Fatty acid transport proteins

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Acronym
FATP

Synonyms
Fatty acid transport protein isotype 1-6; FATP1-6; gene symbols SLC27A1-6; solute carrier family 27A; very long-chain acyl-CoA synthetase; VLCS

Definition
Fatty acid transport proteins (FATPs) are an evolutionary conserved family of integral membrane proteins found at the plasma membrane and on internal membranes. FATPs facilitate the unidirectional uptake and/or intracellular activation of unesterified long-chain and very long-chain fatty acids into a variety of lipid-metabolizing cells and tissues.

Basic Mechanisms
Introduction
Long-chain fatty acids (LCFAs) are vital components of our diet and contribute to cellular processes including metabolic energy generation and storage, plasma membrane synthesis, and protein anchoring. While in some tissues and cell types LCFAs can signal through membrane receptors, they typically have to first cross the plasma membrane to elicit cellular responses. In general, the uptake of fatty acids from the circulation into cells includes the sequence of *1)* localized generation of free fatty acids through hydrolysis of triglycerides (TGs) from lipoproteins by lipases inside the endothelial lumen and rapid binding of fatty acids to albumin, *2)* fatty acid dissociation from albumin followed by passive or active transport across the plasma membrane, and *3)* their association with intracellular binding proteins and subsequent participation in metabolic and signaling processes (Figure 1).

Uptake of LCFAs across the lipid-bilayer of most mammalian cells occurs through both a passive diffusion of LCFAs and a protein-mediated LCFA uptake mechanism. At physiological LCFA concentrations (7.5 nM) the protein-mediated, saturable, substrate-specific, and hormonally-regulated mechanism of fatty acids accounts for the majority (>90%) of fatty acid uptake by tissues with high LCFA metabolism and storage such as skeletal muscle, adipose tissue, liver and heart. The relative contribution of passive diffusion is thought to be higher in cell types that lack fatty acid transporters such as fibroblasts (see Figure 2) and may be elevated as a result of super-physiological fatty acid concentrations. While several fatty acid handling proteins have been identified (see Figure 1), recent *in vivo* studies have particularly highlighted the contribution of FATPs to LCFA uptake, lipid metabolism, and lipid-associated disorders (1).

The Family of Fatty Acid Transport Proteins (FATPs)

In humans and mice, the FATP family is comprised of six structurally related members (FATP1-6; 33-57.1% identity in mice) that are found in all fatty acid-utilizing tissues of the body. While substrate preferences are comparable among the different FATPs paralogues, they differ widely in their tissue expression pattern (2). FATP1 was the first FATP to be identified and is highly expressed in adipose tissue, skeletal muscle and, to a lesser extent, in heart. FATP2 is predominantly expressed in liver and kidney cortex. FATP3 shows a broader expression pattern with high mRNA and protein levels in lung. FATP4 is the only FATP expressed in small intestine and is localized to the apical brush border of the epithelial cells, where it is implicated in the absorption of dietary lipids. FATP4 is also expressed to a lesser extent in other tissues including adipose tissue, liver, skin and heart. Furthermore, FATP4 together with FATP1 are the predominant FATP paralogues in brain. FATP5 is expressed solely in liver. Confocal immunofluorescent microscopy with isolated primary hepatocytes demonstrated that FATP5 is localized to the plasma membrane of these cells, which was confirmed by immunoelectron microscopy of liver sections, showing a predominant localization of FATP5 protein to basal microvilli in the space of Disse. FATP6 is expressed specifically in heart where it is the predominant FATP paralogue. Immunofluorescence microscopy studies of FATP6 in primate and murine hearts have shown that the protein is exclusively located on the sarcolemma, where it is restricted to areas of the plasma membrane juxtaposed to small blood vessels (1). In addition to vertebrates, FATP orthologs have been identified in invertebrate, fungi and prokaryote genomes such as C.*elegans*, S.*cerevisiae*, and M.*tuberculosis*.

