REVIEW ARTICLE

Role of long-chain fatty acyl-CoA esters in the regulation of metabolism and in cell signalling

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The intracellular concentration of free unbound acyl-CoA esters is tightly controlled by feedback inhibition of the acyl-CoA synthetase and is buffered by specific acyl-CoA binding proteins. Excessive increases in the concentration are expected to be prevented by conversion into acylcarnitines or by hydrolysis by acyl-CoA hydrolases. Under normal physiological conditions the free cytosolic concentration of acyl-CoA esters will be in the low nanomolar range, and it is unlikely to exceed 200 nM under the most extreme conditions. The fact that acetyl-CoA carboxylase is active during fatty acid synthesis (K_i for acyl-CoA is 5 nM) indicates strongly that the free cytosolic acyl-CoA concentration is below 5 nM under these conditions. Only a limited number of the reported experiments on the effects of acyl-CoA on cellular functions and enzymes have been carried out at low physiological concentrations in the presence of the appropriate acyl-CoA-buffering binding proteins. Re-evaluation of many of the reported effects is therefore urgently required. However, the observations that the ryanodine-senstitive Ca^{2+}-release channel is regulated by long-chain acyl-CoA esters in the presence of a molar excess of acyl-CoA binding protein and that acetyl-CoA carboxylase, the AMP kinase kinase and the *Escherichia coli* transcription factor FadR are affected by low nanomolar concentrations of acyl-CoA indicate that long-chain acyl-CoA esters can act as regulatory molecules *in vivo*. This view is further supported by the observation that fatty acids do not repress expression of acetyl-CoA carboxylase or Δ^9-desaturase in yeast deficient in acyl-CoA synthetase.

INTRODUCTION

Long-chain acyl-CoA esters serve as important intermediates in lipid biosynthesis and fatty acid degradation. Besides this basal function, a large body of evidence has accumulated indicating that long-chain acyl-CoA esters also have an important function in the regulation of intermediary metabolism and gene expression. Since Bortz and Lynen [1] proposed that acyl-CoA esters are a key regulator of fatty acid synthesis, long-chain acyl-CoA esters have been reported to affect a large number of cellular systems and functions, including ion channels, ion pumps, translocators, enzymes, membrane fusion and gene regulation (Figure 1). In the present article we review the current literature on acyl-CoA as a physiological regulator of cell functions. Special attention is given to the physiological relevance of the reported observations in relation to the cellular levels of long-chain acyl-CoA esters and their respective binding proteins.

PHYSICAL PROPERTIES OF LONG-CHAIN ACYL-CoA ESTERS AND THEIR INTERACTIONS WITH MEMBRANES

Long-chain acyl-CoA esters are amphipathic molecules, consisting of a hydrophilic head-group, the CoA part, and a more hydrophobic region, the acyl chain. Like other detergents, long-chain acyl-CoA esters form molecular solutions at low concentrations, but, as the concentration increases, the critical micelle concentration is reached and association into micelles will occur. Above the critical micelle concentration, the concentration of free molecules will be constant and independent of the total concentration of acyl-CoA [2]. Employing gel-permeation chromatography, analytical centrifugation and spin-label techniques, Powell et al. [3] estimated the critical micelle concentration to be in the range 30–60 μM. However, a thorough study by Constantinides and Stein [2] indicated that the the critical micelle concentration for palmitoyl-CoA can be as high as 70–80 μM under physiological conditions.

Acyl-CoA esters partition into phospholipid vesicles by insertion of the hydrophobic acyl chain into the bilayer [4–7]. The partition constant for palmitoyl-CoA in phospholipid vesicles can be calculated from the original data of Peitzsch and McLaughlin [5] to be 1.5×10^5 M^{-1}, and a value of 5×10^5 M^{-1} has also been determined directly [7]. The partition constant is strongly dependent on the acyl chain length [4,5,7], but is nearly independent of the type of polar head-group, indicating that the acyl chain partitions into the vesicle while the polar head-group is located on the membrane surface in the aqueous environment [4,5]. No transbilayer movement could be observed, even when oleoyl-CoA was preincubated with phosphatidylcholine vesicles for up to 24 h [4].

Partitioning of long-chain acyl-CoA esters into membranes markedly affects membrane integrity [8]. These authors showed that palmitoyl-CoA decreased the latency of β-glucuronidase and UDP-glucuronosyltransferase in rat liver microsomes and increased the permeability of microsomal vesicles to sucrose and citrate, among others. Phosphatidylcholine vesicles can only accommodate 15 mol% oleoyl-CoA without disruption of the bilayer structure. Long-chain acyl-CoA esters were, in comparison with fatty acids and lysophospholipids, the most potent disrupter of membrane bilayers [4]. In contrast, it has been shown that only acylcarnitines, and not acyl-CoAs, are able to

Abbreviations used: ACBP, acyl-CoA binding protein; ANT, adenine nucleotide translocase; CPT, carnitine palmitoyltransferase; FABP, fatty acid binding protein; PKC, protein kinase C.

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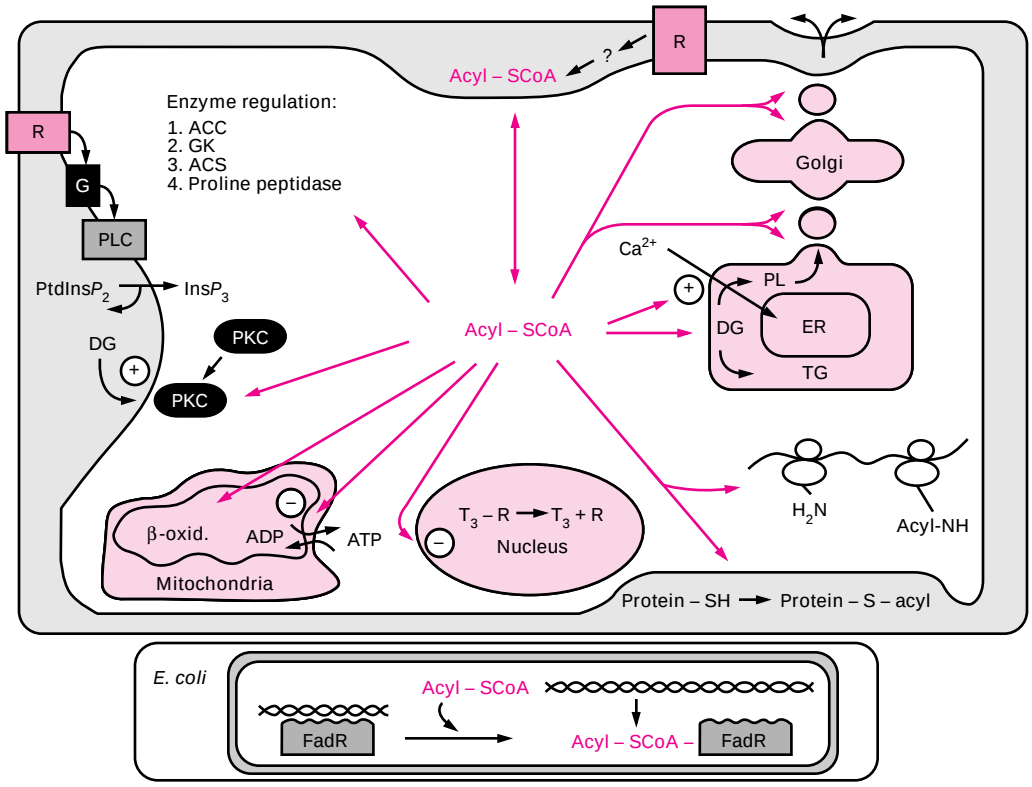

Figure 1 Long-chain acyl-CoA in metabolism and signal transduction

A number of cellular system and processes have been found to be regulated by long-chain acyl-CoA esters, including cytosolic enzymes, ion channels, ion pumps, translocators, membrane fusion and gene regulation. Abbreviations: ACC, acetyl-CoA carboxylase; ACS, acyl-CoA synthetase; acyl-SCoA, acyl-CoA ester; β-oxid., β-oxidation; DG, diacylglycerol; ER, endoplasmic reticulum; G, G-protein; GK, glucokinase; PL, phospholipid; PLC, phospholipase C; R, receptor; T_3, tri-iodothyronine; TG, triacylglycerol.

disrupt membrane barriers to solutes and produce complete membrane solubilization [6,7,9,10].

