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Review Article

Role of oxylipins in cardiovascular diseases

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Abstract
Globally, cardiovascular diseases (CVDs) are the number one cause of mortality. Approximately 18 million people died from CVDs in 2015, representing more than 30% of all global deaths. New diagnostic tools and therapies are eagerly required to decrease the prevalence of CVDs related to mortality and/or risk factors leading to CVDs. Oxylipins are a group of metabolites, generated via oxygenation of polyunsaturated fatty acids that are involved in inflammation, immunity, and vascular functions, *etc*. Thus far, over 100 oxylipins have been identified, and have overlapping and interconnected roles. Important CVD pathologies such as hyperlipidemia, hypertension, thrombosis, hemostasis and diabetes have been linked to abnormal oxylipin signaling. Oxylipins represent a new era of risk markers and/or therapeutic targets in several diseases including CVDs. The role of many oxylipins in the progression or regression in CVD, however, is still not fully understood. An increased knowledge of the role of these oxygenated polyunsaturated fatty acids in cardiovascular dysfunctions or CVDs including hypertension could possibly lead to the development of biomarkers for the detection and their treatment in the future.

Keywords: circulating biomarkers; cardiac oxylipins; plasma oxylipins; adenosine receptors; CYP-epoxygenases; soluble epoxide hydrolase; coronary reactive hyperemia; cardiovascular diseases; hypertension

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Introduction

Cardiovascular Diseases (CVDs) are the number one cause of death globally: more people die annually from CVDs than from any other disease [World Health Organization (WHO)]. An estimated ~18 million people died from CVDs in 2015, representing 31% of all global deaths (WHO). Of these deaths, an estimated 7.4 million were due to coronary heart disease and 6.7 million were due to stroke (WHO). Most cardiovascular diseases can be prevented; if we establish and sort out the precise circulating biomarkers (plasma oxylipins?) for the early detection of CVDs, including hypertension, which may possibly help us to avoid, use of risk factors such as tobacco, unhealthy diet, sedentary life style, and harmful use of alcohol. Also, people with cardiovascular disease or who are considered a high cardiovascular risk (due to the presence of one or more risk factors such as: hypertension, diabetes, hyperlipidemia or an already established disease) need early detection and management using counseling and medicines, as appropriate (WHO). Globally, 1.39 billion persons, representing 31% of all adults (25 years and over), have hypertension [1]. Out of which, 75 million American adults have hypertension; that is 1 out of every 3 adults (CDC, 2016). Surprisingly, the state of West Virginia is ranked #1 (41.0%±1.5%) in the top 10 highest rated states for hypertensive populations in the U.S. (2013, Trust for America's Health). Also, only about half (54%) of people in the US with hypertension have their condition under control; the other half (46%) are not under control and hypertension costs the US $46 billion each year (CDC, 2016). In addition to the hypertensive cases, nearly 1 out of 3 American adults have pre-hypertensive conditions too (CDC, 2016), and pre-hypertension and hypertension are the global major risk factors for the cardiovascular diseases (CVDs)[2]. Therefore, newer early diagnostic tools in the form of circulating biomarkers [plasma oxygenated polyunsaturated fatty acids (oxylipins)?] and therapies are required to decrease the CVDs. Oxylipins are the oxygenated polyunsaturated fatty acids that regulate inflammation, vascular response and coronary hyperemic response[3-12].

Oxylipins

Oxylipins are bioactive lipids generated by the oxidation of polyunsaturated fatty acids (PUFAs)[13]. Since their discovery almost five decades ago, numerous biologic functions have been linked to them and many others are still being elucidated.

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The advancement in detection and quantification rejuvenated both interest and research in oxylipins with accurate nanomolar detection using an array of state-of-the-art mass spectrometry instruments[14]. Still, studying the biological functions of oxylipins is challenged by the sheer number of oxylipins discovered, thus far, over 100 oxylipins have been identified[13], and have overlapping and interconnected roles. Important cardiovascular disease (CVD) pathologies, including: hyperlipidemia, hypertension, thrombosis, hemostasis, and diabetes have been linked to abnormal oxylipin signaling[15].

Oxylipins derived from arachidonic acid (AA) include EETs, HETEs, and prostanoids, whereas those derived from linoleic acid (LA) include EpOMEs and HODEs among others. Figure 1 illustrates some of AA- and LA-derived oxylipins and the main enzymes involved in their generation and breakdown, such as lipoxygenases, CYP-epoxygenases, ω-hydroxylases and cyclooxygenases. Figure 2 illustrates involvement of soluble epoxide hydrolase in changing the ratios of EpOME/DiHOME and EET/DHET, whereas, Figure 3 illustrate involvement of oxylipins in cardiovascular regulation.

Oxylipins biosynthesis

Oxylipins are both potent and short lived. Therefore, they are not stored; rather, they are synthesized *de novo* and regulated closely and exert their effect in a paracrine or autocrine manner[13]. Free PUFAs are mono- or dioxygenated by three families of enzymes: cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP) into distinct classes of oxylipins[16]. The type of oxylipins produced from PUFAs depends on the amount of dietary PUFAs consumed, the oxygenases (COX, LOX or CYP) present for metabolizing PUFAs, and the enzyme's affinity for a specific substrate PUFA[3]. The most well-known oxylipins are the eicosanoids (20-carbon compounds) formed from arachidonic acid (AA) and octadecanoids (18-carbon compounds) derived from linoleic acid (LA)[3]. Cyclooxygenase (COX) enzymes convert AA into prostanoids (PGs and thromboxanes). Also, COX enzymes can produce some hydroxy-metabolites, such as 11-HETE from AA and 9-HODE from LA[17]. Lipoxygenases (LOXs) catalyze the formation of hydroxy fatty acids, including: leukotrienes, lipoxins, resolvins, protectins, maresins, hepoxilins, and eoxins[3]. LOX enzymes also metabolize AA to form mid-chain (5-, 8-, 9-, 11-, 12-, and 15-) HETEs[18]. Cytochrome P450 (CYP) enzymes, which were originally known for their roles in xenobiotic metabolism, could either have epoxygenase or ω-hydroxylase activity[3]. ω-Hydroxylase enzymes (CYP4A and CYP4F) metabolize AA and generate ω-terminal (16-, 17-, 18-, 19-, and 20-) HETEs, whereas CYPs, with epoxygenase activity (CYP2C and CYP2J), metabolize AA and generate epoxyeicosatrienoic acid (EETs), which are further metabolized to dihydroxyeicosatrienoic acids (DHETs) by soluble epoxide hydrolase (sEH)[13]. AA can also be generated from LA metabolism[19]. Metabolism of LA includes the same enzyme families described above; for example, CYP epoxygenases metabolize LA to form EpOMEs, the epoxy compounds of LA. EpOMEs are hydrated by sEH to form DiHOMEs, the dihydroxy form of EpOMEs[18]. LOX enzymes form the hydroxy metabolites of LA: HODEs[13]. Oxylipins have a wide range of biological functions, many of which are still being investigated. They produce their effects through activating PPARs or through GPCRs[20]. Targeting sEH impacted the level of

