VLCAD (EC 1.3.8.9)

Very long-chain acyl-CoA dehydrogenase

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

Contents

SUBCELLULAR LOCALISATION	. 3
*Unexplored kinetic implication: response to membrane microenvironment	. 3
STRUCTURE	. 3
Unique among ACADs	. 3
Substrate specificity	. 4
Catalytic capacity	. 4
\ast STRONG modelling decision: the V _{max} value of VLCAD must be higher than for SCAD and MCA	D 4
Similarity to other ACADs	. 4
* Modelling decision: ACAD9 and VLCAD parameters are interchangeable	. 4
* Modelling decision: catalytic-site-related properties of MCAD might also appear in VLCAD	. 4
FUNCTION	. 5
Oxidase reaction	. 5
* Unexplored activity: acyl-CoA oxidation	. 5
Isomerase activity	. 5
* Unexplored activity: enoyl-CoA isomerase	. 5
Unsaturated fatty acids	. 5
* Unexplored activity: unsaturated acyl-CoAs	. 5
*Unexplored inhibitory kinetics	. 5
* Inhibition by 3-ketodecanoyl	. 5
* Unexplored kinetic implication: semiquinone inhibits ACADs	. 5
MEASURING CONDITIONS	. 6
pH dependence	. 6
* STRONG modelling decision: penalise parameters outside 7.5 < pH < 8.5	. 6
Detergents	. 6

* Modelling decision: penalise parameters measured in the presence of high detegergent	
concentration	6
Buffer dependence	6
* Modelling decision: any buffer in the concentration outside the range 20 mM to 200 mM w be penalised	'ill 7
Temperature	7
* Modelling decision: penalise parameters not measured exactly at 37°C	7
Heterogeneity across species	7
* Modelling decision: penalise non-human parameters, accept mammalian	7
Heterogeneity across tissues	7
* STRONG modelling decision: parameters from all tissues are acceptable, as long as they are protein-corrected	? 7
KINETICS	8
Mechanism	8
* Arbitrary modelling decision: random-order Bi-Bi Michaelis-Menten	8
* Arbitrary modelling decision: ACADS have only one K_m for ETF	8
Reversibility	8
Substrate specificity	9
*Unexplored kinetic implication: VLCAD has activity towards C18, C20, C22, C24	9
Rate equation	9
Variables == initial values	10
Parameters	11
Weighting rule	11
sfvlcad	12
Vvlcad	15
KmvlcadAcylCoAMAT	17
KmvlcadETFox	19
KmvlcadEnoylCoAMAT	21
KmvlcadETFred	23
Keqvlcad	25
REFERENCES	28

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).

Very long-chain acyl-CoA dehydrogenase (VLCAD) has been known only since around 1992 (3). LCAD and VLCAD are the canonical long-chain acyl-CoA dehydrogenases in mammalian mitochondria. ACAD9 was discovered in 2002 (4) and is posited to be responsible for both β -oxidation and molecular chaperonin functions (5). In 2011, He *et al.* (1) also described ACAD10 and ACAD11 – two enzymes that are mainly localized in the brain, as opposed to the other enzymes which are found in energy-generating tissues, like the liver – which seem to have functions other than energy metabolism, i.e. controlling the composition of fatty acids or catabolizing the intermediates of amino acids.

Thus, the five different long chain ACADs are likely specific to the different functions of mitochondrial β -oxidation in various tissues in humans. Aoyama *et al.* (6) report VLCAD to be "unique in its size, structure, and intramitochondrial distribution."

SUBCELLULAR LOCALISATION

VLCAD is loosely associated with the inner-mitochondrial membrane, and requires detergent for extraction, while the others are readily soluble (6). **Does this mean that – like with CPT1 – function changes with changing membrane composition/fluidity? This is probably not easy to exclude.** Boiling the membranes, which should denature the remaining integral membrane proteins, did not reduce later VLCAD binding, suggesting that membrane binding of VLCAD does not require an additional protein (7).

*Unexplored kinetic implication: response to membrane microenvironment

VLCAD, being embedded in the membrane, might respond to changes in membrane composition / fluidity like CPT1.

STRUCTURE

ACADs all contain 1 mol FAD per mol subunit, perhaps contributing to the redox activity of the enzyme (8). The catalytic residue in all known ACADs is glutamate (9). 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 (9). In LCAD and IVD, however, this residue is not conserved. Rater, Glu261 (Glu254 in IVD) in helix G acts as the catalytic residue (9). Fig. 3 in (9) presents this view visually. In VLCAD and ACAD9, the catalytic residue is MCAD-like (9).

