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Unraveling the role of 12- and 20-HETE in cardiac pathophysiology: G-protein coupled receptors, pharmacological inhibitors and transgenic approaches.

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Abstract

Arachidonic acid-derived lipid mediators play crucial roles in the development and progression of cardiovascular diseases. Eicosanoid metabolites generated by lipoxygenases and cytochrome P450 enzymes produce several classes of molecules, including the epoxyeicosatrienoic acid (EET) and hydroxyeicosatetraenoic acids (HETE) family of bioactive lipids. In general, the cardioprotective effects of EETs have been documented across a number of cardiac diseases. In contrast, members of the HETE family have been shown to contribute to the pathogenesis of ischemic cardiac disease, maladaptive cardiac hypertrophy and heart failure. The net effect of 12(*S*)- and 20-HETE depends upon the relative amounts generated, ratio of HETEs/EETs produced, timing of synthesis, as well as cellular and subcellular mechanisms activated by each respective metabolite. HETEs are synthesized by and affect multiple cell types within the myocardium. Moreover, cytochrome P450- (CYP) and lipoxygenase- (LOX) derived metabolites have been shown to directly influence cardiac myocyte growth and the regulation of cardiac fibroblasts. The mechanistic data uncovered thus far has employed the use of enzyme inhibitors, HETE antagonists and the genetic manipulation of lipid-producing enzymes and their respective receptors, all of which influence a complex network of outcomes that complicate data interpretation. This review will summarize and integrate recent findings on the role of 12(*S*)-/20-HETE in cardiac diseases.

Introduction

Heart Failure (HF) remains as a leading cause of morbidity and mortality worldwide. A 2019 report from the American Heart Association estimates a 40–46% increase in global HF prevalence in the next decade. The overall annual cost of HF continues to rise in the US and is projected to reach $69.8 billion[1] by 2030.

HF results from a combination of myocyte death, adverse structural remodeling and progressive contractile dysfunction. Coronary artery disease, hypertension and diabetes all contribute to HF development and progression. Despite recent advances in diagnosis, therapeutic intervention and pharmacotherapy, the prevalence of HF continues to rise, in part due to the aging population and increased incidence of type 2 diabetes, ischemic heart

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disease and metabolic syndrome. A common underlying etiology of these conditions is a pro-inflammatory environment that initiates progressive, maladaptive structural remodeling and systolic/diastolic dysfunction. Recent evidence indicates a causative role of arachidonic acid-derived hydroxyeicosatetraenoic acid (HETE) metabolites in cardiovascular disease progression. This review will focus on their role in cardiac/cardiovascular function and the cellular pathways influenced by these bioactive lipids that culminate in disease activation and progression.

Overview of Arachidonic Acid Metabolism

The metabolic pathways of arachidonic acid (5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenoic acid, AA) are well characterized within the cardiovascular system. AA is cleaved from membrane phospholipids by members of the Phospholipase A2 family. Three major enzymatic pathways metabolize AA to form a variety of biologically active metabolites. Cyclooxygenases (COX-1 and COX-2) produce prostaglandins, thromboxanes and prostacyclins, which have multiple functions including immune surveillance, inflammation, and the regulation of vascular tone and permeability[2]. Lipoxygenases catalyze the formation of hydroperoxide intermediates that are reduced by glutathione peroxidase to form mid-chain hydroxyeicosatetraenoic acid (HETE), lipoxins and leukotrienes[3]. Finally, the cytochrome P450 (CYP) family of enzymes convert AA to a number of cardiovascular mediators. CYPs are a superfamily of NADPH-dependent, heme-containing monooxygenases that metabolize a number of endogenous compounds, steroids and drugs. The CYP450 epoxygenases (e.g. CYP2J2, CYP2C9) convert AA to epoxyeicosatrienoic acids (EETs) while CYP450 hydroxylases (CYP4A, CYP4B and CYP4F) produce HETEs through hydroxylation (e.g. 20-hydroxyeicosatetraenoic acid (20-HETE)) or allylic oxidation (e.g. 12*S*-hydroxy-5*Z*,8*Z*,10*E*,14*Z*-eicosatetraenoic acid (12(*S*)-HETE)).

Hydroxyeicosatetraenoic Acid Synthesis in the Heart

Multiple cell types in the heart synthesize and respond to HETEs, including cardiac myocytes and fibroblasts, infiltrating immune cells (neutrophils and macrophages), coronary artery endothelial and vascular smooth muscle cells, and autonomic nerves[4–6]. This heterogeneity dictates the spatiotemporal pattern of eicosanoid production and the types and amounts of eicosanoids that are generated, which often complicates the interpretation of each mediator as to whether it acts independently or in concert with other members of arachidonic acid metabolites. For example, while several EETs are considered to be anti-inflammatory and cardioprotective, various HETEs are considered to be pro-inflammatory and cardiotoxic.

12-hydroxy-5*Z*,8*Z*,10*E*,14*Z*-eicosatetraenoic acid (12-HETE),12-HETE Synthesis Inhibitors and the Heart.

