the CYP4A11 and CYP4F2 genes have opposite effects on 20-HETE acid excretion [138]. For example in CYP4A11 gene, a thymidine (T)-to-cytosine (C) missense transition at nucleotide 8590 results in a non-synonymous phenylalanine-to-serine amino acid substitution at residue 434 [138]. This CYP4A11 polymorphism reduces catalytic activity by >50% *in vitro* [138]. C carriers compared with T had diminished 20-HETE responses to salt loading [123]; and was associated with a reduction in 20-HETE excretion [138]. Furthermore, some reports indicated that CYP4A11 polymorphism is associated with increased population risk for hypertension [123, 137], however, others stated that it was not associated with blood pressure [138]. As for CYP4F2 gene, a guanine-to-adenine missense transition at nucleotide 1347 results in a non-synonymous valine-to-methionine amino acid substitution at residue 433 [138]. This CYP4F2 polymorphism is associated with the size of arterial wave reflections in Chinese males and individuals with a faster pulse rate [139]. It was also associated with increased 20-HETE excretion and

systolic blood pressure [138], however, *in vitro* it was associated with a reduction in 20- HETE acid production and the reason for this discrepancy remains at large [138].

### 4.9. Role of 20-HETE in Endotoxin-Induced Hypotension

Endotoxic shock is a systemic inflammatory response [38] that induces vascular hyporeactivity and hypotension resulting in multiple organ failure [38-39, 140]. Administration of lipopolysaccharide (10 mg/kg, IP) in male Wistar rats decreased mean arterial blood pressure by 31 mmHg and increase heart rate by 90 beats/min with associated increase in 6-keto-prostaglandin $F_{1\alpha}$, $PGE_2$, $TxB_2$, and nitrite in the serum, kidney, heart, thoracic aorta, and superior mesenteric artery, while systemic and renal 20-HETE and $PGF_{2a}$ levels were decreased [39, 141]. Prostanoids produced during endotoxaemia increased inducible NO synthase (iNOS) protein expression and NO synthesis, and decreased CYP4A1 protein expression and activity [39, 141]. Endotoxic shock is also characterized by decreased expression of constitutive COX-1, CYP4A and eNOS [140]. Dual inhibition of iNOS and COX with a selective or non-selective COX-2 inhibitor restores blood pressure presumably due to a decrease in the expression and activity of COX-2 and iNOS associated with an increase in CYP4A1 expression and 20-HETE synthesis [39, 140-141]. Prevention of hypotension during rat endotoxaemia by a selective COX-2 inhibitor, NS-398, was associated with an increase in 20-HETE and $PGF_{2a}$ levels and with a decrease in the production of $PGI_2$ , $PGE_2$ , and $TxA_2$ , and NO synthesis [141-142]. Administration of a synthetic analog of 20-HETE, 5,14,20-HEDGE prevented the effects associated with endotoxin, whereas the competitive antagonist 6,15,20-HEDE, prevented the effects of 5,14,20-HEDGE on the endotoxin-induced changes in systemic and renal levels of different prostanoids and 20-HETE [38-39, 143].

