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# Carnitine Palmitoyltransferase-I and Regulation of Mitochondrial Fatty Acid Oxidation

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**Summary.** Carnitine palmitoyltransferase-I catalyzes the regulated step in overall mitochondrial fatty acid oxidation. The enzyme is controlled by the steady state level of malonyl-CoA and the enzyme's sensitivity to inhibition by malonyl-CoA. The former is established by the activities of acetyl-CoA carboxylase 2 and malonyl-CoA decarboxylase, while the latter is influenced by post-translational modification (phosphorylation) of CPT-I and/or by changes in the lipid environment of CPT-I.

**Keywords.** Carnitine palmitoyltransferase; Malonyl-CoA; Fatty acids; Lipids; Phosphorylations.

## Introduction

On the occasion of the 100 year anniversary of the discovery of carnitine it seems appropriate to briefly recapitulate the milestones in carnitine research from its discovery to its function.

Carnitine is ubiquitous in nature and is most abundant in muscle from where it was isolated a century ago by *Gulewitsch* and *Krimberg* [1] and *Kutscher* [2]. Its correct structure was determined by *Tomita* and *Sendju* twenty two years later and shown to be *L*-(-)-carnitine [3]. Efforts to determine the physiological function of carnitine were stimulated by the discovery of the neurotransmitter function of acetylcholine. The structural similarity between carnitine (carboxymethyl choline) and choline and acetylcholine, inspired researchers to look for a similar pharmacological role for carnitine [4–7]. While some carnitine compounds gained limited pharmacological significance, the physiological role of carnitine remained unidentified.

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The breakthrough in the elucidation of the physiological function of carnitine came in the 1950s when it was discovered that, (a) carnitine is necessary for fat breakdown in the larvae of the meal worm, *Tenebrio molitor* [8], (b) pigeon liver extract catalyzes the reversible enzymatic acetylation of carnitine with acetyl-CoA [9], and (c) muscle extracts stimulate the oxidation of palmitic acid in liver and the stimulatory factor is carnitine [10]. These discoveries as well as the localization of $\beta$-oxidation within the mitochondrial matrix suggested that carnitine plays a role in transport of activated fatty acids. Indeed, shortly thereafter this carrier function of carnitine was substantiated by the observations (a) that mitochondria isolated from different organs oxidize acetyl- and long-chain acylcarnitines [11, 12], (b) the formation of palmitoylcarnitine by isolated mitochondria is CoA dependent [13], and (c) the oxidation of palmitoyl-CoA is carnitine dependent with palmitoylcarnitine being the obligatory intermediate [14]. In accordance, functional studies on isolated mitochondria identified an outer form of carnitine palmitoyltransferase (CPT-A, CPT-I) that is easily removed with digitonin and an inner form of carnitine palmitoyltransferase (CPT-B, CPT-II) [15–17]. Furthermore, mitochondria devoid of the outer form are no longer able to catalyze the carnitine dependent oxidation of palmitoyl-CoA, yet have retained their full capacity to oxidize palmitoylcarnitine [17]. While the identification of the two forms of carnitine palmitoyltransferases greatly advanced our understanding about the mechanism by which carnitine catalyzes the mitochondrial oxidation of fatty acids, it was not complete until the identification of the carnitine-acylcarnitine translocase (CACT), an antiporter localized in the mitochondrial inner membrane, which catalyzes a reversible heterologous (and homologous) exchange of acylcarnitines and carnitine [18, 19]. Based on these findings and the extramitochondrial localization of long-chain fatty acid activating enzyme, LCAS, [20, 21], the function of carnitine in mitochondrial fatty acid oxidation is depicted in Fig. 1.

Although this function was envisaged about twenty some years ago generally it still holds today. Major advances in the field since then are the localization of CPT-I

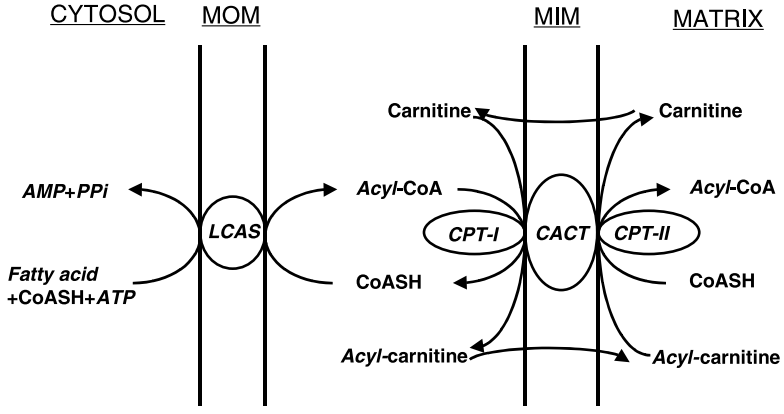


**Fig. 1.** Proposed model of the carnitine-dependent mitochondrial uptake of activated long-chain fatty acids; abbreviations: MOM – mitochondrial outer membrane, MIM – mitochondrial inner membrane, other abbreviations are given in the text

in the mitochondrial outer membrane [22], the finding that CPT-I is a separate entity and is different from CPT-II [23], and by virtue of its inhibition by malonyl-CoA CPT-I catalyzes the regulated step in mitochondrial fatty acid oxidation [24] and these form the topics for this review.

