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Specialized Pro-resolving Mediator Improves Vascular Relaxation via Formyl Peptide Receptor-2

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BACKGROUND: The resolution of inflammation is an active phenomenon important for switching off inflammatory processes once the harmful stimuli are removed and facilitate the return to homeostasis. Specialized pro-resolving mediators (SPMs), such as lipoxin A4, resolvin D1, and resolvin E1, derived from ω-3 or ω-6 polyunsaturated fatty acids, are crucial for the resolution of inflammation. We hypothesized that SPMs are decreased in hypertension which contributes to the acetylcholine-induced contraction in resistance arteries, which are well known to be mediated by leukotrienes and prostaglandins. Moreover, treatment with SPMs will decrease this contraction via formyl peptide receptor-2 (FPR-2) in resistance arteries from spontaneously hypertensive rats (SHR).

METHODS AND RESULTS: We performed a comprehensive eicosanoid lipid panel analysis, and our data showed for the first time that precursors of SPMs are decreased in SHR, limiting the production of SPMs and resolution of inflammation *in vivo*. This phenomenon was associated with an increase in lipid peroxidation in resistance arteries. Although SPMs did not abolish acetylcholine-induced contraction, these lipid mediators improved endothelial function in arteries from SHR via FPR-2 activation at nanomolar concentrations. SPMs also buffered TNF-α-induced reactive oxygen species generation in endothelial cells from C57Bl/6 mice.

CONCLUSIONS: We suggest that FPR-2 and SPMs could be revealed as a new target or therapeutic agent to improve vascular function in arteries from hypertensive rats.

Keywords: blood pressure; endothelial dysfunction; hypertension; resolution of inflammation.

Chronic, low-grade inflammation that leads to vascular dysfunction is a common characteristic of hypertension. The resolution of inflammation is a physiological process to limit the development of chronic inflammation.[1] It was previously believed that inflammatory processes passively returned to homeostasis when danger was eradicated. However, it is now accepted that the resolution of inflammation is an active process that creates specific stop signals for inflammation.[2] Therefore, any imbalance in the resolution of inflammation may contribute to chronic inflammation like that observed in cardiovascular diseases.

Specialized pro-resolving mediators (SPMs) derived from ω-3 or ω-6 polyunsaturated fatty acids are essential in the resolution of inflammation. There are 4 identified specialized pro-resolving lipid mediator groups: resolvins, protectins, lipoxins, and maresins.[2–4] SPMs resolve inflammation by acting on their own G-protein-coupled receptors or modulating G-protein-coupled receptors for other fatty acids.[4] For instance, lipoxin A4 (LXA4), derived from ω-6 polyunsaturated fatty acid arachidonic acid (AA), and resolvin D1 (RvD1), derived from ω-3 polyunsaturated fatty acid docosahexaenoic acid, bind to the formyl peptide receptor-2 (FPR-2).[5,6] Resolvin E1 (RvE1), derived from ω-3 polyunsaturated fatty acid eicosapentaenoic acid binds to the chemokine-like receptor 1, also known as ChemR23 or leukotriene B4 receptor BLT1.[7] After binding their receptors, they enhance phagocytosis, disrupt thromboxane (TXA_2)-mediated platelet aggregation, prevent neutrophil migration and stimulate macrophages to regulate migration and cytokine production.[8,9]

Resolvin effects on the endothelium are described as limiting transendothelial migration of leukocytes and cell–cell interactions with leukocytes to generate resolvins or decreasing leukocyte adhesion.[10] Resolvins have also been found to regulate microvasculature permeability,[10] and protect against endothelial dysfunction induced by systemic inflammation.[11] A study has recently shown that RvD1 and RvE1 have a relaxant effect on rat and human arteries,[12] and treatment with RvD2 partially prevented elevation of systolic blood pressure in angiotensin II (Ang

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II)-induced hypertension.[13] Further, RvD2 partially improved cardiovascular function and structure, decreased fibrosis, and reduced the infiltration of neutrophils in hypertensive animals.[13]

We and others previously observed that resistance arteries from spontaneously hypertensive rats (SHR) have a characteristic contraction to the known vasodilator acetylcholine.[14,15] In arteries from naive animals, acetylcholine binds to G-protein-coupled muscarinic receptors on the endothelial cell membrane which causes an increase in intracellular calcium levels (Ca^{2+}). Increased Ca^{2+} levels will lead to various cellular responses, such as nitric oxide (NO), eicosanoid production,[16–19] and vascular relaxation. In hypertension, a biphasic response is observed in acetylcholine-treated resistance arteries.[14] For instance, at lower concentrations (~1–100 nM) of acetylcholine, the resistance arteries relax as expected mainly due to the release of vasodilators NO, endothelium-dependent hyperpolarization, and/or PGI_2.[14,20] However, the arteries contract at high concentrations (1 µM).[14] This phenomenon was first reported in the 1980s.[20] These contractions have been partly attributed to multiple contractile mediators derived from ω-6 polyunsaturated fatty acids, such as TXA_2, leukotrienes, reactive oxygen species (ROS), and other prostaglandins.[21,22]

Based on these premises and on the fact that low-grade chronic inflammation is present in hypertension, we hypothesized that SPM is decreased in SHR, which contributes to acetylcholine-induced contraction in resistance arteries. On the other hand, an increase in SPMs bioavailability will decrease acetylcholine-induced contraction via FPR-2 in resistance arteries from SHR. This study attempts to distinguish the role of pro-resolving mediators in endothelium-dependent relaxation and contraction in resistance arteries from SHR.

METHODS

For detailed information about Methods, please see Supplementary Material online.

Animals

All animal procedures and protocols used were approved by the Animal Care and Use Committee at both locations. Experiments were conducted following the National Institutes of Health Guide for the Care and Use of Laboratory Animals and Animal Research Reporting of in Vivo Experiments (ARRIVE) guidelines. Male, 12-week-old SHR and Wistar Kyoto rats (WKY) were maintained on a 12-hour light cycle with water *ad libitum* and maintained on a standard chow diet (0.3% NaCl, Harlan Teklad diet TD 7034; Madison, WI). For more information about animals, please see Supplementary Material online.

Radiotelemetry blood pressure measurements

For more information, please see Supplementary Material online.[23]

Treatment

After 1 week of surgery and recovery, male SHR rats (12 weeks old, Charles River Laboratories) were randomly divided to receive Zileuton (Zil; 10 mg/kg/day i.p.), an active inhibitor of 5-lipoxygenase (5-LOX), or vehicle for 10 days. We selected these doses based on a previous study.[24]

Tissue collection

Under isoflurane anesthesia (5% in 100% O_2 administered via nose cone), whole blood was first collected from the abdominal aorta and centrifuged. For serum, whole blood was centrifuged for 15 minutes in 2,000 rcf in a 4°C centrifuge and the supernatant was collected. For plasma, whole blood was centrifuged for 15 minutes at 1,500 rcf in a 4 °C centrifuge and the supernatant was collected. After euthanasia by thoracotomy and exsanguination via cardiac puncture, mesenteric resistance arteries (MRA) were harvested, as described before.[25]

Eicosanoid lipid panel analysis

For more information, please see Supplementary Material online.

