Fatty acid transport proteins: a current view of a growing family

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Long-chain fatty acids (LCFAs) are a major caloric component of our diet and are key metabolites for energy generation and storage. Physiological uptake of LCFAs across cell membranes is a saturable and competable process occurring at low concentrations, indicative of protein-mediated transport. Fatty acid transport proteins are a family of transmembrane proteins that enhance LCFA uptake and are produced in all fatty acid-utilizing tissues. Here, we review our current understanding of the function, expression patterns and regulation and subcellular localization of this interesting family of proteins.

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LCFAS (see Glossary), generated by lingual and pancreatic lipases from dietary triglycerides, contribute over 40% of the caloric content of the Western diet[1]. Efficient absorption of LCFAs, predominantly in the jejunum and also in the ileum, allows < 5% of the ingested lipids to escape with feces. LCFAs are absorbed by the epithelial cells of the small intestinal villi, also termed enterocytes, then re-esterified and incorporated into chylomicrons as triglycerides. Chylomicrons undergo exocytosis at the basolateral side of the cell, where they subsequently enter the lymphatic system[2]. Chylomicrons then travel through the lymphatics to the thoracic duct, which empties into the bloodstream. Capillary-bound lipoprotein lipase, produced by liver, heart, adipose and other tissues, catalyzes the release of FFAS from lipoproteins, of which the vast majority is immediately bound to albumin[3]. Once in the circulatory system, LCFAs are distributed to the various tissues of the body, where they are used for energy storage and production, intracellular signaling, anchoring proteins to the plasma membrane and membrane biosynthesis. In both the intestine and the bloodstream, it is LCFAs and not di- or triglycerides that traverse the plasma membrane and it is this crucial transport step that this review focuses on.

Transport of long-chain fatty acids

There are well-characterized examples of transporter families for amphipathic molecules such as bile acids[4,5], and a clearly defined fatty acid transporter on the outer membrane of *Escherichia coli* is required for LCFA uptake[6]. However, it was initially believed that LCFAs enter eukaryotic cells merely by diffusion through the phospholipid bilayer[7,8]. There is now ample evidence that, in addition to this diffusion component, the intestine[9,10], liver[11], heart[12,13], adipose tissue[14] and other organs possess a saturable and competable LCFA transport system[15]. Investigators have identified several proteins that, when their genes are overexpressed in cultured mammalian cells, increase the uptake of LCFAs. The most prominent and best characterized of these are FAT/CD36, LACS and FATPS. LACS esterify fatty acids to produce acyl-CoA and might enhance the entry of fatty acids into cells by lowering the concentration of free fatty acids in the cytosol, thereby creating a concentration gradient between extra- and intracellular fatty acid pools.

CD36 belongs to the SR-B1 family of scavenger receptors and, in addition to fatty acids, it can bind a variety of molecules, including oxidized LDL, acetylated LDL, maleated bovine serum albumin, collagen, anionic phospholipids, *Plasmodium falciparum*-infected erythrocytes, HDL, LDL and VLDL (Refs 16–20). CD36 is not found in the liver, a tissue that has a large capacity to take up fatty acids, and is present at high levels in tissues such as colon and spleen, which display only low levels of fatty acid uptake, suggesting that CD36 is not the primary fatty acid transporter in all physiologically relevant tissues, and that it performs additional roles unrelated to fatty acid uptake. Although CD36-deficient mice show defects in the uptake of LCFAs in heart, adipose tissue and muscle, these mice have normal fatty acid uptake in the liver, and no defect in the absorption of dietary fatty acids was reported[21]. Similarly, patients with type-I CD36 deficiency show normal levels of accumulation in their liver of an iodinated fatty acid tracer, [^{123}I]-15-(*p*-iodophenyl)-3-(*R*,*S*)-methyl pentadecanoic acid ([^{123}I]–BMIPP)[22], and have not been reported to have fat malabsorption. Interestingly, CD36 appears to be required for fatty acid uptake, primarily under conditions of low FFA concentrations, and one possible role for CD36 could be to bind and concentrate FFA at the cell surface and to transfer LCFAs to FATPs.

This review concentrates on the discussion of FATPs*, a recently identified family of integral membrane proteins that are expressed in all fatty acid-utilizing tissues and cell types, but which are absent from tissues that display only very low rates of fatty acid uptake, such as colon and spleen[23–25].

The FATP family

The first FATP was identified by expression cloning from a murine adipocyte cDNA library as a protein that facilitates the uptake of LCFAs when the gene is overexpressed in adipocytes[14]. This protein, later

*The official nomenclature for the genes encoding the FATPs is *SLC27A1–6* (humans) and *Slc27a1–5* (mice). However, to avoid confusion, we have used *FATP1–6* for all mammalian species. Fatp-a refers to the *Drosophila melanogaster* CG3037 gene product.

