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Acyl-CoA synthesis, lipid metabolism and lipotoxicity

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Abstract

Although the underlying causes of insulin resistance have not been completely delineated, in most analyses, a recurring theme is dysfunctional metabolism of fatty acids. Because the conversion of fatty acids to activated acyl-CoAs is the first and essential step in the metabolism of long-chain fatty acid metabolism, interest has grown in the synthesis of acyl-CoAs, their contribution to the formation of signaling molecules like ceramide and diacylglycerol, and their direct effects on cell function. In this review, we cover the evidence for the involvement of acyl-CoAs in what has been termed lipotoxicity, the regulation of the acyl-CoA synthetases, and the emerging functional roles of acyl-CoAs in the major tissues that contribute to insulin resistance and lipotoxicity, adipose, liver, heart and pancreas.

Introduction

Fatty acids (FA) and their products play diverse cellular roles in metabolism and signaling. Metabolically, in times of nutritional plenty, FA are stored as triacylglycerol (TAG), primarily in adipocytes, and when energy substrates are deficient, FA are released. Recently, FA have come under intense scrutiny because of their purported dysfunctional role when present in excess. Adipocytes are now seen as having an additional role, that of protecting non-adipocytes from excessive FA uptake and limiting the entry of FA into potentially toxic, non-oxidative pathways. Thus, when there is continued nutritional surfeit, FA accumulate as TAG in liver, muscle, heart, and pancreatic β -cells and cause adverse cellular events, collectively termed lipotoxicity.

Lipotoxicity refers to the functional disturbance associated by the presence of excessive TAG stores in non-adipose tissues. Lipotoxicity is manifested by two types of possibly unrelated cellular injuries associated with this excess. One type of injury leads to apoptosis, and the second type leads to impaired insulin signaling. For example, excess TAG in cardiomyocytes is associated with cardiac hypertrophy, apoptosis, and possible progression to cardiomyopathy [1], and in pancreatic islets, TAG accumulation is associated with impaired insulin secretion, type 2 diabetes, and β -cell loss [2,3]. When TAG accumulates in liver and skeletal muscle, however, the primary effect has been to impair the insulin signaling cascade, and apoptosis does not necessarily occur [4,5]. Thus, despite the presence of hepatic steatosis and even with

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long-standing insulin resistance, the progression of non-alcoholic fatty liver disease to steatohepatitis and cell death is not inevitable.

Are these two forms of lipotoxicity entirely different entities, or are they part of a continuum? In neither case is the underlying mechanism of cellular injury clear. Stored within cells in lipid droplets, TAG itself is probably harmless, but both the lipolysis of intracellular TAG stores and the pathway of TAG synthesis can produce FA and lipid intermediates that have deleterious properties. When TAG is lipolyzed, it expands the availability of FA that can enter pathways like peroxidation that increase reactive oxygen species and endoplasmic reticulum stress. Cellular non-esterified FA also regulate death receptor gene expression and apoptosis [6], and can also regulate transcription factors like SREBP, that alter insulin signaling by repressing the transcription of IRS2 in liver and pancreatic β -cells [7]. Conversely, the pathway of TAG synthesis includes potentially toxic lipid intermediates [1,4,8]. Although the formation of long-chain acyl-CoAs may diminish cellular FA content, acyl-CoAs themselves have been considered to be problematic because they are detergents, they alter signaling pathways, and they are precursors of ceramide and diacylglycerol (DAG) [8,9].

