Sex differences in specialized pro-resolving lipid mediators and their receptors in abdominal aortic aneurysms

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ABSTRACT

Objective: In this study, we tested the hypothesis that endogenous expression of specialized pro-resolving lipid mediators (SPMs) that facilitate the resolution of inflammation, specifically Resolvin D1and -D2, as well as Maresin1 (MaR1), can impact abdominal aortic aneurysm (AAA) formation and progression in a sex-specific manner.

Methods: SPM expression was quantified in aortic tissue from human AAA samples and from a murine in vivo AAA model via liquid chromatography-tandem mass spectrometry. mRNA expression for SPM receptors FPR2, LGR6, and GPR18 were quantified by real-time polymerase chain reaction. A Student *t* test with nonparametric Mann-Whitney or Wilcoxon test was used for pair-wise comparisons of groups. One-way analysis of variance after post hoc Tukey test was used to determine the differences among multiple comparative groups.

Results: Human aortic tissue analysis revealed a significant decrease in RvD1 levels in male AAAs compared with controls, whereas FPR2 and LGR6 receptor expressions were downregulated in male AAAs compared with male controls. In vivo studies of elastase-treated mice showed higher levels of RvD2 and MaR1 as well as the SPM precursors, omega-3 fatty acids DHA and EPA, in aortic tissue from males compared with females. FPR2 expression was increased in elastase-treated females compared with males.

Conclusions: Our findings demonstrate that specific differences in SPMs and their associated G-protein coupled receptors exist between sexes. These results indicate the relevance of SPM-mediated signaling pathways in sex differences impacting the pathogenesis of AAAs. (JVS—Vascular Science 2023;4:1-13.)

Clinical Relevance: AAAs are a substantial clinical problem and can lead to sudden aortic rupture and death. Recent studies have shown a critical role for sex disparity during AAA formation, as female sex has a lower incidence of aortic aneurysm disease, but their outcomes following intervention appear to be worse. Few studies have examined the potential causes that underlie these differences. In this study, we tested the hypothesis that sex differences in endogenous SPM expression and their receptors exist between males and females and that this difference could be associated with AAA formation.

Keywords: Abdominal aortic aneurysm; Aortic aneurysms; Lipid mediators; Resolution of inflammation; Resolvins; Sex differences; Specialized pro-resolving mediators

Abdominal aortic aneurysm (AAA) is a chronic inflammatory pathophysiology that is approximately four times more prevalent in males than females.[1] Despite having identical risk factors, females tend to have worse outcomes,[2] largely due to aneurysm rupture rates.[3] The mechanisms that account for these differences in the development and progression of AAA remain elusive,[4] but recent literature has pointed to biological sex-specific responses to the onset and resolution of inflammation as a potential explanation.[5,6] It is clear that biological sex differences exist, but few studies have addressed these disparities relative to endogenous inflammation-resolution mediators in this disease process.

The pathobiology of AAA predominantly involves leukocyte infiltration into the aortic wall and their subsequent production of proinflammatory cytokines.[7] The driving force behind persistent, chronic inflammation is a

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dysregulated "resolution" phase in which the system fails to return to homeostatic conditions. This resolution phase of inflammation is now recognized as an active process mediated by the endogenous production of potent, bio-active families of lipids that are derived from polyunsaturated omega-3 fatty acids. This superfamily, referred to as specialized pro-resolving lipid mediators (SPMs), have been deemed "master regulators" of the resolution phase of inflammation.[8-11] The role of SPMs in the resolution of inflammation in AAAs has yet to be fully elucidated, and there has been very little evaluation of SPMs in regards to sex differences. The unique ability of these bioactive mediators to modulate inflammation could be harnessed as a clinically applicable therapeutic strategy for the treatment of chronic aortic inflammation and vascular remodeling.[12]

In this study, we investigated expression levels of various bioactive SPMs and their respective G-protein coupled receptor (GPCRs), namely FPR2, LGR6, and GPR18, in aortic tissue from humans, as well as experimental murine control aorta and AAA-derived samples. Our hypothesis was that SPM levels are decreased in males during AAA formation, leading to defective inflammation-resolution during this chronic vascular pathology. Thus, we focused mainly on the suspected differential expression of specific SPM isoforms (ie Resolvin D1 and D2 [RvD1/D2] and Maresin1 [MaR1]), the association with sex differences in AAA formation, and how these differences may contribute to the impact of sex on AAA formation.

ARTICLE HIGHLIGHTS

- **Type of Research**: Human tissue and in vivo mouse studies
- **Key Findings**: Human and murine aortic samples with and without abdominal aortic aneurysm (AAA) were collected and analyzed for specialized pro-resolving lipid mediator expressions and their respective receptor expression levels. Human studies demonstrated a decrease in RvD1 and related FPR2 receptor expression in male AAAs compared to male controls. In vivo murine studies showed increased expression of DHA, EPA, RvD2, and MaR1, as well as decrease in FPR2 expression in aortic tissue from male AAAs compared with female AAAs.
- **Take Home Message**: Our findings demonstrate that sex differences exist among specialized pro-resolving lipid mediators and their receptor expressions in human AAA and experimental murine model of AAA.

MATERIALS AND METHODS

Human aortic tissue analysis

Collection of human aortic tissue was approved by the University of Florida's Institutional Review Board (IRB201902782). Consent was obtained from all patients before surgery. Aortic tissue was processed and assayed, as described in the Supplementary Methods.

Animals

Adult male and female 8- to 12-week-old C57BL/6 (wild-type mice) were obtained from Jackson Laboratory and fed regular diet (Teklad global 2016 rodent diet; Envigo). All animal experimentation was approved by the University of Florida's Institutional Animal Care and Use Committee (Protocol #201910902) and maintained compliance with the United States National Research Council's Guide for the Care and Use of Laboratory Animals, the United States Public Health Service's Policy on Humane Care and Use of Laboratory Animals, and Guide for the Care and Use of Laboratory Animals.

Murine elastase AAA model. AAA formation was induced using a topical elastase model, as previously described,[13-15] and aortic diameters were determined by video micrometry using Leica Application Suite 4.3 software (Leica Microsystems). Aortic tissue and plasma were collected on days 7, 14, or 21 for further analyses.

Mass spectrometry analysis of SPMs

Qualitative and quantitative analysis of lipid mediators was performed via liquid chromatography-tandem mass spectrometry (LC-MS/MS) on a ThermoFisher Scientific TSQ Altis triple quadrupole mass spectrometer at the Southeast Center for Integrated Metabolomics, University of Florida.

Quantification of SPM receptors

RNA was isolated from human or murine aortic tissue from patients with AAA and control subjects using Total Exosome RNA and Protein Isolation Kit (Thermo Fisher Scientific). cDNA was synthesized using the iScript cDNA Synthesis Kit (BioRad). Quantitative real-time polymerase chain reaction (RT-PCR) was performed with primer sets (MWG/Operon) in conjunction with SsoFast EvaGreen Supermix (BioRad).

