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The Δ-5 Fatty Acid Desaturase FADS1 Impacts Metabolic Disease by Balancing Pro-Inflammatory and Pro-Resolving Lipid Mediators

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Abstract

Objective—Human genetic variants near the fatty acid desaturase (FADS) gene cluster (*FADS1–2–3*) are strongly associated with cardiometabolic traits including dyslipidemia, fatty liver, type 2 diabetes, and coronary artery disease. However, mechanisms underlying these genetic associations are unclear.

Approach and Results—Here, we specifically investigated the physiologic role of the Δ-5 desaturase FADS1 in regulating diet-induced cardiometabolic phenotypes by treating hyperlipidemic low-density lipoprotein receptor-null mice with antisense oligonucleotides (ASO) targeting the selective knockdown of *Fads1*. *Fads1* knockdown resulted in striking reorganization of both ω-6 and ω-3 polyunsaturated fatty acid (PUFA) levels as well as their associated pro-inflammatory and pro-resolving lipid mediators in a highly diet-specific manner. Loss of *Fads1* activity promoted hepatic inflammation and atherosclerosis, yet was associated with suppression of hepatic lipogenesis. *Fads1* knockdown in isolated macrophages promoted classic M1 activation, while suppressing alternative M2 activation programs, and also altered systemic and tissue

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DISCLOSURES

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inflammatory responses *in vivo*. Finally, the ability of *Fads1* to reciprocally regulate lipogenesis and inflammation may rely in part on its role as an effector of liver X receptor (LXR) signaling.

Conclusion—These results position *Fads1* as an underappreciated regulator of inflammation initiation and resolution, and suggest that endogenously synthesized arachidonic acid (AA) and eicosapentaenoic acid (EPA) are key determinates of inflammatory disease progression and LXR signaling.

Keywords

fatty acids; atherosclerosis; nutrition; macrophage

INTRODUCTION

Human genetic studies have transformed cardiometabolic drug discovery, providing an unparalleled prediction tool for identification of new drug targets. This is exemplified by the recent success story of large-scale genetic studies leading to rapid development of monoclonal antibodies targeting proprotein convertase subtilisin/kexin type 9 for hyperlipidemia and cardiovascular disease.[1] Given the target prediction power of human genetics, large consortium genome-wide association study efforts have identified hundreds of genomic loci linked to cardiometabolic disease traits, providing a refined list of new drug targets. However, many genome-wide association studies have identified loci containing genes of unknown function. Single nucleotide polymorphisms in the *FADS1–2–3* gene cluster have been repeatedly identified in genome-wide association studies across the cardiometabolic disease spectrum, including strong associations with obesity, type 2 diabetes, dyslipidemia, non-alcoholic fatty liver disease, liver enzyme elevation, coronary artery disease, and heart rate.[2–12] However, mechanisms by which *FADS1–2–3* polymorphisms link to these comorbid disease phenotypes are unclear. Given that expression quantitative trait loci studies have revealed altered expression of FADS1 in several of these human genetic studies, we set out to investigate the specific role of *FADS1* in regulating diet-induced cardiometabolic phenotypes in hyperlipidemic mice.

FADS1 is the only mammalian Δ-5 fatty acid desaturase enzyme capable of producing the important polyunsaturated fatty acids (PUFAs) arachidonic acid (AA) and eicosapentaenoic acid (EPA) from substrates dihomo-γ-linolenic acid (DGLA) and eicosatetraenoic acid (ETA), respectively.[13,14] Downstream enzymatic and non-enzymatic oxidation of FADS1 product PUFAs (AA and EPA) generates diverse lipid signaling mediators that coordinate both the initiation and resolution phases of inflammatory processes.[15–18] In general, AA-derived eicosanoids are thought to initiate and potentiate pro-inflammatory responses,[15,16] while EPA- and docosohexaenoic acid (DHA)-derived mediators function in direct opposition to resolve inflammation and initiate wound healing and tissue regenerative responses.[17,18] In particular, lipoxins, resolvins, and protectins are important lipid autacoids with varying structures and functions that are collectively defined as specialized pro-resolving lipid mediators (SPMs) for their ability to actively resolve inflammation.[17,18] Given FADS1's unique position as the sole enzymatic source of endogenous AA and EPA, we hypothesized that *Fads1* loss of function would dramatically alter both pro-inflammatory and pro-resolving lipid mediators to impact diseases of unresolved inflammation such as

atherosclerosis, obesity, insulin resistance, and steatohepatitis. Metabolic phenotyping of global *Fads1*$^{-/-}$ mice has been limited due to the fact that these mice are only viable for approximately 8–12 weeks without supraphysiological supplementation of AA and EPA in the diet.[19,20] To overcome this barrier, here we used second-generation antisense oligonucleotides (ASOs), which predominately target liver, adipose tissue, and cells within the reticuloendothelial system to selectively knock down Fads1 in adult hyperlipidemic mice, thereby circumventing postnatal lethality of global *Fads1* deletion. We hypothesized that the underlying mechanism by which *FADS1* polymorphisms alter cardiometabolic disease phenotypes is by determining the balance of endogenous AA and EPA substrates available for the production of SPMs, thereby impacting the proper resolution of inflammation. To specifically address whether endogenous EPA production by *Fads1* is necessary for SPM generation and inflammation resolution, we provided a subset of mice a diet enriched in ω-3 precursor fatty acids.

MATERIALS AND METHODS

Materials and Methods are available in the online-only Data Supplement.

RESULTS

Fads1 is a Key Determinant of Membrane Phospholipid Composition and Pro-Inflammatory Versus Pro-Resolving Lipid Mediator Balance

To understand the role of *Fads1* in maintaining membrane lipid composition and lipid mediator balance in the context of cardiometabolic disease, we utilized an *in vivo* ASO-mediated knockdown approach[21] in hyperlipidemic low-density lipoprotein receptor-null mice. To selectively alter pro-inflammatory versus pro-resolving lipid mediator balance in the context of *Fads1* loss of function, mice were also fed synthetic diets specifically designed to provide low or high levels of the ω-3 FADS1 substrate fatty acid ETA (Figure SIA) as previously described by Shewale and colleagues.[22] *Fads1* ASO treatment resulted in selective knockdown of hepatic *Fads1* mRNA, without altering *Fads2* or *Fads3* mRNA expression (Figure 1A). In agreement with selective *Fads1* knockdown, *Fads1* ASO treatment resulted in plasma accumulation of the FADS1 ω-6 substrate DGLA and ω-3 substrate ETA in a diet-specific manner (Figures 1B, 1C, and SIB). Reciprocally, FADS1 product fatty acids (AA and EPA) were significantly diminished in the plasma of *Fads1* ASO-treated mice (Figures 1B, 1C, and SIB). Similar diet-specific changes in FADS1 substrate (DGLA and ETA) and product (AA and EPA) fatty acids were seen in the liver of *Fads1* ASO-treated mice, where PUFA biosynthesis is known to be most active (Figure 1D, 1E). Strikingly, when mice were fed a diet enriched in ω-3 PUFA precursors for ETA biosynthesis, *Fads1* knockdown resulted in greater than 80% of the total phospholipid fatty acid pool containing ETA (Figure 1D). This was associated with reciprocal reductions in the ω-3 FADS1 product EPA in total hepatic phospholipids (Figure 1D). This FADS1 substrate/product shift was also seen in several phospholipid classes including phosphatidylethanolamines and phosphatidylcholines (Figures 1E and SIC). These results suggest that *Fads1* is a major contributor to membrane phospholipid remodeling under certain dietary conditions. Given the fundamental roles that AA and EPA play in lipid

mediator production, we next quantified a wide array of pro-inflammatory and pro-resolving lipid mediators in the liver (Figure 1F–1J). Global lipid mediator product profiles were clearly distinct for both dietary and genetic (*Fads1*) interventions. In mice fed the diet enriched ω-3 PUFA precursors, a clear shift in the production of SPMs was observed (Figure 1F–1J). In the presence of the ω-3 PUFA substrate diet, *Fads1* knockdown resulted in a clear imbalance between pro-inflammatory and pro-resolving lipid mediators that would be predicted to oppose proper inflammation resolution. When broadly comparing the ratio of 5-lipoxygenase-derived SPMs to the pro-inflammatory mediator leukotriene B_4, *Fads1* knockdown skews towards a more pro-inflammatory and less pro-resolving mediator profile (Figures 1J, and SII). In fact, *Fads1* knockdown resulted in significantly diminished levels of many of the major bioactive pro-resolving lipid mediators originating from enzymatic conversion of AA (lipoxin A_4, lipoxin B_4), EPA (15-hydroxyeicosapentaenoic acid, lipoxin A_5), and DHA (17*R*-resolvin D1, resolvin D1; protectin D1) (Figures 1F–1J, and SII). Collectively, these results suggest that *Fads1* is a major contributor to diet-induced PUFA remodeling of hepatic membrane lipids, thereby dictating the balance of pro-inflammatory and pro-resolving lipid mediators.