## Results and Discussion

### *Cellular Uptake, Activation, and Mitochondrial Transport of Long-chain Fatty Acids*

Long-chain fatty acids represent a main energy source for many organs, especially for muscle and liver. Following mobilization by lipolysis in adipose tissue and transport in blood bound to albumin, non-esterified fatty acids are taken up by tissues in a process mediated by transport proteins present in the plasma membrane [25]. Once within the cell, non-esterified fatty acids are bound to fatty acid binding proteins (FABP), which are present in the cytosol in high amounts [26]. Depending on the tissue and its metabolic demand, fatty acids either are converted to triglycerides and stored, secreted as VLDL (liver), or are oxidized in the mitochondria for energy production. Before being directed into storage or oxidation, fatty acids are first activated to acyl-CoA esters. This reaction is catalyzed by long-chain acyl-CoA synthetase (LCAS), an enzyme present in the mitochondrial outer membrane and that appears to be a transmembrane protein with the active site exposed to the cytosol [27]. The next step has been proposed to involve the voltage-dependent anion channel (VDAC) or porin [28, 29] and accounts for the movement of activated fatty acids across the mitochondrial outer membrane. The activated fatty acids are then converted to the respective acylcarnitines catalyzed by CPT-I also localized in the mitochondrial outer membrane. The reaction products, long-chain acylcarnitines are then translocated into the mitochondrial matrix in an exchange reaction catalyzed by CACT, an integral inner membrane protein. Within the matrix the acylcarnitines are then reconverted to the respective CoA esters by CPT-II, an enzyme associated with the inner leaflet of the mitochondrial inner membrane. The energy stored in these compounds is then released during their oxidation and conserved as *ATP*. This process involves the sequential oxidative chain shortening of the activated fatty acids by two-carbon units ($\beta$-oxidation) and is catalyzed by the consecutive action of four enzyme families (acyl-CoA dehydrogenase, enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase), each with substrate specificity of short-, medium-, and long-chain acyl-CoA esters [30–32]. The end products of $\beta$-oxidation are acetyl-CoA and reducing equivalents, *FAD*H and *NAD*H. Acetyl-CoA is then oxidized in the tricarboxylic acid cycle to $CO_2$ and reducing agents that are reoxidized in the respiratory chain yielding energy in the form of *ATP*. Alternatively, in the liver acetyl-CoA is converted to ketone bodies (acetoacetate, $\beta$-hydroxybutyrate) which are then exported to peripheral tissues for energy production.

In the overall process of mitochondrial fatty acid oxidation, CPT-I by virtue of its inhibition by malonyl-CoA, represents the key regulatory site controlling the flux through $\beta$-oxidation.

*Carnitine Palmitoyltransferase I (CPT-I)*

Consistent with its central role in mitochondrial fatty acid oxidation, the enzyme exists in at least two isoforms, the liver (CPT-$I_L$) and the muscle type (CPT-$I_M$) [33]. For the muscle isoform two novel splice variants also have been described [34, 35]; however, their quantitative significance is uncertain. Recently a third form, so-called brain-specific isoform (CPT-$I_C$), has been cloned and expressed [36]. Although the protein sequences of the mouse and human CPT-$I_C$ show high similarity to those of CPT-$I_L$ and CPT-$I_M$, only malonyl-CoA binding, but no catalytic activity was observed with the protein expressed in yeast. CPT-$I_L$ and CPT-$I_M$ differ significantly in their kinetic and regulatory properties. The liver type displays higher affinity for carnitine ($K_M$ for carnitine approx. 0.2 m*M*) and lower affinity for the physiological inhibitor malonyl-CoA ($K_i$ for malonyl-CoA approx. 1.0–2.0 $\mu M$), as opposed to the muscle isoform ($K_M$ approx. 1.5–2.0 m*M*, $K_i$ approx. 0.05–0.1 $\mu M$). The isoforms are encoded by genes localized on different chromosomes and exhibit different tissue distribution (reviewed in Refs. [29, 33, 37, 38]). Although neither isoform has been isolated in catalytically active form, the full-length cDNAs have been expressed in *Saccharomyces cerevisiae* and *Pichia pastoris*, and the expressed proteins displayed malonyl-CoA sensitive catalytic activity and kinetic parameters ($K_M$ and $IC_{50}$ for carnitine and malonyl-CoA, respectively) similar to those observed with intact rat liver, heart, and skeletal muscle mitochondria [39–41].

The amino terminus of each CPT-I isoform includes a composite leader sequence consisting of an intermembranous space targeting signal and two 20–30-residue candidate outer-membrane-spanning/stop transfer hydrophobic domains [33] which are retained in the mature protein [42]. The proposed membrane orientation, derived from a multifaceted approach, predicts a topology of CPT-I with two transmembrane domains, separated by a stretch of 27 amino acids on the intermembrane side and both, the N- (short segment) and C-termini (large segment) exposed to the cytosolic side [43].

