CPT1 (EC 2.3.1.21)[i]

Carnitine palmitoyltransferase 1

Author: Johannes C.W. Odendaal

Reviewed: Karen van Eunen

Last Edited: 2023-03-28

Asterisks before titles (*) indicate junctures at which decisions were made or the kinetic implication of a certain set of data disregarded.

[i] Historically, there was debate about whether the cytosolic and mitochondrial matrix CPT activities stem from distinct enzymes or from the same protein differently localized. Until today, they still have one EC number (EC 2.3.1.21). However, it is now established that the two enzymes are genetically and phenotypically distinct (McGarry & Brown, 1997).

Contents

FUNCTION

CPT1 is responsible for the ligation of long-chain cytosolic fatty acids to CoA molecules (1,2). This step activates these carbon-chains, allowing them to enter the mitochondrion and then be catabolized by mFAO. Of the cytosolic sites, CPT1 is thought to exert the highest control over mFAO and ketogenesis in rat liver and – indeed – the highest control of all intrahepatic sites at all metabolic states, including the fed state, starvation, insulin treatment, and refeeding (3,4).

Transcriptional regulation

CPT1 is markedly transcriptionally downregulated by a high-carbohydrate diet, whereas it is strongly upregulated by LCFA (5).

* Modelling decision

The V_{max} of CPT1 cannot be assumed to be the same at different metabolic states. **Assume that the nutritional state of the cells from which CPT1 is taken to be measured, matters (fed, fasted, fat-fed, diabetic).**

SUBCELLULAR LOCALISATION

CPT1 is embedded in the outer mitochondrial membrane (6).

* Unexplored kinetic implication

Changes in membrane fluidity, for example, might result from nutritional or other environmental conditions, and have been shown to influence both carnitine affinity and MalCoA sensitivity in CPT1 (6)**.**

Contact sites

CPT1, as well as its inward-facing cognate, CPT2, seem specifically enriched at contact sites, with about 40% of CPT1 and CPT2 located in only 5-10% of the outer membrane surface area (7,8). Contact sites are points of close apposition between the inner and outer mitochondrial membranes and differ in terms of composition and function from other areas in the membrane (9). They are regulated dynamically based on the functional state of the cell, including by fatty acids which increase the negative surface charge of the membranes, causing repulsion between them and reducing contact site frequency (10,11). Contact sites are thought to be the points of acyl-CoA import for mFAO (8): "The concentration of CPT1 and CPT2 at contact sites raises the possibility that acylcarnitine transfer into the mitochondrial matrix is thereby facilitated."

* Modelling decision

Add a parameter called *contactSiteCPT* to multiply with the V_{max}, which will effectively cause the model to only contain contact site CPT1 and CPT2. This implicit assumption here is that only contact site CPT1 and -2 participate in mFAO and that the others form part of a different module. Physico-chemically, the solubility-related channelling of long-chain acyl-CoAs and -carnitines might be imagined to mean that the intermediates that go to VLCAD would probably pass via contact sites as passive diffusion to the mFAO enzymes, especially of less water-soluble compounds, is unlikely.

IMPORTANT UNCERTAINTIES

(Uncertainty 1) CPT1 *versus* CPT2

As late as the early 1990s, there was still some controversy about whether CPT1 and CPT2 represented separate proteins or the same enzyme catalysing opposite reactions due to differences in their membrane environment (12). Consequently, many older studies do not implement clear strategies to discern between the activities of CPT1 and CPT2 and therefore likely measure combined activity of the two.

* Modelling decision

This makes it difficult to ascertain to which enzymes (CPT1, CPT2, or some mixture of the two) certain measurements apply, e.g. in the work of Finocchiaro *et al.* (13), where no distinction between the isozymes is made. Even in studies where a distinction *is* made, e.g. in McGarry *et al.* (14), the authors note "when released from the membrane, [CPT1] loses its sensitivity to malonyl-CoA." In the latter case, it is tricky to know whether McGarry and colleagues (14) were indeed measuring what they thought they were measuring, as malonyl-CoA-sensitivity is an identifying property of CPT1 (12), and it might be that solubilisation of the mitochondria with the use of detergents might have released CPT2 and inhibited CPT1 in studies done with homogenates study.

Measurement of CPT1 activity in the membrane and some test to identify the enzyme's identity is preferred. **Some authors have systematically distinguished CPT1 and CPT2 activity in their measurements, by, for example, using CPT1- or CPT2-deficient cells** (15,16)**, by assaying intact mitochondria and inhibiting the downstream mFAO to isolate the outward-facing activity** (17)**,** or by recombinantly expressing the enzymes in external vehicles (18–20). **The first two options appear the most appropriate, to our eye, since then the enzymes are assayed in their native membrane environment.** The latter option entails recombinant expression of the proteins in alien environments, which can have an impact on the functioning of particularly CPT1, as will be discussed in the following sections.

In the case of data scarcity, it is possible to use CPT activity from homogenates as an approximation of CPT2 activity if the assay cocktail contained detergents, as previously done (21,22). It has been shown that detergents stimulate CPT2 activity while inhibiting CPT1 (12). This is not a total abrogation of CPT1 activity in favour of CPT2, however – substantial CPT1 activity is also measured at high concentrations of detergent. The same is also true of detergent-free preparations, which have stronger CPT1 activity while still containing substantial CPT2 activity. But this is not recommended.

(Uncertainty 2) Mechanism of malonyl-CoA inhibition (unresolved)

Since the discovery of CPT1's sensitivity to inhibition by malonyl-CoA, many studies have attempted to unravel its mechanism. The mitochondrial outer membrane microenvironment seems to affect the kinetics of CPT1, including by affecting the mechanism of malonyl-CoA inhibition (23). Different isoforms of CPT1 also differ in their sensitivity to malonyl-CoA: it is known, for instance, that the muscle isoform, CPT1b, has a much higher affinity for malonyl-CoA than CPT1 (6). For simplicity, we will focus on the relationship between the liver isoform of CPT1 (CPT1a) and malonyl-CoA.

Malonyl-CoA is the first committed intermediate of the *de novo* lipogenesis, hence its inhibition of mFAO makes intuitive sense as a feedback loop to prevent futile cycling (24). This molecule – both its biochemistry and its physiological role – has been reviewed in detail by previous authors (5,25–27). The mechanism of action of malonyl-CoA appears to contain an allosteric component: proteases have been shown to abrogate malonyl-CoA sensitivity without abrogating the CPT1's catalytic

activity, suggesting that different regions of the protein are involved in catalysis and inhibition (28–31). This would also explain how solubilised CPT1 can still catalyse the transferase reaction despite losing its sensitivity to malonyl-CoA (32).

Most authors have observed the competitive exclusion of palmitoyl-CoA (32–35), or octanoyl-CoA (34), from the active site as the mechanism of inhibition. Competitive allosteric inhibition is known in other enzymes (36): in these mechanisms, inhibitors do not sterically block substrate entry into an active site but induce conformational changes that render substrate-binding less favourable. Indeed, some authors have directly proposed this conformational change model as the mechanism of malonyl-CoA inhibition (5,37).

Another suggestion is that malonyl-CoA is competitive with respect to L-carnitine binding. This is supported by the observation that the same motif that is responsible for malonyl-CoA sensitivity in liver CPT1 is responsible for L-carnitine affinity in muscle (6). Furthermore, Bird and Saggerson (38) found a reduction of the affinity of CPT1 for L-carnitine in rat liver by malonyl-CoA, and also a reduction of malonyl-CoA binding by increased L-carnitine, suggesting competition by steric hindrance between these two molecules. However, Bird and Saggerson (38) also found that increasing the palmitoyl-CoA concentration can reduce the inhibitory effect of malonyl-CoA on L-carnitine binding.

Some authors have suggested that malonyl-CoA binds CPT1 at two different sites (36,39–41). López-Viñas *et al.* (40)Viñas et al. 2007) propose a mixed model of inhibition consisting of the steric hindrance (competitive inhibition) of L-carnitine binding and the simultaneous allosteric modulation (which they classify mechanistically as uncompetitive or non-competitive) of L-carnitine and acyl-CoA binding. The proposal of mixed inhibition raises the question of whether both mechanisms are present in all CPT1 enzymes, or whether distinct subpopulations of CPT1 exist with distinct kinetic properties. In the latter case, a protein sequence would have the potential of exhibiting both mechanisms, with the determining factor being the membrane microenvironment. An example could be that CPT1 is inhibited differently at contact sites *versus* in the rest of the membrane (23). Lloyd *et al*. (42) report different kinetics when malonyl-CoA is added first *versus* when palmitoyl-CoA is added first in an *in vitro* CPT1 activity assay.

If distinct subpopulations of CPT1 do exist and can be isolated, different prior nutritional and environmental conditions, or even different sample preparations, might the membrane structure and, by extension, might induce distinct kinetic features in CPT1. Finally, binding proteins that solubilise and transport fatty acids and their derivatives have also been shown to increase the inhibitory effect of malonyl-CoA (43).

It is clear that CPT1's relationship with malonyl-CoA is complex and multifactorial. For simplicity, we have assumed simple competitive inhibition of acyl-CoA binding. However, much ground remains to be covered towards an accurate characterisation of the relationship between malonyl-CoA and CPT1.

* Arbitrary modelling decision: competitive inhibition against acyl-CoA

We will be selecting a simple competitive inhibition with regards to palmitoyl-CoA binding, not influenced by the presence of binding proteins or the membrane-environment of the enzyme.

(Uncertainty 3) Substrate inhibition

Substrate inhibition – the phenomenon whereby an enzyme is inhibited by the compound that it's meant to convert – has been reported in as many as 10% of enzymes (44). It leads to a rate curve in which the rate increases to a maximum and then starts descending with further substrate increases. Substrate inhibition has often been regarded as an aberration stemming from the artificially high substrate concentration in the laboratory environment, although Reed *et al.* (45) provide an overview of cases which in which substrate inhibition takes place at physiological concentrations and appears to have an evolved biochemical role.

A widely cited study by Bremer and Norum (46) reported substrate inhibition of CPT1 by palmitoyl-CoA, with maximal rates at 10 to 50 µM. This was later echoed by Murthy and Pande (12) and Woldegiorgis *et al.* (47), again with maxima between 10 and 50 µM. Increasing L-carnitine concentrations pushed the maxima to higher palmitoyl-CoA concentrations, leading the authors to conclude a competitive inhibition of L-carnitine binding (46,47).