Vascular function

Third- or fourth-order MRA (<300 µM), 2 mm in length, were mounted on DMT wire myographs (Danish MyoTech, Aarhus, Denmark) for vascular function measurements.[25,26] For more information, please see Supplementary Material online.

Protein expression and lipid peroxidation measurements

Total protein extract of MRA was obtained by mechanical homogenization of the tissue in lysis buffer (cOmplete Lysis-M, Rocher, Germany) containing protease and phosphatase inhibitor cocktail (cOmplete Tablets, Rocher, Germany). Samples were centrifuged (13,000*g* for 15 min at 4 °C), and supernatants were isolated and stored at –80 °C until proceeding to electrophoresis. For more information, please see Supplementary Material online.

Primary MRA endothelial cells

Commercial mouse primary MRA endothelial cells derived from C57BL/6 mice were purchased from Cell Biologics (#C57-6055). For more information, please see Supplementary Material online.

Measurement of ROS generation in endothelial cells

Primary MRA endothelial cells were cultured at 4×10^4 cells/well in a 24-well assay plate in low glucose complete Dulbecco's modified Eagle medium with fetal bovine serum 5%, P/S 1%, and endothelial growth factors (Cell Biologics, M1166) for 24 hours. To induce the generation of ROS, endothelial cells were treated with TNF-α (200 nM) for 1 hour. For more information, please see Supplementary Material online.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 9.1.1 (San Diego, CA, USA). Data are presented as mean ± standard error of the mean and statistical significance was set at $P < 0.05$. Procedures included Student's unpaired *t* test, 1- and 2-way analysis of variance (ANOVA), nonlinear regression analysis (log EC_{50} and E_{max}). Bonferroni *post hoc* testing was used in 1- and 2-way ANOVA. The number (*n*) of independent animals per group is described in the graphs or legends.

RESULTS

Decreased levels of SPM precursors in plasma and increased lipid peroxidation in resistance arteries exemplify exacerbated vascular inflammation in hypertension

We investigated the levels of over 150 eicosanoids in MRA and plasma from WKY and SHR. From this panel, 45 eicosanoids were

detected in the plasma from WKY and SHR. A heat map was generated to show the level of eicosanoids in plasma samples from WKY and SHR (Figure 1a). Since MRA presented with low or undetectable levels of eicosanoids, we could not generate a heat map. Regarding plasma samples, there was a significant decrease in 5 different eicosanoids in SHR: (i) free eicosapentaenoic acid (EPA), a ω-3 polyunsaturated fatty acids (PUFAs); (ii) free adrenic acid, an ω-6 polyunsaturated fatty acid (PUFA) derived from AA[27]; (iii) 20-COOH-AA, a metabolite of 20-HETE produced from AA by cytochrome P450 ω-oxidases[28]; (iv) 5-HETE, an oxylipid derived from AA by 5-LOX[29]; and (5) 14(15)-EET, a cytochrome p450 product of AA[30] (Figure 1b–f). Interestingly, 12,13-dihydroxy-9Z-octadecenoic acid (12,13-DiHOME), a cytochrome P450-derived linoleic acid metabolite,[31] was significantly increased in SHR plasma (Figure 1g). We observed no significant difference in free AA and free DHA in plasma between the groups (Figure 1h,i).

In addition to decreased SPM precursors in plasma from SHR, a significant increase in lipid peroxidation was observed in resistance arteries from SHR (Figure 1j,k). Lipid peroxidation is associated with increased inflammation and might influence the quality and quantity of the bioactive components present in arteries, including SPMs.

SPMs improve vascular relaxation via formyl peptide receptor-2, but do not prevent acetylcholine-induced contraction in hypertension

As expected, we observed acetylcholine-induced contraction in arteries from SHR and total relaxation to acetylcholine in arteries from WKY (Figure 2a). Specifically, at lower concentrations (~1–100 nM) of acetylcholine, the resistance arteries relax mainly due to the release of vasodilators such as NO, endothelium-dependent hyperpolarization, and/or PGI_2 (Figure 2b). However, at high concentrations (1 μM), the arteries start to contract mainly due to the release of TXA_2[21] and other prostaglandins.[21,22] Corroborating these data, we have observed an increase in cyclooxygenase (COX-2) protein expression in arteries from SHR (Figure 2c). Our initial hypothesis was refuted as SPMs (LXA4, RvD1, and/or RvE1) were not able to abolish acetylcholine-induced contraction (Figure 2e,f). However, all of them potentiated acetylcholine-induced relaxation at lower concentrations (Figure 2e,f). SPMs did not change acetylcholine-induced relaxation in arteries from WKY (Supplementary Figure S1A online) or phenylephrine-induced contraction in arteries from WKY and SHR (Supplementary Figure S1B,C online). No changes were observed in endothelium-independent relaxation induced by sodium nitroprusside (Supplementary Figure S1D online). Interestingly, the selective antagonism of FPR-2 (WRW4, 1 μM) prevented the increase in relaxation induced by SPM (Figure 2d–f). No changes were observed in FPR-2 total protein expression in arteries from WKY and SHR (Supplementary Figure S2A online). Also, WRW4 alone did not change contractile function in arteries from WKY and SHR (Supplementary Figure S2B online and Figure 3a), even in the presence of increased extracellular signal-regulated kinases (ERK1/2) phosphorylation in arteries from SHR, which is the main downstream signaling mechanism following FPR-2 activation (Supplementary Figure S3B–D online).

