Chapter 5

The Metabolites of Arachidonic Acid in Microvascular Function

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Additional information is available at the end of the chapter

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Abstract

Arachidonic acid (AA) metabolites have an important role in mediating vascular reactivity to various stimuli, affecting tissue perfusion and tissue supply. In addition, they exert proinflammatory or anti-inflammatory effects on vessels. AA is metabolized by cyclooxygenases (COX) 1 and 2 to prostaglandins (PGs) and thromboxane (TX), by lipooxygenase to leukotrienes; by cytochrome P450 (CYP450)-hydroxylase to 20-hydroxyeicosatetraenoic acid (20-HETE) and by CYP450-epoxygenase to epoxyeicosatrienoic acids (EETs). Increased vascular oxidative stress may induce non-enzymatic production of isoprostanes from AA, which, together with vasoconstrictor metabolites of AA underlie endothelial damage and impaired vascular function. The balance among vasodilator and vasoconstrictor metabolites of AA may be disturbed in cardiometabolic diseases. (e.g. hypertension,obesity,diabetes) Dietary habits significantly affect the metabolism of AA, particularly excessive kitchen salt (NaCl) intake. Control of environmental risks factors, good maintenance of the occurring diseases and balanced nutrition with restricted salt intake can significantly improve the metabolism of AA and alleviate microvascular dysfunction and subsequent organ damage. Current research on pharmacological manipulation of certain components of the AA pathways (such as 20-HETE production inhibition or prolongation of the life of epoxyeicoatrienoic acids(EETs) by inhibitors of soluble epoxide hydrolaze (sEH)promises effective therapy of cardiovascular and cerebrovascular diseases in the future.

Keywords: microcirculation, endothelium, arachidonic acid metabolites, 20-HETE, EETs

1. Arachidonic acid metabolites and their receptors

The polyunsaturated omega-6 fatty acid 20:4(ω-6), arachidonic acid (AA), is a major component of cell membranes which is released from the cell membrane phospholipids primarily by phospholipase A_2 (PLA_2). Phospholipases can be activated by stimulation of vascular endothelial cells with various substances, such as acetylcholine (ACh) or shear stress [1]. AA can be metabolized by series of enzymes to numerous biological active metabolites termed "eicosanoids" or be transformed by reactive oxygen species (ROS) in nonenzymatic manner into isoprostanes [2]. The endothelium has a crucial role in maintenance of their circulatory homeostasis by producing and releasing different vasoactive substances, which regulate the tone of the underlying vascular smooth muscle.

Endothelial cells metabolize AA by three enzymatic pathways: cyclooxygenase (COX), lipoxygenase (LOX), or cytochrome P450 (CYP450) pathway, presented in **Figure 1**.

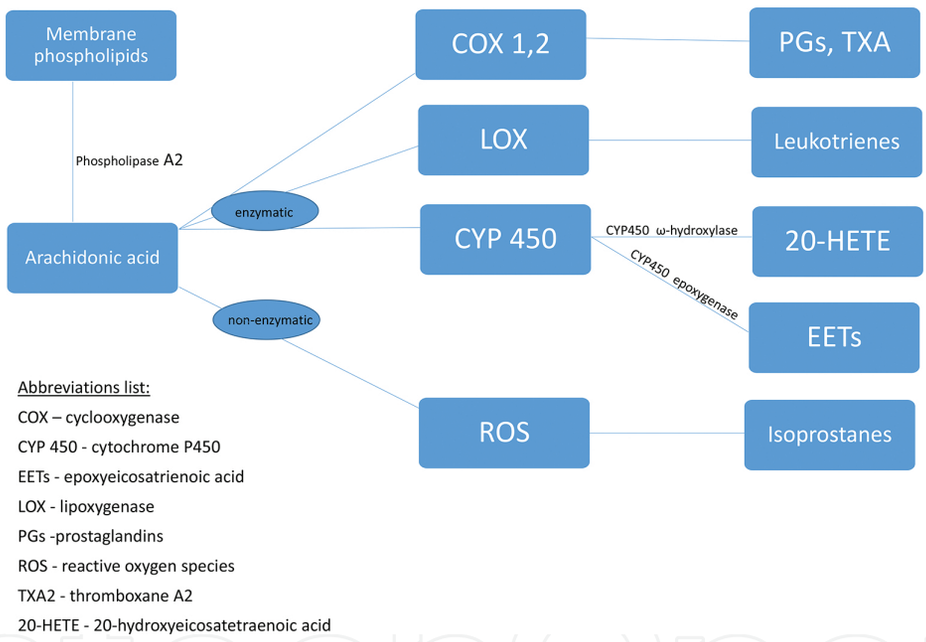

Figure 1. Metabolism of arachidonic acid. COX, cyclooxygenase; CYP 450, cytochrome P450; EETs, epoxyeicosatrienoic acid; LOX, lipoxygenase; PGs, prostaglandins; ROS, reactive oxygen species; TXA2, thromboxane A2; 20-HETE, 20-hydroxyeicosatetraenoic acid.

2. COX pathway

The released AA can be metabolized by COXs into prostanoids, which comprise prostaglandins (PGs) and thromboxanes (TXs). COXs convert AA into endoperoxides (PGH_2), the intermediate of the prostanoid biosynthesis, which can either act as an endothelium-derived contracting factor (EDCF) per se, or be further transformed into prostaglandin PGI_2 (prostacyclin) by prostacyclin synthase, or TXA_2 by thromboxane synthase. It could also be trans-

formed to various other prostaglandins, including PGD_2, PGE_2, and $PGF_2\alpha$ [3]. There are two isoforms of COXs, COX-1 and COX-2, both expressed in physiological and pathological conditions, but their roles, levels of activation, and affinity to AA could be different [2, 4]. In most tissues, COX-1 is constitutively expressed and generates dilator prostaglandins, whereas COX-2 is believed to be primarily an inducible enzyme, activated by pro-inflammatory conditions [2–5]. Oxidative stress can also serve as an initiator of increased COX-2 activity [6].

Numerous studies emphasize the importance of COX-derived metabolites in vascular reactivity regulation: PGs and TXA_2 have a key role in vascular tone expression in both physiological and pathophysiological conditions [2, 7, 8]. The endothelium is the primary source of increased COX activity since endothelial cells contain up to 20 times more COX than vascular smooth muscle cells (VSMC) [9].

3. Prostaglandins and their receptors

Prostanoid receptors, based on their action and signal transduction, can be grouped into three categories: the contractile receptors, the dilatory receptors, and the inhibitory receptors. Prostaglandins and their receptors are presented in **Table 1** [10–13]. The contractile receptors (thromboxane-prostanoid, TP; prostaglandin F, FP; and prostaglandin E_1, EP_1 receptors) mediate Ca^{2+} mobilization and induce smooth muscle contraction. The relaxant receptors (prostacyclin receptor, IP; prostaglandin D_2, DP; prostaglandin E_2, EP_2; and prostaglandin E_4, EP_4) mediate increases in cAMP and induce smooth muscle relaxation. The EP_3 receptor is an inhibitory receptor that mediates decreases in cAMP and inhibits smooth muscle relaxation [14]. Vasodilatory PGs, including PGI_2, PGE_2, and PGD_2 have an important role in regulating renal function; they increase renal blood flow and glomerular filtration rate. PGE_2 is a key regulator of sodium reabsorption in the distal tubules [15]. Therefore, these COX-mediated factors are crucial in the pathogenesis of cardiovascular and kidney diseases.