Acyl-CoAs, insulin resistance, and lipotoxicity

Cellular acyl-CoA content correlates with insulin resistance, and it has been suggested that acyl-CoAs mediate lipotoxicity in non-adipose tissues [5,10]. Although the amphipathic nature of the acyl-CoA molecule is frequently invoked as a potential cause of lipotoxicity, detergent effects have not been reported with excess FA exposure, and lipotoxicity results in apoptosis rather than necrosis. It has been estimated that the free concentration of long-chain acyl-CoAs in cell cytosol is between 1 and 20 μ M, although the local concentration of acyl-CoA within a particular membrane region could theoretically be much higher [11]. On the other hand, the concentrations of acyl-CoA and the acyl-CoA binding protein (ACBP) in liver cell cytosol are similar, so the majority of acyl-CoAs would probably be bound to ACBP, or to liver fatty acid binding protein (LFABP), which can also bind acyl-CoA. In fact, since FABPs comprise as much as 5% of total cytosolic protein, most non-esterified FA within cells is also likely to be bound to an FABP isoform [12]. The evidence for a direct role for acyl-CoA in mediating insulin resistance is strongest in muscle. Compared to controls, intramuscular acyl-CoA content is higher in insulin-resistant animals and humans [10], and the correlation between acyl-CoA content and insulin resistance is stronger than that between insulin resistance and intramuscular TAG [13]. In red quadriceps from high-fat-fed rats, high acyl-CoA content correlates with diminished glucose uptake [14]. Interestingly, fat transplanted into lipodystrophic mice normalizes the content of hepatic acyl-CoA and improves both liver and muscle insulin sensitivity [15]. In a study of acute hyperlipidemia during hyperinsulinemia-induced insulin resistance in rats, muscle insulin resistance develops with an increase in the cumulative exposure to acyl-CoAs, despite the lack of change in the phosphorylation state of multiple insulin signaling intermediates, or in the content of muscle DAG and ceramide [16]. This study was interpreted as indicating that acyl-CoAs must influence insulin signaling metabolically by allosterically inhibiting hexokinase and, consequently, directly diminishing glucose uptake/trapping.

In contrast to muscle, the role of acyl-CoA content in liver is less clear. In mice that over-expressed DGAT2, acyl-CoAs increased, but hepatic insulin sensitivity remained normal [17]. Similarly, in mice lacking glycerol-3-phosphate acyltransferase-1, (*Gpat1*^{-/-}), hepatic acyl-CoA content was 2-fold higher than in control mice, yet insulin suppressed hepatic glucose output appropriately [18]. In the pancreas, acyl-CoA content is believed to regulate insulin secretion, because in β -cells, an increase in acyl-CoA enhances K_{ATP} channel activity and reduces β -cell excitability [19]. Although these studies are suggestive, no study has identified a direct mechanism, and none have ruled out the possibility that other FA metabolites are more

directly involved. Overall, the involvement of acyl-CoAs in insulin resistance might be tissue-specific, depending on the metabolic features of individual tissues and the ability of the specific tissue to convert the acyl-CoA to other toxic or protective downstream lipid metabolites.

Normal roles for acyl-CoAs

Because acyl-CoA and its products have been so closely linked to lipotoxicity, we will focus on the normal roles of long-chain acyl-CoAs in cells and the long-chain acyl-CoA synthetase (ACSL) isoforms that control their production. Long-chain acyl-CoAs are substrates for most pathways that use FA for energy production or for the synthesis of complex lipids like phospholipids, cholesteryl esters, ceramide, and TAG. Acyl-CoAs are substrates for β -oxidation in peroxisomes and mitochondria and for ω -oxidation in the endoplasmic reticulum. In addition to these metabolic functions, acyl-CoAs are required for posttranslational protein acylation, and regulation of enzymes, ion channels, membrane potential, protein trafficking, and transcription [20], as well as cellular budding and fusion [21-23]. Because most acyl-CoA may be bound to ACBP, the functional acyl-CoA unit might be bound, rather than free, acyl-CoA [11].

ACSL isoforms and splice variants

Recognition of the importance of acyl-CoAs as critical for cellular metabolism and their potential involvement in lipotoxicity has intensified interest in the enzymes that catalyze their synthesis. ACSL converts long-chain FA to acyl-CoA via a two-step irreversible reaction that requires ATP. The reaction is driven to completion by the hydrolysis of the pyrophosphate.

ACSL isoforms activate FA of 12 to 22 carbons, the major dietary fatty acids in the human diet, and provide acyl-CoAs for almost all catabolic and anabolic downstream pathway of FA metabolism except for eicosanoid biosynthesis [24]. In rodents and humans, the five *Acs1* genes form a subset of an acyl-CoA synthetase family of 26 members that have been classified according to conserved amino acid sequences [25]. This family also includes the fatty acid transport proteins (FATP or ACSVL), many of which have acyl-CoA synthetase activity. The ACSL nomenclature was revised in 2004 to include the five isoforms ACSL1, 3, 4, 5, and 6 [26]. Each ACSL isoform can be transcribed as one of several different variants that result from differences in 5'-UTRs, the first coding exon, alternative coding exons, and exchangeable motifs and each isoform has one or more distinct subcellular locations [27]. For example, human *Acs13* encodes transcripts that produce the identical protein, although the untranslated regions vary [26,27]. All ACSL isoforms except ACSL1 have two in-frame AUG-translational initiators that encode variants of different length and N-termini; these differences might allow the proteins to associate with a specific subcellular membrane or encode variants that lack a transmembrane domain [26-28]. All of the ACSL isoforms and splice variants have an overlapping range of substrates, but somewhat different substrate preferences and enzyme kinetics [29]. Apart from ACSL6 in which alternative exon use produces protein variants whose affinity for ATP differs [29], little is known about the distinct function of each isoform and its splice variants.

