M/SCHAD (EC 1.1.1.35)

Medium-/short-chain hydroxyacyl-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

NOMENCLATURE

Previously known as short-chain acyl-CoA dehydrogenase (SCHAD), the enzyme is now rather known as *mitochondrial matrix*-associated 3-hydroxyacyl-CoA dehydrogenase (HACD) or medium- and short-chain 3-hydroxyacyl-CoA dehydrogenase (M/SCHAD) (1). SCHAD has come to mean – instead – the 3-hydroxy-2-methylacyl-CoA dehydrogenase (MHBD) of leucine metabolism (2). However, as early as 1971 (3) a distinction was made between "L-3-hydroxyacyl-CoA dehydrogenase" (EC 1.1.1.35) and "acetoacetyl-CoA reductase" (EC 1.1.1.36) This SCHAD is also known as 17β-hydroxysteroid dehydrogenase type 10 (HSD10) or type 2 -3-hydroxyacyl-CoA dehydrogenase (HADII), also has a range of other functions, including acting on steroids and cholic acids (4). SCHAD is also – in contrast to LCHAD and M/SCHAD – a member of the short-chain dehydrogenase/reductase (SDR) family rather than the HACD family (4).

BACKGROUND

3-hydroxyacyl-CoA dehydrogenation is the conversion of 3-hydroxyacyl-CoA to 3-ketoacyl-CoA. Several enzymes catalyse this reaction:

1. 3-hydroxyacyl-CoA dehydrogenase (HADH – what Van Eunen *et al.* (5) refer to as M/SCHAD, or medium/short-chain acyl-CoA dehydrogenase) (6)
2. A short-chain (shorter than C4) 3-hydroxyacyl-CoA dehydrogenase (SCHAD), also known as 3-hydroxy-2-methylacyl-CoA dehydrogenase or 17β-hydroxysteroid dehydrogenase type 10 (HSD10), which acts in leucine metabolism and also the metabolism of steroids and cholic acids (2,4).
3. Mitochondrial trifunctional protein (MTP; (7))
4. Peroxisomal bifunctional protein (PBF; (8))
5. A mitochondrial long-chain 3-hydroxyacyl-CoA dehydrogenase (mLCHAD; (9)) which was reported in bovine and ovine liver, but not in human liver so far.
6. He *et al.* (10) reported the presence of a homotetrameric human short-chain L-3-hydroxyacyl-CoA dehydrogenase with an identical N-termal segment to the bovine mLCHAD (9). This enzyme is identical to an endoplasmic reticulum amyloid β-peptide-binding protein (ERAB) and is not seen in the liver. Whether it is identical or related to HSD10 (2) of mLCHAD (9) is not known.

STRUCTURE

M/SCHAD consists of two identical subunits with molecular mass 35 kDa (9). Xu *et al.* (2): "The crystal structure of human [M/SCHAD] consists of two domains, the N-terminal domain and the C-terminal domain. The former one (catalytic domain) resembles an α/β dinucleotide-binding fold (Rossmann-fold) and comprises a conserved His-Glu pair in the active site. The latter one (dimerization domain) is primarily α-helical and contributes to the [M/SCHAD] dimerization."

Xu *et al.* (2) also observed dimerization to be important for enzyme function in M/SCHAD, by holding the distal substrate-binding C-terminal domain in place.

FUNCTION

M/SCHAD oxidises medium- and short chain hydroxyacyl-CoAs with the concomitant reduction of NAD^+ to NADH (11). Xu *et al.* (2) report that the evidence points towards M/SCHAD's substrate and co-factor binding to the catalytic site, as visualised in Fig. 1B (4).

*Modelling decision: NAD^+/NADH has only one binding site

Xu *et al*. (2) found pretty clear single-site binding for NAD^+/NADH. Previous literature report multiple binding of NADH/NAD^+ (2), but for simplicity I keep to single binding.

*Unexplored kinetic implication: NAD^+/NADH has only one binding site

Byracki *et al.* (12) report the binding of two NADH molecules simultaneously to M/SCHAD. Xu *et al.* (2) argue that this might be an *in vitro* artefact of high NADH concentrations. However, this question remains to be resolved.

*STRONG modelling decision: model only M/SCHAD

We choose to only model the activity of M/SCHAD, as SCHAD contributes only nominally to the HACD activity in the mitochondrial matrix (2) and mLCADH has not been observed in human liver (9).

* Unexplored kinetic implication: substrate inhibition

Kobayashi *et al.* (9) found M/SCHAD and the novel LCHAD to be inhibited at high substrate concentrations, especially with longer chain substrates. This might be a kinetic effect, or simply a detergent effect, perhaps due to micelle formation *in vitro* restricting access to substrates.

* Unexplored cofactor activity: NADH & NADPH

Kobayashi *et al.* (9) found M/SCHAD to low activity with and sensitivity to NADPH, while the novel LCHAD could not use NADPH at all.

* Unexplored enzyme activity: 3-hydroxy-2-methylacyl-CoA dehydrogenase

3-hydroxy-2-methylacyl-CoA dehydrogenase, or short-chain acyl-CoA dehydrogenase, is a mitochondrial matrix-associated dehydrogenase principally associated with leucine metabolism, although it also has activity towards the mFAO intermediate acetoacetyl-CoA (2). This SCHAD is also known as 17β-hydroxysteroid dehydrogenase type 10, also has a range of other functions, including acting on steroids and cholic acids (4). Though its chain-length activity and its expression levels restrict it to a relatively small share of the mitochondrial HACD activity, SCHAD might be important for some kinetic features of the pathway.

* Unexplored long-chain 3-hydroxyacyl-CoA dehydrogenase

Kobayashi *et al.* (9) report a novel HADH enzyme (28 kDa), particularly active towards longer chain substrates, and find an immunological signal for this enzyme only in horse, bovine, and sheep livers, with only weak signals in rat and dog livers, and no signal in human, rabbit, and pig livers. Kobayashi *et al.* (9) themselves mention that the presence of this second HACD enzyme suggests different substrate specificities in mFAO for bovine, rat, and human livers.

* Unexplored inhibition by acetoacetyl-CoA

Schulz (13) reports an inhibition of "L-3-hydroxyacyl-CoA dehydrogenase by acetoacetyl-CoA with a K_I of 7.7 µM", citing unpublished results by Schifferdecker and Schulz.