Prostaglandin I_2 (PGI_2) binds to the prostacyclin receptors (IP) that are located on platelets and vascular smooth muscle cells and mediate inhibition of platelet aggregation and smooth muscle cells relaxation, thus reducing the risk of thrombosis [2, 16, 17]. PGI_2 may represent a compensatory mechanism of vasodilation when the production of nitric oxide (NO) is reduced [18].

The expression of IP receptors was observed by in situ hybridization in various mouse organs showing expression in brain (indicating that IP may be involved in the mediation of pain), in megakaryocytes and the smooth muscles of arteries (consistent with the action of PGI_2 in the cardiovascular system), in afferent arterioles of the glomerulus (indicating its role in regulation of the glomerular filtration rate), and in the thymus and spleen (expressed in mature thymocytes and splenic lymphocytes) [19]. Interaction of PGI_2 with IP receptor plays a central nociceptive role in inflammation. Mice lacking the IP receptor display altered pain perception as well as inflammatory response [20].

AA metabolite	Receptor	Location		Function in health and in the disease
TxA_2	TP-G protein coupled receptor	TPα	Lung, spleen, uterus, placenta, aorta, heart, intestine, liver, eye, thymus, kidney, spinal cord, brain	Thrombosis/hemostasis, modulation of the immune response—*acute myocardial infarction, inflammatory lung disease, hypertension, nephrotic disease*
		TPβ	Human endothelium	
PGD_2	DP_1	Blood platelets, VSMC and nervous tissue, including the central nervous system, gastrointestinal SM, uterine SM		Inhibition of platelet aggregation, SM relaxation, possibly inhibition of autonomic neurotransmitter release
	DP_2			
PGE_2	EP_1	Human myometrium, kidney, lung		Contraction and relaxation of SM, inhibition and enhancement of neurotransmitter release, inhibition of lipolysis, gastric acid secretion, inflammatory mediator release, Ig expression, immunoregulation, inhibition, and enhancement of nonacid (water) secretion—*inflammation, allergy, parturition, and tumorigenesis (colon cancer), endometriosis*
	EP_2	SM, ileum, thymus, lung, spleen, heart, and uterus		
	EP_3	SM of gastrointestinal, uterine, and vascular origin, gastric mucosa kidney, thymus, spleen, lung, and brain		
	EP_4	SM, endothelium, endometrium		
$PGF_{2\alpha}$	PGF (FP)-G protein coupled receptor—the F prostanoid receptor	FPA	Ovary, myometrium, ocular vasculature, iris sphincter, ocular circular muscles Renal distal convoluted tubule, cortical collecting duct, juxtaglomerular apparatus	Luteolysis, parturition, uterine contraction, aqueous humor homeostasis, water, and electrolyte reabsorption Renin secretion, blood pressure regulation—*pregnancy-induced hypertension, pulmonary and myocardial fibrosis, arrhythmias, myocyte hypertrophy, VSMC hypertrophy, vasoconstriction, atherosclerosis*
		FPB		

AA metabolite	Receptor	Location	Function in health and in the disease
		Lung and cardiac fibroblasts; cardiomyocyte, VSMC	
PGI_2	IP	IP_1 IP_2	Blood platelets, VSMC, sensory afferent nerves, thymus (medulla), spleen, heart/aorta, lung — Local control of vascular tone, platelet aggregation—*hypertension*

TXA2, thromboxane A2; TP, thromboxane receptor; PGD2, prostaglandin D2; DP, prostaglandin D2 receptor; PGE_2, prostaglandin E_2; EP, prostaglandin E_2 receptor; $PGF_{2\alpha}$, prostaglandin F_2alpha; FP, prostaglandin F receptor; PGI_2, prostacyclin; IP, prostacyclin receptor.

Table 1. Eicosanoids and their receptors.

The distribution of EP_1 receptors is restricted to kidney, lung, and stomach. In kidney, they are mainly expressed in collecting duct and can be detected in glomerular mesangial cells, podocytes, and proximal tubule cells. The relationship between EP receptor and blood pressure (BP), indicated by Stock et al. [21] in EP_1 null mice, results from the observed increased concentrations of renin and aldosterone, ongoing activation of the renin-angiotensin system (RAS) and disrupted response to angiotensin II [22].

The EP_2 receptors can be found in vascular and interstitial compartments of the kidney and they are the least abundant among the EP receptors but effectively activate in response to stimuli. PGE_2 evokes contractile and/or relaxant responses of vascular smooth muscles in vitro. Lack of EP_2 receptor and dysfunction of PGE_2 pathway may be involved in elicitation of the salt-sensitive hypertension in EPP/2 mice (mice lacking EP2 receptor). Their results indicate that PGE_2, produced in the body in response to a high-salt (HS) diet, evoked considerable hypertension. It is proposed that the absence of the EP_2 receptor abolishes the ability of the mouse vasculature to vasodilate in response to PGE_2 and unmasks the contractile response via the vasoconstrictor EP receptor(s). EP_2 receptors and EP_4 can also be found in endometrium. The amount and localization of these receptors change during pregnancy, which may correspond to changes in uterine contraction during fertilization and implantation [14].

EP_3 receptors are expressed in smooth muscle layer and kidney. Renal EP_3 is mostly recognized for its pressor effects and its diuretic role opposing vasopressin. It is highly expressed in the distal nephron (mostly in the cortical and medullary collecting duct) [23].

DP is the least expressed receptor found in very low levels in small intestine and brain in humans [24] and moderately expressed in the ileum, lung, stomach, brain, and uterus [25] in mice. FP receptors are mainly expressed in corpus luteum and found to be variable during the estrous cycle indicating a close relationship between FP gene expression and luteolysis. PGF2α is a physiological inducer of luteolysis in pregnancy. Independent of estrous cycle, expression of mouse FP mRNA was found in the kidney, heart, lung, and stomach [26, 27].

Thromboxane A_2 (TXA_2) is a potent vasoconstrictor and a pro-aggregatory substance. The balance between PGI_2 and TXA_2 in the circulation is important for cardiovascular homeostasis [17]. TXA_2 binds to the thromboxane-prostanoid (TP) receptors which are located on platelets and their activation causes platelet aggregation, while in vascular smooth muscle cells, TXA_2 causes vasoconstriction and smooth muscle cells proliferation [4, 5].TP receptors appear to be solely responsible for endothelium-dependent contractions. Endoperoxides (PGH_2) and higher concentrations of prostacyclin (in vascular smooth muscle lacking IP receptor sensitivity) and isoprostanes activate this receptor with a varying range of potency. Role of TP receptors in hypertensive process was shown by Tian et al. on renovascular hypertensive (RVH) rats. They showed that TP receptor hyperresponsiveness to vasoconstrictors and IP receptor insensitivity leads to endothelial dysfunction [6].