There are several enzymes implicated in cardiac 12-HETE production. 12-HETE is produced primarily by the12-lipoxygenase enzyme. The 12-lipoxygenase is encoded by the *alox12* gene and catalyzes the stereoselective dioxygenation of arachidonic acid into 12(*S*)-hydroperoxyicosa-5,8,10,14-tetraenoic acid (12-HpETE), which is quickly reduced by

glutathione peroxidase in the cell to form 12(*S*)-HETE[3]. Originally identified as a platelet lipoxygenase, 12-LOX is also expressed in cardiac myocytes[7] and cardiac fibroblasts[8, 9]. Aside from platelets, cardiac myocytes and fibroblasts, 12-LOX can also be found in human islets, adipose tissue, kidneys, macrophages, neutrophils, skin and smooth muscle[10]. Many of the early inhibitors of 12-LOX, including nordihydroguaiaretic acid (NDGA), and 5,6,7-trihydroxyflavone (baicalein) acted as redox inhibitors that blocked oxidation of the non-heme iron in the catalytic site[3]. However, since all lipoxygenases require non-heme oxidation, these inhibitors are non-selective. Fatty acid analogs, such as 5,8,11,14-eicosatetraynoic acid (ETYA), potently block the generation of12(*S*)-HETE but also lack selectivity[3]. Work by the Holman group[11] sought to discover potent and selective 12-LOX inhibitors, identifying several compounds and key attributes to these inhibitors. Additional studies in this field uncovered several selective and potent 12-LOX inhibitors including, ML355[12, 13] and the 12/15-LOX inhibitors ML351[14], 99089[15], and LOXblock-1[16].

The 12/15-lipoxygenase (ALOX15) also produces 12(*S*)- and 15(*S*)-HETE, but the relative proportion of HETEs generated is species specific. In humans, 12/15-LOX produces 10% 12(*S*)-HETE vs 90% 15(*S*)-HETE while in mice the enzyme produces 90% 12(*S)*-HETE vs 10% 15(*S*)-HETE[17]. Due to these species differences, results of studies in mice may overestimate the causative role of 12/15-LOX-derived 12(*S*)-HETE production in cardiac disease as 12/15-LOX is expressed in the heart and is upregulated in HF[18]. 12/15-LOX is also expressed in adipose tissue[19] and across several infiltrating cells including macrophages and neutrophils[20], In fact, the observed changes in 12-HETE across tissues under pathological conditions may be elevated by the mobilization and presence of these infiltrating cells. The benzothiopyranoindole compound (PD-146176) is a potent inhibitor of human 15-LOX[21] and has been shown to inhibit atherosclerosis in hypercholesterolemic rabbits[22].

12-HETE is also produced in the heart by cytochrome P450-dependent mechanisms. CYP1A1 and CYP1B1, both capable of forming mid-chain HETEs (12, 13 or 15-HETE) via lipoxygenase like activity[23], also produce EETs and ω-terminal HETEs[24]. Within the myocardium, CYP1B1 is expressed across coronary blood vessels and cardiac myocytes. CYP1B1 exhibits low cardiac basal expression but is rapidly induced by pressure overload and hypertrophic agonists[25–28] and by hypoxia[29]. CYP1B1 is also involved in the metabolism of sex steroids. Within the cardiovascular system, CYP1B1 protects against angiotensin II (Ang II)-induced hypertension and associated cardiovascular changes in female mice, most likely due to the metabolism of 17-β estradiol into hydroxyestradiols (OHE), especially 2-OHE[30]. Conversely, 6β-hydroxytestosterone, a CYP1B1 metabolite of testosterone, contributes to angiotensin II-induced hypertension in male mice[31].

Although there are at least 4 classes of CYP1B1 inhibitors, the stilbene 2,4,3′,5′-Tetramethoxystilbene (TMS) is the most often used inhibitor across cardiac studies[30, 32–34]. Given the multiple byproducts of CYP1B1 enzymatic activity, one must use caution in interpreting these data with regards to establishing the direct involvement of 12-HETE. Genetic manipulation of CYP1B1 with siRNA or targeted gene deletion have been used to validate a role for the CYP1B1–12-HETE pathway in cardiovascular disease but these

strategies also cannot delineate between steroid metabolism, ROS production and 12-HETE formation[31, 33, 35, 36].

12(*S*)-HETE-mediated Signaling and Receptors.

Recently, a role for G-protein coupled receptors in 12(*S*)-HETE and 20-HETE signaling has been reported. 12-HETE appears to mediate its effects through several receptors, including the low-affinity leukotriene B_4 (BLT2) receptor, since it was shown to compete for radiolabeled leukotriene B4 binding[37]. In addition, 12(*S*)- and 12(*R*)-HETE have also been shown to influence the thromboxane A_2 (TP) receptor as competitive inhibitors that promote the relaxation of mouse mesenteric arteries[38]. Studies by Guo et al.[39] uncovered the orphan receptor GPR31 as a 12(*S*)-HETE receptor (12(*S*)-HETER1) with high binding affinity using the PC3 human prostate cancer cell line[39, 40]. GPR31 belongs to the rhodopsin-like group A subfamily of receptors, coupling to Gα(i) in the G_i/G_o family. In PC3 cells 12-*S*-HETE binding was shown to activate a MEK, ERK1/2 and NF-kB signaling cascade (Figure 1)[39]. Interestingly, GPR31 is also activated by protons[41] and lactic acid[42], further increasing the complex nature of GPR31-mediated receptor signaling and activation. Secondly 12(*S*)-HETE activates a vast array of signaling programs across various cell types with conflicting results. In particular, when looking at 12(*S*)-HETE's effects across the vasculature there are several differences. Prior to the identification of GPR31 as a 12(*S*)-HETER1, 12(*S*)-HETE was shown to be a competitive antagonist of the PGH2/TXA2 thromboxane receptor[43]. While the 12(*S*)-HETER1 was discovered in the context of cancer cell biology, within the vasculature, 12(*S*)-HETE has been observed to mediate increases in endothelial-dependent vasodilation in porcine and human coronary vessels as well as in rat resistant vessels[44–48]. This effect primarily occurs through the activation of large conductance K channels (BK_{Ca}) located across smooth muscle cells[44] as well as through the inhibition of thromboxane receptor-mediated signaling[38]. Conflicting reports suggest that 12(*S*)-HETE serves as a potent vasoconstrictor as exposure to 12(*S*)-HETE constricts isolated dog arcuate arteries[49]. Another study suggests that 12(*S*)-HETE modulates intracellular actions of Ang II in cultured rat vascular smooth muscle cells (VSMC)[50], enhancing Ang II signaling. There are currently no recent reports to suggest the precise mechanisms activated by the interaction of 12(*S*)-HETE and GPR31 on VSMC or the vasodilatory/constrictive effects of 12(*S*)-HETE/GPR31 occurring across the vasculature. In fact, one report suggests that the aorta and mesenteric arteries of mice are devoid of GPR31[38].