Bremer and Norum (46) concluded that two palmitoyl-CoA binding sites must be present to explain substrate inhibition. Indeed, substrate inhibition seems to require multiple substrate-binding sites – a catalytic binding site and a non-catalytic inhibitory site (45). Recently, however, three-dimensional modelling of the enzyme has identified only one binding site suitable for palmitoyl-CoA binding (40). This, of course, raises the question: what is the mechanism responsible for the apparent substrate inhibition of CPT1 by palmitoyl-CoA? Elucidation of the mechanism might also provide clues as to whether this is a biologically relevant phenomenon or an artefact of the laboratory.

Another possible mechanism is suggested by the observation that substrate inhibition in CPT1 is apparently irreversible (12,43). Murthy and Pande (12) referred to a "detergent-type inactivation" because inhibition could not be reversed by subsequently lowering the substrate concentrations. Similarly, bovine serum albumin (BSA) prevented substrate inhibition (12,20,47). However, BSA could not restore the enzyme's activity after palmitoyl-CoA had already inhibited it (12). BSA is a binding protein that sequesters, *inter alia*, acyl-CoAs. These acyl-CoAs, for instance palmitoyl-CoA, then have radically reduced free concentrations (48). Other studies showed that substrate inhibition of CPT1 was also abrogated by the addition of the native binding proteins, fatty-acid binding protein (FABP) and acyl-CoA binding protein (ACBP) (43,49), although they did not test for reversibility.

All of this is in agreement with a model of substrate inhibition as detergent-like (12). Detergent-like behaviour has, indeed, been reported for palmitoyl-CoA (50–52) as well as that the hydrophobic tail of acyl-CoAs inserts into membranes (53–55). A change in membrane lipid composition is known to affect CPT1 function (56,57). Indeed, Pauly and McMillin (58) provided evidence that the palmitoyl-CoA that did not bind to BSA, partitioned into the mitochondrial membrane where, presumably, and in high enough concentrations, it could alter the properties of the membrane. Bhuiyan and Pande (43) showed that octanoyl-CoA does not exhibit this substrate-inhibition behaviour – not even at the supraphysiological concentration of 0.5 mM. Octanoyl-CoA is not a strong detergent like palmitoyl-CoA and has a decreased tendency to partition into membranes (59,60). We are not aware of other acyl-CoA chain lengths that have been assayed for substrate inhibition.

In a previous section (*(Uncertainty 2) Mechanism of malonyl-CoA inhibition (unresolved))* we argued that the membrane interacts with CPT1 in ways that determine the kinetics of the enzyme. High concentrations of palmitoyl-CoA have been observed to affect the activity of virtually all transmembrane substrate transporters (61), suggesting a common mechanism – in this case their interaction with the membrane. CPT2, however, has also not been observed to undergo substrate inhibition (12). This might be explained by the fact that CPT2 is anchored in but never spans the

membrane (1). This would be consistent with a membrane-CPT1 interaction in the observed substrate inhibition.

Substrate inhibition in CPT1 has not been investigated in detail, and most evidence is indirect. The paucity of direct measurements is in itself an indication that substrate inhibition likely only arises under specific experimental conditions. Moreover, our main observation is that substrate inhibition resembles a detergent acting on the membrane rather than an allosteric effect.

Further investigation should focus on identifying whether the inhibitory effect of high concentrations of palmitoyl-CoA is accompanied by changes in membrane composition and fluidity and whether the inhibition can somehow be reversed by restoring the membrane to its original state. Progress can also be made by crystallographically characterising CPT1's structure, something which – to our best knowledge – has thus far only been done computationally (40,62). Crystallography might confirm the absence or presence of allosteric binding sites for palmitoyl-CoA. Trying to replicate the early experiments with other acyl-CoAs might also shed light on the matter: if the detergent effect of palmitoyl-CoA is responsible for the inhibition, then one would also expect other long-chain acyl-CoAs to cause inhibition at high concentrations, with decreasing potency as the acyl chains shorten. If the effect is specific to palmitoyl-CoA, on the other hand, that might indicate an allosteric mechanism.

Finally, even if the inhibition is due to detergent effects, it might still have biological relevance. Changes in membrane composition can also take place *in vivo*. If this is the case, then it might be worthwhile characterising whether this inhibition would occur under physiological conditions, for instance in the presence of cytosolic proteins like fatty acid- and acyl-CoA binding proteins (FABPs and ACBPs). FABPs and ACBPs would drive the free concentration of acyl-CoAs down and might prevent substrate inhibition from ever taking place: if the concentrations of acyl-CoAs that would be required to cause substrate inhibition are never reached, then we could ignore it. We have assumed that this is the case for our model, though it has not been shown unequivocally.

* Modelling decision: no substrate inhibition

Substrate inhibition by palmitoyl-CoA was exclusively observed in experiments where no substrate-binding proteins were added (e.q. (43,46,47)). The direct evidence presented by (43) and (58) that substrate-binding proteins abrogate substrate inhibition and the lack of reported palmitoyl-CoA substrate inhibition of CPT1 in the rest of the literature, where the use of BSA as substrate-binding protein is nearly universal, confirms that **we need not worry about this phenomenon as a confounder of parameters.**

(Uncertainty 4) Acyl-CoA binding proteins (unresolved)

The precise kinetics/dynamics of how substrate availability changes in the presence of substrate binding proteins is still not perfectly understood. Abo-Hashema *et al.* ((49)) showed that CPT1 is able to recognise acyl-CoA binding protein(ACBP)-AcylCoA binary complexes while Pauly and McMillin ((58)) concluded that CPT1 could also use acyl-CoA-BSA complexes as substrates, albeit that they suggest that this would be a slow process. The suggestion that acyl-CoA-BSA complexes are more slowly used by CPT1 as substrates (58) together with the evidence from Bhuiyian *et al.* ((43)) showing how high concentrations of substrate binding proteins lead to a decrease in CPT1 activity, suggest that binding proteins decrease the effective concentration of AcylCoA, though they don't remove them from the substrate pool entirely. They do appear to lower the chance that the substrate and the enzyme will interact favourably for a reaction, however.

In contrast, Bhuiyan *et al.* ((43)) found that octanoyl-CoA was more quickly converted to octanoylcarnitine by CPT1 in the presence of BSA**.**

Pauly and mcMillin (1988) discovered the ratio between the BSA and the palmitoyl-CoA to be determinant with respect to the measured activity (Fig. 1, (58)). At a ratio of 6.1 µM palmitoyl-CoA to 1 µM BSA, the rate was about maxed out:

Pauly and McMillin (58) suggested that about 5 or 6 binding sites on BSA had to be saturated before the mitochondria could compete effectively for the substrate – this explains the suggested 6:1 ratio. Increasing the ratio above 6:1 did not significantly change the maximum measured V_{max}.

ACBP is known to bind acyl-CoAs in a stoichiometry of 1:1 (49). When the *in vivo* concentration of acyl-CoA increases to higher than [ACBP], however, FABP (fatty-acid binding protein which binds acyl-CoA in a stoichiometry of 1:2; (63)) would likely pick up the slack. This might sometimes be the case at the outer membrane environments of mitochondria, for instance during fasting (43).

Assuming an [ACBP] of between 10-50 µM (63), binding with a K_D = 1 nM (1 – 10 nM; (64)), and a [FABP] of 100-300 µM with a K_D = 1 µM (65), very little long-chain acyl-CoA will end up in the free or membrane-bound form *in vivo*, probably.

Finally, in the words of Knudsen *et al.* (63): “It is tempting, therefore, to speculate that, by binding LCACoA, ACBP creates a pool of long-chain acyl-CoA available only for specific purposes.”

* Unexplored kinetic implication

It is therefore fair to expect that the physiological interaction of CPT1 and acyl-CoAs – the real affinity of CPT1 for protein-bound acyl-CoA, the real availability of acyl-CoAs to various processes and organelles, and the levels at which these potential detergents can become toxic – will be projected through the lens of various binding proteins in the cytosol, and that these kinetic effects must be considered if a real picture of CPT1 activity is to be had.

* Arbitrary modelling decision: disregard binding protein effects

For now, the effects of these proteins will be disregarded for the sake of simplicity.

MEASURING CONDITIONS

Use of detergents

Detergents inhibit CPT1 activity and stimulate CPT2 activity (Table 1 from (12)):

In fact, even when claiming to measure the activity of CPT1, for example in (17), detergents are used, which might be inhibiting CPT1 activity.

* Modelling decision: Try to use parameters that were measured in the absence of detergents.

Tissue-specific isoforms

Prip-Buus *et al.* (19): "Tissue analysis from CPT1-deficient patients has shown that human CPT1A is expressed in liver, lymphocytes, and fibroblasts, but not in skeletal muscle [where CPT1B is the expressed isoform]." Cardiac muscle has both liver and muscle isoforms, and exhibits properties that are intermediate to both tissues (4).

* STRONG modelling decision: Only parameters from liver, lymphocytes, and fibroblasts can be used.

Differences across species

Significant parameter differences exist across species, up to an order of magnitude, for example, between Guinea pig liver and human liver; McGarry *et al.* (66). This makes the use of non-human parameters in a human model a non-trivial problem.

* Modelling decision: Try to use human parameters

Subject age

Oey and colleagues (67) show that a factor of 5:1 difference could arise between the activity of CPT1 from adult human liver *versus* that from foetal liver CPT1. When selecting a V_{max} for one's model, this is rather important to consider.

* Modelling decision: Where possible, use CPT1 parameters measured in human tissue of young children of neonates.

Temperature

Kolodziej and Zammit (37) showed a very significant change in malonyl-CoA sensitivity (as much as 40% change in CPT1 activity) when assaying in 37°C vs. 20°C due to membrane fluidization. Whether this magnitude of effect is also seen on, for instance, substrate and product affinity, has not been confirmed but it not out of the question.

* Modelling decision: Try to use parameters measured as close as possible to 37°C, especially Vmax.

Buffer composition

MOPS and HEPES buffer are known to compete with carnitine for binding to CPT1 (49) and any assay carried out in those buffers is to be rejected. Solberg (68) also briefly discusses the effects of salt activation, indicating that a 3.3x increase in [Tris] leads to a 2.5-6.5x increase in CPT1 activity.

* Modelling decision: Do not use parameters measured in HEPES or MOPS buffer.

pH

Mills *et al.* (69) investigated the effect of pH on the binding of malonyl-CoA, substrates, and on the V_{max} of rat liver CPT1. They found an that a modest pH increase from pH = 6.8 to pH = 7.6 increased the [malonyl-CoA] necessary for 50% inhibition (IC_{50}) 8-fold, while the K_m for carnitine decreases 2-fold over the same range, in agreement with Zammit *et al.* (6) and McGarry *et al* (66)'s reports that malonyl-CoA sensitivity and the K_m for carnitine are positively correlated.

* Modelling decision: Try to use parameters measured at pH = 7.2 (cytoplasmic pH).