### 4.10. Natriuretic Effect of 20-HETE

In kidneys, 20-HETE inhibits $Na^+$ transport in the PT and TAL; deficiencies in the renal formation of 20-HETE contributes to sodium retention and development of some salt-sensitive forms of hypertension [28]. 20-HETE has renoprotective actions and opposes the effects of TGF- beta to promote proteinuria and renal end organ damage in hypertension [28]. In addition it also plays a permissive role in the natriuretic response to dopamine [144]. As such, infusion of dopamine (1.5µg/kg/min) in SD rats increased urine flow, sodium excretion, fractional sodium excretion, and proximal and distal delivery of sodium, whereas ABT or HET0016 administration reduced the response to dopamine by 65% [144]. 20-HETE modulates the pressure-natriuretic response [145]. For example the increased renal perfusion pressure resulted in increased renal interstitial pressure, urine flow, 20-HETE levels in renal cortical tissue, and sodium excretion [145-146]. 20-HETE contributes to pressure natriuresis by inhibiting $Na^+$-$K^+$-ATPase activity and promoting internalization of NHE-3 protein from the brush border of the PT [145-146]. The previously mentioned effects were prevented by removal of the renal capsule or by administration of ABT or HET0016 [145-146]. Mice deficient in circadian clock or lacking the circadian transcriptional activator clock gene exhibited significant decrease in the renal and urinary content of 20-HETE, and dramatic changes in the circadian rhythm of renal sodium excretion affecting blood pressure [147]. The renal medullary ET-1 system plays an important role in the control of sodium excretion and arterial pressure through the activation of renal medullary ET-B receptors. It was found that elevation of salt intake activated ET-B receptor within the renal medulla and increased renal medullary production of 20-HETE resulting in a natriuretic effect. Blockade of ET-B receptors resulted in salt sensitive hypertension by reducing renal medullary production of 20-HETE [148]. Although NO, 20-HETE, and EETs contribute to blood pressure and kidney function control, the role of 20-HETE and EETs becomes crucial only under conditions of high sodium intake or after NOS inhibition [149].

## 5. SUMMARY, CONCLUSIONS, AND FUTURE DIRECTIONS

It is largely accepted that the formation of 20-HETE is governed by CYP ω-hydroxylases. Of these hydroxylases, members of the CYP4 family are currently the ones that have proven to be mainly responsible for the production of 20-HETE. However, and more importantly, two processes, the synthesis and the degradation govern the steady state level of 20-HETE in a given organ. As such the enzymatic pathways responsible for its degradation is an interesting point to be investigated in the future. In the current review, various models of cardiovascular disease states such as cardiac hypertrophy, I/R injuries, diabetic cardiomyopathy, and drug-induced cardiotoxicity as well as different models of hypertension were reviewed.

In general, an increment in 20-HETE levels is rather detrimental than beneficial to the cardiovascular system with few exceptions that were model-dependent such as its beneficial effect in salt-dependent and DOCA salt hypertension models. In fact, apparent contradictory findings on the pro- and antihypertensive roles of 20-HETE has been largely resolved recognizing that vascular overproduction of 20-HETE induces vasoconstriction, endothelial dysfunction, and hypertension, whereas tubular deficiency of 20-HETE impairs salt excretion and thus also causes hypertension. Blockade of 20-HETE formation reduces blood pressure and improves renal function in SHR, androgen hypertensive rats as well as, AngII infused rats. In contrast, induction of 20-HETE synthesis improves pressure-natriuresis and lowers blood pressure in Dahl S rats [40, 63].

Targeting 20-HETE synthesis and/or action in heart and vasculature by the use of different synthesis inhibitors or antagonistic analogues might serve as a potential therapeutic strategy in the future to protect the cardiovascular system from 20-HETE detrimental effects. However, an interesting challenge rises with respect to the specificity of these inhibitors and/or analogues towards 20-HETE production in the heart and vasculature without affecting other vital organs. This is because although 20-HETE has detrimental effects in the heart and vasculature it does however produce beneficial effects in other organs.

In any pathophysiological disease state a relationship between cause and effect should be established. Unfortunately, with the current literature very few studies, if any, have directly examined the effect of the direct administration of 20-HETE on the development and progression of various cardiovascular disease states. However, and due to the presence of different difficulties, the direct use of 20-HETE in *in vivo* studies is hindered. These difficulties could be summarized in the following two points: 1- there is no well-established dosing regimen for 20-HETE as there is only one study that infused 20-HETE to mice in the dose of 250 ng/hour [59]. Therefore, knowledge of the pharmacokinetic profile of 20-HETE is required; 2- there is currently no proof of whether or not the action of 20-HETE is within its producing organ or its effect could be radiated outside its producing organ. Thus, administering 20-HETE systemically might not be as effective as inducing its synthesis in a specific organ. Although the previously mentioned difficulties are only applicable for *in vivo* studies, very limited study addressed the direct effect of 20-HETE *in vitro*. Therefore, it would be of interest to examine the direct effect of 20-HETE on cardiotoxicity, apoptosis, hypertrophy, and hypoxia/reoxygenation using cultured cardiomyocytes or cardiac cell lines such as the rat derived cardiomyoblasts, H9c2 cells, and the murine atrial cardiomyocytes, HL-1 cells.