Statistical analysis

Values are means ± standard error of mean, and statistical evaluation was performed with Prism 6 software (GraphPad). A Student t test with nonparametric Mann-Whitney or Wilcoxon test was used for pair-wise comparisons of groups. One-way analysis of variance after post hoc Tukey test was used to determine the differences among multiple comparative groups. A value of $P < .05$ was considered statistically significant.

RESULTS

SPM and associated GPCRs differ in human AAA tissue. We evaluated SPM levels in human AAA tissue samples from male and female patients and comparative controls. Although available data on demographics and

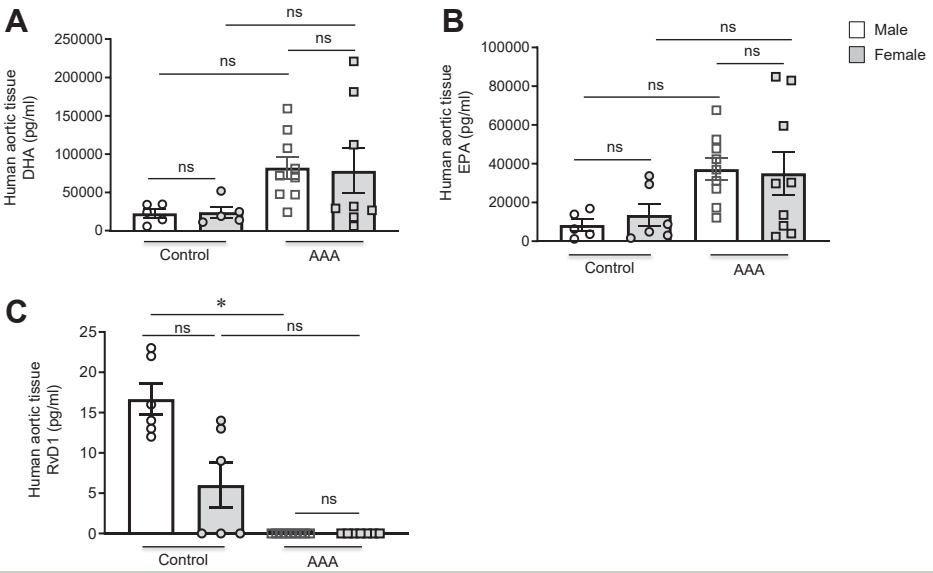

Fig 1. A-B, No significant differences were observed in DHA and EPA levels in male and female aortic tissue from patients with abdominal aortic aneurysms (*AAAs*) compared with controls. *ns*, Not significant; n = 6-10/group. **C,** RvD1 levels were significantly decreased in male patients with AAA compared with controls. *$P < .01$. *ns*, Not significant; n = 6-10/group.

comorbidities from controls (organ transplant donors) were limited, we matched patients with AAA with controls based on available comorbidities and age (Supplementary Table). The ω-3 fatty acid precursors, DHA and EPA, showed trends towards an increase in male and female AAA tissue compared with non-diseased controls (Fig 1, *A-B*). However, there were no significant differences in DHA and EPA expression levels observed between samples from male and female patients with AAA. There was a significant decrease observed in RvD1 expression in male patients with AAA compared to respective male controls (Fig 1, *C*; 1.3 ± 1.3 vs 16.67 ± 1.9 pg/mL; $P = .008$). Moreover, no significant differences were observed in RvD2 expression between aortic tissue from male and female patients with AAA, and MaR1 levels were too low to be detected in both human control and AAA samples (data not shown).

Expression of SPM receptors, FPR2, GPR18, and LGR6, varied in human aortic tissue. FPR2 were downregulated in male AAA compared with controls but showed no differences in female AAA compared with respective controls or male AAA tissue (Fig 2, *A-C*). LGR6 expression was significantly decreased in male AAA samples compared with control but was unchanged in female AAA compared with respective control or male AAA (Fig 2, D-F). Expression of GPR18 showed no differences in both male and female AAA samples compared with respective controls (data not shown).

DHA, EPA, and RvD2 levels differ by sex in experimental murine AAA. SPM levels in aortic tissue were then evaluated using an elastase treatment model of AAAs. Male and female mice underwent topical elastase or heat-inactivated elastase (control) treatment on day 0, and aortas were collected on postoperative day 7 for mass spectrometry analysis to quantify omega-3 fatty polyunsaturated acids, which are metabolic precursors to SPMs, as well as SPM levels. Elastase-treated male mice had a significant increase in DHA levels compared with elastase-treated female mice (86,088 ± 11,783 vs 35,955 ± 6288 pg/mL; $P = .02$) (Fig 3, *A*). Similarly, elastase-treated male mice had a significant increase in EPA levels compared with elastase-treated female mice (6601 ± 1138 vs 2176 ± 398.5 pg/mL; $P = .02$) (Fig 3, *B*).

Interestingly, there was no significant differences in RvD1 levels between male and female controls or elastase-treated males and females compared with their respective controls (Fig 3, *C*). RvD2 levels in aortic tissue was significantly higher in the elastase-treated males compared with the elastase-treated females (272 ± 74 vs 33 ±9 pg/mL; $P = .007$) (Fig 3, *D*). MaR1 levels were increased in aortic tissue of elastase-treated males compared with elastase-treated females (203 ± 30 vs 95.6 ±9.2 pg/mL; $P = .01$). DHA, EPA, and RvD1 plasma levels were significantly increased in elastase-treated males compared with male controls on day 7 (Supplementary Fig 2, *A-C*). Additionally, DHA, EPA and RvD2 plasma levels were significantly increased in female controls compared with male controls (Supplementary Fig 2, *A-C*).

SPM-associated GPCRs, FPR2 and LGR6, display sex-based differences. We sought to evaluate the changes in SPM receptor expression in aortic tissue over disease