BSA

Since by far the most studies make use of BSA in their assays (12,40,70,71), it is important to note that changing [BSA]:[Acyl-CoA] ratios are known to lead to sigmoidicity in the relationship of CPT1 activity to substrate concentration (Fig. 1 from (58)). This is the case in most of the measurements that have been done in the literature: therefore, data from assays with constant [BSA] are to be understood with the caveat that artefacts may be playing a role in the observed kinetics.

* Modelling decision: Try to use parameters measured in BSA-containing assay buffer.

* Unexplored kinetic implication

Measuring CPT2 activity in the presence of different concentrations of BSA almost certainly causes some differences in the observed kinetics. For simplicity, this is disregarded for now.

Order of substrate additions

Lloyd *et al*. (42) report different kinetics when malonyl-CoA is added first *versus* when palmitoyl-CoA is added first in an *in vitro* CPT1 activity assay.

* Arbitrary modelling decision: ignore the effect of adding substrate in different orders

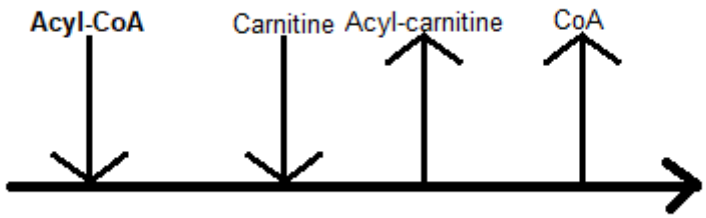
KINETICS

Specificity

CPT1 is specific to long-chain acyl-CoAs (72), though nowhere in the literature wat specified what exactly "long-chain" refers to. According Expasy (E.C. 2.3.1.21), CPT1 is specific to LCFA of chain-length C8 to C16.

Reaction mechanism

Nic a' Bháird *et* al. (73) show a compulsory ordered binding mechanism for CPT2 (acyl-CoA binds first and acylcarnitine released first) and McGarry *et al.* (5) and Ramsay *et al.* (1) extrapolated this to CPT1, though they did not provide direct evidence:



I assume that this can be simplified to the generic random-order bi-bi rate equation (74), with the effects of the ordered mechanism factoring in the fact that the K_m for carnitine varies with the chain length of its co-substrate.

* Unexplored kinetic implication

In the inhibition term of reactions with a conserved co-substrate (CoA or carnitine), the K_i of the carnitine is a competitive inhibitory term against acylcarnitine binding (Car/K_iCar (see highlighted in yellow below)): the carnitine would exclude acylcarnitine binding in a chain-length-independent way, as the carnitine would have to bind before the acylcarnitine in order to keep it out of the active site. When carnitine is a substrate, however, it has a chain-length-dependent binding patters, as the acyl-CoA substrate binds before the carnitine.

In an ordered bi-bi Michaelis-Menten equation, this would be addressed by the fact that a separate K_d and K_i is included for carnitine. In order to fit this mechanism into the random order bi-bi rate equation, however, we need to treat the K_d (in this case K_m) and K_i of carnitine in the equation's denominator as a single value. If we do not do this, then we would select for certain acylcarnitine substrates based on higher K_i of carnitine actually related to the acyl-CoA substrate which doesn't play a role in the inhibition.

** Arbitrary modelling decision*

Take the average of $K_{m,carnitines}$ as the $K_{i,carnitine}$ so there's only one K_i to avoid substrate selection based on the K_m of the enzyme for carnitine.

Rate equation

For n = {8, 10, 12, 14, 16}:

$$vcpt1C_n = \frac{sfcpt1C_n \cdot \frac{Vcpt1 \cdot contactSiteCPT}{VCYT} \cdot \left(\frac{C_nAcylCoACYT[t] \cdot CarCYT[t]}{K_mcpt1C_nAcylCoACYT \cdot K_mcpt1C_nCarCYT} - \frac{C_nAcylCarCYT[t] \cdot CoACYT[t]}{K_{eq}cpt1C_n \cdot K_mcpt1C_nAcylCoACYT \cdot K_mcpt1CarCYT}\right)}{\left(1 + \frac{CarCYT[t]}{K_icpt1CarCYT} + \frac{CoACYT[t]}{K_mcpt1CoACYT}\right) \cdot \left(1 + \frac{MalCoACYT}{K_iMalCoACYT} + \sum_{n=8}^{n=16}\left(\frac{C_nAcylCoACYT[t]}{K_mcpt1C_nAcylCoACYT} + \frac{C_nAcylCarCYT[t]}{K_mcpt1C_nAcylCarCYT}\right)\right)}$$

Variables == initial values

Acyl-CoAs	Acylcarnitines
	C16AcylCarCYT[t] == 0 µM
C14AcylCoACYT[t] == 0 µM	C14AcylCarCYT[t] == 0 µM
C12AcylCoACYT[t] == 0 µM	C12AcylCarCYT[t] == 0 µM
C10AcylCoACYT[t] == 0 µM	C10AcylCarCYT[t] == 0 µM
C8AcylCoACYT[t] == 0 µM	C8AcylCarCYT[t] == 0 µM

Parameters

Weighting rule

I give the parameters weights based on my subjective evaluation. There will be four categories.

1 = credible measurement

0.9 = just short of perfect (e.g. wrong tissue and had to be adjusted, 30°C instead of 37°C)

0.5 = uncertain

0.1 = “I probably wouldn’t choose this if I had another option”

Using the weights, I will reduce the impact of poor measurements.

Weights are given in curly brackets next to parameter values: {} with short reasons

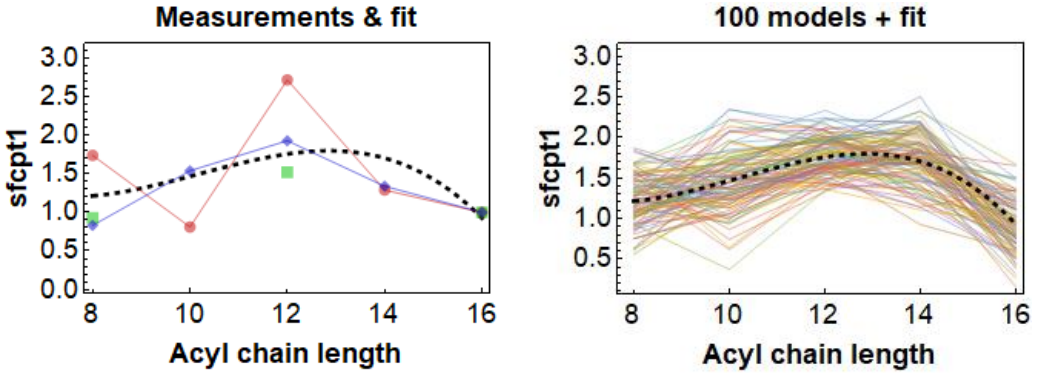
sfcpt1

Satisfactory values found

	Parameter	Chosen value [range]	Alternatives		Comments
sfcpt1		Schaefer *et al.* (1997, (75)) Permeabilised CPT2 deficient patient fibroblasts, sensitive to malonyl-CoA, measure palmitoyl-L-(methyl^{3}H) carnitine from L-(methyl^{3}H) carnitine (540 µM) and palmitoyl-CoA (110 µM) complexed with BSA; 115 mM Tris (pH = 7.4); 4.5 mM reduced glutathione; 70 mM KCl, temp not given; Values taken from Table 2, otherwised read off Figure 3.	West *et al.* (1971, (76)) Ox liver; 30°C; pH = 8.0; 100 mM Tris-HCl; 1% BSA	Finocchiaro *et al.* (1990, (13)) Normal human liver; 25°C; pH = 8; CPT1 and CPT2 not discerned; BSA not used; 91 mM Tris-HCl; Tween-20 included in assay	**1)** These parameters are not purified CPT1 but CPT2, in all likelihood, but I use them as a placeholder while I have no parameters available to me. These parameters are simply called CPT parameters by Finocchiaro *et al.* (13). Murthy *et al.* (12) show that detergents inhibit CPT1 and activate CPT2, and that CPT1 loses activity after solubilization; Finocchiaro *et al.* (13) used Tween-20 both in extracting the enzyme and in the assay cocktail itself. Based on this, and the authors' statement that they see no MalCoA-sensitivity in their extract, I interpret the parameter to be more representative of CPT2. **2)** These parameters are in reasonable agreement with those measured by West *et al.* (76). This shows that our chose parameters might not be entirely unrealistic for CPT1.
	sfcpt1C16	1 *{0.9, no temp}*	1 *{0.5, Ox + old, CPT2 not properly known yet}*	1 *{0.5, temp + CPT2 not distinguished + no BSA}*	
	sfcpt1C14	1.29 *{0.9, no temp}*		1.34 *{0.5, temp + CPT2 not distinguished + no BSA}*	

	sfcpt1C12	2.72 *{0.9, no temp}*	1.52 *{0.5, Ox + old, CPT2 not properly known yet}*	1.93 *{0.5, temp + CPT2 not distinguished + no BSA}* [1.52 - 1.93]	
	sfcpt1C10	0.81 *{0.9, no temp}*		1.54 *{0.5, temp + CPT2 not distinguished + no BSA}*	
	sfcpt1C8	1.74 *{0.9, no temp}*	0.93 *{0.5, Ox + old, CPT2 not properly known yet}*	0.83 *{0.5, temp + CPT2 not distinguished + no BSA}* [0.83 - 0.93]	

Cubic polynomial (multinormal distribution)	
Formula	$a * x^3 + b * x^2 + c * x + d$
Best fit parameters	a = -0.0079339 b = 0.242669 c = -2.30559 d = 8.19036
R^2	0.903259
Covariance matrix	{{0.00015193, -0.00546947, 0.0634863, -0.236767}, {-0.00546947, 0.197408, -2.29769, 8.5924}, {0.0634863, -2.29769, 26.824, -100.622}, {-0.236767, 8.5924, -100.622, 378.738}}
Bounds	{{"bounds: C8", 0.174, 17.4}, {"bounds: C10", 0.081, 8.1}, {"bounds: C12", 0.272, 27.2}, {"bounds: C14", 0.129, 12.9}, {"bounds: C16", 0.1, 10.}}



Comments: n/a

Vcpt1

Semi satisfactory value found, not human liver, but adjusted to human liver proteomics.