Subsequent studies following the identification of the 12(*S*)-HETER1 have identified 12(*S*)-HETE as a mediator of various other signaling cascades including several observations in lymph-endothelial cells, including the release of calcium that occurs in concert with the activation of a RHO-ROCK-MYPT driven signaling cascade that activates myosin light chain-2 (MLC2) (Figure 1)[51, 52]. This particular pathway has been shown to be important for fibroblasts mechanosignaling and in fibroblast transformation to myofibroblasts[53]. The overexpression of 12-LOX is illustrated to be mitogenic for cardiac fibroblasts[9]. Moreover, in lymph-endothelial cells, 12(*S*)-HETE induces the expression of SRY-related HMG-box 18 (SOX18) and prospero homeobox protein 1 (PROX1), two potent influencers of endothelial cells development[54]. In pulmonary artery endothelial cells (PAEC), 12(*S*)-HETE promotes PAEC survival through the activation of a phosphatidylinositol 3-kinase

(PI3K)/Akt-dependent pathway[55]. Interestingly, under levels of high glucose, endothelial cells exposed to 12(*S*)-HETE have dramatic elevations in P-Iκbα, P-P65, ICAM-1 and VCAM-1 alongside impairments in vascular endothelial permeability[56]. Recent reports place the 12-LOX/ 12(*S*)-HETE/GPR31 signaling pathway at the forefront of thrombosis, demonstrating that the 12(S)-HETE/GPR31 pairing enhances the activation of human platelets and thrombosis observed in mouse carotid artery injury models[57].

12-HETE: Antagonists

Currently there are no clearly defined biological or synthetic 12-HETE antagonists. DUP 654 (2-benzyl-1-naphthol), is a potent 5-lipoxygenase inhibitor and has been evaluated as an anti-inflammatory agent[58, 59]. At one point it was characterized and referred to as a 12(*S*)-HETE receptor antagonist in a human epidermal cell line when the 12(*S*)-HETER1 had yet to be identified[60]. Further understanding and characterization of the 12(*S*)-HETE/ GPR31 interaction and its relationship with other receptors may serve beneficial for the development of new 12(*S*)-HETE antagonist and novel pharmacological tools to disrupt or augment 12(*S*)-HETE's bioactions.

20- hydroxyeicosatetraenoic acid (20-HETE) Synthesis in the Heart

The major pathway for 20-HETE production is the ω-hydroxylation of arachidonic acid by the CYP4A and CYP4F sub-families. In mice, CYP4a12a is the primary 20-HETE synthase while CYP4A1,2,3 and CYP4F1 and CYP4F2 produce 20-HETEs in rodents (see Waldman et al.[61] for a comprehensive review). In humans, CYP4A11 and CYP4F2 are the predominant 20-HETE synthases although CYP4F11 and CYP4F3 have limited capacity to produce 20-HETE. The CYP4 enzymes share significant sequence homology and catalytic properties but exhibit distinct tissue distribution and regulation. These enzymes are expressed within the coronary vasculature and cardiac myocytes but not in cardiac fibroblasts[61–63]. Additionally, CYP4A is subject to transcriptional regulation by nuclear receptors, including peroxisomal proliferator-activated receptor α (PPARα) and the androgen receptor (AR)[64–67], and post-translational regulation by microRNAs[63, 68]. Angiotensin II (Ang II) increases CYP4A isoform expression in the kidney[69, 70] and the vasculature[71].

20-HETE-mediated Signaling and Receptors.

20-HETE is a potent vasoactive eicosanoid and elevations in 20-HETE are associated with the onset and progression of various pathologies including myocardial infarction, stroke and hypertension. Most of our current understanding of 20-HETE actions in the cardiovascular system stems from mechanistic studies in endothelial cells and vascular smooth muscle cells. Moreover, 20-HETE activates a wide array of signaling pathways that promote pro-inflammatory and pro-hypertensive signaling programs. In this section we will highlight key findings from these cell types.

Work by Garcia et al. identified the orphan G-protein coupled receptor (GPCR), GPR75, to be a high affinity 20-HETE receptor (20HR)[72]. The characterization of the 20HR included

the use of click chemistry compounds, ligand binding assays, proteomics, bioinformatics, immunoprecipitation and gene silencing in both *in vitro* human endothelial cell experiments and *in vivo* mouse models knocking down GPR75[72, 73]. Previous reports examining 20-HETE's bioactions across the vasculature have clearly identified 20-HETE to be a potent uncoupler of endothelial nitric oxide synthase (eNOS), reducing nitric oxide (NO) bioavailability and a strong inducer of endothelial angiotensin converting enzyme (ACE), increasing circulating angiotensin II levels[74, 75]. These 20-HETE mediated effects rely on an EGFR-MAPK-IKKβ-NFκB-dependent signaling pathway that requires the presence and activation of the 20HR (GPR75) (Figure 2)[72, 76, 77]. More recently GPR40 (also known as (free fatty acid receptor-1 (FFAR-1)) was reported to bind 20-HETE with low affinity and promote glucose-stimulated insulin secretion[78]. It is noteworthy to mention that GPR40 also binds to epoxyeicosatrienoic acids with a similar low affinity and lacks ligand selectivity[79].