MEASURING CONDITIONS

pH

Changing the pH from 6 to 8 can lower the *reverse* Vmax by more than 50% in mitochondrial matrix-associated HACD in various species and tissues (rat liver, Uchida *et al.* (7) and Liu *et al.* (14); human heart, Barycki *et al.* (12); mouse pancreas, Hammar & Berne (15)). What is more, this effect is different for different chain-lengths of substrate, meaning that the specificity of the enzyme for its substrates will also change with changing pH.

In the *forward* direction, He *et al.* (16) found a pH of 10.0 to yield an almost 10 times higher V_{max} than at pH = 8.0.

Kobayashi *et al.* (9), working on bovine liver M/SCHAD, found the forward activity to be strongest at pH = 9.5 (He *et al.* (16) also report a maximum at about pH = 10.0) while the reverse activity was strongest at pH = 4. This is in agreement with Uchida *et al.* (7), Barycki *et al.* (12), Hammar & Berne (15), and Liu *et al.* (14), who found strong M/SCHAD activity at low pH (Fig. 11 in (7)).

* STRONG modelling decision: penalise measurements outside 7.5 – 8.5.

Detergents

Uchida *et al.* (7) also showed different % activities in the presence *versus* the absence of Tween-20, and also in a dose-dependent way. This was the case when palmitoyl-CoA was the substrate but not with octanoyl-CoA.

* Modelling decision: penalise the use of detergents

Try to select parameters that were measured in the absence of detergents, especially when the substrates are longer than 8 carbon atoms.

BSA

El-Fakhri & Middleton (17) report having seen stimulation of reverse HACD activity in the presence of BSA, with a peak at 0.3 mg/mL when assaying rat liver mitochondria for their activity towards palmitoyl-CoA; above 0.6 mg/mL the effect became inhibitory (Fig. 1, (17)). This effect was not seen when the substrate was acetoacetyl-CoA, perhaps suggesting that it has something to do with disrupting micelle formation of longer chain fatty acids at low concentrations and oversequestering them at high concentrations, as was seen for CPT1 (18,19). He *et al.* (16) also saw the stimulatory effect of BSA, noting that the effect was visible when measuring activity in the reverse direction, but not the forward.

* Modelling decision: penalise the use of BSA

For longer substrates for reverse assays (C8-ketoacyl-CoA and longer) try – as far as possible – to use values measured at the same BSA concentration.

Temperature

Xu *et al.* (2) saw increased tendency to denature in the recombinant matrix HACD of *C. elegans.* Though this does not directly say anything about human M/SCHAD, it does suggest that these types of enzymes are vulnerable to denaturation at high temperatures, but to especially to lower temperatures.

* Modelling decision: avoid high temperatures, penalise low ones

Feel free to use K_m values from lower (*but not higher*) temperatures, but be wary of V_{max} values measured at different temperatures.

Tissue-specificity

M/SCHAD was found by Kobayashi *et al.* (9) to be present in all tissues except muscle in bovine samples. However, the database ExPASy reports the enzyme to be present in liver, kidney, pancreas, heart, and skeletal muscle (20). Yang *et al.* (4) shed light on this issue by pointing out that Bennet *et al.* (21) found qualitative and quantitative differences in muscle and liver M/SCHAD. Yang *et al.* (4) note that alternative splicing might be responsible for the tissue specificity of M/SCHAD in liver and skeletal muscle.

El-Fakhri *et al.* (17) investigated the distribution of long-chain and short-chain hydroxyacyl-CoA dehydrogenase activity in rat tissues, and found almost identical distribution between the liver and the heart, whereas in the brain and kidney there was less of both activities. He *et al.* (10) also showed that the amino acid sequence for heart and liver M/SCHAD is identical in both rat and pig. This constitutes nice evidence that heart and liver parameters are interchangeable when looking at HCAD activity.

* Unexplored tissue-distribution: skeletal muscle

It appears that there is some discrepancy in the literature regarding the tissue-specificity of M/SCHAD: future work can focus on determining whether it is – indeed – present in the skeletal muscle or not and – if so – whether it has a different isoform.

* STRONG modelling decision: liver and heart parameters preferred, muscle parameters penalised

For our purposes, however, it is not necessary to know whether the enzyme is – indeed – present in the skeletal muscle or not. We just need to know what we see and when we see it. Since we see some signs of tissue-specificity in M/SCHAD between muscle and liver, we will be wary in selecting parameters that do not come from liver or heart.

Difference across species

M/SCHAD was found by Kobayashi *et al.* (9) to be present in all tested animals, including rats, cows, dogs, horses, rabbits, sheep, and humans. Xu *et al.* (2) found even the mitochondrial matrix-associated HACD in *Caenorhabditis elegans* to be structurally very conserved to human M/SCHAD (49% amino acid identity, also dimerised, Fig. 2B, Xu *et al.* (2)).

* Modelling decision: penalise non-human

Since M/SCHAD is distributed and conserved across species, we will consider parameters from all mammals.

Forward and reverse V_{max}, specificity factors

Note that Kobayashi et al. (9) report very different V_{max} values and specificities for the forward and reverse reactions in bovine liver extract.

* Unexplored kinetic implication: differing forward and reverse specificities

Perhaps there lie some other assumptions in direction-specific substrate-specificity as reported by Kobayashi *et al.* (9), i.e. that equilibrium constants and K_m values should also adapt to changes in assay direction, but I will not explore this too deeply for now.

* STRONG modelling decision: only forward-measured specificities

For M/SCHAD activity, I will penalise specificities measured in the reverse direction

"SCHAD" vs. "M/SCHAD"

Since there was some confusion on the correct nomenclature of the 3-hydroxyacyl-CoA dehydrogenases earlier on, we will also consider studies in which enzymes were called SCHAD but were given the correct EC number (1.1.1.35).

Yang and colleagues (4) point out that M/SCHAD has no activity towards 3-hydroxy-2-methylburyryl-CoA and suggests that this is a sign of contamination with SCHAD. If this is not mentioned. SCHAD contributes only nominally to the HACD activity in the mitochondrial matrix (2), so any shorter-chain hydroxyacyl-CoA activity is likely to be mostly due to M/SCHAD.

