ACSL6 is critical for maintaining brain DHA levels

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Docosahexaenoic acid (DHA; 22:6n3) is the most abundant polyunsaturated fatty acid in the brain, where it is largely esterified to membrane phospholipids. While DHA is found throughout the brain, its levels are relatively higher in gray matter and it is especially enriched at the synapse (1). DHA is a precursor to a series of bioactive molecules, including the specialized proresolving lipid mediators (2) and docosahexaenoyl ethanolamide (1). Collectively, DHA and its mediators regulate, or are involved in, numerous processes in the brain, including the maintenance of membrane fluidity, neuronal survival, synaptic neurotransmission, and regulation of neuroinflammation, among others. Thus, perhaps not surprisingly, disturbances in brain DHA metabolism have been implicated in a host of neurodegenerative and psychiatric diseases (1). The importance of DHA in the brain has led to many nutritional studies examining the role of diet in regulating its levels; however, mechanistic details regarding its uptake into the brain have been relatively scarce. While it is clear that plasma unesterified DHA can enter the brain (3, 4), the molecular mechanisms regulating this process are not clear. In PNAS, Fernandez et al. (5) find that a member of the long-chain acyl-CoA synthetase (ACSL) family, ACSL6, is essential for enriching the brain with DHA.

DHA is a member of the omega-3 family of fatty acids, which cannot be synthesized de novo and are generally considered to be essential. However, DHA itself is not nutritionally essential because it can be synthesized from its dietary precursor α-linolenic acid via a series of reactions that primarily occur in the liver. Importantly, within the brain, the synthesis of DHA from α-linolenic acid is very low. Thus, the brain requires a constant supply of DHA from the blood to replace the DHA consumed in metabolic reactions. There has been considerable debate as to which plasma pools supply the brain with DHA and the mechanisms by which DHA is transported across membranes into the brain parenchyma. Using a variety of kinetic approaches, we and others demonstrated that plasma unesterified DHA enters the brain and is quantitively the major plasma pool supplying the brain with DHA (3, 4, 6), but mechanistic details were lacking. A seminal paper by Kamp and Hamilton (7) demonstrated that unesterified fatty acids readily cross membranes without transporters; however, on its own, this model would rapidly reach an equilibrium, halting a net flux of DHA across the membrane, and it requires acceptor proteins or metabolism of DHA to ensure a continuous flux. The family of fatty acid transport proteins (FATPs), as well as other proteins [fatty acid translocase (CD36) and major facilitator superfamily domain containing 2A (MFSD2A)], clearly facilitates the uptake of fatty acids into tissues (8–10) (Fig. 1). However, despite their name, the FATPs are not classical transporters, but rather possess ACSL activity (9) and facilitate the flux of fatty acids across membranes secondary to metabolic effects (11). Thus, FATPs can quench unesterified fatty acids at or upon crossing the membrane, making them more water soluble, facilitating subsequent metabolism, and decreasing their intracellular concentration. The net effect of many of the FATPs, by metabolizing fatty acids, is to facilitate their flux across the membrane, but not their transport per se. As an analogy, FATPs act similarly to glucokinase or hexokinase, both of which phosphorylate glucose, decreasing its concentration to facilitate its transport by the glucose transporter. While CD36 is not known to have ACSL activity, it increases the esterification of fatty acids into triacylglycerol, subsequently enhancing uptake across the membrane. In the brain, where the amount of triacylglycerol is low, knockout of CD36 has little effect on brain fatty acid levels (1). The mechanism by which MFSD2A facilitates the uptake of lysophospholipids, including those containing DHA, is not yet clear. Along a similar line as the FATPs, several members of the ACSL family have been shown to facilitate fatty acid uptake by influencing the cellular metabolism of