*Regulation of CPT-I by Malonyl-CoA Content*

Since the first description of malonyl-CoA inhibition of CPT-I by *McGarry et al.* [24] numerous studies provided correlative evidence for malonyl-CoA regulation of mitochondrial fatty acid oxidation *via* inhibition of CPT-I (reviewed in Ref. [29]). However, direct evidence for tissue malonyl-CoA in regulation of CPT-I activity has been only recently reported. The first such study was published by *Abu-Elheiga et al.* [44] using transgenic mice which had the muscle specific acetyl-CoA carboxylase (ACC2) deleted. These mice had dramatically reduced malonyl-CoA content in heart and skeletal muscle (reduced by approx. 90 and 96%, respectively), showed increased whole body fatty acid oxidation, including skeletal muscle, and lower amounts of fat in spite of the somewhat greater food intake. Surprisingly, there was also increased hepatic fatty acid oxidation despite the lack of a decrease in hepatic malonyl-CoA content. This latter observation suggests that only a small fraction of malonyl-CoA (probably that produced by ACC2, but not by ACC1, the liver specific isoform) is accessible to CPT-I and consequently the sensitivity of hepatic CPT-I *in vivo* (at least in mice) to malonyl-CoA inhibition is significantly greater than hitherto thought. Secondly, *Harwood et al.* [45] showed recently that

isoenzyme-nonselective acetyl-CoA carboxylase inhibitors, administered orally, decrease tissue malonyl-CoA concentrations in liver, heart, and skeletal muscle of rats and increase whole-body fatty acid oxidation. Along the same line, perfusion of rat hearts with an inhibitor of malonyl-CoA decarboxylase (MCD) which catalyzes the disposal of malonyl-CoA lead to a significant increase in tissue malonyl-CoA concentration and a corresponding decrease in palmitate oxidation. This was accompanied by large increases in glucose oxidation [46].

These data suggest that the steady state tissue content of malonyl-CoA is determined by its formation and its disposal. The formation of malonyl-CoA is catalyzed by acetyl-CoA carboxylase which is expressed in two isoforms (ACC1 and ACC2) encoded by two distinct genes localized on different chromosomes [47, 48]. ACC1 predominates in lipogenic tissues while ACC2 is the predominant form in heart and skeletal muscle [47 and references therein]. The major molecular difference between the two isoforms is that the first 200 amino acid residues of ACC2 are not present in ACC1 and this sequence is thought to be related to its unique function in heart and skeletal muscle to control mitochondrial fatty acid oxidation *via* CPT-I inhibition by malonyl-CoA [49]. Indeed, this N-terminal segment present in ACC2 localizes the enzyme to the mitochondrial outer membrane [50]. This raises the possibility of malonyl-CoA channeling between ACC2 and CPT-I at the mitochondrial outer membrane. Indeed, when rat heart mitochondria are incubated with *ATP*-$Mg^{2+}$, acetyl-CoA, and $HCO_3^-$, they synthesize malonyl-CoA resulting in inhibition of CPT-I [51]. The activity of ACC isoforms is subject to acute control by rapid covalent modification of the enzyme protein (inactivation and activation by phosphorylation and dephosphorylation, respectively) and by feed-forward activation by citrate and feedback inhibition by long-chain acyl-CoA esters [47, 48]. The phosphorylation is catalyzed by *AMP*-activated protein kinase (*AMP*K) which itself is subject to phosphorylation by an upstream protein kinase (*AMP*K-kinase) [52]. It is believed that this protein kinase cascade represents a sensitive system that acts as a cellular fuel gauge. While changes in ACC activity will reciprocally affect tissue malonyl-CoA levels, to substantially decrease malonyl-CoA content, a decreased rate of synthesis must be accompanied by simultaneous degradation of malonyl-CoA by MCD. Therefore, MCD has been suggested as the primary enzyme in the disposal of malonyl-CoA [53] and its presence has been demonstrated in several tissues of different species [54, 55]. Since the activity of MCD is significantly higher than that of ACC2, it is reasonable to assume that the two enzymes are regulated in a reciprocal fashion. This assumption is supported by data presented by *Park et al.* [56] showing that in skeletal muscle ACC2 and MCD are indeed regulated in a reciprocal fashion via phosphorylation. Supportive evidence for phosphorylation of MCD has been presented also for rabbit heart with the phosphorylated form being less active [53].

### *How is Inhibition of CPT-I by Malonyl-CoA Brought About?*

The high affinity malonyl-CoA regulatory site of CPT-I has not been mapped. Studies reported in Ref. [43] suggest that both the catalytic and regulatory sites of CPT-I are in the large carboxy-terminal domain facing the cytosol and the amino terminal domain plays a role in maintaining an optimal conformation of the enzyme required

for both catalytic function and malonyl-CoA sensitivity. Detailed studies on the role of the N-terminal domain in malonyl-CoA inhibition have been published recently [57, 58]. Expression of a series of CPT-$I_L$ deletion mutants ($\Delta$18, $\Delta$35, $\Delta$52, $\Delta$73, $\Delta$83, and $\Delta$129) in *Pichia pastoris* have shown, that with the exception of $\Delta$129 all expressed proteins had significant catalytic activity and only the malonyl-CoA sensitivity was affected. Although deletion of the first 18 amino acid residues was sufficient to abolish malonyl-CoA inhibition, only minimal changes in catalytic properties (*e.g.*, saturation kinetics with respect to both substrates) were observed [57]. Further insight as to which amino acid residue(s) is essential for malonyl-CoA sensitivity has been gained by functional analysis of expressed substitution mutants [58]. These studies demonstrated that a single amino acid substitution (glutamic acid in position 3 replaced by alanine) was as effective in the abrogation of malonyl-CoA inhibition as was the deletion of the first 18 amino acids. Substitution of histidine in position 5 for alanine had a less dramatic effect on malonyl-CoA sensitivity.