Unique among ACADs

The first major difference lies in its composition: while other ACADs are typically homotetramers (subunit 43 – 45 kD), VLCAD is a dimer of a 70kD subunit, each containing an extra 29 kD extra polypeptide (6).

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, (9)).

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 (9).

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

Similarity to other ACADs

"(ACAD9) shares approximately 47% amino acid identity and 65% similarity with human VLCAD. So, the novel molecule is named as acyl-CoA dehydrogenase-9 (ACAD-9), the ninth member of ACADs," Zhang *et al.* (4). It is also membrane-bound – as opposed to LCAD – which further makes it a better cognate to VLCAD (1).

"...suggesting that the novel protein is a homodimer like VLCAD in which the glutamate is replaced by an arginine rather than a tetrameric enzyme like MCAD," Zhang *et al.* (4).

McAndrew *et al.* (9) report that VLCAD and ACAD9 form a class of enzymes that are homodimers of 67-kDa subunits bound to the inner mitochondrial membrane. They contain an additional 180-residues on the C-terminal end, which has been suggested to be responsible for membrane-binding (7).

* Modelling decision: ACAD9 and VLCAD parameters are interchangeable

Considering the similarity of the active sites and overall composition of CAD9 and VLCAD, we will privilege VLCAD parameters but consider ACAD9 parameters as appropriate replacements in the case of no good VLCAD data being available.

* Modelling decision: catalytic-site-related properties of MCAD might also appear in VLCAD

Due to the similarity of the active sites of VLCAD and MCAD (9), any property related to the active site of MCAD must be considered for VLCAD as well, i.e. responsiveness to pH, competitive inhibition, etc.

FUNCTION

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

Oxidase reaction

Like its pig kidney cousin, human liver MCAD (10) was found to also catalyze the oxidase reaction of aliphatic acyl-CoAs.

* Unexplored activity: acyl-CoA oxidation

Considering their similar reaction mechanisms, this might also apply to VLCAD.

Isomerase activity

MCAD was found to also have isomerase activity (3-enoyl-CoA to 2-enoyl-CoA; Fig. (11)).

* Unexplored activity: enoyl-CoA isomerase

This might also apply to VLCA, due to the similarity of MCAD and VLCAD's active sites.

Unsaturated fatty acids

Dommes & Kunau (12) 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

This is therefore also possibly true of VLCAD.

*Unexplored inhibitory kinetics

Though I have not included these in the model, it might be worth keeping in mind for later iterations. I 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 3-ketodecanoyl

Davidson and Schulz (13) report 3-ketodecanoyl to inhibit bovine heart LCAD with a $K_i = 0.075 \ \mu M$. This might be false, it might be species-specific, it might be heart-specific: who knows? Perhaps is it also applicable to VLCAD.

* 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 (14).

MEASURING CONDITIONS

Among the mitochondrial β -oxidation enzymes, the acyl-CoA dehydrogenases are definitely the most difficult to measure accurately (15). 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-FADH₂-linked dehydrogenases. Secondly, there is significant overlap in substrate specificity between the different acyl-CoA dehydrogenases.

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 VLCAD and MCAD (2), you expect a similar pH range. So, values from within this range are probably acceptable. Nguyen *et al.* (16) also observe activity of wild-type SCAD to be consistent between pH = 6.0 and 8.0, with activity declining above or below that range.

Izai *et al.* (3) found higher activity for rat liver VLCAD at pH = 8.0 (1.5 times higher than at pH = 7.5). Also, in mouse liver mitochondria (17), the pH peak was at pH = 7.9.

Küchler *et al.* (18), however, observed human WT MCAD activity to continue to rise beyond pH = 8.0 (Fig. 8 in (18)). 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 doesn't occur much in the literature. *The functional similarity between MCAD and VLCAD suggests that we might be able to draw this lesson for VLCAD as well, though VLCAD is structurally quite different, and any assumptions in this regard must be made with caution.*

* STRONG modelling decision: penalise parameters outside 7.5 < pH < 8.5

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 not be penalized, whereas those measured at 8.0 will be privileged. pH is an important factor for this enzyme, so this will be a main consideration in picking parameters.

Detergents

In rat liver mitochondria, Izai *et al.* (3) found VLCAD activity to be increased in the presence of mild detergents (perhaps due to better isolation of the enzyme from the membrane) and reduced in high concentrations (probably due to denaturation).

* Modelling decision: penalise parameters measured in the presence of high detegergent concentration

If very high (e.g. >2% Triton-100 X) detergent concentrations were used, the parameters will be suspect. I will not, however, penalise parameters that were measured in the absence of detergents – these constitute most of the available parameters for VLCAD and ACAD9 that I have found.