Fads1 Reciprocally Regulates Hepatic Inflammation and Lipogenesis

In parallel to the observed imbalance between pro-inflammatory and pro-resolving lipid mediators, mice treated with *Fads1* ASO develop pathological histology and gene expression signatures that are characteristic of chronic hepatic inflammation (Figure 2A–2E). *Fads1* ASO-treated mice exhibited abnormal appearance of foamy histiocyte clusters that were significantly increased in mice fed the ω-3 PUFA substrate diet (Figure 2A, 2B). *Fads1* knockdown was also associated with increased expression of macrophage-selective genes including cluster of differentiation 68 (*Cd68*), interleukin-6 (*IL-6*), and macrophage inflammatory protein 1 α (*Mip-1α*) (Figure 2C–2E). It is important to note that *Fads1* ASO-driven hepatic inflammation was most apparent in mice fed the ω-3 PUFA substrate diet (Figure 2A–2E), a condition where the ratio of 5-lipoxygenase-derived SPMs to leukotriene B_4 is dramatically decreased (Figure 1J). In addition to effects on hepatic inflammation, *Fads1* knockdown was also associated with reorganization of hepatic lipid metabolism that extends beyond the scope of direct effects on PUFA biosynthesis. *Fads1* knockdown resulted in reduced hepatic triglycerides in mice fed the ω-3 PUFA substrate diet (Figure 2F), while increasing phosphatidylcholine, free cholesterol and cholesteryl ester levels (Figure 2G–I). In conjunction with these alterations in major hepatic lipid species, *Fads1* knockdown was associated with reorganization of lipid metabolic gene expression. *Fads1* knockdown significantly reduced the expression of the master lipogenic transcription factors sterol regulatory element-binding proteins 1c (*Srebp1c*) and 2 (*Srebp2*), and their downstream target genes including acetyl-CoA carboxylase 1 (*Acc1*), and fatty acid synthase (*Fas*) in the liver (Figure 2J–2M). In agreement with suppression of hepatic *de novo* lipogenesis, *Fads1* ASO-treated mice also exhibited reduced body weight and white adipose tissue mass (Figure SIII), despite eating the same amount of food (data not shown). Although *Fads1* ASO treatment produced very minor alterations in oxygen consumption (VO_2) and carbon dioxide production (VCO_2), *Fads1* knockdown did significantly increase the respiratory exchange ratio (RER) in the SFA-fed group (Figure SIVF). In line with alterations in adiposity, *Fads1* ASO-treated mice had improvements in glucose tolerance and modest reductions in fasting

plasma insulin levels (Figure SV). Collectively, these data suggest that *Fads1* knockdown limits hepatic fatty acid synthesis, adipose tissue expansion, and glucose intolerance, while promoting hepatic inflammation under dietary conditions that favor an imbalance of pro-inflammatory and pro-resolving lipid mediators.

Fads1 Knockdown Promotes Dyslipidemia and Atherosclerosis in a Diet-Specific Manner

Polymorphisms in the *FADS1–2–3* gene cluster have been repeatedly associated with plasma lipids and other cardiovascular disease risk factors,[2–12] yet whether FADS1-driven Δ-5 desaturation underlies these genetic associations has remained elusive. Selective ASO-mediated knockdown of *Fads1* trended (p=0.06) towards promoting aortic root atherosclerosis in saturated fat-fed mice, and significantly increased aortic root lesion size in mice fed the ω-3 PUFA substrate diet (Figure 3A, 3B). In agreement with effects in the aortic root, *Fads1* knockdown increased cholesteryl ester levels in whole aortae of mice fed the ω-3 PUFA substrate diet, and also trended towards elevating aortic free cholesterol levels (Figure 3C, 3D). In contrast, *Fads1* knockdown did not significantly alter the total necrotic core area on either dietary background (Figure 3E, 3F). Unlike the significant effects on hepatic triglyceride levels (Figures 2F, and 2J–2M), *Fads1* knockdown resulted in very minor alterations in plasma triglycerides, producing a transient triglyceride lowering effect that was only apparent at 8 weeks of dietary induction (Figure 3G). Given that very low-density lipoprotein triglyceride secretion rates were not significantly altered by *Fads1* knockdown (Figure SVI), the alterations in plasma triglyceride and cholesterol levels observed in *Fads1* ASO-treated mice likely arise from altered intravascular metabolism and turnover of apoB-containing lipoproteins. Interesting, the hepatic expression of lipoprotein lipase (*Lpl*) was increased in *Fads1* ASO treatment (Figure 6R), which may contribute to the dyslipidemia seen in *Fads1* knockdown mice. However, *Fads1* ASO treatment promoted hypercholesterolemia under both dietary settings, characterized by significant increases in low-density lipoprotein cholesterol levels (Figure 3I) without altering very low-density lipoprotein cholesterol levels (Figure 3H). The previously reported ability of the ω-3 PUFA substrate diet to increase high-density lipoprotein cholesterol levels[22] was abolished by *Fads1* knockdown (Figure 3J), indicating that endogenous EPA biosynthesis is necessary for this phenotype. In addition to alterations in plasma lipid levels, *Fads1* knockdown resulted in diet-specific reorganization of circulating leukocyte populations. In saturated fat-fed mice, *Fads1* knockdown reduced $Ly6C^{High}$ monocytes, and similarly reduced both CD4+ and CD8+ T cell populations (Figure 3K–3P). When challenged with the ω-3 PUFA substrate diet *Fads1* ASO-treated mice exhibited elevations in $Ly6C^{High}$ and $Ly6C^{Low}$ monocytes and neutrophils, without significant alterations in T cell populations (Figure 3K–3P). Collectively, these data suggest that *Fads1*-driven Δ-5 desaturation balances pro-atherogenic dyslipidemia and monocytosis in a diet-specific manner (Figure 3).

Fads1 Impacts Macrophage-Driven Inflammation and Resolution Programs Both *In Vitro* and *In Vivo*

Macrophages are requisite players in the pathogenesis of atherosclerosis, playing important roles in both the initiation and resolution phases of the disease progression.[23] Whereas classically M1 activated macrophages are generally pro-inflammatory in nature, alternatively activated M2 macrophages are thought to be involved in the resolution of inflammation