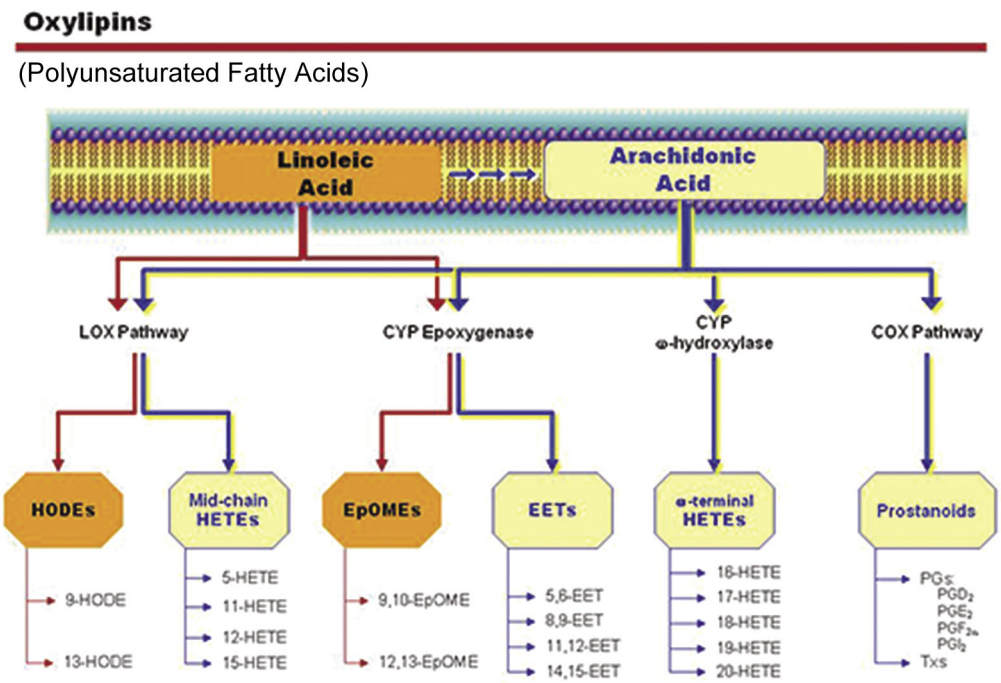


Figure 1. AA- and LA-derived oxylipins and the main enzymes involved in their generation and breakdown.

oxylipins directly affected by its catalytic activity, such as: EETs, DHETs, EpOMEs, and DiHOMEs and indirectly by affecting the other PUFAs pathways, such as; HODEs, and HETEs[21]. The latter observation could be explained by the shift observed in $sEH^{-/-}$ mice through AA metabolism due to EETs accumulation[22], suggesting that the different oxylipin pathways affect one another. Further, compared to n-6 PUFAs, the supplemental dietary n-3 PUFAs have variety of health benefits against cardiovascular diseases by a decrease in the production of inflammatory mediators (eicosanoids, cytokines, and ROS) and the expression of adhesion molecules[23-27]. Also, the beneficial effects of n-3 PUFAs have been well discussed by Lorente-Cebrian *et al* in his review[28], including enhanced vascular functions, cardioprotection, reduction in myocardial infarction, arrhythmia, sudden cardiac death, stroke, *etc*. However, the beneficial effects of n-3 PUFAs are not the interest of the current review article.

Epoxyeicosatrienoic acids (EETs)

EETs are 20-carbon metabolites of arachidonic acid (AA) with numerous physiologic actions. They are generated from AA by the cytochrome P450 epoxygenase pathway. Four distinct regioisomers are produced: 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET. In the heart, EETs exert cardioprotective effects in ischemia/reperfusion injury[29]. EETs are classified as EDHFs; they are produced in endothelial cells and induce hyperpolarization in vascular smooth muscle cells by activating large conductance Ca^{2+}-activated K^+ channels (BKCa)[30, 31]. EETs also cause vasodilation in many vascular beds, such as: the intestines[32], kidney preglomerular vasculature[33], conduit arteries[34-38], and brain[39]. It is worth mentioning that small (KCa2.3) and medium (KCa3.1) conductance Ca^{2+}-activated K^+ channels are important in EETs-induced hyperpolarization[31]. EETs are metabolized rapidly by hydration to their corresponding, less active, DHETs by sEH, which is the main catabolic pathway responsible for EETs breakdown[40]. Not all EETs isomers are substrates for sEH; 5,6-EET is a poor substrate of sEH. In fact, this EET isomer (5,6-EET) along with 8,9-EET are substrates for cyclooxygenase (COX) pathway[41]. The half-life of 14,15-EET was found to be between 7.9–12.3 min[42]. Other catabolic pathways of EETs include ω-oxidation, β-oxidation, and chain elongation. The latter two pathways become more important when the activity of the main pathway, hydration by sEH, is inhibited[41]. Not only were EETs shown to have confirmed beneficial effects in numerous animal studies through their vasodilatory[32-39], cardioprotective[29], and anti-inflammatory effects[43], they were also linked to decreased cardiovascular risk in epidemiological studies in humans[44, 45]. Polymorphism variants where EETs production is decreased, such as decreased CYP2J2 expression (variant G-50T)[45] or EETs breakdown is increased, such as increased sEH activity (variant K55R)[44], had increased risk of coronary