In yeast, fatty acid synthetase is inhibited by its product, long-chain acyl-CoA esters. Sumber and Traüble [11] demonstrated that dimyristoyl-lecithin dispersions, *Escherichia coli* plasma membranes and BSA relieved the feedback inhibition of acyl-CoA on yeast fatty acid synthetase. These authors also demonstrated an interaction between phospholipids and acyl-CoA by employing fluorescent acyl-CoA, and hypothesized that the acyl-CoA esters synthesized are carried to an acceptor membrane by fatty acid synthetase itself, or by another lipid carrier protein. In the membrane the acyl-CoA esters were suggested to be transferred to the site of utilization by lateral diffusion. This mechanism was suggested to permit a much more rapid transfer of an acyl-CoA molecule from the cytosolic compartment to a target protein on the cell membrane compared with free diffusion alone [11]. Long-chain acyl-CoA esters partition into microsomal membranes [12,13]. However, more than 60 % of the bound acyl-CoA esters were hydrolysed during the first 1 h of incubation [13], making the presence and diffusion of acyl-CoA esters in biological membranes under *in vivo* conditions unlikely.

LONG-CHAIN ACYL-CoA ESTERS AS REGULATORS OF ENERGY METABOLISM

The mitochondrial adenine nucleotide translocase (ANT), which catalyses the exchange of ADP and ATP across the mitochondrial inner membrane, is generally accepted to be the overall rate-limiting step in energy metabolism [14,15]. Acyl-CoA esters have been shown to be potent inhibitors of ANT both in intact mitochondria and in submitochondrial particles in a specific and chain-length-dependent manner, with a K_i of 1 μM [16–18]. Reconstitution of ANT, purified from bovine heart mitochondria, into liposomes has demonstrated that the inhibitory effect of acyl-CoA occurred independently of whether the carrier was oriented in the forward or reverse manner in the liposome bilayer, supporting the existence of two separate acyl-CoA inhibitory binding sites on the translocator [18–20]. A specific interaction between acyl-CoA and bovine mitochondrial ANT was demonstrated using an ^{125}I-labelled photoreactive acyl-CoA [21]. Ligating the anterior coronary artery to produce ischaemia resulted in a clear correlation between the increase in the intracellular acyl-CoA level and the decrease in translocase activity *in vitro* [22]. Under these conditions the mitochondrial acyl-CoA concentration was reported to be increased to 1 mM [23]. Consequently, ATP synthesis would be expected to be decreased, resulting in adverse effects on muscle contraction and electrical conduction.

Sequence comparisons show that ANT has extensive sequence identity with the phosphate carrier and the brown-adipose-tissue mitochondrial proton-conducting protein [24,25], which has been shown to be activated by long-chain acyl-CoAs and fatty acids [26]. Interestingly, this effect is opposite to the chain-length-dependent inhibitory action of long-chain acyl-CoA (K_i 2.4 μM) on the pH-dependent anion-conducting channel in the inner membrane of rat liver mitochondria [27].

Rat liver glucokinase is inhibited specifically by palmitoyl-CoA in an allosteric manner at concentrations well below the critical micelle concentration ($K_i \sim 1.8\ \mu M$) [28]. The inhibition of the enzyme by long-chain acyl-CoA esters was instantaneous, reversible and specific; non-esterified fatty acids, short-chain acyl-CoAs, free CoASH or similar amphipathic compounds did not affect enzyme activity [28,29]. The opposing enzyme to glucokinase, glucose-6-phosphatase, has also been found to be inhibited by acyl-CoA esters [30], which may be important in controlling the efflux of glucose during starvation or diabetes.

Bovine heart pyruvate dehydrogenase activity is inhibited by 100 μM palmitoyl-CoA [31], and the activity of this enzyme is also decreased in liver, kidney, adipose tissue and muscle during fasting, concomitant with an increase in the concentration of non-esterified fatty acids in the blood [32–35]. Furthermore, the increase in mitochondrial acyl-CoA level correlated well with the concomitant decrease in pyruvate dehydrogenase activity observed in mitochondria from rat adipocytes, suggesting that long-chain acyl-CoA is a common regulator of this enzyme [36]. However, during starvation the acyl-CoA/CoA, acetyl-CoA/CoA, NADH/NAD^+ and ATP/ADP ratios in the mitochondrial matrix all rise [37–39]. These increases inhibit pyruvate dehydrogenase and result in a switch to energy production from β-oxidation [40]. The inhibition of pyruvate dehydrogenase by long-chain acyl-CoA may, therefore, be indirect, due either to the changes in the above metabolite ratios or to the effect of palmitoyl-CoA on the activity of pH-dependent anion conductance [27] and the steady-state transmembrane potential followed by release of mitochondrial Ca^{2+} [41].

The physiological relevance of acyl-CoA regulation of glucokinase and pyruvate dehydrogenase is not yet known, but a negative effect of these esters on uptake, storage and oxidation of blood glucose is in full accordance with the function of the glucose/fatty acid cycle [42].

Mitochondrial β-oxidation of fatty acids to acetyl-CoA is a major energy source in animals [43,44]. β-Oxidation is inhibited mainly by feedback inhibition. When flux through the tricarboxylic acid cycle is limited, the acetyl-CoA level increases and inhibits β-ketothiolase [45]. Fatty acyl-CoA intermediates accumulate and inhibit the first steps of the pathway catalysed by the chain-length-specific acyl-CoA dehydrogenases [46,47]. The long-chain acyl-CoA dehydrogenase was inhibited by 3-oxopalmitoyl-CoA with a K_i of 0.2 μM when palmitoyl-CoA was the substrate [46].

REGULATION BY LONG-CHAIN ACYL-CoA ESTERS OF ENZYMES INVOLVED IN LIPID SYNTHESIS

Acetyl-CoA carboxylase catalyses the initial and key step in the biosynthesis of long-chain fatty acids [48]. The level of this enzyme in *Saccharomyces cerevisiae* is repressed by the presence of long-chain fatty acids in the growth medium [49]. However, a mutant strain of *S. cerevisiae* defective in acyl-CoA synthetase exhibits little repression of acetyl-CoA carboxylase by fatty acids, indicating that activation by exogenously supplied fatty acids is required for repression of acetyl-CoA carboxylase [49]. Similar observations have been made with regard to repression of the Δ^9-desaturase by Δ^9-unsaturated fatty acids in yeast [50]. Long-chain acyl-CoA esters also inhibit rat liver acetyl-CoA carboxylase directly, with a K_i of 5.5 nM [51]. Acyl-CoA esters of saturated fatty acids with 16–20 carbon atoms are most effective [52]. Citrate not only prevents binding of palmitoyl-CoA to the enzyme, but also dissociates bound palmitoyl-CoA from acetyl-CoA carboxylase in the presence of a long-chain acyl-CoA acceptor such as BSA [53].

Acetyl-CoA carboxylase is also inhibited by reversible phosphorylation by the AMP-activated protein kinase [54]. The AMP-activated kinase is itself activated by phosphorylation by a distinct 'kinase kinase', which is activated by nanomolar concentrations (50–200 nM) of acyl-CoA esters. The potential regulation of lipid metabolism by long-chain acyl-CoA esters is not limited to fatty acid synthesis. 3-Hydroxy-3-methylglutaryl-CoA reductase and hormone-sensitive lipase, which catalyse the key step in cholesterol synthesis and the hydrolysis of cholesterol esters and acylglycerols respectively, are also phosphorylated and regulated by the AMP-activated kinase. 3-Hydroxy-3-methylglutaryl-CoA reductase and hormone-sensitive lipase are also inhibited directly by oleoyl-CoA at concentrations between 0.16 and 1.9 μM [55,56].