4. LOX pathway

The LOX pathways (5, 12, and 15-LOX) of AA metabolism generates eicosanoids (hydroxyeicosatetraenoic acids, HETEs: 12 (S)-HETE, 12 (R)-HETE, and 15 (S)-HETE), lipoxins (LXs), and leukotrienes (LTA_4, LTB_4, LTC_4, LTD_4, and LTE_4) [28]. All of these metabolites have crucial role in pulmonary responses to asthma, inflammation, and atherosclerosis [29–31]. 5-LOX products have been found to be harmful factors in pathological conditions, including cardiovascular and renal diseases [32]. Leukotrienes produced by 12-LOX and 15-LOX cause dilatation, while the 5-LOX generated leukotrienes (LTs), the major LO metabolites associated with vasoconstriction, increased pro-inflammatory cytokines production and also behave as chemotaxins in the blood vessels by recruiting inflammatory cells. LTs are established mediators of pulmonary inflammation and in allergic and pseudoallergic reactions [33] also known as slow-reacting substances of anaphylaxy (SRS-A) which can be produced in basophils and mast cells. Research over the past two decades has established that LTs modulate inflammation in pulmonary arterial hypertension (PAH) [34]. Since LOX pathway does not have much influence on the peripheral vascular function, and pulmonary circulation and pulmonary hypertension are not in the focus of this chapter, the further effects of LOX pathway of AA metabolism will not be discussed here. For comprehensive reading, one may explore many interesting papers, including [35–37].

5. CYP450 pathway

In addition to COX and LOX, CYP450 enzymes (with their numerous isoforms) represent a crucial path in AA metabolism, catalyzing epoxidation reactions (producing EETs) and omega (ω)-hydroxylation reactions (producing 20-HETE) [38]. Two distinct enzymes: CYP-hydroxylase enzymes (CYP4A and CYP4F) generate HETEs (16-, 17-, 18-, 19-, and 20-HETE) while CYP-epoxygenase enzymes generate EETs (5,6-, 8,9-, 11,12-, and 14,15-EET). The CYP epoxygenases, members of CYP2C (predominant in mammals) and CYP2J classes of enzymatic proteins, are primarily located in endoplasmic reticulum of endothelial cells. They convert AA to EETs by

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adding an epoxide across one of the four double bonds in AA and produce the four mentioned EET regioisomers. CYP epoxygenases demonstrate a very high sequence homology among different species (e.g. human CYP2C8, rat CYP2C23 and mouse CYP2c44) [39–41]. Beside CYP2C8, human arteries and arterioles express CYP2C9, CYP2J2, and soluble epoxide hydrolases (sEH) enzymes [42, 43]. sEH converts EETs to their corresponding diols, dihydroxyeicosatrienoic acids (DHETs), which represent the main EET catabolic pathway. EETs produced by the endothelium hyperpolarize vascular smooth muscle cells VSMCs by opening Ca^{2+}-activated K^+ (K_{Ca}) channels leading to vasodilatation. In contrary, 20-HETE is found to be a vasoconstrictor that inhibits the opening of K_{Ca}.

Key vascular effects of CYP epoxygenase-derived EETs, besides regulating vascular tone and angiogenesis, include autocrine anti-inflammatory actions, limitation of leukocyte adhesion and reducing VSMCs proliferation. They have been found to exhibit protective effects in myocardial and cerebral ischemia and in hypertension-induced renal damage [44].

EETs are also able to modulate vascular responses to other stimuli, such as hormonal and paracrine agents. For instance, vasopressin-induced increase in cytosolic Ca^{2+} in renal mesangial cells is amplified by EETs and reduced when EETs synthesis is inhibited [45]. The responses of afferent arterioles to angiotensin II, endothelin-1, and noradrenaline increase when EETs synthesis is inhibited [45]. In transgenic rats with angiotensin II-dependent hypertension, EETs were shown to be antihypertensive and cardioprotective [46]. Inhibition of EETs synthesis reduces glutamate-induced increase in cerebral blood flow response [47]. Streptozocin-induced diabetes in rats (a model for type 1 diabetes mellitus, DM) reduces the levels of protective EETs, and reduced EETs levels lead to exacerbation of stroke [48]. A similar protective role of EETs was demonstrated in diabetic nephropathy [49], atherosclerosis [50], and cardiac ischemic reperfusion injury in diabetic rats [51]. Reduced CYP activity and EETs production caused by high glucose (through elevated superoxide levels) in coronary endothelial cells has also been implicated in impaired endothelium-dependent vasodilation of coronary arterioles [52]. EETs might constitute a key link between insulin resistance and endothelial dysfunction [53]. Upregulation of the CYP2J group of isoforms in mice (which catalyze EETs formation) leads to attenuation of diabetic nephropathy induced by streptozocin [54].

Both EETs and HETE exert numerous biological signaling effects, including important roles in the vasculature, vessel diameter regulation, and tissue perfusion. EETs function as endothelium-derived hyperpolarizing factor and show mostly vasodilator properties, with their vasodilator effects comparable to that of ACh [46], but some EET subtypes can also mediate vasoconstriction—for example, in kidneys where they can cause constriction of the afferent arterioles [46, 55]. 20-HETE is a potent vasoconstrictor and plays an essential role in the myogenic and tubuloglomerular feedback responses in the afferent arteriole, participating in blood pressure control [56].

Many evidences suggest that alteration in EET pathway contributes to the pathophysiology of hypertension, including BP elevation, endothelial dysfunction, and end-organ damage [44]. Also, EETs have anti-inflammatory and angiogenic function [32]. Imbalance of vasoactive

eicosanoids leads to ischemia, thrombosis, coagulopathy, myocardial infarction, and stroke as discussed further in the chapter.

6. Nonenzymatic metabolism of AA

ROS are produced in all layers of the vascular wall by all vascular cell types (endothelium, smooth muscle, and adventitial cells) and by perivascular adipocytes. ROS generated by endothelial cells are superoxide ($O_2\cdot$), hydrogen peroxide (H_2O_2), hydroxyl radicals (HO), and others. Potential sources of endothelial ROS generation include mitochondria, xanthine oxidase (XO), uncoupled NO synthases (NOS), lipoxygenases, cytochrome P450 enzymes, and nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidases [57]. ROS are formed on sites of inflammation and injury; at low concentrations, ROS can act as a signaling molecule involved in the regulation of fundamental cellular activities such as cell growth and cell adjustments and regulation of endothelial function, while at higher concentrations, ROS can cause cell injury and death. Increased concentration of ROS is associated with changes in endothelial signal transmission and redox-regulated transcription factors in inflammation which may be related to endothelial dysfunction and activation of pathological mechanisms [58]; for example, the development of hypertension. This includes promoting the growth of VSMCs, increased contractility and invasion of monocytes and inflammation, increased permeability of vascular endothelium and enhanced adhesion of leukocytes [58].