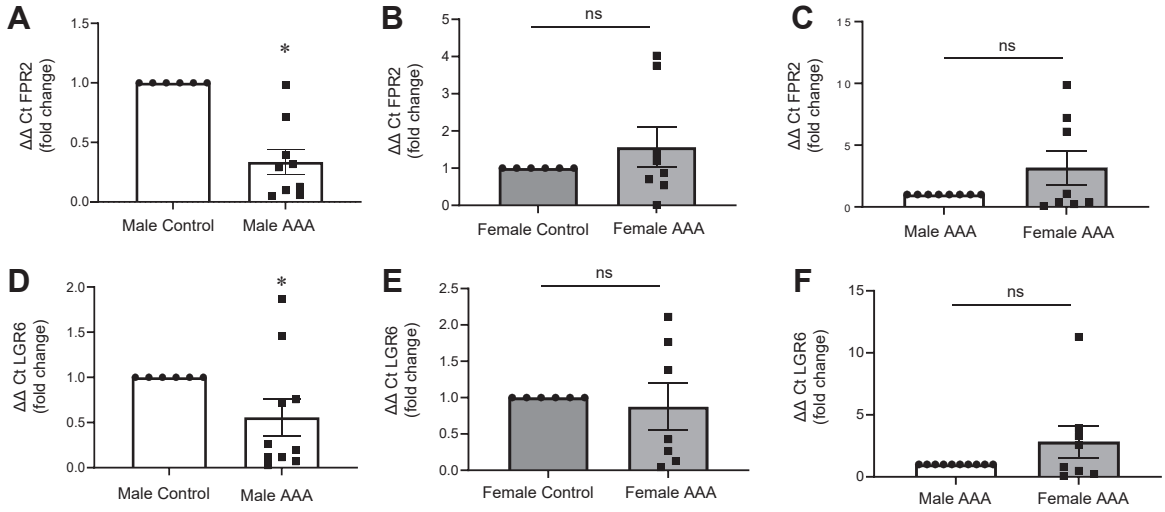

Fig 2. FPR2 **(A-C)** and LGR6 **(D-F)** expressions were significantly decreased in male aortic tissue from patients with abdominal aortic aneurysms (*AAAs*) compared with respective controls. No differences were observed in female AAA tissue compared with female controls or male AAA tissue. *$P < .01$. *ns*, Not significant; n = 5/group.

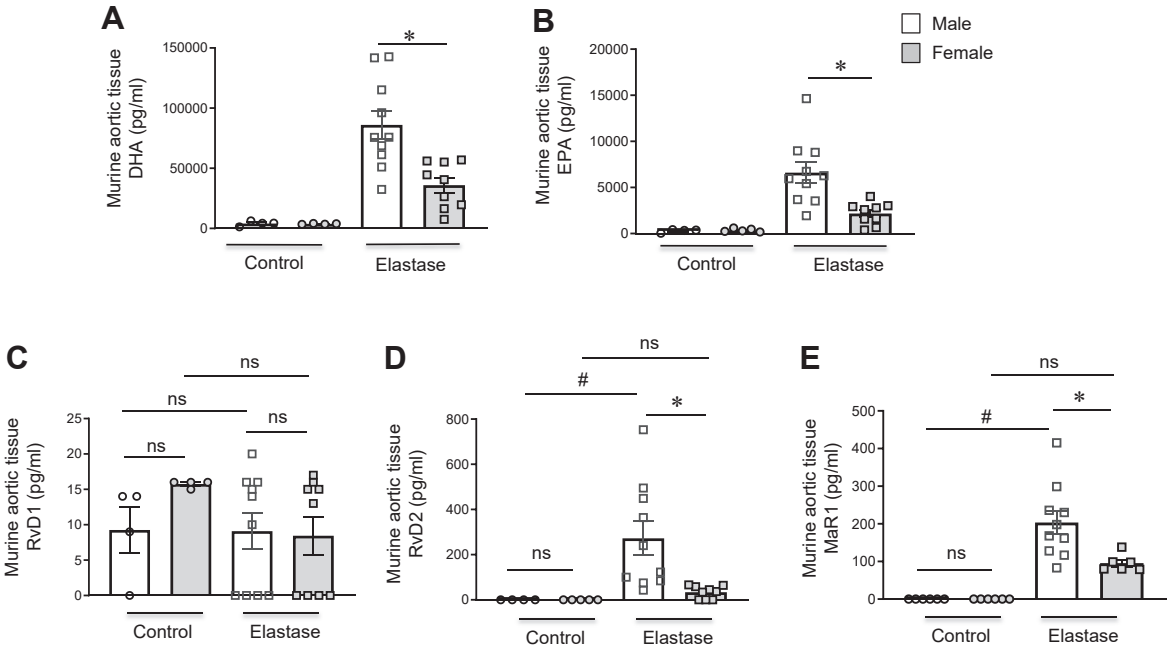

Fig 3. Specialized pro-resolving lipid mediator (SPM) levels vary between males and females in aortic tissue from experimental murine abdominal aortic aneurysm (AAA) model. **A-B**, DHA and EPA levels in elastase-treated male aortic tissue were significantly higher compared with elastase-treated female aortic tissue. *$P < .02$. n = 5-10/group. **C,** No significant differences were observed between the relative groups for aortic tissue expression of RvD1. *ns*, Not significant. n = 5-12/group. **D,** Aortic tissue from elastase-treated male mice showed higher RvD2 levels than all other groups *$P < .01$; #$P < .007$. *ns*, Not significant. n = 5-14/group. **E,** Aortic tissue from elastase-treated males showed a significant increase in MaR1 compared with control male mice, as well as compared with elastase-treated female mice. *$P = .01$; #$P < .0001$. *ns*, Not significant. n = 5-14/group.

progression on days 7, 14, and 21 post-aneurysm induction. As expected, the aortic diameter was significantly increased on days 7, 14, and 21 in elastase-treated male and female mice compared with respective controls (Fig 4). The aortic diameter was significantly increased in elastase-treated male mice compared with respective female mice on days 7 and 14. The RvD1 receptor FPR2, the RvD2 receptor GPR18, and the MaR1 receptor LGR6

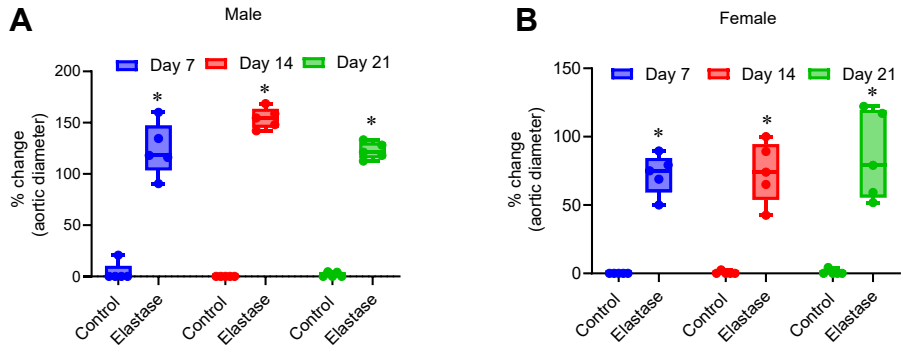

Fig 4. A-B, Elastase-treated wild-type (WT) mice demonstrated a significant increase in aortic diameter on days 7, 14, and 21 compared with respective heat-inactivated elastase-treated (control) mice. *$P < .01$; n = 5 mice/group.

were quantified from aortic tissue using mRNA analysis. FPR2 expression was significantly increased in elastase-treated female mice compared with elastase-treated male on days 7 and 21, but not day 14 (Fig 5, *A-C*). LGR6 expression was significantly decreased in elastase-treated female mice on day 7, increased on day 14, and displayed no difference on day 21 compared with elastase-treated male mice (Fig 5, *D-F*). There were no differences in GPR18 expression in elastase-treated male mice as compared with female mice on any day (data not shown).