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Glossary

ACBP: acyl-CoA-binding protein
ER: endoplasmic reticulum
FABP: fatty acid-binding protein
FATP: fatty acid transport protein
FFA: free fatty acid
HDL: high-density lipoprotein
LACS: long-chain acyl-CoA synthetase (ligase)
LCFA: long-chain fatty acid
LDL: low-density lipoprotein
PPAR: peroxisome proliferative-activated receptor
RXR: retinoid X receptor
VLDL: very low-density lipoprotein
VLACS: very long-chain acyl-CoA synthetase (ligase)
VLCFA: very long-chain fatty acids
YNBD: yeast nitrogen base and dextrose

renamed FATP1 (Ref. 23), is induced during adipocyte differentiation *in vitro* and is found in brain, skeletal muscle, heart, fat and kidney, but not liver. Subsequently, we reported the discovery of a large family of FATPs characterized by the presence of a FATP signature sequence, a 311 amino acid sequence highly conserved among FATP family members[23,26]. So far, five murine and six human FATPs have been identified (FATP 1–6)[14,23–25,27–32]. In mammals, the distinctions between the FATPs are well conserved so that, for example, murine FATP4 is more homologous to human, pig, cow and rat FATP4 than to any other murine FATPs (A. Stahl *et al.*, unpublished; Ref. 23). Apart from mammals, FATPs are found in invertebrates such as *Caenorhabditis elegans* (two FATPs) and *Drosophila melanogaster* (three FATPs), and in fungi such as *Saccharomyces cerevisiae*, which express one FATP family member, called *fat1*. Interestingly, mycobacteria also produce a highly conserved FATP homolog[23], unlike *E. coli*, which utilizes the unrelated *fadL* gene for the transport of fatty acids[33]. So far, no FATP homologs have been found in plants.

FATP expression patterns

To maintain energy homeostasis during fasting–feeding cycles, the body has to be able to move the major metabolites – glucose and LCFAs – from storage to utilizing organs. The differential distribution of glucose among organs is facilitated by tissue-specific expression of a family of different glucose transporters, in addition to different abilities to respond to insulin changes[34]. The mechanisms by which the flux of LCFAs into organs is regulated are less well understood. After the cloning of the first FATP, it was noted that it was absent from the liver and the small intestine, two organs that were known to transport fatty acids actively[9–11]. This seeming contradiction was resolved by the discovery of a large FATP family with one or several members found in every major fatty acid-utilizing organ[23] (Table 1). FATP1 is the major fatty acid transporter in adipose tissue and is also found in the heart, FATP2 is found almost exclusively in liver and kidney cortex[23,35], tissues preferentially metabolizing oleate[36]. *FATP3* shows a broader expression pattern, with notably high mRNA and protein levels in the lung (A. Stahl *et al.*, unpublished). This is of potential importance because the pneumocytes in the adult lung rely on the import of LCFAs to generate dipalmitoylphosphatidylcholine and other phospholipids that form pulmonary surfactant, a phospholipid–protein complex that prevents the collapse of the alveoli by reducing surface tension at the air–liquid interface. FATP4 is the only FATP found in the intestine and is required for LCFA uptake in isolated intestinal epithelial cells[24] and, together with FATP1, is the predominant FATP in the brain[25]. FATP5 has exquisite liver specificity[23,27,32], whereas FATP6 is found almost exclusively in the heart (A. Stahl *et al.*, unpublished).

Transcriptional regulation

Both hormones and cytokines have been reported to regulate FATP expression. Several reports have shown a positive regulation of mouse FATP by ligands that activate either PPAR-γ, PPAR-α or PPAR-γ–RXR heterodimers in hepatoma cell lines, the liver and the intestine[37–39]. Furthermore, a PPAR-binding site was identified in the murine *FATP1* promoter[40]. Because fatty acids and their derivatives, such as prostaglandins,

Table 1. FATP tissue distribution in humans and mice[a,b].

Type of FATP	Lung	Small intestine (enterocytes)	Heart	Liver	Muscle	Pancreas	Brain	Spleen	WAT	Kidney	Refs
FATP1	±[c]	–[d]	++[c]	–[c]	++[d]	ND	+[c]	–[c]	++[d]	+[c]	14,23,24,65
FATP2	–[d]	–[d]	–[d]	++[d]	–[d]	±[d]	–[d]	–[d]	–[c]	++[d]	23,24,29[e]
FATP3	+[c]	–[d]	–[c]	+[c]	–[c]	+[c]	–[c]	–[c]	–[c]	–[c]	23,24[e]
FATP4	±[d]	++[d]	±[d]	+[d]	±[d]	±[f]	+[d]	–[d]	±[c]	+[d]	23–25[e]
FATP5	–[d]	–[d]	–[d]	++[d]	–[d]	ND	–[d]	–[d]	–[d]	–[d]	23,24,27[e]
FATP6	–[d]	–[f]	++[d]	–[d]	–[d]	ND	–[d]	–[d]	–[d]	–[d]	24[e]

[a]Abbreviations: FATP, fatty acid transport protein; ND, experiment not done; WAT, white adipose tissue.
[b]Yellow shading indicates the predominant FATP recorded for each organ. FATP expression (++, high expression; +, expressed; ±, weak expression; –, not expressed) was assessed by a combination of northern blot, western blot and *in situ* hybridization[23,24].
[c]Results from mouse tissue.
[d]Results from mouse and human tissue.
[e]A. Stahl *et al.*, unpublished results.
[f]Results from human tissue.