ROS can induce peroxidation of AA, which gives rise to the isoprostanes. Several scavenger systems are counteracting the ROS, including enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), and nonenzymatic antioxidants such as vitamin E, vitamin C, β-carotene, and heme-binding proteins, and major of them is SOD [59].

ROS can directly act as endothelium-derived contracting factor (EDCF) [60] or indirectly potentiate EDCF-mediated responses by reducing the bioavailability of NO [61] and activating COX in the VSMCs [3]. Their overproduction leads to increased oxidative stress and development of endothelial dysfunction [62]. Thus, endothelial dysfunction impairs vascular function in various diseases, cardiovascular and endocrine-metabolic disorders. Oxidative stress is characterized by reduced bioavailability of NO and enhanced production of ROS [63] which can exhibit both activating and inhibitory effects on the eicosanoid metabolism, and PGI_2 synthesis is more sensitive then TX and LOX pathways. In blood vessel, production of PGI_2 is selectively inhibited by ROS, whereas TXA_2 synthase is unaffected [64]. ROS can modify vascular tone directly by acting as EDCF or indirectly by reducing the bioavailability of NO which potentiate EDCF-mediated responses.

Urinary isoprostane levels are used as biomarkers of oxidative stress in ischemic-reperfusion injury, atherosclerosis, and hepatic disease. An accumulated body of evidence suggests that there is a cross-talk between 20-HETE and ROS production in response to flow- and pressure-induced stimuli in human and experimental animal microcirculation [65, 66]. Novel data show an association between increased CYP4A activity and oxidative stress in human subjects with hypertension. Increased urinary 20-HETE excretion correlated positively with markers of

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oxidative stress and with elevated BP [67]. Similarly, patients recovering from acute ischemic stroke have increased plasma 20-HETE concentrations and elevated plasma oxidative stress markers compared to healthy controls [68].

7. Endothelial dysfunction and metabolites of arachidonic acid

Eicosanoids play an important role in maintenance of vascular reactivity under physiological conditions, but they become deleterious to endothelial function and BP regulation in some conditions with imbalance in the PGI_2/TXA_2 system, in chronic activation of CYP4A and enhanced production of 20-HETE or lack of EETs, as well as in high oxidative stress conditions. At the beginning of the endothelial dysfunction development, there is a compensatory endothelial mechanism of prostacyclin and/or endothelium-derived hyperpolarizing factor (EDHF), which maintains vascular function (**Figure 2**).

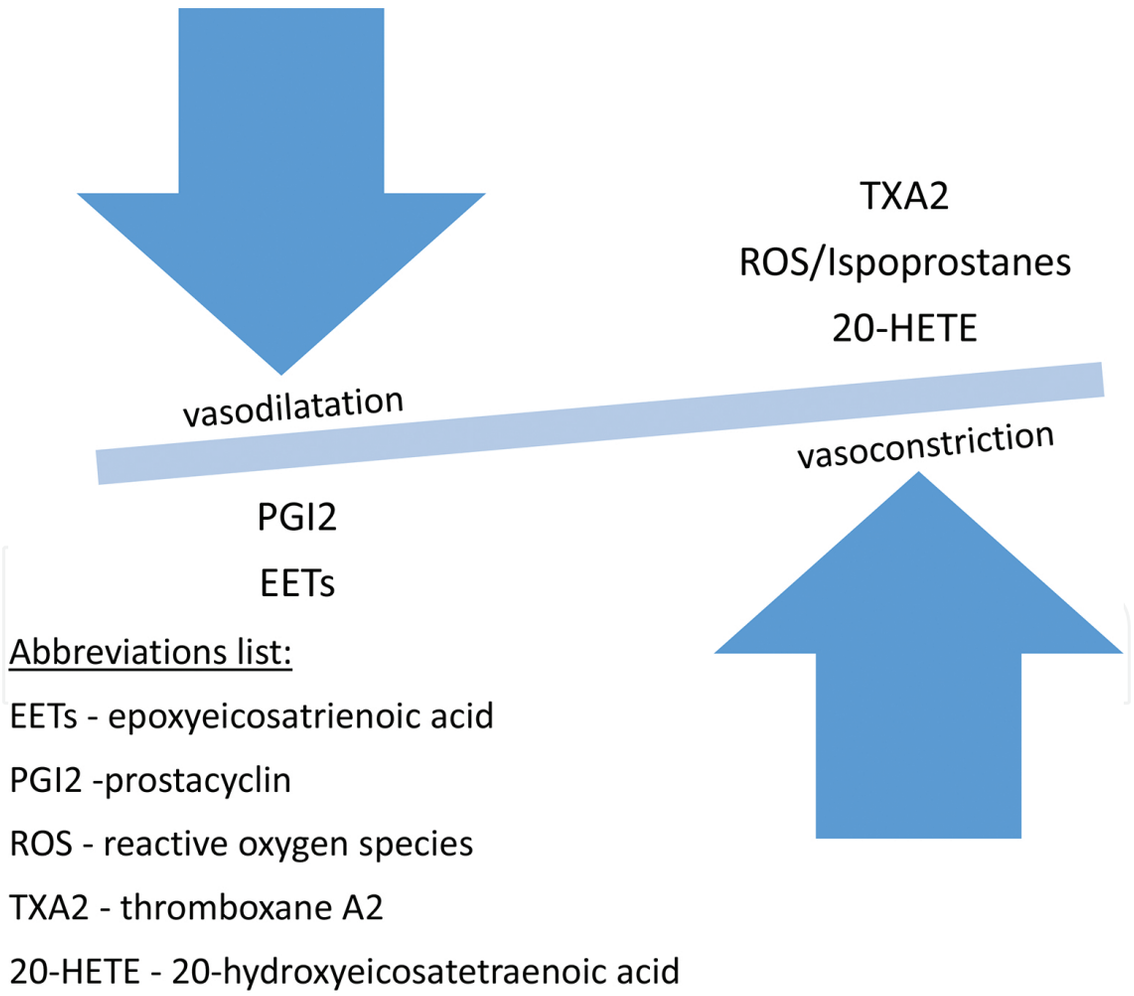

Figure 2. The role of AA metabolites' disbalance in endothelial dysfunction. EETs, epoxyeicosatrienoic acid; PGI2, prostacyclin; ROS, reactive oxygen species; TXA2, thromboxane A2; 20-HETE, 20-hydroxyeicosatetraenoic acid.

Except reduced NO bioavailability and increased ROS production, in some pathological conditions such as hypertension, diabetes, and obesity, there is an overproduction of COX-2-derived prostanoids. Endothelial dysfunction is a hallmark of most cardiovascular and endocrine/metabolic diseases.

8. Obesity

Obesity is associated with microvascular endothelial dysfunction in experimental animal models as well as in obese humans [69, 70]. Human studies have shown that endothelial function of visceral adipose tissue vessels is more damaged compared to subcutaneous adipose tissue vessels [70, 71], and that visceral microenvironment is intrinsically more toxic to the vasculature. Also, COX-derived vasoconstrictors partly contribute to endothelial dysfunction [71, 72]. There is a large body of evidence that endothelial dysfunction precedes and predicts clinical disease [73, 74], suggesting that endothelial dysfunction and impaired vascular reactivity is an initial step in the development of cardiovascular complications caused by obesity. Therefore, endothelial dysfunction in adipose tissue may be a strong prognostic factor for future cardiovascular events [75].