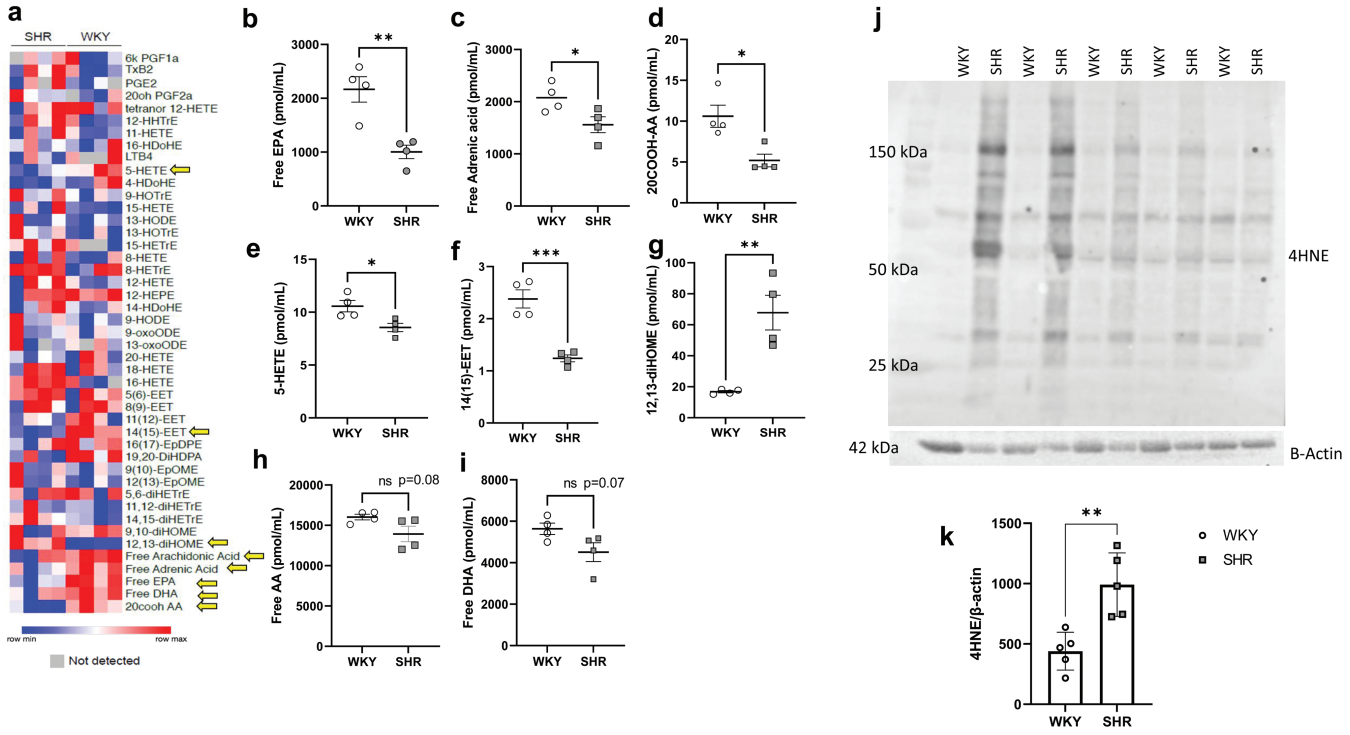

Figure 1. Decreased levels of SPM precursors in plasma and increased lipid peroxidation in resistance arteries. (**a**) Image of heat map analysis of circulating lipids in WKY and SHR. The x-axis represents the different groups, and the *y*-axis represents the different eicosanoids. Arrows denote eicosanoids chosen for further analysis. (**b–i**) Data on polyunsaturated fatty acids and eicosanoid levels in plasma from WKY and SHR. (**j**) Protein expression of lipid peroxidation measured by 4HNE in mesenteric resistance arteries from WKY and SHR with quantitation (**k**). Data presented in mean ± SEM. n = 4–5 for all groups. The number of animals used in each experiment (n) is in parentheses or expressed in dots. The results are expressed as the mean ± SEM. Statistics: t test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). Abbreviations: SEM, standard error of the mean; SHR, spontaneously hypertensive rats; SPM, specialized pro-resolving mediator..

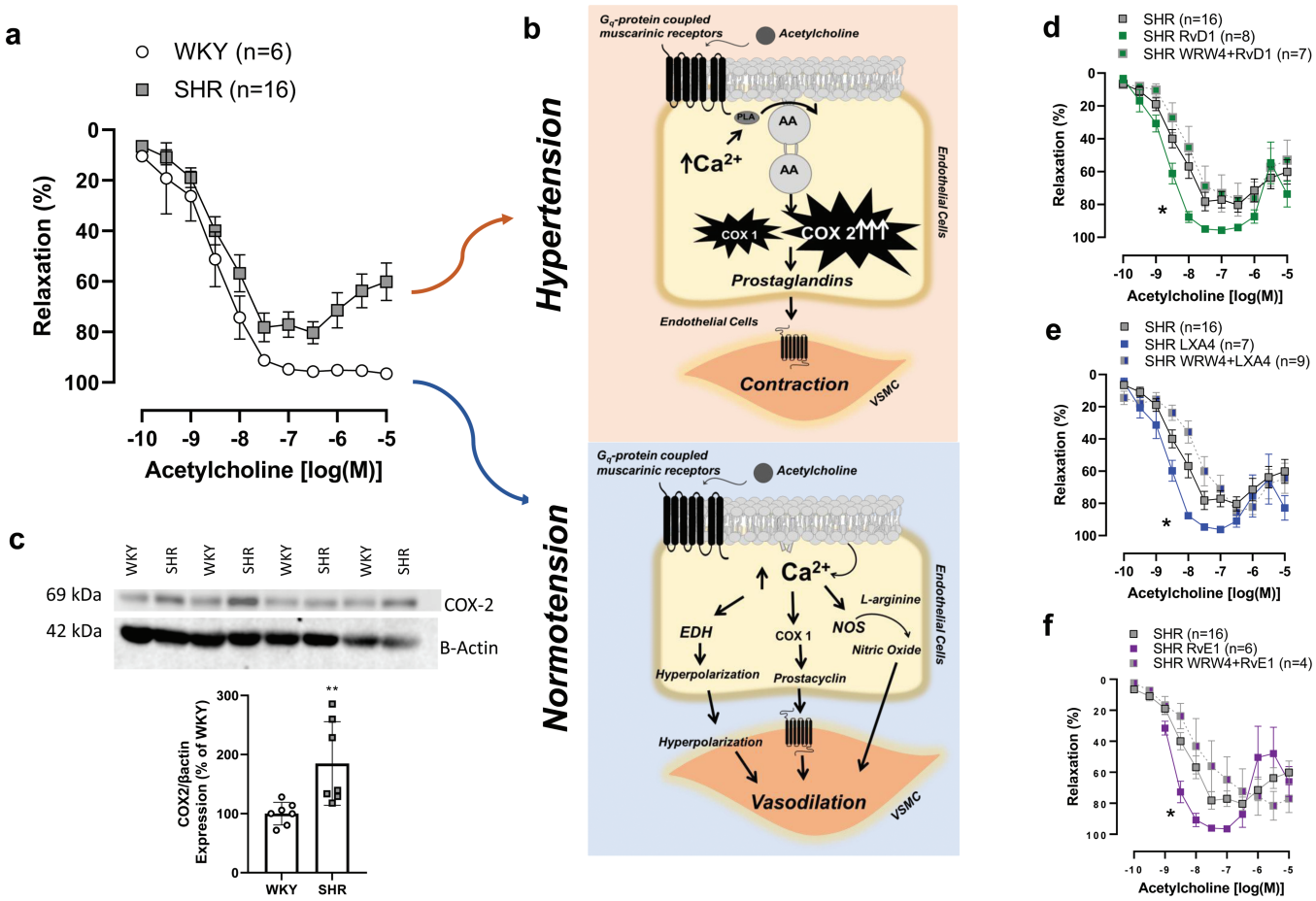

Figure 2. Acetylcholine-induced contractions in hypertension due to increased cyclooxygenase-2 (COX-2) expression. (**a**) Concentration–response curves to acetylcholine in mesenteric resistance arteries from WKY and SHR rats. (**b**) Graphical representation of vasoactivity in mesenteric resistance arteries from hypertensive and normotensive arteries. (**c**) COX-2 expression in mesenteric resistance arteries from WKY and SHR. Concentration–response curves to acetylcholine in mesenteric resistance arteries from SHR rats incubated with RvD1 and RvD1 + WRW4 (**d**), LXA4 and LXA4 + WRW4 (**e**), or RvE1 and RvE1 + WRW4 (**f**). Arteries were precontracted with phenylephrine. The number of animals used in each experiment (n) is in parentheses or expressed in dots. The results are expressed as the mean ± SEM. Statistics: 2-way ANOVA or *t* test (*$P < 0.05$; **$P < 0.01$). Abbreviations: ANOVA, analysis of variance; LXA4, lipoxin A4; RvD1, resolvin D1; RvE1, resolvin E1; SEM, standard error of the mean; SHR, spontaneously hypertensive rats..

