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Deactivating Fatty Acids: Acyl-CoA Thioesterase-Mediated Control of Lipid Metabolism

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Abstract

The cellular uptake of free fatty acids (FFA) is followed by esterification to coenzyme A (CoA), generating fatty acyl-CoAs that are substrates for oxidation or incorporation into complex lipids. Acyl-CoA thioesterases constitute a family of enzymes that hydrolyze fatty acyl-CoAs to form FFA and CoA. Although biochemically and biophysically well characterized, the metabolic functions of these enzymes remain incompletely understood. Existing evidence suggests regulatory roles in controlling rates of peroxisomal and mitochondrial fatty acyl-CoA oxidation, as well as in the subcellular trafficking of fatty acids. Emerging data implicate acyl-CoA thioesterases in the pathogenesis of metabolic diseases, suggesting that better understanding their pathobiology could reveal unique targets in the management of obesity, diabetes and non-alcoholic fatty liver disease.

Keywords

Fatty acyl-CoA; free fatty acid; peroxisomes; mitochondria; energy homeostasis

Acyl-CoA thioesterases deactivate fatty acids within cells

Esters of coenzyme A (CoA) constitute initial as well as intermediate substrates in diverse cellular metabolic pathways. Common examples are free fatty acids (FFA), which upon uptake into cells, are activated by esterification to CoA by members of the acyl-CoA synthetase families, forming **fatty acyl-CoA** molecules that can be oxidized for energy production or incorporated into a variety of complex lipids [1, 2]. For less obvious reasons, cells also express enzymes that hydrolyze these esters, generating FFA free plus CoA. Acyl-

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CoA thioesterase activity, first described in the early 1950's, has been reported widely among organisms ranging from archaea to eukaryotes [3–5].

Acyl-CoA thioesterase activity is largely attributable to a family of enzymes that belong to a broader group of thioester hydrolases (EC 3.1.2) [4]. Thioester hydrolases cleave the thioester bond between a sulfur group and a carboxyl group while, most of the acyl-CoA thioesterases act specifically on molecular substrates that contain CoA [6]. The observation that acyl-CoA thioesterase activity in rat livers was induced by peroxisome proliferators led to the identification and characterization of two types of acyl-CoA thioesterases. Type I acyl-CoA thioesterases were characterized by molecular masses of ~40 kDa and their strong response to peroxisome proliferator treatment, whereas molecular masses of the type II acyl-CoA thioesterase were ~110–150 kDa, with variable responses to peroxisome proliferators [7]. Apart from their similar catalytic activities, types I and II acyl-CoA thioesterases share neither sequence homology nor structural features, so have evolved by convergent evolution and are considered to be analogues rather than homologues [8].

In 2005, a revised nomenclature designated these acyl-CoA thioesterases as ACOTs [9]. At present this group includes 15 genes in mouse (type I *Acots 1–6* and type II *Acots 7–15*) and 12 genes in human (type I *ACOTs 1, 2 and 4* and type II *ACOTs 7–15*) [8]. ACOTs are also categorized within the thioesterase hydrolase family in the Thioester-active enZYme (ThYme) database, which covers a broad array of species [6]. Notwithstanding the detailed biochemical characterizations of several ACOT family members, these enzymes are only beginning to be appreciated as key metabolic regulators in health and disease.

Type I ACOTs

Characteristics

Type I ACOTs belong to the α/β -hydrolase protein family, which also includes many lipases and esterases. These ACOT genes localize to narrow gene clusters in higher mammals and exhibit high sequence homology to each other and among species, suggestive of evolution by gene duplication from a common ancestor [10]. *Acots 1–6* reside on mouse chromosome 12 D3, whereas in humans a cluster on chromosome 14q24.3 contains only 4 genes (*ACOTs 1, 2, 4 and 6*) of which 3 (*ACOTs 1, 2 and 4*) are transcribed and then translated to their respective full-length proteins [10–13]. The fourth gene (*ACOT6*) is translated from a downstream methionine in exon 2, generating a markedly smaller protein. Although ACOT6 contains a catalytic site, it is still unknown whether it possesses enzymatic activity [11]. Type I ACOTs are encoded by 3 exons and the crystal structure of ACOT2 reveals two major domains, an N-terminal β -sandwich domain and a C-terminal hydrolase domain, with the active site located in a hollow space in between the two domains [10, 11, 14, 15]. Type I ACOTs, like the other α/β -hydrolases, utilize a catalytic triad of a serine (located in a so-called nucleophilic elbow and acting as a nucleophile), a histidine and an aspartic acid [16]. Although the amino acid sequence nearby to the catalytic triad is highly conserved, lower sequence similarities in other parts of the C-terminal coding regions may contribute to the differences in substrate specificity and different targeting signals that mediate subcellular localization.

The K_m values for type I ACOTs are mostly in the low μM range for preferred substrates, with $V_{\max}$ values in the low $\mu\text{mol}/\text{min}/\text{mg}$ range. Even at high concentrations, free CoA does not inhibit their activities, suggesting that transcriptional regulation might be more important compared to regulation by end products [16]. Increased expression in response to PPAR α activation suggests that the type 1 ACOTs may play important roles in fatty acid oxidation and energy homeostasis [17].

Metabolic functions

ACOT1 is a cytosolic enzyme with substrate selectivity for long chain (C12–C20) saturated- and monounsaturated acyl-CoAs. It is expressed mainly in liver, kidney, lung and heart in mouse [11, 16]. ACOT1 appears to modulate the cytosolic pool of long chain acyl-CoAs and FFA, potentially controlling ligand availability for the nuclear hormone receptors PPAR α and HNF4 α [17]. In mouse models of diabetes and sepsis-related cardiac-dysfunction, overexpression of ACOT1 reduced reactive oxidative species and improved cardiac function [18, 19].

ACOT2 is also selective for long chain fatty acyl-CoAs. An N-terminal mitochondrial localization sequence targets ACOT2 to the mitochondrial matrix in both rodents and humans [11, 20], suggesting that this enzyme regulates mitochondrial fatty acid oxidation [20]. Overexpression of ACOT2 in mouse liver, where it is normally expressed at low levels, increased hepatic utilization of fatty acids for oxidative phosphorylation *in situ* [21]. It was postulated that ACOT2 promotes fatty acid oxidation via a fatty acid export-activation-uptake cycle. This action may prevent build-up of acyl-CoAs in the mitochondrial matrix and also promote re-utilization of intra-mitochondrial CoA to support high rates of β -oxidation. ACOT2 also introduced a proton leak that may have contributed to some degree of uncoupling of respiration [21].