Although these data clearly demonstrate the significance of the N-terminal domain, specifically glutamate and histidine in positions 3 and 5, respectively in malonyl-CoA inhibition, additional residues must also contribute to malonyl-CoA sensitivity. Deletion of the first 18 amino acid residues, which are identical in both isoforms, from CPT-$I_M$ had a less dramatic effect on malonyl-CoA sensitivity [57]. Furthermore, expression of chimeric CPT-Is in which the amino-termini of the liver and muscle isoforms were switched did not reveal the expected differences in malonyl-CoA sensitivity [59]. Since the muscle and liver CPT-I isoforms differ greatly in their sensitivity to malonyl-CoA inhibition, the expressed chimeric proteins should have reflected a ''phenotypic'' switch if the amino-terminal domains were the sole determinants of malonyl-CoA inhibition of CPT-I.

### *Regulation by Changes in Malonyl-CoA Sensitivity*

It is currently well-established that malonyl-CoA concentration is not the only factor controlling the process of acyl-CoA transport into mitochondria. The liver isoform, in addition to regulation *via* changes in malonyl-CoA concentrations, also is regulated by changes in the enzymes' sensitivity to malonyl-CoA inhibition. Thus diet- and hormone-induced changes in the flux through the fatty acid oxidative pathway are usually accompanied by parallel variations in the specific activity and changes in malonyl-CoA sensitivity of rat liver CPT-I. For example, under ketotic states such as starvation, diabetes, and hyperthyroidism the specific activity of CPT-I increases whereas its sensitivity to malonyl-CoA inhibition decreases [60–63]. The opposite occurs in hypothyroidism, refeeding after starvation, and insulin treatment of diabetes [64–66]. Under these conditions of increased fatty acid oxidation, CPT-I becomes more active and less sensitive to inhibition by malonyl-CoA. The decreased sensitivity of the enzyme coupled with decreased tissue malonyl-CoA content act synergistically thus allowing maximal rates of fatty acid oxidation at any given CPT-I protein abundance. Thus, CPT-$I_L$ is unique in responding to different physiological states, *e.g.*, starvation and insulin deficiency and hypo- and hyperthyroidism, by changing its sensitivity to the inhibitor several fold. These changes amplify the effects of fluctuations in the cytosolic concentration of malonyl-CoA occurring under these conditions.

Although the short-term control of CPT-I in liver mitochondria has been well characterized and is accomplished by both, changes in liver malonyl-CoA content as well as changes in sensitivity of the enzyme to its physiological inhibitor, considerably less is known about the acute control of the muscle isoform, CPT-$I_M$ predominantly or exclusively expressed in heart and skeletal muscle. Most studies using isolated skeletal and heart muscle mitochondria failed to demonstrate changes in sensitivity of CPT-I to malonyl-CoA. It was concluded that, in these tissues, CPT-I is solely regulated by changes in tissue malonyl-CoA content. However, the muscle specific isoform of CPT-I present in heart and skeletal muscle, in contrast to its liver specific counterpart, is extremely sensitive to malonyl-CoA inhibition, with $K_i$ values for malonyl-CoA in the nanomolar range (approx. 0.05–0.1 $\mu M$). Considering that the malonyl-CoA content in these tissues is significantly higher than the reported $K_i$ and $IC_{50}$ values it is not likely that acute or short-term regulation of CPT-I in heart is accomplished by changes in tissue malonyl-CoA content only. In support of this, using the canine model of regional myocardial ischemia *Pauly et al.* [67] reported a decreased malonyl-CoA sensitivity following an ischemic insult with no changes in total CPT-I activity. Additionally, *Sugden et al.* [68] have shown that hyperthyroidism facilitates myocardial fatty acid oxidation by a mechanism(s) that involves increased activity and decreased malonyl-CoA sensitivity of CPT-I. Since insulin infusion reverted the inhibitory properties of CPT-I to that seen with mitochondria isolated from hearts of euthyroid animals but not the activity of CPT-I, these two parameters can apparently be regulated independently from each other. Furthermore, decreased malonyl-CoA sensitivity with no change in CPT-I activity has been documented in hearts from patients with end-stage congestive heart failure [69] and in skeletal muscle mitochondria isolated from trained *vs.* untrained (control) subjects [70]. These conflicting data with respect to changes in malonyl-CoA sensitivity in heart and skeletal muscle can be explained by differences in the isolation conditions, *i.e.*, the use or lack of use of proteases. It is known, that some proteases affect malonyl-CoA sensitivity but not catalytic activity [22].

### *What is the Mechanism of Altered Malonyl-CoA Sensitivity of CPT-I?*

Although changes in malonyl-CoA sensitivity are considered the major mechanism for the regulation of the liver isoform of CPT-I and thus for hepatic mitochondrial fatty acid oxidation, the mechanism for the decrease in malonyl-CoA sensitivity has not been uncovered. In theory, such changes in malonyl-CoA sensitivity could be envisaged as arising directly from modification of CPT-I protein or indirectly as a result of changes in the lipid composition/fluidity of the mitochondrial outer membrane. Both of these possibilities will be briefly discussed below.

It has been suggested that changes in the lipid composition of the membrane microenvironment in which CPT-I resides are important for alteration in malonyl-CoA sensitivity [71 and references therein]. When osmotically swollen rat liver mitochondria were presented with lipids extracted from mitochondrial outer membranes there was a significant increase of inhibition by malonyl-CoA [72]. Surprisingly, the effect of mitochondrial outer membrane lipid extract on malonyl-CoA sensitivity was mimicked by cardiolipin and phosphatidylglycerol, but not by phosphatidylcholine and phosphatidylethanolamine which are the

major lipid components in the mitochondrial outer membrane. Furthermore, cardiolipin, when added to swollen intact liver mitochondria isolated from fasted rats, not only increased but completely restored the malonyl-CoA sensitivity to that seen in mitochondria from fed animals. Cardiolipin is an inner membrane phospholipid and is either absent or present in small amounts in the mitochondrial outer membrane [73, 74], but it is enriched in mitochondrial contact sites [75]. Since CPT-I also is enriched in rat liver mitochondrial contact sites [76–78] these findings suggest the possibility of a specific membrane microenvironment of CPT-I.