SPMs neutralize ROS generation in endothelial cells independent of FPR-2 activation

To investigate whether SPMs can neutralize and decrease ROS generation, we challenged primary endothelial cells from MRA with TNF-α to generate ROS, and subsequently treated these cells with SPMs. All 3 resolvins, RvD1, RvE1, or LXA4 (10 nM) decreased ROS production, however, WRW4 did not prevent these responses (Figure 3a–c).

In vivo inhibition of 5-LOX, an important enzyme for the synthesis of LXA4, worsened endothelial function and acetylcholine-induced contraction in resistance arteries from SHR

Total 5-LOX protein expression (Figure 4a) and the phosphorylation of serine 271 on 5-LOX (Figure 4b) are unchanged in arteries from SHR when compared with WKY. However, we observed an increase in the phosphorylation of serine 523 on 5-LOX in arteries from SHR (Figure 4a). The 15-LOX protein expression is reduced in MRA from SHR animals compared with WKY (Figure 4c). The activity of these enzymes is associated with increased synthesis of SPMs. Because of these data, we treated SHR with Zileuton (Zil, 10 mg/kg/day i.p.), an active inhibitor of 5-LOX, or vehicle for 10 days. No changes in blood pressure were observed (Figure 4d). Alarmingly, some animals treated with Zil presented with an unwell appearance and signs of infection, noted by abscesses in confined tissue spaces. These animals were excluded from the study. Additionally, we observed that treatment with Zil worsened endothelium dysfunction seen in MRA (Figure 4e) and aorta (Supplementary Figure S4A online) from SHR when compared with the control group (vehicle). No changes were observed in the dysfunctional intrarenal arteries from SHR (Supplementary Figure S4B online), which was expected given that these arteries already presented with a relaxation less than 20%–40%.

DISCUSSION

The introduction of lipidomic technology has exponentially increased our understanding of eicosanoid signaling and revealed that it is much more sophisticated than we previously assumed. Here, we ran a full eicosanoid lipidomic panel on the plasma from hypertensive and normotensive animals and found that essential precursors of SPMs are decreased in hypertension. Specifically, there was a significant decrease in EPA and adrenic acid, and a small *P* value ($P = 0.08$ and $P = 0.07$) that should be highlighted for AA and DHA, which leads to a decrease bioavailability of these

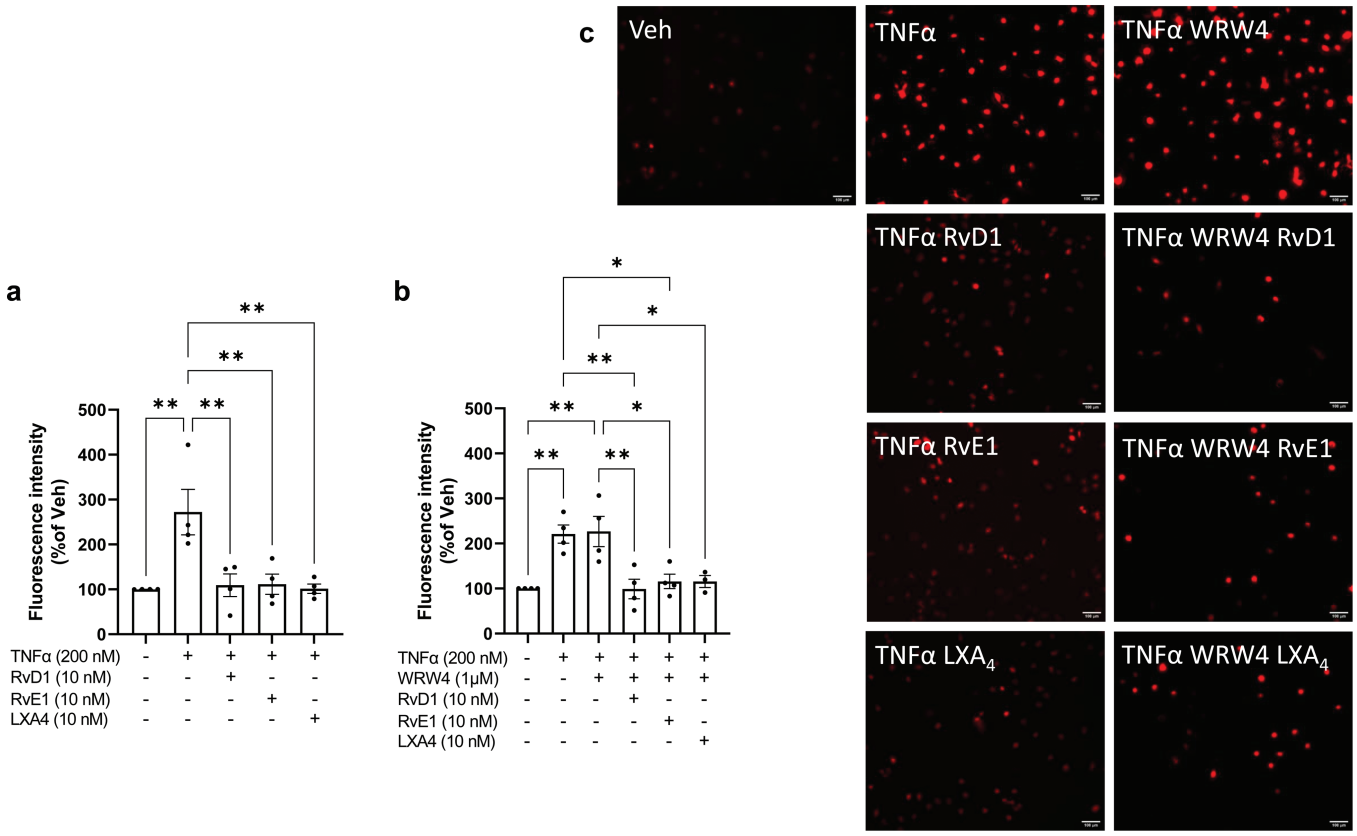

Figure 3. Specialized pro-resolving mediators neutralize oxidative stress in endothelial cells. Quantitation of dihydroethidium fluorescence in primary mesenteric resistance artery endothelial cells treated with TNF-α, with the subsequent addition of RvD1, RvE1, and LXA4 in the absence (**a**) or presence (**b**) of the selective antagonist of FPR-2 (WRW4). (**c**) Representative images of dihydroethidium fluorescence. The number of animals used in each experiment (n) is in parentheses or expressed in dots. The results are expressed as the mean ± SEM. Statistics: 1-way ANOVA (*$P < 0.05$; **$P < 0.01$). Scale bar, 100 μm. Abbreviations: ANOVA, analysis of variance; LXA4, lipoxin A4; RvD1, resolvin D1; RvE1, resolvin E1; SEM, standard error of the mean..