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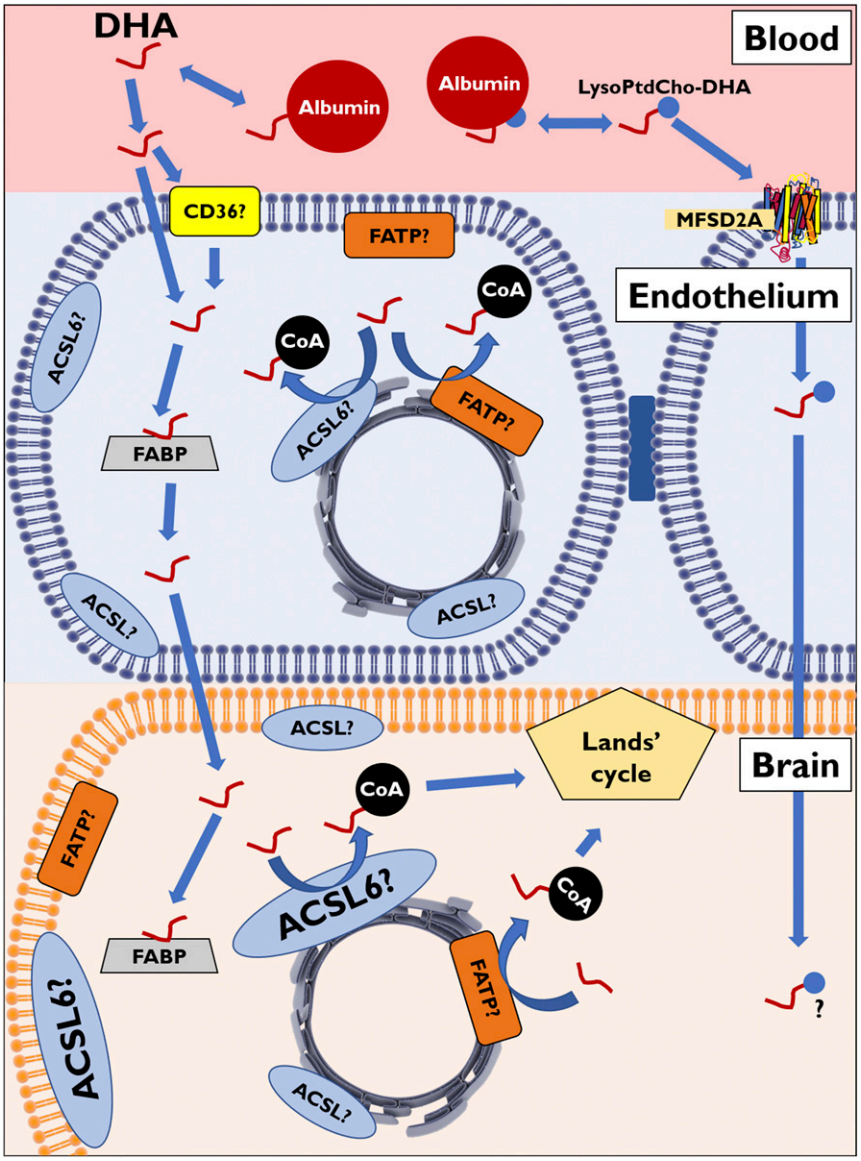

Fig. 1. Schematic representation of the mechanisms regulating brain DHA uptake. Unesterified DHA crosses the endothelium of the BBB by passive diffusion to reach the brain parenchyma. This process is facilitated by a series of proteins and enzymes such as FABPs, FATPs, and ACSLs. As reported in PNAS by Fernandez et al. (5), ACSL6 is critical for the maintenance of brain DHA. *Acsl6* expression is higher in the brain than at the BBB (13), but the intracellular localization of ACSL6 has yet to be fully described. An alternative pathway by which DHA can reach the brain, through the protein MFSD2A, is also shown. LysoPtdCho, lysophosphatidylcholine.

fatty acids in peripheral tissues and cells (9, 11). Another family of proteins involved in fatty acid trafficking are the fatty acid binding proteins (FABPs). FABPs act intracellularly, where they reversibly bind fatty acids and transfer them throughout the cell without ACSL activity (12) (Fig. 1).