Mouse ACOTs 3–6 and human ACOT4 contain functional type 1 **peroxisome** targeting sequences at their C-termini. ACOT3 and ACOT5 exhibit substrate specificities for long- and medium chain acyl-CoAs, respectively. ACOT4 and ACOT6 show more narrow substrate specificities with ACOT4 hydrolyzing short dicarboxylic acyl-CoA esters, particularly succinyl-CoA, and ACOT6 hydrolyzing methyl-branched acyl-CoA esters, such as pristanoyl-CoA [12, 13, 22]. ACOT3 and ACOT5 are not present in human, however, human ACOT4 is active towards both short-chain dicarboxyl-CoAs (e.g. succinyl-CoA) and medium- to long-chain acyl-CoAs and thereby combining activities of ACOTs 3–5 [11]. The functions of these ACOTs may be to promote peroxisomal β -oxidation by redirecting fatty acids and products after chain-shortening by the peroxisomal β -oxidation system to lengths that are suitable for export via specific membrane transporters. These ACOTs also provide a system for recycling of CoA within the peroxisome [5] (see Text Box).

Type II ACOTs

Characteristics

In contrast to the type 1 ACOTs, type II enzymes share low degrees of sequence similarity but rather have common structural features. The defining structural motif of these ACOTs is

the so-called **HotDog domain** [23], which is described as a seven-stranded antiparallel β -sheet ‘bun’ that wraps around a hydrophobic five-turn α -helical ‘sausage’, with a third layer comprising loops over the helix as ‘condiments’ [24]. Because they are identified based on structure, the group of type II enzymes has grown to consist of ACOTs 7–15, with the possibility of additional undiscovered members. Mouse ACOTs 9 and 10 share 96% identity, and *Acot10* is only found in the mouse genome as an intron-less gene, and is most likely a retrogene [8, 25].

An additional distinctive feature of type II ACOTs is that their active sites are located at the dimer interface between two HotDog domains. Moreover, the dimers tend to self-associate, so that functional enzymes exist as oligomers, tetramers or trimers of dimers. Dimers may consist of tandem HotDog domains in the same protomer (e.g. ACOT7), or dimers of single HotDog domain protomers (e.g. ACOT13) [26–28]. Structural studies of ACOT7 have shown that the HotDog domains of the N- and C-termini need to associate to form the catalytic site of the enzyme. The dimerization of these units also creates a second (in)active site with potential regulatory function, which does not contain all the catalytic amino acids found in the active catalytic site. When the inactive site is mutated to contain the proper catalytic amino acids, the thioesterase activity of ACOT7 is increased [28].

In addition to tandem HotDog domains, ACOT11 and ACOT12 each contain a lipid-binding C-terminal **steroidogenic acute regulatory transfer-related (START) domain**, so they are also included in the START domain family of proteins as StarD14 and StarD15 [29]. This inclusion of a START-domain is an occurrence that is so far limited to mammalian ACOTs and is suggested to provide an additional level of intrinsic lipid-mediated regulation of the thioesterase activity [8, 23, 27, 30]. Interestingly, the single HotDog domain ACOT13 was originally identified as a binding partner of the minimal START domain protein phosphatidylcholine transfer protein (PC-TP; synonym StarD2) [31]. PC-TP increases the acyl-CoA thioesterase activity of ACOT13 *in vitro*, supporting the notion of START domains as regulatory units when contained with ACOTs (Figure 1) [3, 8, 31].

As with type 1 ACOTs, the type II enzymes are also localized to various cellular compartments in a wide range of tissues and are active towards a variety of acyl-CoA molecular species. Type II ACOTs have been more extensively studied using genetically engineered mice, so their likely biological roles are somewhat better appreciated.

Metabolic functions

ACOT7 was originally referred to as brain acyl-CoA hydrolase (BACH) due to its high expression and activity in the mammalian central nervous system [32]. ACOT7 accounts for the majority of the thioesterase activity in brain tissue, where it is highly expressed in the cytosol of neurons [33, 34]. Although high levels are found in brain and in testis, ACOT7 isoforms are found in other tissues as well. These isoforms are attributable to alternative splicing events that, in two instances, introduce targeting signals for mitochondrial and nuclear localization, respectively [34, 35]. ACOT7 is active towards a spectrum of long chain acyl-CoAs, including arachidonoyl-CoA and palmitoyl-CoA [28, 34]. Macrophages also express ACOT7, and decreased cellular levels of prostaglandins D2 and E3 upon

overexpression of the enzyme support the likelihood that arachidonoyl-CoA is a preferred metabolic substrate for this enzyme [28].

A role for ACOT7 in neurological function was suggested by the discovery of low to absent levels of an isoform of ACOT7 in the hippocampus of patients with mesial temporal lobe epilepsy [36]. In keeping with this possibility, mice with conditional deletion of ACOT7 in neurons (*Acot7^{N-/-}*) exhibit age dependent neurodegeneration, with decreased cerebellar neuronal cell density giving rise to ataxia, as well as decreased motor coordination and locomotion. Biochemical phenotyping revealed that the loss of neuronal ACOT7 led to a decrease in brain thioesterase activity by >80%. Nevertheless, there were no changes in the tissue levels of long chain fatty acyl-CoAs, nor of free CoA. Interestingly, metabolic changes were observed only after overnight fasting and contrary to expectations, increases in long chain FFA were detected in the *Acot7^{N-/-}* mouse brain. Increased tissue levels of more complex lipids, such as sphingosines, phospholipids and triacylglycerols were also observed in fasting *Acot7^{N-/-}* mice, raising the possibility that ACOT7 might limit lipid accumulation in neurons during periods when FFA are abundant [33]. The relevance of this mouse model was underscored by the discovery of a patient with a homozygous frame shift deletion encompassing part of the first exon in *ACOT7* during a screen for copy number variants in patients with undiagnosed neurodevelopment disorders [37]. This mutation was implicated as causal for the phenotype that was characterized by epilepsy, mild intellectual disability and abnormal behavior, similar to the *Acot7^{N-/-}* mouse [37].

The role of ACOT7 in non-neuronal tissues is less clear. Expression of ACOT7 is normally very low in pancreatic β -cells, but is increased in Zucker diabetic fatty rats, as well as in type 2 diabetic patients [38, 39]. Indeed, ACOT7 seems to contribute to β -cell dysfunction since overexpression in pancreatic β -cells lines and in a transgenic mouse model decreased insulin secretion in response to metabolic stimuli. It was suggested that ACOT7 induces β -cell ATP consumption, thereby decreasing metabolic sensitivity in the endocrine pancreas [40].