Other potential control points for the regulation of fatty acid synthesis by acyl-CoA esters are the mitochondrial citrate transporter and 6-phosphogluconate dehydrogenase, which provide acetyl-CoA and NADPH respectively for fatty acid synthesis respectively [57,58].

The enzymes involved in glycerolipid synthesis are localized on the cytoplasmic surface of microsomes [59]. The addition of increasing amounts of acyl-CoA esters results in a dose-dependent inhibition of many acyltransferases *in vitro*. Moderately high concentrations of oleoyl-CoA were found to be inhibitory to acyl-CoA:phospholipid acyltransferase, and this inhibition could be reversed by the addition of albumin or heat-denatured microsomes [60].

LONG-CHAIN ACYL-CoA ESTERS IN SIGNAL TRANSDUCTION

Membrane trafficking depends on acyl-CoA esters

The regulation and co-ordination of membrane fusion events play a central role in secretion, endocytosis, cell division and transport between intracellular compartments. The molecular mechanism by which transport vesicles bud off from a donor compartment and are transported, recognized and fused to the target membrane was a puzzle until few years ago. Reconstitution of the protein transport pathway from the endoplasmic reticulum to the Golgi in a cell-free system has provided insight into these mechanisms [61]. Acyl-CoA esters have been shown to be essential for either the budding or the fusion process. Palmitoyl-CoA stimulated the release and transport of the transport vesicle [62–64]. Using Triascin C, an acyl-CoA synthetase inhibitor, Pfanner et al. [63] demonstrated that fatty acids had to be activated to fatty acyl-CoAs in order to stimulate transport. It is still unclear how palmitoyl-CoA participates in the formation of transport vesicles and in the fusion process at the target membrane. Protein acylation is most probably involved, since a non-hydrolysable palmitoyl-CoA analogue inhibited both processes [64].

In contrast with the stimulatory effect of palmitoyl-CoA on vesicular transport in the Golgi apparatus, low concentrations of long-chain acyl-CoA and CoA inhibit the GTP-dependent fusion of rat liver microsomal vesicles, with IC_{50} values ranging from 15 to 18 μM for C_{14}-, C_{16}- and C_{18}-CoA esters, whereas high acyl-CoA concentrations (50 μM) favoured the formation of small microsomal vesicles [65]. The authors suggested that GTP-induced membrane fusion in rat liver microsomes depends on an as yet unknown acylation/deacylation mechanism required for complete vesicle sealing. However, this is unlikely, since fatty acyl transfer to proteins is highly specific with regard to amino acid sequence and acyl-chain length [66]. The observation that a non-hydrolysable myristoyl-CoA analogue also blocked the GTP effect [67] further indicates that the effect of acyl-CoA is not caused by protein acylation, but rather by an allosteric effect.

Regulation of ion fluxes during fusion events may play a key role in the recognition process prior to membrane fusion, ensuring that only vesicles with the same ion environment are allowed to fuse. The observed equivalence of palmitoyl- and myristoyl-CoA with regard to the inhibition of GTP-induced Ca^{2+} release supports this suggestion. Thus acyl-CoA esters may both have stimulatory and inhibitory effects during vesicle transport and membrane fusion.

Acyl-CoA regulation of ion fluxes

Glucose is considered as the major physiological stimulus of insulin secretion [68–71]. The molecular mechanism by which glucose stimulates insulin secretion is not well defined, but Deeney et al. [72] presented a model whereby glucose stimulation leads to periodic elevations of the ATP/ADP ratio, closure of ATP-sensitive K^+ channels and hence influx of Ca^{2+} through voltage-gated Ca^{2+} channels.

Transient elevations in medium non-esterified fatty acids have been shown to enhance glucose-induced insulin secretion from pancreatic β-cells, in contrast with prolonged elevations, which resulted in diminished β-cell sensitivity to glucose [72–74]. It was demonstrated that nutrient-stimulated insulin secretion by β-cells was accompanied by increased concentrations of malonyl-CoA and long-chain acyl-CoAs. The observed increase in the intracellular cytosolic acyl-CoA concentration was suggested to be caused by a decrease in β-oxidation due to the inhibition of carnitine palmitoyltransferase I (CPT I) by malonyl-CoA [75]. This is supported by the fact that various CPT I inhibitors (e.g. 2-bromopalmitate) which suppress the oxidation of endogenous fatty acids are potent stimulators of insulin secretion [75,76].

Deeney et al. [72] found that acyl-CoA esters (0.5 μM), but not the corresponding fatty acids, decreased the steady-state medium free Ca^{2+} concentration in clonal permeabilized pancreatic β-cells (HIT-cells) in a chain-length- and concentration-dependent manner. Mitochondrial inhibitors and protein kinase C (PKC) depletion did not affect the ability of acyl-CoA to stimulate Ca^{2+} uptake by the permeabilized cells, whereas thapsigargin, an inhibitor of the endoplasmic-reticulum Ca^{2+}-ATPase, blocked the acyl-CoA effect. Rys-Sikora et al. [67] also observed that palmitoyl-CoA at low concentrations ($< 1\ \mu$M) induced the uptake of Ca^{2+} into saponin-permeabilized DDT_1MF-2 smooth-muscle cells, whereas higher concentrations had an inhibitory effect on Ca^{2+} uptake. The above results indicate that the effects of fatty acids on insulin secretion may be mediated through the effects of acyl-CoA on intracellular Ca^{2+} fluxes.

Larsson et al. [74] demonstrated that the level of long-chain acyl-CoAs increased more than 2-fold when clonal β-cells (HIT-T15) were incubated with palmitate for 18 h. The same authors observed that long-chain acyl-CoA esters induced the rapid and slowly reversible opening of ATP-sensitive K^+ channels in a concentration-dependent manner. Based on these findings, they suggested that long-term exposure to fatty acids, e.g. associated with hyperglycaemia, could by this mechanism prevent the closure of the ATP-sensitive K^+ channel and thus contribute to the development of glucose-insensitivity of β-cells.

Long-chain acyl-CoA esters stimulate Ca^{2+} release by the ryanodine-sensitive Ca^{2+}-release channel in longitudinal tubules and terminal cisternae of sarcoplasmic reticulum from rabbit skeletal muscle [77]. Additionally, long-chain acyl-CoA potentiates the effect of cyclic ADP-ribose on Ca^{2+} release by the ryanodine-sensitive Ca^{2+} channel in sea-urchin eggs [78]. Fatty acyl-CoA esters appeared to interact directly with the ryanodine-sensitive Ca^{2+} channel, since both palmitoyl-CoA and the corresponding non-hydrolysable analogue induced the release of Ca^{2+} with an EC_{50} of 6 μM. Ruthenium Red, a Ca^{2+}-release channel blocker, completely prevented this effect. Furthermore, palmitoyl-CoA increased the affinity of ryanodine binding without changing the binding capacity [77]. Similar effects of long-chain acyl-CoA were observed on the ryanodine receptor from duckling sarcoplasmic reticulum, at concentrations below 20 μM [79,80], and it was suggested to play a role in cold-induced non-shivering thermogenesis.

In liver, in contrast with muscle, low concentrations of acyl-CoA suppressed GTP- and inositol 1,4,5-trisphosphate-induced Ca^{2+} release from rat liver microsomal vesicles, and caused re-uptake of Ca^{2+} into and enlargement of the inositol 1,4,5-trisphosphate-sensitive compartment [65,81].