*Modelling decision: also consider parameters called "SCHAD"

Consider parameters called "SCHAD" that come from studies where the correct EC number (1.1.1.35) is given as belonging to "M/SCHAD". Do not accept M/SCHAD parameters that have where activity towards 3-hydroxy-2-methylburyryl-CoA is reported. When there is clear reason to believe that the authors mean SCHAD according to the definition of (2), then we shall disregard the study at hand.

Forward HACD K_m values

He *et al.* (16) comment on the assays performed by Osumi and Hashimoto (6) noting that they made the faulty assumption of equimolar amounts of enoyl-CoA and hydroxyacyl-CoA in the couple CROT-M/SCHAD assay. In reality, the K_m values should be lower because the real substrate of HACD is less concentrated than the authors assume. Kobayashi *et al.* (9) made no such mistake, as they added the hydroxyacyl-CoA substrate directly.

He *et al.* (16) also note than uncoupled HACD activity assays in the forward direction yield much higher K_m values than should be reality.

*STRONG modelling decision

Our decision is - all else being equal - to err on the aside of lower K_m values, as the problem with this assay seems to be overestimation and not underestimation.

KINETICS

Specificity

Uchida *et al.* (7) in rat liver and Kobayashi *et al.* (9) in pork liver both saw M/SCHAD activity towards substrate of the range C4 to C16.

* STRONG decision: specificity range C4 to C16

Since there is no full data set on the specificity of human M/SCHAD, we will assume that this property is conserved in humans.

Reaction mechanism

Fig. 1B (4) shows that the nicotinamide cofactor and the CoA ester substrate occupy the same binding site. No mention of an ordered binding mechanism is ever made, nor of conformational change in the enzyme upon substrate binding. This suggests that a random order bi-bi Michaelis-Menten type reaction, with the cofactors and substrates having only one affinity.

*STRONG modelling decision: NADH/NAD^+ and KetoacylCoA/HydroxyacylCoA compete

Since these substrate-product pairs share the same binding site, they naturally compete for binding to the enzyme.

Rate equation

The random-order bi-bi Michaelis-Menten according to Rohwer *et al.* (22) is used:

For n = {4, 6, 8, 10, 12, 14, 16}:

$$vmschadC_n = \frac{sfmschadC_n \cdot \frac{Vmschad}{VMAT} \cdot \left(\frac{C_nHydroxyacylCoAMAT[t] \cdot NADMAT}{K_mmschadC_nHydroxyacylCoAMAT \cdot K_mmschadNADMAT} - \frac{C_nKetoacylCoAMAT[t] \cdot NADHMAT}{K_{eq}mschadC_n \cdot K_mmschadC_nKetocylCoAMAT \cdot K_mmschadNADHMAT}\right)}{\left(1 + \frac{NADMAT}{K_mmschadNADMAT} + \frac{NADHMAT}{K_mmschadNADHMAT}\right) \cdot \left(1 + \sum_{n=4}^{n=16}\left(\frac{C_nHydroxyacylCoAMAT[t]}{K_mmschadC_nHydroxyacylCoAMAT} + \frac{C_nKetoacylCoAMAT[t]}{K_mmschadC_nKeotacylCoAMAT}\right)\right)}$$

$$NADMAT = \left(\frac{NAD_tMAT}{\frac{NAD^+}{NADH} + 1}\right) \cdot \frac{NAD^+}{NADH}$$

$$NADHMAT = \left(\frac{NAD_tMAT}{\frac{NAD^+}{NADH} + 1}\right)$$

Variables == initial values

Enoyl-CoAs	Acylcarnitines
C16HydroxyacylCoAMAT[t] == 0 µM	C16KetoacylCoAMAT[t] == 0 µM
C14HydroxyacylCoAMAT[t] == 0 µM	C14KetoacylCoAMAT[t] == 0 µM
C12HydroxyacylCoAMAT[t] == 0 µM	C12KetoacylCoAMAT[t] == 0 µM
C10HydroxyacylCoAMAT[t] == 0 µM	C10KetoacylCoAMAT[t] == 0 µM
C8HydroxyacylCoAMAT[t] == 0 µM	C8KetoacylCoAMAT[t] == 0 µM
C6HydroxyacylCoAMAT[t] == 0 µM	C6KetoacylCoAMAT[t] == 0 µM
C4HydroxyacylCoAMAT[t] == 0 µM	C4KetoacylCoAMAT[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.

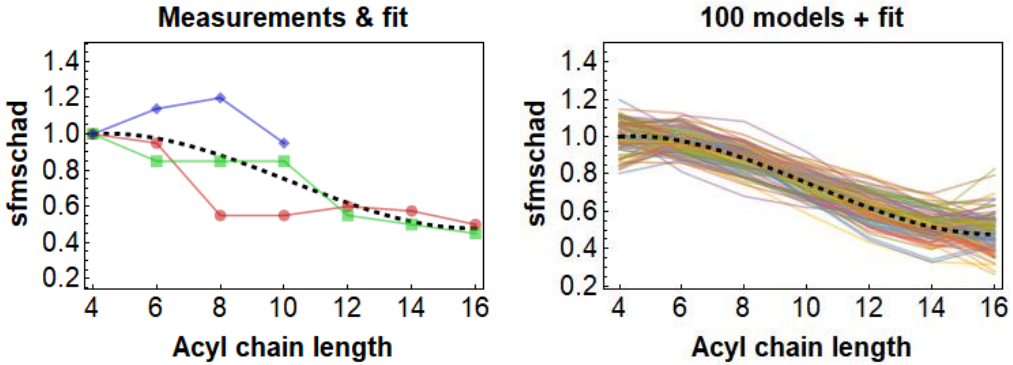
Weights are given in curly brackets next to parameter values: {} with short reasons

sfmschad

Semi-satisfactory values found: no human values, but some consistency in other mammals