Interestingly, other reports have identified an interaction between the chemokine CCL5/RANTES and GPR75, suggesting that CCL5 drives calcium influx[80, 81] and can stimulate insulin secretion from pancreatic islets[82] through GPR75. However, these studies did not provide evidence of direct binding experiments and CCL5/RANTES interaction failed to show receptor activation in repeated β-arrestin recruitment assays[83, 84]. Further studies are necessary to more fully characterize CCL5/RANTES binding to GPR75 and whether it acts as an antagonist for 20-HETE binding.

20-HETE is intimately associated with the promotion of inflammatory signals including the production and release of superoxide/reactive oxygen species (ROS), adhesion molecules and cytokines. Studies by Guo et al. illustrated 20-HETE's potential to stimulate superoxide formation in endothelial cells, an effect that stimulates vascular endothelial growth factor (VEGF) synthesis and promotes cell proliferation[85, 86]. In addition to driving the production of ROS, 20-HETE promotes the expression of adhesion molecules including intracellular cell adhesion molecule 1 (ICAM-1)[87]. In fact, during the identification of this finding, it was also observed that increases in the cytokine IL-6 were also 20-HETE mediated, a finding that was corroborated in transgenic mice expressing CYP4F2 specifically in endothelial cells (Tie2-CYP4F2-Tr)[87, 88].

In vascular smooth muscle cells (VSMC), 20-HETE activates constrictor stimuli and is also involved in the increased sensitization and enhanced responsiveness of vessels to constrictor stimuli[63]. This is accomplished through a coordinated interplay between kinases, channels and changes in calcium. Previous reports identified the activation of protein kinase C (PKC), mitogen-activated protein kinases (MAPK), tyrosine kinase and Rho kinase as the signal cascades that culminate in the phosphorylation and inhibition of the Ca^{2+}-activated K^+ channels (BK_{ca})[63, 89–91]. This results in VSMC depolarization and elevation in cytosolic [Ca^{2+}] through increased Ca^{2+} entry via the L-type Ca^{2+} channels[92, 93]. The 20-HETE-GPR75 pairing initiates a signaling cascade that involves conventional G protein ($G\alpha_{q/11}$) mediated changes alongside the promotion of a GIT1-mediated PKC-stimulated phosphorylation of MaxiKβ (BK_{ca} channel β subunit) that facilitates vasoconstriction[72]. Moreover, studies show 20-HETE to be a mediator of the transient receptor potential (TRP), activating TRPC6 (a non-voltage-gated Ca^{2+} entry/depolarization channel)[94] and transient receptor potential vanilloid 1 (TRPV1) (a nonselective cation channel)[95], increasing

Ca^{2+} mobilization and furthering 20-HETE's pro-vasocontrictor effects[96]. In addition to influencing channels, 20-HETE increases the Ca^{2+} sensitivity of the contractile apparatus through the activation of Rho kinase and subsequent phosphorylation of myosin light chain (MLC20)[63, 97]. Aside from influencing signals that promote vasoconstriction, 20-HETE has also been shown to elicit increases in VSMC mitochondrial superoxide production and may promote a secretory phenotype through the activation of a MAPK1-Elk-1-dependent pathway[98].

Several of these observed signaling pathways are also involved in cardiac myocyte hypertrophy/apoptosis and cardiac-fibroblast dependent extracellular matrix remodeling. Further studies are necessary to elucidate the role of 20-HETE and the 20HR in this context.

20-HETE: Synthesis Inhibitors and Antagonists

One aspect that has truly benefited investigators exploring 20-HETE's role across various pathologies has been the consistent development and characterization of 20-HETE synthesis inhibitors and 20-HETE antagonists. N-hydroxy-N'-(4-butyl-2-methylphenyl)-formamidine (HET0016)[99] and dibromo-dodecenyl-methylsulfimide (*DDMS*)[100] have served as potent 20-HETE synthesis inhibitors across various studies[101, 102]. 20-HETE antagonist compounds such as 20-hydroxyeicosa-6(*Z*),15(*Z*)-dienoic acid (20-HEDE) and *N*-[20-hydroxyeicosa-6(*Z*),15(*Z*)-dienoyl]glycine (20-HEDGE)[77] have served as critical tools in the study of 20-HETE biology, preventing many of 20-HETE's bioactions across the vasculature, including helping to elucidate several of 20-HETE's blood pressure-independent effects[72, 77]. Recent developments by Falck and colleagues[103–106] have provided investigators with new 20-HETE antagonists with increased water solubility. [2,5,8,11,14,17-hexaoxanonadecan-19-yl 20-hydroxyicosa-6(*Z*),15*(Z)*-dienoate] (20-SOLA), was the first water soluble 20-HETE antagonist to be synthesized, showing great efficacy at ameliorating the hypertension and renal injury associated with a diabetic mouse model that display dramatic elevations in circulating 20-HETE levels[103].

HETEs and Cardiac Pathophysiology

Heart disease encompasses a group of disorders involving the myocardium and coronary vasculature. Coronary artery disease, hypertension and diabetes are leading causes of heart disease that contribute to both ischemic (myocardial infarction) and non-ischemic (hypertrophy, fibrosis) heart disease resulting in myocardial infarction, contractile dysfunction and adverse structural remodeling[107]. Based on the cells activated by, and signaling pathways involved, 12(*S*)-HETE and 20-HETE exert their cardiac effects through direct and indirect mechanisms influencing the coronary circulation, inflammation, cardiac myocytes and cardiac fibroblasts.

Myocardial Infarction and Ischemia/reperfusion injury.