The ACSL isoforms contain two highly homologous regions that are conserved from bacteria to humans, a putative ATP-AMP signature motif and an acyl-CoA signature motif [25]. No crystal structure is available for any mammalian ACSL, but analysis of the crystal structure from the distantly related acyl-CoA synthetase from *Thermus thermophilus* HB8 suggests that a fatty acid-binding tunnel exists at the N-terminus [30]. Mutations in rat ACSL4 at residues

that correspond to the entry and termination of this potential FA-binding tunnel showed lower specific activity or diminished affinity for long-chain FA, suggesting that specific residues around the proposed tunnel contribute to the substrate specificity of ACSL4 and its catalytic activity [31]. This may be important in light of the ability of ACSL4 to block apoptosis (see below) [32].

ACSLs in different tissues and organelles

Based on their mRNA abundance, ACSL isoforms differ in their tissue distributions, suggesting that each may play a specific role related to a particular tissue. *Acs14* expression is highest in the adrenal gland and other steroidogenic organs [33], whereas *Acs16* mRNA is most abundant in brain, and to a lesser extent, in skeletal muscle [34]. *Acs15* is expressed most abundantly in small intestine, suggesting that this isoform might be important for the uptake of dietary FA [35]. However, care must be taken in interpreting mRNA data, because the protein abundance does not consistently correspond to the gene expression [36]. Moreover, the expression level of ACSL within the same tissue or organ might well differ in different regions or cell types. For example, *Acs14* is specifically expressed in neurons but not in glial cells in human brain [37]. Knowledge of the tissue-specific distribution of ACSL proteins would provide useful information about the role of ACSL in disturbed lipid metabolism.

Although the function of each of the ACSL isoforms might be related, in part, to its subcellular location, several of the different isoforms have been found on the same organelle membranes and the same isoform may be present on multiple organelles [27]. For example, endogenous ACSL1 is reportedly present on purified outer mitochondrial membrane from liver [38], in gradient-separated endoplasmic reticulum and mitochondrial-associated membrane in liver [39], in adipocyte GLUT4 vesicles [40], and in plasma membrane from 3T3-L1 adipocytes [41]. Immunoprecipitation of over-expressed, tagged ACSL1 and fatty acid transport protein (FATP1) in 3T3-L1 adipocytes suggests that the two proteins interact on the plasma membrane [42]. However, over-expressed, epitope-tagged ACSL1 has been identified by confocal microscopy in mitochondria and endoplasmic reticulum of Ptk-2 and COS cells, but not in plasma membrane, raising the possibility that the subcellular location of ACSL1 might differ in different types of cells [43]. Additionally, proteomics studies have found that several of the ACSL isoforms associate with lipid droplets from different types of cells: ACSL1 in 3T3-L1 adipocytes [44], ACSL4 in 3T3-L1 adipocytes and CHO K2 cells [45,46], and ACSL3 and 4 in hepatoma cell lines [47,48]. It is difficult to understand how a protein with a transmembrane domain might reorganize itself into a monolayer, but perhaps the lipid droplets isolated for study contained fragments of endoplasmic reticulum.