Subtypes of PKC are affected differently by long-chain acyl-CoA

Fatty acids, as well as their CoA derivatives, have been reported to regulate the activity of different PKC subtypes. In contrast with palmitate and oleate, both palmitoyl-CoA and oleoyl-CoA (27 μM) enhanced particulate PKC activity in human skin fibroblasts by 70 % in the presence of Ca^{2+}, phosphatidylserine and diacylglycerol [82]. Partially purified cytosolic PKC activity was enhanced 60–70 % by 13.5 μM palmitoyl- or oleoyl-CoA in the absence of diacylglycerol. Long-chain acyl-CoA esters (20–30 μM) were found to enhance the activity of PKC purified from rat brain, with or without added diacylglycerol but in the presence of phosphatidylserine and Ca^{2+}; the effect was less pronounced in the absence of diacylglycerol [83]. Whether the effect of acyl-CoA on PKC is direct due to binding of acyl-CoA to the enzyme, or indirect through a stimulation of the synthesis of diacylglycerol [84], or is caused by a change in membrane properties due to the accumulation of acyl-CoA, is unknown.

Of the three major PKC isotypes in neutrophils (PKC-β, PKC-ζ and PKCn), only PKCn was affected by 1–10 μM long-chain acyl-CoA [85]. This enzyme was significantly inhibited by acyl-CoA esters, but not by the corresponding fatty acids. Activation of neutrophils with formyl-Met-Leu-Phe triggered within 5 s a 2-fold increase in the concentration of long-chain acyl-CoA which lasted for 120 s; this was inhibited by the acyl-CoA synthetase inhibitor Triascin C [85,86]. Physiological concentrations of long-chain acyl-CoA (1–10 μM) also enhanced formyl-Met-Leu-Phe- and guanosine 5′-[γ-thio]triphosphate-triggered O_2^- generation in permeabilized neutrophils, which could be reversed by Triascin C.

Regulation of gene expression by long-chain acyl-CoA esters

In *E. coli*, fatty acid biosynthesis and degradation are co-ordinately regulated at a transcriptional level by the product of the *fadR* gene, FadR [87,88]. FadR was first identified due to its ability to repress genes encoding proteins required for the transport and degradation of medium- and long-chain fatty acids. These include the *fadL*, *fadD*, *fadE*, *fadA*, *fadB* and *fadH* genes [87,89–93]. In contrast, FadR activates the expression of the *fabA* gene, which encodes β-hydroxydecanoyl thioester dehydrase, involved in the biosynthesis of unsaturated fatty acids [93]. Growth of wild-type *E. coli* cells in minimal medium supplemented with long-chain fatty acids results in induction of *fad* structural genes [89]. This observation led to the suggestion that long-chain fatty acids or a derivative thereof induces the *fad* genes by interacting with FadR to prevent DNA binding and thus inhibiting transcription of the specific gene.

Using DNA–protein gel-retention assays, DiRusso et al. [87] demonstrated that binding of purified FadR to DNA containing the *fadB* promoter was prevented by long-chain acyl-CoA esters,

but not by short-chain acyl-CoA esters or fatty acids. The K_i values for palmitoyl-CoA and oleoyl-CoA were approx. 5 nM, and those for myristoyl-CoA and decanoyl-CoA were 250 nM and 2 μM respectively. These data provide strong evidence that long-chain acyl-CoA esters bind to FadR and thereby inhibit its DNA binding activity. A direct interaction between long-chain acyl-CoA and FadR was shown using a fluorescence quenching assay, and the K_d for binding of oleoyl-CoA to FadR was determined as 12.1 nM [94].

It has been shown that the *S. cerevisiae OLE1* gene, which encodes the Δ^9-desaturase, is repressed when cells are grown in media supplemented with Δ^9-unsaturated fatty acids [95,96], and that deletion of the two fatty acid-activating enzymes, FAA1 and FAA4, blocks repression of this gene by Δ^9-unsaturated fatty acids [50]. This, and the observation that mutation of one of the acyl-CoA synthetases in *S. cerevisiae* substantially decreases the fatty acid-induced repression of acetyl-CoA carboxylase [49], indicates that acyl-CoA esters may also be involved in gene regulation in yeast.

Both saturated and unsaturated long-chain acyl-CoA esters were demonstrated to inhibit the binding of tri-iodothyronine to its nuclear receptor in rat liver ($K_i \sim 0.45$ μM) [97] more efficiently than did the corresponding fatty acids [98]. Furthermore, oleoyl-CoA did not affect ligand binding to the glucocorticoid receptor, indicating that the effect of acyl-CoA on the tri-iodothyronine receptor is specific. Tri-iodothyronine induces lipogenic enzymes [99] and increases liver fatty acid synthesis, which is correlated inversely with the serum fatty acid level [100]. This could indicate that acyl-CoA may contribute to the transcriptional regulation of lipogenic enzymes in a feedback manner, by displacing tri-iodothyronine from its receptor and hence inhibiting transcription of genes encoding lipogenic enzymes [101,102]

INTRACELLULAR ACYL-CoA BINDING PROTEINS (ACBPs)

Ever since it was noted that fatty acid binding proteins (FABPs) bind acyl-CoA esters [103–108], FABPs have been suggested to play an important role in the metabolism and transport of long-chain acyl-CoA esters [109]. However, the identification of a novel cytoplasmic high-affinity ACBP [110–114] has added a new dimension to our understanding of the regulation of metabolism and transport of long-chain acyl-CoA esters and their role as second messengers in signal transduction and gene regulation.

A new look at FABPs in acyl-CoA metabolism

FABPs constitute a family of proteins with molecular masses of 14–15 kDa that are abundantly present in the cytoplasm of tissues involved in the uptake or utilization of fatty acids. All FABP types bind long-chain fatty acids with K_d values in the range 0.1–1.0 μM [103–108]. The binding stoichiometry is 1 mol of ligand per mol of protein, except for liver FABP which binds 2 mol of long-chain fatty acid or long-chain acyl-CoA per mol. Liver FABP, which also binds long-chain acyl-CoA esters with a K_d of 1 μM [108], appears to function as a general hydrophobic anionic ligand-binding protein, whereas the other FABP types evidently are more specific and preferably bind long-chain fatty acids and acylcarnitine esters. FABPs bind acylcarnitine esters with K_d values of the same order of magnitude as for fatty acids [115].

FABPs have been shown to enhance the activity of several microsomal acyl-CoA-utilizing enzymes involved in cholesterol ester, phospholipid and triacylglycerol synthesis, e.g. acyl-CoA:glycerol-3-phosphate acyltransferase [116–118], diacylglycerol acyltransferase [119,120], lysophosphatidic acid acyltransferase [121] and acyl-CoA:cholesterol acyltransferase [122,123]. Rat liver FABP appears to have both stimulatory and inhibitory effects on both mitochondrial and microsomal acyl-CoA synthetases [117,124–126]. Rat liver FABP is able to reverse the inhibitory effect of acyl-CoA on the mitochondrial ANT [127]. FABP has also been shown to reverse the inhibitory effect of palmitoyl-CoA on acetyl-CoA carboxylase activity [128]. To what extent FABP is able to bind and transport long-chain acyl-CoA esters under *in vivo* conditions is not known, but the inability of liver FABP to release product inhibition of the mitochondrial acyl-CoA synthetase *in vitro* [129] questions the extent to which FABP is able to play a role in acyl-CoA metabolism *in vivo*.

The identification of a specific high-affinity ACBP which binds acyl-CoA esters with an affinity 3–4 orders of magnitude higher than that of FABP requires that the role of FABPs in acyl-CoA metabolism and transport be reconsidered.

Role of ACBP in acyl-CoA metabolism and acyl-CoA-mediated cell signalling

ACBP was originally discovered as an impurity in a FABP preparation [110]. ACBP is a widely distributed cytosolic protein of molecular mass 10 kDa that has been found in all eukaryotes tested, from plants to humans [130], and has been identified in liver, adipose tissue, kidney, heart, brain, intestine, skeletal muscle, mammary gland [111], erythrocytes [131] duodenum, testis, adrenal gland, ovary, lung and spleen [132,133]. Recently an ACBP isoform (endozepine-like peptide) was identified in mouse testes [134]. The highest concentration of ACBP is found in liver, where it is evenly distributed in all hepatocytes [133]. In other tissues ACBP is reported to be be present at high concentrations in specialized cells such as steroid-producing cells of the adrenal cortex and testis, and in epithelial cells specialized in secretion and in water and electrolyte transport, which are all characterized by high energy metabolism. In *Drosophila melanogaster*, ACBP has been found primarily expressed in tissues that are associated with high energy production or fat metabolism [135]. ACBP has been purified, cloned and sequenced from a large number of different species and tissues, and shows a high degree of similarity among the different species (Figure 2). The broad range of distribution throughout the animal and plant kingdoms, and the high degree of similarity among the different tissues and species, suggest that ACBP is a housekeeping protein. This was further supported by Mandrup et al. [136], who demonstrated that the gene of ACBP has all the characteristics of a housekeeping gene.