during plaque regression and stabilization.[24] To address the role of *Fads1* in macrophage phenotype switching, we elicited peritoneal macrophages from ASO-treated mice, and acutely induced polarization programs with M1 (lipopolysaccharide, LPS) or M2 (interleukin 4, IL-4) stimuli (Figure 4). It is important to note that *Fads1* mRNA expression was suppressed by lipopolysaccharide treatment (Figure 4A), yet was increased by IL-4 treatment (Figure 4F). *Fads1* knockdown in macrophages was associated with skewing towards M1 and away from M2 polarization (Figure 4). In support of this, *Fads1* knockdown resulted in augmented lipopolysaccharide-driven pro-inflammatory gene expression (Figure 4A–4E), yet was associated with diminished IL-4-driven alternative activation gene signatures (Figure 4F–4J). The expression of AA and EPA oxidizing enzymes cyclooxygenases 1 (*Cox-1*) and 2 (*Cox-2*) and 12-lipoxygenase (*Alox12*) were differentially expressed with *Fads1* knockdown under certain stimulated conditions (Figure 4D, 4E, 4I, 4J). *Fads1* knockdown in macrophages was also associated with generally lower levels of lipid mediators originating from oxidation of AA, EPA, and DHA (Figure 4K). Given that *Fads1* knockdown was associated with such striking diversification of lipid mediators both in isolated macrophages (Figure 4K) and in the liver (Figures 1F–1J and SII), we performed linear regression analysis to correlate lipid mediator concentrations in the liver to the major *in vivo* phenotypes under study. The EPA-derived lipid mediator lipoxin A_5 was negatively associated (R^2=0.5299, p=0.0003) with atherosclerotic lesion area (Figure SVIIC), while the AA-derived mediator 5(S),15(S)-dihydroxyeicosatetraenoic acid was positively associated (R^2=0.2467, p=0.026) with atherosclerosis (Figure SVIID). Circulating monocyte numbers were positively correlated with the AA-derived mediator leukotriene B_4 (R^2=0.6743, p<0.0001) and the poorly studied DHA-derived lipid mediator 21-hydroxydocosahexaenoic acid (R^2=0.612, p=0.0003) (Figure SVIIE, SVIIF). Also, blood glucose levels were negatively associated with the DHA-derived lipid mediators 17-hydroxydocosahexaenoic acid (R^2=0.5001, p=0.0005) and 21-hydroxydocosahexaenoic acid (R^2=0.3468, p=0.006) (Figure SVIIG, SVIIH). Collectively, these data suggest that *Fads1* plays an underappreciated role in macrophage polarization and lipid mediator production, and provide initial clues into potential *Fads1*-regulated lipid mediators driving diverse cardiometabolic phenotypes.

To further investigate the role of *Fads1* in orchestrating pro-inflammatory and pro-resolving macrophage programs *in vivo*, we treated diet-fed control and *Fads1* knockdown mice with M1 (LPS) or M2 (IL-4) skewing stimuli and followed acute inflammatory responses in the circulation, liver, and aortic arch (Figure 5). *Fads1* knockdown did not appreciably alter plasma cytokine levels under IL-4-stimulated conditions (data not shown), but did significantly alter LPS-stimulated plasma cytokine responses. Although *Fads1* knockdown did not alter the early LPS-induced burst of circulating tumor necrosis factor α (TNFα), *Fads1* ASO treatment was associated with significantly elevated LPS-stimulated levels of several interleukins (IL-1β, IL-12p70, and IL-10) after six hours (Figure 5A–5D). In the liver, *Fads1* knockdown was associated with increased expression of the macrophage marker genes *F4/80* and *Cd68*, and this effect was particularly apparent in mice fed the ω-3 substrate diet (Figure 5F, 5G). *Fads1* knockdown in the liver was associated with reduced expression of the M2 marker gene arginase 1 under all conditions (Figure 5H). In contrast, *Fads1* knockdown enhanced LPS-stimulated expression of macrophage-derived cytokines

including MCP-1, MIP-1α, and TNFα (Figure 5I–5K), yet modestly blunted expression of the acute phase protein serum amyloid A (SAA) in ω-3 substrate diet-fed mice (Figure 5L). In the aortic arch, there was a roughly 50% knockdown of *Fads1* with *Fads1* ASO treatment, and much like what was seen in elicited macrophages (Figure 4A), LPS alone suppressed *Fads1* expression by 50% when compared to saline injected mice (Figure 5M). *Fads1* knockdown in the aortic arch was associated with increased expression genes encoding macrophage markers such as *F4/80* and *Cd11b*, particularly in the ω-3 substrate diet groups (Figure 5N, 5O). Furthermore, *Fads1* knockdown was associated with diet-specific effects on both LPS- and IL-4-stimulated cytokine expression in the aortic arch (Figure 5P–5T). Collectively, these results suggest that *Fads1* regulates systemic inflammation, tissue macrophage abundance, and macrophage polarization *in vivo* in a highly gene- and tissue-specific manner.

Fads1 Determines the Reciprocal Regulation of Inflammation and Lipogenesis Driven by Liver X Receptor (LXR)

The nuclear hormone receptor LXR is a well-known regulator of tissue inflammation, atherosclerosis, cholesterol balance, *de novo* lipogenesis, and membrane PUFA-diversification.[25–30] Given that our results here link *Fads1* to these same phenotypes we hypothesized that *Fads1* may be an important effector of LXR signaling. To test this, we examined LXR agonist-induced metabolic reprogramming in control and *Fads1* knockdown mice maintained on a chow diet (Figure 6). *Fads1* knockdown was associated with enhanced basal and LXR-stimulated fecal neutral sterol loss, yet was associated with blunted basal and LXR-stimulated liver triglyceride levels (Figure 6A, 6B). In contrast to these LXR-associated lipid phenotypes, the ability of *Fads1* knockdown to alter hepatic cholesterol levels was largely independent of LXR activation state (Figure 6C–6E). However, *Fads1* knockdown significantly increased plasma cholesterol only in animals treated with pharmacological LXR agonist (Figure 6F). Knockdown of *Fads1* did not significantly alter the hepatic expression of *LXRα* itself (data not shown), but did result in selective alterations in LXR-stimulated gene expression (Figure 6G–6R). For instance, the ability of the LXR agonist T0901317 to increase the expression of *Srebp1c* and its target genes (*Fas*, *Scd1*, and *Acc1*) was blunted in *Fads1* knockdown mice (Figure 6K–6N). In parallel, LXR-stimulated expression of genes involved in cholesterol efflux (ATP-binding cassette transporters G5 and G8) and phosphatidylcholine remodeling (lysphosphatidylcholine acyltransferase) were also blunted in *Fads1* knockdown mice (Figure 6O–6Q). However, not all LXR-stimulated gene expression was blunted in *Fads1* knockdown mice, as was the case for lipoprotein lipase (*Lpl*) (Figure 6R). The hepatic expression of HMG-CoA reductase (*Hmgcr*) and HMG-CoA synthase (*Hmgcs1*) were increased in both the basal and LXR-stimulated state (Figure 6I, 6J). However, *Fads1* knockdown only increased hepatic expression of *Srebp2* in the T0901317-treated mice (Figure 6H). These results suggest that *Fads1* is an effector of LXR agonist-driven transcriptional control of fatty acid and cholesterol metabolic programs in the liver.

DISCUSSION

Cardiometabolic diseases including obesity, insulin resistance, atherosclerosis, and steatohepatitis all share tissue-specific features of chronic unresolved inflammation.[31–34] Although often underappreciated, membrane PUFA-derived lipid mediators play requisite roles in both the initiation and resolution phase of inflammation. There is a wealth of evidence showing beneficial effects of dietary PUFA supplementation on cardiometabolic diseases,[35–37] yet the specific contribution of endogenous PUFA synthesis driven by FADS1 (i.e. AA and EPA generation) has been elusive. Here we provide new evidence that *Fads1*-driven PUFA biosynthesis plays a role in cardiometabolic diseases associated with chronic unresolved inflammation (obesity, insulin resistance, atherosclerosis, and steatohepatitis). The main findings of the current study are: (1) *Fads1* is a major contributor to diet-driven enrichment of PUFAs in membrane phospholipids; (2) *Fads1* loss of function results in diminished levels of AA-, EPA-, and DHA-derived pro-resolving lipid mediators; (3) *Fads1* knockdown promotes hepatic inflammation in a diet-specific manner; (4) *Fads1* knockdown promotes atherosclerosis in a diet-specific manner; (5) *Fads1* knockdown results in the suppression of hepatic *de novo* lipogenesis; (6) *Fads1* knockdown is associated with reduced adiposity and improved glucose tolerance; (7) *Fads1* knockdown results in atherogenic dyslipidemia; (8) *Fads1* activity impacts circulating monocyte and T cell levels in a diet-specific manner; (9) *Fads1* reciprocally regulates M1 and M2 polarization programs in macrophages; (10) *Fads1* is a tissue-specific effector of LPS- and IL-4-driven reprogramming of systemic inflammatory responses *in vivo*; and (11) *Fads1* reciprocally regulates fatty acid and cholesterol reprogramming driven by the liver X receptor (LXR). Collectively, these data support a model in which *Fads1*-driven AA and EPA production diversifies both pro-inflammatory and pro-resolving lipid mediator production to dictate proper inflammation initiation and resolution in cardiometabolic disease.