Progressive coronary artery disease involves chronic inflammation that often results in unstable atherosclerotic plaques that undergo abrupt rupture and thrombus formation. This results in coronary arterial obstruction, diminished blood flow and resultant ischemia and myocyte necrosis[108]. Cardiomyocyte death triggers the activation of innate immune system

and a surge in inflammatory responses, which can be sub-divided into an acute phase (1–3 days), a resolution phase (3–14 days) and a remodeling phase. Multiple lipid mediators have been implicated in these stages and inflammatory responses. Human cardiac ischemia and MI studies examining changes in eicosanoid levels identified elevations in 12-HETE and 20-HETE in diseased patients compared to patients without cardiovascular events[109, 110]. Their effects are often accentuated or modified in the presence of chronic hypertension and or Type II diabetes. For example, 12(*S*)-HETE has been implicated in the pathogenesis of atherosclerosis[111] and diabetes[112], with studies showing strong correlations between 12(*S*)-HETE and Type 2 diabetic patients with and without coronary artery disease (CAD)[113]. A direct link between 12(*S*)-HETE and Ang II has also been made in the context of diabetes wherein hyperglycemia increases renal Ang II levels that promote subsequent 12(*S*)-HETE production through the activation of the AT_1 receptor[114]. This interplay between 12(*S*)-HETE and the components of RAS may prove vital for identifying 12(*S*)-HETE's full contribution to cardiovascular related pathologies. Further evidence is necessary to better dissect these relationships and also determine how the 12(*S*)-HETER1 (GRP31) plays a role under these conditions.

Similarly, 20-HETE levels have been correlated with MI, and a recent study looking at a male cohort of patients undergoing carotid endarterectomy revealed 20-HETE to be a significant metabolite associated with carotid atheroma plaque when compared to healthy subjects[115]. This study also identified positive correlations between 20-HETE, body mass index and diastolic blood pressure in these patients[115]. This human study suggests a potential role for 20-HETE in the development and progression of ischemic heart disease, although the spatiotemporal patterns for 20-HETE expression have not been fully defined. Such studies would necessitate continuous sampling of plasma 20-HETE levels that could be linked to cardiac performance, additional biomarkers or structural indicators.

12(S)-HETE, Coronary Artery Disease, Myocardial infarction, and post-infarct remodeling.

12(*S*)-HETE has context-specific effects within the myocardium. For example, 12(*S*)-HETE exerts a cardioprotective effect during hypoxia-induced preconditioning via TRPVI-dependent vasodilation[116], but is deleterious during long-term remodeling following myocardial infarction[117]. 12-HETE contributes to sustained inflammation and increased cardiomyocyte death, elevating pro-inflammatory markers such as MCP-1 and IL-6, resulting in monocyte and neutrophil recruitment during the acute phase post MI[118, 119]. Elevations in 12-HETE have been observed in patients 24–40 hours post-MI[120]. 12/15-LOX appears to be the major source of 12(*S*)-HETE since both inhibition and genetic manipulation of 12/15-LOX has been shown to alter cardiac function and inflammation post MI. For example, Kayama et al.[18] reported that 12/15-LOX overexpression in mice resulted in increased 12(*S*)-HETE levels, macrophage infiltration and systolic dysfunction compared to wild-type controls. Recent work from Kain et al.[117] indicated that genetic deletion of 12/15-LOX improved post-MI survival and LV function following permanent coronary artery ligation[117]. Less is known about the role of CYP1B1 derived 12-HETE in myocardial ischemic injury.

Mechanistically, 12-HETE induces cardiac apoptosis during the acute and chronic phases of myocardial infarction and HF. Early reports by Nazarewicz et al.[121] indicated that 12-HETE stimulates mitochondrial NO synthase activity, resulting in dissipation of the mitochondrial transmembrane potential, cytochrome c release and apoptosis. In a series of studies, Gross and colleagues demonstrated that distinct phospholipase A_2 isoforms are localized to mitochondria and provide AA that is subsequently metabolized to produce local eicosanoids (12-HETE, 20-HETE, 14,15-EET)[122]. Under non-pathological conditions, cPLA2ζ is the predominant isoform, and AA is shunted into a CYP-dependent pathway that favors the generation of cardioprotective EETS. However, in mouse models of cardiac ischemia/reperfusion (I/R) injury or in cardiac tissue from HF patients, $iPLA_2\gamma$ is the predominant isoform and AA are shunted into the CYP- and 12-LOX pathways to favor the release of cardiotoxic 12-HETE and 20-HETE, resulting in pathologic mPTP opening, mitochondrial swelling, cytochrome c release and apoptosis[122]. These studies underscore the contribution of subcellular compartmentalization and disease-induced changes in synthetic enzymes in determining the role of HETEs in heart failure progression following ischemia/reperfusion injury.

Conversely, several studies demonstrated a potential cardioprotective role for 12-LOX-derived 12(*S*)-HETE in myocardial ischemia reperfusion injury. Gabel et al.[123] used isolated heart preparations from 12-LOX deficient mice to examine the effects of 12-HETE on preconditioning-induced cardioprotection. Their results suggested that preconditioning decreases cardiac necrosis and improves post-ischemic recovery via a 12-HETE-dependent mechanism[123]. These effects are likely mediated by transient receptor potential vanilloid 1 (TRPV1) activation in a PKC-dependent manner[116]. A potential caveat to both studies is that isolated hearts were used and these preparations do not measure the impact of infiltrating 12-HETE releasing immune cells that occurs *in vivo* upon reperfusion of an ischemic myocardium. It is possible that the amount of 12-HETE differs between invading immune cells and cells of the cardiovascular system.

20-HETE: Coronary Artery Disease, Myocardial infarction, and post-infarct remodeling

Extensive data implicate 20-HETEs as a potent mediator in the development and progression of hypertension, coronary artery disease and ischemic cardiomyocyte injury. The role of 20-HETE in coronary artery disease and hypertension was briefly touched upon in previous sections, but the reader is directed to several excellent review articles on these topics[63, 91]. This section will focus on 20-HETE and myocardial injury.

