SCAD (EC 1.3.99.2)

Short-chain acyl-CoA dehydrogenase

Author: Christoff Odendaal

Reviewed: Emmalie A. Jager

Last Edited: 2024-02-27

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

Contents

Acyl-CoA dehydrogenases (ACADs) are mitochondrial flavoproteins that desaturate acyl-CoA substrates. They are encoded in the nucleus, translated in the cytoplasm, and imported into and matured in the mitochondria (1). Among them, four form a part of mammalian mFAO (SCAD, MCAD, LCAD, VLCAD) and three are involved in amino acid degradation (isovaleryl- (IVD), isobutyryl-(IBD), glutaryl-CoA dehydrogenase (GCAD); on top of this, various peroxisomal and microsomal acyl-CoA oxidases can be regarded as performing the same reaction: substrate dehydrogenation at positions α,β (2).

The results from Furuta *et al.* (3), Davidson & Schulz (4), and Dommes & Kunau (5) in rat liver, bovine liver, and bovine heart, respectively, were suggestive of the existence of short-chain, general, and long-chain acyl-CoA dehydrogenases in each tissue. This might be considered the first evidence of the existence of SCAD.

SUBCELLULAR LOCALISATION

Mature SCAD enzymes are found in the mitochondria (1). SCAD is water soluble and is therefore located in the matrix of the mitochondrion (6).

STRUCTURE

ACADs all contain 1 mol FAD per mol subunit, perhaps contributing to the redox activity of the enzyme (7). The catalytic residue in all known ACADs is glutamate (8). This amino acid has been shown to initiate catalysis by abstracting the substrate α-hydrogen as H^+ (2). In MCAD, the residue in question is Glu376 (2), and there are homologues for this residue in SCAD, IBD, GCAD, and SCAD (8). In LCAD and IVD, however, this residue is not conserved. Rater, Glu261 (Glu254 in IVD) in helix G acts as the catalytic residue (8). Fig. 3 in (8) presents this view visually. In VLCAD and ACAD9, the catalytic residue is MCAD-like (8).

Substrate specificity

The basis for substrate specificity basically hinges on the length of a hydrophobic pocket which, ending in a glutamine and glutamate residue (Gln95 & Glu99) in MCAD and SCAD, contains glycine residues in VLCAD, allowing for much deeper protrusion of the acyl-CoA into the active site:

Shorter chain lengths are precluded from binding to VLCAD by the absence of a Ser166 residue which, in MCAD, forms a hydrogen bond with the 3'-phosphate of CoA. In VLCAD, this loss is offset by additional hydrophobic interactions by longer acyl chains (Fig. 3, (7)).

Catalytic capacity

The absence of Ser166 – which forms a hydrogen bond with the 3'-phosphate of CoA – from VLCAD reduces the limiting step of product release, leading to a higher Vmax for VLCAD (and, presumably, for ACAD9) than for SCAD and MCAD (8).

* STRONG modelling decision: the V_{max} value of VLCAD must be higher than for SCAD and MCAD

FUNCTION

Acyl-CoA dehydrogenases (ACADs) are mitochondrial flavoproteins that desaturate acyl-CoA substrates (1).

Oxidase reaction

Like its pig kidney cousin, human liver MCAD (9) was found to also catalyze the oxidase reaction of aliphatic acyl-CoAs.

* Unexplored activity: acyl-CoA oxidation

SCAD and MCAD are structurally similar at their catalytic sites, and it is possible that SCAD also has oxidase activity.

Isomerase activity

Zeng and Li (10) found MCAD to also have isomerase activity (3-enoyl-CoA to 2-enoyl-CoA; Fig. 1 in (10)).

* Unexplored activity: enoyl-CoA isomerase

SCAD and MCAD are structurally similar at their catalytic sites, and it is possible that SCAD also has oxidase activity.

Unsaturated fatty acids

Dommes & Kunau (5) also show, quite extensively, that SCAD, MCAD, and LCAD have – at least in bovine liver – activity towards unsaturated CoA esters.

* Unexplored activity: unsaturated acyl-CoAs

*Unexplored inhibitory kinetics

Though we have not included these in the model, it might be worth keeping in mind for later iterations. we omit these kinetics as they concern either metabolites that the model does not contain or they have not been confirmed in human cells.

* Inhibition by Propionyl-CoA

Finocchiaro *et al.* (7) report a mild inhibition of human SCAD activity by propionyl-CoA, echoing the sharp inhibition of bovine SCAD by acetoacyl-CoA (Shaw and Engel (11), Ki = 1 uM)

* Inhibition by straight-, long-chain acyl-CoAs

Ikeda *et al.* ((12) - rat liver) report straight long-chain acyl-CoAs like PalCoA and stearoyl-CoA inhibiting SCAD and MCAD by 30-50% of their total activity at 100 µM concentration. The mechanism is not discussed, but the same is not seen for shorter chains and LCAD.

* Inhibition by acetoacetyl-CoA

Davidson and Schulz (1982) report acetoacyl-CoA to inhibit bovine heart SCAD with a Ki = 1.0 µM. This might be false, it might be species-specific, it might be heart-specific: who knows?

* Unexplored kinetic implication: semiquinone inhibits ACADs

ETF-semiquinone, the partially reduced form of ETF, can accumulate when the coenzyme Q pool is reduced and is a potent inhibitor of acyl-CoA dehydrogenase (13).

MEASUREMENT CONDITIONS

Measuring challenges

Among the mitochondrial β-oxidation enzymes, the acyl-CoA dehydrogenases are the most difficult to measure accurately (14). This is due firstly to the fact that the acyl-CoA dehydrogenases are flavoproteins in which the reduction of FAD^+ to cannot be followed directly as in NAD-$FADH_2$-linked dehydrogenases. Secondly, there is significant overlap in substrate specificity between the different acyl-CoA dehydrogenases.

Temperature dependence

Lucas *et al.* (15) echo a combination of the points above, by indicating that physiological concentrations of cofactors and substrates can stabilize SCAD and MCAD significantly at 40°C – they suggest that this could be a rescue mechanism during fever.

Wild-type SCAD activity is reported to be relatively consistent under varying temperatures, for instance in Nguyen *et al.* (16), where only 15% of activity lost after prolonged incubation at 45°C. Two mutants of SCAD (R147W and G185S), however, were much less thermostable, losing 50% and 85% of their activity at 45°C.

*Modelling decision: activity down to 30°C is not penalised

Since SCAD activity is reported to be stable at varying temperatures, we can be less strict on temperature cutoffs for measures V_{max} values. However, note that this thermostability was shown for upwards temperature changes and not for decreases.

pH dependence

Since Glu376 initiates catalysis by H^+-abstraction from the substrate, pH is expected to be important for the catalysis. The pK of this amino acid in MCAD lies between 7.5 and 8.5 (2), which is the range within which Glu376 is in its reduced state and its activity is high. Since this catalytic residue is shared by SCAD and MCAD (2), you expect a similar pH range. So, values from within this range are probably acceptable. Nguyen *et al.* (16) observe activity of wild-type SCAD to be consistent between pH = 6.0 and 8.0, with activity declining above or below that range.