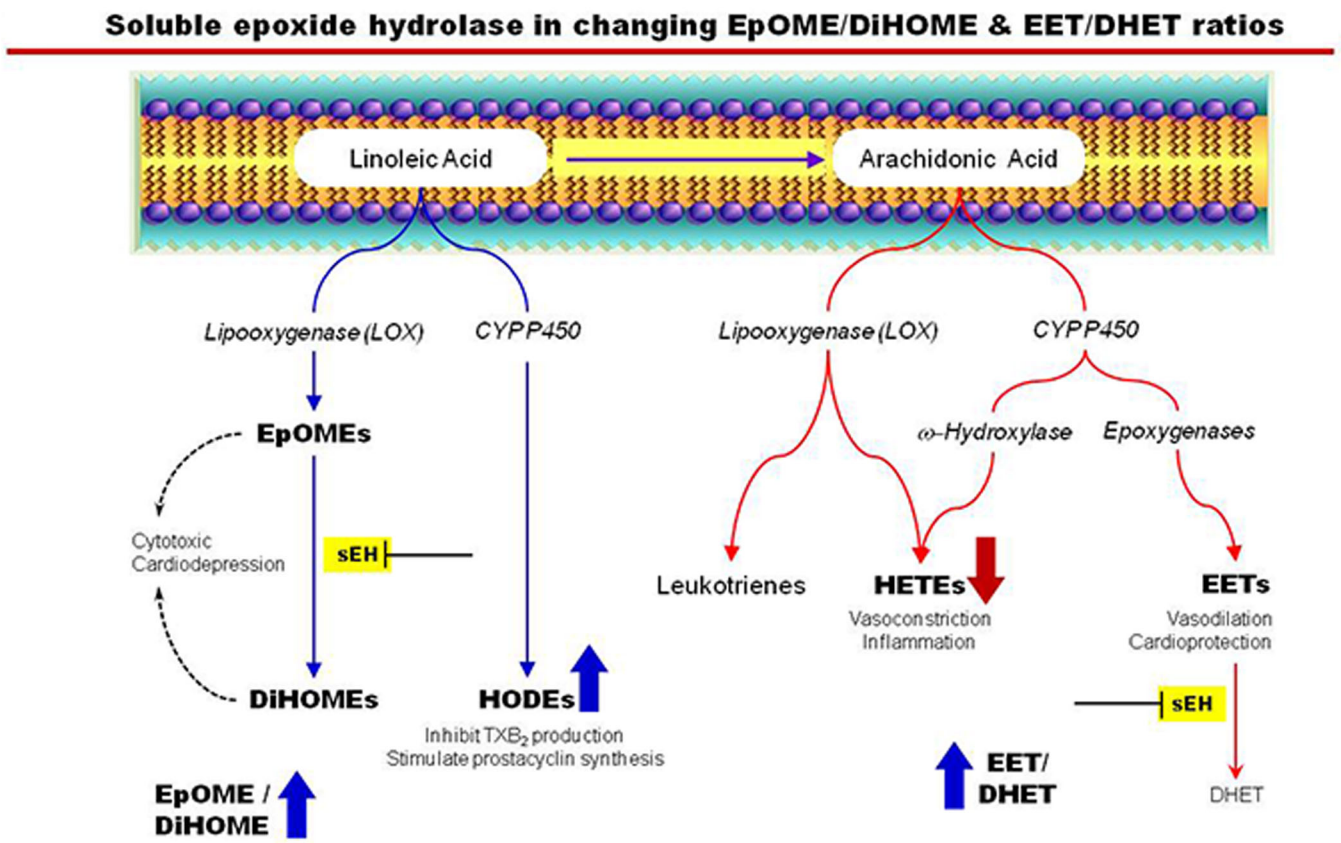


Figure 2. Soluble epoxide hydrolase in changing EpOME/DiHOME & EET/DHET ratios.

artery disease. It was speculated by some researchers that EETs exert their effects through specific cell surface receptors, which is supported by the finding that different responses were elicited by different stereoisomers and regioisomers of EETs[46]. However, it is worth mentioning that numerous reports linked EETs' signaling pathway with protein kinase A (PKA) and cAMP[47].

Mid-chain hydroxyeicosatetraenoic acids (HETEs)

Mid-chain HETEs are produced through allylic oxidation of AA by lipoxygenase (LOX)[22]. They were shown to have chemotaxis effects, change in vascular tone, and induced the production of vascular endothelial growth factors[48-51]. Also, the increased formation of mid-chain HETEs was involved in cardiovascular dysfunction[52-55]. Also, mid-chain HETEs like 5-, 8-, 9-, 11-, and 12-HETE stimulate migration, chemotaxis and chemokinesis in leukocytes[56-59], whereas, 15-HETE appears to have opposite effects[60, 61]. Also, 15-HETE can be converted into lipoxins (LXs) which play a role in the resolution of inflammation [62]. Unlike the vasodilatory effect of EETs in the kidneys[33], 12-HETE caused vasoconstriction in small renal arteries[63]. Also, the generation of mid-chain HETEs is increased in essential hypertension[64] suggesting that they could be involved in its pathogenesis. These reports point to opposite effects of EETs *vs* HETEs in vascular biology. Maayah *et al* reported that mid-chain HETEs blocked the synthesis of EETs and increased their conversion to DHETs in RL-14 cells[65]. Moreover, although sEH is not directly involved in the generation or breakdown of mid-chain HETEs, sEH was found to be essential for mid-chain HETE–mediated induction of cellular hypertrophy[65]. Therefore, not only do EETs and mid-chain HETEs have opposite effects, they seem to affect the level of each other.

ω-Terminal hydroxyeicosatetraenoic acids (ω-terminal HETEs)

16-, 18-, 19-, and 20-HETEs are produced by cytochrome P450 ω-hydroxylase activity. 16-HETE is produced and released by polymorphonuclear leukocytes upon angiotensin II stimulation[66]. Of the ω-terminal HETEs, 20-HETE, which is a potent vasoconstrictor in mouse mesenteric arteries, mouse aorta and mouse coronary artery, is produced by CYP4A from arachidonic acid[34, 35, 37, 38, 67-71]. Also, 20-HETE is a potent vasoconstrictor in porcine coronary arteries[72].

Epoxyoctadecaenoic acid (EpOMEs)

EpOMEs and DiHOMEs were reported to increase oxidative stress in vascular endothelial cells[73-75]; DiHOMEs were toxic to renal proximal tubular cells[76] and intravenously injected 9,10-EpOME had cardiodepressive effects in dogs[77]. Pretreatment with 12,13-EpOME protected primary cultures of rabbit renal proximal tubular cells against hypoxia/reoxygenation injury[78]. Also, effects of EpOME/DiHOME ratio, induced by sEH inhibition using AUDA, improved renal recovery in response to ischemia/reperfusion injury in C57BL/6 mice[79].

9-, 13-Hydroxyoctadecadienoic acids (9-, 13-HODEs)

Linoleic acid is also metabolized through hydroxylation by CYP epoxygenases to form hydroxyl-LA metabolites known as hydroxyoctadecadienoic acids (HODEs)[22]. HODEs are associated with oxidative stress[80, 81], and the 9-HODE induces macrophage IL-1β [82]. Also, 9- and 13-HODE activate plasminogen activator inhibitor type-1 via PPARγ activation in endothelial cells [83], and 13-HODE is also suggested to have an anti-inflammatory role in inflammatory diseases through its effect as a PPARγ-agonist[84-88]. Also, 13-HODE increased prostacyclin (PGI2) biosynthesis, which was involved in splenic and coronary artery relaxation in smooth muscle cells in Mongrel dogs[89]. 9-HODE, unlike 13-HODE, was described as pro-inflammatory in an experimental wound-healing model in rats[90, 91], whereas, 13-HODE prevents platelets adhering in human vascular endothelial cells[92, 93].