Interestingly, *Acot7^{N-/-}* mice exhibit additional metabolic abnormalities including increased adiposity, dyslipidemia and increased liver triglycerides after fasting, but the mechanistic basis for these effects remain unclear. To test for direct metabolic effects, an ACOT7 protein engineered to harbor gain-of-function mutations [28] was overexpressed in liver and adipose tissue [41]. Despite measurable increases in thioesterase activity, overexpression in liver did not alter the flux of fatty acids for oxidation or incorporation into complex lipids. Nor were any metabolic changes observed after the chronic metabolic stress of high fat feeding or an acute metabolic stress of 18h of food restriction. Similarly, there were no changes observed in body weight, adipose tissue mass or glucose tolerance after overexpression in adipose tissue. By contrast, increased thioesterase activity in **brown adipose tissue (BAT)** was associated with decreased capacity to maintain body temperature during an acute cold exposure at 4°C [41].

ACOT8 is a peroxisomal protein with broad substrate specificity, including medium-to long-chain and methyl branched acyl-CoAs, intermediates of β -oxidation and bile acid-CoAs [5] (see Text Box). The activity is inhibited by CoA and expression is upregulated by fasting

and PPAR α activation [42] and ACOT8 overexpression leads to peroxisomal proliferation in murine and human cell lines [43]. ACOT8 was first identified as an interacting partner of the HIV-1 Nef protein, a protein that is important for viral replication and infectivity [44, 45]. This interaction is thought to protect against proteasome degradation of Nef [46]. Interestingly, Nef increased the thioesterase activity of ACOT8 *in vitro* [44]. ACOT8 expression is increased in human hepatocellular carcinoma, and cell culture studies suggest a pathogenic role for the enzyme [47]. In lung adenocarcinoma, ACOT8 is upregulated in lymph node metastases and is associated with a poorer prognosis [48]. Whether ACOT8 contributes to tumorigenesis via its enzymatic activity or its capacity to induce peroxisomal proliferation is unclear.

ACOT9 is localized to mitochondria and is widely expressed, with high levels observed in mouse kidney, adipose tissue and brain, and very low expression in liver [25, 49]. ACOT9 is most active towards long-chain saturated acyl-CoAs, but also towards saturated and branched short-chain acyl-CoAs, many of which serve as intermediates in the pathways of amino acid degradation [25, 49]. These findings suggest that ACOT9 provides a regulatory connection, or metabolic crosstalk, between mitochondrial fatty acid and amino acid metabolism [25].

ACOT11, commonly referred to as Thioesterase superfamily member 1 (THEM1) or StARD14, was first described as brown fat-inducible thioesterase (BFIT), which is upregulated in BAT by cold ambient temperatures [50]. THEM1 hydrolyzes a broad range of substrates from acetyl-CoA to long chain fatty acyl-CoAs, with long chain acyl-CoAs being preferred *in vitro* [30, 51]. The lipid ligand of the START domain remains unknown, although the crystal structure suggests FFA may be accommodated within the lipid binding pocket [52]. Whereas the START domain exhibited binding to phosphatidylinositol 4-phosphate in a protein-lipid overlay assay, evidence is lacking for its specific binding within the lipid binding pocket [51]. Although not required for thioesterase activity, the START domain is essential for optimal enzyme activity. ATP stimulates, whereas ADP and CoA inhibit THEM1 activity, while each of these metabolites induce dimerization of the enzyme [30]. The cellular localization of THEM1 remains unclear. Studies in cell lines suggest that THEM1 is imported into mitochondria, whereas tissue fractionation studies in mouse BAT localize THEM1 to the cytosol, endoplasmic reticulum and nucleus [30, 51].

Due to its induction by cold exposure and the finding of increased mRNA expression in obesity-resistant mouse strains, it was suggested that THEM1 would promote energy expenditure [50]. In contrast to expectations, *Them1*^{-/-} mice exhibited increased energy expenditure and resistance to diet induced-obesity. Increased fatty acyl-CoAs in BAT and decreased thioesterase activity in BAT homogenates revealed that THEM1 functions as an ACOT *in vivo* [53]. The higher energy expenditure observed in *Them1*^{-/-} mice was explained by an enhanced cold thermogenesis, with THEM1 directly repressing lipolysis within BAT [54] (Figure 2).

In line with increased heat production and O₂ consumption, increased rates of fatty acid oxidation and glucose uptake were observed in BAT homogenates and primary brown adipocytes from *Them1*^{-/-} mice, respectively. *Them1*^{-/-} mice exhibit improved glucose

homeostasis, which could be explained by the increased heat production by BAT. However, THEM1 is also upregulated by high fat feeding in liver and WAT, so additional studies are needed to ascertain the potential contributions of THEM1 to insulin resistance in these tissues.

ACOT12 was first purified as a cold labile acetyl-CoA hydrolase from the cytosol of rat liver [55]. It demonstrates substrate specificity for acetyl-CoA and to lesser extents, propionyl-, butyryl- and acetoacetyl-CoA. Like ACOT11, ACOT12 is activated by ATP and inhibited by ADP [55]. Although this ATP/ADP-dependent regulation was previously shown to influence oligomerization [56, 57], recent studies suggest that these nucleotides bind to a distinct site, and are not necessary for multimerization [27]. Interestingly, phosphatidic acids decrease the thioesterase activity of ACOT12 putatively by interaction with the START-domain, suggesting regulation that is linked to metabolic conditions within the cell [57]. ACOT12 expression is induced by metabolic stimuli including starvation and the administration of peroxisome proliferators, and is decreased by refeeding and insulin administration [57, 58]. Although ACOT12 harbors a putative peroxisomal targeting sequence at its C-terminus, only a minor fraction of the protein associates with peroxisomes [59, 60]. ACOT12 is primarily expressed in liver, kidney and small intestine [57], suggesting a possible role in gluconeogenesis, although its biological functions remain unknown.