DISCUSSION

In this study, significant differences in RvD1 expression and SPM receptors (FPR2 and LGR6) were observed in human aortic tissue from male and female controls and AAAs. In vivo studies of elastase-treated mice showed higher levels of DHA, EPA, RvD2, and MaR1 and significant differences in FPR2 and LGR6 expressions in aortic tissue from males as compared with females, in the inflammation-resolution process. Our results suggest that sex-based differences in the levels of SPMs and their receptors could play an important sequential role in the resolution of aortic inflammation and vascular remodeling in the progression of AAAs.

The biogenesis of SPMs begins with the local release of ω-3 and ω-6 polyunsaturated fatty acids in response to tissue injury,[16] such as the initial inflammatory response, that preludes the development of AAAs. These fatty acids are then taken up by invading immune cells, like neutrophils and macrophages,[11] or by local cells, such as vascular endothelial and smooth muscle cells[17] in the context of vascular diseases, where they undergo enzymatic processing that eventually leads to the production of SPM families. These families include Lipoxins, D- and E-series Resolvins, Protectins, and Maresins. The primary role of SPMs in inflammation is to serve as master regulators of the resolution process, and the exact mechanisms they elicit to achieve this are context- and tissue-dependent. In the context of vascular inflammation, various families of SPMs have been show to inhibit AAA formation, promote polarization of invading immune cells to a pro-resolving phenotype,[7] and attenuate inflammatory signaling and activation pathways of endothelial cells and vascular smooth muscle cells.[18,19]

Female sex has been shown to have an increased capacity to produce EPA and DHA from alpha-linolenic acid, and a recent meta-analysis found females to have higher plasma concentrations of DHA than males.[20,21] Therefore, we were surprised to find that SPMs were lower in female mice compared with male mice. In 2015, Barden et al compared plasma concentrations of SPMs in men and women with features of the metabolic syndrome and health-matched control subjects and identified that the plasma concentrations of the SPM precursors 18-hydroxy-EPA, 14-hydroxy DHA, and 17 hydroxy-DHA were higher in females than males, but that there were no sex differences in the concentrations of RvD1, RvD2, or MaR1.[22] More recently, Barden et al evaluated plasma concentrations of SPMs in healthy men and women randomly allocated to receive saline or varying concentrations of intravenous dexamethasone and reported that plasma RvD1 was lower in females compared with males, and that plasma RvD2 was not different between the sexes. Similar to our study, plasma MaR1 levels were unable to be detected in plasma from healthy volunteers.[23] Rathod et al profiled lipid mediators in the fluid of skin blisters 24 hours after their induction by cantharidin.[6] They identified distinct clusters of lipid mediators in females and males, and only protectin D1 was significantly different between sexes, although the sum of D-series resolvins was significantly higher in females. In a separate experiment, plasma SPMs were measured in females and males 8 hours following typhoid vaccination, and no EPA- or DHA-derived SPMs reported (including RvD1, RvD2) were different between

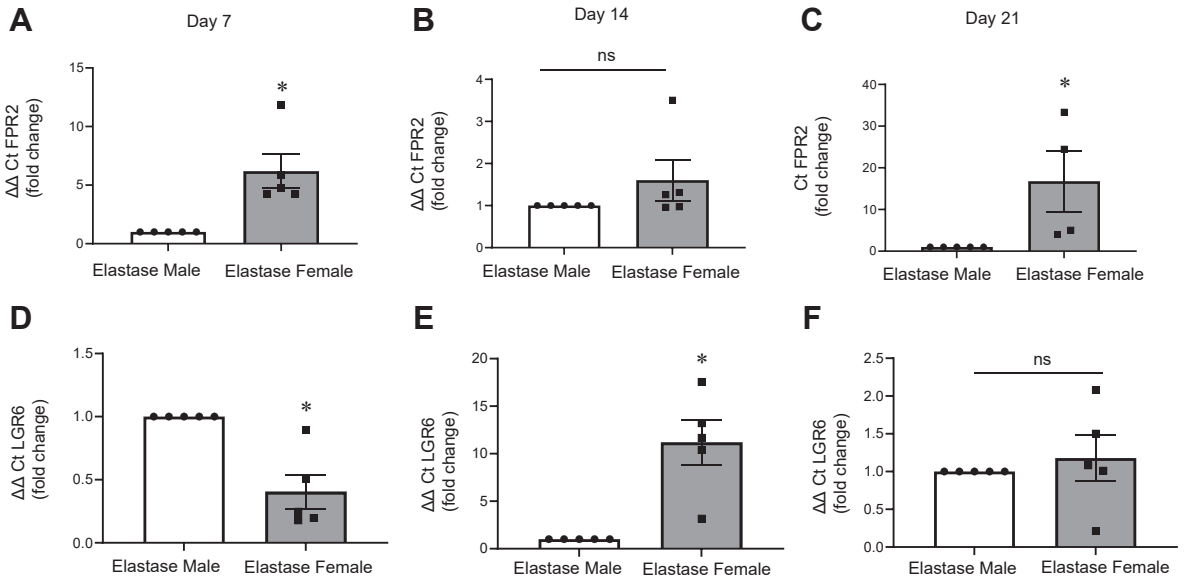

Fig 5. A-C, Elastase-treated female mice displayed a significant increase in FPR2 receptor expression on day 7 and day 21. *$P < .01$. *ns*, Not significant. n = 5/group**. D-F**, LGR6 receptor expression was significantly decreased in elastase-treated female mice on day 7, which was increased on day 14, between elastase-treated male and female mice. *$P < .01$. *ns*, Not significant. n = 5/group.

the sexes, although the sum of E-series resolvins was significantly higher in females.[6] In a study by Prashar et al, investigators found a sex mediated elevation of SPMs in a Sjogren's syndrome mouse model, where RvD1, RvD2, and MaR1 production were elevated in plasma collected from mice after disease onset, but not in male mice. They also found an increase in the expression of the ALX/FPR2 and GPR18 receptors in submandibular glands, which may explain why additional RvD1 amounts enhance the resolution of inflammation in female mice after disease onset in this model.[24] Altogether, the above findings suggest that differences in concentrations of specific SPMs exist in a sex-specific manner.