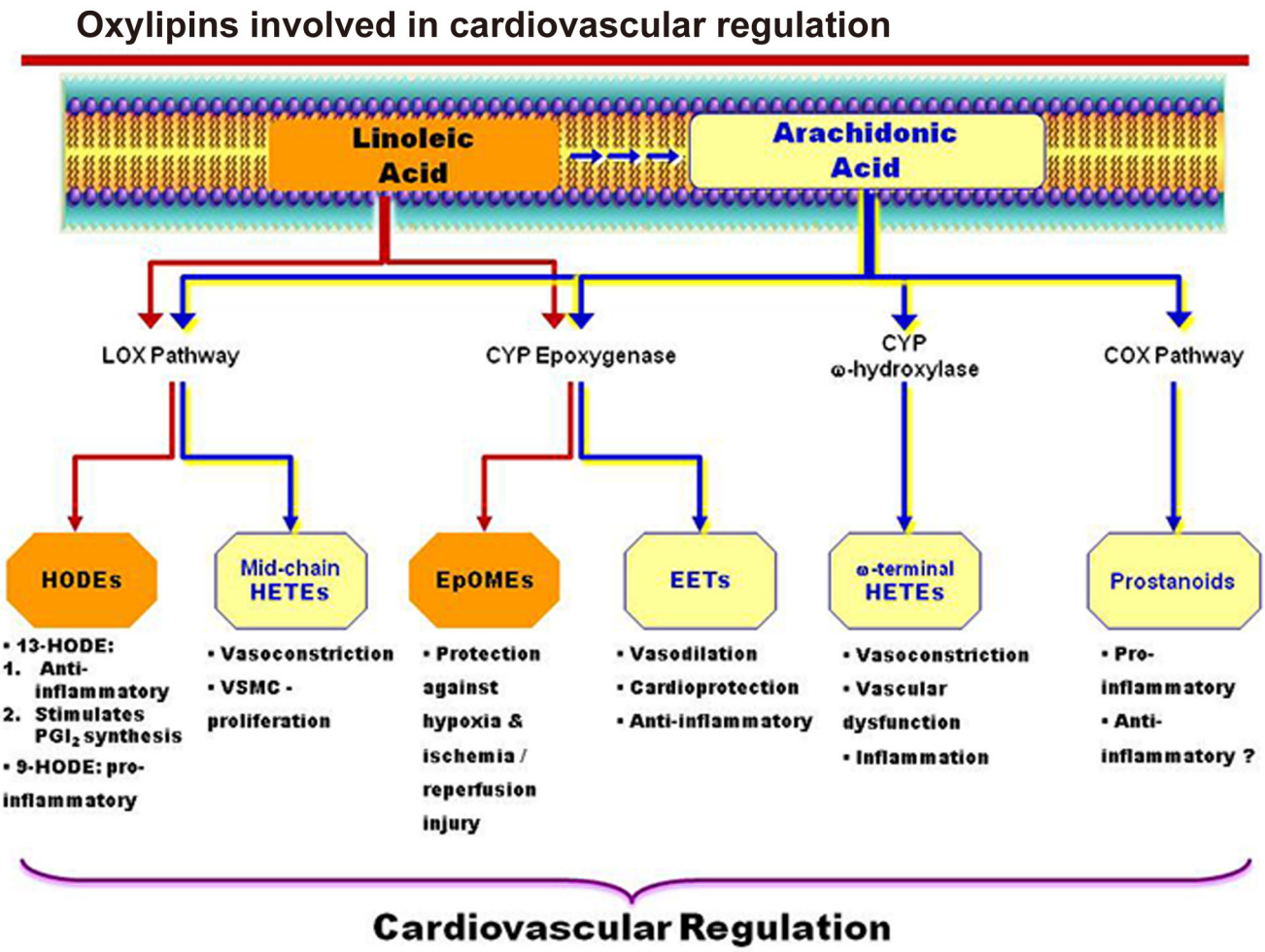
Prostanoids

The term prostanoids comprises of two distinct groups: prostaglandins and thromboxanes. Prostaglandins G2 and H2, which are AA metabolites formed by COX isoforms (1 and 2), get converted into the 4 main bioactive PGs (D2, E2, I2, and F2α) and thromboxanes (TXA2 and TXB2)[94, 95]. Most PGs have pro-inflammatory effects. However, PGE2 was found to have an anti-inflammatory role as well by up-regulating cAMP and inducing secretion of the anti-inflammatory IL-10[96]. Similarly, PGD2 attenuated inflammation in experimental models of pleuritis and colitis [95]. 6-keto-PGF1α is a stable metabolite and marker for PGI2 that is produced via cyclooxygenase (COX) signaling. 6-keto-PGF1α in humans is inversely related to cardiovascular events and high blood pressure[97, 98]. PGF2α induces vasoconstriction in bovine, canine, and human coronary arteries[99]. PGF2α is associated with cardiac dysfunction and cardiac hypertrophy[100, 101]. TXA2 induces vasoconstriction and aggregation of platelets [102]. Also, TXB2 is positively associated with high blood pressure and multiple cardiovascular dysfunctions[97, 98].

Discussion

Oxylipins regulate inflammation, vascular response and coronary hyperemic response[3-7, 9-12]. Recently, in our laboratory, oxylipins were analyzed in both blood plasma and heart perfusates of adenosine A2A receptor knockout ($A2AAR^{-/-}$) mice, where soluble epoxide hydrolase (sEH) was overexpressed and CYP2C was underexpressed [69, 103] compared to its wild-type mice ($A2AAR^{+/+}$); Tie2-sEH Tr (sEH-overexpressed mice) compared to C57Bl/6; sEH knockout ($sEH^{-/-}$) mice compared to its wild-type mice ($sEH^{+/+}$); Tie2-CYP2J2 Tr (CYP2J2-overexpressed) compared to C57Bl/6 mice, and *trans*-4-[4-(3-adamantan-1-ylureido)cyclohexyloxy]benzoic acid (t-AUCB, sEH-inhibitor) treated C57Bl/6 compared to non-treated C57Bl/6 mice [8-12]. The heart perfusate data have demonstrated positive/negative effects of a brief ischemia on cardiac oxylipin levels (local) with enhanced or reduced coronary reactive hyperemic responses depending upon the mice ($A2AAR^{-/-}$, $sEH^{-/-}$, Tie2-sEH Tr, CYP2J2 Tr, C57Bl/6, and sEH-inhibitor-treated C57Bl/6 and other transgenic mice), as they are assessed before and after the ischemia *in vitro* mouse model of isolated heart[9-12]. Also, blood plasma oxylipins pro-