In obesity, chronic exposure of endothelial cells to high levels of circulating fatty acids increases the formation of ROS, which further leads to a disbalance between vasodilation and vasoconstriction leading to net vasoconstriction. Vascular production of PGs can be also altered in obesity because endothelial production of the superoxide anion contributes to enhanced COX expression [76].

Vasodilation in response to flow is reduced in visceral compared to subcutaneous arterioles, and the COX metabolites have been shown to participate in the mechanisms of endothelium-dependent dilation in subcutaneous adipose tissue resistance arteries [70, 71]. Furthermore, ROS (mainly H_2O_2) participate in that response, while the metabolites of the CYP450 partially contribute to dilation of microvessels from both subcutaneous and visceral adipose tissues [70].

In pathological conditions, such as cardiovascular disease and obesity, NO bioavailability is reduced and other endothelium-derived dilator substances compensate for the lack of NO release during flow or agonist activation [70, 77]. The inhibition of NOS augments the contribution of CYP450 metabolites to vasodilation [78], suggesting that EDHF may function as a compensatory mechanism when NO synthesis is impaired in obesity. It is also possible that CYP450 metabolites and COX enzymes are sources of ROS [79], whose production is enhanced in obesity and cardiovascular disease [70, 77].

Taken together, data suggest that endothelium-derived dilator substances other than NO (i.e. H_2O_2 and metabolites of CYP450) may contribute to vasodilation in obesity [70]. Previous studies in the coronary circulation have shown that the P450 component to dilation is present during coronary disease when NO levels are reduced [80]. Therefore, it is possible that the P450 component to dilation in adipose tissue is conserved during disease in the presence and the absence of NO-dependent vasodilation.

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9. Arachidonic acid metabolites and hypertension

High BP represents a major risk factor for coronary artery disease, stroke, heart failure, peripheral vascular disease, vision loss, and chronic kidney disease [81, 82]. Reduced releasing of NO and enhanced production of EDCF increase contraction and lead to vascular diseases such as hypertension [83]. Chronically increased arterial BP presumably can cause premature aging of the intima, arterial remodeling, and smooth muscle cells dysfunction. Proper functioning of the endothelium and delayed appearance of vascular complications caused by hypertensive process can be managed by increasing the availability of NO, favoring the EDHF-mediated responses, and preventing the release or action of EDCFs.

Ever since the 1990s when the COX-pathway-independent EDHFs were discovered, substantial number of studies demonstrated CYP450 metabolites of AA to be that other source and these newly discovered EDHFs (EETs and HETEs) were proven to have influence in pathophysiology of many diseases including hypertension. Production of vasoconstrictor cyclooxygenase products, especially ROS, contributes to the development of endothelial dysfunction in hypertension [84, 85]. Until today, wide array of techniques and different types of blood vessels have been comprehensively investigated to discover EET-mediated VSMC signaling mechanisms. Prevailing conclusion of these studies is that EETs activate K^+ channels in vascular smooth muscle cell and in particular the large-conductance K_{Ca} channels causing cell membrane hyperpolarization [86–88]. Another consistent finding has been increase in cAMP in response to EETs, and this signaling pathway has been associated with vasodilation [86–88]. To achieve vasorelaxations, EETs and other EDHFs also require a guanine nucleotide binding protein (protein G) [89, 90]. Capdevila and Falck showed in their study that the EDHF-mediated response (therefore EETs) becomes evident only in the state of reduced NO bioavailability meaning that physiological concentration of NO is adequate to moderate EDHF generation and, under physiological conditions, endothelium-dependent vasodilatation seems to be mainly dependent on NO production [91]. EETs are also contributing to shear stress-dependent hyperpolarization and dilation of skeletal muscle arterioles in mice [92–94]. eNOS-deficient mice have had intact vasorelaxation to shear stress and a reduced vasodilation to ACh also suggesting that EETs can possibly enlarge their contribution to attenuate vascular resistance when NO levels are reduced demonstrating inhibitory interaction between NO and EETs [95]. Another study on essential hypertensive patients showed also connection between impaired NO availability and alternative mechanism of endothelium-dependent vasodilatation, related mainly to compensatory CYP2C9-derived EDHF [96]. Experimental findings in humans have also determined that NO and CYP epoxygenases regulate arterial stiffness in response to flow variations [97]. Early observation on the possible contribution of EETs to BP control and hypertension came from studies on rats treated with an epoxygenase enzyme inhibitor who became hypertensive when fed high salt [98–100]. Study in transgenic mice which expressed human CYP2J2 and CYP2C8 epoxygenases in endothelium and increased endothelial EET biosynthesis has proved that endothelial CYP epoxygenases regulate BP [101]. In fact, these mice exhibit enhanced afferent arteriolar dilation, lower BP, and attenuated hypertension-induced renal injury compared to wild type [101]. These findings suggest the potential therapeutic utility of antihypertensive strategies that may increase CYP-derived

EETs. Recently, a protective role of CYP2J2-derived EETs was found in heart failure [102], suggesting that CYP2J2-derived EETs may be a target for the development of drugs to prevent cardiac hypertrophy and cardiomyocyte apoptosis in heart failure [44]. Sinal et al. showed that mice lacking the sEH gene (epoxide hydrolase 2, Ephx2-/-) have significantly higher circulating EET levels and lower BP compared to wild-type mice. Renal production of DHETs was decreased and EET formation increased in the Ephx2-/-mice, also suggesting an important role for epoxygenase metabolism in the regulation of BP [103]. In addition, the administration of a sEH inhibitor (sEHI) significantly lowers BP in various rodent models of hypertension [103, 104]. The administration of a single dose of an sEHI (*N*,*N*-dicyclohexylurea, DCU) to ANG II-infused rats greatly increased the level of EETs, decreased the urinary DHET excretion, and lowered systolic BP, thus reversing the hypertensive phenotype typical of the spontaneously hypertensive rats (SHR) [105, 106]. However, adverse events may occur in the pulmonary vasculature. EETs, generated in VSMCs of pulmonary blood vessels, increase intracellular Ca^{2+}, thus inducing vasoconstriction and increasing pulmonary artery pressure [107].