The *FADS1–2–3* genetic locus is unique in that it shares genome-wide significant associations with almost all cardiometabolic phenotypes across the metabolic syndrome spectrum.[2–12] However, the relative roles of the three separate enzymes (FADS1, FADS2, and FADS3) encoded at this locus in driving cardiometabolic disease has been elusive. This study provides the first evidence that specific loss of the Δ-5 desaturase FADS1 can dynamically alter many of the cardiometabolic phenotypes originally identified in human genome-wide association studies. However, it is important to compare and contrast our findings to a recent manuscript describing cardiometabolic phenotypes in a gene trap *Fads1* loss of function mouse model.[38] The work by Powell and colleagues reported similar improvements in body weight and glucose tolerance with *Fads1* loss of function, but also reported modest reductions in atherosclerotic burden in *Fads1* loss of function mice on an apolipoprotein E-null background.[38] Given these discrepant results, it is essential to note that hepatic expression levels of *Fads1* were not reported in the work by Powell and colleagues,[38] and AA and EPA levels were very modestly reduced in their *Fads1* targeted mice. In fact, the authors speculate that this model produced a hypomorphic allele rather than a complete loss of function allele.[38] Furthermore, the work by Powell and colleagues is complicated by the fact that mice were studied on diets containing supplemental AA, thereby potentially masking the phenotypes driven by lack of endogenous AA production.

Furthermore, a major difference between the current studies and the work by Powell and colleagues[38] is the use of different hyperlipidemic mouse models. Whereas atherosclerosis in the *Ldlr*$^{-/-}$ model used here is mainly driven by VLDL and LDL accumulation, the more rapid atherosclerosis progression in apoE$^{-/-}$ mice is driven largely by the accumulation of intestinally-derived apoB-containing lipoproteins. Given early postnatal lethality of global *Fads1* knockout mice,[19,20] and obvious limitations of genetic hypomorphs and ASO-mediated knockdown, additional tissue-specific genetic approaches will ultimately be necessary to fully understand the cell autonomous roles of *Fads1* in cardiometabolic disease. It is important to note that the ASO knockdown approach used here does not accurately reflect the anticipated effect of common *FADS1* polymorphisms. In fact, ASOs typically only achieve appreciable knockdown in the liver, adipose, kidney, and some cells with the reticuloendothelial cell system, whereas the human *FADS1* variants likely affect expression across all tissues. Moving forward it will be important to study each individual *FADS1* SNP using non-ASO genetic approaches to fully understand the functional consequences of the cardiometabolic disease-associated variants.

Given the fact that *Fads1* knockdown dramatically reorganizes membrane phospholipids as well as diversifies downstream oxidation products of AA, EPA, and DHA (Figures 1 and 2), it is challenging to define a single unifying mechanistic link between Δ-5 desaturation and cardiometabolic disease phenotypes. In fact, it is most likely that certain lipid mediator alterations seen in *Fads1* knockdown mice may drive specific aspects of the phenotype. For instance, recent work has shown that vulnerable regions within human atherosclerotic plaques exhibit a lower SPM to leukotriene ratio similar to what is seen with *Fads1* knockdown (Figure 1J).[31,39] Moreover, direct administration of resolvin D1 (which is reduced in *Fads1* ASO-treated mice, Figure 1F–1J) promotes features of atherosclerotic plaque stability (e.g. increased fibrous cap thickness) in mice.[31] Furthermore, the AA-derived lipid mediator lipoxin A_4, which is severely reduced in *Fads1* ASO-treated mice (Figure. 1F–1J and SIIC), has been shown to limit chronic inflammation in adipose tissue and liver.[40–42] Importantly, *Fads1*'s ability to alter lipid mediator balance is very dependent on dietary provision of substrate fatty acids (Figures 1F–1J and SII). In support of this concept, *Fads1* knockdown has minimal effects on phospholipid acyl chain composition when a primary saturated fat source is provided, yet when a diet enriched in 18 carbon length ω-3 fatty acid precursors is fed, Fads1 knockdown is associated with striking alterations in membrane phospholipids (Figures 1D, 1E, and SIC) and downstream lipid mediators (Figures 1F–1J, and SII). These results suggest that FADS1 activity becomes particularly important in lipid mediator balance under dietary conditions where precursor essential fatty acid substrates for long-chain PUFA biosynthesis are provided.

Another important finding of this work is that *Fads1* is novel effector of LXR signaling *in vivo*, reciprocally balancing LXR-driven increases in fecal cholesterol disposal and *de novo* lipogenesis (Figure 6). Additional work is needed to understand how *Fads1*-driven AA- and EPA-derived lipid mediators impact reverse cholesterol transport and fatty acid biosynthesis, but in line with our observations a recent report linked the AA-derived metabolome to reverse cholesterol transport in mice and humans.[43] Furthermore, AA has also been shown to inhibit LXR-driven transactivation of the SREBP1c promoter, thereby impacting a number of downstream lipid signaling pathways in cells.[44] Based on these collective

observations it is tempting to speculate that AA itself, or more likely some downstream oxidative metabolite of AA, allows for coordinated regulation of LXR, SREBP1c, and PPARα signaling to balance lipid metabolic and inflammatory transcriptional programs. However, when considering the ability of *Fads1* to alter LXR signaling in our studies it is important to note that we studied LXR activation in different dietary cholesterol levels and genetic backgrounds. For instance, all studies examining effects of *Fads1* knockdown on atherosclerosis and hepatic inflammation (Figures 1–5) were done in $Ldlr^{-/-}$ mice fed synthetic diets supplemented with 0.2% cholesterol, whereas the exogenous LXR agonist studies (Figure 6) were done in chow-fed C57BL/6 mice. It has previously been demonstrated that dietary cholesterol levels can dramatically alter LXR target gene expression, with high dietary cholesterol providing substrate for endogenous oxysterol ligand production[26,45,46]. Therefore, the ability of Fads1 to alter LXR signaling is likely determined in part by dietary cholesterol levels. It is also important to note that the ability of *Fads1* to alter hepatic cholesterol levels does not solely rely on its ability to alter LXR-driven transcriptional programs. In support of this, *Fads1* ASO-driven increases in hepatic free cholesterol levels are apparent in both basal (vehicle) and LXR-stimulated (T0901317-treated) conditions (Figure 6). Therefore, *Fads1* likely impacts cholesterol homeostasis by LXR-dependent and independent mechanisms. One potential way that *Fads1* could impact hepatic free cholesterol levels is by altering membrane phospholipid fatty acid composition to secondarily alter the stability of cholesterol-rich lipid rafts. It is well known that PUFA-enrichment in membrane phospholipids can have profound effects on lipid raft formation and stability.[47,48] Our data suggest that under certain dietary conditions *Fads1* can be a major determinant of phospholipid fatty acid composition (Figure 1D). Therefore, we hypothesize that one plausible way *Fads1* could alter hepatic FC levels in a LXR-independent manner may be by altering the stability of cholesterol-rich lipid raft domains. In summary, this work demonstrates that ASO-mediated knockdown of the Δ-5 desaturase Fads1 impacts many of the cardiometabolic phenotypes that were originally genetically linked to the *FADS1–2–3* locus in a highly diet-specific manner. These findings highlight *Fads1* as a regulator of inflammation initiation and resolution, and highlight the interplay between endogenous and exogenous (dietary) fatty acids in diseases characterized by unresolved inflammation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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A.D.G., A.L.B., J.S.P. and J.M.B. planned the project, designed diets, designed experiments, analyzed data, and wrote the manuscript; A.D.G., R.C.S., A.L.B., R.N.H., A.C.B., D.F., B.E.S., R.E.M., and D.S.A. conducted mouse experiments, performed biochemical workup of mouse tissues, analyzed data, and aided in manuscript preparation; A.D.G., R.Z., B.E.S. and M.S. performed lipid mass spectrometry studies; R.G.L. provided antisense oligonucleotides; All authors were involved in the editing of the final manuscript.