file data of A2AAR$^{-/-}$ *vs* A2AAR$^{+/+}$ have supported the data of heart perfusates (cardiac oxylipins profile) of A2AAR$^{-/-}$ *vs* A2AAR$^{+/+}$ mice[8]. Further, there are evidences that show a relationship exists between endothelial dysfunctions involving cardiovascular diseases/hypertension and genetic polymorphisms in A2AAR, CYP2J2 and sEH genes in humans[44, 104-108]. Similarly, A2AAR$^{-/-}$ have increased hypertension compared to A2AAR$^{+/+}$ mice[109]. Hypertension was also observed in CYP2J5$^{-/-}$ compared to CYP2J5$^{+/+}$ mice[110], and sEH overexpression in rats have increased blood pressure compared to their respective control[111], while sEH$^{-/-}$ mice had lower blood pressure[40]. Further, the up-regulation of angiotensin II is directly proportional to sEH up-regulation[112], whereas, sEH inhibition blocks Angiotensin II-induced hypertension in rats[113]. As we mentioned earlier, the plasma levels of both EETs (oxylipins) and DHETs (oxylipins) levels in A2AAR$^{-/-}$ *vs* A2AAR$^{+/+}$ mice supported the findings of the heart perfusate samples (cardiac oxylipins) of A2AAR$^{-/-}$ *vs* A2AAR$^{+/+}$ mice; EETs/DHETs ratio was decreased by the elevated DHET levels which leads to significant reduction in coronary reactive hyperemic response in A2AAR$^{-/-}$ *vs* A2AAR$^{+/+}$ mice [8]. Similarly, in the heart, EETs exert cardioprotective effects against ischemia/reperfusion injury[29], and EETs are classified as EDHFs; they are produced in endothelial cells and induce hyperpolarization in vascular smooth muscle cells by activating large conductance BKCa[30, 31]. EETs also cause vasodilation in many vascular beds, such as: the intestines[32], kidney preglomerular vasculature[33], conduit arteries[34-38, 69, 103, 114], and brain[39]. EETs are metabolized rapidly by hydration to their corresponding, less active, dihydroxyeicosatrienoic acids (DHETs) by soluble epoxide hydrolase sEH, which is the main catabolic pathway responsible for EETs breakdown[40]. Earlier, we reported that sEH knockout (sEH$^{-/-}$ mice) and pharmacologic inhibition of sEH decreased DHETs in heart perfusate (cardiac oxylipins) leading to significantly increased coronary reactive hyperemic response in sEH$^{-/-}$ and t-AUCB (sEH-inhibitor)-treated C57BL/6 *vs* their respective controls[11, 12]. The increased expression of sEH and decreased expression of CYP2C in A2AAR$^{-/-}$mice[69, 103] is probably the cause of an increase in DHETs' generation and drove the decrease in EET/DHET ratios in A2AAR$^{-/-}$ *vs* A2AAR$^{+/+}$ mice[8]. Also, EETs have anti-inflammatory properties[41], the higher the sEH activity [like K55R (genetic polymorphism in humans)] alters the ratio between EpOME:DiHOME (oxylipins) in the athero-



sclerosis risk in communities, where Caucasians carry K55R variant allele[44]. Also, we found out recently an enhanced coronary reactive response with the change in the isolated hearts perfusate oxylipins (cardiac) and plasma oxylipins (increased the ratio between EETs:DHETs, EpOMEs:DiHOMEs, and decreased in HETEs, prostanoids, TXB2, *etc*) in sEH$^{-/-}$, Tie2-CYP2J2 Tr and sEH inhibitor-treated C57BL6 *vs* Tie2-sEH Tr and A2AAR$^{-/-}$ mice, and these oxylipins play an important role in the cardiovascular functions in mice[8-12]. Our observation in A2AAR$^{-/-}$ and Tie2-sEH Tr compared to its respective wild-type mice cardiac as well as plasma oxylipin data are very interesting since it suggests a possible decrease in the anti-inflammatory role of EETs and increase in the pro-inflammatory role of DHETs with the reduction in coronary reactive hyperemic response[8, 9]. Also, effects have been reported on arachidonic acid metabolism leading to cardiovascular diseases due to cytochrome P450 polymorphism[115], and this CYP450 polymorphisms are considered to be one of the determinants of susceptibility to cardiovascular diseases. CYP-epoxygenases, CYP2C and CYP2J2 metabolize arachidonic acid to form EETs, which are involved in vascular relaxation, anti-inflamation, anti-apoptosis, anti-thrombosis, and cardioprotective activities. Therefore, it is strongly suggested that genetic polymorphisms in CYP2C and CYP2J2 cause lower activities which are associated with an increased risk of several cardiovascular diseases like, hypertension and coronary artery disease[116-126]. Soluble epoxide hydrolase (sEH) metabolizes CYP-epoxygenases derived active epoxyeicosatrienoic acids from arachidonic acids to the less active dihydroxyeicosatrienoic acids (DHETs). Therefore, polymorphism in sEH gene has also been associated with cardiovascular diseases, such as the higher the sEH activity [K55R (genetic polymorphism in humans)] alters the ratio between EpOME:DiHOME (oxylipins) in the atherosclerosis risk in communities, where Caucasians carry K55R variant allele[44, 127]. Also, genetic polymorphisms in ω-hydroxylases (CYP4A11 & CYP4F2) are associated with higher risk of hypertension through 20-HETE[128-130]. As we mentioned earlier, a relationship exists between endothelial dysfunctions involving cardiovascular diseases/hypertension and genetic polymorphisms in A2AAR, CYP2J2 and sEH genes in humans[44, 104-108]. Therefore, we observed recently an enhanced coronary reactive response with the change in isolated hearts perfusate oxylipins (cardiac) and plasma oxylipins (increased the ratio between EETs:DHETs, EpOMEs:DiHOMEs, and decreased in HETEs, prostanoids, TXB2, *etc*) in sEH$^{-/-}$,Tie2-CYP2J2 Tr and sEH inhibitor-treated C57BL6 compared to Tie2-sEH Tr and A2AAR$^{-/-}$ mice, and these oxylipins play an important role in the cardiovascular functions in mice[8-12]. The attenuated coronary reactive hyperemia (CRH) in A2AAR$^{-/-}$ and Tie2-sEH Tr mice could be due to decreased EET/DHET ratio, which could be attributed to the increased expression of sEH and decreased expression of CYP2C[69, 103], and this may indicate an overall reduction in EETs vasodilatory and anti-inflammatory activity in A2AAR$^{-/-}$ and Tie2-sEH Tr mice compared to their respective wild-type mice[8, 9].

In addition to EETs and DHETs, mid-chain (5-, 8-, 11-, 12- and 15-) HETEs were also detected in both blood plasma and heart perfusates, and they were increased with reduced coronary reactive hyperemic response in A2AAR$^{-/-}$ and Tie2-sEH Tr compared to their respective wild-type mice[8, 9]. Lipoxygenases produce mid-chain HETEs from arachidonic acid (AA)[22]. Also, CYP-epoxygenase 1B1 produces mid-chain HETEs through oxidation of AA, and mid-chain HETEs have pro-inflammatory and vasoconstriction properties[22, 65, 131]. Further, cardiovascular dysfunction associated with increased formation of mid-chain HETEs have been reported[52-55]. As we mentioned earlier, heart perfusates and blood plasma demonstrated an increase in mid-chain HETEs in A2AAR$^{-/-}$ and Tie2-sEH Tr with reduced coronary reactive hyperemic response compared to their respective wild-type mice[8, 9]. Therefore, the increase in mid-chain (5-, 8-, 11-, 12- and 15-) HETEs in A2AAR$^{-/-}$ and Tie2-sEH Tr mice, along with the decreased EET/DHET ratios, indicated an increase in the pro-inflammation and vasoconstriction associated with A2AAR–deletion (A2AAR$^{-/-}$) and sEH overexpression (Tie2-sEH Tr) in mice, and might have contributed to the decrease or reduction of coronary reactive hyperemic response in both A2AAR$^{-/-}$ and Tie2-sEH Tr compared to their respective wild-type mice[8, 9].