ACBP binds medium- and long-chain acyl-CoA esters with very high affinity, with a preference for C_{14}–C_{22} acyl-CoA esters [137–139]. ACBP shows only a low affinity for CoA (K_d 2 μM), and does not bind fatty acids, acylcarnitines, cholesterol and a number of nucleotides [137]. Employing isothermal microcalorimetry, the K_d values for palmitoyl-CoA binding to yeast and bovine ACBPs were determined to be 0.6×10^{-10} M and 0.5×10^{-13} M by direct and indirect titration microcalorimetry respectively [138,140]. The reason for using indirect microcalorimetry for measurement of palmitoyl-CoA binding to bovine ACBP was that the binding affinity was too high to be determined by direct microcalorimetry. The K_d value for palmitoyl-CoA binding obtained by indirect titration of the octanoyl-CoA–ACBP complex with palmitoyl-CoA was calculated using the method of Sigurskjold et al. [141]. However, calculation of the dissociation constant from the same data using a different method published by Hu and Eftink [142] yields a dissociation constant of 2 nM. A near-linear relationship between chain length and

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Human-1      WGDLWLLPPASANOGTGTEAEFEKAAEEVRHLKTKPSDEEMLFIYGHYKQATVGDIN-TER  60
Human-2      ----------------SQAEFEKAAEEVRHLKTKPSDEEMLFIYGHYKQATVGDIN-TER  43
Rat          ----------------SQADFDKAAEEVKRLKTQPTDEEMLFIYSHFKQATVGDVN-TDR  43
Mouse        ----------------SQADFDKAAEEVKRLKTQPTDEEMLFIYSHFKQATVGDVN-TDR  43
Bovine       ----------------SQAEFDKAAEEVKHLKTKPADEEMLFIYSHYKQATVGDIN-TER  43
Pig          ----------------SQAEFEKAAEEVKNLKTKPADDEMLFIYSHYKQATVGDIN-TER  43
Dog          ----------------SQAEFDKAAEDVKHLKTKPADDEMLYIYSHYKQATVGDIN-TER  43
Tortoise     ----------------SQAEFDKAAEEVKQLKSQPTDEEMLYIYSHFKQATVGDIN-TER  43
Duck         ----------------AEAAFQKAAEEVKQLKSQPSDQEMLDVYSHYKQATVGDVN-TDR  43
Chicken      ----------------SEAAFQKAAEEVKELKSQPTDQEMLDVYSHYKQATVGDVN-TDR  43
Frog         ---------------MSPQADFDKAAGDVKKLKTKPTDDELKELYGLYKQSTVGDINIEC-  45
M. secta     ---------------MSLQAQFDQAASNVRNLKSLPSDNDLLELYALFKQASAGDADPANR  46
Drosophila   ---------------MVSE-QFNAAAEKVKSLTKRPSDDEFLQLYALFKQASVGDND-TAK  43
Yeast-1      ----------------VSQ-LFEEKAKAVNELPTKPSTDELLELYALYKQATVGDND-KEK  43
Yeast-2      ----------------VSQ-LFEEKAKAVNELPTKPSTDELLELYGLYKQATVGDND-KEK  43
B. napus     ---------------MGLKEDFEEHAEKVKKLTASPSNEDLLILYGLYKQATVGPVT-TSR  45
A. thaliana  ---------------MGLKEEFEEHAEKVNTLTELPSNEDLLILYGLYKQAKFGPVD-TSP  45
Cotton       ---------------MGLKEEFEEHAEKVKTLPAAPSNDDMLILYGLYKQATVGPVNT-SR  44
ELP          ----------------MSQVEFEMACASLKQLKGPVSDQEKLLVYSFYKQATQGDCNIP-V  44

Human-1      ---------------PGMLDFTGKAKWDAWNELKGTSKEDAMKAYINKVEELKKKYGI-  103
Human-2      ---------------PGMLDFTGKAKWDAWNELKGTSKEDAMKAYINKVEELKKKYGI-   86
Rat          ---------------PGLLDLKGKAKWDSWNKLKGTSKENAMKTYVEKVEELKKKYGI-   86
Mouse        ---------------PGLLDLKGKAKWDSWNKLKGTSKESAMKTYVEKVDELKKKYGI-   86
Bovine       ---------------PGMLDFKGKAKWDAWNELKGTSKEDAMKAYIDKVDELKKKYGI-   86
Pig          ---------------PGILDLKGKAKWDAWNGLKGTSKEDAMKAYINKVDELKKKYGI-   86
Dog          ---------------PGLLDLRGKAKWDAWNQLKGTSKEDAMKAYVNKVDELKKKYGI-   86
Tortoise     ---------------PGFLDFKGKAKWDAWDALKGMAKEEAMKAYIAKVEELKGKYGI-   86
Duck         ---------------PGMLDFKGKAKWDAWNALKGMSKEDAMKAYVAKVEELKGKYGI-   86
Chicken      ---------------PGMLDFKGKAKWDAWNALKGMSKEDAMKAYVAKVEELKGKYGI-   86
Frog         ---------------PGMLDLKGKAKWDAWNLKKGLSKEDAMSAYVSKAHELIEKYGL-   88
M. secta     ---------------PGLLDLKGKAKFDAWHKKAGLSKEDAQKAYIAKVESLIASLGLQ   90
Drosophila   ---------------PGLLDLKGKAKWEAWNKQKGKSSEAAQQEYITFVEGLVAKYA--   86
Yeast-1      ---------------PGIFNMKDRYKWEAWENLKGKSQEDAEKEYIALVDQLIAKYSS-   86
Yeast-2      ---------------PGIFNMKDRYKWEAWENLKGKSQEDAEKEYIAYVDNLIAKYSS-   86
B. napus     ---------------PGMFSMKERAKWDAWKAVEGKSTDEAMSDYITKVKQLLEAEASSASA  92
A. thaliana  ---------------PGMFSMKERAKWDAWKAVEGKSSEEAMNDYITKVKQLLEVAASKAST  92
Cotton       ---------------PGMFNMREKYKWDAWKAVEGKSKEEAMGDYITKVKQLFEAAGSS    89
ELP          ---------------PPATDVRAKAKYEAWMVNKGMSKMDAMRIYIAKVEELKKKEPC-    87
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Figure 2 Comparison of amino acid sequences of ACBPs from 16 different species

Identical residues are emboldened. Sources: human-1 [171], human-2 [172], rat [112], mouse [173], bovine [113], pig [174], dog, tortoise, duck, chicken, *Arabidopsis thaliana* [130], frog [175], *Manduca sexta* [130], *Drosophila melanogaster* [135], yeast-1, yeast-2 [140], *Brassica napus* [176], cotton [177] and endozepine-like peptide (ELP) [134].

relative binding affinity was observed with acyl-CoA esters up to a chain length of C_{18} using EPR spectroscopy [137] and up to a chain length of C_{14} using direct titration microcalorimetry [139]. Calculation of the dissociation constant for palmitoyl-CoA binding to ACBP by linear extrapolation of the microcalorimetry data yields a K_d of approx. 0.5 nM. These values are in good agreement with the value (5 nM) obtained using a membrane partitioning assay (John R. Silvius, personal communication). Increasing the salt concentration reduces the binding of dodecanoyl-CoA to ACBP about 10-fold [139]. The binding affinities also depend on the buffer used. Re-estimation by microcalorimetry of the affinity of binding of bovine ACBP to palmitoyl-CoA in 25 mM ammonium acetate (pH 6.0)/150 mM NaCl or in 20 mM Mops/10 mM potassium phosphate (pH 7.2)/100 mM KCl/20 mM NaCl/3.5 mM $MgCl_2$/3 mM ATP yielded K_d values of 0.4 nM and 2 nM respectively (J. Knudsen, unpublished work). However, it should be noted that these binding affinities are at the upper limit of what can be determined by direct titration calorimetry.