Buffer dependence

Izai *et al.* (3) did not find a noteworthy difference between the activities of the enzyme at varying buffer concentrations (20 mM – 200 mM) or types (Tris vs. Potassium Phosphate).

* Modelling decision: any buffer in the concentration outside the range 20 mM to 200 mM will be penalised

Temperature

Takusa *et al.* (19) report – in human fibroblast extract – 2 times higher activity when measured at 30° C as opposed to 37° C. Any measured VLCAD activity can be viewed through this lens.

* Modelling decision: penalise parameters not measured exactly at 37°C Double penalise them at temperatures lower than 25°C.

Heterogeneity across species

Human VLCAD was found to be slightly smaller (migrated faster in SDS-PAGE) than rat VLCAD (6), so there is some cross-species variation. How this impacts the kinetics, is not clear.

* Modelling decision: penalise non-human parameters, accept mammalian

Heterogeneity across tissues

"The size of the VLCAD protein detected in human liver, heart, and skin fibroblast, respectively, was identical to that of the purified VLCAD protein. This is consistent with the previous data that the size and content of both the VLCAD protein and mRNA in various rat organs were identical, suggesting the existence of a single species of VLCAD in various organs", according to Aoyama *et al.* (6).

* STRONG modelling decision: parameters from all tissues are acceptable, as long as they are protein-corrected

KINETICS

Mechanism

Peterson *et al.* (10) 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.* report this mechanism to be valid for all ACADs (Scheme 1, (20)).

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 (21). Thorpe (22) report a compulsory ordered mechanism: acyl-CoA binds first, and enoyl-CoA leaves last (shown for MCAD):



Given the similarity of the catalytic residues of VLCAD and MCAD (9), a similar reaction mechanisms can be expected from VLCAD.

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

For convenience, a random-order bisubstrate-biproduct Michaelis-Menten reaction will be assumed (23).

* 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 in any case) 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 (21), we are skeptical about whether this reaction is, in practice, reversible. To our 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 (24), but this might be simple competitive binding. However, in terms of convenience kinetics (23), we 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.

* Unexplored model check: forward and reverse V_{max} values should be similar.

Kumar and Srivastava (25) 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 V_{max} values should be the same, should you ever wish to calculate them.

Substrate specificity

VLCAD in humans is specific for the C8 to C24-chain length acyl-CoAs (6) with a maximum around C16.

*Unexplored kinetic implication: VLCAD has activity towards C18, C20, C22, C24

Rate equation

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

 $vvlcadC_n$

$$=\frac{sfvlcadC_{n}\cdot\frac{Vvlcad}{VMAT}\cdot(\frac{C_{n}AcylCoAMAT[t]\cdot ETF_{ox}}{K_{m}vlcadC_{n}AcylCoAMAT\cdot K_{m}vlcadETF_{ox}}-\frac{C_{n}EnoylCoAMAT[t]\cdot ETF_{red}}{K_{eq}vlcadC_{n}\cdot K_{m}vlcadC_{n}AcylCoAMAT\cdot K_{m}vlcadETF_{ox}})}{(1+\frac{ETF_{ox}}{K_{m}vlcadETF_{ox}}+\frac{ETF_{red}}{K_{m}vlcadETF_{red}})\cdot(1+\sum_{n=8}^{n=16}(\frac{C_{n}AcylCoAMAT[t]}{K_{m}vlcadC_{n}AcylCoAMAT}+\frac{C_{n}EnoylCoAMAT[t]}{K_{m}vlcadC_{n}EnoylCoAMAT}))}$$

where:

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

Variables == initial values

Acyl-CoAs	Enoyl-CoAs
C16AcylCoAMAT[t] == 0 μM	C16EnoylCoAMAT[t] == 0 μM
C14AcylCoAMAT[t] == 0 μM	C14EnoylCoAMAT[t] == 0 μM
C12AcylCoAMAT[t] == 0 µM	C12EnoylCoAMAT[t] == 0 μM
C10AcylCoAMAT[t] == 0 μM	C10EnoylCoAMAT[t] == 0 μM
C8AcylCoAMAT[t] == 0 μM	C8EnoylCoAMAT[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

sfvlcad

No satisfactory values found: ACAD9 parameters had to be taken as substitutes for VLCAD.