Ikeda *et al.* (12) on the rat ACADs: “The three enzymes were stable at pH 5.5 through 8.7 for at least 10 min at 37°C”

Finocchiaro *et al.* (7) echo this idea by testing the pH dependence of SCAD, MCAD, and IVD (Fig. 5, (7)). pH = 8.0 us a clear maximum, with 8.5 and 7.5 lysing close by.

Küchler *et al.* (17) however, observed human WT MCAD activity to continue to rise beyond pH = 8.0 (Fig. 8, (17)). Perhaps this indicates that we can conclude with some certainty that pH < 8.0 is lower than the physiological state, but that we are uncertain about the effects of higher pH. Luckily, pH < 8.0 does not occur much in the literature. *The similarity between MCAD and SCAD suggests that we might be able to draw this lesson for SCAD as well.*

* STRONG modelling decision: 7.5 < pH < 8.5 is the acceptable range

Considering the conflicting reports, and to allow for a certain range of pH variation so as to not exclude too much literature, any parameters measured at a pH of between 7.5 and 8.5 will be accepted, whereas those not measured at pH = 8.0 will be penalised. pH is an important factor for this enzyme, so this will be a main consideration in picking parameters.

Heterogeneity across species

There is definitely a difference between the enzymes of different species: the substrate specificity and affinity of SCAD is explicitly reported to be different between rat and human (7,12) and this probably holds across other species as well. The exact factors with which these parameters differ, are probably not so important, as I have ample human liver data.

Peterson *et al.* (9) showed that – besides for a few minor differences – pig kidney and human liver MCAD are basically identical. This lends credence to the idea that MCAD kinetics from pigs and humans and from different tissues can be used interchangeably. *Considering the similarity of SCAD and MCAD, it might be reasonable to expect this also to be the case for SCAD.*

* Modelling decision: porcine parameters not penalised

However, if neither human nor porcine parameters can be found, others might still be considered.

Heterogeneity across tissues

The same isoform of MCAD seems to be present in all tissues – at least I have not encountered any evidence to the contrary. The impact of this on kinetics is not immediately obvious to me, nor is it important, I think, as I have ample parameters from human liver available.

Peterson *et al.* (9) showed that – besides for a few minor differences – pig kidney and human liver MCAD are basically identical. This lends credence to the idea that MCAD kinetics from pigs and humans and from different tissues can be used interchangeably. *Considering the similarity of SCAD and MCAD, it might be reasonable to expect this also to be the case for SCAD.*

* Modelling decision: all tissues are, so long as they are protein-adjusted

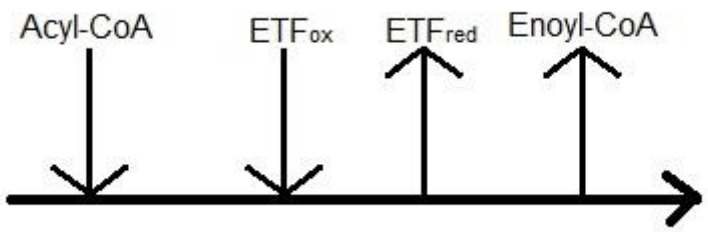
KINETICS

Mechanism

Peterson *et al.* (9) note that the mechanisms for MCAD-FAD + Octanoyl-CoA (forward) and for MCAD-FAD + Octenoyl-CoA (reverse) were microscopically similar, proceeding in three steps: binding and two isomerization steps. During the first isomerization step, reducing equivalents are transferred to the enzyme, reducing its prosthetic FAD group, which is the re-oxidised by interaction with the ETF, which becomes reduced in its stead (second isomerization step). Goetzman *et al.* (18) report this mechanism to be valid for all ACADs (Scheme 1, (18)).

The enoyl-CoA product is extremely tightly bound to the reduced enzyme (e.g. K_d = 13 pM compared to the 200 nM of the octanoyl-CoA substrate) and is released only due to the re-oxidation of the enzyme's FAD prosthetic group by ETF (19).

Thorpe and Kim (19) report a compulsory ordered mechanism: acyl-CoA binds first, and enoyl-CoA leaves last (shown with MCAD), byturyl-CoA, with crotonyl-CoA produced. However, considering the similarity of the reactions, this can be assumed for SCAD as well.



* Arbitrary modelling decision: random-order Bi-Bi Michaelis-Menten

For convenience, a random-order bisubstrate-biproduct Michaelis-Menten reaction will be assumed (20).

* Arbitrary modelling decision: ACADS have only one K_m for ETF

ACADs are reported as having a single K_m value for ETF, even though it binds to the ACAD after the binding of the acyl-CoA: this might change the binding kinetics for ETF in different conditions. However, it might be that the size of ETF (being a protein) renders the change in active site composition (which is kinetically unfavourable anyway) less consequential for its kinetics. This might be an explanation for why we only see one K_m value for ETF.

Reversibility

Due to the lack of data on reverse ACAD reactions and the redox-system (ETF-ACAD interaction) that overcomes thermodynamic unfavourability of product release (19), I am skeptical about whether this reaction is, in practice, reversible. To my eye, there is no good reason why the concomitant oxidation of the ACAD and release of the product should be able to take place in reverse. The enoyl-CoA product of these reactions is known to inhibit the reaction (12), but this might be simple competitive binding. However, in terms of convenience kinetics (20), I will regard this reaction as reversible until further notice.

** Unexplored kinetic implication: ACAD activity might not be reversible*

** Arbitrary modelling decision: the reaction will be treated as reversible.*

Reverse Vmax

Kumar and Srivastava (21) also found the observed relaxation rate constants of the corresponding fast and slow steps of the forward and reverse reactions to be nearly the same, suggesting that forward and reverse Vmaxes should be the same, should you ever wish to calculate them. *Considering the similarity of SCAD and MCAD, it might be reasonable to expect this also to be the case for SCAD.*

* Model assumption: forward and reverse V_{max} values should be similar.

Substrate specificity

SCAD in humans is specific for the C4 and C6-chain length acyl-CoAs (16). Though there is slight activity towards C8-acyl-CoAs, this is very low when measured and probably does not contribute a lot to the total activity towards those metabolites.