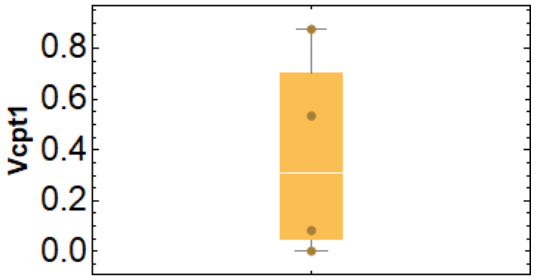
Parameter		Chosen value [range]	Alternatives			Comments
Vcpt1		Vlies *et al.* (2007, (17)) Human fibroblast, 37°C, pH = 7.0, 150 mM KCL, 25 mM Tris, 20 mM KPi, 5 mM KCN, 1 mg/mL BSA, did use detergent	Brown *et al.* (2001, (21)) Human fibroblast, 30°C, pH = 7.2, 150mM KCl and 5 mM Tris-HCl, 1% BSA	Schaefer *et al.* (1997, (75)) Permeabilised CPT2 deficient patient fibroblasts, sensitive to malonyl-CoA, measure palmitoyl-L-(methyl^{3}H) carnitine from L-(methyl^{3}H) carnitine (540 µM) and palmitoyl-CoA (110 µM) complexed with BSA; 115 mM Tris (pH = 7.4); 4.5 mM reduced glutathione; 70 mM KCl, temp not given; Values taken from Table 2, otherwise read off Figure 3.	Murthy *et al.* (1987, (12)) Rat liver, isolated mitochondria, separated into IMV and OMV, 30°C, pH = 7.4, with 1.3mg/250µL BSA	**1)** An adjusted value according to the proteomics on ProteomicsDB (77) yields adjusted human liver CPT1a activity. **2)** Van Vlies *et al.* (17) presented a sophisticated assay which also accounted for downstream accumulation of products. **3)** Van Vlies *et al.* (17) explicitly note that they have measured V_{max} values up to 7x bigger than in previous studies, so this observation should not surprise us.
	Vcpt1	0.872 µmol.min^{-1}.mg-mito-Protein^{-1} *{0.9, fibroblast}* [0.0047 – 0.18094] *, **	0.536 µmol.min^{-1}.mg-mito-Protein^{-1} *, ** *{0.5, temp + fibroblast}*	0.084 µmol.min^{-1}.mg-mito-Protein^{-1} *, ** *{0.5, temp + fibroblast}*	0.0047 µmol.min^{-1}.mg-mito-Protein *** *{0.5, temp + rat}*	

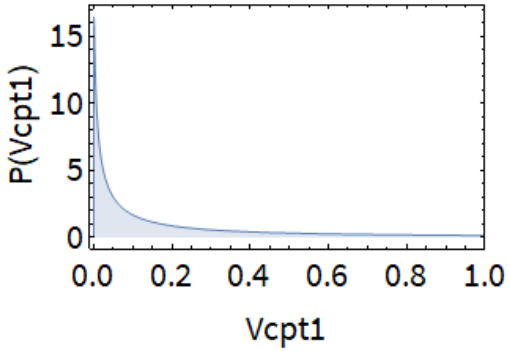
* Converted from fibroblast protein to hepatocyte protein: 10Log(iBAQ) - liver/ 10Log(iBAQ) – fibroblast for CPT1a expression yielded a ratio of 100 (77).

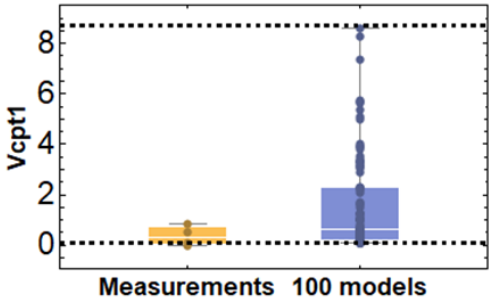
** Converted to mitochondrial protein by multiplying by 4 (78)

*** Converted to mitochondrial protein by dividing by 20; OMV protein is 5%of mitochondria, protein (12)

log-normal distribution	
Parameters (of the normal distribution)	μ = -1.80963 σ = 2.35101
Bounds	{"bounds", 0.0872, 8.72}





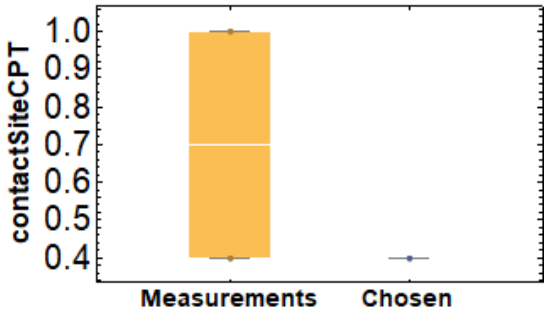


contactSiteCPT

Semi satisfactory value.

	Parameter	Chosen value [range]	Alternatives	Comments
contactSiteCPT		Fraser *et al.* (1998, (8)) Rat liver mitochondria, marker enzymes used to identify the fractions were adenylate kinase (intermembrane space), rotenone-insensitive NADPHcytochrome c reductase (rNCR; outer membrane) and cytochrome c oxidase (cyt-ox; inner membrane) according to the method of (79)	Assume all CPTs can participate in mFAO.	1) Contact sites are points of close apposition between the inner and outer mitochondrial membranes and differ in terms of composition and function from other areas in the membrane (9). 2) They are regulated dynamically based on the functional state of the cell, including by fatty acids which increase the negative surface charge of the membranes, causing repulsion between them and reducing contact site frequency (10,11). So the 40% might change 3) This assumption helps to restore CPT1's flux control at low substrate concentrations, which would otherwise be helped by CACT
	contactSiteCPT	0.4 (0.5, *simple uncertainty*)	1.0 (0.5, *simple uncertainty*)	

Unique	
Values	0.4



Comments: No variation allowed, unique value

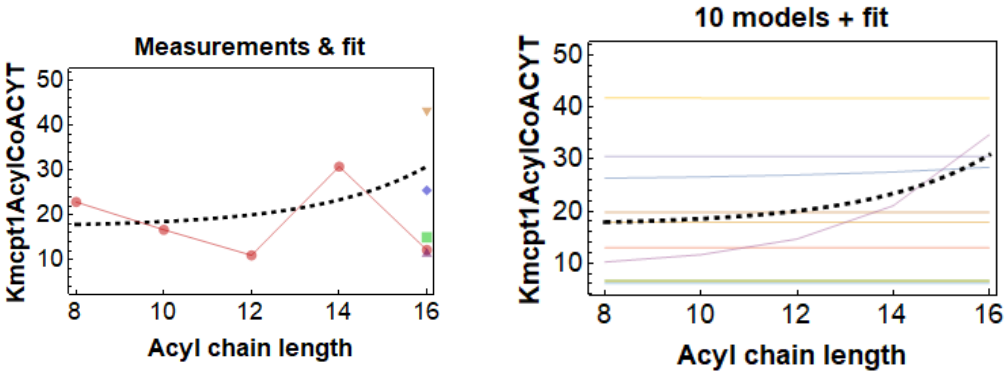
Kmcpt1AcylCoA

No satisfactory value: no values were found that expressed the change of the K_m for chain lengths and which are also unambiguously CPT1 parameters

Parameter	Chosen value [range]	Alternatives					Comments
Kmcpt1AcylCoACYT	Finocchiaro *et al.* (1990, (13)) Normal human liver; 25°C; pH = 8; CPT1 and CPT2 not discerned; BSA not used; 91 mM Tris-HCl; Tween-20 included in assay	Vlies *et al.* (2007, (17)) human fibroblast, 37°C, pH = 7.0, 150 mM KCL, 25 mM Tris, 20 mM KPi, 5 mM KCN (inhibits downstream enzymes), 1 mg/mL BSA, did use detergent	Demaugre (1988, (80)) Permeabilised CPT2 deficient patient fibroblasts, sensitive to malonyl-CoA, measure palmitoyl-L-(methyl^{3}H) carnitine from L-(methyl^{3}H) carnitine (540 µM) and palmitoyl-CoA (110 µM) complexed with BSA; 115 mM Tris (pH = 7.4); 4.5 mM reduced glutathione; 70 mM KCl, temp not given; take average of two controls	Chen *et al.* (2020, (18)) recombinant rat liver CPT1, in *e. Coli* ; 30°C, pH = 7.3, 25 mM HEPES (**HEPES competes with carnitine**), 75 mM KCl, BSA in a ratio of 6.1:1 with PalCoA	Prip-Buus (2001, (19)) human fibroblasts expressed in yeast, 30°C, pH = 7.3, 25 mM HEPES (**HEPES competes with carnitine**), 75 mM KCl, BSA in a ratio of 6.1:1 with PalCoA	Gobin *et al.* (2003, (20)) human fibroblasts expressed in yeast, 30°C, pH = 7.3, 25 mM HEPES (**HEPES competes with carnitine**), 75 mM KCl, BSA in a ratio of 6.1:1 with PalCoA	**1)** Murthy and Pande (12) show that detergents inhibit CPT1 and activate CPT2, and that CPT1 loses activity after solubilization; Finocchiaro *et al.* (13) used Tween-20 both in extracting the enzyme and in the assay cocktail itself. Based on this, and the authors' statement that they see no MalCoA-sensitivity, I expect the parameter to be more representative of CPT2. **2)** Between Van Vlies *et al.* (17) , Prip-Buus *et al* (19) and Gobin *et al.* (20) there is quite some variation. The latter two use Hepes buffer, which is known to inhibit carnitine binding and can therefore lead to artifacts; also Van Vlies *et al.* (17) use detergents in their assay, which inhibits CPT1 activity. McGarry *et al.*
Kmcpt1C16AcylCoACYT	12.2 µM	15 µM	25.5 µM	11.7 µM	43 µM	88.8 µM	

		{0.5, temp + CPT2 not distinguished + no BSA} [11.7 – 88.8]	*{0.9, fibroblast}*	*{0.5, pH + no temp}*	*{0.5, rat + temp}*	*{0.5, temp + HEPES + fibroblast expressed in yeast}*	*{0.5, temp + HEPES + fibroblast expressed in yeast}*	(66) report Km values for rat muscle and liver (30°C, pH = 7.4) between 30 µM and 60 µM. **The take-home message:** all values are between 0 and 10 and 100 µM, which us the range into which FInocchiaro *et al.* (17)'s parameters also fall.
	Kmcpt1C14AcylCoACYT	30.8 µM *{0.5, temp + CPT2 not distinguished + no BSA}*						
	Kmcpt1C12AcylCoACYT	11 µM *{0.5, temp + CPT2 not distinguished + no BSA}*						
	Kmcpt1C10AcylCoACYT	16.7 µM *{0.5, temp + CPT2 not distinguished + no BSA}*						
	Kmcpt1C8AcylCoACYT	22.9 µM *{0.5, temp + CPT2 not distinguished + no BSA}*						

Exponential growth/decay (multinormal distribution)	
Formula	$a * e^{x*b} + c$
Best fit parameters	a = 0.0219735 b = 0.401344 c = 17.3323
R^2	0.62
Covariance matrix	{{1.16017, -3.16294, -30.9189}, {-3.16294, 8.63096, 83.1205}, {-30.9189, 83.1205, 1062.57}}
Bounds	{{"bounds: C8", 2.29, 229.}, {"bounds: C10", 1.67,167.}, {"bounds: C12", 1.1, 110}, {"bounds: C14", 3.08, 308.}, {"bounds: C16", 1.22, 122.}}



Comments: mostly flat, sometimes curved upwards, sometimes downwards. Upwards slopes more frequent and steeper than downwards slopes.