In the brain, relatively little work has been completed examining the regulation of fatty acid uptake by enzymes with ACSL activity, let alone the ACSL family of proteins. At the blood–brain barrier (BBB), CD36, FATP1, FATP4, FABP3, FABP5, ACSL1, ACSL3, ACSL4, and ACSL6 are the most characterized proteins involved in fatty acid trafficking (1, 13, 14). Both FATP1 and FABP5 have recently been suggested as important for DHA uptake through the BBB (15–18), although both are not selective for DHA (16–18). Nicolazzo and coworkers (16) recently identified FABP5 in the BBB as critical for the uptake of unesterified DHA, with its ablation leading to a decrease not only in unesterified DHA uptake but also in brain DHA levels. Furthermore, DHA exposure increases the expression of FAPB5 (as well as FATP1 and FATP4) at the BBB (19), suggesting a feed-forward mechanism by which DHA can up-regulate its own uptake. However, compared with the FATPs and FABPs, the role of ACSL in brain fatty acid, and especially in DHA uptake, has not been as extensively studied. Elegant work using recombinant ACSL demonstrated that both ACSL4 and a variant of ACSL6 had a relatively low DHA-mediated IC_{50} for inhibiting palmitoyl-CoA formation (20). Interestingly *Acsl6* mRNA is most abundant in the brain, followed by the testis (another DHA-rich organ), whereas *Acsl4* mRNA is primarily expressed in the adrenal gland and liver. In their paper, Fernandez et al. (5) demonstrate first that ACSL6 protein is most abundant in the brain. Furthermore, because ACSL6 levels in their mouse model continued to increase in the brain with age, it would appear to be more relevant for fatty acid metabolism during

adulthood as opposed to development. In their report, *Acsl6* deficiency led to an ∼50% decrease in DHA within the brain phospholipid pools, as well as to a series of neurochemical and behavioral abnormalities (5). Fernandez et al. (5) demonstrate that *Acsl6* is necessary for DHA enrichment in the brain, but as they note, it is not clear exactly where this step might occur. ACSL6 could be critical for quenching unesterified DHA entering the endothelium or the parenchyma (Fig. 1). However, beyond uptake, another important pathway for maintaining brain DHA levels is Lands' cycle, in which DHA released from phospholipid membranes by phospholipase A_2 requires activation of DHA with CoA before being reesterified, or recycled, into the phospholipid. Lands' cycle is quite active in the brain and has been estimated to consume ∼0.1 to 0.3% of the brain's ATP for the recycling of DHA alone (1). Given the vast number of energy-dependent reactions that occur in the brain, 0.1 to 0.3% of the ATP is likely significant. For instance, fatty acids such as the omega-3 fatty acid eicosapentaenoic acid, which is also taken up into the brain at a similar rate as DHA, are not extensively recycled within brain phospholipids via Lands' cycle and are not enriched in the brain. While it was demonstrated that overexpression of *Acsl6* in PC12 cells, a neuronal cell line, led to increased DHA uptake and levels (21), the exact cell types and steps by which ACSL6 acts upon DHA to enrich brain phospholipids in vivo remained unknown. Interestingly, Fernandez et al. (5) generated an astrocyte-specific knockout of *Acsl6*, which was associated with lower brain DHA, demonstrating that astrocytic ACSL6 is critical for brain DHA enrichment. Future studies examining ACSL6 intracellular localization are still needed, however.

In accordance with Fernandez et al. (5) who find behavioral abnormalities concurrent with low brain DHA, others report that deletion of several proteins involved in brain fatty acid uptake as well as chronic dietary studies leading to lower brain DHA levels are also associated with neurochemical and behavioral abnormalities (10, 16, 22). While one might think that dietary studies would be the gold standard for determining the effects of DHA in the brain, they are often confounded by other dietary factors and, unlike *Acsl6* deficiency in which decreases in DHA levels were specific to the brain, by concurrent decreases in peripheral tissue DHA levels. Overlapping effects between studies that are able to target brain DHA without concurrent peripheral effects on DHA metabolism and dietary studies will be important for elucidating the roles of DHA in the brain in vivo. Unfortunately, different approaches, assays, and outcomes have often been used; thus, caution must be taken when trying to assess whether DHA-mediated effects are consistent between studies. One clear area of overlap is that Fernandez et al. (5) observed an increase in microglia activity in response to lipopolysaccharide administration upon *Acsl6* deficiency-induced brain DHA depletion, which is consistent with other genetic and dietary approaches that lower brain DHA levels (23). It is also possible that genetic approaches could lead to cellular or subcellular differences in DHA levels that have yet to be elucidated or to the finding that, in some cases, lower DHA is secondary to other cellular or metabolic effects. *Acsl6*-deficient mice might be a useful tool to elucidate not only the mechanisms by which the brain is enriched with DHA but also the role DHA plays in regulating brain homeostasis.