FATP structure and mechanism of transport

Due to the high overall sequence similarity, the presumed secondary structure of all FATP members is assumed to be similar, with an extracellular N- and an intracellular C-terminus. Because of the hydrophobic nature of a protein that strongly interacts with fatty acids, the exact topology of the FATPs is difficult to predict. Studies with FATP1 revealed at least one α-helical transmembrane domain and several membrane-associated domains (3). However, FATPs do not show any obvious similarities to other transporter families, e.g. the polytopic membrane transporters for hydrophilic substrates such as members of the → GLUT family, or transporters of amphipatic and hydrophobic substrates, e.g. bile and cholesterol transporters. It has been demonstrated that FATP1 forms homo-dimers, and possibly higher-order complexes, most likely by interactions in the cytoplasmatic loop. Co-expression experiments of non-functional FATP1 mutants with wild-type FATP1 revealed that oligomerization is required for their transport function. A 311-amino acid sequence motif is conserved in all mammalian FATP family members and is essential for the function of these proteins. An AMP-binding sequence (IYTSGTTGXPK), likely facing the cytosol, is found at the beginning of this sequence motif. This 11-amino acid motif is conserved in a number of proteins that either bind ATP or catalyze reactions that proceed through adenylated intermediates.

In vitro and *ex vivo* studies have shown that FATPs transport LCFAs and very long-chain fatty acids (VLCFAs) but no medium-chain fatty acids, fatty acid esters, or lipid-soluble vitamins (4). LCFA transport is inhibited by prior protease treatment. Synthetic substrates for FATPs include ^{14}C-labeled fatty acids and the fluorescently labeled fatty acid analogue C1-BODIPY-C12. Using the latter substrate, differences in

fatty acid uptake kinetics between FATP expressing 3T3 L1 adipocytes and 3T3 L1 fibroblasts, which are devoid of FATPs, can be readily appreciated (Figure 2).

It has been shown that FATP1, -2, -3, -4, and -5 can also catalyze the formation of CoA thioesters of hydrophobic substrates such as VLCFAs and unconjugated bile acids. However, it is still an ongoing debate whether FATPs are (*a*) solely transmembrane transport proteins mediating LCFA uptake, possibly in close association with other proteins such as long-chain acyl-CoA synthetases, or (*b*) are themselves membrane bound long-chain and very long-chain acyl-CoA synthetases that trap LCFAs inside the cell following fatty acid diffusion across the plasma membrane, or (*c*) combine the transport with acyl-CoA synthetase activity for optimal uptake, or (*d*) are multifunctional proteins that mediate LCFA uptake independently of their esterification activities. Clearly, further investigations of the enzymatic and transport activities of FATPs are needed to resolve these important questions. Extrapolating from our current knowledge, it is likely that *in vivo* several fatty acid handling proteins such as fatty acid translocase (FAT/CD36), long-chain acyl-CoA synthetases (ACSLs), fatty acid binding proteins (FABPs), and acyl-CoA binding proteins (ACBPs) interact with FATPs to facilitate efficient uptake of fatty acids (Figure 1). In accordance with this hypothesis, FATP1 has been shown to associate with ACSL1 (5), suggesting that both proteins are essential for FFA uptake. As a model for LCFA uptake we have suggested the following mechanism (3). In addition to a small, diffusional component, LCFAs are either directly transported by FATP complexes across the plasma membrane or, alternatively are first accumulated on the plasma membrane by binding to CD36, which subsequently hands on the fatty acids to FATPs. Within the cells, LCFAs are rapidly activated and metabolized by ACSLs or FATPs. Subsequent binding of fatty acids to intracellular LCFAs handling proteins facilitates the unloading of transporters and synthetases and acts as an intracellular fatty acid buffer.

FATP null mutants and FATP polymorphisms

Knockout (KO) mice have been reported for several FATPs (1). As insulin desensitization has been closely linked to excessive fatty acid uptake and intracellular diacylglycerol and TG accumulation, these animal models were particularly evaluated in the context of protection from diet-induced → type 2 diabetes. In addition, studies on human subjects have also established genetic links between polymorphisms in FATP genes and metabolic alterations (1).