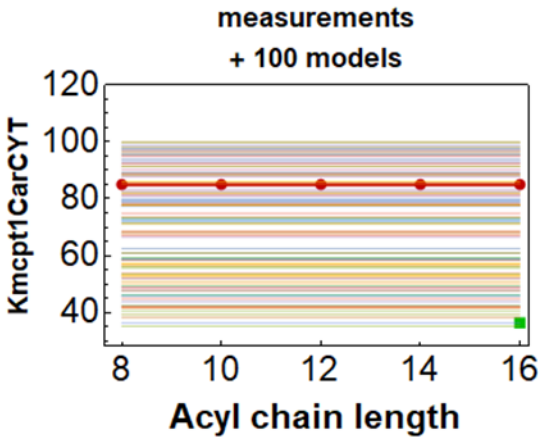
Kmcpt1CarCYT

No satisfactory value: no values were found that expressed the change of the K_m for chain lengths

	Parameter	Chosen value	Alternatives	Comments
Kmcpt1CarCYT		Van Vlies *et al.* (2007, (17)) Human fibroblast, 37°C, pH = 7.0, 150 mM KCL, 25 mM Tris, 20 mM KPi, 5 mM KCN, 1 mg/mL BSA, did use detergent. This is quite a reliable parameter, as they went to some length to discern between CPT1 and CPT2.	Demaugre *et al.* (1988, (81)) Permeabilised CPT2 deficient patient fibroblasts, sensitive to malonyl-CoA, measure palmitoyl-L-(methyl^{3}H) carnitine from L-(methyl^{3}H) carnitine (540 µM) and palmitoyl-CoA (110 µM) complexed with BSA; 115 mM Tris (pH = 7.4); 4.5 mM reduced glutathione; 70 mM KCl, temp not given; take average of two controls	**1)** Since the parameters of Finocchiaro *et al.* (13) are probably CPT2 parameters (see Kmcpt1AcylCoACYT for details), I do not consider them here. I choose the values from Van Vlies *et al.* (17). Though these were measured in human fibroblasts, K_m values are not dependent of expression levels, so fibroblast data is more easily translatable to the liver context. **2)** I choose to use the K_m for carnitine for C16 chain-length substrate as a temporary placeholder for all the K_m values for carnitine, even though the fact that carnitine binds only after acyl-CoA to CPT1 (73) probably means that the carnitine would have different affinities for CPT1 depending on the other substrate.
	Kmcpt1C16CarCYT	85 µM *{0.9, fibroblast}*	36.5 µM *{0.5, pH + no temp}*	
	Kmcpt1C14CarCYT	85 µM *{0.1, wrong chain-length}*		
	Kmcpt1C12CarCYT	85 µM *{0.1, wrong chain-length}*		

	Kmcpt1C10CarCYT	85 µM *{0.1, wrong chain-length}*		
	Kmcpt1C8CarCYT	85 µM *{0.1, wrong chain-length}*		

Linear (flat - pick one value for all the chain-lengths)	
Formula	$a * (x + b) + c$
Chosen parameters	a = {0,0} b = 0 c = {35, 100}
R^2	n/a
Covariance matrix	n/a
bounds	{{"bounds: C8", 17/2, 850}, {"bounds: C10", 17/2, 850}, {"bounds: C12", 17/2, 850}, {"bounds: C14", 17/2, 850}, {"bounds: C16", 17/2, 850}} 425}, {"bounds: C16", 17, 425}}



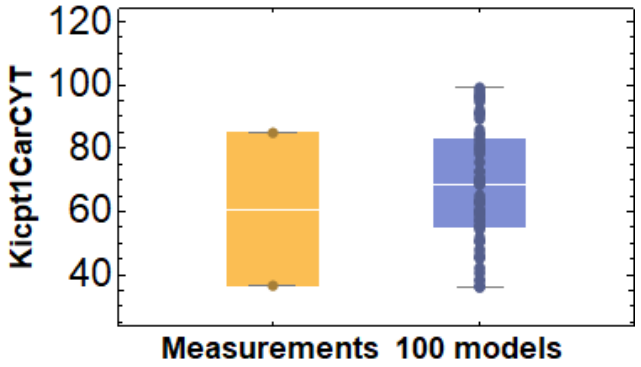
Comments: n/a

Kicpt1CarCYT

Semi-satisfactory values found: the form of this equation doesn't allow us to accurately capture the inhibitory effects of carnitine against the binding of acylcarnitines, as it would be dependent first on the binding of an acyl-CoA. We take the average of the whole range of chain length-specific carnitine K_m

	Parameter	Chosen value	Alternatives	Comments
Kmcpt1CarCYT		Van Vlies *et al.* (2007, (17)) Human fibroblast, 37°C, pH = 7.0, 150 mM KCL, 25 mM Tris, 20 mM KPi, 5 mM KCN, 1 mg/mL BSA, did use detergent. This is quite a reliable parameter, as they went to some length to discern between CPT1 and CPT2.	Demaugre *et al.* (1988, (81)) Permeabilised CPT2 deficient patient fibroblasts, sensitive to malonyl-CoA, measure palmitoyl-L-(methyl^{3}H) carnitine from L-(methyl^{3}H) carnitine (540 µM) and palmitoyl-CoA (110 µM) complexed with BSA; 115 mM Tris (pH = 7.4); 4.5 mM reduced glutathione; 70 mM KCl, temp not given; take average of two controls	**1**) In the inhibition term of reactions with a conserved co-substrate (CoA or carnitine), the Ki of the carnitine is a competitive inhibitory term (Car/KiCar (see highlighted in yellow below)): the carnitine would exclude acylcarnitine binding, but this would be chain-length-independent. Hence I take the Km value as the Ki value (the average, if the chain lengths differ).
	Kicpt1CarCYT	85 µM *{0.1, not true K_i}*	36.5 µM *{0.1, not true K_i}*	

Calculate	
equals	Kmcpt1CarMAT



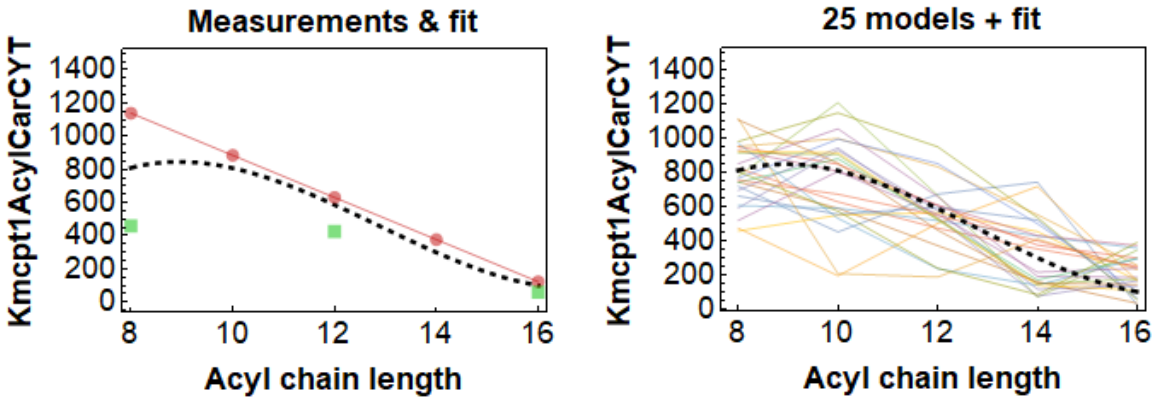
Comments: n/a

Kmcpt1AcylCarCYT

No satisfactory value: no values were found that expressed the change of the K_m for chain lengths and which are unambiguously CPT1 parameters

	Parameter	Chosen value	Alternatives	Comments
Kmcpt1AcylCarCYT		Finocchiaro *et al.* (1990, (13)) Normal human liver; 25°C; pH = 8; CPT1 and CPT2 not discerned; BSA not used; 91 mM Tris-HCl; Tween-20 included in assay	West *et al.* (1971, (76)) Ox liver; 30°C; pH = 8.0; 100 mM Tris-HCl; 1% BSA	**1)** These parameters are not purified CPT1 but CPT2, in all likelihood, but I use them as a placeholder while I have no parameters available to me. These parameters are simply called CPT parameters by Finocchiaro *et al.* (13). Murthy *et al.* (12) show that detergents inhibit CPT1 and activate CPT2, and that CPT1 loses activity after solubilization; Finocchiaro *et al.* (13) used Tween-20 both in extracting the enzyme and in the assay cocktail itself. Based on this, and the authors' statement that they see no MalCoA-sensitivity in their extract, I interpret the parameter to be more representative of CPT2. **2)** These parameters show some agreement with those measured by West *et al.* (76), i.e. an increase from C16 (in the 10s) through C12 (middle 100s). This shows that our chose parameters might not be entirely unrealistic for CPT1.
	Kmcpt1C16AcylCarCYT	123 µM *{0.5, temp + CPT2 not distinguished + no BSA}* [60 – 123]	60 µM *{0.5, Ox + old, CPT2 not properly known yet}*	
	Kmcpt1C14AcylCarCYT	377 µM *{0.5, temp + CPT2 not distinguished + no BSA}*		
	Kmcpt1C12AcylCarCYT	631 µM *{0.5, temp + CPT2 not distinguished + no BSA}* [426 – 631]	426 µM *{0.5, Ox + old, CPT2 not properly known yet}*	
	Kmcpt1C10AcylCarCYT	885 µM *{0.5, temp + CPT2 not distinguished + no BSA}*		
	Kmcpt1C8AcylCarCYT	1139 µM *{0.5, temp + CPT2 not distinguished + no BSA}* [460 – 1139]	460 µM *{0.5, Ox + old, CPT2 not properly known yet}*	