ACOT13 (THEM2) is a small (~15 kDa) single HotDog domain thioesterase. The crystal structure of THEM2 reveals that the single HotDog structure self-associates as a homotetramer, with two homodimers stacked “back-to-back”. The physiological substrate for THEM2 appears to be long-chain fatty acyl-CoAs, although it is also active towards short-chain aromatic acyl-CoAs [26, 61, 62]. THEM2 is expressed in highly oxidative tissues, including liver, heart, kidney and BAT [31, 62, 63], and its expression is regulated by PPAR α [62]. Although human THEM2 was demonstrated to co-localize to microtubules in cell culture [63], no influence of microtubules on mouse THEM2 activity was observed [62]. THEM2 is enriched in mouse mitochondria [62, 64] and liver mitochondria from *Them2*^{-/-} mice exhibit reduced thioesterase activity, further supporting the association of THEM2 with mitochondria [65]. Increased long chain fatty acyl-CoA and reduced FFA concentrations in livers of *Them2*^{-/-} mice confirm the function of THEM2 as an ACOT *in vivo*.

Ablation of THEM2 protected mice against high fat diet-induced hepatic steatosis and impaired glucose homeostasis [65]. The phenotype of improved glucose homeostasis is shared with *Pctp*^{-/-} mice, supporting the metabolic relevance of THEM2-PC-TP interactions [66]. Mechanistic studies in cell culture systems revealed that the PC-TP-THEM2 complex suppresses insulin signaling by reducing the abundance and stability of insulin receptor substrate 2 and by interacting with tuberous sclerosis complex (TSC) 2 to stabilize the mTORC1-inhibiting TSC1-TSC2 complex. It was therefore postulated that PC-TP functions as an intracellular sensor for changes in membrane phosphatidylcholines that vary in response to metabolic stimuli such as fasting, that in turn activates THEM2 [67]. Studies in primary hepatocytes have demonstrated that the THEM2-PC-TP complex promotes fatty acid oxidation by mitochondria, [68], providing an increased flux of TCA cycle intermediates that supports gluconeogenesis under fasting conditions. In the setting of overnutrition, THEM2 instead promotes liponeogenesis by supplying the glycerolipid-

pathway with substrates for ACSL1 and GPAT [68, 69] (Figure 2). THEM2 is also expressed in BAT [62], and increased adaptive thermogenesis is observed in *Them2*^{-/-} mice, supporting the hypothesis that THEM2 regulates energy substrate utilization in BAT [70].

ACOT14 (THEM4) was discovered as an interaction partner and inhibitor of the C-terminus of Akt, leading to its initial designation as the C-terminal modulator protein (CTMP) [71]. Bioinformatics and biochemical characterization of the recombinant protein revealed a HotDog domain with thioesterase activity towards medium and long chain fatty acids [72]. Like ACOT13, THEM4 forms a tetramer consisting of two homodimers [72, 73]. An N-terminal mitochondrial localization sequence targets the enzyme to mitochondria, where it can exit the organelle in response to apoptotic signals, potentially contributing to apoptosis by inhibiting Akt [74]. Another mechanism by which CTMP might sensitize the cell to apoptosis is by controlling mitochondrial dynamics [75]. Due to its inhibition of Akt activity, THEM4 may suppress insulin signaling and cancer cell growth [76, 77]. However depending on cell and cancer type, THEM4 has also been reported to activate Akt [78, 79]. Additional studies are needed to elucidate the regulatory effects that occur in response to THEM4-Akt interactions. It is notable that this interaction does not influence the thioesterase activity *in vitro* [72], and *Them4*^{-/-} mice only exhibit mild mitochondrial changes without evidence of altered lipid metabolism [75]. Consequently, the biological role for THEM4 as an acyl-CoA thioesterase remains unclear.

ACOT15 (THEM5) was included in the ACOT-family based on its sequence similarity to THEM4 [8]. It consists of a single HotDog domain that forms homodimers in solution, and potentially larger oligomers *in vivo*, where it localizes to the mitochondrial matrix [73]. THEM5 displays substrate specificity towards medium and long chain saturated and unsaturated acyl-CoAs, particularly linoleoyl-CoA (18:2-CoA). Its enzymatic function *in vivo* is evidenced by decreased contents of long chain fatty acids, especially linoleic acid, in mitochondria from livers of *Them5*^{-/-} mice. In addition, alterations in cardiolipin synthesis, with increased concentrations of the precursor monolysocardiolipin lead to elongation and enlargement of mitochondria, without associated changes in the mitochondrial DNA copy number [73]. Reduced respiratory capacity and β -oxidation in mitochondria were associated with the age-dependent development of hepatic steatosis, with increased fasting plasma FFA concentrations and hypoketosis [73]. Taken together, these findings illustrate a key role for THEM5 in regulating mitochondrial function.

Conclusions and future perspectives

Upon uptake into cells, FFAs are activated by esterification to CoA, allowing them to become substrates for oxidation or for incorporation into complex lipids. Cells also express enzymes that hydrolyze fatty acyl-CoAs to form FFA and CoA. Two very different types of ACOTs have evolved by convergent evolution to mediate the same activity. Whereas over the past several decades these enzymes have been relatively well characterized biochemically and biophysically, an understanding of their metabolic functions has lagged and key questions remain unaddressed (see Outstanding Questions). Existing evidence suggests that type I enzymes may function in peroxisomes and mitochondria to control rates of fatty acyl-CoA oxidation. For example, fatty acyl-CoA oxidation in peroxisomes may be terminated by

ACOT-mediated hydrolysis, so that the foreshortened FFAs can be transferred to mitochondria for subsequent β -oxidation. Type I ACOTs may also function within mitochondria to recycle CoA under conditions that necessitate high rates of mitochondrial β -oxidation. By contrast, type II ACOTs appear to play more direct roles in the cellular trafficking of fatty acyl-CoA molecules, controlling their dispositions towards oxidation versus complex lipid synthesis. Emerging evidence in mouse models and humans suggest key roles of ACOTs in the pathogenesis of metabolic diseases, suggesting that further research into the biological roles of the enzymes could reveal unique targets in the management of common disorders, including obesity, diabetes and non-alcoholic fatty liver disease.

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Glossary

Fatty acyl-CoA

Activated form of fatty acids that is formed when an free fatty acid molecule is esterified to CoA by way of a thioester bond. Fatty Acyl-CoAs are substrates for oxidation or incorporation into complex lipids.

α/β -Hydrolase domain

A protein structure that for is commonly found in enzymes that hydrolyze lipids. This structure forms the active site of Type I ACOTS.

Brown adipose tissue (BAT)

A thermogenic type of mammalian adipose tissue that is highly enriched in mitochondria which accounts for the brown appearance. It is mainly localized to distinct depots, including the interscapular region in mice. Mitochondria in BAT mitochondria are characterized by the expression of UCP1, which uncouples oxidative phosphorylation from proton flux, leading to the generation of heat

HotDog domain

A protein structure that is characterized by a set of antiparallel beta-sheets that creates a structure with an appearance of a hot dog bun. The bun wrap around a five-turn alpha-helix that takes on the appearance of a hot dog. HotDog domains form the structural basis for Type II ACOTs in which the enzyme active site is found at the interface between domains.