In hyperinsulinemic-hyperglycemic clamp studies, FATP1 KO mice were protected from the insulin-desensitizing effects of lipid injections. FATP1 KO mice were completely resistant to long-term diet-induced obesity, insulin-desensitization, and other parameters of the metabolic syndrome. Loss of FATP1 function reduced muscle TG content and prevented a lipid bolus induced reduction of IRS-1 tyrosine phosphorylation and PI-3 kinase association. FATP1 is also expressed on the plasma membrane of brown adipose tissue (BAT) and FATP1 KO mice showed reduced basal fatty acid uptake and displayed smaller lipid droplets in BAT. As a consequence FATP1 KO mice failed to up-regulate fatty acid uptake and to defend their core body temperature following cold exposure suggesting that FATP1 is required for thermogenesis (6).

As yet, no human diseases have been identified as a result of FATP1 mutations. However, genetic polymorphisms in the human FATP1 gene have been linked to dyslipidemia. An A/G exchange at position +48 in intron 8 of the FATP1 gene has been shown to result in increased TG concentrations in female but not in male subjects. In a second study, the

same polymorphism was linked to increased postprandial TG concentrations and smaller low density lipoprotein (LDL) particles. To date, it is still unknown if this polymorphism is associated with altered levels of FATP1 expression and/or function.

A FATP2 KO mouse has been generated and investigated in the context of the neurodegenerative endocrine disorder X-linked adrenoleukodystrophy. However, no association between FATP2 function and X-linked adrenoleukodystrophy was found. FATP2 KO mice exhibited a decreased peroxisomal very long-chain acyl-CoA synthetase activity and decreased peroxisomal VLCFA β-oxidation in liver and kidney. However, no VLCFA accumulation in either of these organs was observed. The consequences of FATP2 loss for hepatic and renal LCFA uptake are presently unknown.

To date, four studies on murine FATP4 deletions have been published. Both the introduction of a premature stop codon in exon 3 due to a spontaneous mutation as well as the deletion of exon 3 by gene manipulation resulted in an early neonatal lethality due to symptoms strikingly similar to restrictive dermopathy, a rare human genetic disorder. Neonate mice exhibited thickened, tight skin and a disrupted epidermal barrier as well as facial deformations and breathing difficulties. Additional analyses of the genetically engineered mice demonstrated reduced esterification activities for C24:0, but not C16:0 or C18:1 (very) long-chain fatty acids in dermal and intestinal lysates from FATP4 null mice. No effects on LCFA uptake have been examined in these two studies. A third study described a deletion of exons 1 and 2 of the murine FATP4 gene resulting in embryonic lethality occurring before day 9.5 of gestation. This phenotype was ascribed to the absence of FATP4 in the yolk sac where it is normally expressed by the cells of the extra-embryonic endoderm, likely resulting in an impaired absorption of maternal lipids by the embryo during early embryogenesis. Isolated primary enterocytes from heterozygote mice had a 48% reduction in FATP4 expression and a 40% reduction in LCFA uptake. However, no malabsorption of lipids was detected *in vivo*. A fourth report describing an epidermal-specific conditional FATP4 KO mouse showed that these mice develop a hyperkeratosis with a disturbed epidermal barrier suggesting that epidermal FATP4 is essential for the maintenance of a normal skin structure and function. In humans, a polymorphism in exon 3 of the FATP4 gene leads to an amino acid exchange (G209S) that has been linked to decreased parameters for TG and insulin levels, body mass index (BMI), and systolic blood pressure.

FATP5 KO mice have been characterized in two studies focusing on the role of FATP5 in hepatic lipid and bile metabolism. LCFA uptake in primary hepatocytes isolated from FATP5 KO mice was reduced by 50% and hepatic lipid content in the KO mice was significantly reduced despite an increased fatty acid *de novo* biosynthesis. Detailed analysis of the hepatic lipidome of FATP5 KO mice revealed significant quantitative and qualitative alterations among lipid classes. Similarly to FATP1 KO mice, homozygote deletion of FATP5 resulted in resistance to high-fat diet-induced weight gain and insulin-resistance. FATP5 KO mice displayed both decreased caloric intake and increased energy expenditure on this diet. How changes in hepatic lipid metabolism lead to altered feeding behavior is currently unknown. While the total bile pool in FATP5 KO mice was unchanged, a distinct shift from conjugated to unconjugated bile acids occurred as a result of FATP5 deletion. The remaining conjugated bile acids were exclusively derived from *de novo* synthesis, implying a role of FATP5 in the reconjugation of bile acids during enterohepatic recirculation.

