Editorial

Knockdown of Δ-5 Fatty Acid Desaturase Is More Than Just a Fad

Nadya M. Morrow, Murray W. Huff

Human genome-wide association studies have revealed a large number of genomic loci linked to cardiometabolic disease traits.[1–3] However, for many of the gene variants identified, it remains unknown how they functionally influence the disease phenotype or whether these genes represent new therapeutic targets. Single-nucleotide polymorphisms in *FADS1*, which encodes Δ-5 fatty acid desaturase, have been identified in many large genome-wide association studies as strongly linked to cardiometabolic diseases including obesity, type 2 diabetes mellitus, dyslipidemia, fatty liver, liver enzyme elevation, and coronary artery disease.[1–9] The underlying mechanisms by which variants of *FADS1* link to these phenotypes is unknown. FADS1 is the only mammalian Δ-5 fatty acid desaturase enzyme capable of introducing a double bond at carbon 5 (from the carboxyl group) leading to the synthesis of the critically important polyunsaturated fatty acids arachidonic acid (AA) and eicosapentaenoic acid (EPA) from dihomo-γ-linolenic acid (20:3, ω-6) and eicosatetraenoic acid (20:4, ω-3), respectively (Figure).[10]

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In turn, AA and EPA can be converted by downstream enzymatic and nonenzymatic oxidation reactions to a diverse group of lipid-signaling mediators that are capable of initiating or resolving inflammatory processes.[11,12] In general, AA-derived eicosanoids are thought to primarily initiate or potentiate proinflammatory responses, with fewer AA-derived proresolving lipid mediators.[11] On the contrary, EPA-derived and docosahexaenoic acid–derived mediators primarily resolve inflammation and initiate wound healing and tissue regenerative responses.[12] Specifically, lipoxins, resolvins, and protectins are important autocrine or paracrine lipids, and collectively, they are defined as specialized proresolving mediators named for their ability to actively resolve inflammation.[12] Because ω-6 and ω-3 fatty acids or their derivatives cannot be interconverted by mammals, it is entirely possible that diets containing different amounts of precursor ω-6 or ω-3 fatty acids are capable of influencing the balance of specialized proresolving mediators.

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In this edition of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Gromovsky et al[13] pose 2 interesting and biologically important questions: (1) In *Ldlr*$^{-/-}$ mice, does the knockdown of *Fads1*, which would mimic a complete loss-of-function *FADS1* human variant, dramatically impact diet-induced tissue inflammation and related cardiometabolic phenotypes? (2) Does a diet rich in AA-precursor ω-6 fatty acids versus a diet rich in EPA-precursor ω-3 fatty acids shift the balance of substrates available for specialized proresolving mediator production, thereby altering the phenotypic response to *Fads1* deletion? Model development shifted to second-generation antisense oligonucleotides because global deletion of mouse *Fads1* results in early postnatal lethality.[14,15] Injection of *Fads1* antisense oligonucleotides into adult mice predominately targets liver, adipose tissue, and reticuloendothelial cells, resulting in >90% knockdown of *Fads1* mRNA in these tissues. Of significance, although diets differed in ω-6 versus ω-3 fatty acid precursors, diets contained no AA, and the content of EPA and docosahexaenoic acid was held constant across all dietary groups.[16]

This well-controlled study revealed that FADS1 makes a substantial contribution to polyunsaturated fatty acids in hepatic membrane phospholipids, an effect quantitatively more pronounced in ω-3–fed mice (Figure). *Fads1* knockdown also diminished hepatic levels of AA-derived, EPA-derived, and docosahexaenoic acid–derived proresolving and proinflammatory lipid mediators. With the ω-3 diet, the balance of these lipid mediators was markedly shifted in that the loss of proresolving mediators was more pronounced than the loss of proinflammatory mediators. *Fads1* depletion promoted hepatic inflammation with both diets. However, in ω-3–fed mice, in which hepatic inflammation was low under control conditions, *Fads1* depletion induced a greater proinflammatory response, compared with that in ω-6–fed mice. *Fads1* knockdown increased hepatic cholesterol content in both dietary groups, despite decreased expression of *Srebp2* (sterol response element–binding protein 2) and increased fecal neutral sterol excretion. *Fads1* knockdown enhanced hypercholesterolemia, systemic lipopolysaccharide-induced inflammation, aortic inflammation, and atherosclerosis in both dietary groups. However, in the ω-3–fed group, in which systemic and aortic inflammation, plasma monocytes, and atherosclerosis were lower in controls, *Fads1* knockdown induced increases of greater magnitude in these parameters than those observed in ω-6–fed mice.