Alternatively, covalent modification of CPT-I protein itself could also be responsible for these changes in the enzymes' malonyl-CoA sensitivity. *Pegorier et al*. reported that glucagon or dibutyryl-*cAMP* treatment of fetal hepatocytes decreases the malonyl-CoA sensitivity of CPT-I in isolated mitochondria five- and ten-fold, respectively with no effect on the catalytic activity [79]. These data suggest that the changes observed in malonyl-CoA sensitivity of CPT-I are brought about by a *cAMP*-dependent signaling event. In addition to malonyl-CoA sensitivity, phosphorylation based regulation of CPT-$I_L$ catalytic activity also has been suggested. In isolated rat hepatocytes glucagon or dibutyryl-*cAMP* increased CPT-I activity without affecting malonyl-CoA sensitivity, an effect which was mimicked by the protein phosphatase inhibitor okadaic acid [80]. Subsequent studies by the same authors indicated that phosphorylation of cytoskeletal components rather than phosphorylation of CPT-$I_L$ is responsible for the increased catalytic activity [81, 82]. Studies in bovine aortic endothelial cells by *Yamagishi et al*. [83] also suggested that CPT-I is regulated by phosphorylation/dephosphorylation and involves PKA and/or tyrosine kinase signaling. In these cells, leptin induced a doubling of CPT-I activity and a 50% decrease in ACC activity. Changes in activities of both enzymes were prevented by H-89, an inhibitor of protein kinase A and by genistein, a tyrosine kinase inhibitor. Similarly, treatment of bovine aortic endothelial cells with the *cAMP* antagonist Rp-*cAMP*S (the Rp diastereoisomer of adenosine 3′,5′-cyclic monophosphothionate) prevented the leptin-induced increase in CPT-I and the decrease in ACC activity. Since the CPT-I activity was determined in permeabilized cells with little or no malonyl-CoA present the data are consistent with a mechanism that involves phosphorylation of CPT-I and ACC. Phosphorylation of the CPT-$I_L$ isoform also has been suggested by *Wang et al*. [84] in neonatal cardiac myocytes which abundantly express the liver form. These authors showed that hypoxia increases CPT-$I_L$ activity with no effect on the muscle isoform. This activation by hypoxia was prevented by pervanadate, an inhibitor of protein tyrosine phosphatases. These data suggest activation of the liver isoform by dephosphorylation.

In addition to the above indirect data, reports of purported phosphorylation/dephosphorylation based regulation of CPT-$I_L$ were published as early as 1985 by *Harano et al.* [85]. Studies on isolated hepatocytes suggested that the stimulatory effect of glucagon on CPT activity was due to phosphorylation of CPT. Using $^{32}P$ labeling and immunoprecipitation with CPT antibodies, the authors detected a radiolabeled polypeptide with a molecular weight of approx. 66–68 kDa. Because of the use of CPT-II antibodies to immunoprecipitate radiolabeled polypeptides, the interpretation of these findings has been rejected.

Recently we have initiated a proteomic approach to address the post-translational modification of CPT-$I_L$ The enzyme was isolated from rat liver mitochondrial outer membranes by harvesting the 88 kDa protein by semi-preparative SDS-PAGE and electroelution. The isolated CPT-$I_L$ was digested with trypsin and the resulting digest examined by both MALDI-TOF MS and HPLC-ESI-MS using a linear quadrupole ion trap interfaced with a nanospray source. These studies identified three potential phosphorylation sites by MALDI-TOF one of which has been sequenced by HPLC-ESI-MS. This diphosphopeptide represents the amino acid sequence $^{740}$FSpSPETDpSHR$^{749}$ in the C-terminal region of CPT-I with serine residues 741 and 747 phosphorylated. The phosphorylation consensus sequence in this diphosphopeptide corresponds to protein kinase CKII. Consistent with this, protein kinase CKII catalyzes the incorporation of $^{32}$P from [$\gamma$-$^{32}$P]-*ATP* into CPT-I and phosphorylation of CPT-I with unlabeled *ATP* renders malonyl-CoA inhibition from competitive to uncompetitive type and increases its catalytic activity [86].

## Questions and Future Directions

In this short review we briefly recapitulated and discussed experimental data presented in the literature with respect to regulation of CPT-I by malonyl-CoA. While in essence these data are compatible with one or the other model depicted in Figs. 1 and 2 in Ref. [29], they also raise new questions concerning the protein components involved in mitochondrial fatty acid uptake as well as a more detailed mechanism of this process.

As shown earlier [29], polyanion 22 (PA22), an inhibitor of VDAC, inhibits the carnitine dependent oxidation of palmitoyl-CoA but not that of palmitoylcarnitine in isolated rat liver mitochondria. The fact that in rat liver all three VDAC isoforms (VDAC1, VDAC2, VDAC3) are expressed at the protein level (unpublished observation from the authors' laboratory) raises the question which VDAC isoform is involved in the transport of activated fatty acids? Furthermore, is this porin-dependent step subject to regulation? In preliminary unpublished data we have shown that in rat liver mitochondria VDAC1 is phosphorylated *in vivo*. Consistent with this, it has been shown that VDAC1 isolated from rat liver mitochondria can be phosphorylated by protein kinase A and that phosphorylation alters the gating properties of VDAC1 [87]. We are currently using an immunological and proteomic approach to elucidate the function of VDAC in mitochondrial uptake of activated fatty acids.