precursors to synthesize resolvins and lipoxins in SHR (Figure 1b, c, h, i). Adrenic acid has recently been implicated to antagonize production of leukotriene B4, a potent inflammatory molecule, and enhance the resolution of inflammation *in vivo*.[32] Lipidomic analysis also revealed that 20-COOH-AA, 5-HETE, and 14(15)-EET synthesized from AA were decreased in SHR plasma (Figure 1d–f). It has been shown that these lipid mediators and precursors of SPMs also present a direct effect on the cardiovascular system. Accordingly, it has been shown that 20-COOH-AA causes relaxation of porcine coronary microvessels constricted with endothelin,[33] 14,15-EET relaxes bovine coronary arterial rings in a concentration-related manner via activation of potassium channels and hyperpolarization of the membrane.[34] Interestingly, the vasodilation produced by 14,15-EET was not attenuated by the removal of the endothelium, which indicates a direct action of 14,15-EET on microvascular smooth muscle.[35] On the other hand, 5-HETE induces pulmonary vasoconstriction and edema.[36] At this point, we do not know the reason why these important precursors of SPM are decreased in hypertension.

Interestingly, we also observed a significant increase in 12,13-DiHOME metabolite (Figure 1g). 12,13-DiHOME has recently been identified as an oxylipin,[37] which is a bioactive lipid derived from PUFA metabolism after mono- or dioxygenases action. It has been shown that 12,13-DiHOME increases fatty acid uptake.[37,38] Scientific evidence has shown that increased serum levels of 12,13-DiHOME was induced by physical exercise and exposure to cold. This metabolite induces absorption of fatty acids by brown adipose tissue and stimulates the browning process in white adipose tissue.[37,38] The increase of this metabolite could be one of the reasons that we have observed overall decreased levels of polyunsaturated fatty acids in the present study.

In addition to the decreased levels of SPM precursors in the plasma from SHR, we also observed a significant increase in lipid peroxidation in resistance arteries from SHR (Figure 1j,k). The combined decreased levels of SPM precursors in plasma and increased lipid peroxidation in resistance arteries may result in exacerbated vascular inflammation in hypertension. Lipid peroxidation is a complex reaction process resulting from ROS-mediated attack on the cell membrane lipids, including PUFA, resulting in cell damage and dysfunction and lipid mediator imbalance, including SPMs. It has been shown that lipid mediator imbalance leads to production of COX-derived prostaglandins over SPMs that ultimately leads to cardiovascular dysfunction.[39] In line, COX-derived products are one of the main factors released in response to high concentrations to acetylcholine in arteries from SHR. Therefore, the decrease in SPM bioavailability, due to its lipid peroxidation and/or reduction in their production, could be one of the reasons of why high concentrations of acetylcholine induce contraction in arteries from SHR (Figure 2a). Based on these data, we show that the potential for SPM therapy in hypertension is promising.

To further investigate the underlying mechanisms leading to the improvement of vasodilation induced by resolvins, we treated the arteries from both strains with an FPR-2 antagonist. FPR-2, a receptor modulated by some of these lipid mediators, has been shown to be one of the receptors involved in the resolution of inflammation (Figure 2d–f).[5,6] Accordingly, LXA4 binds FPR-2 with high affinity,