In humans, 20-HETE has been associated with increased incidences of myocardial infarction (MI)[124], an observation that has been dissected further through various studies. In animal models, the heart contains the CYP4A and 4F isoforms necessary for 20-HETE synthesis[63, 106, 125, 126]. Gross and colleagues reported that 60 min of ischemia followed by a 60 min reperfusion period resulted in a significant increase in 20-HETE levels in coronary venous plasma[127]. 20-HETE synthesis inhibitors 17-ODYA and DMMS reduced 20-HETE levels that correlated with a marked reduction in myocardial infarct size[126, 128]. Studies using the 20-HETE antagonist 20-HEDE confirmed that 20-HETE exacerbates cardiac injury[129]. Moreover, studies in rodents have implicated a role for CYP4A-20-HETE

in ischemia (40 min)/reperfusion (30 min) in diabetic rats. Interestingly, pretreatment with HET0016 resulted in significant improvement in cardiac function in the hearts obtained from diabetic but not in control rats, while an inhibitor of soluble epoxide hydrolase, improved cardiac functional recovery in both control and diabetic animals[130]. This was one of the first studies to suggest that the overall ratio of EET/HETE dictates the degree of ischemia-reperfusion injury.

The site of 20-HETE synthesis and release during ischemia/reperfusion may also account for the observed deleterious effects. For example, rats subjected to a repetitive ischemia protocol revealed marked increases in 20-HETE production across ischemic collateral-dependent zones (CZ) of the heart as opposed to nonischemic zones (NZ)[106]. Interestingly these elevations in 20-HETE across the CZ were exacerbated in a rat model of metabolic syndrome (JCR:LA-cp, JCR), corroborating studies that correlate 20-HETE with metabolic syndrome parameters such hypertension[72], insulin signaling/resistance[131] and obesity[131–133]. Further studies in JCR rats demonstrated that elevations in 20-HETE promote large artery stiffness and decreased arterial compliance as a consequence of increased elastin degradation, MMP12 activation and pronounced systolic hypertension[134]. 20-SOLA has also proven to be effective with regards to the heart as it can promote the restoration of coronary collateral growth (CCG) after ischemic injury, preventing endothelial dysfunction and apoptosis[106].

At the cellular level, 20-HETE is involved in mediating cardiac myocyte apoptosis. Early reports indicate that the addition of 20-HETE to neonatal cardiac myocytes resulted in dissipation of the mitochondrial membrane potential, increased the expression of pro-apoptotic Bax and caspase 3 activity[135]. Additional studies by this group[136] reported that 20-HETE mediated Ang II-induced apoptosis occurs via the regulation of the mitochondrial permeability transition pore and increased production of reactive oxygen species (ROS). 20-HETE also participates in β-adrenergic induced cardiac myocyte apoptosis through a calmodulin dependent kinase pathway[137]. Moreover, increased advanced glycation end products in diabetes induced- HF promotes cardiac myocyte apoptosis through a 20-HETE-dependent activation of the NADPH oxidase-2 isoform[138].

Heart Failure (HF).

Chronic responses to I/R injury and hemodynamic overload involves a complex interplay of inflammation, mechanical stress and hormone that result in pathological hypertrophy and remodeling of the extracellular matrix[139]. HETEs have both indirect and direct actions on HF pathogenesis. The indirect actions are mediated by their roles in the promotion of hypertension and coronary artery disease[140–142]. Myocardial changes in expression of CYP and LOX enzymes and subsequent changes in metabolite generation are associated with the pathogenesis of cardiac hypertrophy, fibrosis and HF via direct effects on cardiac myocyte growth and the regulation of cardiac fibroblast phenotypes[143, 144].

The role of 12-HETE in cardiac remodeling has been well established. Increased 12-HETE-levels were observed in response to pressure overload hypertrophy[26] and in animal models of HF[145, 146]. 12-HETE has been shown to play an important role in Ang II and isoproterenol (ISO) induced cardiac myocyte hypertrophy. Several studies using the

human cardiomyocyte RL-14 cells from El-Kadi's group demonstrated that pharmacological inhibition of CYP1B1 with TMS or genetic knockdown with CYP1B1-siRNA prevented ISO-induced increases in cell volume and pro-hypertrophic gene expression[147]. In this model, 12-HETE increased superoxide production and ERK1/2 and NF-kB signaling[33]. The same pathways appear to be regulated by 12-HETE in response to Ang II-induced hypertrophy[148, 149]. Interestingly, resveratrol has been shown to prevent the Ang II-mediated increases in cardiac myocyte hypertrophy, which correlated with a reduction in CYP1B1 protein expression[149]. 12-HETE derived from 12/15-LOX regulate cardiac fibroblast growth transformation to a myofibroblast phenotype and resultant fibrosis[143, 150]. Thus, 12-HETE plays a significant role in extracellular matrix remodeling and myocyte hypertrophy that drives HF progression.

Little is known about the role of 20-HETE in mediating pathological remodeling mechanisms of the heart. Elevated cardiac 20-HETE levels have been observed during Ang II-induced cardiac hypertrophy[151]. 20-HETE's ability to increase ACE expression[76] may likely be involved in the amplification of hypertrophic and fibrotic signaling within the myocardium as previously mentioned. Support for this notion comes from a recent study showing that N-disodium succinate-20-hydroxyeicosa-6(Z),15(Z)-diencarboxamide (AAA), another water soluble 20-HETE antagonist, attenuated the development of cardiac hypertrophy in Cyp1a1-Ren-2 transgenic rats, a model of ANG II-dependent malignant hypertension[104]. Consequently, future transgenic and pharmacological approaches targeting GPR75 are warranted to address the role of 20-HETE in pathological remodeling of the heart.