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NONSTANDARD ABBREVIATIONS AND ACRONYMS

FADS	fatty acid desaturase
FADS1–2–3	*FADS1, FADS2, FADS3* gene cluster
ASO	antisense oligonucleotide
PUFA	polyunsaturated fatty acid
AA	arachidonic acid
DGLA	dihomo-γ-linolenic acid
EPA	eicosapentaenoic acid
ETA	eicosatetraenoic acid
DHA	docosahexaenoic acid
LXR	liver X receptor
SPM	specialized pro-resolving lipid mediator

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Highlights

- FADS1 activity determines lipid mediator balance in a diet-specific manner
- Knockdown of *Fads1* promotes hepatic inflammation and atherosclerosis
- *Fads1* knockdown results in suppression of lipogenic gene expression
- *Fads1* regulates M1 and M2 activation programs in macrophages
- *Fads1* is a novel effector of LXR-driven reorganization of lipid metabolism

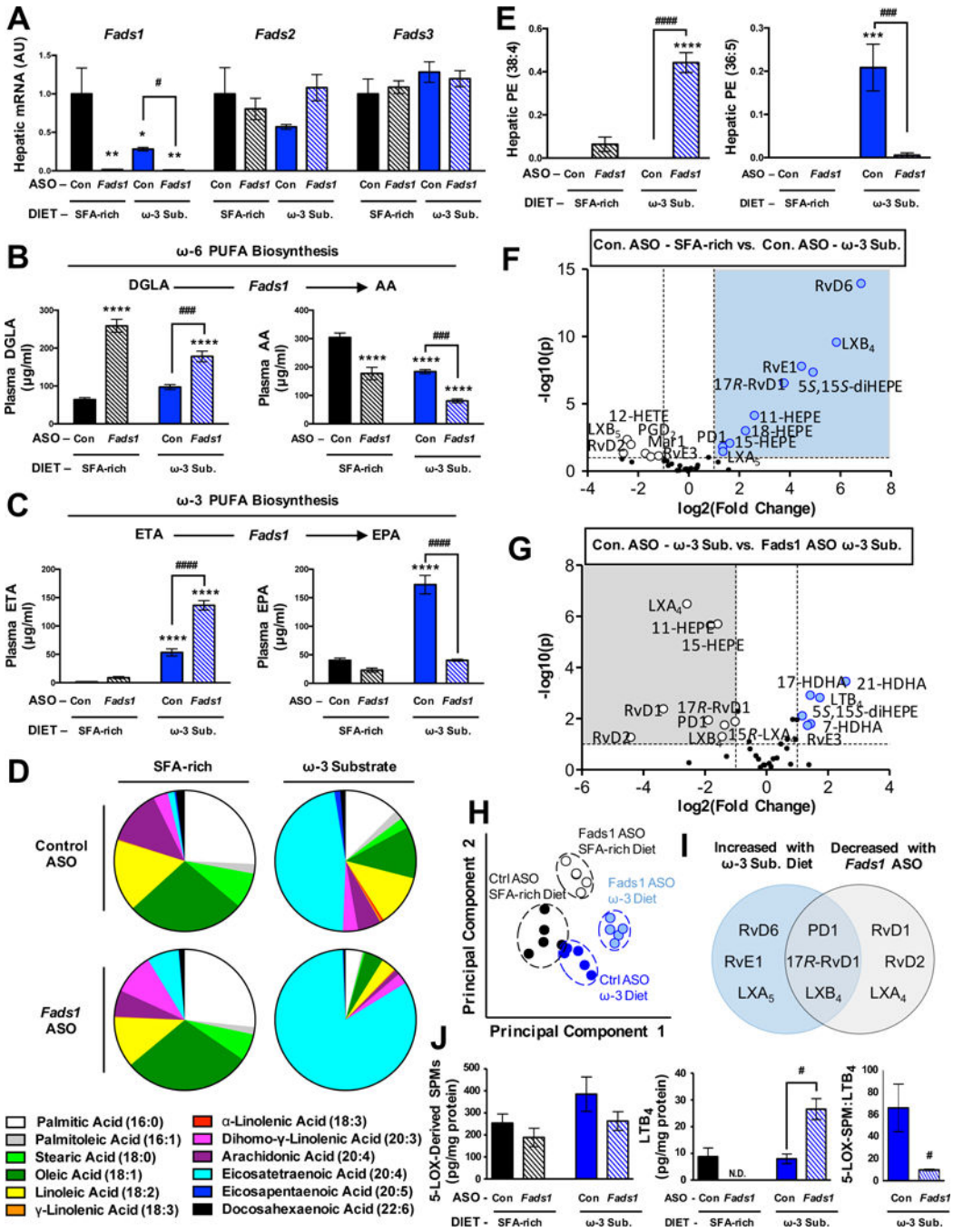

Figure 1. *Fads1* is a Key Determinant of Membrane Phospholipid Composition and Pro-Inflammatory Versus Pro-Resolving Lipid Mediator Balance

(A) qPCR quantification of hepatic *Fads1*, *Fads2*, and *Fads3* mRNA; *n=5 per group*. **(B–C)** FADS1 substrate (ω-6 dihomo-g-linolenic acid, DGLA and ω-3 eicosatetraenoic acid, ETA) and product (ω-6 arachidonic acid, AA and ω-3 eicosapentaenoic acid, EPA) fatty acid concentrations in plasma; *n=6 per group*. **(D)** Total hepatic phospholipid fatty acid composition, *n=5 per group*. **(E)** Hepatic phosphatidylethanolamine (PE) species 38:4 and 36:5, *n=5 per group*. **(F)** Volcano plot of all metabolites detected with the larger circles that

are labeled representing metabolites that exceed the minimum thresholds for significance ($p<0.05$) and fold change (> or <2). Lipid mediators that were significantly increased in ω-3 diet-fed animals are blue and appear in the shaded region while those that were significantly decreased are white; *n=5 per group*. **(G)** Volcano plot of all metabolites detected with the larger circles that are labeled representing metabolites that exceed the minimum threshold for significance ($p<0.05$) and fold change (> or <2). Lipid mediators that were significantly decreased by *Fads1* ASO are white and appear in the shaded region while those that significantly increased are blue; *n=5 per group*. **(H)** Partial least squares-discriminant analysis two-dimensional scores plot demonstrated clustering of samples into distinct and separate groups based on both diet and ASO. **(I)** Venn diagram displaying the lipid mediators that were significantly increased by ω-3 diet with control ASO in the blue circle and those that were significantly decreased by *Fads1* ASO while on ω-3 diet in the gray circle. Those metabolites in the overlapping region both increased on ω-3 diet and were decreased by *Fads1* ASO; *n=5 per group*. **(J)** (*Left*) Combined levels of 5-lipoxygenase (5-LOX)-derived specialized pro-resolving lipid mediators (SPM) are shown for each diet and treatment group. SPM included in this index are resolvin (Rv) D1, 17*R*-RvD1, RvD2, 17*R*-RvD3, RvD4, RvD6, RvE1, RvE2, lipoxin (LX) A_4, 15*R*-LXA_4, LXA_5, LXB_4 and LXB_5; *n=5 per group*. Graph displays mean ± S.E.M. (*Middle*) Levels of leukotriene B_4 (LTB_4). Only one of the five samples in the group fed a control diet and treated with *Fads1* ASO contained LTB_4 above the limit of detection (0.1 pg) and thus the entire group was omitted; *n=3–5 per group*. Graph displays mean ± S.E.M. Statistical significance is determined by *t*-test. # $p<0.05$ control ASO vs *Fads1* ASO. (*Right*) The ratio of 5-LOX-derived SPM to LTB_4 is shown for mice on ω-3 rich diet exposed to control or *Fads1* ASO; *n=5 per group*. Graph displays mean ± S.E.M. Statistical significance is determined by *t*-test. # $p<0.05$ control ASO vs *Fads1* ASO). For panels A–C and E, graphs display mean ± S.E.M. Statistical significance is determined by 2-way ANOVA. * = significantly different from the control ASO – saturated and monounsaturated fatty acid (SFA)-rich diet group (*$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$); # = significantly different from control ASO group within the ω-3 substrate diet group ($^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$, $^{\#\#\#\#}p<0.0001$).