Parameter		Chosen value [range]	Alternatives		Comments
sfmschad		Kobayashi et al. (1996, (9)) bovine liver extract, 30°C, 0.1 M Tris, 0.1 M KCl, pH = 10., 1 mM NAD, 40 uM hydroxyacyl-CoA for only the **forward** reaction	Uchida et al. (1992, (7)) rat liver mitochondrial extract, 30°C, pH = 7.5, 100 mM KCl, 100 mM Tric-Cl, **reverse** assay	Osumi & Hashimoto (1980, (6)) C4-reaction as 1.0 in rat liver homogenate, called "rat liver mitochondrial HACD", which I assume as equal to M/SCHAD, **forward** reaction, 100 mM Tris, 100mM KCl, pH = 10.2, 0.22 ug crotonase, 1 mM NAD, 1 mM 2-enoyl-CoA, 30°C	**1)** Kobayashi *et al.* (9) report different specificities for the forward and reverse reactions in bovine liver extract. This suggests that a modelling decision needs to be made, and I would rather err on the side of choosing forward specificities for modify the forward V_{max} of this reaction, which would exclude the parameters of Uchida *et al.* (7). **2)** It is nonetheless encouraging to see that the specificities of rat liver M/SCHAD for the reverse reaction as measured by Uchida *et al.* (7) have similar values. **3)** Osumi *et al.* (6) measure much higher specificity up until the C10 substrate in rat mitochondrial homogenate, but we select against it as the other two sets of specificities are in relative agreement.
	sfmschadC16	0.5 *{0.1, bovine + temp + pH}* [0.45 – 0.5]	0.45 *{0.1, rat + temp + reverse}*		
	sfmschadC14	0.575 *{0.1, bovine + temp + pH}* [0.5 – 0.575]	0.5 *{0.1, rat + temp + reverse}*		
	sfmschadC12	0.6 *{0.1, bovine + temp + pH}* [0.55-0.6]	0.55 *{0.1, rat + temp + reverse}*		
	sfmschadC10	0.55 *{0.1, bovine + temp + pH}* [0.55 – 0.95]	0.85 *{0.1, rat + temp + reverse}*	0.95 *{0.1, rat + temp + pH}*	
	sfmschadC8	0.55 *{0.1, bovine + temp + pH}* [0.55 – 1.2]	0.85 *{0.1, rat + temp + reverse}*	1.20 *{0.1, rat + temp + pH}*	
	sfmschadC6	0.95 *{0.1, bovine + temp + pH}* [0.85 – 1.14]	0.85 *{0.1, rat + temp + reverse}*	1.14 *{0.1, rat + temp + pH}*	
	sfmschadC4	1.0 *{0.1, bovine + temp + pH}*	1.0 *{0.1, rat + temp + reverse}*	1.0 *{0.1, rat + temp + pH}*	

Cubic polynomial (multinormal distribution)	
Formula	$a * x^3 + b * x^2 + c * x + d$
Best fit parameters	a = 0.000703944 b = -0.021478 c = 0.149614 d = 0.700122
R^2	0.97
Covariance matrix	{{7.51912*10^{-7}, -0.0000222295, 0.000197997, -0.000516012}, {-0.0000222295, 0.000664561, -0.0059982, 0.0158519}, {0.000197997, -0.0059982, 0.0550692, -0.148459}, {-0.000516012, 0.0158519, -0.148459, 0.411967}}
Bounds	{{"bounds: C4", 0.1, 10.}, {"bounds: C6", 0.095, 9.5}, {"bounds: C8", 0.055, 5.5}, {"bounds: C10", 0.055, 5.5}, {"bounds: C12", 0.06, 6.}, {"bounds: C14", 0.0575, 5.75}, {"bounds: C16", 0.05, 5.}}



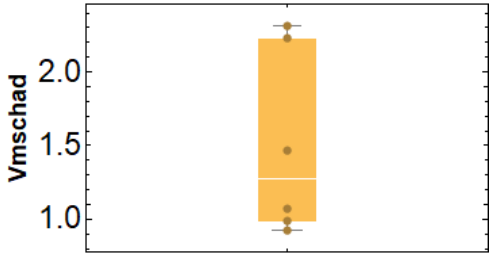
Comments: Some of the data points are not captured by the function.

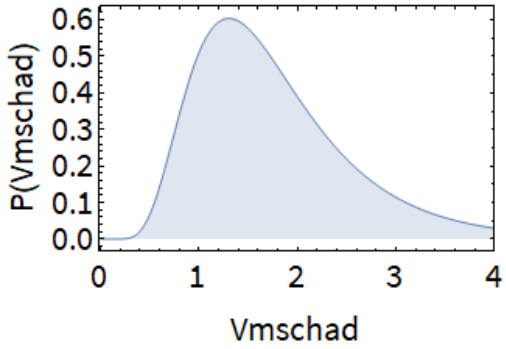
Vmschad

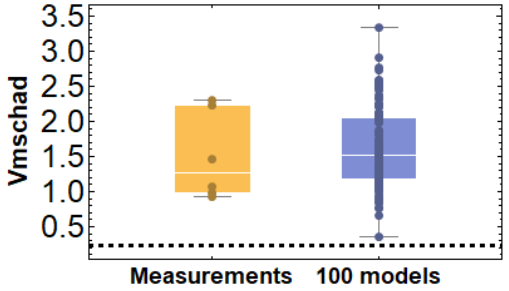
Satisfactory value found.