Future Directions

As the eicosanoid field continues to explore and expand its understanding of the etiology of HF and concurrent cardiovascular diseases, it is highly likely that it will begin to uncover new signaling mediators and novel therapeutic targets. Presently, data suggest that both 12(*S*)- and 20-HETE are key mediators influencing various signaling mechanisms that promote and contribute to HF progression. These studies rely heavily upon pharmacological inhibitors and transgenic deletion/overexpression of enzymes and receptors. Although these approaches have served as invaluable tools in the assessment and characterization of HETE's bioactions across a multitude of biological conditions and scenarios, they are not without limitations. Pharmacological inhibitors of biosynthetic enzymes often result in changes across several eicosanoids and could impact the overall ratios of EET/HETEs. As discussed throughout the review, there are several exciting observations with regards to newfound lipid-receptor interactions. It will be vital to understand whether or not these lipids exert conventional "unbiased" or "biased" ligand signals and the ramifications of these cascades. This concept is particularly important as the preservation of unique signaling mechanisms may provide a benefit to the myocardium. The exploration of biased agonism has yielded a host of new therapeutically relevant compounds including the AT_1R-β-arrestin biased ligand TRV120027 which targets the AT_1R and favors reductions in blood pressure and increases in cardiac performance[152].

The chemical and functional diversity of cardiac eicosanoids requires new systems-based biological approaches, from analytical methods to sophisticated computational approaches to model lipid metabolism and lipid-receptor interactions. The identification of signaling nodes as well as multivariate data analytics of lipidomic profiles will allow investigators to capture metabolic snapshots at different stages of heart disease development and progression[153]. Likewise, mechanistic characterization of lipid metabolite-GPCR interactions could lead to the generation of novel therapeutics that target specific receptors to treat cardiovascular diseases.

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Non-standard Abbreviations and Acronyms:

EET	Epoxyeicosatrienoic acid
HETE	Hydroxyeicosatetraenoic acids
CYP	Cytochrome P450
LOX	Lipoxygenase
HF	Heart Failure
AA	Arachidonic Acid
COX	Cyclooxygenases
12(S)-HETE	12*S*-hydroxy-5*Z*,8*Z*,10*E*,14*Z*-eicosatetraenoic acid
20-HETE	20-hydroxy-5,8,11,14-eicosatetraenoic acid
GPCR	G-protein Coupled Receptor
NO	Nitric Oxide
ACE	Angiotensin Converting Enzyme
AAA	N-disodium succinate-20-hydroxyeicosa-6(*Z*),15(*Z*)-diencarboxamide
MI	Myocardial infarction

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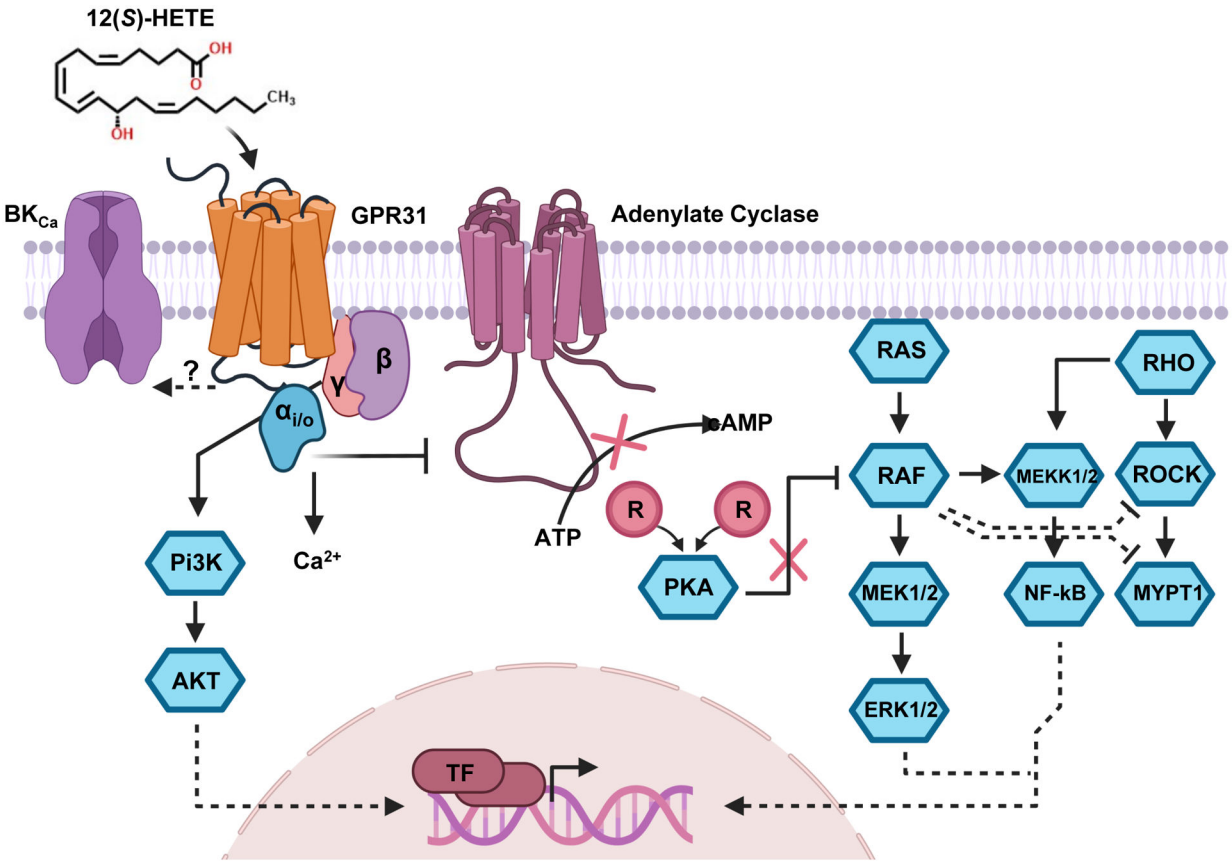

Figure 1: 12(*S*)-HETE/GPR31 Signaling Pathways.