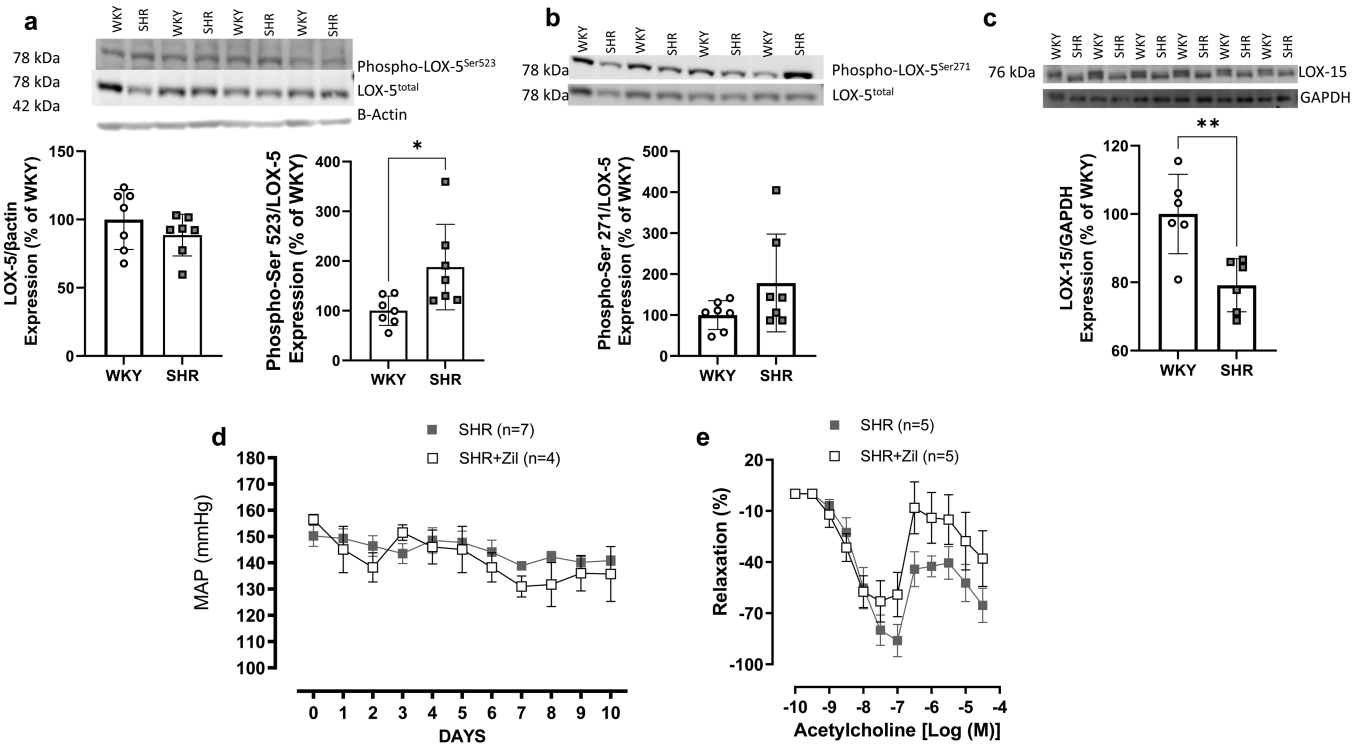

Figure 4. Administration of lipoxygenase 5 (5-LOX) inhibitor Zileuton worsened endothelial dysfunction and acetylcholine-induced contraction observed in arteries from SHR. 5-LOX (**a**), phospho-lipoxygenase-5 at serine 523 (**a**), and phospho-lipooxygenase-5 at serine 271 (**b**) and 15-lipoxygenase (15-LOX) (**c**) expression in mesenteric resistance arteries from WKY and SHR. (**d**) Mean arterial pressure (MAP) derived from systolic and diastolic pressures measured by telemetry, from SHR treated with Zileuton (Zil) or vehicle for 10 days. (**e**) Concentration–response curves to acetylcholine in mesenteric resistance arteries from SHR rats treated with Zil or vehicle. The number of animals used in each experiment (n) is in parentheses or expressed in dots. The results are expressed as the mean ± SEM. $P < 0.05$. Statistics: *t* test (**a**–**c**) and 2-way ANOVA (**d** and **e**). Abbreviations: ANOVA, analysis of variance; SEM, standard error of the mean; SHR, spontaneously hypertensive rats.

thereby stimulating arachidonate release and GTPase.[40] RvD1 is known to act via GPR32, but also via ALX/FPR-2 and increase adenylyl cyclase activity leading to increased intracellular cAMP levels and activation of Protein Kinase A, as with the LXA4.[41] Based on prior research, RvE1 binds ChemR23 and BLT-1, however, the main studies were performed in immune cells.[7] ALX/FPR-2 is the most studied receptor and has proven binding with at least LXA4 and RvD1. It is essential to mention that part of the receptors involved in the binding of SPM are orphan receptors and need further studies. Therefore, we chose to use the ALX/FPR-2 antagonist due to the extensive studies already showing FPR-2 efficiency as a receptor for some of the SPM. Despite the lack of previous data demonstrating the ability of RvE1 in binding FPR-2/ALX, our data suggest that this phenomenon needs further analysis.[5,6]

The FPR is represented by 3 isoforms, FPR-1, -2, and -3 and they belong to the G-protein-coupled receptors family.[42] FPRs are expressed at high levels on leukocytes, and they play an important role in immune defense. Although knowledge of the biological function of FPR-1 in other tissues is emerging, the role of FPR-2 in the vascular system is not fully understood. Also, the precise mechanism linking FPR-2 activation in the vascular-immune network in hypertension remains unknown. Here, we showed that resistance arteries from hypertensive animals could sense SPMs, LXA4, RvD1, or RvE1, via FPR-2. Although, there was no difference in the FPR-2 protein expression in MRA from SHR and WKY, there was an increase in ERK1/2, the main downstream signaling mechanism following FPR-2 activation (Supplementary Figures S2A and S3D online). This suggests that the increase in FPR-2 sensitivity to SPMs in resistance arteries from SHR, may be a compensatory mechanism in response to decreased synthesis and/or bioavailability of SPM in hypertension. It is well known that the endothelial dysfunction observed in resistance arteries from hypertensive animals partially occurs due to the decrease in NO bioavailability via exacerbated ROS production. Increased ROS production also leads to an increase in lipid peroxidation as we observed in Figure 1j,k. SPM treatment was able to abolish TNF-α-induced ROS generation in endothelial cells (Figure 3a). However, FPR-2 did not change this response (Figure 3b). These data suggest that these lipid mediators may directly neutralize ROS generation independent of receptor signaling. For instance, polyunsaturated fatty acids, including SPMs, serve as excellent substrates for lipid peroxidation because of the presence of active bis-allylic methylene groups. It is possible that the increase in vascular lipid peroxidation, as seen in arteries from SHR, is important to protect the vascular tissue against exacerbated ROS generation, and, subsequently to maintain NO bioavailability.

When compared with the other SPMs, the involvement of LXA4 in the cardiovascular system is still controversial. LXA4 is a biologically active product generated from AA by lipoxygenase, and it seems to have opposite effects to leukotrienes, which are lipid mediators associated with the initiation of inflammation. The generation of LXA4 is a rapid process and aspirin does not inhibit its formation. In fact, aspirin triggers the production of LXA4 through acetylation of COX-2, also known as aspirin-triggered LXA4.[43] We previously observed that LXA4 induces contraction in the aorta[44] and it is not inhibited by a TXA2/ PGH2 receptor antagonist or a specific inhibitor of COX-1. However, an inhibitor of COX-2 decreases this contraction, suggesting that LXA4 may have positive feedback on COX-2.[44] We observed an increase in

the phosphorylation of serine 523 on 5-LOX in arteries from SHR (Figure 4a). The activity of this enzyme is associated with elevated synthesis of LXA4. Because of these data, we treated SHR with Zil, an active inhibitor of 5-LOX, or vehicle for 10 days. Although there were no changes observed in blood pressure, the treatment with Zil worsened endothelium dysfunction seen in MRA and aorta from SHR (Figure 4d and Supplementary Figure S4A online). Overall, these data suggest that the increase in the phosphorylation of serine 523 on 5-LOX to increase LXA4 synthesis in arteries may be a compensatory mechanism and its inhibition leads to an exacerbated vascular dysfunction in arteries from SHR.

Limitations of the study

A limitation of the present study was that we were only able to detect the precursors of the SPM used in the present study. Another limitation is that pharmacological treatments could have unexpected side effects. For instance, Zileuton treatment induced infection noted by the presence of abscesses in confined tissue spaces. While knockout and knockin mice are powerful tools for probing the functions of specific genes, we decided not to use mice in the present study to avoid variations between different species. The SHR has been widely used to study hypertension, and these rats present with spontaneous hypertension. Previously, we also observed that arteries from female SHR present with contraction to acetylcholine,[45] but this response was less exacerbated than male. Therefore, we did not include females rats in the present study because we are investigating the sex differences in another study.

Collectively, our ` show for the first time that SPMs can improve endothelial function via FPR-2 in hypertensive animals at nanomolar concentrations. We have also shown that the levels of free ω-3 and ω-6 polyunsaturated fatty acids are decreased in the plasma from SHR, potentially contributing to the decreased production of SPMs *in vivo*. Multiple downstream intermediates of AA are also decreased in SHR plasma. Therefore, the combined decreased levels of SPM precursors in plasma, and increased lipid peroxidation in resistance arteries may result in impaired resolution of inflammation and/or chronic hyperinflammation in arteries from SHR. Overall, this study suggests that SPMs could be revealed as a new therapeutic agent to improve vascular function in arteries from hypertensive rats.

SUPPLEMENTARY MATERIAL

Supplementary materials are available at *American Journal of Hypertension* (http://ajh.oxfordjournals.org).

Supplementary Figure S1. Concentration–response curves to acetylcholine (**A**) or phenylephrine (**B** and **C**) in mesenteric resistance arteries from WKY or SHR rats incubated with RvD1, RvE1, and LXA4. (**D**) Concentration–response curve to sodium nitroprusside (SNP) in mesenteric resistance arteries from WKY and SHR. The number of animals used in each experiment (*n*) is in parentheses. The results are expressed as the mean ± SEM.