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1 Lacombe RJS, Chouinard-Watkins R, Bazinet RP (2018) Brain docosahexaenoic acid uptake and metabolism. *Mol Aspects Med* 64:109–134.
2 Serhan CN (2014) Pro-resolving lipid mediators are leads for resolution physiology. *Nature* 510:92–101.
3 Chen CT, et al. (2015) Plasma non-esterified docosahexaenoic acid is the major pool supplying the brain. *Sci Rep* 5:15791.
4 Umhau JC, et al. (2009) Imaging incorporation of circulating docosahexaenoic acid into the human brain using positron emission tomography. *J Lipid Res* 50:1259–1268.
5 Fernandez RF, et al. (2018) Acyl-CoA synthetase 6 enriches the neuroprotective omega-3 fatty acid DHA in the brain. *Proc Natl Acad Sci USA* 115:12525–12530.
6 Chouinard-Watkins R, et al. (2017) Phospholipid class-specific brain enrichment in response to lysophosphatidylcholine docosahexaenoic acid infusion. *Biochim Biophys Acta Mol Cell Biol Lipids* 1862(10 Pt A):1092–1098.
7 Kamp F, Hamilton JA (1992) pH gradients across phospholipid membranes caused by fast flip-flop of un-ionized fatty acids. *Proc Natl Acad Sci USA* 89:11367–11370.
8 Son NH, et al. (2018) Endothelial cell CD36 optimizes tissue fatty acid uptake. *J Clin Invest* 128:4329–4342.
9 Watkins PA (2008) Very-long-chain acyl-CoA synthetases. *J Biol Chem* 283:1773–1777.
10 Nguyen LN, et al. (2014) Mfsd2a is a transporter for the essential omega-3 fatty acid docosahexaenoic acid. *Nature* 509:503–506.
11 Mashek DG, Li LO, Coleman RA (2007) Long-chain acyl-CoA synthetases and fatty acid channeling. *Future Lipidol* 2:465–476.
12 Furuhashi M, Hotamisligil GS (2008) Fatty acid-binding proteins: Role in metabolic diseases and potential as drug targets. *Nat Rev Drug Discov* 7:489–503.
13 Pélerin H, et al. (2014) Gene expression of fatty acid transport and binding proteins in the blood-brain barrier and the cerebral cortex of the rat: Differences across development and with different DHA brain status. *Prostaglandins Leukot Essent Fatty Acids* 91:213–220.
14 Mitchell RW, et al. (2011) Fatty acid transport protein expression in human brain and potential role in fatty acid transport across human brain microvessel endothelial cells. *J Neurochem* 117:735–746.
15 Pan Y, et al. (2015) Fatty acid-binding protein 5 facilitates the blood-brain barrier transport of docosahexaenoic acid. *Mol Pharm* 12:4375–4385.
16 Pan Y, et al. (2016) Fatty acid-binding protein 5 at the blood-brain barrier regulates endogenous brain docosahexaenoic acid levels and cognitive function. *J Neurosci* 36:11755–11767.
17 Ochiai Y, et al. (2017) The blood-brain barrier fatty acid transport protein 1 (FATP1/SLC27A1) supplies docosahexaenoic acid to the brain, and insulin facilitates transport. *J Neurochem* 141:400–412.
18 Lee GS, Pan Y, Scanlon MJ, Porter CJH, Nicolazzo JA (2018) Fatty acid-binding protein 5 mediates the uptake of fatty acids, but not drugs, into human brain endothelial cells. *J Pharm Sci* 107:1185–1193.
19 Pan Y, et al. (2018) Dietary docosahexaenoic acid supplementation enhances expression of fatty acid-binding protein 5 at the blood-brain barrier and brain docosahexaenoic acid levels. *J Neurochem* 146:186–197.
20 Van Horn CG, et al. (2005) Characterization of recombinant long-chain rat acyl-CoA synthetase isoforms 3 and 6: Identification of a novel variant of isoform 6. *Biochemistry* 44:1635–1642.
21 Marszalek JR, Kitidis C, Dirusso CC, Lodish HF (2005) Long-chain acyl-CoA synthetase 6 preferentially promotes DHA metabolism. *J Biol Chem* 280:10817–10826.
22 Joffre C, Nadjar A, Lebbadi M, Calon F, Laye S (2014) n-3 LCPUFA improves cognition: The young, the old and the sick. *Prostaglandins Leukot Essent Fatty Acids* 91:1–20.
23 Trépanier MO, Hopperton KE, Orr SK, Bazinet RP (2016) N-3 polyunsaturated fatty acids in animal models with neuroinflammation: An update. *Eur J Pharmacol* 785:187–206.