Peroxisome

An intracellular organelle that plays an important role in a variety of anabolic and catabolic biochemical pathways. Peroxisomes are critical for the oxidation of very long chain- and complex-fatty acids and certain amino acids as well as for the synthesis of bile acids and plasmalogens

Steroidogenic acute regulatory protein-related lipid transfer (START) domain

START domains are ~ 210 amino acids conserved protein motifs that create a hydrophobic pocket that is well suited for lipid binding. START domains function in lipid sensing and transfer within cells.

References

- Cooper DE, et al. Physiological Consequences of Compartmentalized Acyl-CoA Metabolism. *J Biol Chem.* 2015; 290:20023–20031. [PubMed: 26124277]
- Anderson CM, Stahl A. SLC27 fatty acid transport proteins. *Mol Aspects Med.* 2013; 34:516–528. [PubMed: 23506886]
- Cohen DE. New players on the metabolic stage: How do you like Them Acots? *Adipocyte.* 2013; 2:3–6. [PubMed: 23700546]
- Hunt MC, Alexson SE. The role Acyl-CoA thioesterases play in mediating intracellular lipid metabolism. *Prog Lipid Res.* 2002; 41:99–130. [PubMed: 11755680]
- Hunt MC, et al. Regulation of peroxisomal lipid metabolism: the role of acyl-CoA and coenzyme A metabolizing enzymes. *Biochimie.* 2014; 98:45–55. [PubMed: 24389458]
- Cantu DC, et al. Thioesterases: a new perspective based on their primary and tertiary structures. *Protein Sci.* 2010; 19:1281–1295. [PubMed: 20506386]
- Svensson LT, et al. Very long chain and long chain acyl-CoA thioesterases in rat liver mitochondria. Identification, purification, characterization, and induction by peroxisome proliferators. *J Biol Chem.* 1995; 270:12177–12183. [PubMed: 7744868]
- Brocker C, et al. Evolutionary divergence and functions of the human acyl-CoA thioesterase gene (ACOT) family. *Hum Genomics.* 2010; 4:411–420. [PubMed: 20846931]
- Hunt MC, et al. A revised nomenclature for mammalian acyl-CoA thioesterases/hydrolases. *J Lipid Res.* 2005; 46:2029–2032. [PubMed: 16103133]
- Hunt MC, et al. Peroxisome proliferator-induced long chain acyl-CoA thioesterases comprise a highly conserved novel multi-gene family involved in lipid metabolism. *J Biol Chem.* 1999; 274:34317–34326. [PubMed: 10567408]
- Hunt MC, et al. Analysis of the mouse and human acyl-CoA thioesterase (ACOT) gene clusters shows that convergent, functional evolution results in a reduced number of human peroxisomal ACOTs. *FASEB J.* 2006; 20:1855–1864. [PubMed: 16940157]
- Westin MA, et al. The identification of a succinyl-CoA thioesterase suggests a novel pathway for succinate production in peroxisomes. *J Biol Chem.* 2005; 280:38125–38132. [PubMed: 16141203]
- Westin MA, et al. Peroxisomes contain a specific phytanoyl-CoA/pristanoyl-CoA thioesterase acting as a novel auxiliary enzyme in alpha-and beta-oxidation of methyl-branched fatty acids in mouse. *J Biol Chem.* 2007; 282:26707–26716. [PubMed: 17613526]
- Kirkby B, et al. Functional and structural properties of mammalian acyl-coenzyme A thioesterases. *Prog Lipid Res.* 2010; 49:366–377. [PubMed: 20470824]
- Mandel CR, et al. Crystal structure of human mitochondrial acyl-CoA thioesterase (ACOT2). *Biochem. Biophys Res Commun.* 2009; 385:630–633.
- Huhtinen K, et al. The peroxisome proliferator-induced cytosolic type I acyl-CoA thioesterase (CTE-I) is a serine-histidine-aspartic acid alpha /beta hydrolase. *J Biol Chem.* 2002; 277:3424–3432. [PubMed: 11694534]
- Dongol B, et al. The acyl-CoA thioesterase I is regulated by PPARalpha and HNF4alpha via a distal response element in the promoter. *J Lipid Res.* 2007; 48:1781–1791. [PubMed: 17485727]
- Xia C, et al. Cardiomyocyte specific expression of Acyl-coA thioesterase 1 attenuates sepsis induced cardiac dysfunction and mortality. *Biochem Biophys Res Commun.* 2015; 468:533–540. [PubMed: 26518651]
- Yang S, et al. Protective effects of Acyl-coA thioesterase 1 on diabetic heart via PPARalpha/PGC1alpha signaling. *PLoS One.* 2012; 7:e50376. [PubMed: 23226270]
- Svensson LT, et al. Molecular cloning and characterization of a mitochondrial peroxisome proliferator-induced acyl-CoA thioesterase from rat liver. *Biochem J.* 1998; 329(Pt 3):601–608. [PubMed: 9445388]