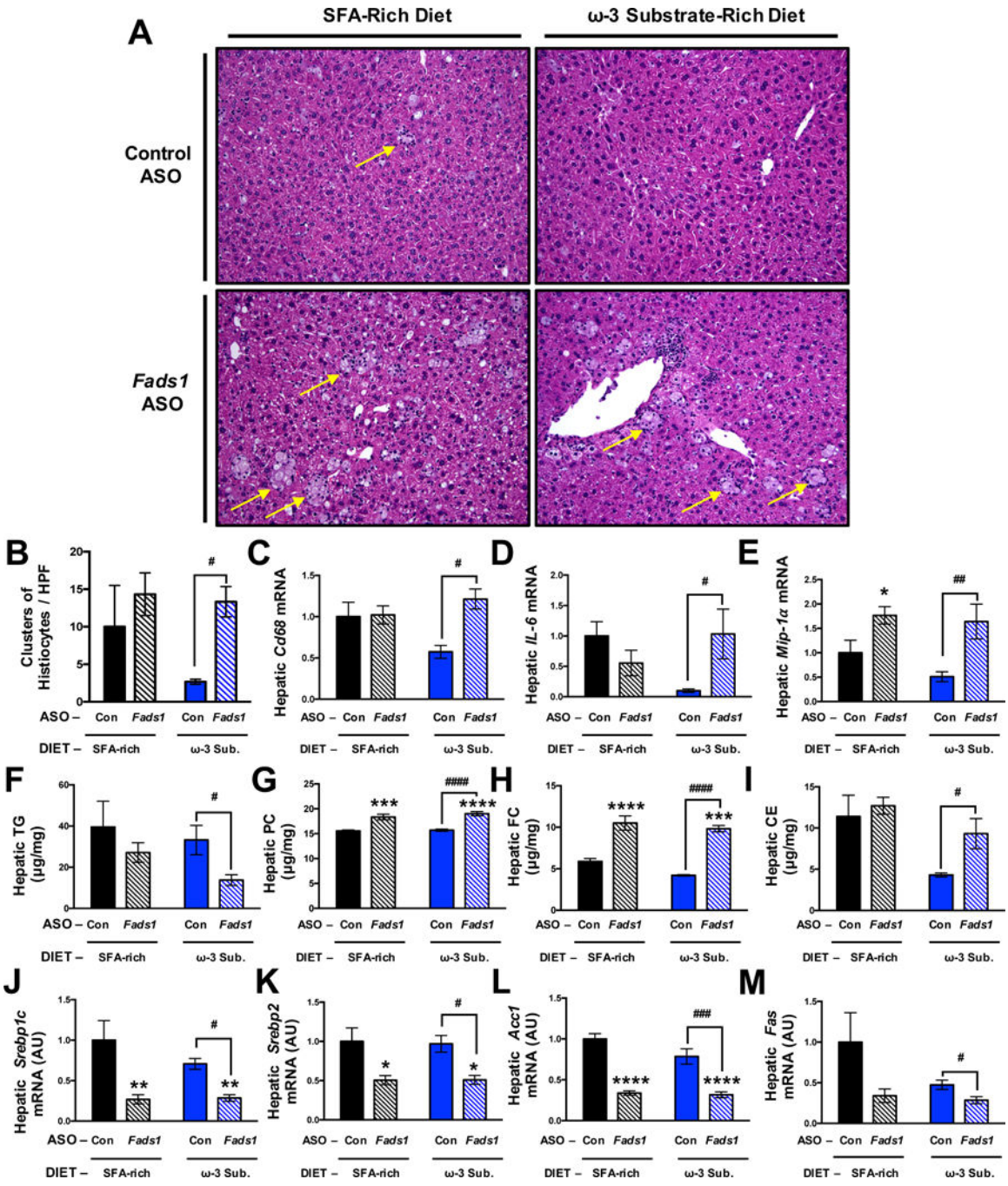

Figure 2. *Fads1* is a Critical Regulator of Hepatic Inflammation and Lipogenesis

(A) H&E-stained liver sections show enhanced immune cell invasion characterized mainly by foamy histiocytes in the context of *Fads1* knockdown (shown at 200x magnification); Arrows indicate areas of foamy histiocytes. **(B)** Pathologist quantified foamy histiocyte clusters per 40x field, *n=3 per group*. **(C–E)** Hepatic expression of macrophage genes including cluster of differentiation 68 (*Cd68*), interleukin 6 (*IL-6*), and macrophage inflammatory protein 1α (*Mip-1α*); *n=5 per group*. **(F–I)** Total hepatic triglycerides (TG), phosphatidylcholine (PC), free cholesterol (FC), and cholesteryl ester (CE); *n=6 per group*.

(J–M) Hepatic expression of lipogenic genes including sterol regulatory element-binding proteins 1c (*Srebp1c*) and 2 (*Srebp2*), acetyl-CoA carboxylase 1 (*Acc1*), and fatty acid synthase (*Fas*); *n=5 per group*. All graphs display mean ± S.E.M. Statistical significance is determined by 2-way ANOVA. * = significantly different from the control ASO – saturated and monounsaturated fatty acid (SFA)-rich diet group (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001); # = significantly different from control ASO group within the ω-3 substrate diet group ($^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001, $^{\#\#\#\#}$p<0.0001).

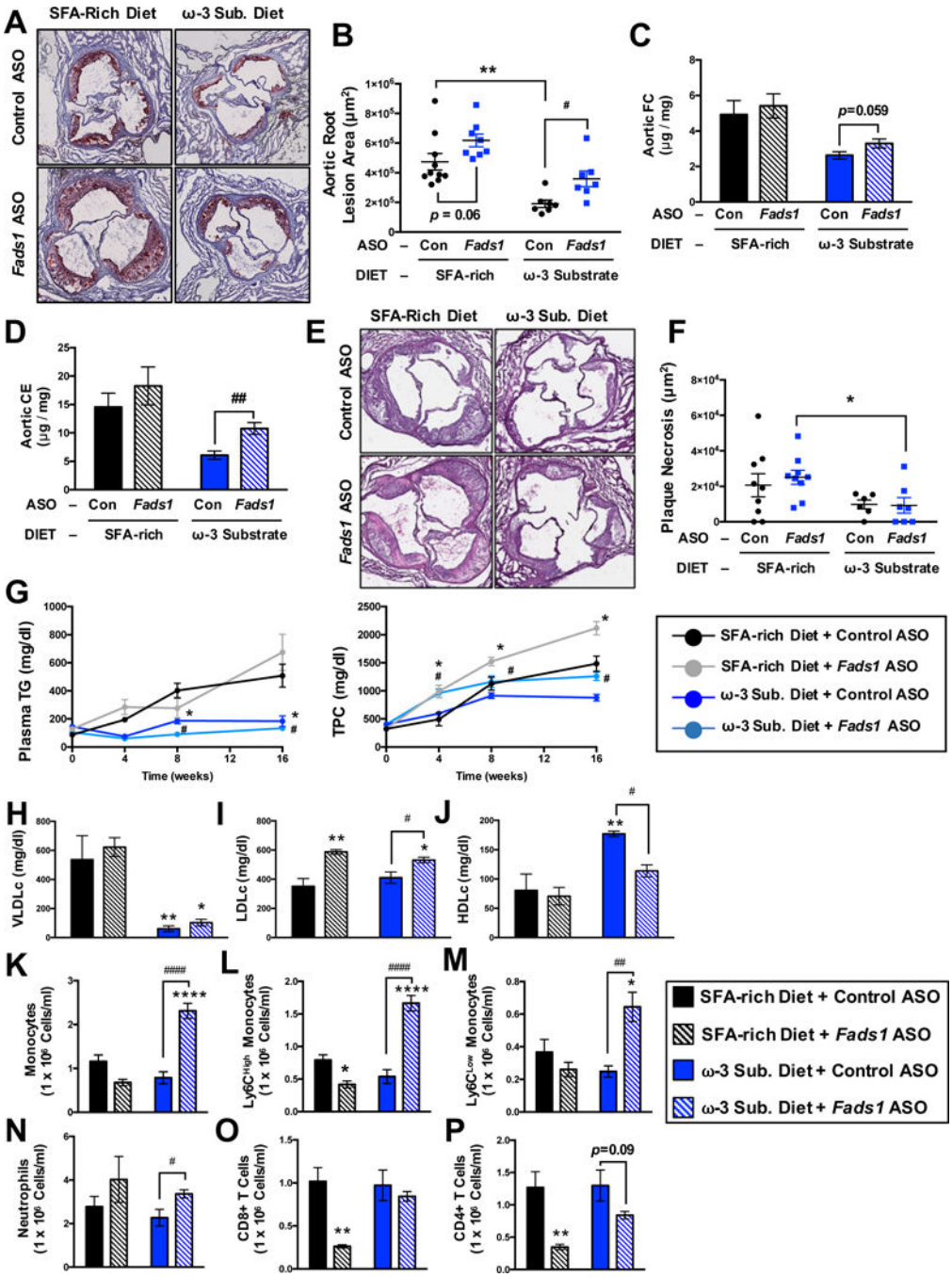

Figure 3. *Fads1* Knockdown Promotes Dyslipidemia, Alters Leukocyte Populations, and Increases Atherosclerosis in a Diet-Specific Manner