Omega-terminal HETEs are also generated from AA, but through ω-hydroxylases (CYP4A, CYP4F)[132]. The primary oxylipin of ω-terminal HETEs is 20-HETE[132], which promotes hypertension, vascular contraction, and vascular dysfunction[133, 134]. Further, blocking 20-HETE synthesis reduced mean arterial pressure in old spontaneously hypertensive (SHR) female rats[135]. Our lab has recently reported that blocking ω-hydroxylases by DDMS/HET0016 enhanced coronary reactive hyperemic response and vascular relaxation in mesenteric arteries of sEH-overexpressed (Tie2-sEH Tr) and control mice[9, 67, 136]. Similarly, targeting the ω-terminal–HETEs pathway, reversed the decreased coronary reactive hyperemic response in A2AAR$^{-/-}$ mice[8], because A2AAR$^{-/-}$ mice have ω-hydroxylases upregulated[34, 71]. Therefore, ω-hydroxylases–inhibition reversed the decreased coronary reactive hyperemic response as well as vascular relaxation in A2AAR$^{-/-}$ mouse aorta and mesenteric arteries[9, 34, 67, 71, 136]. Further, we were able to detect these ω-terminal (19- and 20)-HETEs in plasma samples through LC-MS/MS and found increased levels of 20-HETE[8]. Therefore, the increased 20-HETE formation through ω-hydroxylases in A2AAR$^{-/-}$ compared to A2AAR$^{+/+}$ mice, along with the other changes in plasma/cardiac oxylipins, suggests an increase in the pro-inflammation state, vascular contraction, and vascular dysfunction associated with A2AAR–deletion (A2AAR$^{-/-}$) or sEH-overexpression (Tie2-sEH Tr) in mice may have contributed to the attenuated coronary reactive hyperemic response in A2AAR$^{-/-}$ and Tie2-sEH Tr compared to their respective wild-type mice. Similarly, in humans, 20-HETE is produced by CYP4A and CYP4F, and CYP4A11 genes, and polymorphisms in these genes are associated with essential hypertension in the male western Chinese Han population[137]. Also, CYP4A11 gene polymorphism is involved in coronary artery disease as well as myocardial infarction in Han and Uygur populations in China[138, 139].

Deletion of A2AAR in A2AAR$^{-/-}$ mice was associated with

increased plasma/cardiac prostanoid levels too, including: PGF2α, PGE2, PGD2, and TXB2 compared to their respective wild type mice[8]. Prostanoids include two groups of metabolites (oxylipins): 1) thromboxanes (TXA2 and TXB2) and 2) bioactive prostaglandins (PGF2α, PGE2, PGI2, PGD2), and they are generally pro-inflammatory[94, 95]. Therefore, the increased prostanoid levels in A2AAR$^{-/-}$ mice may have contributed to an increase in the proinflammatory state in A2AAR$^{-/-}$ compared to their respective wild-type mice[8]

Soluble epoxide hydrolase (sEH) role in cardiovascular biology extends beyond its role in the conversion of 5,6-, 8,9-, 11,12- and 14,15-EETs into 5,6-, 8,9-, 11,12- and 14,15-DHETs; it also plays a central role in the metabolism of arachidonic-, linoleic- and omega-3-derived oxylipins [22]. Like EpOMEs, whose parental fatty acid is linoleic acid, they are hydrolyzed to DiHOMEs by sEH. Also, we reported earlier that the deletion of sEH (sEH$^{-/-}$ mice)[12] and inhibition of sEH by t-AUCB[11] increased EpOMEs, decreased DiHOMEs, and increased EpOME/DiHOME ratio in heart perfusate. The increased EpOME/DiHOME and EETs/DHETs ratios were believed to contribute to the enhancement of coronary reactive hyperemic response[11], which suggested that increased EpOME/DiHOME and EETs/DHETs ratios may have had a positive role in mediating vasodilation in coronary and aortas[11, 36-38, 69, 103]. Also, a protective effect against hypoxia/reoxygenation injury by EpOMEs in primary cultures of rabbit renal proximal tubular cells was reported by Nowak *et al*[78], an effect which was faded away with DiHOMEs[78]. Further, endothelium-dependent vasodilation in the cerebral circulation was impaired by decreased EpOME/DiHOME ratio in Tie2-sEH Tr mice[140]. Therefore, in A2AAR-null mice had decreased EpOME/DiHOME ratio due to increased DiHOMEs in the plasma[8]. Whereas, sEH-null and C57Bl/6 mice treated with sEH inhibitor (t-AUCB) had an increased EpOME/DiHOME ratio due to increased EpOMEs and decreased DiHOMEs in the heart perfusates[12]. Also, DiHOMEs were reported to have deleterious effects, including: cytotoxic, cardiodepressive and vascular contraction[141, 142]. Because of that, the decreased EpOME/DiHOME ratio and increased DiHOMEs in A2AAR-null mice may have contributed to the blunted coronary reactive hyperemic response. As mentioned earlier, sEH was overexpressed and CYP-epoxygenase was underexpressed A2AAR-null compared to wild-type mice[69, 103]. Further, polymorphism in sEH gene has been associated with cardiovascular diseases, such as the higher the sEH activity [K55R (genetic polymorphism in humans)] alters the ratio between EpOME:DiHOME (oxylipins) in the atherosclerosis risk in communities, where Caucasians carry K55R variant allele[44, 127].

In addition to EpOMEs, HODEs are generated through hydroxylation of LA by CYP epoxygenases or lipoxygenases[22]. 9-HODE, but not 13-HODE, was increased in A2AAR$^{-/-}$ *vs* A2AAR$^{+/+}$ mice. Role of 9-HODE is pro-inflammatory[90, 91], whereas role of 13-HODE could be anti-inflammatory[84-88]. The opposite effects of these two isomer oxylipins make this observation consistent with our earlier reports of an increased 13–HODE level in sEH–deleted (sEH$^{-/-}$) and C57Bl/6 mice treated with sEH inhibitor (t-AUCB)[11, 12]. The increased 9-HODE levels in A2AAR$^{-/-}$ mice plasma may be linked to the reported increase in sEH-expression and decreased expression of CYP-epoxygenase in A2AAR$^{-/-}$ compared to its wild-type mice[69, 103]. Thus, the increased 9-HODE levels in A2AAR$^{-/-}$ and the decreased 9-HODE levels in sEH$^{-/-}$ and C57Bl/6 mice treated with sEH inhibitor (t-AUCB) may have contributed to the reduction of coronary reactive hyperemic response in A2AAR$^{-/-}$ and increased coronary reactive hyperemic response (CRH) in sEH$^{-/-}$ and C57Bl/6 mice treated with sEH inhibitor (t-AUCB)[11, 12].