Regulation of ACSL

Transcriptional control

Transcription has been studied in detail only for *Acs11*. The identical ACSL1 protein is encoded by three forms of mRNA that differ in their 5' untranslated region, and it was proposed that this 5' UTR allows differential transcription by physiological activators in different tissues [49]. Form A of *Acs11* mRNA, which is highly expressed in rat liver and adipose, contains a steroid response element-1 sequence, and was hypothesized to be used during lipogenesis, because it is upregulated in rat liver by diets containing 20% soybean oil plus 49% sucrose or by a fat-free diet with 69% sucrose [50]. The steroid response element-1 element may be responsible for the decrease in liver TAG accumulation which parallels the decreased *Acs11* mRNA in mice fed a high safflower oil diet that contained 0.5% cholate [51]. It was proposed that *Acs11* Form B, which is prominent in liver, is related to oxidation [49] because of the PPRE sites in the *Acs11* promoter. *Acs11* Form C mRNA, the major ACSL1 transcript in rat heart, is also expressed in liver; it contains PPRE sequences and is induced by peroxisomal proliferator

drugs in rats and in cultured cells [49,52]. In vivo, however, PPAR α agonists upregulate both ACSL activity and *Acs11* mRNA in rat liver and adipose, but not in heart [53].

***Acs11* mRNA is also affected by other physiological treatments**

High carbohydrate and high fat diets increase *Acs11* mRNA 7- to 8-fold in rat liver [50], so it was not surprising to find that *Acs11* mRNA is strongly induced by the PPAR γ ligand BRL-49653 in epididymal adipose tissue [54]. In liver, heart, muscle, and adipose, *Acs11* mRNA is decreased by lipopolysaccharide [55]. In 3T3-L1 adipocytes, it is stimulated by insulin and T3 [56], and in adipose depots from hyperphagic, obese Zucker (*fa/fa*) rats or rats with ventromedial hypothalamic lesions, *Acs11* mRNA and activity are higher than in lean [57,58] controls, and ACSL1 activity and mRNA decrease in visceral fat after exercise training [59].

Transcriptional control of the other ACSL isoforms has not been as well studied

Oncostatin, an Interleukin-6 family member, reduces TAG in hyperlipidemic hamsters [60] and increases *Acs13* and *Acs15* expression in HepG2 cells via ERK signaling. Oncostatin treatment also results in increased FA β -oxidation [61]. When fed to Zucker diabetic fatty rats, the PPAR γ agonist GW1929 increases *Acs15* in BAT [62]; *Acs15* in BAT also increases in mice kept at 4 °C for 48 h [63]. Like *Acs11*, *Acs15* mRNA in liver is induced by carbohydrate feeding and by insulin via SREBP1c [64]. In summary, transcriptional regulation only suggests possible functions, and it should be remembered that ACSL isoform mRNA correlates poorly with protein and activity [36].

Post-translational regulation

As the enzyme that catalyzes the requisite step for FA activation, ACSL lies at an important potential regulatory point in the control of lipid metabolism and signaling. Evidence was presented more than 30 years ago that ACSL activity is regulated acutely by insulin [65], epinephrine and ACTH [66], and norepinephrine and glucagon [67]. However, because these observations were made before the five ACSL isoforms were cloned, and because direct evidence is lacking for acute regulation of any isoform, the identity of the affected isoforms remains unknown. ACSL1 recovered from the rat liver mitochondrial outer membrane is acetylated at both lysine633 and at the N-terminus and is phosphorylated at tyrosine85 [68]. Our lab also found that ACSL5 in rat primary hepatocytes is phosphorylated on a threonine residue (Li and Coleman, unpublished data). The physiological roles of these post-translational modifications are unknown, but they may alter activity or interactions with other proteins. Acute regulation of ACSL might change the subcellular pools of FA and acyl-CoA and thereby alter a broad range of cellular processes that are controlled by this ratio or by the availability of either as a ligand for a PPAR or HNF4 α .

Physiological functions for ACSL4

The physiological functions of ACSL4 have been studied and include possible roles in polyunsaturated FA metabolism in brain, in steroidogenesis, and in eicosanoid metabolism related to apoptosis. ACSL4 expression is highest in adrenal cortex, ovary and testis, and was first cloned from rat adrenal [33]. Although ACSL4 can activate FAs of 12-22 carbons, it prefers polyunsaturated FAs, and its specific activity is 5-fold higher with 20:4n6 and 20:5n6 than with 18:1 [33]. Unlike the other ACSL isoforms, ACSL4 is encoded on the X chromosome, and is highly expressed in mouse and human cerebellum and hippocampus [69] (<http://www.brain-map.org>), where it may be required for dendritic spine formation [70]. Deficiency of ACSL4 causes about 1% of X-linked mental retardation [37,70,71].