Another observation of great significance is that hepatic fatty acid oxidation is increased in ACC2 knockout mice without a decrease in tissue malonyl-CoA content [44]. In line with this is the observation that isolated mitochondria are able to synthesize malonyl-CoA leading to inhibition of CPT-I [51]. Is hepatic malonyl-CoA compartmentalized and only the malonyl-CoA produced by ACC2 accessible to CPT-I? If so, then the development of selective ACC2 inhibitors could provide new avenues for therapeutic treatment of diseases characterized by excessive fatty acid oxidation.

Based on these new observations we propose a model for the structural and functional organization of mitochondrial fatty acid oxidation enzymes as depicted in Fig. 2.

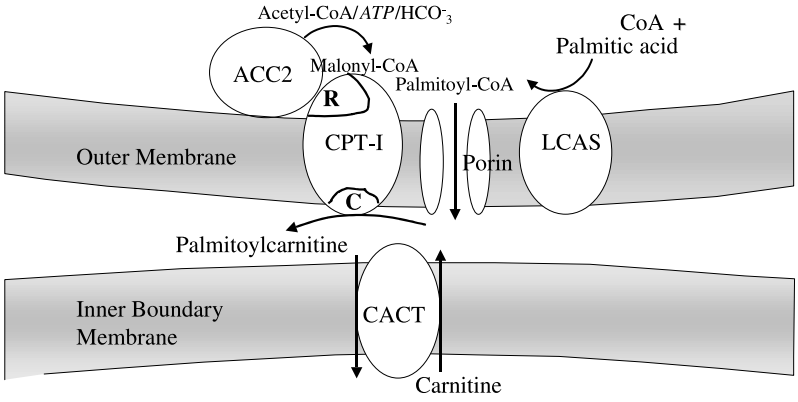


**Fig. 2.** Proposed structural and functional organization of mitochondrial outer membrane enzymes involved in mitochondrial uptake of long-chain fatty acids; the mitochondria associated ACC2, the key enzyme for malonyl-CoA production for regulation of CPT-I, is incorporated into this model; abbreviations: R and C – regulatory or malonyl-CoA binding site and catalytic site of CPT-I, other abbreviations are given in the text

A further unanswered question regards the enrichment of CPT-I in rat liver mitochondrial contact sites [76–78] and the altered inhibitory properties of CPT-I in contact sites as opposed to the enzyme in the bulk outer membrane [77]. It is well documented that covalent attachment of lipophilic moieties to proteins affects reversible subcellular targeting, influences interaction with membranes and membrane microdomains [88–90], and phosphorylation after membrane targeting, as shown for the endothelial isoform of nitric oxide synthase [91]. In addition to protein acylation, a similar effect on protein targeting has been shown also for

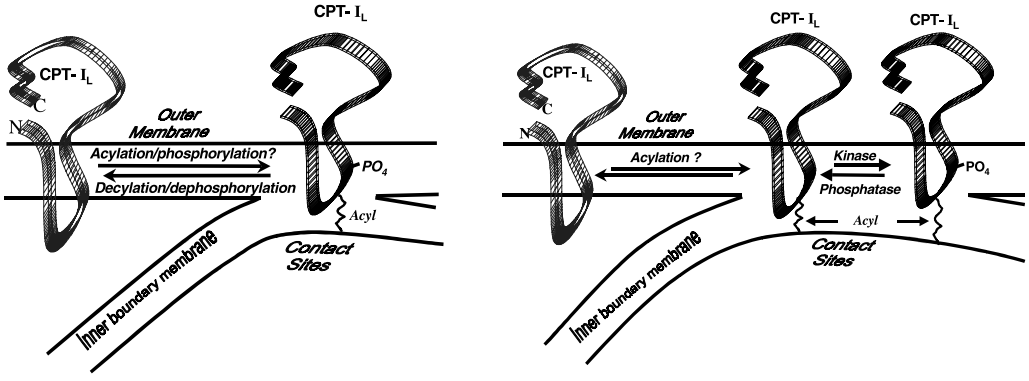


**Fig. 3.** Hypothetical mechanisms for enrichment of CPT-$I_L$ in rat liver mitochondrial contact sites; *left*: translocation and enrichment of CPT-I in contact sites induced by reversible post-translational modification (*i.e.*, phosphorylation, acylation) of the enzyme protein; in this model the altered inhibitory properties of the enzyme could be due to interaction of CPT-I with other proteins present in the contact sites (*i.e.*, mitochondrial inner membrane protein(s)), interaction with specific inner membrane lipids, such as cardiolipin, and to phosphorylation itself; *right*: in this two step model acylation (*i.e.*, palmitoylation) induces the lateral movement of CPT-I and its enrichment in contact sites; once in contact sites, the reversible phosphorylation of CPT-I by specific kinases/phosphatases then alters the inhibitory properties of the enzyme

protein modification *via* reversible phosphorylation [90]. Could post-translational modification, as proposed in Fig. 3, either by phosphorylation or/and acylation, be the underlying mechanism for recruitment of CPT-I in contact sites as well as for its altered inhibitory properties? These questions are unanswered at present and their answer will require a detailed comparative proteomic study of the enzymes present in these two submitochondrial locations, *i.e.*, mitochondrial contact sites and bulk mitochondrial outer membrane. Examination for post-translational modification of CPT-I isolated from contact sites and bulk outer membrane from fed, fasted, and diabetic animals and determinations of the enzyme's kinetic properties should provide answers to the questions raised above.