Supplementary Figure S2. Formyl peptide receptor-2 (FPR-2) expression in mesenteric resistance arteries from WKY and SHR (**A**). Concentration–response curves to acetylcholine in mesenteric resistance arteries from SHR rats incubated with or without WRW4 (**B**). The number of animals used in each experiment (*n*) is in parentheses or expressed in dots. The results are expressed as the mean ± SEM. The results are expressed as the mean ± SEM. $P < 0.05$. Statistics: 2-way ANOVA or *t* test.

Supplementary Figure S3. (**A**) Concentration–response curves to acetylcholine in mesenteric resistance arteries from WKY rats incubated with WRW4. (**B** and **D**) phospho-ERK1/2 at (Thr202/Tyr204) expression in mesenteric resistance arteries from WKY and SHR. (**C** and **D**) Total ERK1/2 expression in mesenteric resistance arteries from WKY and SHR. The number of animals used in each experiment (*n*) is in parentheses or expressed in dots. The results are expressed as the mean ± SEM. $P < 0.05$. Statistics: 2-way ANOVA (**A**) and *t* test (**D**).

Supplementary Figure S4. Concentration–response curves to acetylcholine in (**A**) aorta and (**B**) and intrarenal arteries from SHR treated with Zileton (Zil) or vehicle. The number of animals used in each experiment (*n*) is in parentheses or expressed in dots. The results are expressed as the mean ± SEM. $P < 0.05$. Statistics: 2-way ANOVA.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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REFERENCES

1. Schett G, Neurath MF. Resolution of chronic inflammatory disease: universal and tissue-specific concepts. *Nat Commun* 2018; **9**:3261–3269.
2. Serhan CN. Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. *Annu Rev Immunol* 2007; **25**:101–137.
3. Serhan CN, Chiang N. Lipid-derived mediators in endogenous anti-inflammation and resolution: lipoxins and aspirin-triggered 15-epi-lipoxins. *ScientificWorldJournal* 2002; **2**:169–204.
4. Serhan CN, Chiang N, Dalli J. The resolution code of acute inflammation: novel pro-resolving lipid mediators in resolution. *Semin Immunol* 2015; **27**:200–215.
5. Chiang N, Arita M, Serhan CN. Anti-inflammatory circuitry: lipoxin, aspirin-triggered lipoxins and their receptor ALX. *Prostaglandins Leukot Essent Fatty Acids* 2005; **73**:163–177.
6. Krishnamoorthy S, Recchiuti A, Chiang N, Yacoubian S, Lee CH, Yang R, Petasis NA, Serhan CN. Resolvin D1 binds human phagocytes with evidence for proresolving receptors. *Proc Natl Acad Sci U S A* 2010; **107**:1660–1665.
7. Arita M, Ohira T, Sun YP, Elangovan S, Chiang N, Serhan CN. Resolvin E1 selectively interacts with leukotriene B4 receptor