Because ACSL4 has a marked preference for 20:4 [33], several groups have examined its effects on eicosanoid-mediated functions. To determine whether unesterified 20:4 caused apoptosis in colon cancer cell lines, Cao et al. [32] over-expressed COX-2 and ACSL4 in EcR293 cells. Either of these manipulations decreased apoptosis, so the authors concluded that intracellular unesterified arachidonate may regulate apoptosis and COX-2, and hypothesized that ACSL4 promotes carcinogenesis by decreasing arachidonate and, thus, apoptosis [32]. This interesting finding has not been pursued. A single report of a mouse heterozygous for ACSL4 deficiency showed an abnormal uterus with polycysts and increased uterine prostaglandins [72]. ACSL4 is also present in adipocytes where it may activate 20:4. Enhanced production of 20:4-CoA would be important in adipocytes to replenish phospholipids lipolyzed by the insulin-activated Ad-PLA2 [73].

In the adrenal, *Acsl4* mRNA and protein are induced by ACTH [74]. Pursuing this finding, Podestá and his colleagues have published a series of fascinating papers that describe an essential role for ACSL4 and its preferred substrate, 20:4n6, in steroidogenesis. When Leydig cells are stimulated with chorionic gonadotropin, they both release 20:4n6 and synthesize steroid hormone. If ACSL4 is blocked, steroid hormone production is inhibited [75]. Thus, it was hypothesized that ACSL4 activates 20:4 and sequesters the 20:4-CoA product into a specialized pool that enters the mitochondrial matrix [76], where it is required for the StAR-mediated transport of cholesterol into the mitochondria prior to its conversion to a steroid hormone [75]. Interestingly, acute hormonal regulation of this process occurs by enhancing ACSL4 protein synthesis rather than via mRNA expression [77].

ACSL, TAG synthesis, and lipid channeling

Increased synthesis of TAG is associated with lipotoxicity, yet sequestering FA in lipid droplets as TAG may be protective. ACSLs are involved in both processes. The isoform that has been most closely linked to TAG synthesis is ACSL1. In obese and hypertriglyceridemic rats with hepatic steatosis, hepatic ACSL activity is increased together with increased *Acs11* mRNA [57,78]. ACSL activity is also increased in the gastrocnemius muscle of high-fat-fed rats compared with lean controls, and the increased activity may contribute to the accumulation of intramuscular lipid [79]. Further, in differentiating 3T3-L1 adipocytes, *Acs11* mRNA increases 160-fold [80], while ACSL specific activity increases 100-fold [81].

ACSL1 over-expression studies have convincingly linked ACSL1 to TAG and, by association, to lipotoxicity. Transgenic overexpression (6- to 11-fold increases) of *Acs11* in mouse heart results in cardiac myocyte TAG accumulation and lipotoxic cardiomyopathy [82]. When *Acs11* is transiently overexpressed in C57Bl6 mice, liver TAG content increases [83]. NIH 3T3 *Acs11*/FATP1 cells incubated with radiolabeled oleate accumulate more TAG than NIH 3T3 cells alone [84]. Additionally, when *Acs15* is overexpressed in rat hepatoma McArdle-RH7777 cells, it channels exogenously derived oleate towards TAG synthesis and storage [85]. These observations are consistent with a function for high ACSL activity in TAG synthesis and storage. However, there are discrepancies as to the exact roles of the various isoforms of ACSL when it comes to channeling acyl-CoAs into TAG synthesis. Thus, in contrast to adenovirus-mediated overexpression of ACSL1 in mouse liver [83], when *Acs11* is overexpressed in primary rat hepatocytes, exogenous FA is incorporated into DAG and phospholipids, with no changes in TAG synthesis or accumulation [86]. Further, pulse-chase experiments reveal that overexpressed *Acs11* decreases the turnover of intracellular TAG and phospholipids, possibly through altering the reacylation of lysophospholipids. In contrast, overexpressed ACSL5 in McArdle -RH7777 cells increases FA incorporation into TAG, but not phospholipid [61]. Taken together, these overexpression studies suggested that, ACSL1 and ACSL5 channel FA toward different lipid pathways and that the channeling might be cell specific. Additionally,

there appears to be a link between the channeling of FA by ACSL and the development of lipotoxicity.