With the results of these future directed projects, we will have a better understanding regarding the direct effect of 20-HETE and its potential targets in the heart and thus providing better prognosis and therapeutic targets for patients with cardiovascular diseases.

## CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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Declared none.

## ABBREVIATIONS

| | | |
|---|---|---|
| 10-SUYS | = | acetylenic fatty acid sodium 10-undecynyl sulfate |
| 17-ODYA | = | 17-octadecynoic acid |
| 1K1C | = | one kidney and one constriction model |
| 20-OH PG | = | 20-hydroxyprostaglandin |
| 2K1C | = | two kidneys and one constriction model |
| 5,14,20-HEDE; WIT003 | = | 20-hydroxyeicosa-5(Z),14(Z)-dienoic acid |
| 5,14,20-HEDGE | = | N-[20-hydroxyeicosa-5(Z),14(Z)-dienoyl]glycine |
| 6,15,20-HEDE; WIT002 | = | 20-hydroxyeicosa-6(Z),15(Z)-dienoic acid |
| 6,15,20-HEDGE | = | 20-hydroxyeicosa-6(Z),15(Z)-dienoyl]glycine |
| AA | = | Arachidonic acid |
| ABT | = | 1-aminobenzotriazole |
| ACE | = | angiotensin-converting enzyme |
| AhR | = | aryl hydrocarbon receptor |
| AngII | = | angiotensin II |
| BaP | = | benzo(a)pyrene |
| CO | = | carbon monoxide |
| CO2 | = | carbon dioxide |
| CoCl2 | = | cobalt (II) chloride |
| COX | = | cyclooxygenase |
| CYPs | = | cytochrome P450s |
| Dahl S | = | Dahl salt-sensitive |
| DBDD | = | dibromododec-11-enoic acid |
| DDMS | = | N-methylsulfonyl-12,12-dibromododec-11-enamide |
| DHETs | = | dihydroxyeicosatrienoic acids |
| DHT | = | 5 alpha-dihydrotestosterone |
| DOCA | = | deoxycorticosterone acetate |
| dTGRs | = | Double transgenic rats |
| ED50 | = | median effective dose |
| EETs | = | epoxyeicosatrienoic acids |
| ET | = | endothelin |
| GFR | = | glomerular filtration rate |
| HET0016 | = | N-hydroxy-N'-(4-butyl-2methylphenyl)formamidine |
| HETEs | = | hydroxyeicosatetraenoic acids |
| HO-1 | = | heme oxygenase-1 |
| I/R | = | ischemia-reperfusion |
| iNOS | = | inducible NO synthase |
| $K_{Ca}$ | = | $Ca^{2+}$-activated $K^+$-channels |
| L-NAME | = | N-nitro-L-arginine methyl ester |
| LOX | = | lipoxygenase |
| LTs | = | leukotrienes |
| LXs | = | lipoxins |
| $Na^+$-$K^+$-$2Cl^-$ | = | sodium-potassium-2 chloride co-transporter |
| $Na^+$-$K^+$-ATPase | = | sodium potassium ATPase pump |
| NHE-3 | = | sodium hydrogen exchanger-3 |
| NO | = | nitric oxide |
| NOS | = | NO synthase |
| PaCO2 | = | partial pressure of carbon dioxide in the arterial blood |
| PGs | = | prostaglandins |
| PPARα | = | peroxisome proliferator-activated receptor α |
| PT | = | proximal tubule |
| ROMK | = | renal outer medullary K+ channel |
| SD | = | Sprague Dawley |
| sEH | = | soluble epoxide hydrolases |
| SHR | = | spontaneously hypertensive rats |
| TAL | = | thick ascending limb of the loop of Henle |
| TGF-beta | = | Transforming growth factor-beta |
| TGRs | = | Ren-2 renin transgenic rats |
| TS011 | = | N-(3-Chloro-4-morpholin-4-yl)Phenyl-N'-hydroxyimido formamide |
| VSM | = | vascular smooth muscle |
| WKY | = | Wistar Kyoto rats |

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