The actions of SPMs are facilitated through activation of specific GPCRs. Specific bioactive SPM isoforms like RvD1 and lipoxin A4 (LXA_4) are ligands for the ALX/FPR2 receptor that can be found on neutrophils, lymphocytes, epithelial cells, macrophages, etc. Key actions attributed to ALX/FPR2 activation include blocking neutrophil migration through downregulation of chemokine receptors and reducing cytokine and chemokine production, which work to reduce inflammation and return the system back to homeostatic conditions.[25] Other resolvins, like RvD2, act through another GPCR (ie, GPR18),[26] and have been shown to be expressed on macrophages and monocytes and regulates their phagocytic function/clearance to assist in phagocytic function.[27] The pro-resolving actions of MaR1 have been demonstrated to be carried out via LGR6 signaling. This GPCR is found throughout various tissues and vasculature. LGR6 activation by MaR1 has been shown to induce protection in vascular inflammatory conditions by modulating the efferocytic clearance of apoptotic smooth muscle cells.[14] Our results suggest that the SPM-receptor expressions contribute to the pathogenesis of human AAAs, especially in males, as the downregulation of FPR2 and LGR6 suggests that the anti-inflammatory signaling via these GPCRs is decreased thereby contributing to dysregulated inflammation-resolution of AAAs.

There are several limitations of our study. The isoforms of the SPMs such as RvD2 and MaR1 levels in the human female and male aortic tissue controls were below detectable limits using LC-MS-MS quantification methods. However, the experimental models suggest that SPM isoform expressions are altered especially during the early phases of inflammation-resolution. This indicates that sequential analyses of aortic tissue especially in diseased conditions will be more prudent to analyze lipid mediators due to their short half-life. Concomitant analysis of pro-inflammatory lipid mediators such as arachidonic acid-derived prostaglandins and thromboaxanes also needs to be performed for analyzing the kinetics of inflammation and resolution during vascular injury. Additionally, immune cells, like neutrophils and macrophages, are important sources of SPMs, like resolvins and maresins,[11] and are upregulated early on in the AAA pathogenesis, so analysis of aortic tissue through the development and progression of vascular inflammation, like in AAA, would provide insight into the correlation between SPM presence, immune cell infiltration, and disease progression. Further studies are

required to characterize the cell-specific expression of SPM receptor expressions via spatial immunostaining of aortic tissue. Moreover, sex differences in SPM formation span both human and experimental models, but to date have been inconsistent and influenced by disease context, experimental settings, microbiome, inflammatory mediators, species, race, age, comorbidities, and additional factors.[6,24,28,29] The limited sociodemographic clinical data and differences in characteristics between the human subjects should be acknowledged as a limitation for the comparison between control and AAA aortas.

Our findings demonstrate that differences exist among SPM expression inhuman and murine AAA tissue and plasma samples. Males appear to have higher levels of certain SPMs compared with females in our in vivo murine AAA models, despite females having higher expression of some SPM-associated GPCRs. However, these differences were not observed in the human AAA samples, suggesting that the sequential expression of SPMs and their specific receptors may help define the dysregulation of inflammation-resolution in this vascular pathology. Defining the early mechanisms and the balance between SPM and receptor expression underlying sex-related differences in AAA formation is critical, as understanding the differences in disease patterns based on sex may allow for the development of new translational approaches to the prevention and treatment of patients with aortic aneurysms. Further investigation with a therapeutic combination of specific SPMs is required in chronic AAA and aortic rupture experimental models to delineate these pathways.

AUTHOR CONTRIBUTIONS

Conception and design: AS, GU
Analysis and interpretation: AF, VL, AS, GU, TG
Data collection: AF, VL, ZL, GS, CE, EP, GL, JH, AZ, TG
Writing the article: AF, VL, AS, GU
Critical revision of the article: AF, VL, ZL, GS, CE, EP, GL, JH, AZ, AS, GU, TG
Final approval of the article: AF, VL, ZL, GS, CE, EP, GL, JH, AZ, AS, GU, TG
Statistical analysis: AF, VL, AS
Obtained funding: AF, AS, GU
Overall responsibility: GU
AF and VL contributed equally to this article and share co-first authorship.

REFERENCES

1. Singh K, Bonaa KH, Jacobsen BK, Bjork L, Solberg S. Prevalence of and risk factors for abdominal aortic aneurysms in a population-based study: the Tromso Study. Am J Epidemiol 2001;154:236-44.
2. O'Donnell TFX, Verhagen HJ, Pratesi G, et al. Female sex is associated with comparable 5-year outcomes after contemporary endovascular aneurysm repair despite more challenging anatomy. J Vasc Surg 2020;71:1179-89.
3. Grootenboer N, Bosch JL, Hendriks JM, van Sambeek MR. Epidemiology, aetiology, risk of rupture and treatment of abdominal aortic aneurysms: does sex matter? Eur J Vasc Endovasc Surg 2009;38:278-84.
4. Villard C, Hultgren R. Abdominal aortic aneurysm: sex differences. Maturitas 2018;109:63-9.
5. Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol 2016;16:626-38.
6. Rathod KS, Kapil V, Velmurugan S, et al. Accelerated resolution of inflammation underlies sex differences in inflammatory responses in humans. J Clin Invest 2017;127:169-82.
7. Pope NH, Salmon M, Davis JP, et al. D-series resolvins inhibit murine abdominal aortic aneurysm formation and increase M2 macrophage polarization. FASEB J 2016;30:4192-201.
8. Serhan CN. Novel pro-resolving lipid mediators in inflammation are leads for resolution physiology. Nature 2014;510:92-101.
9. Basil MC, Levy BD. Specialized pro-resolving mediators: endogenous regulators of infection and inflammation. Nat Rev Immunol 2016;16:51-67.
10. Serhan CN. Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. Annu Rev Immunol 2007;25:101-37.
11. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature 2014;510:92-101.
12. Filiberto AC, Ladd Z, Leroy V, et al. Resolution of inflammation via RvD1/FPR2 signaling mitigates Nox2 activation and ferroptosis of macrophages in experimental abdominal aortic aneurysms. FASEB J 2022;36:e22579.
13. Laser A, Lu G, Ghosh A, et al. Differential gender- and species-specific formation of aneurysms using a novel method of inducing abdominal aortic aneurysms. J Surg Res 2012;178:1038-45.
14. Elder CT, Filiberto AC, Su G, et al. Maresin 1 activates LGR6 signaling to inhibit smooth muscle cell activation and attenuate murine abdominal aortic aneurysm formation. FASEB J 2021;35:e21780.
15. Filiberto AC, Spinosa MD, Elder CT, et al. Endothelial pannexin-1 channels modulate macrophage and smooth muscle cell activation in abdominal aortic aneurysm formation. Nat Commun 2022;13:1521.
16. Kasuga K, Yang R, Porter TF, et al. Rapid appearance of resolvin precursors in inflammatory exudates: novel mechanisms in resolution. J Immunol 2008;181:8677-87.
17. Chatterjee A, Komshian S, Sansbury BE, et al. Biosynthesis of pro-resolving lipid mediators by vascular cells and tissues. Faseb j 2017;31:3393-402.
18. Chatterjee A, Sharma A, Chen M, Toy R, Mottola G, Conte MS. The pro-resolving lipid mediator maresin 1 (MaR1) attenuates inflammatory signaling pathways in vascular smooth muscle and endothelial cells. PLoS One 2014;9:e113480.
19. Miyahara T, Runge S, Chatterjee A, et al. D-series resolvin attenuates vascular smooth muscle cell activation and neointimal hyperplasia following vascular injury. FASEB J 2013;27:2220-32.
20. Lohner S, Fekete K, Marosvolgyi T, Decsi T. Gender differences in the long-chain polyunsaturated fatty acid status: systematic review of 51 publications. Ann Nutr Metab 2013;62:98-112.
21. Baker EJ, Miles EA, Burdge GC, Yaqoob P, Calder PC. Metabolism and functional effects of plant-derived omega-3 fatty acids in humans. Prog Lipid Res 2016;64:30-56.
22. Barden AE, Mas E, Croft KD, Phillips M, Mori TA. Specialized pro-resolving lipid mediators in humans with the metabolic syndrome after n-3 fatty acids and aspirin. Am J Clin Nutr 2015;102:1357-64.
23. Barden A, Phillips M, Hill LM, et al. Antiemetic doses of dexamethasone and their effects on immune cell populations and plasma mediators of inflammation resolution in healthy volunteers. Prostaglandins Leukot Essent Fatty Acids 2018;139:31-9.
24. Parashar K, Schulte F, Hardt M, Baker OJ. Sex-mediated elevation of the specialized pro-resolving lipid mediator levels in a Sjögren's syndrome mouse model. FASEB J 2020;34:7733-44.
25. Serhan CN, Krishnamoorthy S, Recchiuti A, Chiang N. Novel anti-inflammatory–pro-resolving mediators and their receptors. Curr Top Med Chem 2011;11:629-47.
26. Chiang N, Dalli J, Colas RA, Serhan CN. Identification of resolvin D2 receptor mediating resolution of infections and organ protection. J Exp Med 2015;212:1203-17.
27. Chiang N, de la Rosa X, Libreros S, Serhan CN. Novel resolvin D2 receptor Axis in Infectious inflammation. J Immunol 2017;198:842-51.