In contrast to cholesterol metabolism, loss of *Fads1* suppressed hepatic de novo lipogenesis and reduced hepatic triglyceride content and plasma triglycerides, which were associated with decreased adiposity and improved glucose tolerance, even though hepatic and systemic inflammation were increased. In a separate study of *Fads1* knockdown in chow-fed C57BL/6 mice, the authors observed that decreased hepatic

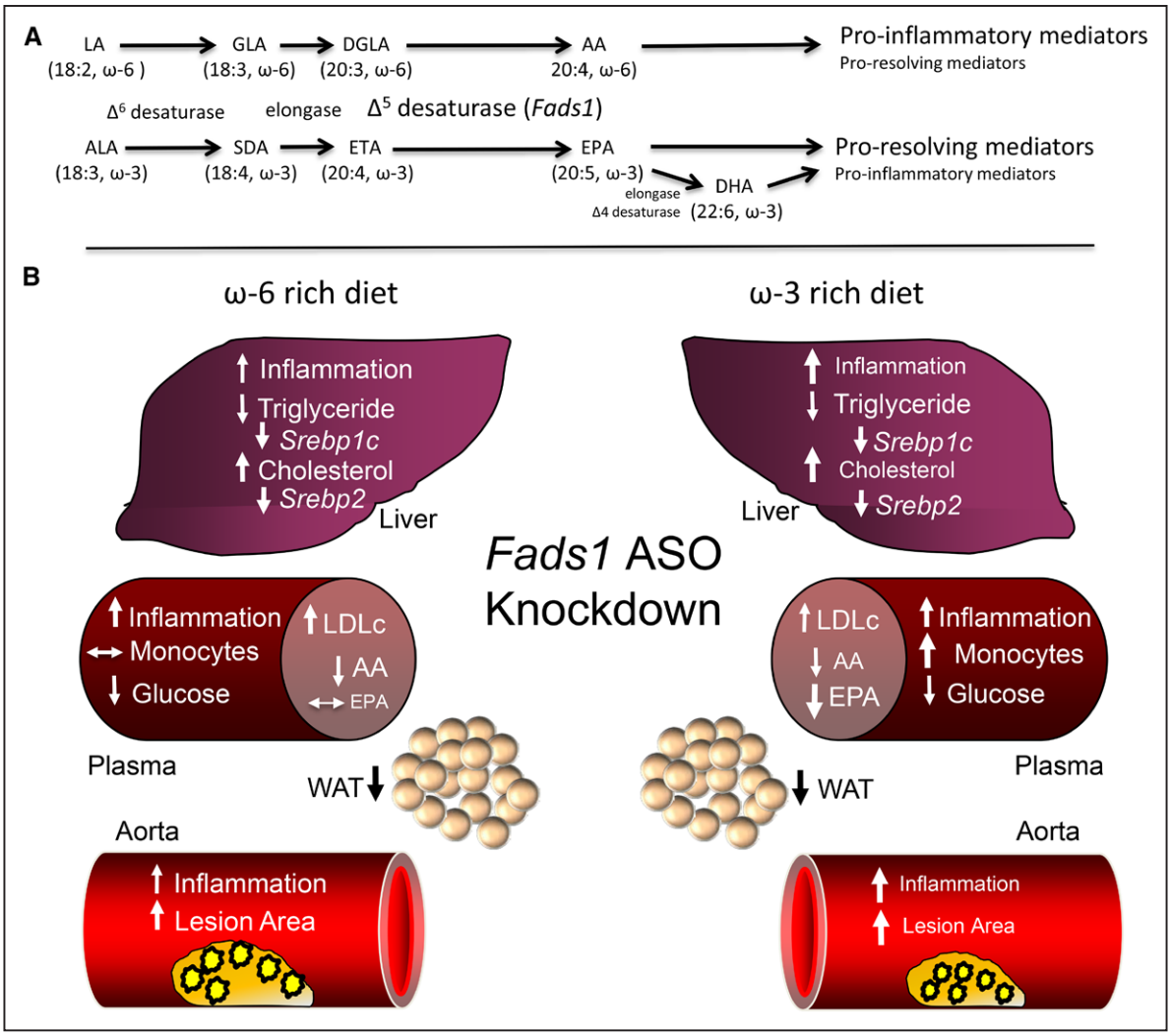

Figure. The knockdown of Δ-5 fatty acid desaturase *Fads1* impacts metabolic disease parameters by altering the balance of proinflammatory and proresolving lipid mediators in a diet-specific manner. **A**, Enzymes and fatty acids that comprise the ω-6 and ω-3 fatty acid pathways leading to the synthesis of proinflammatory and proresolving lipid mediators. The murine fatty acid desaturase 1 gene (*Fads1*) encodes the enzyme Δ-5 fatty acid desaturase and catalyzes the conversion of dihomo-γ-linolenic acid (DGLA, 20:3, ω-6) or eicosatetraenoic acid (ETA, 20:4, ω-3) to arachidonic acid (AA, 20:4, ω-6) or eicosapentaenoic acid (EPA, 20:5, ω-3), respectively. **B**, The impact of *Fads1* knockdown by antisense oligonucleotides (ASO) in *Ldlr*$^{-/-}$ mice fed diets enriched in ω-6 AA precursors (**left**) or diets enriched in ω-3 EPA precursors (**right**). Inflammation, mRNA expression, metabolic parameters, inflammatory markers, and atherosclerosis were assessed primarily in liver, plasma, white adipose tissue (WAT), and the aortic sinus. ALA indicates α-linoleic acid; DHA, docosahexaenoic acid; GLA, γ-linolenic acid; LA, linoleic acid; LDLc, low-density lipoprotein cholesterol; SDA, stearidonic acid; *Srebp1c*, sterol response element–binding protein 1c; and *Srebp2*, sterol response element–binding protein 2. The font size indicates the relative magnitude of each parameter in control mice (intact *Fads1*). The size and direction of the arrows indicate the magnitude of the change in each parameter resulting from *Fads1* knockdown.

lipogenesis and triglyceride content (and likely adiposity and glucose intolerance) were driven by deactivation of liver X receptor (LXR)-mediated fatty acid programming, because of the loss of FADS1-synthesized LXR ligands. Overall, the authors conclude that synthesis of FADS1-driven AA and EPA results in diversified production of proinflammatory and proresolving lipids that mediate appropriate inflammation initiation and resolution in cardiometabolic disease. Furthermore, a diet rich in ω-3 EPA precursors shifts the balance in favor of proresolving lipid mediators to a greater extent than a ω-6–rich diet.

These well-conducted comprehensive and complex studies reveal several unique and important results. FADS1 activity determines lipid mediator balance in a diet-specific manner. Furthermore, lipid mediator synthesis is largely driven by diet-derived precursors of the FADS1 reaction (dihomo-γ-linolenic acid, ω-6 and eicosatetraenoic acid, ω-3) rather than preformed dietary EPA, a fatty acid downstream of the FADS1 reaction. This is highlighted by the observation that both diets contained similar amounts of EPA, yet *Fads1* knockdown induced large functional changes in EPA-derived specialized proresolving mediators for which EPA is the precursor. Fads1 products suppress hepatic inflammation, systemic monocytosis and atherosclerosis. Paradoxically, FADS1 products increase hepatic lipogenesis, adipose tissue accumulation, and glucose intolerance, driven by enhanced LXR-activated gene expression. This study clearly establishes in vivo that FADS1 is a novel effector of LXR-mediated reorganization of fatty acid metabolism. The authors provide strong evidence that many of the