Pharmacological Intervention

Insulin

Insulin stimulates FATP1-mediated fatty acid transport on the protein level but negatively regulates FATP1 mRNA transcription through a *cis*-acting insulin response promoter sequence, albeit no changes in FATP1 protein levels have been reported following insulin exposure. In basal adipose and muscle cells, most of the FATP1 protein is sequestered in an intracellular perinucelar compartment, where it co-localizes with the insulin-sensitive glucose transporter GLUT4 (→ Glucose Transporters). Insulin stimulation induces the translocation of FATP1-containing vesicles to the plasma membrane resulting in an increase in cellular LCFA uptake.

Phloretin

Phloretin inhibits FATP-mediated traversing of fatty acids across lipid bilayers. Phloretin is the aglycon of phlorizin and has been used to terminate the uptake of LCFAs and VLCFAs in timed *in vitro* uptake assays with cultured cells or in *ex vivo* uptake assays with isolated primary cells.

Thiazolidinedions

A peroxisome proliferator-activated receptor (PPAR) binding site was identified in the murine FATP1 promoter. Several reports have shown a positive regulation of mouse FATPs by ligands that activate PPAR-α, PPAR-γ, or PPAR-γ/RXR hetero-dimers.

TNFα

Tumor necrosis factor alpha (TNFα) is a negative regulator of FATP expression and down-regulates FATP mRNA and protein levels in several tissues.

Aknowledgments

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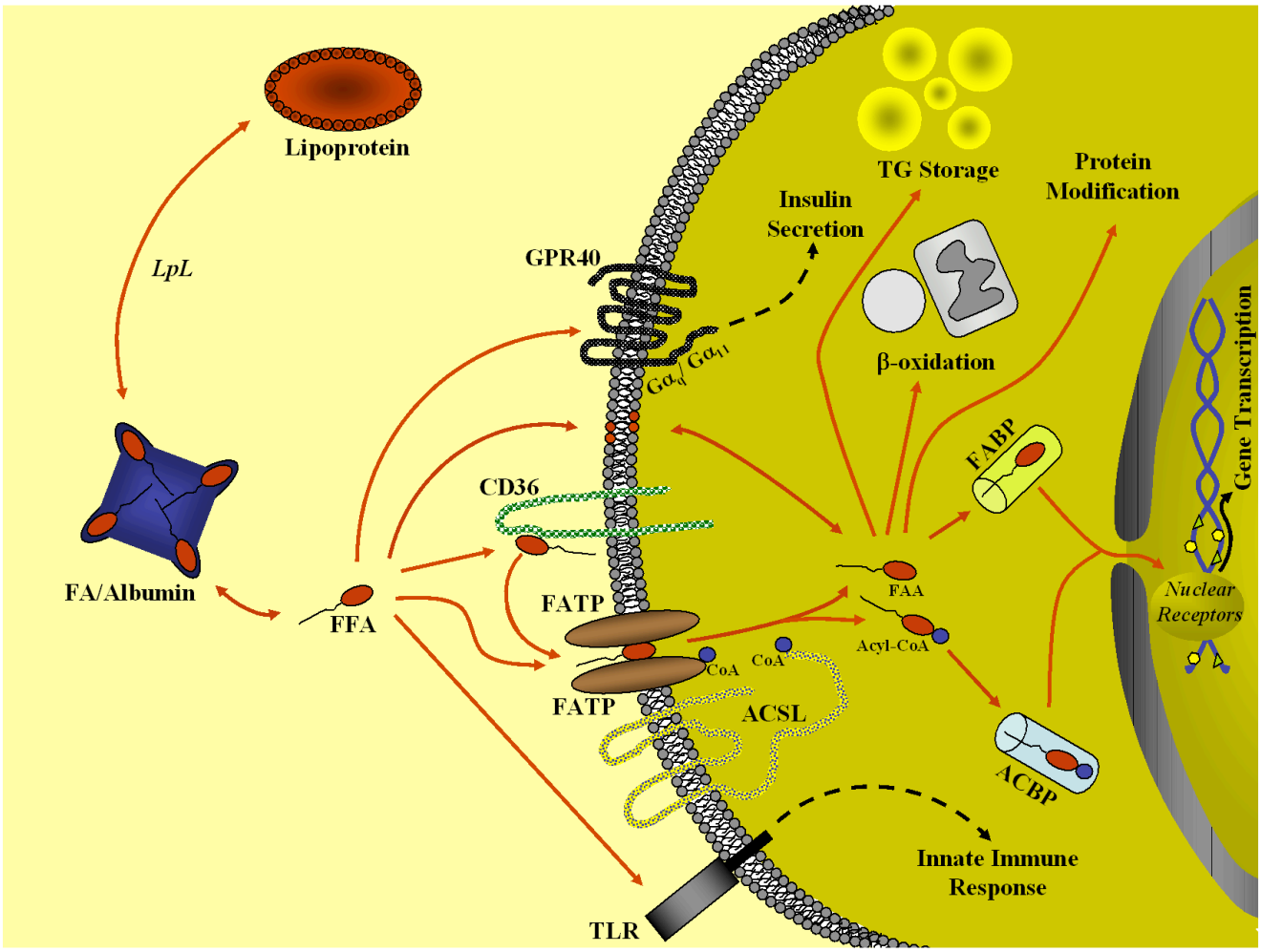
Figure Legends