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## References

[1] Gulewitsch VS, Krimberg R (1905) Hoppe-Seylers Z Physiol Chem **45**: 326
[2] Kutscher F (1905) Untersuch Nahr- und Genussmitt **10**: 528
[3] Tomita M, Sendju Y (1927) Hoppe-Seyler's Z Physiol Chem **169**: 263
[4] Strack E, Forsterling K (1937) Naunyn-Schmiedebergs Arch Exp Path Pharmak **185**: 612
[5] Strack E, Forsterling K (1950) Hoppe-Seyler's Z Physiol Chem **285**: 207
[6] Strack E, Forsterling K (1953) Hoppe-Seyler's Z Physiol Chem **295**: 377
[7] Binon F, Deltour G (1957) Experientia **12**: 357
[8] Fraenkel G, Friedman S (1957) Carnitine. In: Marrian RS, Thiman KV (eds) Vitamins and Hormones vol. 15. Academic Publ, New York, p 73
[9] Friedman S, Fraenkel G (1955) Arch Biochem Biophys **59**: 491
[10] Fritz IB (1955) Acta Physiol Scand **34**: 367
[11] Bremer J (1962) J Biol Chem **237**: 2228
[12] Bremer J (1962) J Biol Chem **237**: 3628
[13] Bremer J (1962) J Biol Chem **238**: 2774
[14] Fritz IB, Yu KTN (1963) J Lipid Res **4**: 3279
[15] Skrede S, Bremer J (1970) Eur J Biochem **14**: 465
[16] Yates DW, Garland PB (1970) Biochem J **119**: 547
[17] Hoppel CL, Tomec RJ (1972) J Biol Chem **247**: 832
[18] Pande SVA (1975) Proc Natl Acad Sci USA **72**: 883
[19] Ramsay RR, Tubbs PK (1975) FEBS Lett **54**: 21
[20] Norum KR, Farstad M, Bremer J (1966) Biochem Biophys Res Commun **24**: 797
[21] Garland PB, Yates DW, Haddock BA (1970) Biochem J **119**: 553
[22] Murthy MSR, Pande SV (1987) Proc Natl Acad Sci **84**: 378
[23] Esser V, Britton CH, Weiss BC, Foster DW, McGarry JD (1993) J Biol Chem **268**: 5817
[24] McGarry JD, Leatherman GF, Foster DW (1978) J Biol Chem **253**: 4128
[25] van Nieuwenhoven FA, van der Vusse GJ, Glatz JFC (1966) Lipids **31**: S223
[26] Bernlohr DA, Simpson MA, Vogel-Hertzel AV, Banaszak LJ (1997) Annu Rev Nutr 273
[27] Hesler CB, Olymbios C, Haldar DJ (1990) J Biol Chem **265**: 6600
[28] Turkaly P, Kerner J, Hoppel C (2001) FEBS Letters **460**: 241
[29] Kerner J, Hoppel C (2000) Biochim Biophys Acta **1486**: 1
[30] Kunau W-H, Dommes V, Schulz H (1995) Prog Lipid Res **34**: 267
[31] Eaton S, Bartlett K, Pourfarzam M (1996) Biochem J **320**: 345