Rate equation

For n = {4, 6}:

$$vscadC_n = \frac{sfscadC_n \cdot \frac{Vscad}{VMAT} \cdot \left(\frac{C_nAcylCoAMAT[t] \cdot ETF_{ox}}{K_mscadC_nAcylCoAMAT \cdot K_mscadETF_{ox}} - \frac{C_nEnoylCoAMAT[t] \cdot ETF_{red}}{K_{eq}scadC_n \cdot K_mscadC_nAcylCoAMAT \cdot K_mscadETF_{ox}}\right)}{\left(1 + \frac{ETF_{ox}}{K_mscadETF_{ox}} + \frac{ETF_{red}}{K_mscadETF_{red}}\right) \cdot \left(1 + \sum_{n=4}^{n=6}\left(\frac{C_nAcylCoAMAT[t]}{K_mscadC_nAcylCoAMAT} + \frac{C_nEnoylCoAMAT[t]}{K_mscadC_nEnoylCoAMAT}\right)\right)}$$

where:

$$ETF_{ox} = \left(\frac{ETF_tMAT}{ETF_{\frac{ox}{red}} + 1}\right) \cdot ETF_{\frac{ox}{red}}$$

$$ETF_{red} = \frac{ETF_tMAT}{ETF_{\frac{ox}{red}} + 1}$$

Variables == initial values

Acyl-CoAs	Enoyl-CoAs
C6AcylCoAMAT[t] == 0 µM	C6EnoylCoAMAT[t] == 0 µM
C4AcylCoAMAT[t] == 0 µM	C4EnoylCoAMAT[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.

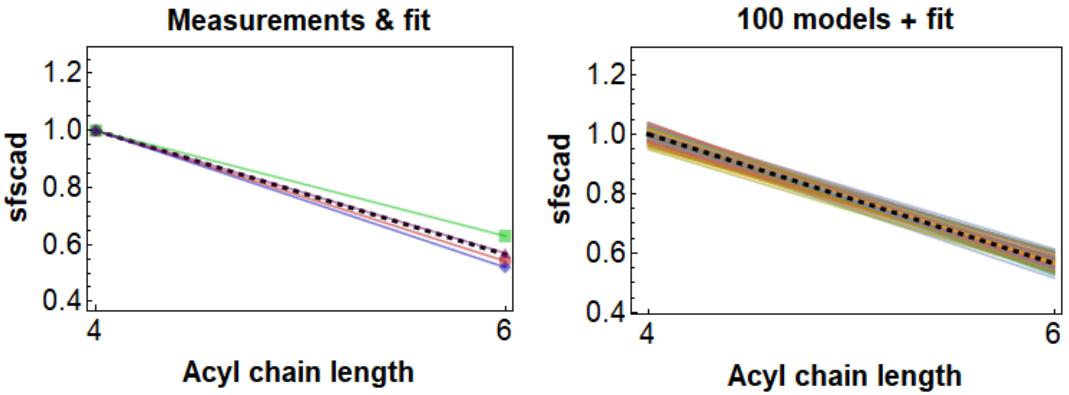
Weights are given in curly brackets next to parameter values: {} with short reasons

sfscad

Satisfactory values found.

	Parameter	Chosen value [range]	Alternatives			Comments
sfscad		Nguyen *et al.* (2002, (16)) human recombinant SCAD expressed in E. Coli, pH = 8.0, 20 mM Tris, 18.5 mM glucose, 3uM ETF, 20U glucose oxidase, 0.5U catalase, 60uM acyl-CoA, 32°C	Finocchiaro *et al.* (1987, (7)) human liver homogenate, 100 M KPi bufferm pH = 8.0, 5.6 µM ETF (intermediate electron acceptor), 0.048 mM DCIP (terminal electron acceptor), 0.1 FAD, 0.1 mM acyl-CoA, 32°C, spectrophotometer	Dommes & Kunau (1984, (5)) bovine liver mitochondria, 200 mM Bicone2/KOH, pH = 8.0, Triton X-100 0.15%, 0.1 mM meldoblau, 0.25 mM iodonitrotetrazolium chloride, A, 30°C	Shaw & Engel (1984, (11)) bovine liver mitochondria, 120 mM KPi, pH = 7.1, 0.001% dichlorophenol-indophenol, 50uM butyryl-CoA, 25°C	**1)** The specificity factors for bovine liver (5,11) and for human liver (Nguyen *et al*. (16) - recombinant; Finnochiaro *et al*. (7) - native) are very similar. This gives high confidence in the selected parameter from Nguyen *et al.* (16), which is chosen babove the value of Finocchiaro *et al.* (7) only because it is more recent. **2)** There seems to be some very slight activity towards the OctCoA substrate in human studies (and in all the bovine and porcine studies that I have looked at - Shaw & Engel (11) , Dommes & Kunau (5), Wu *et al.* (22)), but this is not reported across all human studies (7). I would investigate this in subsequent experiments.
	sfscadC6	0.542 *{1.0}* [0.542 – 0.63]	0.63 *{1.0}*	0.52 *{0.9, bovine}*	0.57 *{0.1, bovine + temp + pH}*	
	sfscadC4	1.0 *{1.0, temp}*	1.0 *{1.0}*	1.0 *{0.9, bovine}*	1.0 *{0.1, bovine + temp + pH}*	

Linear (multinormal distribution)	
Formula	$a * (x + b) + c$
Best fit parameters	a = -0.217167 b = 0.538282 c = 1.98556
R^2	0.998
Covariance matrix	{{0.000219256, 0.000251829, -0.00115961}, {0.000251829, 0.000298671, -0.00137531}, {-0.00115961, -0.00137531, 0.00633296}}
Bounds	{{"bounds: C4", 0.1, 100.}, {"bounds: C6", 0.0542, 54.2}}



Comments: n/a

Vscad

Semi-satisfactory value: human VLCAD, MCAD, and SCAD measured together would be a stronger parameter set.