Parameter		Chosen value [range]	Alternatives					Comments
Vmschad		Treacy *et al.* (2000, (23)) human foetal liver, reverse reaction, 37°C, 100 mM KPi, pH = 6.3, 0.1 mM DTT, 0.1% Triton X-100, 100 uM NADH, 0.01 mg/mL protein, n=11, C4 ketoacyl-CoA substrate; ***, ****	Kobayashi *et al.* (1996, (9)) bovine liver extract, 30°C, 50 mM KPi, pH = 7.5, 0.1 mM NADH, 20 uM acetoacetyl-CoA); ratio of M/SCHAD to LCADH is roughly 78/(78+22) = 78; *,**	El-Fakhri & Middleton (1982, (17)): rat liver mitochondrial extract, reverse activity, 100 mM KPi, pH = 7.0, 40 uM 3-acetoacetyl-CoA, 0.1 mg/mL NADH, 30°C; *	Bennett *et al.* (1999, (21)) human foetal liver homogenate, reverse reaction, 37°C, 100 mM KPi, pH = 6.3, 0.1 mM DTT, 0.1% Triton X-100, 100 uM NADH, 0.01 mg/mL protein, n=11, C4 ketoacyl-CoA substrate; *, **	Osumi & Hashimoto (1980, (6)) "rat liver HACD", which I assume as equal to M/SCHAD, reverse reaction, 50 mM KP1, pH = 7.4, 0.1 mM NADH, 1 mM acetoacetyl-pantetheine, 30°C); forward reaction with hydroxyacyl-pantetheine/reverse reaction with ketoacyl-CoA = 270/714; **	Kapoor et al. (2009, (24)) human fibroblast homogenate, reverse activity, 100 mM KPi, pH = 7.0, 40 uM 3-acetoacetyl-CoA, 0.1 mg/mL NADH, 0.3mg/mL BSA, no temp given) converted liver by multiplying with 10 (Treacy *et al.*, 2000), *, **	**1)** I seem to be at least in the right order of magnitude with my chose value, since all estimated Vmax values are within about a factor of two of each other. **2)** I choose the value from Treacy *et al.* (23) due to the fact that it was measured at 37°C, and on human foetal liver directly. It stems from 11 samples and is in very good agreement with the value from Bennet *et al.* (21) which was also directly measured on human foetal liver tissue and on 11 samples though it is not clear whether these control liver samples might overlap, as the two papers are from related research projects.
	Vmschad	2.31 (± 1.46) µmol.min^{-1}.mg-mito-Protein^{-1} *{0.5, pH + detergent}* [0.928 – 2.31] **range: (0.85, 3.77)**	0.988 µmol.min^{-1}.mg-mito-protein^{-1} *{0.5, bovine + temp}*	0.928 µmol.min^{-1}.mg-mito-prot^{-1} *{0.1, rat + temp + pH}*	2.23 (± 1.46) µmol.min^{-1}.mg-mito-Protein *{0.5, pH + detergent}* **range: (0.76, 3.69)**	1.47 µmol.min^{-1}.mg-mito-Protein *{0.1, rat + temp + pH}*	1.07 µmol.min^{-1}.mg-mito-Protein *{0.1, pH + temp + BSA}*	
	* = reverse activity converted to forward activity according to the ratio 400/720 from Kobayashi *et al.* (9) ** = scaled from cellular protein to mitochondrial protein using Wisniewski *et al.* (25)'s protein ruler							

Log-normal distribution	
Parameters **(of the normal distribution)**	μ = 0.473004 σ = 0.456904
Bounds	{"bounds", 0.231, 23.1}







Comments: n/a

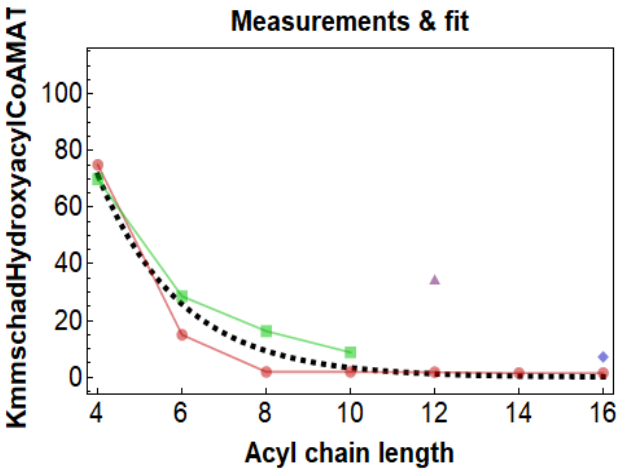
KmmschadHydroxyacylCoAMAT

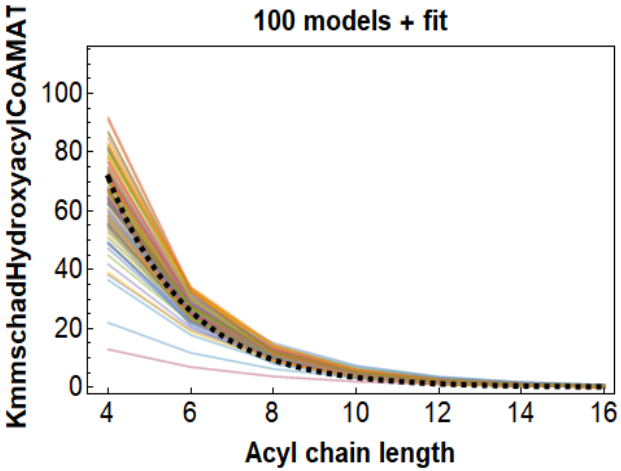
Semi-satisfactory values. No human values available, but mammalian values can be used as substitutes.

	Parameter	Chosen value [range]	Alternatives		Comments
KmmschadHydroxyacylCoAMAT		Kobayashi et al. (1996, (9)) bovine liver extract, 30°C, 0.1 M Tris, 0.1 M KCl, pH = 10., 1 mM NAD, 40 uM hydroxyacyl-CoA for only the **forward** reaction	Osumi & Hashimoto. (1980, (6)) C4-reaction as 1.0 in rat liver homogenate, called "rat liver mitochondrial HACD", which I assume as equal to M/SCHAD, **forward** reaction, 100 mM Tris, 100mM KCl, pH = 10.2, 0.22 ug crotonase, 1 mM NAD, 1 mM 2-enoyl-CoA, 30°C	Liu et al. (2007, (11)) rat liver MSCHAD expressed in *E. coli*, forward direction, assumed conditions the same as He *et al.* (1988, (16)) He *et al.* (1989, (16)) Pig heart homogenate, 25°C, forward assay, 200 mM KPi, pH = 8.0, 0.2 mg.mL BSA, 0.5 mM NAD, 0.25 CoASH, 16.7 uM hydroxyacyl-CoA, 79 mU ketothiolase ~~Disregarded to get the data to fit the other complete sets better. Otherwise it messes with the shape of the data~~	**1)** He *et al.* (16) comment on the assays performed by Osumi *et al.* (6), noting that they made the faulty assumption of equimolar amounts of enoyl-CoA and hydroxyacyl-CoA in the coupled CROT-M/SCHAD assay. In reality, the K_m values should be lower because the real substrate of HACD is less concentrated than the authors assume. Kobayashi et al. (9) made no such mistake, as they added the hydroxyacyl-CoA substrate directly. *He et al.* (16) also note that uncoupled HACD activity assays in the forward direction yield much higher K_m values than should be reality. Our decision is - all else being equal - to err on the side of lower Km values, as the problem with this assay seems to be overestimation and not underestimation. **2)** The values measured by Osumi *et al.* (6), He *et al.* (16) and Liu *et al.* (11) are in many place quite different from those measured by Kobayashi *et al.* (9); however, at least we consistently see values in the low micromolar range and not, say, in the hundreds of micromolars or millimolar range.
	KmmschadC16HydroxyacylCoAMAT	1.5 µM *{0.1, bovine + temp + pH}*			
	KmmschadC14HydroxyacylCoAMAT	1.5 µM *{0.1, bovine + temp + pH}*			
	KmmschadC12HydroxyacylCoAMAT	1.8 µM *{0.1, bovine + temp + pH}*			
	KmmschadC10HydroxyacylCoAMAT	1.9 µM *{0.1, bovine + temp + pH}* [1.9 – 8.8]	8.8 µM *{0.1, rat + temp + pH + faulty assumption}*		
	KmmschadC8HydroxyacylCoAMAT	1.9 µM *{0.1, bovine + temp + pH}*	16.3 µM *{0.1, rat + temp + pH*	34.6 µM (11) *{0.1, rat + temp + BSA}*	