Parameter Alternatives					Comments	
cfulcadC16	Ensenauer <i>et al.</i> (2005, (26)) recombinant human ACAD9 expressed in human embryonic kidney cells, Tris 200 mM, pH = 8.0, D-glucose 0.5% (w/v), ETF porcine liver, 50 µM acylCoA, 32°C	Izai <i>et al.</i> (1992, (3)) rat liver mitochondria- this might be especially inappropriate for substrate specificity, as this is really a differentiating characteristic between rats and humans; 50 mM Potassium Phosphate, pH = 7.4, 30 μM PalCoA, 35 μM 2,6- dichloroindophenol, 1 mM N- ethylmaleimide, and enzyme, 30°C	Zhang <i>et al.</i> (2002, (4)) recombinant human ACAD9 expressed in COS7 cells, 100 mM potassium phosphate, pH = 7.6, 50 μ DCPIP, 2 mM PES, 0.2 mM N- ethylmaleide, 50 μM acyl-CoA, 37°C - C18:0 was also tried as substrate, and was found to have a much lower specificity (5 times) than PalCoA	Goetzman <i>et</i> <i>al.</i> (2007, (7)) recombinant human VLCAD expressed in C43 <i>E. coli</i> , Tris 200 mM, pH = 8.0, D- glucose 0.5% (w/v), ETF porcine liver, 50 µM acylCoA, 32°C	Aoyama <i>et al.</i> (1995, (6)): "very low activity towards substrates with 10- and 12 carbon" human liver, whole cells incubated in FCS- free DMEM with 4 nmol [1-14C]-palmitic acid dissolved in α- cyclodextrin; 37C; reaction was stopped with 10% BSA and 3 N perchloric acids, and the products measured with scintillation	 No good VLCAD specificities for the whole range could be found. From Aoyama <i>et al.</i> (6) we infer the C12-C8 activities ("very low") to be 10% of Vmax.
sfvlcadC16	1.0 {0.1, ACAD9 + recomb. expr. In kidney + temp}	1.0 {0.1, rat + pH + temp}	1.0 {0.1, ACAD9 (double penalty) + recomb. expr}	1.0 {0.9, temp}		

sfvlcadC14	0.26 {0.1, ACAD9 + recomb. expr. In kidney + temp} [0.26 - 0.511]	0.45 {0.1, rat + pH + temp}		0.511 {0.9, temp}	
sfvlcadC12	0.14 {0.1, ACAD9 + recomb. expr. In kidney + temp} [0.13 - 0.14]	0.13 {0.1, rat + pH + temp}			0.2 {0.1, values guessed from the statement "very low" activity detected}
sfvlcadC10	1.14 {0.1, ACAD9 + recomb. expr. In kidney + temp} [0 - 1.14]	0 {0.1, rat + pH + temp}			0.2 {0.1, values guessed from the statement "very low" activity detected}
sfvlcadC8	0.11 {0.1, ACAD9 + recomb. expr. In kidney + temp} [0.0 - 0.11]	0 {0.1, rat + pH + temp}	0.1 {0.1, ACAD9 (double penalty) + recomb. expr}		0.1 {0.1, values guessed from the statement "very low" activity detected}

Cubic polynomial (multinormal distribution)				
Formula	$a * x^3 + b * x^2 + c * x + d$			
Best fit parameters	a = 0.00690989			
	b = -0.235061			
	c = 2.6593			
	d = -9.66863			
R ²	0.94			
Covariance matrix	{{0.0000144243, -0.000533004, 0.00636031, -0.0243268},			
	{-0.000533004, 0.0197519, -0.236409, 0.906902},			
	{0.00636031, -0.236409, 2.83877, -10.9265},			
	{-0.0243268, 0.906902, -10.9265, 42.2134}}			
Bounds	{{"bounds: C8", 0.01, 1.}, {"bounds: C10", 0.02, 2.}, {"bounds: C12", 0.02, 2.}, {"bounds: C14",			
	0.0511, 5.11}, {"bounds: C16", 0.1, 10.}}			



<u>Comments</u>: The substantial bump at C10 for the ACAD9 that was chosen as the value in the deterministic model disappears almost completely if you fit a function to these data. This, even though I explicitly chose a cubic polynomial which contains more that one minimum/maximum. I think the simulated data represent the data points quite well.