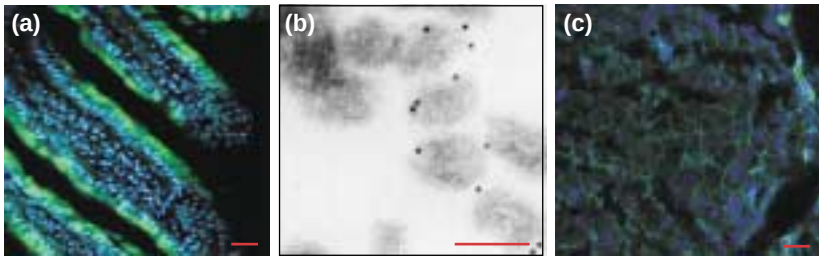

Fig. 1. Subcellular localization of mammalian FATPs. (a) Deconvolution microscopy of a small intestinal thin section stained with anti-FATP4 (green) and DAPI (blue); scale bar = 40 mm. Reproduced, with permission, from Ref. 24. (b) Immunoelectron microscopy of fresh-frozen murine intestinal cells. Fresh-frozen unfixed microsections of murine ileum were incubated with FATP4-specific antiserum, which was detected by 10 nm gold-conjugated secondary antibodies. A cross-section through the microvilli of the brush border is shown; scale bar = 0.12 mm. Reproduced, with permission, from Ref. 24. (c) Confocal microscopy of a mouse liver thin section stained with anti-FATP2 (green) and DAPI (blue); scale bar = 20 mm. Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole; FATP, fatty acid transport protein.

are ligands for PPARs (Ref. 41), it is possible that a positive feedback loop regulates the expression of FATPs, allowing cells to import LCFAs as long as they are present in the circulation. Surprisingly, the adipogenic hormone insulin was reported to increase the expression of *Lacs* in murine adipocytes[42], but to downregulate the expression of *FATP1* in the same cells[43]. *FATP1* has also been reported to be downregulated in the adipose tissue of *ob/ob* mice, which have increased adipogenesis[44]. Other negative regulators of FATPs include endotoxin, tumor necrosis factor α and interleukin 1, which can mediate a dramatic reduction of hamster liver *FATP1* expression[45]. One caveat is that these studies are based on the analyses of mRNA levels and many were performed with probes that have the potential to crossreact with several FATP family members. In addition, so far only the regulation of *FATP1* has been examined, and the extent to which other FATPs are regulated in these paradigms examined is unclear. Additional work is needed at both the RNA and the protein level to understand the regulation of FATP expression.

FATP subcellular localization

FATPs are not cytosolic fatty acid transporter proteins but rather integral membrane proteins and resistant to alkaline extraction[14]. The exact membrane topology is unclear, but it has been speculated that FATPs have between one and six transmembrane domains[30].

The subcellular localization of FATP1 in 3T3L1 adipocytes revealed that the protein is found on the plasma membrane and in small vesicles distributed throughout the cytoplasm[14]. Similarly, we found FATP4 to be localized primarily to the apical plasma membrane of mouse enterocytes, with small amounts present in vesicles close to the apical plasma membrane[24]. The rat *FATP2* gene was originally cloned after purification of the protein from a peroxisomal fraction[46], and more recently was reported to be localized to the ER (Ref. 29). However, we found that FATP2 is clearly localized to specific domains of the plasma membrane of monkey hepatocytes (A. Stahl *et al.*, unpublished). Although overexpression of epitope-tagged human *FATP2* and *FATP5* resulted in an accumulation of protein in the ER (Refs 29,32), this is probably a result of massive protein overproduction because the endogenous proteins in the liver are localized almost exclusively to the hepatic plasma membrane (Fig. 1c). FATP2 (Fig. 1c), FATP3 (A. Stahl *et al.*, unpublished), FATP4 (Ref. 24; Figs 1a and 1b), FATP5 (A. Stahl *et al.*, unpublished) and FATP6 (A. Stahl *et al.*, unpublished), are all targeted to specific domains on the murine plasma membrane, making it unlikely that they contribute to LCFA metabolism in peroxisomes or the ER.