Cubic polynomial (multinormal distribution)	
Formula	$a * x^3 + b * x^2 + c * x + d$
Best fit parameters	a = 3.20833 b = -123.708 c = 1443.17 d = -4461.5
R^2	0.91
Covariance matrix	{{66.5479, -2395.72, 27772.7, -103282.}, {-2395.72, 86413.1, -1.00382*10^6, 3.74071*10^6}, {27772.7, -1.00382*10^6, 1.16876*10^7, -4.36557*10^7}, {-103282., 3.74071*10^6, -4.36557*10^7, 1.63483*10^8}}
Bounds	{{"bounds: C8", 1139/10, 11390}, {"bounds: C10", 177/2, 8850}, {"bounds: C12", 631/10, 6310}, {"bounds: C14", 377/10, 3770}, {"bounds: C16", 123/10, 1230}}



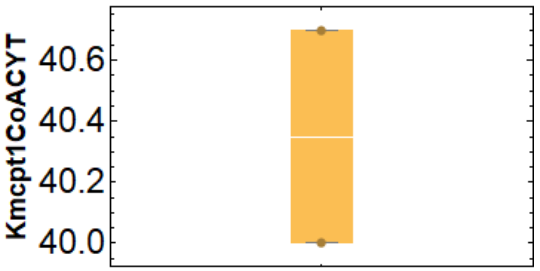
Comments: n/a

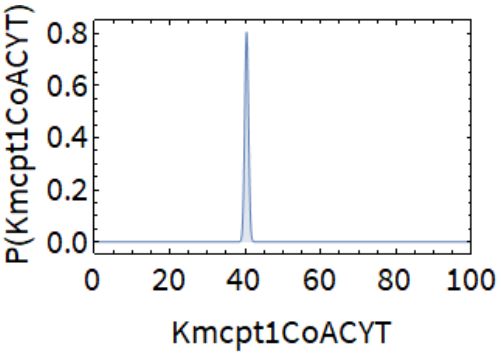
Kmcpt1CoACYT

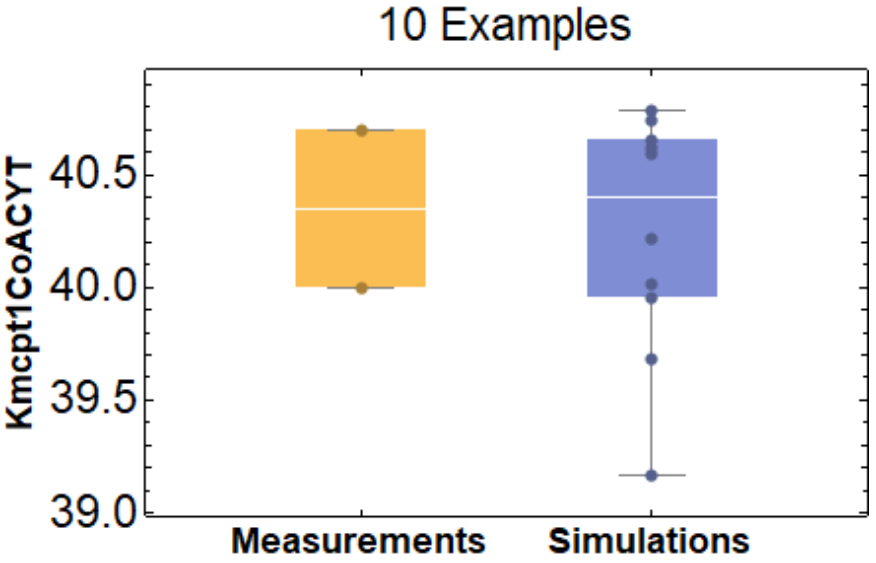
Satisfactory value found.

	Parameter	Chosen value	Alternatives	Comments
Kmcpt1CoACYT		Ramsay *et al.* (2001, (1)) Rat liver (taken from literature, but I don't have access to the original paper).	Kohn & Garfinkel (1983, (82)) "Average of literature values – literature not indicated" Rat heart, did not carefully distinguish between CPT1 and CPT2.	**1)** The value from Kohn & Garfinkel (82) was calculated as the "average" of literature values. They never indicate which literature values were used for their average, so it is not possible to ascertain what the origin and measuring conditions of this parameter were. What we do know, is that Kohn & Garfinkel (82) constructed the model for rat heart and that they did not distinguish carefully between CPT1 and CPT2. Therefore, we choose the value from Ramsay *et al.* (1), even though it suffers from its own opacity in the sense that the underlying source is not open access. **2)** CoA does not exhibit cooperative binding: acyl-CoA binds before carnitine ((73) showed this for CPT2; Ramsay *et al.* and McGarry *et al.* (1,5) extrapolated this to CPT1, though they did not provide direct evidence), and - depending on the chain-length of the acyl-CoA, the carnitine might well see varying degrees of affinity increase. In the reverse direction, however, CoA binds first, meaning that it is not differentially affected depending on its acylcarnitine co-substrate. **3)** Ramsay *et al.* (1) reports K_m values for CoA for various carnitine acyltransferases (CPT1A, CPT2, COT, CrAT and CPT for the endoplasmic reticulum) that are between 16 µM and 300 µM (in fact, if the CPT from the endoplasmic reticulum is excluded, they fall in the even narrower range of 16 µM to 112 µM). This suggests that the K_m for CoA among carnitine acyltransferases does not vary too much (remains in the lower micromolar range), which suggests we cannot be too off.
	Kmcpt1CoACYT	40 µM *{0.5, rat + uncertain origin}* [40 – 40.7]	40.7 µM *{0.5, rat + uncertain origin}*	

log-normal distribution	
Parameters **(of the normal distribution)**	μ = 3.69755 σ = 0.0122673
Bounds	{"bounds", 4, 400}







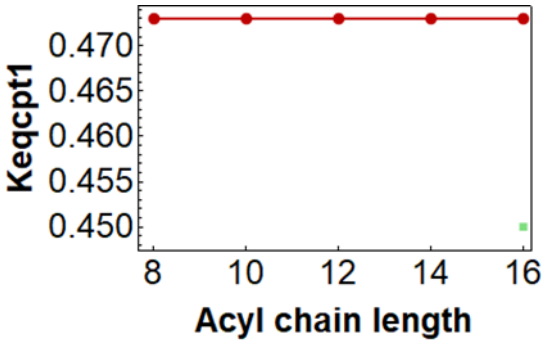
Comments:

Keqcpt1

eQuilibrator yields relatively reliable and systematic value, and they even agree with literature: even so, the effect of chain-length cannot be taken into account, as the reaction in eQuilibrator is only defined for the C16:0 substrate

	Parameter	Chosen value	Alternatives	Comments
Keqcpt1		Estimated using eQuilibrator (83) Ionic strength = 0.125 mM, pH = 7.2, temperature correction estimate using Van 't Hoff relation	Norum (1964, (84)) calf liver, 30°C, pH = 7.4, 100 mM Tris-HCl, no BSA, CPT1 and CPT2 not discerned, assayed in both directions	**1)** Since eQuilibrator could only perform its estimation for the C16-chain for this reaction, the K_{eq} was assumed identical for all the other chain lengths **2)** I choose to use the K_m for carnitine for C16 chain-length substrate as a temporary placeholder for all the K_m values for carnitine, even though the fact that carnitine binds only after acyl-CoA to CPT1 (73) probably means that the carnitine would have different affinities for CPT1 depending on the other substrate.
	Keqcpt1C16	0.473 *{1.0}* [0.45 – 0.473]	0.45 *{0.5, temp}*	
	Keqcpt1C14	0.473 *{0.1, assumed equivalent to Keqcpt1C16}*		
	Keqcpt1C12	0.473 *{0.1, assumed equivalent to Keqcpt1C16}*		
	Keqcpt1C10	0.473 *{0.1, assumed equivalent to Keqcpt1C16}*		
	Keqcpt1C8	0.473 *{0.1, assumed equivalent to Keqcpt1C16}*		

Unique	
Values	{{8, 0.473}, {10, 0.473}, {12, 0.473}, {14, 0.473}, {16, 0.473}}



Comments: No variation allowed, unique values from eQuilibrator.

Kicpt1MalCoACYT

Much remains still be confirmed about this effect, both mechanistically and kinetically. A decent placeholder value has been found for the meantime, though.

	Parameter	Chosen value	Alternatives	Comments
Kicpt1MalCoA		Fraser *et al.* (2001, (23)) Rat liver, pH = 7.1, temperature not indicated, assume room temperature, 70 mM KCl, 1% defatted BSA		**1)** Based on a lack of consensus in the literature, I model this as a simple competitive inhibition effect against acyl-CoA binding (see "Malonyl-CoA (unresolved)"). Fraser *et al.* (23) suggest a simple 1:1 stoichiometry and K_i values as indicated. **2)** Mills *et al.* (34) showed that octanoyl-CoA and palmitoyl-CoA were both competitively excluded by malonyl-CoA, lending some credence to the idea that malonyl-CoA acts on all chain lengths. **3)** Fraser *et al.* (23): Ki = 9.2 µM (outer membrane) Ki = 9.1 µM (contact sites) (rat liver, pH = 7.1, temperature not indicated, assume room temperature, 70 mM KCl, 1% defatted BSA). They indicate that CPT1 at contact sites is inhibited by competition against palmitoyl-CoA (and cites changed K_m values, while omitting data on whether malonyl-CoA changes the K_m for carnitine - I think there was an a priori assumption here) while, in outer membrane fractions, inhibition is principally exerted through V_{max} decrease (non-competitive inhibition). **4)** Alteration of the number of contact sites by changing the energisation state of the mitochondria (85) might be another example of a short-term, membrane-mediated regulatory mechanism which is not accounted for by our model. **5)** Changes in CPT1's sensitivity for malonyl-CoA can probably also take place: Saggerson (25) discusses how changes in CPT1's affinity for malonyl-CoA occurs more slowly that the changes in malonyl-CoA concentration – i.e. cannot be effected over 30 minutes – but that it does play a role over the medium-term. **6)** Malonyl-C is produced by acetyl-CoA carboxylase (ACC) from acetyl-CoA and converted back to acetyl-CoA by malonyl-CoA decarboxylase (MCD) or consumed during lipogenesis by
	Kicpt1MalCoACYT	9.1 µM *{0.1, rat + temp + mechanism not elucidated}* [9.1 – 9.2]		

fatty acid synthase (FAS) (25). The activity of these enzymes is enough to replenish cytosolic malonyl-CoA 4-5 times a minute (86), so this effect should be felt over the very short term.

Unique	
Values	9.1

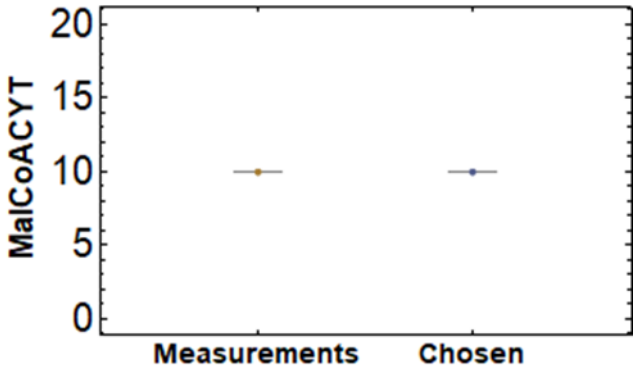
Comments: No variation allowed, unique value.