		[1.9 – 34.6]	*+ faulty assumption}*		
	KmmschadC6HydroxyacylCoAMAT	15 µM *{0.1, bovine + temp + pH}* [15 – 28.6]	28.6 µM *{0.1, rat + temp + pH + faulty assumption}*		
	KmmschadC4HydroxyacylCoAMAT	75 µM *{0.1, bovine + temp + pH}* [7.2 – 75]	69.9µM *{0.1, rat + temp + pH + faulty assumption}*	7.2 µM (16) *{0.1, pig heart + temp + BSA}*	

Exponential growth/decay_2vars (multinormal distribution)	
Formula	$a * e^{x*b}$
Best fit parameters	a = 545.25 b = -0.508369
R^2	0.89
Covariance matrix	{{92332.9, -37.9538}, {-37.9538, 0.0161841}}
Bounds	{{"bounds: C4", 15/2, 750}, {"bounds: C6", 3/2, 150}, {"bounds: C8", 0.19, 19.}, {"bounds: C10", 0.19, 19.}, {"bounds: C12", 0.18, 18.}, {"bounds: C14", 0.15, 15.}, {"bounds: C16", 0.15, 15.}}





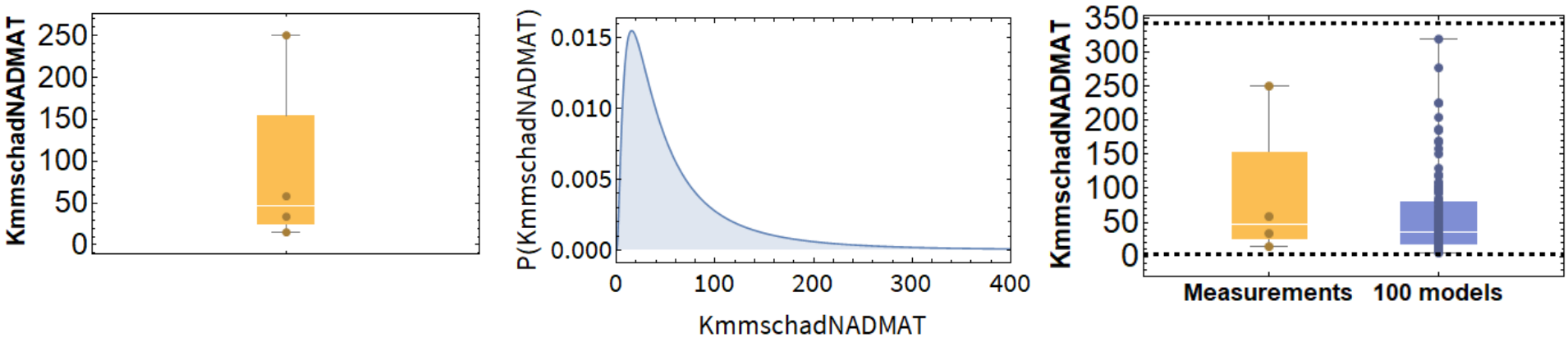
Comments: n/a

KmmschadNADMAT

Satisfactory parameter value found.

	Parameter	Chosen value [range]	Alternatives			Comments
KmmschadNADMAT		Barycki et al. (1999, (12)) recombinant human heart enzyme, 100 mM potassium phosphate, **pH = 8.0**, fluorescence titration (excitation & emission wavelengths 285 and 335 nM), no temp given.	Kobayashi et al. (1996, (9)) bovine liver extract, 30°C, 0.1 M Tris, 0.1 M KCl**, pH = 10**, 40 uM hydroxyacyl-CoA, forward reaction	Osumi & Hashimoto (1980, (6)) "rat liver mitochondrial HACD", forward reaction, 100 mM Tris, 100mM KCl, **pH = 10.2**, 0.22 ug crotonase, 1 mM NAD, 1 mM 2-enoyl-CoA, 30°C	He et al. (1989, (16)) pig heart homogenate, assume parameters measured with C4-substrate as belonging to M/SCHAD, 25°C, 200 mM KPi, **pH = 8.0**, 0.2 mg/mL BSA, 0.5 mM NAD, 0.25 mM CoASH, 79 mU pig heart 3-ketothiolase	**1)** Though the Km given by Barycki *et al.* (12) is technically a thermodynamic K_d and not a kinetic K_m, the assumption can be made that the thermodynamic hurdle of binding is determinant to the kinetic hurdle of metabolite affinity. **2)** Barycki *et al.* (12)'s value is also is the only human value, and the only value measured at physiological pH (pH = 8.0). **3)** pH seems to be very important for determining NAD+ binding to M/SCHAD - which makes sense, since it is part of a redox reaction - and the range of K_d values reported by Barycki *et al.* (12) at varying pH values (5.0 < pH < 10.0 ➔ 7.3 < K_m < 143.0 µM) is in pretty close agreement with the spread of K_m values measured by other authors
	KmmschadNADMAT	34.2 µM *{0.5, no temp + K_i}* [15.4 – 250]	250 µM *{0.1, bovine + pH + temp}*	58.5 µM *{0.1, rat + temp + pH}*	15.4 µM *{0.1, pig + temp + BSA}*	

Log-normal distribution	
Parameters (of the normal distribution)	μ = 3.74825 σ = 1.00094
Bounds	{"bounds", 3.42, 342.}