Parameter		Chosen value [range]	Alternatives			Comments
Vscad		Finocchiaro et al. (1987, (7)) 0.0146 µmol.min^{-1}.mg-liver-protein^{-1} (human liver homogenate after ammonium sulphate precipitation, 100 M KPi buffer, pH = 8.0, 1.5 or 3.0 mM PMS (phenazine methosulphate - intermediate electron acceptor), 0.048 mM DCIP (terminal electron acceptor), 0.1 FAD, 0.1 mM acyl-CoA, 32°C, spectrophotometer, 0.1mM ButCoA); [1],[2]	Ikeda et al. (1985, (12)) 0.081 µmol.min^{-1}.mg-mito-protein^{-1} (rat liver mitochondrial isolate after ammonium sulphate precipitation - likely an overestimation, therefore, 100 M KPi buffer pH = 8.0, 1.5 or 3.0 mM PMS (phenazine methosulphate - intermediate electron acceptor), 0.048 mM DCIP (terminal electron acceptor), 0.1 FAD, 0.1 mM acyl-CoA, 32°C, spectrophotometer, 0.1mM ButCoA), multiply by 0.2 to get the Vmax(app) when ETF is used as electron acceptor (Ikeda et al., 1995, themselves saw this) [1], [2]	Dommes & Kunau (1984, (5)) bovine liver mitochondria, 200 mM Bicone2/KOH, pH = 8.0, Triton X-100 0.15%, 0.1 mM meldoblau, 0.25 mM iodonitrotetrazolium chloride, 0.1 mM ButCoA, 30°C	Shaw & Engel (1984, (11)) bovine liver mitochondria, 120 mM KPi, pH = 7.1, 0.001% dichlorophenol-indophenol, 50uM butyryl-CoA, 25°C	**1)** We choose the value from Finnochiaro *et al.* (7) as it is the only SCAD value measured in human cells. SCAD activity is defined as butyryl-CoA dehydrogenase activity, which is a slight issue, as MCAD can also dehydrogenate butyryl-CoA. Relying, however, on the mitochondrial proteomics by Wolters *et al.* (23) we see that SCAD is expressed about 6.5 times more lowly than MCAD. If the SCAD V_{max} chosen here is divided by the V_{max} of MCAD (0.0759 µmol.min^{-1}.mg-mito-Protein^{-1}, the value from Aoyama *et al.* (24) multiplied by 3 to adjust it to conform more to the partitioning of octanoyl-CoA and palmitoyl-CoA dehydrogenation activity, then we get an SCAD V_{max} which is about 9 times smaller than the MCAD V_{max}. This is close to the 6.5 times lower expression. The absence of Ser166, which forms a hydrogen bond with the 3'-phosphate of CoA, from VLCAD reduces the limiting step of product release, leading to a higher V_{max} for VLCAD (and, presumably, for ACAD9) than for SCAD and MCAD (8). The active sites of SCAD and MCAD therefore don't differ structure-chemically in the same way that VLCAD differs from MCAD and SCAD, which lends credence to the view that the differences between SCAD and MCAD V_{max} should align more with differences in expression. Therefore this is a realistic parameter.
	Vscad	0.00834 µmol.min^{-1}.mg-mito-Protein^{-1} (*, **, ***) *{1.0}* 0.01668 µmol.min^{-1}.mgmito-Protein^{-1} (*, **, ***, *****) *{0.9, post facto adjusted to get C8 accumulation}* [0.00463 – 0.213]	0.00463 µmol.min^{-1}.mg-mito-Protein^{-1} (**) *{0.9, rat}*	0.213 µmol.min^{-1}.mg-mito-Protein^{-1} *{0.5, bovine + very high for an ACAD}*	0.043 µmol.min^{-1}.mg-mito-protein^{-1} *{0.1, bovine + temp + pH}*	

* Scaled to mitochondrial protein (25% of cellular protein) according to Wiśniewski *et al.* (25)

** Multiplied by 3.5 according to the purification factor of the enzyme during ammonium sulphate precipitation (24)

***, Divided by 2: specific activity with PMS as primary electron acceptor gives about 2x the activity of what you see with the natural primary acceptor, ETF (7)

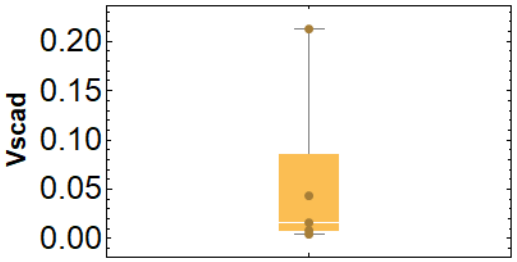
****, Multiplied by 2: to get an accumulation of C8 CoA- and carnitine esters in the patient model, as you see in real patients. This is still within the range of values investigated, but it prevents the unusual accumulation of C6 CoA and carnitine esters. This is a top-down decision, therefore.

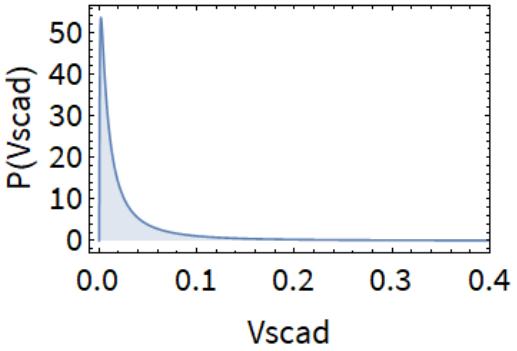
[1] assumption 1: PalCoA is only oxidised by VLCAD (in reality it's only 90%-ish of the time), OctCoA only by MCAD (only true about 90% of the time), and ButCoA only by SCAD

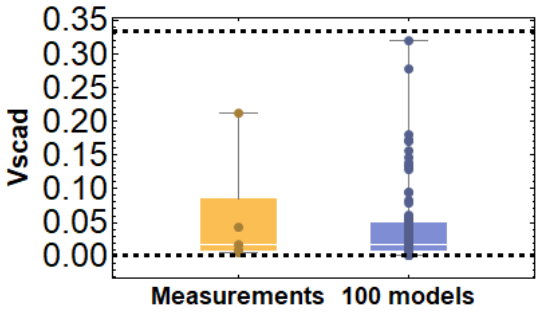
[2] assumption 2: all liver cells are hepatocytes (in reality it's only 80% of the cells)

[3] assumption 3: the VLCAD:MCAD ratio is the same in fibroblasts and hepatocytes.

log-normal distribution	
Parameters **(of the normal distribution)**	μ = -4.23425 σ = 1.43062
Bounds	{"bounds", 0.000834, 0.3336}







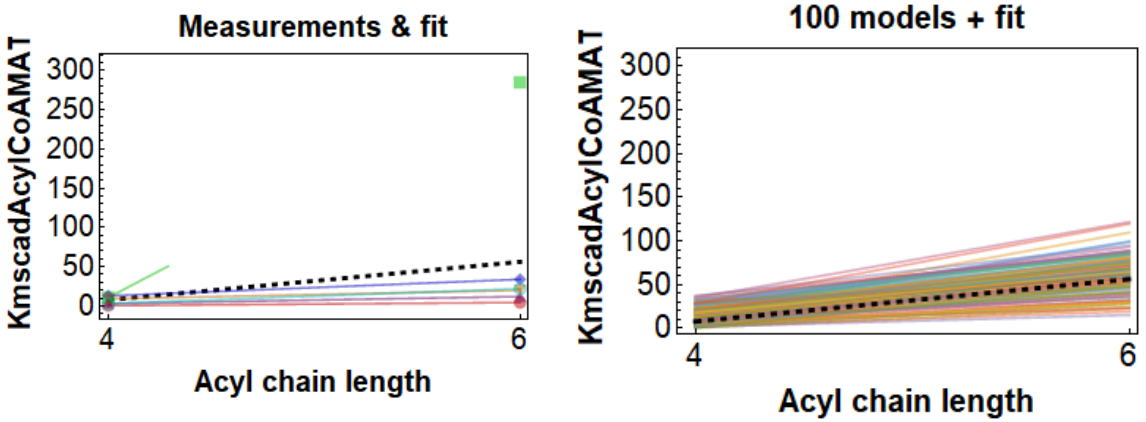
Comments: Note how few values actually get to the value that we chose for the deterministic model.

KmscadAcylCoAMAT

Satisfactory values found.