Vvlcad

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

Parameter	Chosen value [range]		Comments	
	Aoyama <i>et al.</i> (1995, (6)) 0.019 μmol.min ⁻¹ .mg-liver-protein ⁻¹ human liver, whole cells incubated in FCS-free DMEM with 4 nmol [1-14C]- palmitic acid dissolved in α- cyclodextrin, presumably at 37°C; reaction was stopped with 10% BSA and 3 N perchloric acids, and the products measured with scintillation, [1], [2]	Izai et al. (1992, (3)) $0.069 \ \mu mol.min^{-1}.mg-mito-protein^{-1}$ rat liver mitochondria, 50 mM Potassium Phosphate, pH = 7.4, 30 μ M PalCoA, 35 μ M 2,6-dichloroindophenol, 1 mM N-ethylmaleimide, and enzyme, 30°C, ^[2]	Oey <i>et al.</i> (2005, (27)) 0.000760 μmol.min ⁻¹ .mg-liver-protein ⁻¹ 6-week-old baby liver, assay conditions not specified (Wanders <i>et al.</i> (1999) notes multiple possible measurement techniques). However, assuming that the measurement is accurate and that the protein of the normalisation is total liver protein and not mitochondrial protein (because they never mention isolated mitochondria) ^[1] , ^[2]	 We choose the value from Aoyama et al. (6) as is it is the only value that both comes from human VLCAD and has clearly specified assay conditions. It is more than 25 times larger than the human value from Oey et al. (27), but the assay conditions and the extraction procedure of the latter are
Vvlcad	0.076 μmol.min ⁻¹ .mg-mito-Protein (*) {1.0} [0.003 – 0.076]	0.0345 μmol.min ⁻¹ .mg- mito-protein ⁻¹ (**, ***) {0.5, rat + pH + temp}	0.003 µmol.min ⁻¹ .mg-mito-protein ⁻¹ (*) {0.1, measurement conditions unclear}	not clear to me.

* Scaled to mitochondrial protein (25% of cellular protein) according to Wiśniewski et al. (28).

**, Divided by 4: specific activity with PMS as primary electron acceptor gives about 4x the activity of what you see with the natural primary acceptor, ETF (3)

***, Takusa et al. (19) report – in human fibroblast extract – 2 times higher activity when measured at 30°C

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

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

log-normal distribution				
Parameters $\mu = -3.02583$				
(of the normal distribution)	σ = 1.12823			
Bounds {"bounds", 0.0076, 0.76}				



Comments: n/a

KmvlcadAcylCoAMAT

Satisfactory values not found: no human VLCAD values for these ranges are available.

	Parameter	Chosen	Alte	ernatives		Commonte
		value				comments
ЛАТ		Nandy <i>et al.</i> (1996, (2)) LCAD parameters, 100 mM potassium phosphate buffer, wild-type human expressed in E. coli, pH = 7.6, 200 μM ferricenium, 25°C	Izai <i>et al.</i> (1992, (3)) rat liver mitochondrial VLCAD , 50 mM Potassium Phosphate, pH = 7.4, 30 μM PalCoA, 35 μM 2,6- dichloroindophenol, 1 mM N-ethylmaleimide, and enzyme, 30°C	Kakimoto <i>et al.</i> (2015, (17)) purified mouse liver VLCAD , 100 mM KH2PO4, 0.1 mM EDTA, pH = 7.2, 37° C, 150 μ M ferricenium hexafluorophosphat e, PalCoA (0 - 1400 μ M)	Ensenauer <i>et al.</i> (2005, (26)) recombinant human ACAD9 expressed in human embryonic kidney cells, Tris 200 mM, pH = 8.0, D- glucose 0.5% (w/v), ETF porcine liver, 50 µM acylCoA, 32°C	1) I select Nandy <i>et al.</i> (4)'s values even though they
lcadAcylCoAN	KmvlcadC16AcylCoAMAT	14 μM {0.1, LCAD (double penalty) + temp} [0.04 – 339.3]	0.04 μM {0.1, rat + pH + temp}	339.3 μM {0.5, mouse + pH}	2.8 μM {0.1, ACAD9 + recomb. expr. In kidney + temp}	 originate from LCAD, as I could not find any ranges for VLCAD which could be used here. 2) It bears mentioning that the Van Eunen <i>et al.</i> (29) used the same LCAD parameters as VLCAD
Kmv	KmvlcadC14AcylCoAMAT	10 μM {0.1, LCAD (double penalty) + temp}				model.
	KmvlcadC12AcylCoAMAT	7 μM {0.1, LCAD (double penalty) + temp}				
	KmvlcadC10AcylCoAMAT	10 μM {0.1, LCAD (double penalty) + temp}				
	KmvlcadC8AcylCoAMAT	8 μM {0.1, LCAD (double penalty) + temp}				

Cubic polynomial (multinormal distribution)					
Formula	$a * x^3 + b * x^2 + c * x + d$				
Best fit parameters	a = 2.17186				
	b = -70.7764				
	c = 751.701				
	d = -2590.58				
R ²	0.64				
Covariance matrix	{{51.764, -1880.76, 22023.9, -82863.9},				
	$\{-1880.76, 68503.1, -804433., 3.03549*10^{6}\},$				
	{22023.9, -804433., 9.47716*10 ⁶ , -3.58856*10 ⁷ },				
	$\{-82863.9, 3.03549^{*}10^{6}, -3.58856^{*}10^{7}, 1.36412^{*}10^{8}\}\}$				
Bounds	{{"bounds: C8", 4/5, 400}, {"bounds: C10", 1, 500}, {"bounds: C12", 7/10, 350},				
	{"bounds: C14", 1, 500}, {"bounds: C16", 7/5, 700}}				



<u>Comments</u>: Not a great fit, but something of the variation in the data is explained.