28. Troisi F, Pace S, Jordan PM, et al. Sex Hormone-Dependent lipid mediator formation in male and female mice during Peritonitis. Front Pharmacol 2021;12:818544.
29. Calder PC. Eicosapentaenoic and docosahexaenoic acid derived specialised pro-resolving mediators: concentrations in humans and the effects of age, sex, disease and increased omega-3 fatty acid intake. Biochimie 2020;178:105-23.

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Supplementary Methods

Materials and methods

Human aortic tissue analysis. Collection of human aortic tissue was approved by the University of Florida's Institutional Review Board (IRB201902782). Consent was obtained from all patients before surgery. Aortic tissue from male and female patients was resected during open surgical abdominal aortic aneurysm (AAA) repair as well as during organ transplant donor surgery (controls). Available demographic data and comorbidities from controls and patients with AAA were collected, as described in Supplementary Table I. Tissue was homogenized in Trizol, and RNA was purified per manufacturer's protocol (Qiagen). cDNA was synthesized using the iScript cDNA Synthesis Kit (BioRad). Quantitative real-time polymerase chain reaction was performed with primer sets (MWG/Operon) in conjunction with SsoFast EvaGreen Supermix (BioRad), as previously described.[2] Gene expression was calculated by using the relative quantification method according to the following equation: $2(-\Delta CT)$, where ΔCT = (Average gene of interest) − (Average reference gene), where GAPDH was used as the reference gene.

Animals. Adult male and female 8- to 12-week-old C57BL/6 (wild-type mice) were obtained from The Jackson Laboratory and fed regular diet (Teklad global 2016 rodent diet; Envigo). All animal experimentation was approved by the University of Florida's Institutional Animal Care and Use Committee (Protocol #201910902) and maintained compliance with the United States National Research Council's Guide for the Care and Use of Laboratory Animals, the United States Public Health Service's Policy on Humane Care and Use of Laboratory Animals, and Guide for the Care and Use of Laboratory Animals. All animals had free access to food (regular composition) and water.

Murine elastase AAA model. AAA formation was induced using a topical elastase model, as previously described.[3-5] A midline laparotomy was made and the infrarenal aorta was exposed circumferentially just distal to the renal arteries down to the aortic bifurcation. Five μl of porcine pancreatic elastase (Sigma-Aldrich; 0.3 mg protein/mL, 7 units/mg protein) or 5 μl heat-inactivated elastase (at 90 °C for 30 minutes) as a control was applied topically to the exposed aortic adventitia for 5 minutes. Aortic tissue or plasma were collected at day 7, 14, or 21. Prior to euthanizing the mouse, the recent laparotomy incision was opened, and the abdominal aorta was exposed. The infrahepatic inferior vena cava just lateral to the abdominal aorta was identified. A needle was used to extract ∼0.8mL of whole blood and was immediately placed in a heparinzed Eppendorf tube. This was later centrifuged at 2200 RPM for 15 minutes, and ∼200μL plasma was extracted and frozen at −80 °C. Aortic diameters were determined by video micrometry using Leica Application Suite 4.3 software (Leica Microsystems). The aortas were collected and flash frozen in liquid nitrogen and stored at −80 °F for liquid chromatography-mass spectrometry analysis.