BLT1 and ChemR23 to regulate inflammation. *J Immunol* 2007; **178**:3912–3917.
8. Spite M, Serhan CN. Novel lipid mediators promote resolution of acute inflammation: impact of aspirin and statins. *Circ Res* 2010; **107**:1170–1184.
9. Dona M, Fredman G, Schwab JM, Chiang N, Arita M, Goodarzi A, Cheng G, von Andrian UH, Serhan CN. Resolvin E1, an EPA-derived mediator in whole blood, selectively counterregulates leukocytes and platelets. *Blood* 2008; **112**:848–855.
10. Sansbury BE, Spite M. Resolution of acute inflammation and the role of resolvins in immunity, thrombosis, and vascular biology. *Circ Res* 2016; **119**:113–130.
11. Rathod KS, Kapil V, Velmurugan S, Khambata RS, Siddique U, Khan S, Van Eijl S, Gee LC, Bansal J, Pitrola K, Shaw C, D'Acquisto F, Colas RA, Marelli-Berg F, Dalli J, Ahluwalia A. Accelerated resolution of inflammation underlies sex differences in inflammatory responses in humans. *J Clin Invest* 2017; **127**:169–182.
12. Jannaway M, Torrens C, Warner JA, Sampson AP. Resolvin E1, resolvin D1 and resolvin D2 inhibit constriction of rat thoracic aorta and human pulmonary artery induced by the thromboxane mimetic U46619. *Br J Pharmacol* 2018; **175**:1100–1108.
13. Díaz Del Campo LS, García-Redondo AB, Rodríguez C, Zaragoza C, Duro-Sánchez S, Palmas F, de Benito-Bueno A, Socuéllamos PG, Peraza DA, Rodrigues-Díez R, Valenzuela C, Dalli J, Salaices M, Briones AM. Resolvin D2 attenuates cardiovascular damage in angiotensin II-induced hypertension. *Hypertension* 2023; **80**:84–96.
14. Edwards JM, McCarthy CG, Wenceslau CF. The obligatory role of the acetylcholine-induced endothelium-dependent contraction in hypertension: can arachidonic acid resolve this inflammation? *Curr Pharm Des* 2020; **26**:3723–3732.
15. Lüscher TF, Vanhoutte PM. Endothelium-dependent contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. *Hypertension* 1986; **8**:344–348.
16. Lückhoff A, Busse R. Calcium influx into endothelial cells and formation of endothelium-derived relaxing factor is controlled by the membrane potential. *Pflugers Arch* 1990; **416**:305–311.
17. Lückhoff A, Pohl U, Mülsch A, Busse R. Differential role of extra- and intracellular calcium in the release of EDRF and prostacyclin from cultured endothelial cells. *Br J Pharmacol* 1988; **95**:189–196.
18. Smani T, Zakharov SI, Csutora P, Leno E, Trepakova ES, Bolotina VM. A novel mechanism for the store-operated calcium influx pathway. *Nat Cell Biol* 2004; **6**:113–120.
19. Mitchell JA, Ali F, Bailey L, Moreno L, Harrington LS. Role of nitric oxide and prostacyclin as vasoactive hormones released by the endothelium. *Exp Physiol* 2008; **93**:141–147.
20. Gluais P, Lonchampt M, Morrow JD, Vanhoutte PM, Feletou M. Acetylcholine-induced endothelium-dependent contractions in the SHR aorta: the Janus face of prostacyclin. *Br J Pharmacol* 2005; **146**:834–845.
21. Vanhoutte PM. Endothelium-dependent contractions in hypertension: when prostacyclin becomes ugly. *Hypertension* 2011; **57**:526–531.
22. Komori K, Vanhoutte PM. Endothelium-derived hyperpolarizing factor. *Blood Vessels*. 1990; **27**:238–245.
23. Szasz T, Davis RP, Garver HS, Burnett RJ, Fink GD, Watts SW. Long-term inhibition of xanthine oxidase by febuxostat does not decrease blood pressure in deoxycorticosterone acetate (DOCA)-salt hypertensive rats. *PLoS One* 2013; **8**:e56046.
24. Ee MT, Kantores C, Ivanovska J, Wong MJ, Jain A, Jankov RP. Leukotriene B4 mediates macrophage influx and pulmonary hypertension in bleomycin-induced chronic neonatal lung injury. *Am J Physiol Lung Cell Mol Physiol* 2016; **311**:L292–L302.
25. Wenceslau CF, McCarthy CG, Szasz T, Calmasini FB, Mamenko M, Webb RC. Formyl peptide receptor-1 activation exerts a critical role for the dynamic plasticity of arteries via actin polymerization. *Pharmacol Res* 2019; **141**:276–290.
26. Wenceslau CF, McCarthy CG, Earley S, England SK, Filosa JA, Goulopoulou S, Gutterman DD, Isakson BE, Kanagy NL, Martinez-Lemus LA, Sonkusare SK, Thakore P, Trask AJ, Watts SW, Webb RC. Guidelines for the measurement of vascular function and structure in isolated arteries and veins. *Am J Physiol Heart Circ Physiol* 2021; **321**:H77–H111.
27. Singh N, Barnych B, Wagner KM, Wan D, Morisseau C, Hammock BD. Adrenic acid-derived epoxy fatty acids are naturally occurring lipids and their methyl ester prodrug reduces endoplasmic reticulum stress and inflammatory pain. *ACS Omega* 2021; **6**:7165–7174.
28. Fang X, Dillon JS, Hu S, Harmon SD, Yao J, Anjaiah S, Falck JR, Spector AA. 20-carboxy-arachidonic acid is a dual activator of peroxisome proliferator-activated receptors alpha and gamma. *Prostaglandins Other Lipid Mediat* 2007; **82**:175–184.
29. Austin Pickens C, Yin Z, Sordillo LM, Fenton JI. Arachidonic acid-derived hydroxyeicosatetraenoic acids are positively associated with colon polyps in adult males: a cross-sectional study. *Sci Rep* 2019; **9**:12033.
30. Falck JR, Koduru SR, Mohapatra S, Manne R, Atcha KR, Atcha R, Manthati VL, Capdevila JH, Christian S, Imig JD, Campbell WB. 14,15-Epoxyeicosa-5,8,11-trienoic acid (14,15-EET) surrogates: carboxylate modifications. *J Med Chem* 2014; **57**:6965–6972; e-pub ahead of print 19 August 2014. Erratum in: J Med Chem. 2014 Nov 13;57(21):9218. Atcha, Krishnam Raju [added].
31. Zimmer B, Angioni C, Osthues T, Toewe A, Thomas D, Pierre SC, Geisslinger G, Scholich K, Sisignano M. The oxidized linoleic acid metabolite 12,13-DiHOME mediates thermal hyperalgesia during inflammatory pain. *Biochim Biophys Acta Mol Cell Biol Lipids* 2018; **1863**:669–678.
32. Brouwers H, Jónasdóttir HS, Kuipers ME, Kwekkeboom JC, Auger JL, Gonzalez-Torres M, López-Vicario C, Clària J, Freysdottir J, Hardardottir I, Garrido-Mesa J, Norling LV, Perretti M, Huizinga TWJ, Kloppenburg M, Toes REM, Binstadt B, Giera M, Ioan-Facsinay A. Anti-inflammatory and proresolving effects of the omega-6 polyunsaturated fatty acid adrenic acid. *J Immunol* 2020; **205**:2840–2849.
33. Kaduce TL, Fang X, Harmon SD, Oltman CL, Dellsperger KC, Teesch LM, Gopal VR, Falck JR, Campbell WB, Weintraub NL, Spector AA. 20-Hydroxyeicosatetraenoic acid (20-HETE) metabolism in coronary endothelial cells. *J Biol Chem* 2004; **279**:2648–2656.
34. Falck JR, Krishna UM, Reddy YK, Kumar PS, Reddy KM, Hittner SB, Deeter C, Sharma KK, Gauthier KM, Campbell WB. Comparison of vasodilatory properties of 14,15-EET analogs: structural requirements for dilation. *Am J Physiol Heart Circ Physiol* 2003; **284**:H337–H349.
35. Oltman CL, Weintraub NL, VanRollins M, Dellsperger KC. Epoxyeicosatrienoic acids and dihydroxyeicosatrienoic acids are potent vasodilators in the canine coronary microcirculation. *Circ Res* 1998; **83**:932–939.
36. Burhop KE, Selig WM, Malik AB. Monohydroxyeicosatetraenoic acids (5-HETE and 15-HETE) induce pulmonary vasoconstriction and edema. *Circ Res* 1988; **62**:687–698.
37. Leiria LO, Tseng YH. Lipidomics of brown and white adipose tissue: implications for energy metabolism. *Biochim Biophys Acta Mol Cell Biol Lipids* 2020; **1865**:158788.

38. Macêdo APA, Muñoz VR, Cintra DE, Pauli JR. 12,13-diHOME as a new therapeutic target for metabolic diseases. *Life Sci* 2022; **290**:120229.
39. Bruno AS, Lopes PDD, de Oliveira KCM, de Oliveira AK, de Assis Cau SB. Vascular Inflammation in hypertension: targeting lipid mediators unbalance and nitrosative stress. *Curr Hypertens Rev* 2021; **17**:35–46.
40. Cattaneo F, Parisi M, Ammendola R. Distinct signaling cascades elicited by different formyl peptide receptor 2 (FPR2) agonists. *Int J Mol Sci* 2013; **14**:7193–7230.
41. Tavares LP, Negreiros-Lima GL, Lima KM, Silva PMR E, Pinho V, Teixeira MM, Sousa LP. Blame the signaling: role of cAMP for the resolution of inflammation. *Pharmacol Res* 2020; **159**:105030.
42. Edwards JM, Roy S, Galla SL, Tomcho JC, Bearss NR, Waigi EW, Mell B, Cheng X, Saha P, Vijay-Kumar M, McCarthy CG, Joe B, Wenceslau CF. FPR-1 (formyl peptide receptor-1) activation promotes spontaneous, premature hypertension in dahl salt-sensitive rats. *Hypertension* 2021; **77**:1191–1202.
43. Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K. Novel functional sets of lipid-derived mediators with anti-inflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. *J Exp Med* 2000; **192**:1197–1204.
44. Wenceslau CF, McCarthy CG, Szasz T, Webb RC. Lipoxin A4 mediates aortic contraction via RHOA/RHO kinase, endothelial dysfunction and reactive oxygen species. *J Vasc Res* 2014; **51**:407–417.
45. Costa TJ, Ceravolo GS, dos Santos RA, de Oliveira MA, Araújo PX, Giaquinto LR, Tostes RC, Akamine EH, Fortes ZB, Dantas AP, Carvalho MH. Association of testosterone with estrogen abolishes the beneficial effects of estrogen treatment by increasing ROS generation in aorta endothelial cells. *Am J Physiol Heart Circ Physiol* 2015; **308**:H723–H732.