10. Role of 20-HETE in hypertension

20-HETE is a CYP450–derived omega-hydroxylation metabolite of arachidonic acid and plays a complex role in blood pressure regulation. 20-HETE biosynthesis is primarily localized to the VSMCs [108], with the exception of endothelium in the pulmonary circulation which may also produce 20-HETE [109]. In physiological conditions, NO, carbon monoxide (CO) and superoxide inhibit the formation of 20-HETE by binding to the heme binding site of the CYP450 pathway enzymes. A role of 20-HETE in NO homeostasis was first suggested by Frisbee et al [110]. They showed that 20-HETE decreases the effect of ACh-induced relaxation in cremasteric arterioles. Studies using endothelial cells demonstrated that 20-HETE stimulates superoxide production by mechanisms that include eNOS uncoupling and activation of NAD(P)H oxidase-dependent and -independent pathways [110–113]. The rate of 20-HETE biosynthesis is inversely proportional to the blood vessel diameter [114]. 20-HETE is not detected in large conduit vessels [115].Therefore, it is largely believed that 20-HETE is an eicosanoid of the microcirculation [116]. In the microvasculature, 20-HETE has been shown to play a pressor role by sensitizing VSMCs to constrictor stimuli and increasing myogenic tone and by acting on the endothelium to further promote endothelial dysfunction and endothelial activation [116].While the formation of 20-HETE in VSMC is stimulated by angiotensin II and endothelin and is inhibited by NO CO, inhibition of 20-HETE synthesis attenuates the vascular responses to angiotensin II, endothelin, noradrenaline, NO, and CO [115]. Other autacoids can also stimulate 20-HETE production, for example, serotonin (5-hydroxytryptamine, 5-HT], and other growth factors [117, 118]. Liu et al. showed that inhibitors of COX-2 increase the levels of 20-HETE [119]. The report by Sacerdoti et al. [120] was the first to implicate 20-HETE in the pathogenesis of hypertension showing that depletion of renal CYP450 normalizes BP in SHR rats. ANG II-mediated hypertension in rats can be decreased by 40% by inhibition of 20-HETE synthesis [116]. Contribution of 20-HETE to blood pressure regulation include diet-, age-, and sex-specific alterations in the expression of CYP enzymes that produce 20-HETE [121]. In

pregnancy-induced hypertensive women, the urinary excretion of DHETs is increased compared to healthy pregnant women, which may implicate an increased degradation of EETs [122].

On the contrary, 20-HETE is contributing to antihypertensive mechanisms too; it is involved in the regulation of the pressure-natriuretic response by inhibitory acting on sodium reabsorption and promoting natriuresis in the kidney tubules [123]. HET0016, which is cytochrome P450 ω-hydroxylase inhibitor, attenuates cerebrovascular inflammation, attenuates oxidative stress, and improves vasomotor function in spontaneously hypertensive rats [124].

RAS is the crucial in regulation of body fluid volume and blood pressure [125]. ANG II increases blood pressure by (1) vasoconstriction via AT1R activation, increased sympathetic tone, and the release of arginine-vasopressin and (2) modulation of renal sodium and water reabsorption by stimulating renal AT1R, the production and release of aldosterone, or the sensation of thirst in the central nervous system [116].

ANG II stimulates 20-HETE synthesis in renal microvessels and decreases EET levels by downregulating epoxygenases and increasing their degradation by increasing expression and activity of sEH [117, 126, 127]. In conditions, such as renovascular disease (RVD), there is an increase in the expression of the renin-angiotensin system, associated with enhanced lipid peroxidation related to activation of the renin-angiotensin system [125], elevated levels of ANG II, which parallels an increase in plasma 20-HETE and a decrease in EET plasma levels that supports a pivotal role of EETs in vascular homeostasis [125]. RVD is a relatively rare form of secondary hypertension [128, 129]. The interactions between 20-HETE and the RAS occur at several levels. Increased production of 20-HETE in the peripheral vasculature contributes to the acute vasoconstrictor response to ANG II, whereas acute and chronic inhibition of 20-HETE synthesis attenuates the renal pressor response to ANG II and the development of ANG II-dependent hypertension [116], respectively. The infusion of angiotensin II (ANG II), a potent vessel constrictor, elevates blood pressure in various animal models [106].

In RVD, plasma 20-HETE significantly correlated with plasma renin activity, thus suggesting its role in the elevation of blood pressure through the possible increase of vasomotion and vascular reactivity [130]. All of presented studies suggest that there is a communication network among various eicosanoids. In physiological conditions, eicosanoids are important in the maintenance of vascular tone and reactivity, but in chronic activation of CYP4A/20HETE system or lack of EETs and high oxidative stress, they become deleterious to endothelial function and blood pressure regulation.

11. Arachidonic acid metabolites and high-salt diet

It is well known that increased NaCl intake is an important risk factor for development and progression of hypertension [131, 132], while a reduction in dietary sodium is associated with lowering of BP in many patients with essential hypertension [133]. Furthermore, some studies on normotensive animal models have shown that changes in NaCl intake determined vascular

responses to various physiological stimuli, in conduit vessels and resistance arteries, as well as in the microcirculation [134–136].

In contrast to studies which demonstrated deleterious effects of high levels of ANG II and RAS activation on the BP levels [125], there are studies demonstrating that decrease in ANG II circulating levels leads to impaired microvascular endothelial function. This has been recently extensively reported in the paper by Boegehold et al. [137]. One of the most deleterious effects of HS intake is impaired endothelial function [132], and it is crucial to evaluate the altered vascular function in microcirculation because it is a target of the pathological events. In animal models, even a short-term HS diet impairs vascular function by altering the responses to both vasoconstrictor and vasodilator stimuli in different vascular beds [134–136], that is associated with overproduction of vasoconstrictor factors, TXA_2, and PGF2α [134, 136].

In their study, Cavka et al. have shown that one week of HS diet increased plasma levels of potent vasoconstrictor, TXB_2, and that the nonselective COX antagonist indomethacin restored blood flow, whereas the selective COX-2 inhibitor did not cause any change in the impaired hyperemic blood flow in healthy young women [138]. Further, both inhibitors reduce plasma TXA_2 levels, suggesting that some other vasoconstrictor dominantly derived by COX-1 may play important role in impaired microvascular reactivity in subjects on the HS diet. Short-term exposure to a HS diet alter AA metabolism, and COX enzymes (mainly COX-1) play an important role in the development of microvascular endothelial dysfunction [138].

As already mentioned, in vascular pathogenesis, there may be a disbalance where COX-derived constrictors become dominant over the prostacyclin which is usually responsible for vasodilatation under physiological conditions [3, 139]. In endothelial dysfunction, endothelial cells became a source of COX-derived constrictors and enhanced oxidative stress may modify COX-dependent function leading to damaged vascular tone [9] due to decreased NO bioavailability and an increased formation of EDCFs [140]. In animal models, COX-1 metabolites are responsible for endothelium-dependent contractions, but with aging or disease, COX-2 can be induced contributing to EDCF-mediated responses [140, 141].

HS-induced endothelial dysfunction is caused by decreased plasma concentration of ANG II which leads to increased oxidative stress [139, 142, 143], as demonstrated in SS.BN13 consomic rats studies [139], leading to impaired relaxation of middle cerebral artery (MCA) in response to hypoxia and ACh due to decrease in vascular antioxidative capacity [144]. Importance of ANG II is further supported by intravenous infusion of suppressor dose of ANG II during HS diet which restores normal vascular relaxation and restores ROS concentration to normal values [145].