Figure 1. Free fatty acid uptake and action in mammalian cells.
Serum free fatty acids (FFA) are generated from lipoproteins by the action of endothelial lipoprotein lipase (*LpL*). At physiological conditions, the majority of FFAs is bound to albumin while the concentration of unbound FFAs in this equilibrium is low. Plasma membrane traversing of FFAs into the cell under these conditions occurs mainly by a protein-mediated mechanism, either by interaction of the FFAs directly with FATP complexes or by a preceding binding to cell-surface proteins, such as CD36, which subsequently hands the FFAs on to the FATPs. On the cytosolic site, FFAs are quickly activated and coupled to coenzyme A (CoA) by the catalysis of long-chain fatty acyl-CoA synthetases (ACSLs) or the by FATPs itself. Fatty acid binding proteins (FABPs) or acyl-CoA binding proteins (ACBPs) facilitate an intracellular unloading of the transporters and the synthetases and can also function as an intracellular fatty acid buffer. In the cell, FFAs can act at different subcellular localizations and have functions in energy generation and storage, membrane synthesis, protein modification, and activation of nuclear transcription factors. In addition to acting intracellularly, unbound FFAs can also signal extracellularlay in certain cell types (e.g. by stimulating the G-protein coupled receptor GPR40 in β-cells to induce insulin secretion, or by activating toll-like receptors [TLRs) to initiate the innate immune response).

Figure 2. Quencher-based real-time fatty acid uptake assay with a fluorescently labeled FFA analogue (C1-Bodipy-C12).
Predominantly protein-mediated fatty acid uptake by 3T3-L1 adipocytes (diamonds) was compared to diffusion-driven uptake by fibroblasts (squares) using the QBT Fatty Acid Uptake reagent (Molecular Devices Corp., CA, USA), which contains C1-Bodipy-C12 as substrate in conjunction with a cell impermeable quencher. Uptake kinetics were recorded using a Gemini fluorescence plate reader. Error bars indicate the standard deviations from 12 independent wells. RFU: relative fluorescence units.