KmvlcadETFox

Semi-satisfactory value found: no human parameter found, but it seems to agree nicely with the other values – measurements and estimates.

Parameter	Chosen value		Alterna	atives	Comments
	Izai <i>et al.</i> (1992, (3)) rat liver mitochondria, VLCAD , 50 mM Potassium Phosphate, pH = 7.4, 30 μM PalCoA, 35 μM 2,6- dichloroindophenol, 1 mM N-ethylmaleimide, and enzyme, 30°C	Wainio <i>et al.</i> (1970, (30)) pig and monkey, conditions unclear 0.7 to 2.6 µM, take average.	Van Eunen et al. (2016, (29)) estimated based on constant ACAD parameters from Nandy et al. (2) and Finnochiaro et al. (2) and Finnochiaro et al. (8) where, for instance, VLCAD's parameters were assumed to be the same as those measured for LCAD, and half an order of magnitude variation up- and downwards from the rat parameters from Van Eunen et al (2013), and also only forward parameters were re- estimated - this might be quite a weak estimation.	Thorpe (1991, (22)) reports a range of K _m values, from 0.2 to 2 μ M 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 VLCAD. Also, we do not know whether this is in the forward or reverse direction. Other values in Thorpe (1991, (22)): 1.1) <i>P. denitrificans</i> : K _m = 2.5 μ M for glutaryl-CoA dehydrogenase (direction and conditions not given); methylotrophic bacteria: 1.2) K _m = 7 μ M for trimethylamine dehydrogenase (direction and conditions not given).	 I use Izai <i>et al.</i> (3)'s parameters for rat liver, as it is the only measured affinity of ETF for VLCAD that is available from literature. The estimate of Van Eunen <i>et al.</i> (29) and the ranges from Wainio <i>et al.</i> (4) and Thorpe (22) indicate that we are in the right range.
KmvlcadETFox	0.83 μM {0.1, rat + temp + pH} [0.1 – 2.6]	1.65 μM 0.7 to 2.6 μM, take average {0.1, pig and monkey + conditions unclear}	0.10 μM {0.1, computational estimate based on other faulty parameters}	1.1 μM Take average of 0.2 – 2.0 range {0.1, unclear origin of enzyme and assay conditions}	

log-normal distribution				
Parameters $\mu = -0.473207$				
(of the normal distribution)	σ = 1.25177			
Bounds	{"bounds", 0.083, 8.3}			



Comments: n/a

KmvlcadEnoylCoAMAT

No satisfactory values found: no direct product affinity measurements for VLCAD are available, that I am aware of.

Parameter	Chosen value	Alternatives	Comments
	Average between K _d values: 0.6 μM from Goetzman <i>et al.</i> (20) & 0.153 μM from Peterson <i>et al.</i> (1995, (21))		 These are all K_d values, not K_ms, 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.
KmvlcadEnoylCoAMAT	Goetzman et al. (2006, (20)) recombinant human VLCAD expressed in E. coli, 10 mM HEPES, pH = 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% surfactant P20, BIAcore; Kd for C4AcylCoA = 0.6 μM, perhaps indicating that it is reasonable to just assume the Km for the substrate as the Km for the product: this is not the case for isovaleryl-CoA dehydrogenase, however, so I cannot just extrapolate this to other enzymes {0.1, recomb. + pH + no temp + only for C4AcylCoA} Peterson et al. (1995, (21)) recombinant human liver in E. coli, 25°C, pH = 7.6, 50 mM KPi, 0.3 mM EDTA, ferrocenium hexafluoraphosphate NOTE: similar to the Ki for Octynoyl-CoA = 0.36 μM) {0.1, recomb. + pH + temp + substrate unclear}	Ikeda <i>et al.</i> (1985, (24)) K _i = 28.5 μM inhibition of LCAD activity by 2- hexadecenoyl-CoA; (rat liver mitochondrial extract, 100 M KPi buffer pH = 8.0, 0.1 mM OctCoA, 0.048 mM DCIP (terminal electron acceptor), 0.1 FAD, 0.1 mM Pal- CoA, 32°C, spectrophotometer)	2) A There are no parameters available for VLCAD's product affinity of product inhibition, as far as I am aware. The parameters that Ikeda <i>et al.</i> (24) measured for LCAD, MCAD, and SCAD in rat mitochondria are quite high (100x higher, in some cases) in comparison the the values measured in other studies (see the parameter choices for MCAD and SCAD). Therefore, I will not just be taking the LCAD parameter over from Ikeda <i>et al.</i> (24), as this will leave product inhibition much stronger for VLCAD that for MCAD and SCAD, and even in the work of Ikeda et al. (24), the K _i for the various enzymes did not differ by much. Since I can find parameters for SCAD and MCAD which are in the same order of magnitude (in the 0.1 range), I will take the average of the SCAD and MCAD parameter as the VLCAD parameter in order to avoid an unfounded asymmetry.
KmvlcadC16EnoylCoAMAT	0.377 μM [0.153 – 28.5]	28.5 μM {0.1, LCAD + only for C16}	between the ACADs' responses to product
	(see above for weight)		accumulation.
KmvlcadC14EnoyICoAMAT			2) A big accumption have is that the different
KmvlcadC12EnoylCoAMAT	0.377 µM		enovi-CoA chain-lengths all have the same
KmvlcadC10EnoylCoAlvIA1	0.377 µW		affinity for VI CAD, which seems highly unlikely
KINVICADUSENOVICOAIVIAT	0.377 µIVI		if the same is not true for the acyl-CoAs. But
KmvlcadC6EnoylCoAMAT	0.377 μM		this is, again, a consequence of parameter
			scarcity.