	Parameter	Chosen value	Alternatives					Comments
KmscadAcylCoAMAT		Nguyen *et al.* (2002, (16)) human recombinant MCAD expressed in E. Coli, pH = 8.0, 20 mM Tris, 18.5 mM glucose, 3uM ETF, 20U glucose oxidase, 0.5U catalase, 60uM acyl-CoA, 32°C	Ikeda et al. (1985, (12)) rat liver mitochondrial isolate, 100 M KPi buffer pH = 8.0, 1.5 or 3.0 mM PMS (phenazine methosulphate - intermediate electron acceptor), 0.048 mM DCIP (terminal electron acceptor), 0.1 FAD, 0.1 mM acyl-CoA, 32°C, spectrophotometer, 0.1mM ButCoA	Finnochiaro et al. (1987, (7)) human liver homogenate, 100 M KPi buffer pH = 8.0,1.5 or 3.0 mM PMS (intermediate electron acceptor), 0.048 mM DCIP (terminal electron acceptor), 0.1 FAD, 0.1 mM acyl-CoA, 32°C, spectrophotometer	Davidson & Schulz's (1982, (4)) bovine heart mitochondria, 0.1 mM KPi, pH = 7.6, 28 2,6-dichlorophenolindophenol, 0.65 mM PMS, 20 uM acyl-CoA, 0.2 mM N-ethylmaleimide, 25°C	Dommes & Kunau (1984, (5)) bovine liver mitochondria, 200 mM Bicone2/KOH, pH = 8.0, Triton X-100 0.15%, 0.1 mM meldoblau, 0.25 mM iodonitrotetrazolium chloride, 0.1 mM ButCoA, 30°C	Shaw & Engel (1984, (11)) bovine liver mitochondria, 120 mM KPi, pH = 7.1, 0.001% dichlorophenol-indophenol, 50uM butyryl-CoA, 25°C	1) The two sets of human parameters (Finnochiaro *et al.* (7) and Nguyen *et al.* (16)) differ quite a bit: almost by a factor of 100. The bovine liver SCAD parameters (4,5,11) were consistently smaller than what Finnochiaro *et al.* (7) reported with human liver. The rat parameters (12) did not follow this pattern. Since Nguyen *et al.* (16) published their results more recently, I will take them.
	KmscadC6AcylCoAMAT	4.4 µM *{1.0}* [4.4 – 285]	285 µM *{0.5, rat + outlier}*	33.9 µM *{1.0}*	12 µM *{0.1, bovine + pH + temp}*	20 µM *{0.9, bovine}*	22 µM *{0.1, bovine + temp + pH}*	
	KmscadC4AcylCoAMAT	0.38 µM *{1.0}* [0.38 – 12.9]	10.7 µM *{0.5, rat + outlier}*	12.9 µM *{1.0}*	3 µM *{0.1, bovine + pH + temp}*	9.0 µM *{0.9, bovine}*	3 µM *{0.1, bovine + temp + pH}*	

Linear (multinormal distribution)	
Formula	$a * (x + b) + c$
Best fit parameters	a = 24.2875 b = -2.23975 c = -35.1604
R^2	0.27
Covariance matrix	{{477.438, -54.1685, -2.2303}, {-54.1685, 6.95242, 0.286255}, {-2.2303, 0.286255, 0.0117861}}
Bounds	{{"bounds: C4", 0.038, 38.}, {"bounds: C6", 0.44, 440.}}



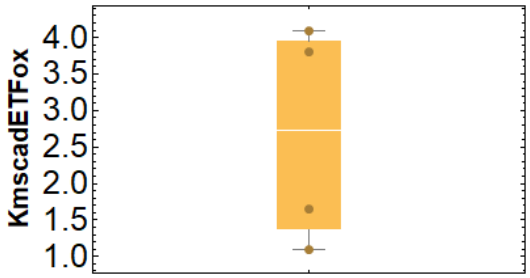
Comments: Higher values are overemphasized in this function, but I could not find a nice fit the describe the data and capture the variation simultaneously, and I do want to not blind the ensemble model to the potential of high $K_{m,SCAD}$ values for hexanoyl-CoA.

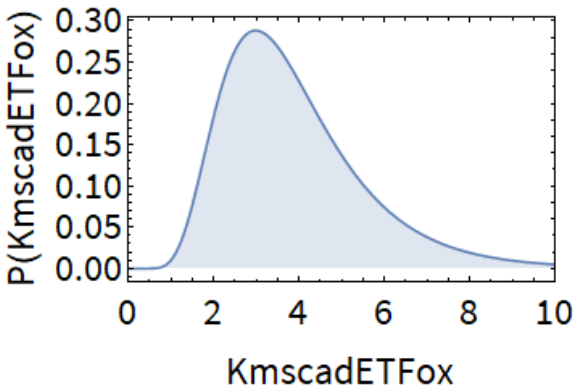
KmscadETFox

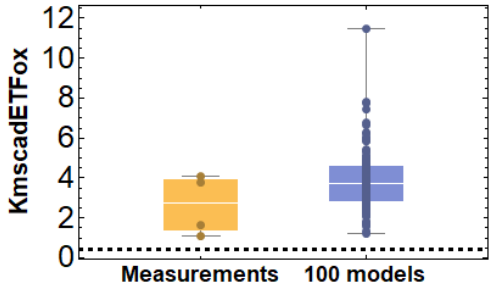
Satisfactory value found.

	Parameter	Chosen value	Alternatives			Comments
KmscadETFox		Finocchiaro et al. (1987, (7)) human liver homogenate, 100 M KPi buffer pH = 8.0, 1.5 or 3.0 mM PMS (intermediate electron acceptor), 0.048 mM DCIP (terminal electron acceptor), 0.1 FAD, 0.1 mM acyl-CoA, 32°C, spectrophotometer	Wainio (1970, (26)) pig and monkey, conditions unclear **0.7 to 2.6 µM, take average.**	Ikeda et al. (1985, (12)) rat liver mitochondrial isolate, 100 M KPi buffer pH = 8.0, 1.5 or 3.0 mM PMS (phenazine methosulphate - intermediate electron acceptor), 0.048 mM DCIP (terminal electron acceptor), 0.1 FAD, 0.1 mM acyl-CoA, 32°C, spectrophotometer, 0.1mM ButCoA	Thorpe reports a range of K_m values, from 0.2 to 2 µM having been measured in a variety of buffers, ionic strengths, and pH values. Though it is not explicitly mentioned, this seems to be independent of the ACAD in question, though it is mentioned in connection to MCAD. Also, we do not know whether this is in the forward or reverse direction. Other values in Thorpe (1991, (27)): 1.1) *P. denitrificans*: K_m = 2.5 µM for glutaryl-CoA dehydrogenase (direction and conditions not given); methylotrophic bacteria: 1.2) K_m = 7 µM for trimethylamine dehydrogenase (direction and conditions not given). **Take average of 0.2 – 2.0 range.**	**1)** I use Finocchiaro *et al.*'s (7) parameters, as they are the only human parameters available. **2)** The general similarity of the parameters and ranges proposed here are good confirmations of the chosen parameter.
	KmscadETFox	4.1 µM *{1.0}* [0.2 – 4.1]	1.65 µM **0.7 to 2.6 µM, take average** *{0.1, pig and monkey + conditions unclear}*	3.8 µM *{0.9, rat}*	1.1 µM **Take average of 0.2 – 2.0 range** *{0.1, unclear origin of enzyme and assay conditions}*	

log-normal distribution	
Parameters **(of the normal distribution)**	μ = 1.27243 σ = 0.423962
Bounds	{"bounds", 0.41, 41.}