Lipoprotein
LpL
FA/Albumin
FFA
GPR40
Gαq / Gα11
CD36
FATP
FATP
CoA
CoA
ACSL
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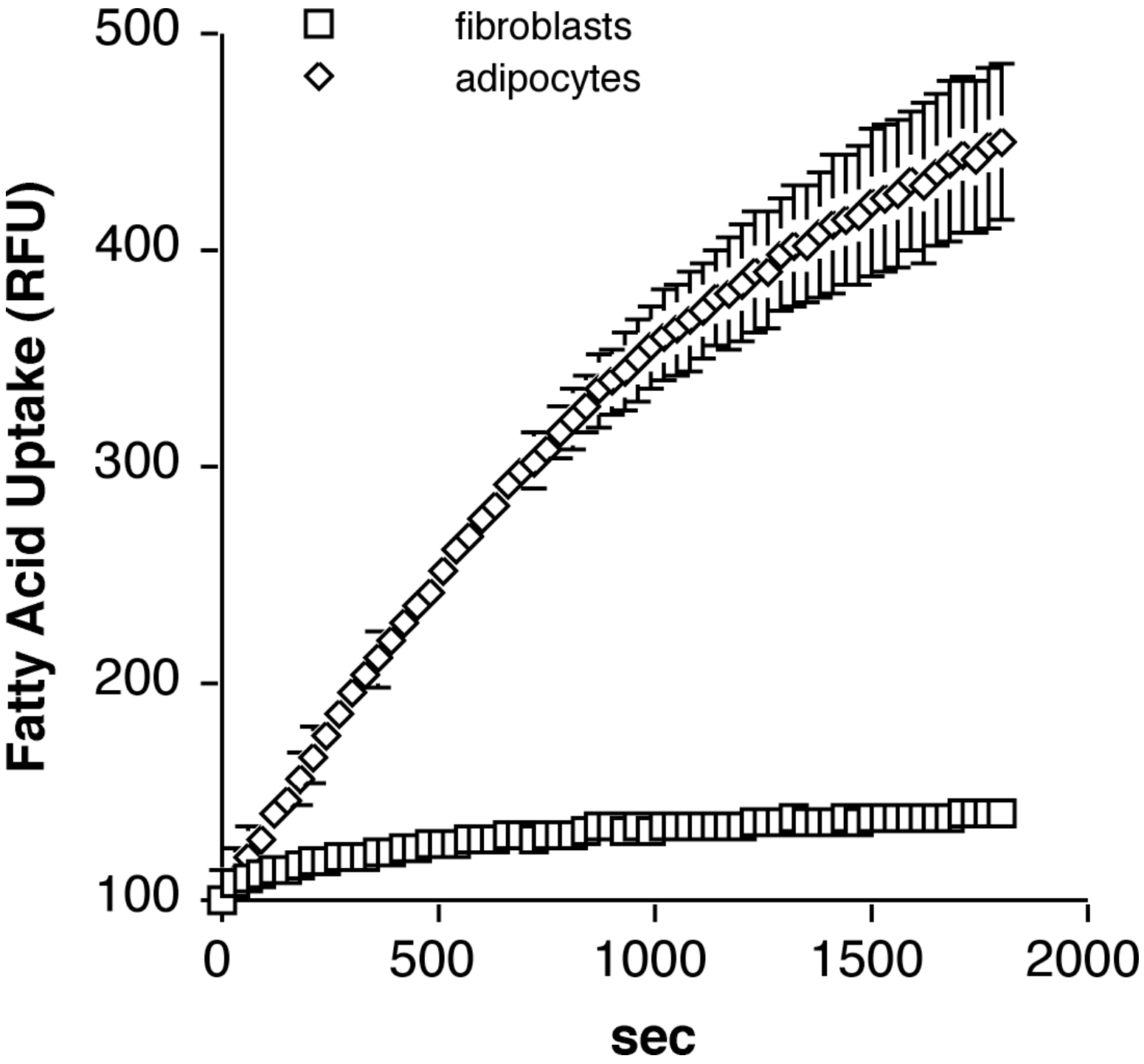
Acyl-CoA
TG Storage
Insulin Secretion
β-oxidation
Protein Modification
FABP
ACBP
Gene Transcription
Nuclear Receptors
Innate Immune Response
TLR


fibroblasts
adipocytes
Fatty Acid Uptake (RFU)
500
400
300
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100
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