21. Moffat C, et al. Acyl-CoA thioesterase-2 facilitates mitochondrial fatty acid oxidation in the liver. *J Lipid Res.* 2014; 55:2458–2470. [PubMed: 25114170]
22. Westin MA, et al. Molecular cloning and characterization of two mouse peroxisome proliferator-activated receptor alpha (PPARalpha)-regulated peroxisomal acyl-CoA thioesterases. *J Biol Chem.* 2004; 279:21841–21848. [PubMed: 15007068]
23. Dillon SC, Bateman A. The Hotdog fold: wrapping up a superfamily of thioesterases and dehydratases. *BMC Bioinformatics.* 2004; 5:109. [PubMed: 15307895]
24. Leesong M, et al. Structure of a dehydratase-isomerase from the bacterial pathway for biosynthesis of unsaturated fatty acids: two catalytic activities in one active site. *Structure.* 1996; 4:253–264. [PubMed: 8805534]
25. Tillander V, et al. Acyl-CoA thioesterase 9 (ACOT9) in mouse may provide a novel link between fatty acid and amino acid metabolism in mitochondria. *Cell Mol Life Sci.* 2014; 71:933–948. [PubMed: 23864032]
26. Cheng Z, et al. Crystal structure of human thioesterase superfamily member 2. *Biochem Biophys Res Commun.* 2006; 349:172–177. [PubMed: 16934754]
27. Swarbrick CM, et al. Structural basis for regulation of the human acetyl-CoA thioesterase 12 and interactions with the steroidogenic acute regulatory protein-related lipid transfer (START) domain. *J Biol Chem.* 2014; 289:24263–24274. [PubMed: 25002576]
28. Forwood JK, et al. Structural basis for recruitment of tandem hotdog domains in acyl-CoA thioesterase 7 and its role in inflammation. *Proc Natl Acad Sci U S A.* 2007; 104:10382–10387. [PubMed: 17563367]
29. Alpy F, Tomasetto C. START ships lipids across interorganelle space. *Biochimie.* 2014; 96:85–95. [PubMed: 24076129]
30. Han S, Cohen DE. Functional characterization of thioesterase superfamily member 1/Acyl-CoA thioesterase 11: implications for metabolic regulation. *J Lipid Res.* 2012; 53:2620–2631. [PubMed: 22993230]
31. Kanno K, et al. Interacting proteins dictate function of the minimal START domain phosphatidylcholine transfer protein/StarD2. *J Biol Chem.* 2007; 282:30728–30736. [PubMed: 17704541]
32. Yamada J. Long-chain acyl-CoA hydrolase in the brain. *Amino Acids.* 2005; 28:273–278. [PubMed: 15731883]
33. Ellis JM, et al. Acyl coenzyme A thioesterase 7 regulates neuronal fatty acid metabolism to prevent neurotoxicity. *Mol Cell Biol.* 2013; 33:1869–1882. [PubMed: 23459938]
34. Kuramochi Y, et al. Characterization of mouse homolog of brain acyl-CoA hydrolase: molecular cloning and neuronal localization. *Brain Res Mol Brain Res.* 2002; 98:81–92. [PubMed: 11834298]
35. Hunt MC, et al. Alternative exon usage selectively determines both tissue distribution and subcellular localization of the acyl-CoA thioesterase 7 gene products. *Cell Mol Life Sci.* 2007; 64:1558–1570. [PubMed: 17514357]
36. Yang JW, et al. Aberrant cytosolic acyl-CoA thioester hydrolase in hippocampus of patients with mesial temporal lobe epilepsy. *Amino Acids.* 2004; 27:269–275. [PubMed: 15592755]
37. Asadollahi R, et al. The clinical significance of small copy number variants in neurodevelopmental disorders. *J Med Genet.* 2014; 51:677–688. [PubMed: 25106414]
38. Marselli L, et al. Gene expression profiles of Beta-cell enriched tissue obtained by laser capture microdissection from subjects with type 2 diabetes. *PLoS One.* 2010; 5:e11499. [PubMed: 20644627]
39. Parton LE, et al. Limited role for SREBP-1c in defective glucose-induced insulin secretion from Zucker diabetic fatty rat islets: a functional and gene profiling analysis. *Am J Physiol Endocrinol Metab.* 2006; 291:E982–994. [PubMed: 16772326]
40. Martinez-Sanchez A, et al. Disallowance of Acot7 in beta-Cells Is Required for Normal Glucose Tolerance and Insulin Secretion. *Diabetes.* 2016; 65:1268–1282. [PubMed: 26861785]
41. Ellis JM, et al. Metabolic and tissue-specific regulation of acyl-CoA metabolism. *PLoS One.* 2015; 10:e0116587. [PubMed: 25760036]

42. Hunt MC, et al. Characterization of an acyl-coA thioesterase that functions as a major regulator of peroxisomal lipid metabolism. *J Biol Chem.* 2002; 277:1128–1138. [PubMed: 11673457]
43. Ishizuka M, et al. Overexpression of human acyl-CoA thioesterase upregulates peroxisome biogenesis. *Exp Cell Res.* 2004; 297:127–141. [PubMed: 15194431]
44. Watanabe H, et al. A novel acyl-CoA thioesterase enhances its enzymatic activity by direct binding with HIV Nef. *Biochem. Biophys Res Commun.* 1997; 238:234–239.
45. Liu LX, et al. Binding of HIV-1 Nef to a novel thioesterase enzyme correlates with Nef-mediated CD4 down-regulation. *J Biol Chem.* 1997; 272:13779–13785. [PubMed: 9153233]
46. Serena M, et al. Molecular characterization of HIV-1 Nef and ACOT8 interaction: insights from in silico structural predictions and in vitro functional assays. *Sci Rep.* 2016; 6:22319. [PubMed: 26927806]
47. Hung YH, et al. Fatty acid metabolic enzyme acyl-CoA thioesterase 8 promotes the development of hepatocellular carcinoma. *Oncol Rep.* 2014; 31:2797–2803. [PubMed: 24788990]
48. Jung WY, et al. Acyl-CoA thioesterase 8 is a specific protein related to nodal metastasis and prognosis of lung adenocarcinoma. *Pathol Res Pract.* 2013; 209:276–283. [PubMed: 23540296]
49. Poupon V, et al. Molecular cloning and characterization of MT-ACT48, a novel mitochondrial acyl-CoA thioesterase. *J Biol Chem.* 1999; 274:19188–19194. [PubMed: 10383425]
50. Adams SH, et al. BFIT, a unique acyl-CoA thioesterase induced in thermogenic brown adipose tissue: cloning, organization of the human gene and assessment of a potential link to obesity. *Biochem J.* 2001; 360:135–142. [PubMed: 11696000]
51. Chen D, et al. Human brown fat inducible thioesterase variant 2 cellular localization and catalytic function. *Biochemistry.* 2012; 51:6990–6999. [PubMed: 22897136]
52. Thorsell AG, et al. Comparative structural analysis of lipid binding START domains. *PLoS One.* 2011; 6:e19521. [PubMed: 21738568]
53. Zhang Y, et al. Targeted deletion of thioesterase superfamily member 1 promotes energy expenditure and protects against obesity and insulin resistance. *Proc Natl Acad Sci U S A.* 2012; 109:5417–5422. [PubMed: 22427358]
54. Okada K, et al. Thioesterase superfamily member 1 suppresses cold thermogenesis by limiting the oxidation of lipid droplet-derived fatty acids in brown adipose tissue. *Mol Metab.* 2016; 5:340–351. [PubMed: 27110486]
55. Prass RL, et al. Purification and characterization of an extramitochondrial acetyl coenzyme A hydrolase from rat liver. *J Biol Chem.* 1980; 255:5215–5223. [PubMed: 6102995]
56. Isohashi F, et al. Effects of nucleotides on a cold labile acetyl-CoA hydrolase from the supernatant fraction of rat liver. *Biochemistry.* 1983; 22:584–590. [PubMed: 6132615]
57. Horibata Y, et al. Enzymatic and transcriptional regulation of the cytoplasmic acetyl-CoA hydrolase ACOT12. *J Lipid Res.* 2013; 54:2049–2059. [PubMed: 23709691]
58. Matsunaga T, et al. Physiological changes in the activities of extramitochondrial acetyl-CoA hydrolase in the liver of rats under various metabolic conditions. *Eur J Biochem.* 1985; 152:331–336. [PubMed: 2865134]
59. Nakanishi Y, et al. Subcellular distribution of ATP-stimulated and ADP-inhibited acetyl-CoA hydrolase in livers from control and clofibrate-treated rats: comparison of the cytosolic and peroxisomal enzyme. *J Biochem.* 1994; 115:328–332. [PubMed: 7911463]
60. Westin MA, et al. Short- and medium-chain carnitine acyltransferases and acyl-CoA thioesterases in mouse provide complementary systems for transport of beta-oxidation products out of peroxisomes. *Cell Mol Life Sci.* 2008; 65:982–990. [PubMed: 18264800]
61. Cao J, et al. The mechanisms of human hotdog-fold thioesterase 2 (hTHEM2) substrate recognition and catalysis illuminated by a structure and function based analysis. *Biochemistry.* 2009; 48:1293–1304. [PubMed: 19170545]
62. Wei J, et al. Thioesterase superfamily member 2 (Them2)/acyl-CoA thioesterase 13 (Acot13): a homotetrameric hotdog fold thioesterase with selectivity for long-chain fatty acyl-CoAs. *Biochem J.* 2009; 421:311–322. [PubMed: 19405909]
63. Cheng Z, et al. Human thioesterase superfamily member 2 (hTHEM2) is co-localized with beta-tubulin onto the microtubule. *Biochem Biophys Res Commun.* 2006; 350:850–853. [PubMed: 17045243]