A number of experimental results obtained *in vitro* and *in vivo* clearly indicate that ACBP is able to act as an intracellular acyl-CoA transporter and pool former. *In vitro*, ACBP has a strong attenuating effect on the inhibition of acetyl-CoA carboxylase and of mitochondrial ANT by long-chain acyl-CoA [129]. In addition, it readily protects acyl-CoA against hydrolysis by microsomal hydrolases and stimulates mitochondrial long-chain acyl-CoA synthetase [129]. ACBP was found to desorb acyl-CoA esters immobilized in multilamellar liposomes on a nitrocellulose membrane, and was able to transport and donate acyl-CoA to mitochondrial β-oxidation and to microsomal glycerolipid synthesis. Overexpression of either bovine or yeast ACBP in *S. cerevisiae* led to an increased concentration of intracellular acyl-CoA, indicating that ACBP is able to act as an acyl-CoA pool former *in vivo* [140,143].

Compelling evidence that ACBP participates in acyl-CoA transport *in vivo* has been obtained from yeast. Disruption of the ACBP gene in *S. cerevisiae* results in a dramatic perturbation of the acyl-CoA level and composition [144]. The levels of total acyl-CoA and stearoyl-CoA were increased 2.5- and 7.0-fold respectively. Despite this, the Δ^9-desaturase mRNA level in the

ACBP knock-out strain was increased 3-fold, and no changes in the synthesis of monounsaturated fatty acids or in the overall fatty acid composition in the knock-out strain could be observed. These results strongly suggest that the increased stearoyl-CoA pool in the ACBP knock-out strain was not available to the Δ^9-desaturase, and that the ACBP knock-out strain has a defect in intracellular acyl-CoA transport.

INTRACELLULAR ACYL-CoA CONCENTRATIONS

The total cellular concentration of long-chain acyl-CoA esters has been reported to be in the range 5–160 μM, depending on the tissue and its metabolic state (Table 1). The levels of acyl-CoA esters are found to vary significantly in different metabolic conditions such as fasting [1,145,146], diabetes [145], fat/glucose feeding [145] and ingestion of hypolipidaemic drugs [146–148]. For example, in 48 h-fasted rats the total concentration of acyl-CoA esters has been found to be increased at least 2–4-fold [1,146].

The compartmentation of long-chain acyl-CoA esters is an important unresolved issue, and the actual free cytosolic concentration of long-chain acyl-CoA esters is not known for any tissue. Only a few attempts to estimate the intracellular distribution of long-chain acyl-CoAs have been reported [23,36,129]. The concentration of long-chain acyl-CoA in rat adipocyte mitochondria was determined to be 205 μM in the fed state, and to be increased upon 24 h starvation to 258 μM. However, that study did not yield any information on the purity of the mitochondria used [36]. Using hearts from both control and mildly ischaemic rats, Idell-Wenger et al. [23] found that 95 % of total free and esterified cellular CoA was mitochondrial. Likewise, Kobayashi and Fujisawa [149] found 92 % of the cellular long-chain acyl-CoA to be located in the mitochondria in dog heart. From the original data of Rasmussen et al. [129] it can be calculated that the long-chain acyl-CoA concentration in rat liver mitochondria is 230 μM, which constitutes only 15 % of the total long-chain acyl-CoA. It has been suggested that 20–40 % of the total acyl-CoA pool is cytosolic [150]. Deeney et al. [72] estimated the cytosolic long-chain acyl-CoA level in a clonal β-cell line to constitute approx. 78 % of the total long-chain acyl-CoA, giving a cytosolic concentration of 90 μM. Sensitive methods for readily estimating the relative distributions of acyl-CoA esters in different cellular compartments are not currently available. A major problem in this context is the instability of acyl-CoA in cell homogenates due to the presence of acyl-CoA hydrolases and CPTs. An additional problem is that most methods that are available for the determination of acyl-CoA concentrations in tissues are based on the determination of CoA released from acid-precipitated long-chain acyl-CoA, and therefore may not give information on the nature of the individual acyl-CoA species present. A further complication is that the existing methods of acyl-CoA analysis yield different results [151]. Extensive comparisons of HPLC methods and the enzymic method in our laboratory have shown that the enzymic method overestimates the cellular acyl-CoA concentration approx. 2-fold.

REGULATION OF THE INTRACELLULAR ACYL-CoA CONCENTRATION

The concentrations of ACBP and long-chain acyl-CoA in fed rat liver have been determined to be 40–50 nmol/g and 40–60 nmol/g of tissue respectively [129,146], indicating that the acyl-CoA/ACBP ratio might be close to unity. Calculation of the concentration of free long-chain acyl-CoA under these conditions, assuming that long-chain acyl-CoA esters bind to ACBP with a K_d of 1 nM and that the intracellular ACBP concentration is

Table 1 Concentrations of long-chain acyl-CoA esters in different tissues, cell types and organelles

LC-acyl-CoA, long-chain acyl-CoA.

Tissue/cell type/organelle	Concentration (μM)	Measured compound	Reference
Rat liver	18.8 (fed state)	CoA by HPLC	[1]*
	53.9 (fed state)	LC-acyl-CoA by HPLC	[178]*
	64.4 (fed state)	LC-acyl-CoA by HPLC	[179]*†
	55.0 (cytosolic)	LC-acyl-CoA by HPLC	[129]*†
	230 (mitochondrial)	LC-acyl-CoA by HPLC	[129]*†
	23.6 (fed state)	LC-acyl-CoA by HPLC	[180]*
	54.6 (48 h-fasted)	LC-acyl-CoA by HPLC	[180]*
	60.0 (fed state)	CoA by HPLC	[146]*
	164 (48 h-fasted)	CoA by HPLC	[146]*
Rat kidney	29.8 (fed state)	LC-acyl-CoA by HPLC	[179]*
Rat heart	27.4 (fed state)	LC-acyl-CoA by HPLC	[179]*
Rat skeletal muscle	12.3 (fed state)	LC-acyl-CoA by HPLC	[179]*
Rat brain	33.1 (fed state)	LC-acyl-CoA by HPLC	[179]*
Neutrophils	5–10	Enzymic measurement of CoA	[85]
Pancreatic β-cells	25 (nutrient-stimulated)	Enzymic measurement of CoA	[75]
	100 (unstimulated)	Enzymic measurement of CoA	[75]
	115	Enzymic measurement of CoA	[72]‡
	90 (cytosolic)	Enzymic measurement of CoA	[72]‡
Mitochondria (rat adipocytes)	205 (fed state)	Enzymic measurement of CoA	[36]†
	258 (24 h-fasted)	Enzymic measurement of CoA	[36]†

* Original data converted from nmol/g wet weight into μM, assuming that 80% of the tissue was water [181].

† Mitochondrial acyl-CoA concentrations were changed from nmol/mg of protein (original data) to matrix concentration (μM) based on a mitochondrial matrix space of 1 μl/mg of mitochondrial protein [23].

‡ Original data converted into μM based on the assumptions that the cytosolic water space is 2 ml/g of protein and that the cytosol represents 78% of the cellular total [72].