Vascular endothelial overexpression of human CYP2J2 in mice (Tie2-CYP2J2 Tr) increased CRH after brief ischemia compared to respective wild-type mice[10]. As we described earlier, ischemic insult to the heart is likely to cause damage if not corrected within a short period of time[8, 9, 11, 12]. The heart responds to ischemic insult by increasing coronary flow through CRH to reduce the deleterious effects of ischemia-induced damage[8, 9, 11, 12, 143]. Also, we previously described that the role of EETs and DHETs, as well as other oxylipins, in correlation with the changes in CRH in mice in response to brief ischemic insult[8, 9, 11, 12]. More generation of EETs compared to DHETs have well-established their beneficial cardiovascular effects[8, 9, 11, 12, 47, 142, 144, 145]; more EETs generation compared to DHETs protect from myocardial and cerebral ischemia/reperfusion injury[29, 146] and relaxes vascular beds, including: the intestines, preglomerular and brain[32, 33, 39]. Vascular endothelial overexpression of human CYP2J2 in mice (Tie2-CYP2J2 Tr) generates more EETs compared to their respective WT mice from AA through epoxidation[11, 147]. The strategy to increase EETs level is to create the endothelial overexpression of CYP2J2 (Tie2-CYP2J2 Tr) in mice[141, 148]. Endothelial overexpression of human CYP2J2 protected from cerebral ischemia in male mice, and 11,12- and 14,15-EET levels were increased in aortic endothelial cell (isolated from Tie2-CYP2J2 Tr mice) culture medium[148]. Jia *et al* also suggested that the protection against cerebral ischemia was linked to increase in blood flow and anti-inflammatory activities in endothelial overexpression of human CYP2J2 in mice (Tie2-CYP2J2 Tr)[148], both of which are recognized effects of EETs[32, 33, 39, 149]. In our lab, these Tie2-CYP2J2 Tr mice had increased CRH compared to its wild-type mice[10], and this study also suggests that CYP2J2-derived EETs do play a significant role in CRH after a brief ischemic insult[10]. The CYP2J2*7 polymorphism in humans, which is associated with reduced CYP2J2 activity, was linked to higher risk of adverse cardiovascular outcomes including myocardial infarction[125, 150]. Also, hypertension was observed in CYP2J5$^{-/-}$ compared to CYP2J5$^{+/+}$ mice[110].

In addition to EETs and DHETs in Tie2-CYP2J2 Tr mice, ω-terminal HETEs are generated from AA by CYP4A and CYP4F subfamilies[132]. The 19- and 20-HETEs are potent oxylipins typically found at very low levels, and these ω-terminal HETEs were below detectable levels in our mouse heart perfusate samples in both Tie2-CYP2J2 Tr and their respective wild-type mice[10]. Since the 20-HETE is a potent vasoconstrictor and involved with the renin-angiotensin system to produce hypertension, vasoconstriction, and vascular dysfunction[132-134],

we analyzed the effect of inhibiting ω-hydroxylases (CYP4A & CYP4F) activities by DDMS. DDMS enhanced CRH more in Tie2-CYP2J2 Tr *vs* WT mice [10].

Another group of oxylipins produced by AA through cyclooxygenase pathway is prostanoids, which include prostaglandins (PGs) and TXB2. Vascular endothelial overexpression of CYP2J2 in mice (Tie2-CYP2J2 Tr) did not have significant changes in the cardiac prostanoid levels, but we observed a trend towards lower in Tie2-CYP2J2 Tr *vs* WT mice[10]. PGs are generally pro-inflammatory, but PGD2 and PGE2 have anti-inflammatory properties and secretes anti-inflammatory IL-10[95, 96]. We described earlier that by targeting the EETs pathway (sEH deletion or sEH inhibition), the levels of cardiac prostanoids were decreased in isolated mouse heart perfusates[11, 12]. Though, mice overexpressing the EETs-generating CYP2J2 in this study did not have significant change in prostanoid levels[10], but interestingly, the levels of cardiac PGF2α and cardiac PGE2 were decreased in response to ischemia in Tie2-CYP2J2 Tr *vs* WT mice[10].

Interestingly, 8-iso-PGF2α was significantly lower in Tie2-CYP2J2 Tr *vs* WT mice, and 8-iso-PGF2α is one of the isoprostanes, which are produced through lipid peroxidation of AA[151]. 8-iso-PGF2α, produced under the elevated level of reactive oxygen species (ROS), act as a surrogate marker for ROS production[141]. 8-iso-PGF2α is a potent vasoconstrictor in isolated guinea pig hearts[151]. The level of 8-iso-PGF2α was not changed by ischemic insult in Tie2-CYP2J2 Tr and WT mice, possibly because of brief ischemic insult, but the level of 8-iso-PGF2α was lower in Tie2-CYP2J2 Tr *vs* WT mice pre- and post-ischemia[10]. The decrease in 8-iso-PGF2α levels in Tie2-CYP2J2 Tr *vs* WT mice, and the subsequent decrease in vasoconstrictive activity, may have contributed to the enhanced CRH observed in Tie2-CYP2J2 Tr *vs* WT mice[10].

Therefore, an increase in knowledge of plasma oxylipins profile in CVDs including hypertensive individuals, and these plasma oxylipins could be the predictors of cardiovascular diseases and hypertension. Recently, we observed increased 8,9-,11,12-,14,15-DHETs; increased 5-,11-,15-, 19-, 20-HETEs; increased prostanoids (6-keto-PGF1α, PGF2α, PGD2, PG-E2, and TxB2) levels; increased 9,10-,12,13-DiHOMEs; increased 9-, 13-HODEs; decreased in the ratio of 8,9-EETs:8,9-DHETs; 11,12-EETs:11,12-DHETs; 14,15-EETs: 14,15-DHETs and decreased 9,10- and 12,13- EpOME/DiHOME ratios in the plasma of A2AAR$^{-/-}$ *vs* A2AAR$^{+/+}$ mice[8]. Further, A2AAR$^{-/-}$ mice have reduced CRH response, reduced vasodilation, hypertension with overexpression of soluble epoxide hydrolase and underexpression of CYP-epoxygenases in A2AAR$^{-/-}$ *vs* A2AAR$^{+/+}$ mice[8, 69, 103, 109]. These plasma oxylipins may be used as circulating biomarkers possibly detecting cardiovascular dysfunctions or cardiovascular diseases in future. Because, these oxygenated polyunsaturated fatty acids (oxylipins) regulate inflammation, vascular response, atherosclerosis, hypertension, and coronary hyperemic response[3-12, 44, 109, 110, 127]. Also, a strong relationship exists between endothelial dysfunctions involving cardiovascular diseases/hypertension and genetic polymorphisms in A2AAR, CYP2J2 and sEH genes in humans[44, 104-108].