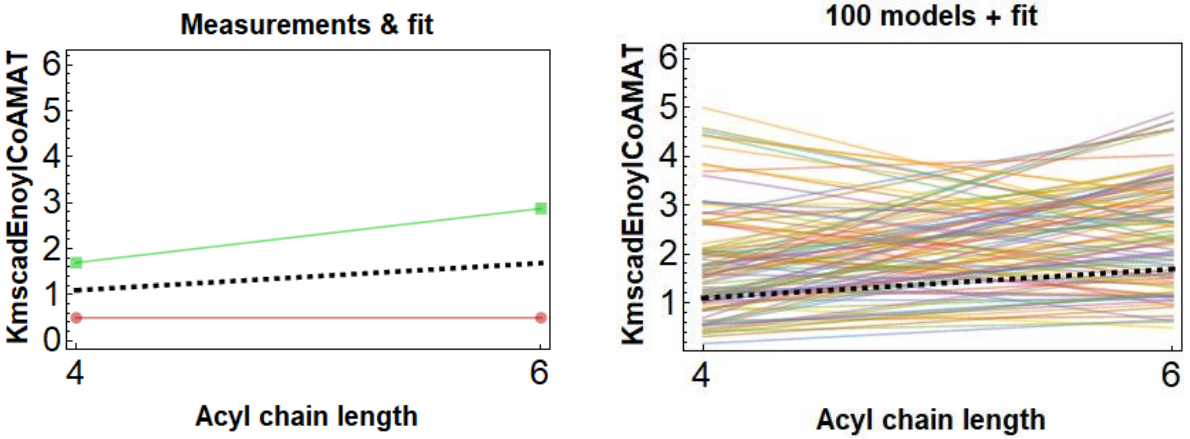
Comments: n/a

KmscadEnoylCoAMAT

Semi-satisfactory values found. It would be nice to confirm that K_d values and K_m values are interchangeable, if at all possible.

	Parameter	Chosen value	Alternatives	Comments
KmscadEnoylCoAMAT		Goetzman *et al.* (2006, (18)) K_d = 0.5 µM for C4-enyol-CoA, recombinant human SCAD expressed in E. coli, 10 mM HEPES, pH = 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% surfactant P20, BIAcore; K_d for C4AcylCoA = 0.6 µM, perhaps indicating that it is reasonable to just assume the K_d for the substrate as the K_m for the product: this is not the case for acyl-CoA dehydrogenases, for instance isovaleryl-CoA, which has different K_d values for its substrates and its products	Saenger *et al.* (2005, (28)) K_d values, recombinant mutant - kinetically dead - human E368Q SCAD expressed in E.coli with activity knocked out specifically to be able to see what the binding affinity is; 25°C, pH = 7.6, 50 mM KPi buffer, 0.1mM ButCoA	**1)** This is a K_d, not a K_m, but it does the work of K_i in most cases anyway, so I assume rapid equilibrium binding which would make $K_d = K_m$. **2)** An assumption here is that the same affinity for Enoyl-CoA is applicable for all chain lengths, which is unlikely is the K_m changes for different AcylCoA chain-lengths. However, the K_d values proffered by Saenger *et al.* (28) suggest that they are similar for C4 and C6,. **3)** The parameter from Goetzmann *et al.* (18) lies with a factor 3 of the corresponding parameters in the only other human measurement, from Saenger *et al.* (28) **4)** I choose Goetzman *et al.* (18), however, because Saenger *et al.* (28) performed their assays on a mutant SCAD (kinetically dead), which renders it a bit more suspect. **5)** Saenger *et al.* (28): K_d for C8-enoyl = 21.3 µM == also, K_d for C8AcylCoA = 125.3 µM (recombinant mutant - kinetically dead - human E368Q SCAD expressed in *E.coli* with activity knocked ou specifically to be able to see what the binding affinity is; 25°C, pH = 7.6, 50 mM KPi buffer); however since there is no substantial activity for the C8 and C10-acyl-CoA reactions, we omit these values.
	KmscadC6EnoylCoAMAT	0.5 µM (assumed) *{0.1, pH + no temp + K_d + assumed same as C4}* [0.5 – 2.88]	2.88 µM *{0.1, kinetically dead enzyme + pH + temp + K_d}*	
	KmscadC4EnoylCoAMAT	0.5 µM *{0.1, pH + no temp + K_d}* [0.5 – 1.7]	1.7 µM *{0.1, kinetically dead enzyme + pH + temp + K_d}*	

Linear (multinormal distribution)	
Formula	$a * (x + b) + c$
Best fit parameters	a = 0.295 b = -9.27681 c = 2.65666
R^2	0.70
Covariance matrix	{{0.88805, 1.03072, 3.49396}, {1.03072, 1.26171, 4.27697}, {3.49396, 4.27697, 14.4982}}
Bounds	{{"bounds: C4", 0.05, 5.}, {"bounds: C6", 0.05, 5.}}



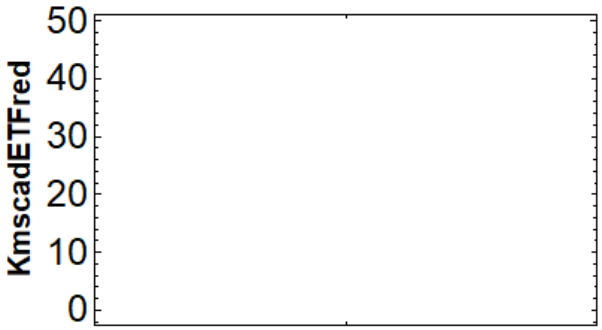
Comments: A much wide distribution is assumed than suggested by the data, but the data aren't very compelling, so that's what we want.