positive, improved, or suppressed metabolic indices associated with ω-3 fatty acid–rich diets (tissue inflammation, monocytosis, and atherosclerosis) are directly linked to lipid mediators synthesized by FADS1 and derived from dietary ω-3 fatty acids.

Several unanswered questions remain. Although these experiments represent functional validation of any *FADS1* complete loss-of-function variant, no insights are provided as to the metabolic impact of partial loss-of function or gain-of-function variants of *FADS1* identified in human genome-wide association studies. It is intriguing to speculate that target tissues preferentially use endogenously synthesized FADS1-derived EPA for synthesis of proresolving lipid mediators, rather than diet-derived EPA. Elucidation of this apparent metabolic channeling mechanism would represent an exciting revelation. The antisense oligonucleotide approach restricts *Fads1* knockdown to specific tissues, such that the impact of *Fads1* depletion in other *Fads1*-sensitive tissues, including the brain, could not be elucidated. However, the antisense oligonucleotide approach may be advantageous from a therapeutic perspective because liver and adipose tissue are organs most likely to be targeted by an intervention. FADS1 synthesizes a wide variety of proinflammatory and proresolving lipid species. Although it remains unknown how these Fads1 products regulate cholesterol metabolism, it will be important to determine the mechanism underlying the *Fads1*-knockdown cholesterol effect. Is it a direct effect on genes or proteins involved in cholesterol metabolism (other than LXR targets) or is the effect secondary to tissue inflammation? It will also be valuable for therapeutic development to determine the relative contribution of each individual lipid mediator to the cardiometabolic phenotypes under investigation. Some may be the prime determinants of the inflammatory response, whereas others may be specific for modulating the LXR response. In addition, in *Fads1*-knockdown mice, concentrations of the FADS1 substrate eicosatetraenoic acid (20:4, ω-3) increase substantially in liver membrane phospholipids. It is possible that eicosatetraenoic acid accumulation per se contributes to some of the observed changes in metabolic and inflammatory indices.

Finally, it will be important to establish whether FADS1 is a viable therapeutic target. With suppression of *Fads1*, worsening of cholesterol metabolism and inflammation eclipses the improvement in triglyceride and glucose metabolism with respect to atherosclerosis. It remains to be determined whether *Fads1* activation, perhaps mimicking human gain-of-function variants, improves cholesterol metabolism and attenuates atherosclerosis, without any downside with respect to triglyceride and carbohydrate metabolism.

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