Conclusion

A possible relationship exists between positive/negative changes in oxylipins profile and positive/negative changes in cardiovascular function in A2AAR$^{-/-}$, sEH$^{-/-}$, Tie2-sEH Tr, CYP2J2 Tr, C57Bl/6, and sEH-inhibitor-treated C57Bl/6 mice[8-12]. Also, a relationship exists between cardiovascular diseases and polymorphisms in adenosine receptors including A2A, CYP-epoxygenases (CYP2C, CYP2J2) and soluble epoxide hydrolase genes in humans[44, 104-108]. Further, A2A AR$^{-/-}$ mice had sEH overexpressed and CYP2C underexpressed[69, 103], and the sEH gene, EPHX2, is called a susceptibility gene for CVDs in humans[44]. Higher sEH activity (K55R) due to polymorphism altered the ratio between EpOMEs: DiHOMEs with elevated atherosclerosis risk in Caucasian populations[44]. Furthermore, mid-chain (5-, 11-, 12- and 15-) HETEs (oxylipins) have chemotaxis effects, change vascular tone, and produce vascular endothelial growth factors[48-51]. Also, increased levels of mid-chain HETEs have been linked with cardiovascular dysfunctions[52-55]. Unlike the vasodilation, cardioprotective and anti-hypertensive effects of EETs in the reno-cardiovascular system[33], the mid-chain HETEs are involved in essential hypertension[64]. Recently, we discovered an enhanced CRH response with a change in cardiac and plasma oxylipins (increase in the ratio between EETs:DHETs and EpOMEs:DiHOMEs, and decreases in HETEs, HODEs, prostanoids, TxB2, *etc*) isolated from mouse (sEH$^{-/-}$,Tie2-CYP2J2 Tr and sEH inhibitor-treated C57BL/6) heart perfusates and blood plasma *vs* A2AAR$^{-/-}$ and Tie2-sEHTr mice (reduced CRH response, decreased ratio between EETs:DHETs and EpOMEs:DiHOMEs, and increases in HETEs, HODEs, prostanoids, TXB2, *etc*)[8-12]. These cardiac and plasma oxylipins play a pivotal role in the regulation of cardiovascular functions in mice[8-12]. DHETs are the sEH-catalyzed metabolic breakdown products of EETs; EETs have anti-inflammatory properties[41], but A2AAR$^{-/-}$mice have increased DHETs *vs* EETs due to increased expression of sEH and decreased expression of CYP2C with more vasoconstriction in A2AAR$^{-/-}$ *vs* A2AAR$^{+/+}$ mice[8, 69, 103]. Whereas, sEH$^{-/-}$ mice have increased EETs *vs* DHETs with more A2AAR upregulation and vasodilation in sEH$^{-/-}$ *vs* sEH$^{+/+}$ mice[12, 36]. Increased ω-HETEs (20-HETE) and mid-chain HETEs are observed in blood plasma of A2AAR$^{-/-}$ *vs* A2AAR$^{+/+}$ mice[8]. Whereas, 20-HETE and mid-chain HETEs were decreased in sEH$^{-/-}$ *vs* sEH$^{+/+}$ mice[12]. Also, 20-HETE promotes hypertension, vasoconstriction, and vascular dysfunction[133, 134]. A2AAR$^{-/-}$ mice had increased plasma prostanoid levels, including 6-keto-PGF1α, PGE2, PGF2α, and TXB2[8]. Also, prostaglandins are pro-inflammatory[95], and TXB2 is the inactive degradation product of TXA2, which mediates platelet aggregation, smooth muscle contraction, and endothelial inflammation[95]. In A2AAR$^{-/-}$ mice, a decrease in EpOME/DiHOME ratio was driven by increased DiHOMEs and decreased EpOMEs in the blood plasma with reduced CRH[8], whereas, an increase in EpOME/DiHOME ratio was driven by increased EpOMEs and decreased DiHOMEs with enhanced CRH[12], and DiHOMEs have deleterious effects, including cytotoxic, car-

diodepressive, and vasoconstrictive properties[141, 142]. HODEs were increased in A2AAR$^{-/-}$ *vs* A2AAR$^{+/+}$ mice[8], and HODEs are thought to be pro-inflammatory[90, 91]. All these changes in cardiac and plasma oxylipins profile in A2AAR$^{-/-}$ and Tie2-sEHTr compared to their respective wild-type and sEH$^{-/-}$, Tie2-CYP2J2 Tr and sEH inhibitor-treated C57BL/6 mice may be responsible for the significantly reduced CRH response, increase in blood pressure, increase in vasoconstriction, and cardiovascular dysfunctions[8-12, 44, 48-55, 64, 103, 109, 133, 134]. Therefore, an increase in knowledge of plasma oxylipins profile in CVDs including hypertensive individuals may be used as circulating biomarkers possibly detecting cardiovascular dysfunctions or cardiovascular diseases in future.

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Abbreviations

6-keto-PGF1α: 6-keto prostaglandin-F1α; AA: Arachinodic acid; A2AAR: A2A adenosine receptor; BK: Large conductance potassium channels; COX: Cyclooxygenase; CRH: coronary reactive hyperemia; CYP2J2: Cytochrome P450 2J2; CYP450: cytochrome P450; DDMS: dibromo-dodecenyl-methylsulfimide; DHETs: dihydroxyeicosatrienoic acids; DiHOMEs: dihydroxyoctadecaenoic acids; EDHF: endothelium-derived hyperpolarizing factor; EETs: epoxyeicosatrienoic acids; EpOMEs: epoxyoctadecaenoic acids; HETEs: hydroxyeicosatetraenoic acids; HODEs: hydroxyoctadecadienoic acids; K_{ATP}: ATP-sensitive K^+ channels; KCa: calcium-acitvated K^+ channels; LC-MS/MS: liquid chromatography, tandem mass spectroscopy; LOX: Lipoxygenase; PG: prostaglandin; PHF: peak hyperemic flow; PUFAs: polyunsaturated fatty acids; sEH: soluble epoxide hydrolase; sEH$^{-/-}$: sEH–knockout mice; sEH$^{+/+}$: wild type mice normally expressing sEH; t-AUCB: *trans*-4-[4-(3-adamantan-1-ylureido)cyclohexyloxy] benzoic acid; TXA2: thromboxane A2; TXB2: thromboxane B2; PGF2α: prostaglandin-F2α; PGD2: prostaglandin-D2; PGE2 prostaglandin-E_2; LA: linoleic acid.

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