MalCoACYT

Satisfactory value found.

	Parameter	Chosen value	Alternatives	Comments
MalCoACYT		[Acetyl-CoA] = 0.9 nmol.mg-protein^{-1} (87) Convert to 158.82 µM using the total cell volume (3000 µm3) and protein (600 pg) (78) Convert to malonyl-CoA using the ratio [malonyl-CoA]/[acetyl-CoA] = 0.0526 in starved rat liver homogenate from Guynn *et al.* (88) [Malonyl-CoA] = 8.35 µM Round up to 10 µM		**1)** Similar to K_i of CPT1 for malonyl-CoA reported by Fraser *et al.* (23)
	MalCoACYT	10.0 µM		

Unique	
Values	10



Comments: No variation allowed, unique value.

REFERENCES

1. Ramsay RR, Gandour RD, Van Der Leij FR. Molecular enzymology of carnitine transfer and transport. Biochimica et Biophysica Acta - Protein Structure and Molecular Enzymology. 2001;1546(1):21–43.

2. Cooper GM. Mitochondria. In: Cooper GM, editor. The Cell: A Molecular Approach. 8th ed. Sunderland, MA: Sinauer Associates; 2019. p. 434–7.

3. Drynan L, Quant PA, Zammit VA. Flux control exerted by mitochondrial outer membrane carnitine palmitoyltransferase over β-oxidation, ketogenesis and tricarboxylic acid cycle activity in hepatocytes isolated from rats in different metabolic states. Biochemical Journal. 1996;317(3):791–5.

4. Eaton S. Control of mitochondrial β-oxidation flux. Prog Lipid Res. 2002;41(3):197–239.

5. McGarry JD, Brown NF. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. Eur J Biochem. 1997;244(1):1–14.

6. Zammit VA, Price ND, Fraser F, Jackson VN. Structure-function relationships of the liver and muscle isoforms of carnitine palmitoyltransferase I. Biochem Soc Trans. 2001;29(2):287–91.

7. Pfanner N, Rassow J, Klei IJ, Neupert W. A dynamic model of the mitochondrial protein import machinery. Cell. 1992;68(6):999–1002.

8. Fraser F, Zammit VA. Enrichment of carnitine palmitoyltransferases I and II in the contact sites of rat liver mitochondria. Biochemical Journal. 1998;329(2):225–9.

9. Brdiczka D. Contact sites between mitochondrial envelope membranes. Structure and function in energy- and protein-transfer. BBA - Reviews on Biomembranes. 1991;1071(3):291–312.

10. Wojtczak L, Adams V, Brdiczka D. Effect of oleate on the apparent Km of monoamine oxidase and the amount of membrane-bound hexokinase in isolated rat hepatocytes: Further evidence for the controlling role of the surface charge in hexokinase binding. Mol Cell Biochem. 1988;79(1):25–30.

11. Klug GA, Krause J, Östlund AK, Knoll G, Brdiczka D. Alterations in liver mitochondrial function as a result of fasting and exhaustive exercise. Biochimica et Biophysica Acta (BBA) - Bioenergetics. 1984;764(3):272–82.

12. Murthy MS, Pande S V. Some differences in the properties of carnitine palmitoyltransferase activities of the mitochondrial outer and inner membranes. Biochem J. 1987;248(3):727–33.

13. Finocchiaro G, Colombo I, DiDonato S. Purification, characterization and partial amino acid sequences of carnitine palmitoyl-transferase from human liver. FEBS Lett. 1990;274(1–2):163–6.

14. McGarry JD, Leatherman GF, Foster DW. Carnitine palmitoyltransferase I. The site of inhibition of hepatic fatty acid oxidation by malonyl-CoA. Journal of Biological Chemistry. 1978;253(12):4128–36.

15. Schaefer J, Jackson S, Dick DJ, Turnbull M. Trdimctional Enzvme Deficienw Adult Presenta & of a UsuaU ; Fatal P-Oxidation Defect. Ann Neurol. 1996;597–602.

16. Bonnefont JP, Taroni F, Cavadini P, Cepanec C, Brivet M, Saudubray JM, et al. Molecular analysis of carnitine palmitoyltransferase II deficiency with hepatocardiomuscular expression. Am J Hum Genet. 1996;58(5):971–8.

17. van Vlies N, Ruiter JP, Doolaard M, Wanders RJ, Vaz FM. An improved enzyme assay for carnitine palmitoyl transferase I in fibroblasts using tandem mass spectrometry. Molecular Gene. 2007;90(1):24–9.

18. Chen X, Shang L, Deng S, Li P, Chen K, Gao T, et al. Peroxisomal oxidation of erucic acid suppresses mitochondrial fatty acid oxidation by stimulating malonyl-CoA formation in the rat liver. J Biol Chem. 2020;295(30):10168–79.

19. Prip-Buus C, Thuillier L, Abadi N, Prasad C, Dilling L, Klasing J, et al. Molecular and enzymatic characterization of a unique carnitine palmitoyltransferase 1A mutation in the Hutterite community. Mol Genet Metab. 2001;73(1):46–54.

20. Gobin S, Thuillier L, Jogl G, Faye A, Tong L, Chi M, et al. Functional and Structural Basis of Carnitine Palmitoyltransferase 1A Deficiency. Journal of Biological Chemistry. 2003;278(50):50428–34.

21. Brown NF, Mullur RS, Subramanian I, Esser V, Bennett MJ, Saudubray JM, et al. Molecular characterization of L-CPT I deficiency in six patients: insights into function of the native enzyme. J Lipid Res. 2001;42(7):1134–42.

22. Woeltje KF, Kuwajima M, Foster DW, McGarry JD. Characterization of the mitochondrial carnitine palmitoyltransferase enzyme system. II. Use of detergents and antibodies. Journal of Biological Chemistry. 1987;262(20):9822–7.

23. Fraser F, Padovese R, Zammit VA. Distinct Kinetics of Carnitine Palmitoyltransferase I in Contact Sites and Outer Membranes of Rat Liver Mitochondria. Journal of Biological Chemistry. 2001;276(23):20182–5.

24. McGarry JD, Woeltje KF, Kuwajima M, Foster DW. Regulation of ketogenesis and the renaissance of carnitine palmitoyltransferase. Diabetes Metab Rev. 1989;5(3):271–84.

25. Saggerson D. Malonyl-CoA, a key signaling molecule in mammalian cells. Annu Rev Nutr. 2008;28:253–72.

26. Zammit VA. Malonyl-CoA, a Key Signaling Molecule in Mammalian Cells. Biochemical Journal. 1999;343(2):505–15.

27. Ruderman NB, Saha AK, Vavvas D, Witters LA. Malonyl-CoA, fuel sensing, and insulin resistance. Am J Physiol Endocrinol Metab. 1999;276(1):E1–18.

28. Kashfi K, Cook GA. Proteinase treatment of intact hepatic mitochondria has differential effects on inhibition of carnitine palmitoyltransferase by different inhibitors. Biochemical Journal. 1992;282(2):909–14.

29. Murthy MS, Pande S v. Malonyl-CoA binding site and the overt carnitine palmitoyltransferase activity reside on the opposite sides of the outer mitochondrial membrane. Proceeding sof the National Academy of Sciences. 1987;84(2):378–82.

30. Fraser F, Corstorphine CG, Zammit VA. Evidence that both the acyl-CoA-and malonyl-CoA binding sites of mitochondrial overt carnitine palmitoyltransferase (CPT I) are exposed on the cytosolic face of the outer membrane. Biochem Soc Trans. 1996;24(2):184S.

31. McGarry JD, Brown NF, Inthanousay PP, Park DI, Cook BA, Foster DW. Insights into the topography of mitochondrial carnitine palmitoyltransferase gained from the use of proteases. Progress in cClinical and Biological Research. 1992;375:47–61.

32. McGarry JD, Leatherman GF, Foster DW. Carnitine palmitoyltransferase I. The site of inhibition of hepatic fatty acid oxidation by malonyl-CoA. Journal of Biological Chemistry. 1978;253(12):4128–36.

33. Bremer J. The effect of fasting on the activity of liver carnitine palmitoyltransferase and its inhibition by malonyl-CoA. Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism. 1981;665(3):628–31.

34. Mills SE, Foster DW, McGarry JD. Interaction of malonyl-CoA and related compounds with mitochondria from different rat tissues. Relationship between ligand binding and inhibition of carnitine palmitoyltransferase I. Biochemical Journal. 1983;214(1):83–91.

35. Saggerson ED, Carpenter CA. Effects of fasting and malonyl CoA on the kinetics of carnitine palmitoyltransferase and carnitine octanoyltransferase in intact rat liver mitochondria. FEBS Lett. 1981;132(2):166–8.

36. Cook GA, Mynatt RL, Kashfi K. Yonetani-Theorell analysis of hepatic carnitine palmitoyltransferase-I inhibition indicates two distinct inhibitory binding sites. Journal of Biological Chemistry. 1994;269(12):8803–7.

37. Kolodziej MP, Zammit VA. Sensitivity of inhibition of rat liver mitochondrial outer-membrane carnitine palmitoyltransferase by malonyl-CoA to chemical- and temperature-induced changes in membrane fluidity. Biochemical Journal. 1990;272(2):421–5.

38. Bird MI, Saggerson ED. Interacting effects of L-carnitine and malonyl-CoA on rat liver carnitine palmitoyltransferase. Biochemical Journal. 1985;230(1):161–7.

39. Bird MI, Saggerson ED. Binding of malonyl-CoA to isolated mitochondria. Evidence for high- and low-affinity sites in liver and heart and relationship to inhibition of carnitine palmitoyltransferase activity. Biochemical Journal. 1984;222(3):639–47.

40. López-Viñas E, Bentebibel A, Gurunathan C, Morillas M, De Arriaga D, Serra D, et al. Definition by functional and structural analysis of two malonyl-CoA sites in carnitine palmitoyltransferase 1A. Journal of Biological Chemistry. 2007;282(25):18212–24.

41. Zammit VA, Corstorphine CG, Gray SR. Changes in the ability of malonyl-CoA to inhibit carnitine palmitoyltransferase I activity and to bind to rat liver mitochondria during incubation in vitro. Differences in binding at 0° C and 37° C with a fixed concentration of malonyl-CoA. Biochemical Journal. 1984;222(2):335–42.