Additional evidence for the ability of the ACSL isoforms in channeling acyl-CoAs towards different metabolic fates is provided by *in vitro* studies that used the fatty acid analog triacsin C to competitively inhibit ACSL1, ACSL3 and ACSL4 [29,87,88]. In fibroblasts, triacsin C blocks the incorporation of labeled glycerol into TAG, DAG and cholesterol esters by more than 93% and into phospholipid by 83%; although incorporation of exogenous FA into TAG was similarly blocked 95%, the incorporation of FA into phospholipid was not impaired [87]. In an analogous experiment in rat hepatocytes, triacsin C blocked TAG synthesis, but had little or no effect on oleate incorporation into phospholipid, cholesterol esters or β -oxidation end products [89]. It was tentatively concluded from these studies that the triacsin C-sensitive ACSL isoforms contribute to *de novo* lipid synthesis, whereas another ACSL isoform, perhaps ACSL5 or ACSL6, channels acyl-CoAs towards the reacylation of lysophospholipids and β -oxidation.

Overexpression of ACSL isoforms and lipotoxicity: interpretative problems

Interpreting metabolism

Although in most of the studies cited above, over-expression was used to determine the function of ACSL1 or ACSL5, two difficulties have emerged in interpretation. First, by converting FA to acyl-CoA, the ACSLs effectively trap FA within the cell via vectorial transport [90]. Thus, FA entry into cells may be facilitated by plasma membrane proteins, but to retain FAs within the cell, they must be converted to acyl-CoAs or down-stream metabolites that cannot exit [91-93]. Consistent with this view, both ACSL1 and FATP1, a member of the ACS super-family with ACSL activity, were identified as proteins that facilitate cell import of FA [94, 95]. This view of vectorial transport is further supported by the fact that both the presence of an ACSL on an intracellular membrane and enhanced down-stream FA metabolism can increase cellular FA uptake [96]. Thus, because the rate of entry of FA into cells is coupled to metabolism [96], overexpressing ACSL may merely allow more FA to enter cells. The excess FA may then be incorporated into lipid metabolic pathways that have little to do with normal enzymatic function.

In rat hepatoma cells, overexpressed ACSL5 increases exogenous FA uptake, despite the intracellular location of ACSL5 on endoplasmic reticulum and mitochondria [85]. In COS cells, mitochondria-located ACSL1 enhances oleate uptake [43]. However, FA uptake does not always change in concert with the change of ACSL activity. For example, overexpression of ACSL1 in rat primary hepatocytes does not increase the uptake of labeled oleate [86]. Taken as a whole, ACSL activation of FA to acyl-CoA acts as a key step linking FA uptake and metabolism. To fully understand ACSL function in promoting the uptake and storage of excess FA, it will be important to examine protein-protein interactions between ACSL and downstream lipid metabolic enzymes.

Palmitate toxicity

The second difficulty in evaluating the role of FA uptake is that metabolism is usually measured using a single labeled FA species, something that never occurs physiologically. If the FA used experimentally is palmitate (16:0), the resulting toxic effects may have more to do with issues specific to palmitate than to the enzymatic function of the ACSL isoform or to any normal physiological process. Palmitate as the sole FA offered to a cell is problematic for at least two reasons. Normally, cells cope with the presence of excess FA by sequestering it in TAG molecules within lipid droplets until the FA can be released from TAG and oxidized or used for a synthetic purpose. However, 16:0-CoA is an extremely poor substrate for diacylglycerol

acyltransferase, the final step in TAG synthesis (Figure 1) [97]. Thus, in lieu of TAG synthesis, the end products of 16:0-CoA metabolism could be ceramide or DAG, both of which are believed to activate signaling pathways that harm cell viability. Secondly, in addition to impaired TAG formation, adding palmitate alone to cells results in the synthesis of *sn*-1,2-di16:0-phosphatidylglycerol, a cardiolipin precursor. Because cardiolipin synthase has a low affinity for saturated phosphatidylglycerol, cardiolipin synthesis decreases, and cells undergo apoptosis [98,99]. It is for these reasons that adding 16:0 plus a monounsaturated FA like 18:1 or 16:1 in a ratio of 1:2 protects against apoptosis; it both provides 18:1-CoAs that can be esterified by the diacylglycerol acyltransferases, and allows the synthesis of phosphatidylglycerol that is a better substrate for cardiolipin synthase.