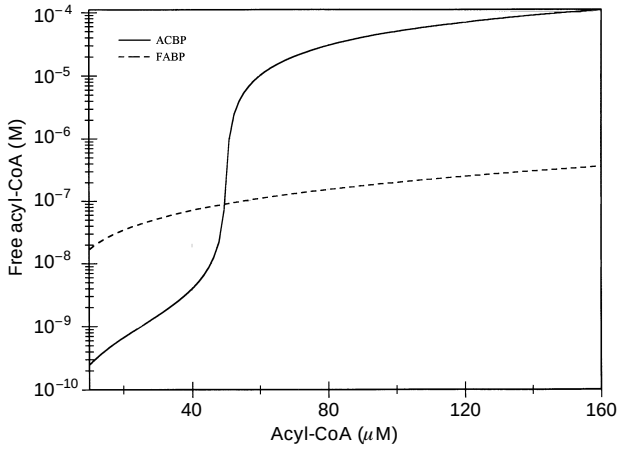

Figure 3 Calculation of the free concentration of acyl-CoA esters in the presence of ACBP or FABP

Calculations are based on the presence of 50 μM ACBP and 0.3 mM liver FABP (0.6 mM binding sites), and K_d values for the binding of long-chain acyl-CoA esters of 1 nM (ACBP) and 1 μM (FABP).

50 μM, is shown in Figure 3. At acyl-CoA/ACBP molar ratios below 1, the calculated free concentration of long-chain acyl-CoA is < 10 nM. As the ratio approaches 1, the concentration of unbound acyl-CoA will increase dramatically, and will approach the total acyl-CoA concentration when the ratio exceeds 1. At this point FABP will be expected to take over the buffering function. Liver FABP binds long-chain acyl-CoA with a K_d of approx. 1.0 μM [108]. Assuming that the maximal obtainable acyl-CoA concentration in liver cytosol is 150 μM and that the cytosolic FABP concentration is 0.3 mM (0.6 mM binding sites) [152], the calculated free concentration of acyl-CoA will never be expected to exceed 0.2 μM.

However, long-chain acyl-CoA esters will be expected to bind to a number of other proteins in the cell, including the high-affinity binding site on acyl-CoA synthetase and acyl-CoA-utilizing enzymes. Furthermore, by screening a bovine brain cDNA library using a degenerate ACBP oligonucleotide probe, Webb et al. [153] isolated a related clone encoding a 533-amino-acid protein containing an ACBP-like domain, termed bovine brain factor. Based on the amino acid sequence, it was suggested that this protein could be associated with either the cell surface or the mitochondrial membrane [154]. It is tempting to speculate that membrane-associated bovine brain factor binds fatty acyl-CoA with high affinity, and thus by competing with the cytosolic ACBP is able to create a local pool of membrane-bound acyl-CoA esters. In addition, long-chain acyl-CoA esters readily partition into membranes. The partition constant for palmitoyl-CoA into phospholipid vesicles can be calculated from the original data of Peitzsch and McLaughlin [5] as 1.5×10^5 M^{-1}, and a value of 5×10^5 M^{-1} has also been determined [7]. If it is assumed that these value can be applied to *in vivo* conditions, the calculated free concentration of acyl-CoA in a liver cell in the absence of binding proteins would be about 1 μM. The cytosolic concentration of long-chain acyl-CoA in a liver cell *in vivo* can therefore be expected to be well below 0.2 μM and never to exceed 1 μM.

An additional control factor for assuring low concentrations of long-chain acyl-CoA under *in vivo* conditions is the high activity of acyl-CoA hydrolases found in most subcellular compartments [155–158]. These enzymes include short-, medium- and long-chain acyl-CoA hydrolases, but our knowledge of the physiological role of these enzymes is sparse, although termination of fatty acid synthesis has been ascribed to a medium-chain acyl-CoA hydrolase in the mammary gland [159,160]. The acyl-CoA hydrolases usually display K_m values ranging from 0.1 to 6 μM for long-chain acyl-CoA esters [155,161,162]. The sizes of the different acyl-CoA pools have been postulated to be

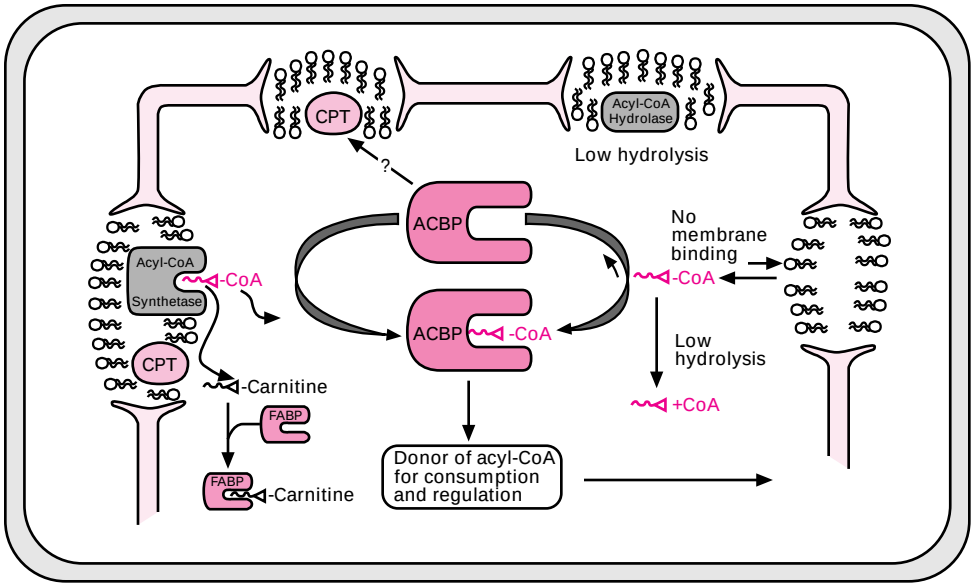

Figure 4 Role of ACBP in long-chain acyl-CoA ester pool formation

ACBP is able to stimulate acyl-CoA synthetase activity and to prevent hydrolysis and membrane partitioning of long-chain acyl-CoA esters, and thereby form a pool of long-chain acyl-CoA that is available for specific purposes.

Table 2 Proposed effects of acyl-CoA in cellular regulation and signal transduction

TG, triacylglycerol; CE, cholesteryl ester; ER, endoplasmic reticulum.

Acyl-CoA regulation of:	Effect	Effect of binding protein	Refs.
Lipid metabolism			
Fatty acid synthesis			
Acetyl-CoA carboxylase	Inhibitory; K_i 5.5 nM	Reversed by ACBP and FABP	[51,129]
AMP-activated kinase kinase	Stimulatory; nanomolar range		[54]
Mitochondrial acyl-CoA synthetase	Inhibitory; K_i 4 μM	Reversed by ACBP	[129,164]
Citrate transporter	Inhibitory; K_i 32 μM		[57]
TG/phospholipid/CE synthesis			
HMG-CoA reductase	Inhibitory; $K_i \leqslant 1.9$ μM		[55]
β-Oxidation			
CPT I	Inhibitory; $K_i \sim 20$ μM		[182]
Long-chain acyl-CoA dehydrogenase	Inhibitory; K_i 0.2 μM		[47]
CE/TG hydrolysis			
Hormone-sensitive lipase	Inhibitory; K_i 0.5 μM		[56]
Energy metabolism			
ANT	Inhibitory; $K_i < 1$ μM	Reversed by ACBP and FABP	[17,127,129]
Glucokinase	Inhibitory; K_i 0.5 μM		[28,29]
Glucose-6-phosphatase	Inhibitory; K_i 50 μM		[30]
Pyruvate dehydrogenase	Inhibitory; K_i 30 μM		[36,57]
Signal transduction			
Ion channels/pumps			
Ca^{2+} release from sarcoplasmic reticulum	Stimulatory; EC_{50} 6 μM	Potentation by acyl-CoA/ACBP*	[77,80]
Ca^{2+} release from sea urchin eggs	Stimulatory		[78]
GTP-dependent Ca^{2+} release from ER	Inhibitory; K_i 0.5 μM		[67]
Ca^{2+}-induced Ca^{2+} accumulation	Inhibitory; $K_i \sim 3$ μM		[67]
Ca^{2+} release from ER ($Ins P_3$-insensitive)	Stimulatory; EC_{50} 50 μM		[81]
Ca^{2+} re-uptake by Ca^{2+}-ATPases	Stimulatory; EC_{50} 0.5 μM		[72]
$\Delta\Psi$ and Mg^{2+} in mitochondria	Inhibitory		[183]
Plasma-membrane Na^+/K^+-ATPase	Stimulatory; EC_{50} 3 μM		[184]
pH-dependent anion-conductance channel	Inhibitory; K_i 2.4 μM		[27]
ATP-sensitive K^+ channel	Stimulatory		[74]
PKC subtypes			
Ca^{2+}/phospholipid/diacylglycerol-dependent PKC	Stimulatory; $EC_{50} \leqslant 15$ μM		[83]
PKCn	Inhibitory; $K_i < 10$ μM		[85]
Gene expression			
Nuclear thyroid hormone receptor	Inhibitory; K_i 0.12 μM		[97,98]
FadR (*E. coli* transcription factor)	Inhibitory; K_i 5 nM		[87]
Protein sorting			
Vesicular transport in Golgi apparatus	Stimulatory		[63,64]
GTP-dependent vesicle fusion in ER	Inhibitory		[65]
Proteolysis			
Proline endopeptidase	Inhibitory; K_i 9 μM		[185]