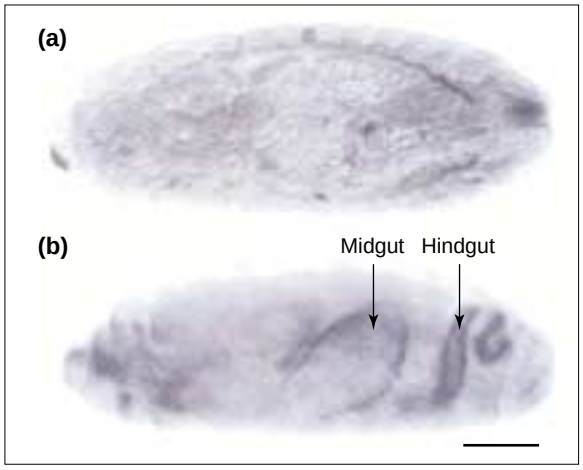

Fig. 2. Subcellular localization of dmFATP. Staining of a *Drosophila* embryo by *in situ* hybridization with (a) a sense control or (b) a dmFATP-specific probe; scale bar = 0.15 mm. Abbreviation: dmFATP, *Drosophila melanogaster* fatty acid transport protein.

FATP gain/loss of function

Given the high degree of homology between the different FATP members, it is not surprising that all of them enhance the uptake of LCFA when transiently or stably expressed in cells[23,24]. In addition, FATP members, even from evolutionarily quite different species, can functionally replace each other – the gene encoding a nematode FATP will enhance LCFA uptake when expressed in mammalian cells[23] and a gene encoding murine FATP1 can restore the phenotypic and biochemical deficiencies of yeast lacking the endogenous fatty acid transporter[47]. Although FATP-knockout and transgenic mice are still under construction, it has been shown that reduction of FATP4 protein levels translates directly into an inhibition of LCFA uptake in primary intestinal epithelial cells[24]. In addition, it is interesting to note that a single P-element insertion disrupting the transcription of a *Drosophila Fatp* results in lethality [Berkeley Fly Database, line L(2)k10307]. Curiously, the *Drosophila Fatp-a* gene* is expressed in the mid- and hindgut of the embryo (Fig. 2), reminiscent of the mammalian *FATP4*. No mutations in human FATP genes have been reported so far. However, an intronic polymorphism in the human *FATP1* gene has been linked recently with increased plasma triglyceride levels[48].

Catalytic activity and mechanism of transport

The mechanisms and requirements for LCFA uptake through FATPs are poorly understood. FATPs do not show any obvious similarities to other transporter

families and, so far, the evidence for them being *bona fide* transporters remains circumstantial, namely: increased fatty acid transport upon overexpression of the gene, decreased fatty acid transport upon reduction or deletion in yeast or primary cells and plasma membrane localization as expected from a transporter.

However, one important clue can be derived from analysis of the primary amino acid sequence. An AMP-binding sequence (Prosite PDOC00427: [LIVMFY]-x (2)-[STG]-[STAG]-G-[ST]-[STEI]-[SG]-x- [PASLIVM]-[KR]) is found in all known FATPs located at the beginning of the 300 amino acid-long FATP signature sequence[23]. This sequence is also present in several other prokaryotic and eukaryotic proteins with a variety of different functions and subcellular localizations. It has been suggested that many of the reactions catalyzed by enzymes with an AMP-binding domain involve ATP-dependent covalent binding of AMP to their substrates. An incomplete list, reflecting the diversity of the more than 128 proteins in the Swiss–Prot database containing one or several AMP-binding domains, is shown in Table 2.

An alignment based on full-length sequences from bacterial, invertebrate and human FATPs with nine other AMP-binding domain-containing proteins of similar size (Fig. 3; Table 3) does not suggest that FATPs are homologous to any particular group of enzymes, including fatty acyl-CoA synthetases.

Table 2. AMP-binding domain-containing proteins[a]

Protein	Number of AMP-binding domains	Organism
Fatty acid transport protein	1	*Homo sapiens*
Bubblegum/Lipidosin	1	*H. sapiens*
4-Coumarate-Coenzyme A ligase 1	1	*Arabidopsis thaliana*
Acetyl-Coenzyme A synthetase 1	1	*Saccharomyces cerevisiae*
NADH-dependent butanol dehydrogenase A	1	*Clostridium acetobutylicum*
Medium-chain fatty-acid-Coenzyme A ligase	1	*Pseudomonas oleovorans*
ANGR protein	1	*Vibrio anguillarum*
Bile acid-Coenzyme A ligase	1	*Eubacterium* spp.
Transcription factor BTF3 homolog 3	1	*H. sapiens*
Crotonobetaine/Carnitine-Coenzyme A ligase	1	*Escherichia coli*
2,3-Dihydroxybenzoate-AMP ligase	1	*Bacillus subtilis*
Ser-activating enzyme	1	*B. subtilis*
D-alanine-activating enzyme	1	*B. subtilis*
Long-chain fatty acid transport protein	1	*S. cerevisiae*
Peroxisomal-Coenzyme A synthetase	1	*S. cerevisiae*
Gramicidin S synthetase I	1	*B. brevis*
Homeobox protein cut	1	*Drosophila melanogaster*
High-molecular-weight protein 2	1	*Yersinia enterocolitica*
Interleukin 1β convertase	1	*H. sapiens*
Keratin, type II cytoskeletal 8	1	*H. sapiens*
Long-chain fatty acid-Coenzyme A ligase 1	1	*S. cerevisiae*
Aminoadipate-semialdehyde dehydrogenase large subunit	1	*Candida albicans*
Chemoreceptor mcpa	1	*Caulobacter crescentus*
O-succinylbenzoic acid–Coenzyme A ligase	1	*B. subtilis*
Nucleolar protein NOP56	1	*H. sapiens*
Pectinase gene transcriptional regulator	1	*Erwinia chrysanthemi*
Surfactin synthetase subunit 3	1	*B. subtilis*
Hc-toxin synthetase	3	*Cochliobolus carbonum*
Surfactin synthetase subunit 1	3	*B. subtilis*
δ-(l-α-aminoadipyl)-L-cysteinyl-D-Val synthetase	3	*Cephalosporium acremonium*
Gramicidin S synthetase II	4	*B. brevis*
Bacitracin synthetase 1	5	*B. licheniformis*
Tyrocidine synthetase III	6	*B. brevis*
Cyclosporin synthetase	10	*Tolypocladium inflatum*