A more physiological method of examining the toxicity that can occur with an increased entry of FA into cells is to infuse rodents with mixtures of FA or to enhance FA uptake by over-expressing ACSL, although the extent of FA uptake should optimally be similar to that which might occur in vivo. Excessive overexpression, as achieved by transgenically overexpressing ACSL1 to a level 11-fold higher than in control hearts, caused hearts to accumulate TAG, become hypertrophic, and develop left-ventricular dysfunction [82]. In this model, ACSL activity, acyl-CoA and DAG were not measured, but ceramide content increased 3-fold, and the cardiac myocytes died by apparent apoptosis. A similar cardiac-specific over-expression of FATP1, which also has ACSL activity, increases heart FA uptake 4-fold and causes impaired left ventricular filling and bi-atrial enlargement with preserved systolic function [100]. Adenovirus-mediated ACSL1 expression in liver increased ACSL activity 5-fold, but TAG content increased only 2-fold, and acyl-CoA content did not change [83]. Insulin signaling was not examined in this study. Although these studies were useful in studying lipoapoptosis, they may not be physiologically relevant to obesity and the development of hepatic steatosis and insulin resistance.

In contrast to results in mice, cells in culture have not shown apparent harm from ACSL over-expression. When ACSL5 was over-expressed in McArdle-RH7777 cells, the epitope-tagged protein was present on both mitochondrial and endoplasmic reticulum membranes, ACSL specific activity was 2-fold higher than in control cells, and oleate and glycerol incorporation into TAG increased, indicating the use of FA derived from both exogenous and reacylation pathways [85]. Adenovirus-mediated over-expression of ACSL1 in rat primary hepatocytes increased ACSL specific activity 3.7-fold and increased oleate incorporation into DAG and phospholipids, but not TAG, and decreased incorporation into cholesterol esters [86]. In a comparison of over-expressed ACSL6 and ACSL1 in PC12 neuronal cells, ACSL6 overexpression resulted in greater uptake of 20:4 and 22:6, together with an increase in neurite outgrowth, but the relative increases in ACSL activity were not ascertained [80]. Although these cell culture studies do not support the hypothesis that acyl-CoAs (apart from 16:0) are toxic, conclusions must be tempered because the transfected protein might have been mislocated. Further, if downstream pathways are unable to cope normally with the unaccustomed excess flux of acyl-CoA substrates, the acyl-CoAs might be directed into metabolic pathways different than those normally followed.

Conclusion

In summary, over-expression of ACSL isoforms has been used to promote increased uptake of FA into cells and to examine the effects of FA on insulin resistance and apoptosis. We are beginning to view over-expression as a method that may not provide physiological information about normal ACSL enzymatic activity and function. Care should be taken to avoid cellular uptake of FA that exceeds what would occur during starvation or with obesity and insulin resistance. To prevent lipotoxicity, cells must either decrease FA influx, expand glycerolipid storage capacity, or increase acyl-CoA catabolism. It remains unclear at this time whether

targeting one or several of the ACSL isoforms would result in diminished FA uptake and protection from lipotoxicity.

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Fig. 1. Palmitate (16:0) is poorly incorporated into triacylglycerol and cardiolipin

Palmitic acid (16:0) is activated by an acyl-CoA synthetase and esterified to glycerol-3-phosphate to produce 16:0-LPA. Addition of a second 16:0-CoA produces di-16:0-PA, which is a precursor for the formation of PG and cardiolipin. The di-16:0-PA can also be hydrolyzed to form a DAG which is a precursor for the synthesis of triacylglycerol; however, 16:0-CoA is a poor substrate for DGAT and di-16:0-phosphatidylglycerol is a poor substrate for cardiolipin synthase (gray bars).

ACBP, acyl-CoA binding protein; BSA, bovine serum albumin; CDP, cytidine diphosphate; CLS, cardiolipin synthase; DAG, diacylglycerol; DGAT, diacylglycerol acyltransferase; G3P, glycerol-3-phosphate; FABP, fatty acid binding protein; LPA, lysophosphatidic acid; PA, phosphatidic acid; PG, phosphatidylglycerol; PI, phosphatidylinositol; TAG, triacylglycerol