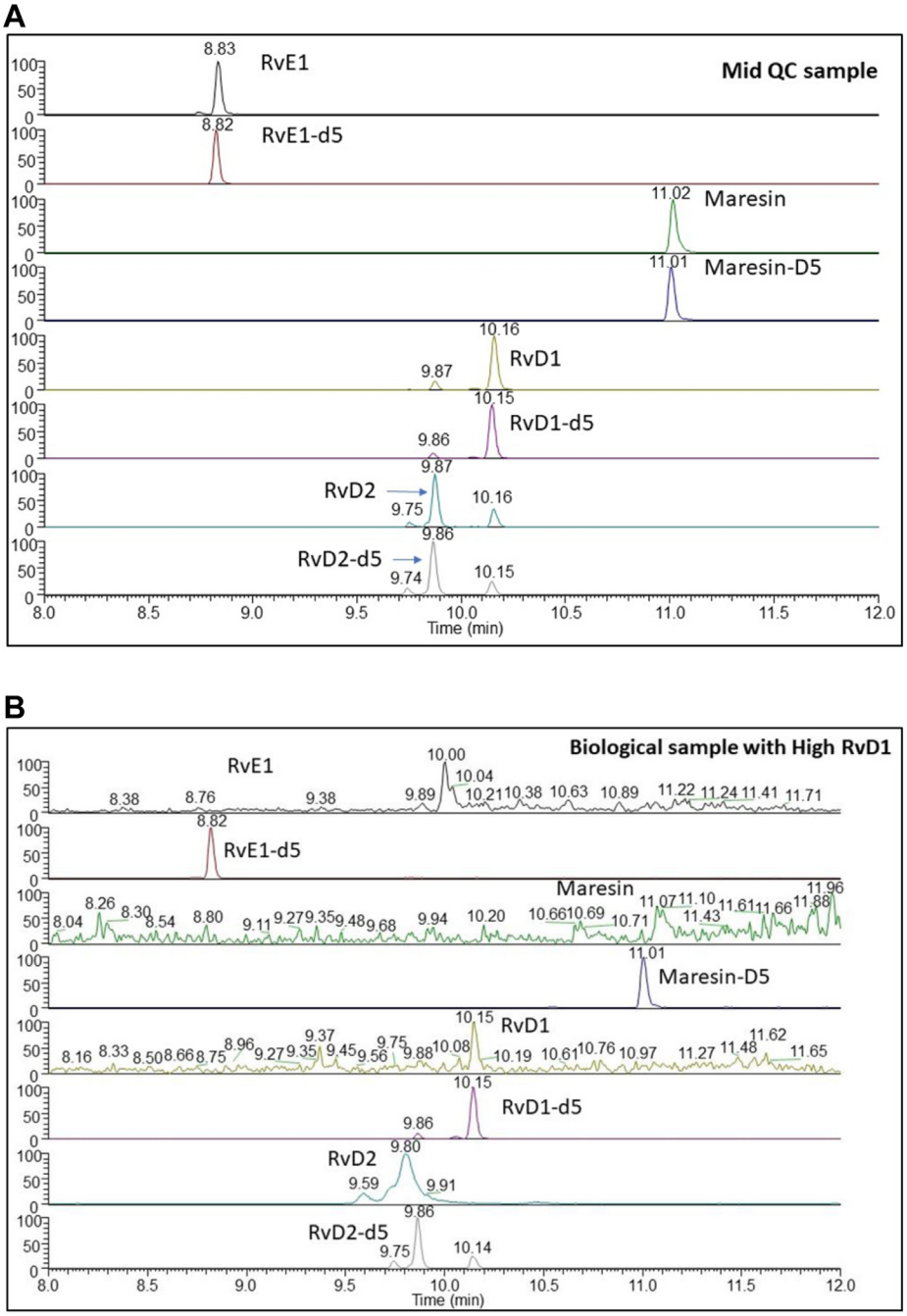
Mass spectrometry analysis of specialized pro-resolving lipid mediators. Resolvins and deuterated standards were purchased from Cayman Chemical. Liquid chromatography-mass spectrometry grade acetonitrile, water with 0.1% formic acid, methanol, water, and ammonium acetate were purchased from Fisher (Fisher Scientific). Methyl formate and hexane were purchased from Sigma. Five hundred mg capacity C18 solid phase extraction columns were purchased from Biotage. Three sets of calibration curves (25-1000 pg/mL) and QCs (50, 500, 1000 pg/mL) were obtained for resolvins D1, D2, D3, D5, 17(R)-resolvin D1, E1, docosahexanoic acid, (±)18-HEPE, eicosapentanoic acid and Maresin 1. Calibrators and QC were prepared in ethanol. Deuterated internal standards (IS) working solution in ethanol consisted of Resolvin D1-d5, Resolvin D2-d5, Resolvin D3-d5, 17(R)-resolvin D1-d5, docosahexaneoic acid-d5, Resolvin E1-d4, and eicosapentanoic acid-d5, Maresin 1-d5 was spiked into all calibrators and QCs at 40 μl, dried down under nitrogen, and reconstituted with 50μl 1:1 methanol: 5mM ammonium acetate, pH 9.The tissue homogenates were extracted by solid phase extraction following previously published methods.[1] Briefly, each sample was spiked with 4 μL deuterated internal standard solution. Proteins were precipitated by addition of 4 mL of chilled methanol, incubated for 45 minutes at 4 °C. The supernatant was collected after centrifugation and dried to about 0.5 ml under nitrogen. The remaining samples were acidified with 9 mL water, pH 3.5. SPE columns were equilibrated with 3 mL methanol and 6 mL water. The acidified samples were rapidly loaded on the conditioned SPE columns. The SPE columns were then washed with 4 mL water, followed by 5 mL hexane. Captured resolvins were eluted with 9 mL methyl formate. Samples were dried completely under nitrogen and reconstituted with 50 μL 1:1 methanol: 5 mM ammonium acetate, pH 9. Liquid chromatography-tandem mass spectrometry quantitation by selected ion monitoring (SRM) was performed on a Thermo TSQ Altis triple quadrupole mass spectrometer with Vanquish UPLC system. All samples were analyzed in negative heated electrospray ionization. Separation was achieved on an ACE 18-pfp 100 × 2.1 mm, 2 μm column with mobile phase A as 0.1% formic acid in water and mobile phase B as acetonitrile. The flow rate was 350 μL/min with a column temperature of 25 °C. All calibrators, QCs, and samples were injected at 15 μL. The quantification was normalized to starting tissue weight. Representative chromatograms of SPM analysis are shown in Supplementary Fig 1.

Quantification of specialized pro-resolving lipid mediator receptors. RNA was isolated from murine and human aortic tissue from patients with AAAs or control