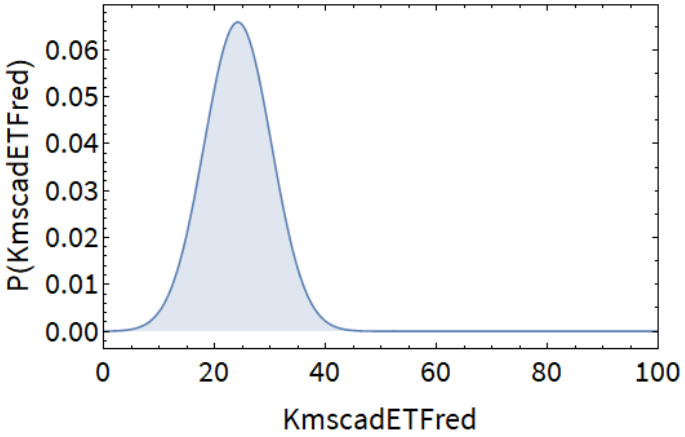
KmscadETFred

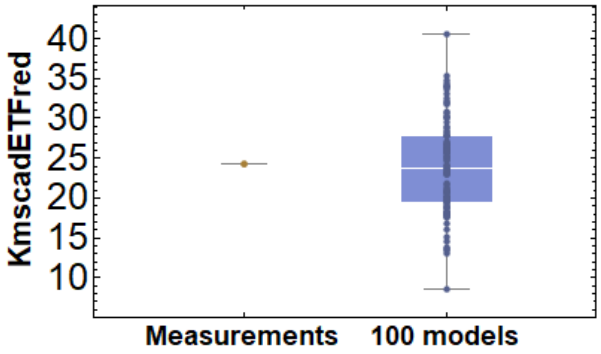
No satisfactory value found. This isn't something that has been measured in the literature, as far as I know,.

	Parameter	Chosen value	Alternatives	Comments
KmscadETFred		Modre-Osprian *et al.* (2009, (29)) From a generic (tissue-unspecific, animal-unspecific) model of mFAO	**If you want a range, you can use the range of ETFox, as it is not clear that ETFred and ETFox should have different K_m values.**	**1)** The sources that I have (26,27,30) that mention ETF affinity for ACAD enzymes, either do not mention the direction in which the activity was measured (in which case it is reasonable to assume that they refer to the forward reaction, with oxidized ETF as substrate; i.e. Thorpe, (27), Waino *et al.* (26)) or explicitly refer to the forward direction. For this reason, Modre-Osprian *et al.* (29)'s value, taken from Van Eunen *et al*. (31), seems more suitable, since they actually considered reversible kinetics and it makes intuitive sense that the K_m would be higher for the product. In any case, the difference between the K_m values of the reduced and oxidised ETF isn't huge (both in low micromolar range), so I doubt it is going to make a big difference. You would also not expect a massive difference between the two states of ETF, since ETF is a protein, and therefore very large, making the proportional effect of one charge on the ETF relatively smaller. *The alternative is that the K_m for ETF is the same in both directions*, but I yield to the expertise of Modre-Osprian *et al*. (29) in this case even though they didn't do the measuring themselves.
	KmscadETFred	24.2 µM *{0.1, from model, original source/tissue/conditions unclear}*		

normal distribution (constructed	
Parameters **(of the normal distribution)**	μ = 24.2 σ = 6.05
Bounds	{"bounds", 2.42, 242.}







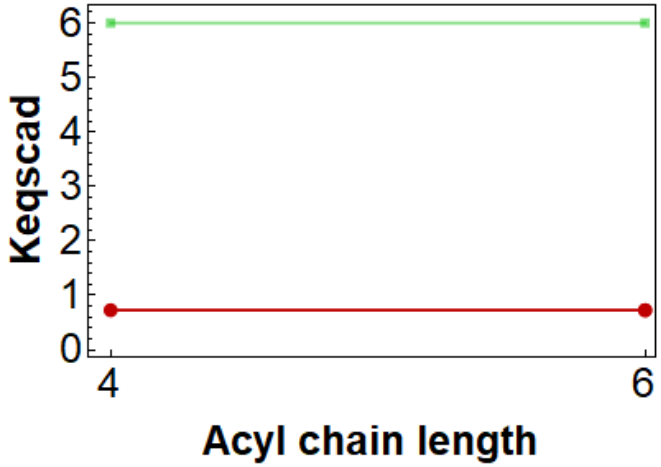
Comments: n/a

Keqscad

Satisfactory value found.

	Parameter	Chosen value	Alternatives	Comments
Keqscad		Thorpe *et al*. (1979, (32)) and Gustafson *et al*. (1986, (33)) report the same value: 0.7 for C4-acyl-CoA. 1.4 mM PMS (phenazine methasulphate, a more user-friendly substitute for ETF), 30 µM DCI (2,6-dichlorophenolindophenol, a redox due to track the assay), 20 mM phosphate buffer, 60 µM EDTA, pH = 7.6, T = 25°C 1.4 mM PMS (phenazine methasulphate, a more user-friendly substitute for ETF), 30 µM DCI (2,6-dichlorophenolindophenol, a redox due to track the assay), 20 mM phosphate buffer, 60 µM EDTA, *adjusted for temperature using the Van 't Hoff equation.*	Modre-Osprian *et al.* (2009, (29)) From a generic (tissue-unspecific, animal-unspecific) model of mFAO	**1)** Thorpe *et al.* (32) and Gustafson *et al.* (33). **2)** eQuilibrator, which is used elsewhere in this model, does not allow for ETF to be a reagent in its reactions. It does allow FAD/$FADH_2$ to be, but those give unrealistically small Keq values - more than orders of magnitude smaller than 1, which cannot be for a working reaction - suggesting that ETF plays some important thermodynamic stabilisation role. **3)** No more than an order of magnitude from the K_{eq} value proposed by Van Eunen *et al.* (31) based on Modre-Osprian *et al.* (29).
	KeqscadC6	0.728 (assumed the same as for C4). *{0.5, second-hand source, original inaccessible + assumed the same for all chain lengths}* [0.728 – 6.0]	6.0 *{0.1, from model, original source/tissue/conditions unclear}*	
	KeqscadC4	0.728 *{0.5, second-hand source, original inaccessible + assumed the same for all chain lengths}* [0.728 – 6.0]		

Unique	
Values	{{4, 0.728}, {6, 0.728}}



Comments: No variation allowed, unique values from Thorpe *et al*. (32) and Gustafson *et al*. (33).

REFERENCES

1. He M, Pei Z, Mohsen AW, Watkins PA, Murdoch G, Van Veldhoven PP, et al. Identification and characterization of new long chain acyl-CoA dehydrogenases. Mol Genet Metab. 2011;102(4):418–29.

2. Nandy A, Kieweg V, Kräutle FG, Vock P, Küchler B, Bross P, et al. Medium-long-chain chimeric human acyl-CoA dehydrogenase: Medium-chain enzyme with the active center base arrangement of long-chain acyl-CoA dehydrogenase. Biochemistry. 1996;35(38):12402–11.

3. Furuta S, Miyazawa S, Hashimoto T. Purification and properties of rat liver acyl-CoA dehydrogenases and electron transfer flavoprotein. The Journal of Biochemistry. 1981;90(6):1739–50.

4. Davidson B, Schulz H. Separation, properties, and regulation of acyl coenzyme A dehydrogenases from bovine heart and liver. Arch Biochem Biophys. 1982;213(1):155–62.