64. Mootha VK, et al. Integrated analysis of protein composition, tissue diversity, and gene regulation in mouse mitochondria. *Cell*. 2003; 115:629–640. [PubMed: 14651853]
65. Kang HW, et al. Thioesterase superfamily member 2/acyl-CoA thioesterase 13 (Them2/Acot13) regulates hepatic lipid and glucose metabolism. *FASEB J*. 2012; 26:2209–2221. [PubMed: 22345407]
66. Shishova EY, et al. Genetic ablation or chemical inhibition of phosphatidylcholine transfer protein attenuates diet-induced hepatic glucose production. *Hepatology*. 2011; 54:664–674. [PubMed: 21538437]
67. Ersoy BA, et al. Phosphatidylcholine transfer protein interacts with thioesterase superfamily member 2 to attenuate insulin signaling. *Sci Signal*. 2013; 6:ra64. [PubMed: 23901139]
68. Kawano Y, et al. Thioesterase superfamily member 2 (Them2) and phosphatidylcholine transfer protein (PC-TP) interact to promote fatty acid oxidation and control glucose utilization. *Mol Cell Biol*. 2014; 34:2396–2408. [PubMed: 24732803]
69. Tillander V, et al. Thioesterase superfamily member 2 (Them2) enables glycerol-3-phosphate acyltransferase 1 (GPAT1) to promote glucose intolerance in the setting of hepatic steatosis. *Hepatology*. 2016; 63:127A.
70. Kang HW, et al. Thioesterase superfamily member 2/Acyl-CoA thioesterase 13 (Them2/Acot13) regulates adaptive thermogenesis in mice. *J Biol Chem*. 2013; 288:33376–33386. [PubMed: 24072708]
71. Maira SM, et al. Carboxyl-terminal modulator protein (CTMP), a negative regulator of PKB/Akt and v-Akt at the plasma membrane. *Science*. 2001; 294:374–380. [PubMed: 11598301]
72. Zhao H, et al. The Akt C-terminal modulator protein is an acyl-CoA thioesterase of the Hotdog-Fold family. *Biochemistry*. 2009; 48:5507–5509. [PubMed: 19453107]
73. Zhuravleva E, et al. Acyl coenzyme A thioesterase Them5/Acot15 is involved in cardiolipin remodeling and fatty liver development. *Mol Cell Biol*. 2012; 32:2685–2697. [PubMed: 22586271]
74. Parcellier A, et al. Carboxy-Terminal Modulator Protein (CTMP) is a mitochondrial protein that sensitizes cells to apoptosis. *Cell Signal*. 2009; 21:639–650. [PubMed: 19168129]
75. Parcellier A, et al. The Carboxy-Terminal Modulator Protein (CTMP) regulates mitochondrial dynamics. *PLoS One*. 2009; 4:e5471. [PubMed: 19421406]
76. Park J, et al. New players in high fat diet-induced obesity: LETM1 and CTMP. *Metabolism*. 2014; 63:318–327. [PubMed: 24333006]
77. Hwang SK, et al. Repeated aerosol delivery of carboxyl-terminal modulator protein suppresses tumor in the lungs of K-rasLA1 mice. *Am J Respir Crit Care Med*. 2009; 179:1131–1140. [PubMed: 19286625]
78. Chang JW, et al. Carboxyl-Terminal Modulator Protein Positively Acts as an Oncogenic Driver in Head and Neck Squamous Cell Carcinoma via Regulating Akt phosphorylation. *Sci Rep*. 2016; 6:28503. [PubMed: 27328758]
79. Chen YC, et al. CTMP, a predictive biomarker for trastuzumab resistance in HER2-enriched breast cancer patient. *Oncotarget*. 2016

Trends Box

Upon uptake into cells, fatty acids are activated by conversion to fatty acyl-CoAs, which are substrates for both oxidation and complex lipid biosynthesis.

Acyl-CoA thioesterases (ACOTs) are enzymes that convert fatty acyl-CoAs to free fatty acids plus CoA molecules. Type 1 ACOTs and Type 2 ACOTs are two distinct types of enzymes, with only their enzymatic activities in common.

ACOTs appear to regulate fatty acid metabolism by controlling substrate availability during oxidation and by regulating the intracellular trafficking of fatty acyl-CoAs.

ACOTs play key roles in diverse metabolic processes, including energy expenditure, hepatic gluconeogenesis and neuronal function.