[a]The Swiss Prot database was searched for proteins containing the AMP-binding domain consensus sequence (Prosite PDOC00427): [LIVMFY]-x (2)-[STG]-[STAG]-G- [ST]-[STEI]-[SG]-x- [PASLIVM]-[KR] using the ExPaSy web-based tool ScanProcit-Pattern against SWISS PROT (http://ca.expasy.org/tools/scnpsit2.html). A subset of the search results, reflecting the diversity of proteins with this sequence motive, is shown together with the number of AMP-binding domains and the organism name.

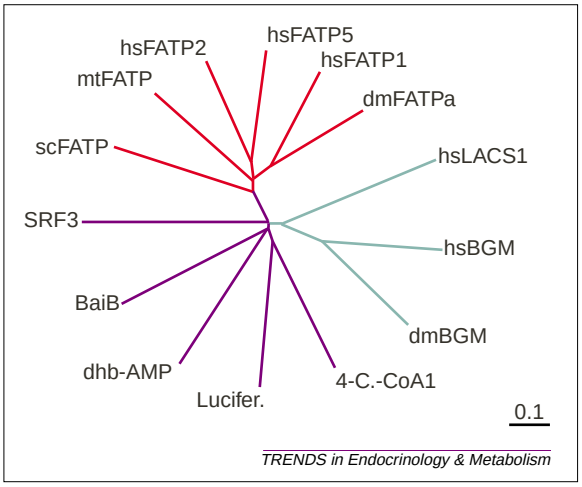

Fig. 3. Alignment of AMP-binding domain containing proteins with the fatty acid transport protein (FATP) family. Full-length sequences of AMP-binding domain-containing proteins were aligned using ClustalW. Based on the alignment data, a radial tree was drawn using TreeViewPPC. The bar indicates the number of substitutions per residue (i.e. 0.1 corresponds to ten substitutions per 100 residues). Abbreviations: 4-C.-CoA, 4-coumarate-CoA ligase; AMP, adenosine monophosphate; BaiB, bile-CoA ligase; BGM, bubblegum protein; dhb-AMP, 2,3-dihydrosybenzoate-AMP ligase; dm, *Drosophila melanogaster*; FATP, fatty acid transport protein; hs, *Homo sapiens*; LACS, long-chain acyl-CoA synthetase; Lucifer., luciferin 4-monooxygenase; mt, *Myobacterium tuberculosis*; pep., peptide; sc, *Saccharomyces cerevisiae*; SRF, surfactin synthetase subunit. Accession numbers for AMP-binding proteins: 4-C.-CoA1, AAG50881; BaiB, P19409; SRF3, Q08787; dhb-AMP lig., AAB40794; dmBGM, AAF53368; hsBGM, NP055977; hsLACS1, P4125; Lucifer., S62787.

Instead, all FATPs, in spite of the considerable species differences between mycobacteria and humans, form a distinct subgroup (between 23% and 45% amino acid identity; Fig. 3; Table 3) that is equally distantly related to most other members of the group (between 6% and 17% identity; Fig. 3; Table 3). However, it seems clear that the AMP-binding motif is important for FATP-mediated fatty acid transport because mutations of Ser250 and Thr252 within the AMP-binding motif in murine FATP1 abolish transport activity and impair the binding of 8-azido-ATP to the protein[31,49].