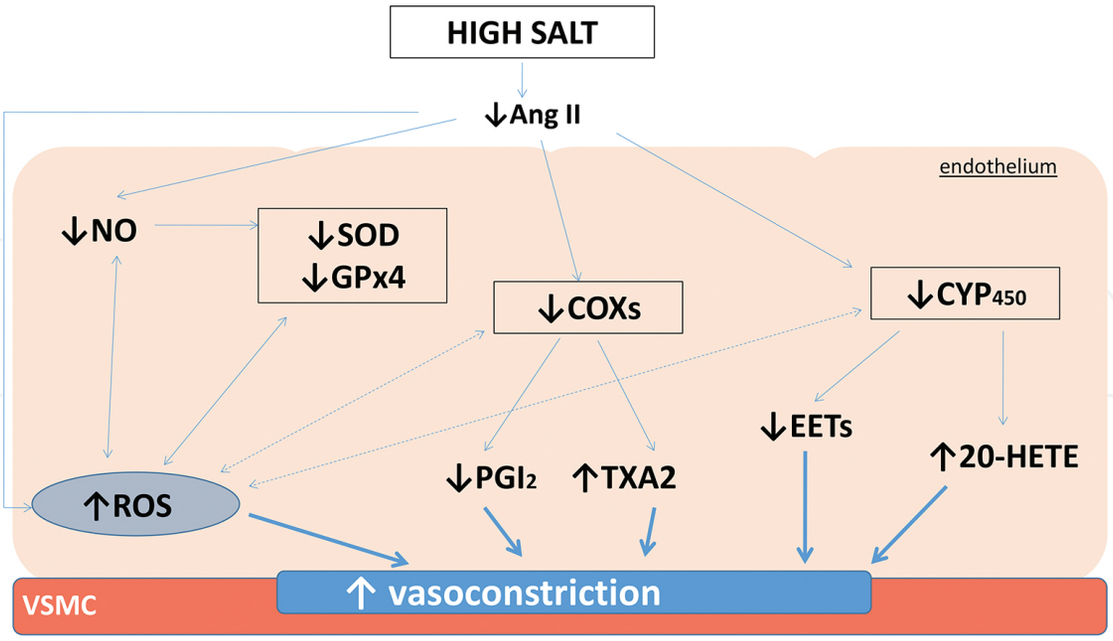
Antioxidative systems are very important in the maintenance of cellular redox homeostasis and prevent excessive accumulation of $O_2^{-}\cdot$ and its reactive metabolites [146]. Reduced activity of antioxidant mechanism, alone or in combination, with increased O_2^{-} production, may contribute to increased vascular $O_2^{-}\cdot$ level associated with a high intake of salt [144]. Several ubiquitous primary antioxidant enzymes such as SOD, catalase, and peroxidase catalyze the conversion of ROS in more stable molecules such as O_2 and water. Until now, research of the influence of high salt intake was based exclusively on changes in the level of SOD isoforms.

Lenda et al. presumed that HS diet decreased the protein expression or activity of the antioxidant enzymes (SOD isoforms) leading to increase oxidative stress, and consequently, reduced dilatation of blood vessels [143, 147]. However, this effect is not uniform at each vascular bed; for example, HS intake has no effect on the expression of CuZn SOD or MnSOD in mesenteric arteries [146] or the expression of CuZn SOD in the arteries of skeletal muscle [141]. Studies on Ren1-BN congenic rats showed that a short-term increase in dietary salt intake reduces the expression of the Cu/Zn SOD and Mn SOD in the cerebral vasculature and that ANG II infusion prevents the reduction of Cu/Zn SOD expression, but not Mn SOD expression, in HS-fed animals [143]. Recent studies showed that, except for reduced protein levels of SOD isoforms, HS intake also significantly reduced the level of mRNA expression of glutathione peroxidase 4 GPx4, very important enzyme in maintaining reduced levels of lipid peroxidation and oxidative stress [142]. Treatment with TEMPOL [136, 148] (which is a SOD mimetic) returns the NO level to the concentrations similar to the ones in the animals on a normal salt diet which indicates that the $O2^{-}\cdot$ is responsible for the oxidation of NO under these conditions. HS diet promotes increased generation of superoxide anion from NOS in spinotrapezius muscle arterioles of C57BL/6J mice, thus impairing endothelium-dependent dilation through reduced NO bioavailability [149].

There are several possible sources of $O_2^{-}\cdot$ in the vascular wall such as mitochondrial respiratory chain, NAD(P)H xanthine oxidase, COX, CYP-450 enzyme, and the NOS [150]. When the bioavailability of NO is greatly reduced, as is the case during HS diet, endothelium activates various compensatory physiological pathways. Impaired endothelium-dependent vasodilation is maintained partially by the production and release of other endothelial vasodilator other than NO, such as prostanoids (prostacyclin) and other endothelium hyperpolarizing factors (EDHFs). In endothelial dysfunction, besides ROS, other harmful metabolites of the arterial wall are formed, that is, TXA_2 and PGH_2 [151]. Endothelial dysfunction is related to pathogenesis of thrombosis and atherosclerosis [151, 152]. mRNA expression of COXs (COX-1 and COX-2) showed a significant reduction of both isoforms in the brain blood vessels after a week of HS intake [153]. New functional studies demonstrated that the flow-mediated dilatation in isolated cerebral arteries of Sprague-Dawley (SD) rats on a high salt is not mediated by COXs neither with EETs [154], which is a further evidence of the abovementioned results of the molecular studies.

Lombard et al. reported that the production of TXA_2 and PGI_2 was altered by HS diet, TXA_2 contributed to impaired vascular response to reduced oxygen partial pressure (PO_2) in animals on HS diet, and that MCA of animals on a HS diet decreased the production of PGI_2 in hypoxic conditions [155].

Taken together, there is a cross-talk between the enzymes producing the vasoactive metabolites and ROS—ROS may be the side-product of impaired activation of COX, NOS, or CYP450 enzymes together with NAD(P)H oxidase activation, and simultaneously, ROS may affect the production of vasoactive metabolites of COX, shifting the production of them from vasodilators to vasoconstrictors and affecting the bioavailability of NO [153, 156, 157] (**Figure 3**).



Abbreviations list:
Ang II - angiotensin II
CAT - catalase
COXs – cyclooxygenases
CYP 450 - cytochrome P450
EETs - epoxyeicosatrienoic acid
GPx 4- glutathione peroxidase 4
NO – nitric oxide
PGI2 -prostacyclin
ROS - reactive oxygen species
SOD - superoxide dismutase
TXA2 - thromboxane A2
VSMC- vascular smooth muscle cell
20-HETE - 20-hydroxyeicosatetraenoic acid

Figure 3. Schematic overview of the influence of high salt intake to increased oxidative stress and reduced vasodilation. Ang II, angiotensin II; CAT, catalase; COXs, cyclooxygenases; CYP 450, cytochrome P450; EETs, epoxyeicosatrienoic acid; GPx 4, glutathione peroxidase 4; NO, nitric oxide; PGI2, prostacyclin; ROS, reactive oxygen species; SOD, superoxide dismutase; TXA2, thromboxane A2; VSMC, vascular smooth muscle cell; 20-HETE, 20-hydroxyeicosatetraenoic acid.