Linear		
Formula	a * (x + b) + c	
Chosen parameters	a = {0,0}	
	b = 0	
	c = {0.01, 30}	
R ²	n/a	
Covariance matrix	n/a	
Bounds	{{"bounds: C8", 0.0125667, 37.7}, {"bounds: C10", 0.0125667, 37.7}, {"bounds:	
	C12", 0.0125667, 37.7}, {"bounds: C14", 0.0125667, 37.7}, {"bounds: C16",	
	0.0125667, 37.7}}	



<u>Comments</u>: I have no certainty even about any one of the values – the information that I have is almost all chain-length independent. Therefore, I assume the same value for all the chain lengths. Future work can investigate the impact of the relationship between these.

KmvlcadETFred

No satisfactory value found. This is not something that has been measured in the literature, as far as I know.

Parameter	Chosen	Alternatives	Comments
KmvlcadETFred	value Modre-Osprian <i>et al.</i> (2009, (31)) From a generic (tissue- unspecific, animal- unspecific) model of mFAO 24.2 μM {0.1, from model, original source/tissue/conditio ns unclear}	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 (3,22,30) 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 (22), Waino <i>et al.</i> (30)) or explicitly refer to the forward direction. For this reason, Modre-Osprian <i>et al.</i> (31) 's value, taken from Van Eunen <i>et al.</i> (32), 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. <i>The alternative is that the K_m for ETF is the same in both directions</i> , but I yield to the measuring themselves.

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



Comments: n/a

Keqvlcad

Satisfactory values found.

Parameter	Chosen value	Alternative s	Comments
	 Thorpe <i>et al.</i> (1979, (33)) and Gustafson <i>et al.</i> (1986, (34)) report the same value: 9.0 for C8-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, 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, <i>adjusted for temperature using the Van 't Hoff equation</i>. 	Mordre- Osprian <i>et</i> <i>al.</i> (2009, (31)) From a generic (tissue- unspecific, animal- unspecific) model of mFAO	 Thorpe <i>et al.</i> (31) and Gustafson <i>et al.</i> (1986, (31)): chains longer than C8 chosen to be the same as the reaction with the C8 substrate and those shorter that C6 to be the same as C4. This is according to the pattern I saw with the other reactions: shorter chains tend to have divergent K_{eq} values from medium and longer chains. eQuilibrator, which is used elsewhere in this model, does not allow
KeqvlcadC16	 8.27 (assumed the same as for C8) {0.5, second-hand source, original inaccessible + assumed the same for all chain lengths} [6.0 - 8.27] 		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
KeqvlcadC14	 8.27 (assumed the same as for C8) {0.5, second-hand source, original inaccessible + assumed the same for all chain lengths} [6.0 - 8.27] 	6.0 {0.1, from model, original	smaller than 1, which cannot be for a working reaction - suggesting that ETF plays some important thermodynamic stabilisation role.
KeqvlcadC12	 8.27 (assumed the same as for C8) {0.5, second-hand source, original inaccessible + assumed the same for all chain lengths} [6.0 - 8.27] 	source/tiss ue/conditio ns unclear}	3) No more than a factor or 0.3 removed from the K _{eq} value proposed by Van Eunen <i>et al.</i> (2013, (32)) based
KeqvlcadC10	8.27 (assumed the same as for C8) {0.5, second-hand source, original inaccessible + assumed the same for all chain lengths}		on Mordre-Osprian <i>et al.</i> (2009, (31)).