(A) Representative Oil Red O stained (with hematoxylin counterstain) aortic root cross-sections. **(B)** Quantification of aortic root lesion area; *n=7–10 per group*. **(C)** Total free cholesterol (FC) content in mouse aortas; *n=8–10 per group*. **(D)** Total cholesteryl ester (CE) content in mouse aortas; *n=8–10 per group*. **(E)** Representative hematoxylin & eosin stained aortic root cross-sections. **(F)** Quantification of necrotic core area; *n=8–10 per group*. **(G)** Plasma total triglyceride (TG) and total plasma cholesterol (TPC); *n=6 per group*. **(H–J)**

Very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol levels after 16 weeks of diet and ASO treatment; *n=4 per group*. **(K–P)** Flow cytometric detection of circulating leukocyte populations; *n=6 per group*. All graphs display mean ± S.E.M. Statistical significance is determined by 2-way ANOVA. * = significantly different from the control ASO – saturated and monounsaturated fatty acid (SFA)-rich diet group (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001); # = significantly different from control ASO group within the ω-3 substrate diet group ($^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001, $^{\#\#\#\#}$p<0.0001).

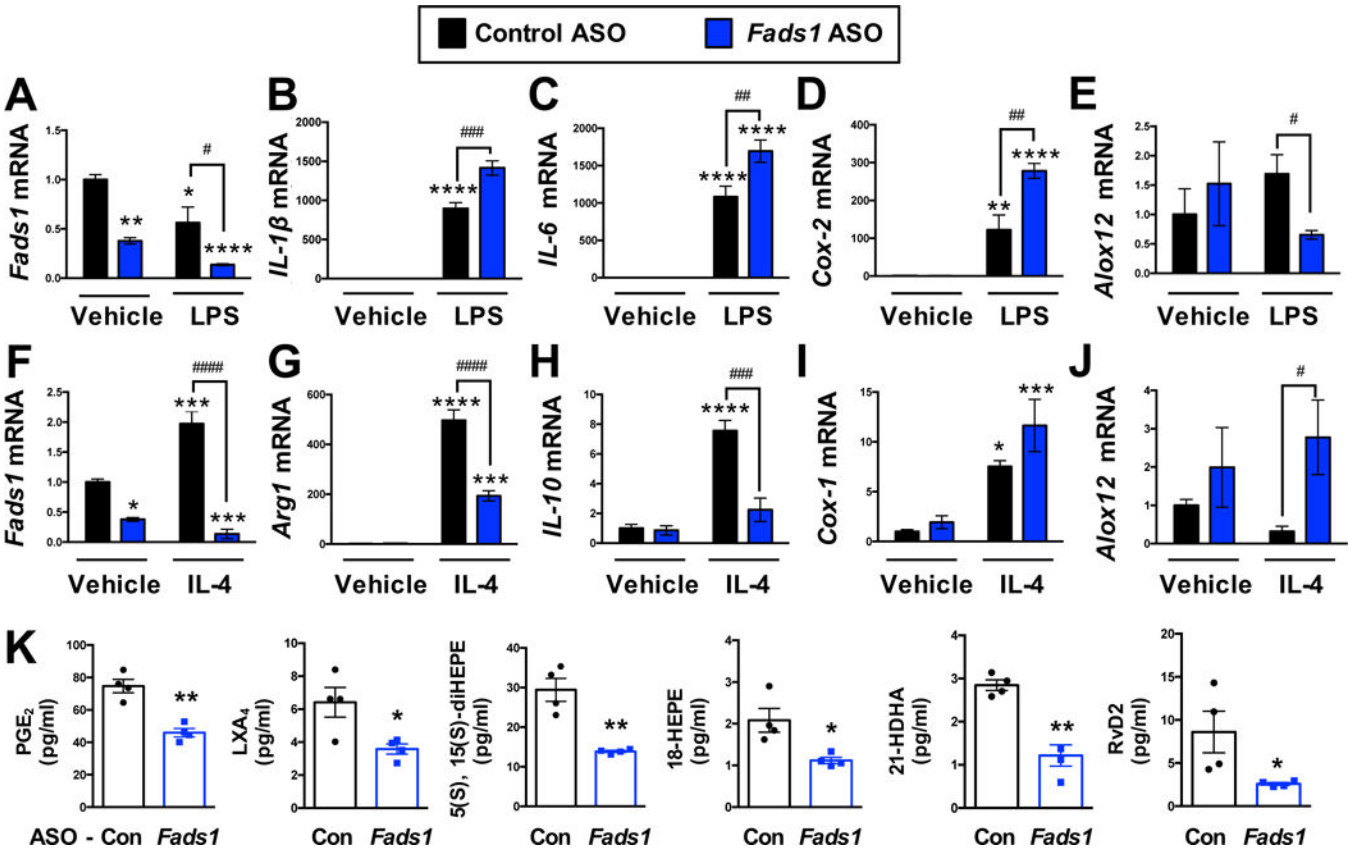

Figure 4. ***Fads1*** **Knockdown Promotes Classic M1 Activation and Suppresses Alternative M2 Activation Programs in Macrophages**

Low-density lipoprotein receptor-knockout mice were treated with ASOs and the saturated and monounsaturated fatty acid (SFA)-rich diet for 8 weeks, and then primary macrophages were elicited from the peritoneal cavity for functional characterization. **(A–E)** Macrophages isolated from control and *Fads1* ASO-treated mice were stimulated with 50 ng/ml lipopolysaccharide (LPS). 6 hours later gene expression was quantified by qPCR; *n=4 per group*. **(F–J)** Macrophages isolated from control and *Fads1* ASO-treated mice were stimulated with 10 ng/ml interleukin 4 (IL-4). 6 hours later gene expression was quantified by qPCR; *n=4 per group*. **(K)** Conditioned media was collected from non-stimulated macrophages for LC-MS/MS detection of lipid mediators; *n=4 per group*. All graphs display mean ± S.E.M. For panels A–J, statistical significance is determined by 2-way ANOVA. * = significantly different from the control ASO – vehicle stimulus group (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001); # = significantly different from control ASO group within the LPS or IL-4 treatment group (#p<0.05, ##p<0.01, ###p<0.001, ####p<0.0001). For panel K, statistical significance is determined by *t*-test. * = significantly different from control ASO group (*p<0.05, **p<0.01).