12. Arachidonic acid metabolites and diabetes mellitus

It is clearly recognized that elevated plasma concentration of glucose is responsible for the pathogenesis of vascular complications associated with DM; hyperglycemia can modify vascular function—it compromises the endothelium-dependent relaxation, increases the contractile response of vascular smooth muscle and the development of inflammatory, thrombotic, and atherosclerotic events.

Impaired endothelium-dependent vasodilatation has been shown in various vascular beds of different animal models and in human with DM [62]. In patients with DM type 2 and in diabetic mice, reduced production on NO, increased generation of ROS, and enhanced vasoconstrictor

tone were related to impaired endothelium-dependent vasodilation [158]. This attenuated vascular response includes multiple mechanisms, but it seems that increased oxidative stress is the first alteration that triggers more others. Similar to findings in hypertension and high salt diet, endothelial dysfunction in diabetes could also be related to the release of vasoconstrictor mediators, for example, increased production of 20-HETE leading to activation of ROS through an NAD(P)H-dependent pathway. This may have an important therapeutic potential in the treatment of diabetic vascular complications, for example, nephropathy [159].

The impaired endothelium-dependent dilation to ACh in diabetic animals is due to the accompanying release of EDCF and can be attributed to the exposure of the endothelial cells to high blood glucose level, causing increased oxidative stress and overexpression of both COX-1 and COX-2 [160]. Also, as previously mentioned in the paragraph on COXs metabolites and ROS, increased ROS production may determine vasoconstrictor response. Exogenous administration of AA in diabetic dogs induces TXA_2-mediated contraction, while increases the prostacyclin-mediated vasodilation in the arteries of control dogs [161]. ACh-induced vasodilation of diabetic aortas, mesenteric arteries, and femoral arteries is reduced, but COX inhibitors improve that response [162]. Some studies have shown that hyperglycemia increases the expression of COX-2, in large blood vessels and in microcirculation, leading to increased production of vasoconstrictor prostanoids which modify vascular reactivity [162]. Numerous data indicate that there is an increase the release of ROS from endothelial cells in DM, especially superoxide anion, which is thought to be particularly responsible for the increased COX-2 expression [62]. Hyperglycemia increases the release of AA, modifies the formation and function of prostanoids, and thus induces modification of vasomotor tone [62].

Other enzymes, which metabolize AA, are affected by diabetes. Diabetes alters CYP expression and 20-HETE formation, leading to upregulation of CYP4A isoforms and to elevated levels of 20-HETE [115]. The 20-HETE inhibitor HET0016 attenuates the development of diabetes-induced vascular dysfunction, suggesting a contribution of 20-HETE to endothelial dysfunction in diabetes and other insulin-resistant conditions [163]. Recent findings also suggest that 20-HETE impairs insulin-stimulated vasodilator effects that are mediated by the IRS-1/PI3K/AKT/eNOS pathway [163]. Elevated levels of CYP-derived 20-HETE in diabetic patients with cardiac ischemia are associated with dysfunction of circulating endothelial progenitor cells and angiogenic capacity [164]. On the other hand, experiments have indicated that in streptozocin-induced diabetic rats (with impaired endothelial function and contractile responses), the vascular CYP2E1 is significantly increased, leading indirectly to a reduction in the levels of the potent vasoconstrictor 20-HETE (by inhibiting CYP4A enzymes). Preincubation of vessels in vitro with 20-HETE rescued contractile functions, suggesting that the role of 20-HETE in diabetes-induced vascular dysfunction is complex, although those experiments were conducted on aortic vascular models and not in microcirculation [165].

In experiments conducted on two animal models (streptozocin-treated rats) with different levels of glucose metabolism impairment—glucose intolerance model and diabetic model, the expressions of CYP enzymes involved primarily in production of EETs (CYP2J4, CYP2C23) and HETE(CYP4A2 and CYP4A3) were compared, as well as sEH (which degrades EETs) [166]. In the glucose intolerance model, increased degradation of EETs by elevated expression of

soluble epoxide hydrolase might contribute to endothelial dysfunction. Findings in the diabetic model suggest a different mechanism, primarily a shift in the balance between EETs and 20-HETE production caused by changes in CYP2J4 and CYP4A3 expression [166–168].

13. Arachidonic acid metabolites and stroke

In recent years, studies suggest that hypertension, a risk factor for stroke, is associated with an increased production of 20-HETE in the wall of cerebral vessels [169]; 20-HETE has a crucial role in cerebral blood flow regulation [170] and is a well-described mediator or neural tissue damage in stroke [169]. 20-HETE increases the production of ROS [171–174], has a role in increasing vasospasm following subarachnoid hemorrhage (SAH), and also affects infarct volume after t-MCAO in rats. Its inhibitors, such as HET0016 [175], reduce vasospasm after SAH and reduce stroke volume and neurological outcome after stroke. Dunn et al. showed that in spontaneously hypertensive rats the CYP4A expression and 20-HETE production were raised in the cerebral vasculature and that inhibition of 20-HETE production noticeably reduced the infarct size and endothelial dysfunction presented in stroke [169]. In humans, excretion of 20-HETE is associated with hypertension and endothelial dysfunction. Ward et al. showed that plasma concentrations of 20-HETE, EETs, and DiHETEs were elevated in patients with acute ischemic stroke and increased oxidative stress was present noticeable by increased plasma F2-isoprostanes [68]. It is considered that free radicals formation that accompanies ischemic brain injury is an acute response [68].

Pretreatment and treatment with 20-HETE inhibitors have been proposed as a new potential approach for stroke treatment [176]. Recently, beside 20-HETE, other metabolites of AA, such as EETs, have been shown to have a potential to alleviate the impairment of tissue perfusion and detrimental outcome of stroke [177–179]. Several studies demonstrated that stabilizing the levels of EETs is important, and that the inhibition of sEH is cerebroprotective against ischemic stroke and SAH [177, 178]. Thus, CYP metabolites could play an important role as new targets for the pharmaceutical industry in managing brain damage that occurs with cerebral ischemia and stroke [180].

14. Conclusion remarks

In conclusion, it is obvious that metabolites of AA play an extremely important role in the mechanisms of microvascular responses and microvascular regulation of tissue blood flow and perfusion. Balance between vasodilator and vasoconstrictor metabolites of AA may be disturbed in various cardiometabolic diseases (such as hypertension, stroke, obesity, diabetes) and underlies endothelial dysfunction which is related to many complications accompanying these diseases. Dietary habits significantly affect the metabolism of AA, particularly excessive NaCl intake or high blood glucose levels lead to endothelial dysfunction, as well. Control of environmental risks factors, good maintenance of the occurring diseases, and balanced

nutrition with restricted salt intake can significantly improve metabolism of AA and alleviate possible microvascular dysfunction and subsequent organ damage. Current research on alternative pathways of AA metabolism and pharmacological manipulation with certain components of the AA pathways (such as 20-HETE production inhibition or prolongation of the life of EETs by sEH inhibitors) promises effective therapy of cardiovascular and cerebrovascular diseases in the future.

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