42. Lloyd AC, Carpenter CA, Saggerson ED. Intertissue differences in the hysteretic behaviour of carnitine palmitoyltransferase in the presence of malonyl-CoA. Biochemical Journal. 1986;237(1):289–91.

43. Bhuiyan AKMJ, Pande S v. Carnitine palmitoyltransferase activities: Effects of serum albumin, acyl-CoA binding protein and fatty acid binding protein. Mol Cell Biochem. 1994;139(2):109–16.

44. Chaplin MF, Bucke C. Enzyme Technology. Cambridge: Cambridge University Press; 1990.

45. Reed MC, Lieb A, Nijhout HF. The biological significance of substrate inhibition: A mechanism with diverse functions. BioEssays. 2010;32(5):422–9.

46. Bremer J, Norum KR. The mechanism of substrate inhibition of palmityl coenzyme A: carnitine palmityltransferase by palmityl coenzyme A. Journal of Biological Chemistry. 1967;242(8):1744–8.

47. Woldegiorgis G, Bremer J, Shragoa E. Substrate inhibition of carnitine palmitoyltransferase by palmitoyl-CoA and activation by phospholipids and proteins. Biochimica et Biophysica Acta (BBA)/Lipids and Lipid Metabolism. 1985;837(2):135–40.

48. Færgeman NJ, Knudsen J. Role of long-chain fatty acyl-CoA esters in the regulation of metabolism and in cell signalling. Biochemical Journal. 1997;323(1):1–12.

49. Abo-Hashema KAH, Cake MH, Lukas MA, Knudsen. The interaction of acyl-CoA with acyl-CoA binding protein and carnitine palmitoyltransferase I. The International Journal of Biochemistry adn Cell Biology. 2001;33(8):807–15.

50. Duszyński J, Wojtczak L. Effect of detergents on ADP translocation in mitochondria. FEBS Lett. 1974;40(1):72–6.

51. Halle-Smith SC, Murray AG, Selwyn MJ. Palmitoyl-CoA inhibits the mitochondrial inner membrane anion-conducting channel. FEBS Lett. 1988;236(1):155–8.

52. Grevengoed TJ, Klett EL, Coleman RA. Acyl-CoA Metabolism and Partitioning. Annu Rev Nutr. 2018;34(1):1–30.

53. Goñi FM, Requero MA, Alonso A. Palmitoylcarnitine , a surface-active metabolite. 1996;390:1–5.

54. Juguelin H, Bessoule JJ, Cassagne C. Interaction of amphiphilic substrates (acyl-CoAs) and their metabolites (free fatty acids) with microsomes from mouse sciatic nerves. BBA - Biomembranes. 1991;1068(1):41–51.

55. Brecher P. The interaction of long-chain acyl CoA with membranes. Mol Cell Biochem. 1983;57(1):3–15.

56. Zammit VA, Corstorphine CG, Kolodziej MP, Fraser F. Lipid molecular order in liver mitochondrial outer membranes, and sensitivity of carnitine palmitoyltransferase I to malonyl-CoA. Lipids. 1998;33(4):371–6.

57. Broadway NM, Saggerson E. D. Effect of membrane environment on the activity and inhibitability by malonyl-CoA of the carnitine acyltransferase of hepatic microsomal membranes. Biochemical Journal. 1997;322(2):435–40.

58. Pauly DF, McMillin JB. Importance of acyl-CoA availability in interpretation of carnitine palmitoyltransferase I kinetics. Journal of Biological Chemistry. 1988;263(34):18160–7.

59. Boylan JG, Hamilton JA. Interactions of Acyl-Coenzyme A with Phosphatidylcholine Bilayers and Serum Albumin. Biochemistry. 1992;31(2):557–67.

60. Requero MA, González M, Goñi FM, Alonso A, Fidelio G. Differential penetration of fatty acyl-coenzyme A and fatty acylcarnitines into phospholipid monolayers. FEBS Lett. 1995;357(1):75–8.

61. Morel F, Lauquin G, Lunardi J, Duszynski J, Vignais P v. An appraisal of the functional significance of the inhibitory effect of long chain acyl-CoAs on mitochondrial transports. FEBS Lett. 1974;39(2):133–8.

62. Lopes VG, Filho A de BC, Yoshinaga MY, Hirata MH, Ferreira GM. Carnitine palmitoyl transferase I: Conformational changes induced by long-chain fatty acyl CoA ligands. J Mol Graph Model. 2022;112(November 2021).

63. Knudsen J, Neergaard TBF, Gaigg B, Jensen MV, Hansen JK. The Role of Acyl-CoA Binding Protein in Acyl-CoA Metabolism and Acyl-CoA-Mediated Cell Signalling. America Society for Nutrotional Sciences. 2000;130(2 SUPPL.):294–8.

64. Neess D, Bek S, Engelsby H, Gallego SF, Færgeman NJ. Long-chain acyl-CoA esters in metabolism and signaling: Role of acyl-CoA binding proteins. Prog Lipid Res [Internet]. 2015;59:1–25. Available from: http://dx.doi.org/10.1016/j.plipres.2015.04.001

65. Rolf B, Oudenampsen-Krüger E, Börchers T, Færgeman NJ, Knudsen J, Lezius A, et al. Analysis of the ligand binding properties of recombinant bovine liver-type fatty acid binding protein. Biochimica et Biophysica Acta (BBA)/Lipids and Lipid Metabolism. 1995;1259(3):245–53.

66. McGarry JD, Mills SE, Long CS, Foster DW. Observations on the affinity for carnitine, and malonyl-CoA sensitivity, of carnitine palmitoyltransferase I in animal and human tissues. Demonstration of the presence of malonyl-CoA in non-hepatic tissues of the rat. Biochemical Journal. 1983;214(1):21–8.

67. Oey NA, Den Boer MEJ, Wijburg FA, Vekemans M, Augé J, Steiner C, et al. Long-chain fatty acid oxidation during early human development. Pediatr Res. 2005;57(6):755–9.

68. Solberg HE. Different carnitine acyltransferases in calf liver. Biochim Biophys Acta. 1972;280(1972):422–34.

69. Mills SE, Foster DW, McGarry JD. Effects of pH on the interaction of substrates and malonyl-CoA with mitochondrial carnitine palmitoyltransferase I. Biochemical Journal. 1984;219(2):601–8.

70. Tein I, Demaugre F, Bonnefont JP, Saudubray JM. Normal muscle CPT1 and CPT2 activities in hepatic presentation patients with CPT1 deficiency in fibroblasts: Tissue specific isoforms of CPT1? J Neurol Sci. 1989;92(2–3):229–45.

71. Fraser F, Zammit VA. Submitochondrial and subcellular distributions of the carnitine-acylcarnitine carrier. FEBS Lett. 1999;445(1):41–4.

72. Zammit VA. Carnitine palmitoyltransferase 1: Central to cell function. IUBMB Life. 2008;60(5):347–54.

73. Nic a' Bháird N, Kumaravel G, Gandour RD, M.J. Krueger, Ramsay RR. Comparison of the active sites of the purified carnitine acyltransferases from peroxisomes and mitochondria by using a reaction-intermediate analogue. Biochemical Journal. 1993;294(3):645–51.

74. Rohwer JM, Hanekom AJ, Crous C, Snoep JL, Hofmeyr JH. Evaluation of a simplified generic bi-substrate rate equation for computational systems biology. IEE Proceedings-Systems Biology. 2006;153(5):338–41.

75. Schaefer J, Jackson S, Taroni F, Swift P, Turnbull DM. Characterisation of carnitine palmitoyltransferases in patients with a carnitine palmitoyltransferase deficiency: Implications for diagnosis and therapy. J Neurol Neurosurg Psychiatry. 1997;62(2):169–76.

76. West DW, Chase JFA, Tubbs PK. The Seperation and Properties of two forms of carnitine palmitoylstransferase from ox liver mitochondria. Biochem Biophys Res Commun. 1971;42(5):912–8.

77. Schmidt T, Samaras P, Frejno M, Gessulat S, Barnert M, Kienegger H, et al. ProteomicsDB. Nucleic Acids Res. 2018;46(D1):D1271–81.

78. Wiśniewski JR, Vildhede A, Norén A, Artursson P. In-depth quantitative analysis and comparison of the human hepatocyte and hepatoma cell line HepG2 proteomes. J Proteomics. 2016;136:234–47.

79. Fraser F, Corstorphine CG, Zammit VA. Topology of carnitine palmitoyltransferase I in the mitochondrial outer membrane. Biochemical Journal. 1997;323(3):711–8.

80. Demaugre F, Bonnefont JP, Mitchell GA, Nguyen-Hoang N, Pelet A, Rimoldi M, et al. Hepatic and muscular presentations of carnitine palmitoyl transferase deficiency: two distinct entities. Pediatr Res. 1988;25(3):308–11.

81. Demaugre F, Bonnefont JP, Mitchell GA, Nguyen-Hoang N, Pelet A, Rimoldi M, et al. Hepatic and muscular presentations of carnitine palmitoyl transferase deficiency: two distinct entities. Pediatr Res. 1988;25(3):308–11.

82. Kohn MC, Garfinkel D. Computer simulation of metabolism in palmitate-perfused rat heart. I. Palmitate oxidation. Ann Biomed Eng. 1983;11(5):361–84.

83. Flamholz A, Noor E, Bar-Even A, Milo R. EQuilibrator - The biochemical thermodynamics calculator. Nucleic Acids Res. 2012;40(D1):770–5.

84. Norum KR. Palmityl-CoA: carnitine palmityltransferase: purification from calf-liver mitochondria and some properties of the enzyme. Biochimica et Biophysica Acta (BBA) - Specialized Section on Enzymological Subjects. 1964;89(1):95–108.

85. Bücheler K, Adams V, Brdiczka D. Localization of the ATP/ADP translocator in the inner membrane and regulation of contact sites between mitochondrial envelope membranes by ADP. A study on freeze-fractured isolated liver mitochondria. Vol. 1056, Biochimica et Biophysica Acta. 1991.

86. Abo Alrob O, Lopaschuk GD. Role of CoA and acetyl-CoA in regulating cardiac fatty acid and glucose oxidation. Biochem Soc Trans. 2014;42(4):1043–51.

87. Shurubor YI, D'Aurelio M, Clark-Matott J, Isakova EP, Deryabina YI, Beal MF, et al. Determination of coenzyme A and acetyl-coenzyme A in biological samples using HPLC with UV detection. Molecules. 2017;22(9).

88. Guynn RW, Veloso D, Veech RL. The concentration of malonyl-coenzyme A and the control of fatty acid synthesis in vivo. Journal of Biological Chemistry [Internet]. 1972;247(22):7325–31. Available from: http://dx.doi.org/10.1016/S0021-9258(19)44633-4