The 12(*S*)-HETE receptor (GPR31) is $G\alpha_{i/o}$ coupled and inhibits adenylate cyclase from converting ATP to cAMP. This action causes the reassociation of the regulatory subunits of PKA to its catalytic subunits, inhibiting the kinase activity of PKA. RAF, a member of the MAPK pathway, is capable of being inhibited through phosphorylation by PKA. RAF has been shown to phosphorylate MEK which subsequently activates ERK1/2 which can translocate to the nucleus and promotes gene expression through the activation of nuclear transcription factors. Additionally, RAF has also been shown to induce NF-κB through MEKK1 which also promotes gene expression. Additionally, through alterations of RAF activity, 12(*S*)-HETE acting on GPR31 may also exert an inhibitory effect on ROCK and MYPT1 related signaling. Lastly, the $G_{\beta\gamma}$ effects of GPCRs like GPR31 have been demonstrated to influence intracellular calcium levels as well as drive PI3K/AKT signaling pathways with downstream consequences to alter gene expression. It is still unknown how the pairing of 12(S)-HETE and GPR31 alters the activation of the large conductance K channels (BK_{Ca}).

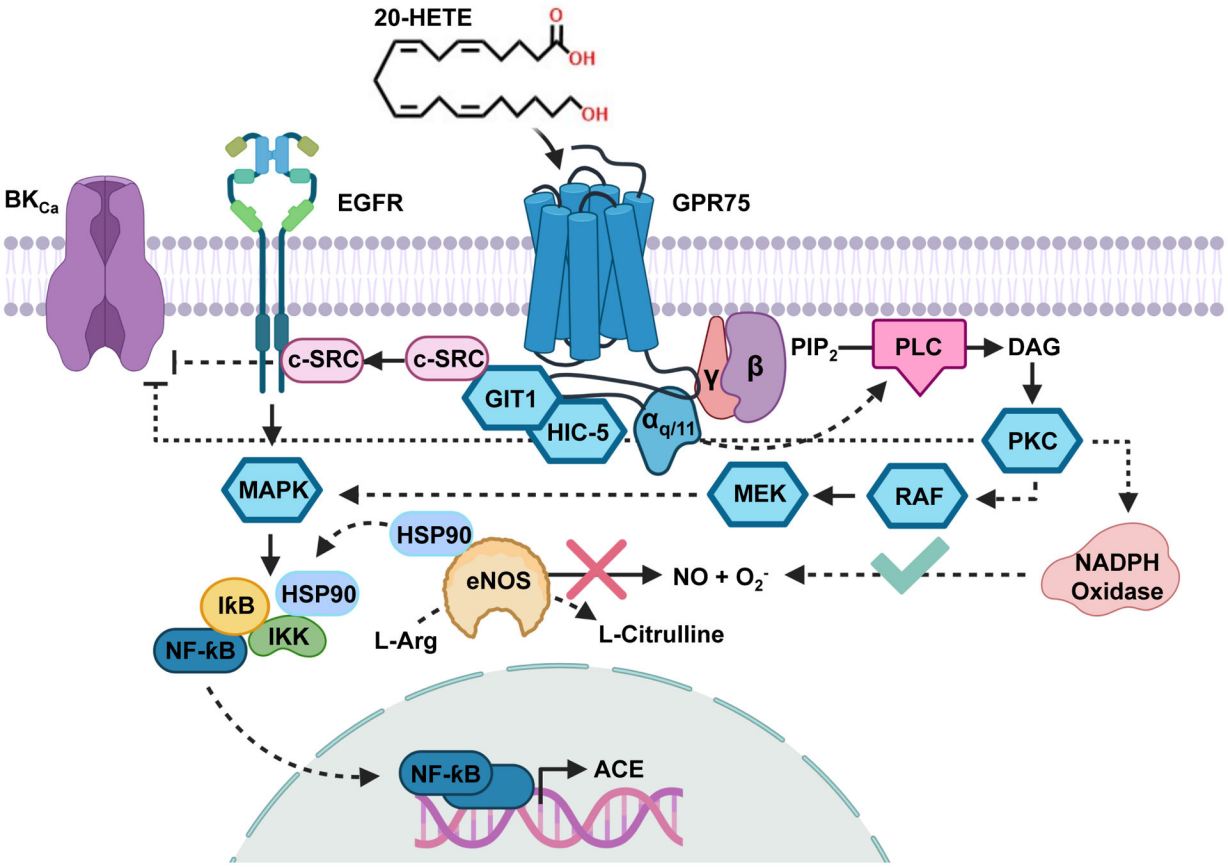

Figure 2: 20-HETE/GPR75 Signaling Pathways.

The 20-HETE receptor (20HR) (GPR75) is G$\alpha_{q/11}$ coupled and promotes changes in intracellular calcium. In endothelial cells, the activation of GPR75 via 20-HETE promotes the transactivation of the epidermal growth factor receptor (EGFR) through a GIT1-/c-SRC-dependent mechanism. Activation of the EGFR results in sequential activation of a MAPK/IκB/IKK and the translocation of NF-κB to stimulate the promoter regions of angiotensin converting enzyme (ACE). Simultaneously, the activation of IKK promotes the recruitment of the chaperone protein HSP90 towards IKK and away from endothelial nitric oxide synthase (eNOS), resulting in the uncoupling of eNOS and a reduction in NO production/bioavailability. Additionally, phospholipase C (PLC) stimulation driven by GPR75 G$\alpha_{q/11}$ results in the activation of PKC and subsequent increases in NADPH oxidase-derived reactive oxygen species (ROS) generation. The phosphorylation of the large conductance K channels (BK_{Ca}) by PKC also promotes a vasoconstrictor stimuli. These changes promote endothelial dysfunction and set into motion a pro-inflammatory signaling program that elevate various mediators including the synthesis of the chemokine IL-6.