It has been suggested that FATP1, -2, -5 and the yeast fat1 have one or several of the enzymatic activities demonstrated for other AMP-binding proteins. Rat FATP2 was originally purified from liver peroxisomes as a protein associated with VLACS activity[46]. The full-length human cDNA was cloned subsequently and named human *VLACS* because transient transfection of this gene into COS cells increased acyl-CoA synthetase activity towards VLCFAS fivefold, LCFAs twofold and branched-chain fatty acids 2.7- to fivefold[29]. Coe *et al.*[50] showed that a Myc–His-tagged murine FATP1 also increased VLACS, but not LACS, activity when the gene was transiently overexpressed in COS cells or partially purified by chromatography on nickel-chelate resin. This activity was abrogated by a 6-amino acid substitution in the AMP-binding motif (amino acids 249–254) and by a 59 amino acid deletion in a highly conserved part of the FATP signature sequence (amino acids 464–523). In support of these data, yeast strains with a disrupted *fat1* gene showed decreased VLACS activity and elevated intracellular VLCFA levels[51,52].

The notion that the protein expressed by *fat1* acts solely as a VLACS rather than as a FATP was subsequently challenged by DiRusso *et al.*[26,47], who showed that the *fat1* disruptants grown in the presence of oleate showed no depression in VLACS activity (cells grown on YNBD showed a reduced but not abolished VLACS activity), but rather demonstrated a diminished uptake and β-oxidation of LCFAs, both of which could be

Table 3. Percentage identity (below the diagonal) and percentage homology (above the diagonal) among the AMP-binding domain-containing proteins and a hypothetical peptide of 650 amino acids with an average amino acid composition[a,b]

	dmFATPa	hsFATP1	hsFATP5	hsFATP2	mtFATP	scFATP	Lucifer.	4-C.-CoA1	dhb-AMP	BaiB	SRF3	hsLACS1	dmBGM	hsBGM	Peptide
dmFATPa		56.8	43.7	46.0	41.9	37.1	25.5	28.3	24.4	21.2	14.0	23.2	20.4	21.9	17.8
hsFATP1	45.1		46.1	47.5	44.2	36.0	25.6	28.3	23.5	23.7	12.4	20.9	22.0	22.4	15.0
hsFATP5	30.8	35.5		57.2	41.2	35.1	23.9	26.2	23.4	21.6	15.2	23.2	21.5	22.9	13.6
hsFATP2	33.2	35.5	40.6		45.6	39.3	27.2	29.5	27.3	25.0	13.1	24.9	25.0	23.0	15.2
mtFATP	31.4	32.4	29.3	34.0		34.0	26.0	27.8	27.1	22.8	12.4	22.5	21.7	20.9	14.5
scFATP	24.7	25.2	23.3	26.4	23.0		26.0	25.9	21.9	21.9	11.9	22.6	20.9	19.4	14.0
Lucifer.	12.8	15.3	14.3	15.2	14.1	15.4		42.6	30.8	28.4	12.2	23.3	25.2	19.8	14.5
4-C.-CoA1	16.4	15.9	13.3	15.8	16.7	13.7	27.9		32.6	30.0	13.1	25.9	27.1	23.2	16.3
dhb-AMP	14.9	14.2	13.5	13.4	16.1	11.0	18.6	19.8		28.5	12.8	19.2	23.1	17.3	14.2
BaiB	13.2	15.1	12.8	13.2	13.1	14.2	17.4	17.1	18.1		10.7	16.8	22.3	18.6	13.0
SRF3	8.2	7.4	8.1	7.4	6.6	6.2	6.2	7.1	8.6	5.8		11.2	11.4	11.8	8.4
hsLACS1	11.9	12.0	12.6	13.4	12.6	10.1	11.7	15.9	11.6	7.5	6.6		29.8	28.1	15.0
dmBGM	10.4	9.7	11.4	11.8	11.3	10.2	14.5	14.8	11.5	10.5	5.5	16.7		51.6	16.1
hsBGM	11.2	11.4	12.5	11.5	10.9	9.8	11.5	13.2	8.6	9.3	5.8	16.0	36.3		13.1
Peptide	7.9	6.1	5.4	6.2	5.6	4.6	5.2	7.3	5.8	6.1	4.0	6.3	5.8	5.0	

[a]Alignment of AMP-binding domain containing proteins with the fatty acid transport protein (FATP) family. Full-length sequences of AMP-binding domain-containing proteins were aligned using ClustalW. Compiled with the use of MacBoxshade. Relations among the FATP family are highlighted in yellow and among BGM/LACS in green. Accession numbers for AMP-binding proteins: 4-C.-CoA1, AAG50881; BaiB, P19409; SRF3, Q08787; dhb-AMP, AAB40794; dmBGM, AAF53368; hsBGM, NP055977; hsLACS1, P4125; Lucifer., S62787.

[b]Abbreviations: 4-C.-CoA, 4-coumarate-CoA ligase; AMP, adenosine monophosphate; BaiB, bile-CoA ligase; BGM, bubblegum protein; dhb-AMP, 2,3-dihydrosybenzoate-AMP ligase; dm, *Drosophila melanogaster*; FATP, fatty acid transport protein; hs, *Homo sapiens*; LACS, long-chain acyl-CoA synthetase; Lucifer., luciferin 4-monooxygenase; mt, *Myobacterium tuberculosis*; sc, *Saccharomyces cerevisiae*; SRF, surfactin synthetase subunit.