5. Dommes V, Kunau WH. Purification and properties of acyl coenzyme A dehydrogenases from bovine liver. Formation of 2-trans,4-cis-decadienoyl coenzyme A. Journal of Biological Chemistry. 1984;259(3):1789–97.

6. Liang X, Le W, Zhang D, Schulz H. Impact of the intramitochondrial enzyme organization on fatty acid oxidation. Biochem Soc Trans. 2001;29(2):279–82.

7. Finocchiaro G, Ito M, Tanaka K. Purification and properties of short chain acyl-CoA, medium chain acyl-CoA, and isovaleryl-CoA dehydrogenases from human liver. Journal of Biological Chemistry. 1987;262(17):7982–9.

8. McAndrew RP, Wang Y, Mohsen AW, He M, Vockley J, Kim JJP. Structural basis for substrate fatty acyl chain specificity: Crystal structure of human very-long-chain acyl-CoA dehydrogenase. Journal of Biological Chemistry. 2008;283(14):9435–43.

9. Peterson KL, Sergienko EE, Wu Y, Kumar NR, Strauss AW, Oleson AE, et al. Recombinant Human Liver Medium-Chain Acyl-CoA Dehydrogenase: Purification, Characterization, and the Mechanism of Interactions with Functionally Diverse C8-CoA Molecules. Biochemistry. 1995;34(45):14942–53.

10. Zeng J, Li D. Intrinsic isomerase activity of medium-chain Acyl-CoA dehydrogenase. Biochemistry. 2005;44(17):6715–22.

11. Shaw L, Engel PC. The purification and properties of ox liver short-chain acyl-CoA dehydrogenase. Biochem J. 1984;218(2):511–20.

12. Ikeda Y, Okamura-Ikeda K, Tanaka K. Purification and characterization of short-chain, medium-chain, and long-chain acyl-CoA dehydrogenases from rat liver mitochondria. Isolation of the holo-and apoenzymes and conversion of the apoenzyme to the holoenzyme. Journal of Biological Chemistry. 1985;260(2):1311–25.

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

14. Wanders RJA, Vreken P, Den Boer MEJ, Wijburg FA, Van Gennip AH, IJlst L. Disorders of mitochondrial fatty acyl-CoA β-oxidation. In: Journal of Inherited Metabolic Disease. 1999. p. 442–87.

15. Lucas TG, Henriques BJ, Rodrigues J V., Bross P, Gregersen N, Gomes CM. Cofactors and metabolites as potential stabilizers of mitochondrial acyl-CoA dehydrogenases. Biochim Biophys Acta Mol Basis Dis [Internet]. 2011;1812(12):1658–63. Available from: http://dx.doi.org/10.1016/j.bbadis.2011.09.009

16. Nguyen T V., Riggs C, Babovic-Vuksanovic D, Kim YS, Carpenter JF, Burghardt TP, et al. Purification and characterization of two polymorphic variants of short chain acyl-CoA dehydrogenase reveal reduction of catalytic activity and stability of the Gly185Ser enzyme. Biochemistry. 2002;41(37):11126–33.

17. Küchler B, Abdel-Ghany AG, Bross P, Nandy A, Rasched I, Ghisla S. Biochemical characterization of a variant human medium-chain acyl-CoA dehydrogenase with a disease-associated mutation localized in the active site. Biochem J. 1999;337(2):225–30.

18. Goetzman ES, Nguyen T V., Vockley. Functional analysis of acyl-CoA dehydrogenase catalytic residue mutants using surface plasmon resonance and circular dichroism. Mol Genet Metab. 2006;87(3):233–42.

19. Thorpe C, Kim JJ. Structure and mechanism of action of the acyl-CoA dehydrogenases. The FASEB Journal. 1995;9(9):718–25.

20. 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.

21. Kumar NR, Srivastava DK. Reductive Half-Reaction of Medium-Chain Fatty Acyl-CoA Dehydrogenase Utilizing Octanoyl-CoA/Octenoyl-CoA as a Physiological Substrate/Product Pair: Similarity in the Microscopic Pathways of Octanoyl-CoA Oxidation and Octenoyl-CoA Binding. Biochemistry [Internet]. 1994;33(29):8833–41. Available from: https://pubs.acs.org/sharingguidelines

22. Wu J, Bell AF, Luo L, Stephens AW, Stankovich MT, Tonge PJ. Probing hydrogen-bonding interactions in the active site of medium-chain acyl-CoA dehydrogenase using Raman spectroscopy. Biochemistry. 2003;42(40):22846–11856.

23. Odendaal JCW, Jager EA, Martines ACMF, Vieira Lara MA, Huijkman NCA, Kiyuna LA, et al. Towards the digital twin: personalised modelling of clinical heterogeneity between medium-chain acyl-CoA dehydrogenase patients. 2023;

24. Aoyama T, Souri M, Ushikubo S, Kamijo T, Yamaguchi S, Kelley RI, et al. Purification of human very-long-chain acyl-coenzyme A dehydrogenase and characterization of its deficiency in seven patients. Journal of Clinical Investigation. 1995;95(6):2465–73.

25. 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.

26. Wainio WW. Energy conservation. In: The Mammalian Mitochondrial Respiratory Chain. New York and London: Academic Press; 1970. p. 331.

27. Thorpe C. Electron-transferring flavoproteins. In: Muller F, editor. Chemistry and Biochemistry of Flavoenzymes. 1st ed. Boca Raton: CRC Press; 1991. p. 471–86.

28. Saenger AK, Nguyen T V., Vockley J, Stankovich MT. Thermodynamic regulation of human short-chain acyl-CoA dehydrogenase by substrate and product binding. Biochemistry. 2005;44(49):16043–53.

29. Modre-Osprian R, Osprian I, Tilg B, Schreier G, Weinberger KM, Graber A. Dynamic simulations on the mitochondrial fatty acid Beta-oxidation network. BMC Syst Biol. 2009;3:1–15.

30. Izai K, Uchida Y, Orii T, Yamamoto S, Hashimoto T. Novel fatty acid β-oxidation enzymes in rat liver mitochondria: I. Purification and properties of very-long-chain acyl-coenzyme A dehydrogenase. Journal of Biological Chemistry. 1992;267(2):1027–33.

31. van Eunen K, Simons SMJ, Gerding A, Bleeker A, den Besten G, Touw CML, et al. Biochemical Competition Makes Fatty-Acid β-Oxidation Vulnerable to Substrate Overload. PLoS Comput Biol. 2013;9(8):2–9.

32. Thorpe C, Matthews RG, Williams CH. Acyl-Coenzyme A Dehydrogenase from Pig Kidney. Purification and Properties. Biochemistry. 1979;18(2):331–7.

33. Gustafson WG, Feinberg BA, McFarland JT. Energetics of β-Oxidation. Reduction potentials of general fatty acyl-CoA dehydrogenase, electron transfer flavoprotein, and fatty acyl-CoA substrates. Journal of Biological Chemistry. 1986;261(17):7733–41.