	[6.0 – 8.27]	
KeqvlcadC8	8.27	
	{0.5, second-hand source, original inaccessible + assumed the same for all chain lenaths}	
	[6.0 - 8.27]	

Unique		
Values	{{8, 8.27}, {10, 8.27}, {12, 8.27}, {14, 8.27}, {16, 8.27}}	



Comments: No variation allowed, unique values from Thorpe et al. (33) and Gustafson et al. (34)

REFERENCES

- 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.
- 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.
- Zhang J, Zhang W, Zou D, Chen G, Wan T, Zhang M, et al. Cloning and functional characterization of ACAD-9, a novel member of human acyl-CoA dehydrogenase family. Biochem Biophys Res Commun. 2002;297(4):1033–42.
- He M, Rutledge SL, Kelly DR, Palmer CA, Murdoch G, Majumder N, et al. A new genetic disorder in mitochondrial fatty acid β-oxidation: ACAD9 deficiency. Am J Hum Genet. 2007;81(1):87–103.
- 6. 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.
- Goetzman ES, Wang Y, He M, Mohsen AW, Ninness BK, Vockley J. Expression and characterization of mutations in human very long-chain acyl-CoA dehydrogenase using a prokaryotic system.pd. Mol Genet Metab. 2007;91(2):138–47.
- 8. 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.
- 9. 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.
- 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.
- 11. Zeng J, Li D. Intrinsic isomerase activity of medium-chain Acyl-CoA dehydrogenase. Biochemistry. 2005;44(17):6715–22.
- 12. 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.
- 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.
- 14. Eaton S. Control of mitochondrial β-oxidation flux. Prog Lipid Res. 2002;41(3):197–239.

- Wanders RJA, Vreken P, Den Boer MEJ, Wijburg FA, Van Gennip AH, IJIst L. Disorders of mitochondrial fatty acyl-CoA β-oxidation. In: Journal of Inherited Metabolic Disease. 1999. p. 442–87.
- 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. Kakimoto PAHB, Tamaki FK, Cardoso AR, Marana SR, Kowaltowski AJ. H2O2 release from the very long chain acyl-CoA dehydrogenase. Redox Biol [Internet]. 2015;4:375–80. Available from: http://dx.doi.org/10.1016/j.redox.2015.02.003
- 18. 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.
- 19. Takusa Y, Fukao T, Kimura M, Uchiyama A, Abo W, Tsuboi Y, et al. Identification and characterization of temperature-sensitive mild mutations in three japanese patients with nonsevere forms of very-long-chain acyl-CoA dehydrogenase deficiency. Mol Genet Metab. 2002;75(3):227–34.
- 20. 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.
- 21. Thorpe C, Kim JJ. Structure and mechanism of action of the acyl-CoA dehydrogenases. The FASEB Journal. 1995;9(9):718–25.
- 22. Thorpe C. Electron-transferring flavoproteins. In: Muller F, editor. Chemistry and Biochemistry of Flavoenzymes. 1st ed. Boca Raton: CRC Press; 1991. p. 471–86.
- Rohwer JM, Hanekom AJ, Crous C, Snoep JL, Hofmeyr JH. Evaluation of a simplified generic bisubstrate rate equation for computational systems biology. IEE Proceedings-Systems Biology. 2006;153(5):338–41.
- 24. Ikeda Y, Okamura-Ikeda K, Tanaka K. Purification and characterization of short-chain, mediumchain, 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.
- 25. 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
- Ensenauer R, He M, Willard JM, Goetzman ES, Corydon TJ, Vandahl BB, et al. Human Acyl-CoA dehydrogenase-9 plays a novel role in the mitochondrial β-oxidation of unsaturated fatty acids. Journal of Biological Chemistry. 2005;280(37):32309–16.
- 27. 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.

- 28. 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.
- 29. van Eunen K, Volker-Touw CML, Gerding A, Bleeker A, Wolters JC, van Rijt WJ, et al. Living on the edge: Substrate competition explains loss of robustness in mitochondrial fatty-acid oxidation disorders. BMC Biol. 2016;14(1).
- 30. Wainio WW. Energy conservation. In: The Mammalian Mitochondrial Respiratory Chain. New York and London: Academic Press; 1970. p. 331.
- 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.
- 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.
- 33. Thorpe C, Matthews RG, Williams CH. Acyl-Coenzyme A Dehydrogenase from Pig Kidney. Purification and Properties. Biochemistry. 1979;18(2):331–7.
- 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.