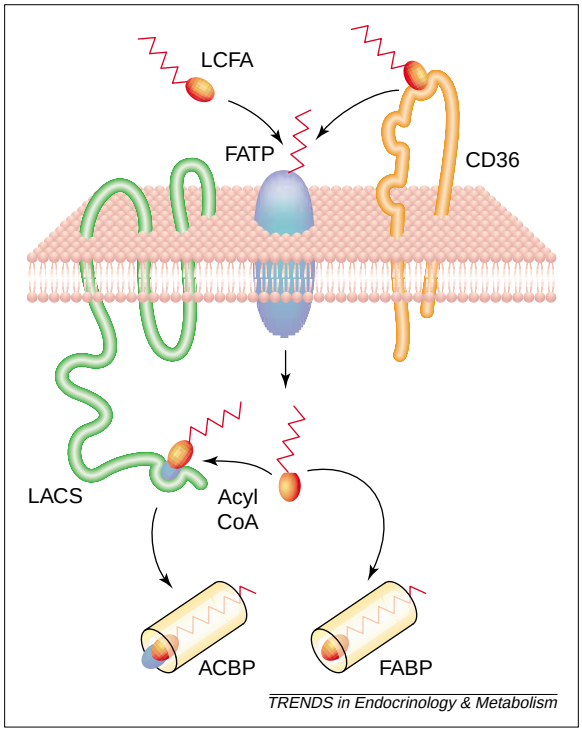

Fig. 4. A model for cellular fatty acid uptake. FATPs, CD36, LACS and FABPs could cooperate to facilitate efficient LCFA uptake. Extracellular LCFAs might bind directly to FATPs and be transported into cells. Alternatively, LCFAs could first bind to CD36 and be passed on to FATP. Intracellular LCFAs would then be coupled to CoA by LACS, preventing their efflux, with FABPs and ACBPs acting as a cytoplasmic buffer for incorporated LCFAs and their CoA esters. The depicted membrane topology and oligomerization states of certain proteins are purely schematic. Abbreviations: ACBP, acyl-CoA-binding protein; FABP, fatty acid binding protein; FATP, fatty acid transport protein; LACS, long-chain acyl-CoA synthetase (ligase); LCFA, long-chain fatty acid.

restored by expression of murine *FATP*. To explain both transport and VLACS activity of FATP1, it was suggested that the transport process could be coupled with acyl-CoA production[50]. However, murine *FATP1* overexpression clearly increases uptake of LCFAs such as oleate and palmitate[14], whereas no significant acyl-CoA activity towards these fatty acids was found in cells overexpressing murine *FATP1* (Ref. 50). Furthermore, increased intracellular fatty acid concentrations can upregulate the expression of LACS and VLACS (Refs 53–56). *FATP* expression could therefore lead indirectly, via elevation of intracellular fatty acid levels, to the observed increase in VLACS activity. This effect would be abolished by mutations that affect fatty acid transport by FATP, such as the Ser250A and Thr252A substitutions within the AMP-binding motif in murine FATP1(Refs 31,49). In bacteria, fatty acid uptake and activation to acyl-CoA by *fadL* and *fadD*, respectively, are closely coupled[57], and it seems probable that a similarly linked system is present in eukaryotes to prevent the efflux of LCFAs after uptake. If this hypothesis is true, one would expect that LACS and VLACS enzymes would copurify in crude preparations with FATP.

Recently, proteins other than FATPs with VLACS activity have been identified. An AMP-binding protein in *Drosophila*, termed bubblegum, has VLACS activity and its gene is similar in sequence to *LACS* (41% identity to the fly *Lacs* gene) but not to any FATP (9–13% identity with three *Drosophila Fatps*)[58]. The gene encoding a human bubblegum homolog has been cloned and the protein it synthesizes also demonstrates VLACS activity[59,60].

Another enzymatic activity was reported for human FATP5 (termed VLACS homolog 2)[32]. Steinberg *et al.*[61] concluded that human FATP5 is a cholate-CoA ligase after observing that transient expression of *FATP5* in COS cells resulted in a twofold increase in VLACS activity but a 200-fold increase in cholate-CoA ligase activity. By contrast, overexpression of human *FATP2* resulted only in elevated VLACS activity[61]. A sequence comparison between human FATP5 and a known bacterial bile-CoA ligase (another protein with an AMP-binding domain) shows only 13% identity between the two proteins (Fig. 3; Table 3), which is comparable to the sequence relatedness of human FATP5 to firefly luciferase, but much lower than the 30% identity between human FATP5 and the mycobacterial FATP. How and if the observed cholate-CoA ligase activity is related to the robust enhancement of LCFA uptake observed after *FATP5* overexpression[23] is unclear. Furthermore, we routinely do LCFA uptake assays in the presence of a 100-fold excess of the bile acid taurocholate, which should compete with LCFA uptake and/or CoA activation if FATP5 is indeed a cholate-CoA ligase. However, FATP5-mediated LCFA transport is unaffected by taurocholate.