Outstanding Questions Box

- Do Type I and II ACOTs play distinct roles in fatty acid metabolism?
- What are the cellular and molecular mechanisms responsible for the organelle-specific compartmentalization of ACOTs?
- Do START domains function *in vivo* to regulate the enzymatic activity of ACOTs?
- Why do START domains coexist with HotDog domains within ACOT11/THEM1 and ACOT12, whereas StARD2/PC-TP and ACOT13/THEM2 are distinct proteins that interact?
- Certain ACOTs appear to become maladaptive in the setting of overnutrition, contributing to the pathogenesis of common metabolic diseases, such as diabetes, obesity and nonalcoholic fatty liver disease. Can the enzymatic activities of these individual ACOTs be selectively targeted with small molecule inhibitors?

Text box**Peroxisomal lipid metabolism**

Peroxisomes are nearly ubiquitous organelles essential for degradation of very long chain fatty acids, long chain dicarboxylic fatty acids generated by ω -oxidation in the endoplasmic reticulum and long chain methyl branched fatty acids, and for the synthesis of bile acids from cholesterol. Peroxisomes contain their own complement of enzymes to catabolize lipids that are poor substrates for β -oxidation in mitochondria. Unlike the mitochondria, which catalyze the complete oxidation of fatty acids, peroxisomes partially shorten the fatty acyl chains of acyl-CoA esters, typically by promoting 2–5 cycles of β -oxidation, which remove 2 carbons per cycle. The shortened products are then exported from the organelle for further use by the cell, such as continued oxidation in the mitochondria. The final steps in bile acid synthesis are an example of partial peroxisomal β -oxidation, whereby the CoA-ester side-chain of C₂₇ bile acid intermediates is shortened by one cycle of peroxisomal β -oxidation to produce the corresponding C₂₄ bile acid-CoA and propionyl-CoA. The C₂₄ bile acid-CoA is then conjugated to taurine or glycine by the type 1 ACOT-related enzyme bile acid-CoA:amino acid **VV**-acyltransferase (BAAT), transported out of the peroxisome and excreted in bile [5]. Peroxisomes also initiate the oxidation of 3-methyl branched long chain fatty acids (e.g. from dairy products) because they contain additional enzymes that catalyze α -oxidation, which is required for the conversion of 3-methyl FA to a 2-methyl FA prior to chain shortening by β -oxidation. Because peroxisomes catalyze only a few cycles of β -oxidation, a variety of metabolites are formed that must be exported. Due to the ‘multitude’ of substrate specificities of the peroxisomal ACOTs collectively, these enzymes may function to terminate the chain-shortening process. The peroxisomal ACOTs may also contribute to maintain a pool of free CoA for the ongoing oxidation in which the thiolase step requires a CoA molecule for each cycle of β -oxidation [5] (see text box figure).

Peroxisome

Text box figure. Role of ACOTs in peroxisomal lipid metabolism

Acyl-CoA esters enter peroxisomes via different dimers of the ATP-binding cassette D (ABCD1-3) transporters located in the membrane. Imported acyl-CoA esters (including CoA esters of bile acid intermediates and various very long- and long-chain acyl-CoAs) are shortened by β-oxidation to achieve acyl chain-lengths that are suitable substrates for the various ACOTs contained within the organelle. Not shown are that peroxisomes also contain short- and medium-chain acylcarnitine transferases that can convert acyl-CoAs to the corresponding carnitine esters, which are transported out of peroxisomes. Adopted with permission from reference [5]. Abbreviations: ABCD; ATP-binding cassette D transporter; BA, bile acid; BA(I), bile acid intermediate; BAAT, bile acid-CoA:amino acid *N*-acyltransferase; FFA, free fatty acid; Gly, glycine; LCA, long chain acyl; LCDCA,

long chain dicarboxylic acyl; LFA, long chain fatty acyl; MCA, medium chain acyl; MFA, medium chain fatty acyl; Tau, taurine; SCDCA, short chain dicarboxylic acyl; SDCFA, short chain dicarboxylic fatty acid; VLCA, very long chain acyl.

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Figure 1. Relationships between the ACOT and START domain protein families

The ACOT and START-domain protein families are divided into sub-groups. ACOTs are each divided into Type I and Type II ACOTs based on structure, with Type I enzymes containing an α/β Hydrolase catalytic domain and Type II enzymes containing single or tandem HotDog domains. START domain proteins are sub-grouped as multidomain and minimal, which denotes the presence of the START domain as the only domain. ACOT11/THEM1 (StARD14) and ACOT12 (StARD15) are shared in ACOT and START protein families because they each harbor both a tandem HotDog domain and a START domain. The dashed arrow indicates an additional connection between the ACOT and START domain protein families: ACOT13/THEM2 and StARD2/PC-TP are interacting proteins. Abbreviations: ACOT, acyl-CoA thioesterase; M, MLN64 N-terminal domain of StARD3; Mt mitochondrial targeting signal of StARD1; ND, N-terminal domain; PH, pleckstrin

homology; RHO, Rho GTPase activating protein; SAM, sterile alpha motif; START, steroidogenic acute regulatory protein-related lipid transfer

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Figure 2. ACOT11/THEM1 and ACOT13/THEM2 function in brown adipose tissue and liver
 (Top panel) THEM1 is highly expressed in brown adipose tissue and is upregulated by cold exposure. Upon adrenergic stimulus that occurs in response to cold exposure, lipolysis of lipid droplets leads to the release of fatty acids that are directed towards mitochondria for β -oxidation in order to support thermogenesis. THEM1 functions to reduce energy expenditure by limiting the oxidation of fatty acids derived from the lipolysis of lipid droplets. Adopted with permission from reference [54]. (Bottom panel) THEM2 is enriched in liver together with its interacting partner PC-TP. During fasting, THEM2 directs fatty acids towards

mitochondria for β -oxidation, where the increased TCA cycle flux can support gluconeogenesis. In the setting of overnutrition, THEM2 may also promote an increase in flux of fatty acids towards glycerolipid synthesis, which can lead to the production of lipids that suppress insulin signaling. Adopted with permission from reference [68]. Abbreviations: ACSL1, Long chain acyl-CoA synthetase 1; ACSLx, Acyl-CoA synthetase that is as yet unidentified; CPT, Carnitine palmitoyl transferase; ER, endoplasmic reticulum, ETC, electronic transport chain; GPAT1, glycerol-3-phosphate acyltransferase 1; LD, lipid droplet; LPA, lysophosphatic acid; PC, phosphatidylcholine; TAG, triacylglycerol; TCA; tricarboxylic acid cycle; UCP, uncoupling protein; VLDL, very low density lipoprotein.