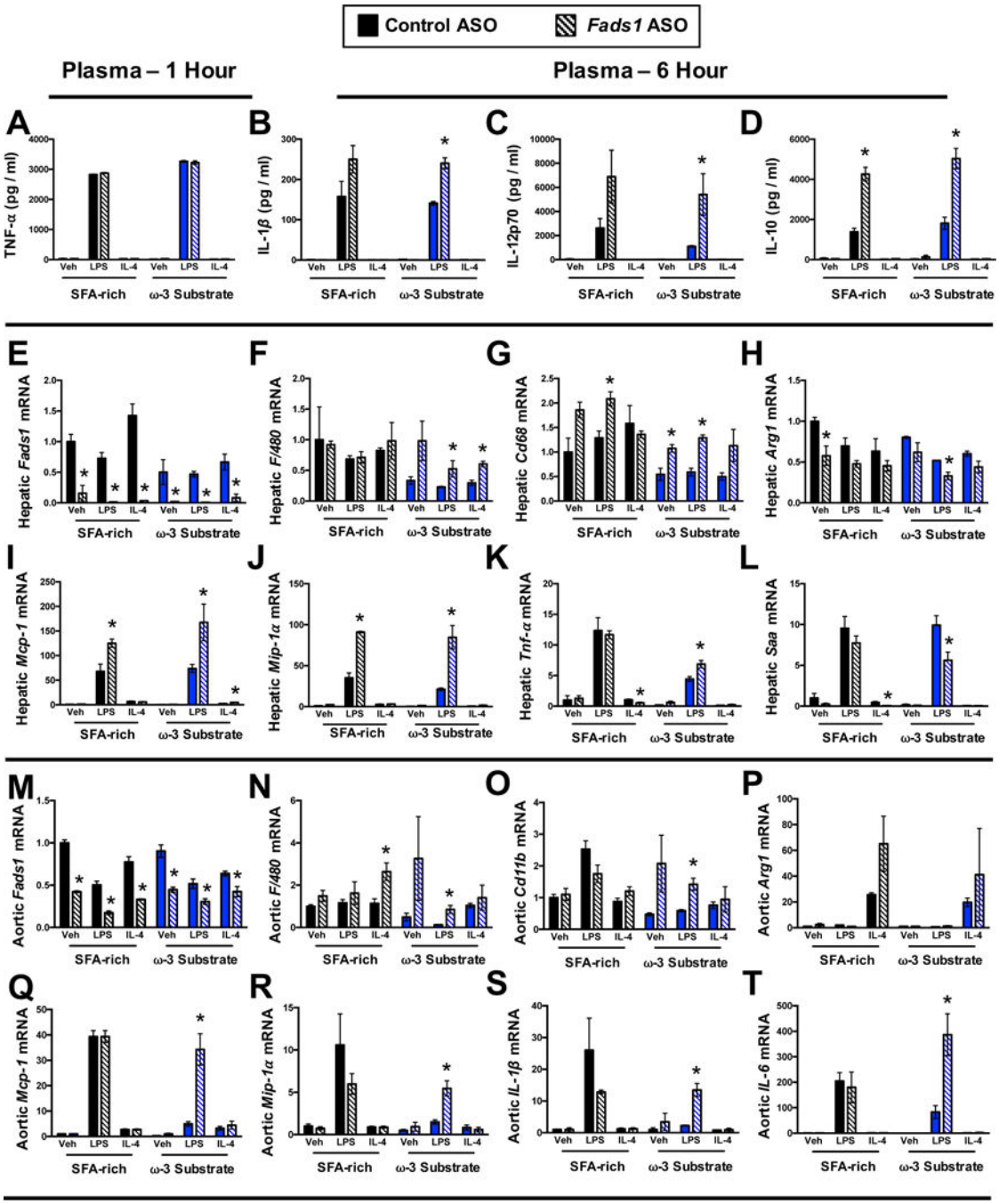

Figure 5. ***Fads1*** **Knockdown Alters Systemic Inflammation** ***In Vivo***

(A) Acute phase TNF-α levels in plasma 1 hour after Veh/LPS/IL-4 stimulation in mice fed diet and treated with ASOs for 8 weeks; *n=3 per group.* **(B–D)** Terminal cytokine levels in plasma 6 hours after Veh/LPS/IL-4 stimulation; *n=3 per group.* **(E–L)** qPCR quantification of hepatic gene expression; *n=3 per group.* **(M–T)** qPCR quantification of aortic arch gene expression; *n=3 per group.* All graphs display mean ± S.E.M. Statistical significance is determined by *t*-test. * = significantly different from control ASO group within the same diet group (*p<0.05).

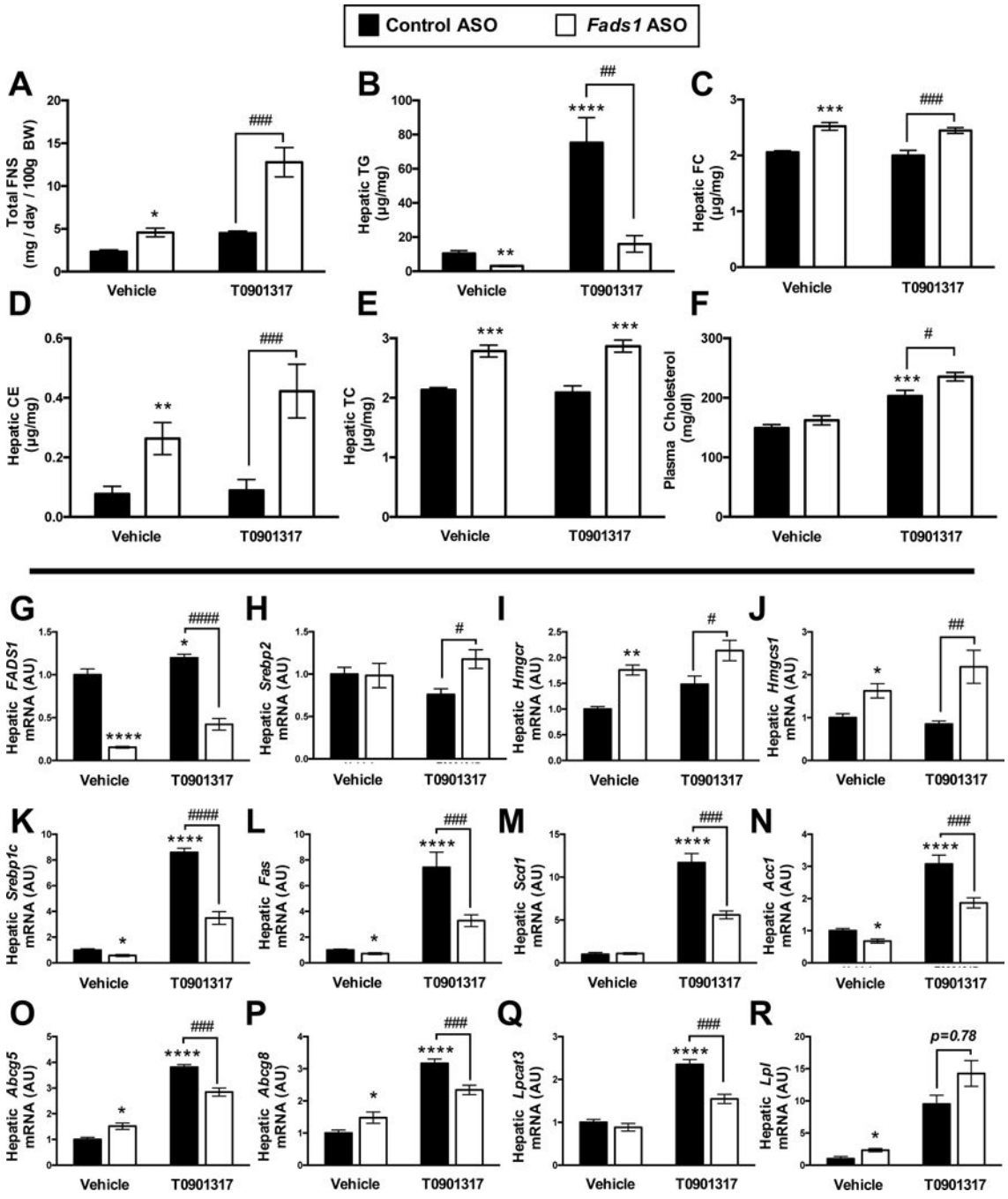

Figure 6. *Fads1* is an Effector of LXR-Driven Reorganization of Lipid Metabolism

Chow-fed C57BL/6J mice were treated with control or Fads1 ASO for 10 consecutive weeks, and then were also gavaged daily with Vehicle or T0901317 (25 mg/kg/day) during the tenth week; *n=6 per group*. **(A)** Total fecal neutral sterol loss from mice fed chow and treated with ASOs for 10 weeks. **(B)** Total hepatic triglycerides (TG); *n=6 per group*. **(C)** Hepatic free cholesterol (FC); *n=6 per group*. **(D)** Hepatic cholesteryl ester (CE); *n=6 per group*. **(E)** Hepatic total cholesterol (TC); *n=6 per group*. **(F)** Total plasma cholesterol in mice fed chow and treated with ASOs for 10 weeks; *n-6 per group*. **(G–R)** qPCR

quantification of hepatic gene expression, *n=6 per group*. All graphs display mean ± S.E.M. Statistical significance is determined by 2-way ANOVA. * = significantly different from the control ASO – vehicle gavage group (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001); # = significantly different from control ASO group within the T0901317 gavage group ($^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001, $^{\#\#\#\#}$p<0.0001).