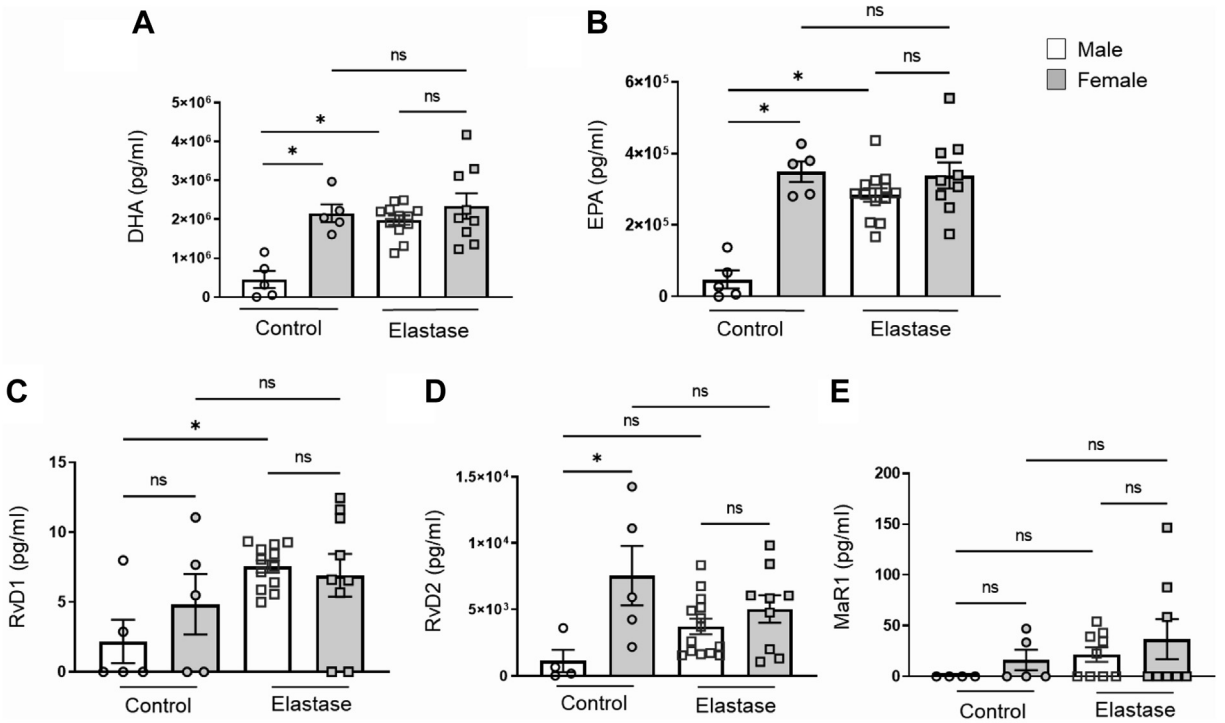
subjects using Total Exosome RNA and Protein Isolation Kit (Thermo Fisher Scientific). cDNA was synthesized using the iScript cDNA Synthesis Kit (BioRad). Quantitative real-time polymerase chain reaction was performed with primer sets (MWG/Operon) in conjunction with SsoFast EvaGreen Supermix (BioRad). To evaluate receptor expression of FPR2, LGR6, and GPR18 in human aortic tissue, the following primers were used: FPR2 Fwd: GGCTACACTGTTCTGCGGAT, FPR2 Rev: CACCCAGATCACAAGCCCAT, LGR6 Fwd: ACGGCTTACCTGGACCTCA, LGR6 Rev: TGCTTGTCCTGGGATGTGTG, GPR18 Fwd: GCCAAGCGTTACACTGGAAA, GPR18 Rev: TGATACTTAGAAACTCCTGTCCATC GAPDH Fwd: TTGATGGCAACAATCTCCAC, GAPDH Rev: CGTCCCGTAGACAAAATGGT.

The following primers were used for murine analysis: FPR2 Fwd: CACAGGAACCGAAGAGTGTAAG, FPR2 Rev: CACCATTGAGAGGATCCACAG, LGR6 Fwd: GAGGACGGCATCATGCTGTC, LGR6 Rev: GCTCCGTGAGGTTGTTCATACT, GPR18 Fwd: TCATGATCGGGTGCTACGTG, GPR18 Rev: CTTGTAGCATCAGGACGGCA, β-Actin Fwd: GGCTGT ATTCCCCTCCATCG and β-Actin Rev: CCAGTTGGTAACAATGCCATGT. Gene expression was calculated by using the relative quantification method according to the following equation: $2(-\Delta CT)$, where ΔCT = (average gene of interest) − (average reference gene), where GAPDH was used as the reference gene for human tissue and β-Actin was used as the reference gene for murine tissue. Each polymerase chain reaction was carried out in triplicate, and the relative quantification of gene expression was quantified as fold change.

Statistical Analysis. Values are means ± standard error of mean, and statistical evaluation was performed with Prism 6 software (GraphPad). A Student *t* test with nonparametric Mann-Whitney or Wilcoxon test was used for pair-wise comparisons of groups. One-way analysis of variance after post hoc Tukey test was used to determine the differences among multiple comparative groups. A value of $P < .05$ was considered statistically significant.



Supplementary Fig 1. Representative chromatograms **(A-B)** of aortic tissue analysis from the liquid chromatography-tandem mass spectroscopy system.



Supplementary Fig 2. Expression of DHA **(A)**, EPA **(B)** and specialized pro-resoloving lipid mediators (SPMs; **C-E**) in plasma on day 7 of in vivo murine abdominal aortic aneurysm (AAA) model. $^{*}P < .05$. *ns*, Not significant. n = 4-12/group.

Supplementary Table. Characteristics and comorbidities of male and female patients with abdominal aortic aneurysms (*AAAs*) and comparative controls

Characteristics	Male control (n = 6)	Female control (n = 5)	Male AAA (n = 10)	Female AAA (n = 9)
Age, years	57.5 ±3.33	41.8 ±4.47	70.4 ±2.53	66.1 ±4.53
Comorbidities				
Hypertension	100 (2)	100 (3)	90 (9)	78 (7)
Smoking (current/former)	50 (1)	33 (1)	100 (10)	89 (8)
Peripheral artery disease	0 (0)	0 (0)	50 (5)	11 (1)
Dyslipidemia	0 (0)	67 (2)	60 (6)	56 (5)
COPD	0 (0)	0 (0)	20 (2)	22 (2)
CAD	50 (1)	0 (0)	40 (4)	11 (1)
MI	0 (0)	20 (24)	30 (3)	44 (4)
CHF	0 (80)	0 (0)	10 (1)	0 (0)
Cerebrovascular disease	0 (0)	33 (1)	30 (3)	0 (0)
Renal insufficiency	0 (0)	0 (0)	30 (3)	0 (0)
Missing	67 (4)	40 (2)	0 (0)	0 (0)
Aneurysm size, cm	NA	NA	6.05 ±0.37	6.92 ±0.82
Missing	NA	NA	20 (2)	33 (3)

CAD, Coronary artery disease; *CHF,* congestive heart failure; *COPD,* chronic obstructive pulmonary disease; *MI,* myocardial infarction.
Data are presented as percent (number) or mean ± standard error of the mean.

SUPPLEMENTARY REFERENCES

1. Norris PC, Serhan CN. Metabololipidomic profiling of functional immunoresolvent clusters and eicosanoids in mammalian tissues. Biochem Biophys Res Commun 2018;504:553-61.
2. Sharma AK, Lu G, Jester A, et al. Experimental abdominal aortic aneurysm formation is mediated by IL-17 and attenuated by mesenchymal stem cell treatment. Circulation 2012;126(11 Suppl 1):S38-45.
3. Laser A, Lu G, Ghosh A, et al. Differential gender- and species-specific formation of aneurysms using a novel method of inducing abdominal aortic aneurysms. J Surg Res 2012;178:1038-45.
4. Elder CT, Filiberto AC, Su G, et al. Maresin 1 activates LGR6 signaling to inhibit smooth muscle cell activation and attenuate murine abdominal aortic aneurysm formation. FASEB J 2021;35:e21780.
5. Filiberto AC, Spinosa MD, Elder CT, et al. Endothelial pannexin-1 channels modulate macrophage and smooth muscle cell activation in abdominal aortic aneurysm formation. Nat Commun 2022;13:1521.