Comments: n/a

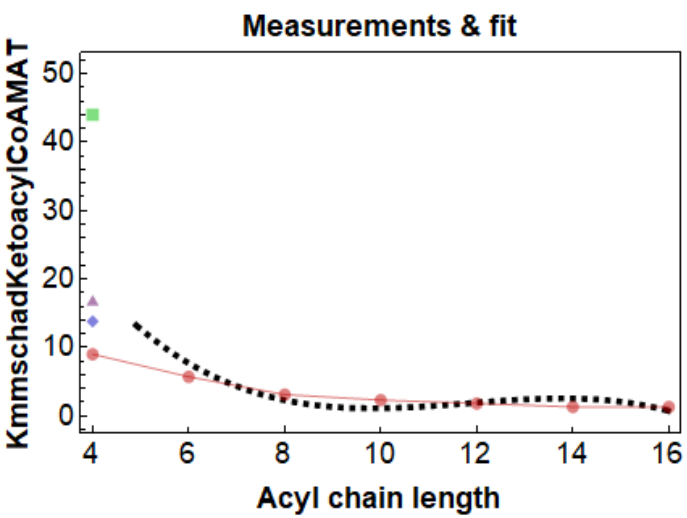
KmmschadKetoacylCoAMAT

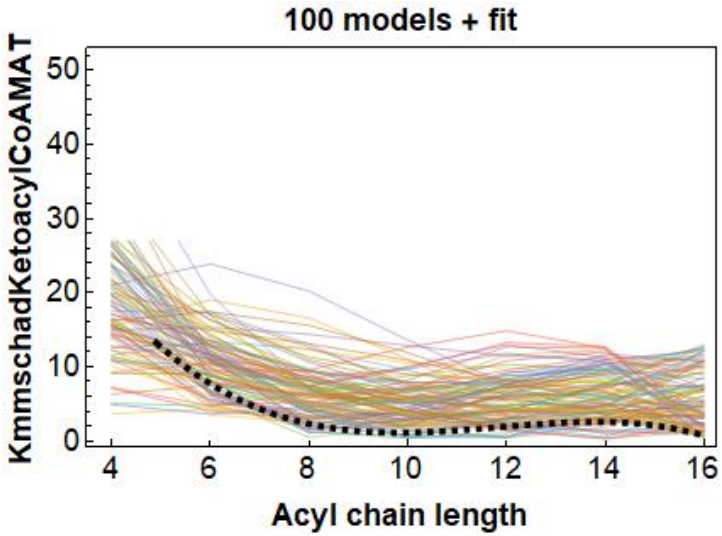
Semi-satisfactory values. No human values available for C4< substrates, but other mammalian values can be used as substitutes.

	Parameter	Chosen value [range]	Alternatives				Comments
KmmschadAnoylCoACYT		Kobayashi et al. (1996, (9)) bovine liver extract, 30°C, 50 mM KPi, pH = 7.5, 0.1 mM NADH, reverse reaction **(Barycki *et al.* (12) for the C4 substrate)**	Liu et al. (2004, (14)): recombinant rat liver enzyme, 0.1 mM KPi (must be a mistake, they must have meant 100 mM), pH = 5.0, 0.1 mM DTT, 40 µM acetylacytyl-CoA, 100 uM NADH, 0.2 µg purified enzyme, no temp given	Barycki et al. (1999, (12)) recombinant human heart enzyme, they say "SCHAD" but I assume they are looking at M/SCHAD, 100 mM sodium phosphate buffer, pH = 8.0, 0.1 mM DTT, 0.1% Triton-X 100, 100 µM NADH, no temp given	Osumi & Hashimoto (1980, (6)) rat liver homogenate, called "rat liver mitochondrial HACD", which I assume as equal to M/SCHAD, reverse reaction, 50 mM KP1, pH = 7.4, 0.1 mM NADH, 1 mM acetoacetyl-CoA, 30°C	Noyes & Bradshaw (1973, (3)) pig heart homogenate, reverse assay, pH = 7.3, 12.5 mM sodium pyrophosphate, pH = 7.3, NADH = 0.25 mM, 25°C	**1)** Bovine parameters from Kobayashi *et al.* (9) are the only available parameters for chain-lengths other than C4, so we use them. **2)** We use the C4-substrate value from Barycki *et al.* (12), as it is the only human value available. It is good support for the chose value as well as for the other chain-lengths that it is in reasonable agreement with the value measured in bovine liver by Kobayashi *et al.* (9). **3)** Barycki *et al.* (12): range 13.8 - 45.0 µM between pH = 8.0 and pH = 5.0 (recombinant human heart enzyme, 100 mM sodium phosphate buffer, 0.1 mM DTT, 0.1% Triton-X 100, 100 µM NADH, temp not given); this might partially explain the higher K_m values reported by the other authors, who all measured their values at sub-physiological pH. **4)** Encouragingly, for the chain-lengths for which there are multiple parameters available, the values are all in the lower 10s of micromolars.
	KmmschadC16KetoacylCoAMAT	1.3 µM *{0.5, bovine + temp}*					
	KmmschadC14KetoacylCoAMAT	1.3 µM *{0.5, bovine + temp}*					
	KmmschadC12KetoacylCoAMAT	1.8 µM *{0.5, bovine + temp}*					
	KmmschadC10KetoacylCoAMAT	2.3 µM *{0.5, bovine + temp}*					
	KmmschadC8KetoacylCoAMAT	3.1 µM *{0.5, bovine + temp}*					
	KmmschadC6KetoacylCoAMAT	5.7 µM *{0.5, bovine + temp}*					
	KmmschadC4KetoacylCoAMAT	9.0 µM *{0.5, bovine + temp}* [9.0 – 60]	44 µM *{0.1, rat + no temp + pH + apparent error*	13.8 µM *{0.1, heart + "SCHAD" assume "M/SCHAD" +*	16.9 µM *{0.1, rat + temp + pH}*	60 µM *{0.1, pig + pH + temp}*	

			in their description of conditions}	*detergent + no temp}*			

Cubic polynomial (multinormal distribution)	
Formula	$a * x^3 + b * x^2 + c * x + d$
Best fit parameters	a = -0.0462185 b = 1.63702 c = -18.7794 d = 71.4198
R^2	0.57
Covariance matrix	{{0.00547873, -0.162417, 1.44431, -3.6926}, {-0.162417, 4.86683, -43.8239, 113.423}, {1.44431, -43.8239, 400.864, -1055.68}, {-3.6926, 113.423, -1055.68, 2852.66}}
Bounds	{{"bounds: C4", 1.38, 138.}, {"bounds: C6", 0.57, 57.}, {"bounds: C8", 0.31, 31.}, {"bounds: C10", 0.23, 23.}, {"bounds: C12", 0.18, 18.}, {"bounds: C14", 0.13, 13.}, {"bounds: C16", 0.13, 13.}}