* R. Fulceri, J. Knudsen and A. Benedetti, unpublished work.

regulated by acyl-CoA hydrolases [163]. Some acyl-CoA hydrolases have been shown to respond to metabolic stresses, e.g. by showing increased activity after ingestion of hypolipidaemic drugs; this treatment also resulted in an increased acyl-CoA level in rat liver [147]. Moore et al. [36] have shown that acyl-CoA hydrolase activity in rat adipocytes decreased upon starvation, while the acyl-CoA level increased. Whether the intracellular acyl-CoA concentration is regulated by acyl-CoA hydrolase activity is not known. Nevertheless, it is very likely that acyl-CoA hydrolases could act as 'scavengers' if the free long-chain acyl-CoA pool increases to micromolar concentrations. Furthermore, large fluctuations in the concentration of long-chain acyl-CoA esters in the cell will be prevented by the fact that the acyl-CoA synthetase is product-inhibited by long-chain acyl-CoA esters, e.g. palmitoyl-CoA is inhibitory with a K_i of 4 μM [164].

The most prominent intracellular fatty-acyl derivatives are the acylcarnitine esters, and evidence for a direct link between the long-chain acyl-CoA pool and the long-chain acylcarnitine pool was provided by Arduini et al. [165]. Long-chain carnitine acyltransferases constitute a family of enzymes present in the outer membrane of mitochondria, peroxisomes and endoplasmic reticulum (reviewed in [166]). The equilibrium constant for CPT I has been reported to be approx. 1 [166]. It must, therefore, be expected that there will be a free flow of acyl chains between the acyl-CoA and acylcarnitine pools, and the acylcarnitine pool will in this way act as buffer for activated acyl chains *in vivo* (Figure 4).

In rat heart the intracellular cytosolic concentrations of carnitine and free CoA are approx. 2.5 mM and 0.014 mM respectively, and the cytosolic acylcarnitine concentration is 25 μM [23].The K_d for palmitoylcarnitine binding to FABP is in the range 0.4–0.6 μM [107]. The calculated free concentration of acylcarnitine under *in vivo* conditions in the presence of FABP will, therefore, be in the nanomolar range, i.e. the same range as the concentration of free acyl-CoA (2–200 nM). It is therefore

not unlikely that the acylcarnitine pool can act as a sink for activated fatty acids *in vivo*. The observation that acyl-CoA and acylcarnitine levels increase proportionally in both ischaemic and hypoxic rat hearts [167] supports this suggestion. Parallel increases in the acyl-CoA and acylcarnitine concentrations were observed both in the mitochondria and in the cytosol from rat heart [23].

If the total concentration of long-chain acyl-CoA exceeds the binding capacity of ACBP, long-chain acyl-CoA and acylcarnitine will start competing with each other for binding sites on FABP. Assuming that the total concentrations of cytosolic acylcarnitine and long-chain acyl-CoA are 0.4 mM and 150 μM respectively, then even under the most extreme conditions (Table 1) the calculated free concentration of long-chain acyl-CoA will not exceed 1 μM if the FABP and ACBP concentrations are 0.3 mM and 50 μM respectively. At this point the activity of the acyl-CoA hydrolases will be expected to be very high, preventing further increases in the intracellular concentration of long-chain acyl-CoA.

REGULATION OF PHYSIOLOGICAL FUNCTIONS *IN VIVO* BY LONG-CHAIN ACYL-CoA

Taking all of the above considerations into account, we conclude that the intracellular free acyl-CoA concentration will be in the range 0.1–200 nM under normal physiological conditions. If the cytosolic ACBP/acyl-CoA ratio stays below 1, the free acyl-CoA concentration will be in the range 2–10 nM. The fact that fatty acid synthesis occurs despite the fact that the K_i for acetyl-CoA carboxylase is 5.5 nM strongly indicates that the free concentration of liver cytosolic long-chain acyl-CoA is below 5.5 nM under these conditions.

Immunohistochemical studies in yeast show that ACBP is not found in mitochondria and peroxisomes (C. Børsting, R. Hummel, A. Stoop, M. van den Berg, H. F. Tabak, J. Knudsen and K. Kristiansen, unpublished work). It cannot be excluded, therefore, that large local changes in the free concentration of long-chain acyl-CoA esters may occur, for example in the mitochondria, where the total level of acyl-CoA can increase to extremely high levels (1 mM) [23], and processes and enzymes such as β-oxidation, the ANT, the citrate transporter and pyruvate dehydrogenase could be inhibited. However, mitochondria also contain acyl-CoA hydrolases, and this situation is therefore unlikely to occur [168].

If the total free acyl-CoA concentration under normal physiological conditions is well below 200 nM, and most likely below 10 nM, the role of acyl-CoA as a physiological regulator of the cellular processes shown in Table 2 will be expected to be limited to the regulation of acetyl-CoA carboxylase, the AMP-activated kinase kinase and gene expression in *E. coli*, unless acyl-CoA can be donated directly from a binding protein (ACBP and perhaps FABP) to the proteins in question.

That this indeed might occur is suggested by the observation that the acyl-CoA–ACBP complex at molar ratios below 1 can donate acyl-CoA for β-oxidation [138] to the acyl-CoA:lysophospholipid acyltransferase in red blood cells [131], and can regulate sarcoplasmic ryanodine-sensitive Ca^{2+}-release channels (R. Fulceri, J. Knudsen and A. Benedetti, unpublished work). This situation is not unique for ACBP-bound ligands. It has recently been demonstrated that the retinal/cellular retinol-binding-protein complex, rather than free retinal, is the preferred substrate for lecithin:retinol acyltransferase [169] and for the microsomal retinol dehydrogenase [170].

The ability of the acyl-CoA–ACBP complex to donate acyl-CoA to acyl-CoA-utilizing or -regulated systems is not universal. Acetyl-CoA carboxylase was completely protected against inhibition by acyl-CoA at all concentrations up to 5 μM at acyl-CoA/ACBP ratios below 0.8 [138]. It is therefore tempting to speculate that ACBP, by binding long-chain acyl-CoA, creates a pool of long-chain acyl-CoA that is available for specific purposes only (Figure 4).

Most of the reported effects of long-chain acyl-CoAs shown in Table 2 have been obtained by the addition of acyl-CoA directly to the system in question, without the presence of any kind of ACBP to buffer the concentration and prevent membrane binding of acyl-CoA. Partitioning of acyl-CoA esters into membranes or non-specific binding to proteins in the absence of ACBPs could result in extremely high local concentrations, making the result meaningless. Re-investigation of the regulatory role of acyl-CoA in these systems, in the presence of physiological concentrations of ACBP and FABP, is therefore urgently required.

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