[32] Bartlett K, Eaton S (2004) Eur J Biochem **271**: 462
[33] McGarry JD, Brown NF (1997) Eur J Biochem **244**: 1
[34] Yu G-S, Lu Y-C, Gulick T (1998) Biochem J **334**: 225
[35] Yu G-S, Lu Y-C, Gulick T (1998) Biochim Biophys Acta **1393**: 166
[36] Price NT, van der Leij FR, Jackson VN, Corstophine CG, Thomson R, Sorensen A, Zammit VA (2002) Genomics **80**: 433
[37] Kerner J, Hoppel C (1998) Annu Rev Nutr **18**: 179
[38] Van der Leij FR, Huijkman NCA, Boomsma C, Kuipers JRG, Bartelds B (2000) Molec Genetics Metab **71**: 139
[39] deVries Y, Arvidson DN, Waterham HR, Cregg JM, Woldegiorgis G (1997) Biochemistry **36**: 5285
[40] Zhu H, Shi J, deVries Y, Arvidson DN, Cregg JM, Woldegiorgis G (1997) Arch Biochem Biophys **347**: 53
[41] Prip-Buus C, Cohen I, Kohl C, Esser V, McGarry JD, Girard J (1998) FEBS Lett **429**: 173
[42] Kolodziej MP, Zammit VA (1993) FEBS Lett **327**: 294
[43] Fraser F, Corstorphine CG, Zammit VA (1997) Biochem J **323**: 711
[44] Abu-Elheiga L, Matzuk MM, Abo-Hashema KAH, Wakil SJ (2001) Science **291**: 2613
[45] Harwood HJ, Petras SF, Shelly LD, Zaccaro LM, Perry DA, Makowski MR, Hargrove DM, Martin KA, Tracey WR, Chapman JG, Magee WP, Dalvie DK, Soliman VF, Martin WH, Mularski CJ, Eisenbeis SA (2003) J Biol Chem **278**: 37099
[46] Dyck JRB, Cheng J-F, Stanley WC, Barr R, Chandler MP, Brown S, Wallace D, Arrhenius T, Harmon C, Yand G, Nadzan AM, Lopaschuk GG (2004) Circ Res **94**: e78
[47] Kim K-H (1997) Ann Rev Nutr **17**: 77
[48] Brownsey RW, Zhande R, Boone AN (1996) Biochem Soc Trans **25**: 232
[49] Ha J, Lee J-K, Kim K-S, Witters LA, Kim K-H (1996) Proc Natl Acad Sci USA **93**: 11466
[50] Abu-Elheiga L, Brinkley WR, Zhong L, Chirala SS, Woldegiorgis G, Wakil SJ (2001) Proc Natl Acad Sci USA **97**: 1444
[51] Kerner J, Minkler P, Hoppel C (2004) Faseb J **18**: A863
[52] Hardie DG, Carling D (1997) Eur J Biochem **246**: 259
[53] Dyck JRB, Barr AJ, Barr RL, Kolattukudy GD, Lopaschuk GD (1998) Am J Physiol Am J Physiol **275**: H2122
[54] Scholte HR (1953) Biochim Biophys Acta **309**: 457
[55] Kim YS, Kolattakudy PE (1978) Arch Biochem Biophys **190**: 234
[56] Park H, Kaushik VK, Constant S, Prentki M, Przybytkowski E, Ruderman NB, Saha AK (2002) J Biol Chem **277**: 32571
[57] Shi J, Zhu H, Arvidson DN, Cregg JM, Woldegiorgis G (1998) Biochemistry **37**: 11033
[58] Shi J, Zhu H, Arvidson DN, Woldegiorgis G (1999) J Biol Chem **274**: 9421
[59] Swanson ST, Foster DW, McGarry JD, Brown NF (1998) Biochem J **335**: 513
[60] Robinson N, Zammit VA (1982) Biochem J **206**: 177
[61] Bremer J (1981) Biochim Biophys Acta **665**: 628
[62] Saggerson ED, Carpenter CA (1981) FEBS Lett **129**: 225
[63] Stakkestad JA, Bremer J (1983) Biochim Biophys Acta **750**: 244
[64] Saggerson ED, Carpenter CA (1986) Biochem J **236**: 137
[65] Grantham BD, Zammit VA (1986) Biochem J **239**: 485
[66] Gamble MS, Cook GA (1985) J Biol Chem **260**: 9516
[67] Pauly DF, Kirk A, McMillin JB (1991) Circ Res **68**: 1085
[68] Sugden MC, Priestman DA, Orfali KA, Holness MJ (1999) Horm Metab Res **31**: 300
[69] Martin MA, Gomez MA, Guillen F, Bornstein B, Campos Y, Rubio JC, de la Calzada CS, Arenas J (2000) Biochim Biophys Acta **1502**: 330
[70] Starritt E, Howlett RA, Heigenhauser GJF, Spriet LL (2001) Am J Physiol **278**: E462

[71] Zammit VA, Corstorphine C, Kolodziej M, Fraser F (1998) Lipids **33**: 371
[72] Mynatt RL, Greenhaw JJ, Cook GA (1994) Biochem J **299**: 761
[73] Daum G (1985) Biochim Biophys Acta **822**: 1
[74] Daum G, Vance J (1997) Prog Lipid Res **36**: 103
[75] Ardail D, Privat J-P, Egret-Charlier M, Levrat C, Lerme F, Louisot P (1990) J Biol Chem **265**: 18797
[76] Hoppel CL, Kerner J, Turkaly P, Turkaly J, Tandler B (1998) J Biol Chem **273**: 495
[77] Fraser F, Zammit VA (1998) Biochem J **329**: 225
[78] Hoppel C, Kerner J, Turkaly P, Tandler B (2001) Arch Biochem Biophys **392**: 321
[79] Pegorier J-P, Garcia-Garcia M, Prip-Buus C, Duee P-H, Kohl C, Girard J (1989) Biochem J **264**: 93
[80] Guzman M, Geelen MJH (1992) Biochem J **287**: 487
[81] Guzman M, Kolodziej MP, Caldwell A, Corstorphine CG, Zammit VA (1994) Biochem J **300**: 693
[82] Velasco G, Geelen MJH, Gomez del Pulgar T, Guzman M (1999) Adv Exp Med Biol **466**: 43
[83] Yamagishi S, Edelstein D, Du X, Kaneda Y, Guzman M, Brownlee M (2001) J Biol Chem **276**: 25096
[84] Wang D, Xia Y, Buja ML, McMillin JB (1998) Mol Cell Biochem **180**: 163
[85] Harano Y, Kashiwagi A, Kojima H, Suzuki H, Hashimoto T, Shigeta Y (1985) FEBS Lett **188**: 267
[86] Kerner J, Distler AM, Minkler P, Parland W, Peterman SM, Hoppel CL (2004) J Biol Chem **279**: 41104
[87] Bera AK, Ghosh S (2001) J Struct Biol **135**: 67
[88] Blenis J, Resh MD (1993) Curr Opin Cell Biol **5**: 984
[89] Resh MD (1999) Biochim Biophys Acta **145**: 1
[90] Resh MD (2004) Subcell Biochem **37**: 217
[91] Gonzales E, Kon R, Lin AJ, Golan DE, Michel T (2002) J Biol Chem **277**: 39554