Clearly, more research is needed to determine which (if any) enzymatic activities are associated with FATPs and which proteins are responsible for VLACS activity in mammalian cells.

A model for LCFA uptake

Although FATP overexpression alone leads to an increase in LCFA uptake, it is likely that *in vivo* several proteins interact to facilitate efficient uptake of fatty acids, and that the combination of FATPs and other proteins will vary from organ to organ. In fact, two of the proteins implicated in LCFA uptake, LACS and FABPs, have tissue-specific isoforms[62] and could form organ-specific complexes with FATPs. Figure 4 illustrates two models for a LCFA-uptake protein complex. LCFAs generated by lipases, either from lipid droplets in the intestine or from lipoproteins in the circulation, could be directly transported by FATPs across the plasma membrane. Alternatively, LCFAs might first bind to CD36, which would transfer them to FATPs. This last scenario could be especially important under conditions of low fatty acid to albumin ratios, in which CD36 has been shown to be more effective in facilitating LCFA transport[21], probably by accumulating LCFAs on the plasma membrane. After uptake, rapid esterification of LCFAs by LACS would prevent efflux whereas rapid binding of LCFAs and acyl-CoA by FABPs and acyl-CoA-binding proteins would help the unloading of FATPs and LACS and act as an intracellular LCFA buffer[63]. In accordance with this hypothesis, LACS have been shown to be membrane-bound proteins and to colocalize with FATP1 on the adipocyte plasma membranes[64]. Although this model is in accordance with our current understanding of LCFA uptake into cells, it is purely hypothetical. Clearly, much work remains to be done to determine the molecular mechanisms of this important physiological process.

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In vivo assessment of the effects of estrogen on human brain

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There is increasing evidence from animal and *in vitro* studies to suggest that estrogen might have neuroprotective effects, and several plausible physiological mechanisms have been proposed. However, it is not yet fully understood how estrogen affects the human brain. There are several techniques that are currently employed for *in vivo* assessment of brain structure and function in humans, including neuropsychological and neuroendocrine testing, computerized tomography, structural and functional magnetic resonance imaging, magnetic resonance spectroscopy, single photon emission spectroscopy and positron emission tomography. Results from studies investigating the effects of estrogen on the female brain using the above techniques are reviewed here. The current data from humans suggest that the use of estrogen hormone-replacement therapy (HRT) in healthy, postmenopausal women might reduce the risk of developing Alzheimer's disease (AD) and preserve certain aspects of cognitive function. The use of HRT in postmenopausal women might also modulate neurotransmitter function and can increase cerebral blood flow in a regionally specific and task-dependent manner. In addition, the neuroprotective effects of HRT might depend on the length of its use. However, there is very little evidence at present that HRT is an effective treatment for established AD.

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Animal and *in vitro* studies have shown that estrogen has numerous effects on the central nervous system (CNS), including increasing spine density, enhancing expression of neuronal growth factor, regulating several neurotransmitter systems, influencing gene transcription via intracellular receptors, exerting antioxidant effects, hyperpolarizing neuronal membranes, and reducing neuronal generation of β amyloid [which is present in plaques in Alzheimer's disease (AD)][1–4]. In addition, there is increasing evidence from epidemiological studies that estrogen might have protective effects on human brain. The use of hormone-replacement therapy (HRT) in healthy postmenopausal women reduces the risk of developing, and delays the onset of AD, and this effect might depend on the duration of HRT use[5]. Estrogens might also decrease vulnerability to depression and serve as an adjunct therapy to conventional antidepressants[6]. In addition, in women suffering from psychosis, estrogens can modulate illness severity and affect side-effect profiles of antipsychotic medication[7]. However, the exact underlying mechanism for the neuroprotective effect of estrogens in humans is not completely understood. Recently, more *in vivo* assessment techniques for the investigation of brain structure and function have become available. We review the current literature investigating the effects of estrogen on human brain structure and function.

Assessment of cognitive function

Neuropsychological tests indirectly measure brain function by assessing cognitive ability (e.g. memory). Gender differences in cognitive ability have been well described: women perform better than men do on verbal and memory tasks, whereas men tend to excel at spatial tasks[8,9]. In addition, in women endogenous estrogen can affect cognition during the menstrual cycle. Some women perform better on spatial and abstract reasoning tasks during menstruation (when estrogen levels are low) and better on verbal, articulatory and fine motor tasks during midcycle (when estrogen levels are high)[10].

Aging is associated with deficits in verbal and visual memory, attention, concentration and visuospatial function, and consequently many studies have investigated the effect of HRT on age-related cognitive decline in postmenopausal women. Overall, the results suggest that HRT can improve some aspects of cognitive function in healthy postmenopausal women, including (verbal) memory, attention, reaction-time speed and abstract reasoning. However, not all researchers support this view, and differences in methodology (such as cross-sectional versus longitudinal design, tests employed, age of

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