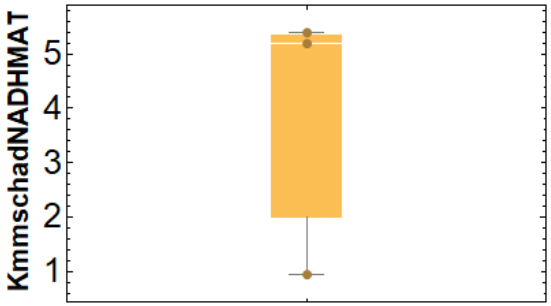
Comments: n/a

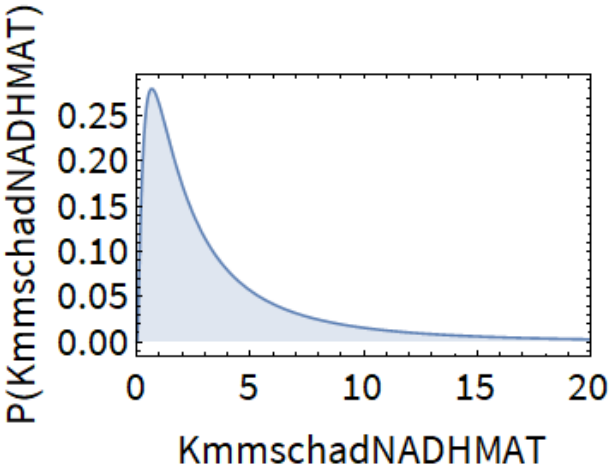
KmmschadNADHMAT

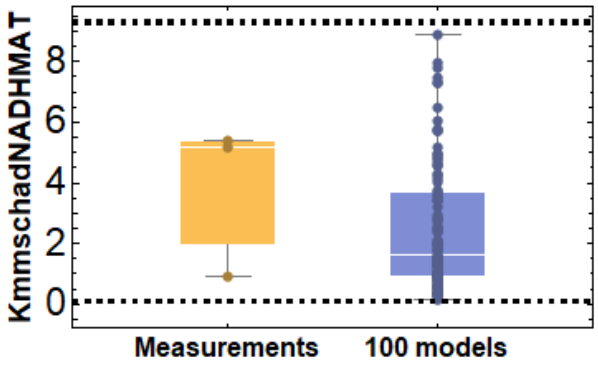
Satisfactory parameter value found.

	Parameter	Chosen value [range]	Alternatives		Comments
KmmschadNADHMAT		Barycki et al. (1999, (12)) recombinant human heart enzyme, 100 mM potassium phosphate, **pH = 8.0**, fluorescence titration (excitation & emission wavelengths 285 and 335 nM), no temp given.	Kobayashi et al. (1996, (9)) bovine liver extract, 30°C, 50 mM Kpi, **pH = 7.5**, 20 uM ketoacyl-CoA, reverse reaction	Osumi & Hashimoto (1980, (6)) "rat liver mitochondrial HACD", which I assume as equal to M/SCHAD if the activity is measured using acetoacetyl-CoA as substrate, reverse reaction, 50 mM KP1, **pH = 7.4**, 0.1 mM NADH, 1 mM acetoacetyl-pantetheine, 30°C	**1)** Though the Km given by Barycki *et al.* (12) is technically e thermodynamic K_d and not a kinetic K_m, the assumption can be made that the thermodynamic hurdle of binding is determinant to the kinetic hurdle of metabolite affinity. **2)** Barycki *et al.* (12)'s value is also the only human value, and the only value measured at physiological pH (pH = 8.0). **3)** pH seems to be very important for determining NADH binding to M/SCHAD - which makes sense, since it is part of a redox reaction - and the range of K_d values reported by Barycki *et al.* (12) at varying pH values ($5.0 < pH < 10.0$ ➔ $0.34 < K_m < 30.0$ µM) is in pretty close agreement with the spread of K_m values measured by other authors
	KmmschadNADHMAT	0.93 µM *{0.9, no temp}* [0.93 – 5.4]	5.2 µM *{0.5, bovine + temp}*	5.4 µM *{0.1, rat + pH + temp}*	

Log-normal distribution	
Parameters **(of the normal distribution)**	μ = 0.618437 σ = 1.1687
Bounds	{"bounds", 0.093, 9.3}







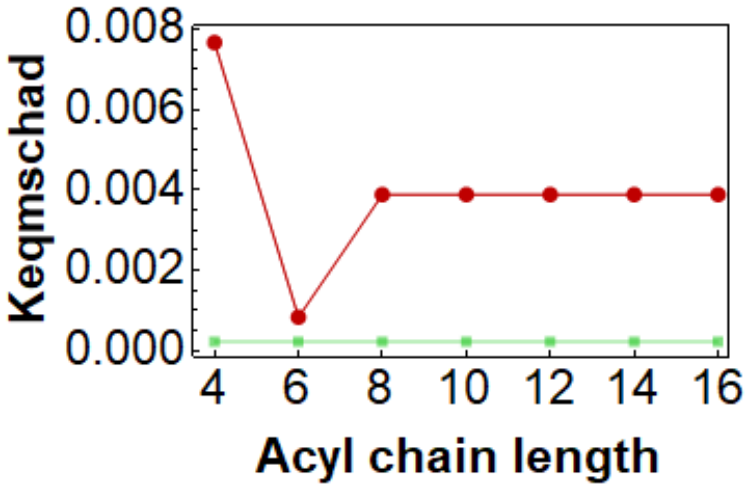
Comments: n/a

Keqmschad

eQuilibrator yields reliable and systematically determined values values.

	Parameter	Chosen value	Alternatives	Comments
Keqmschad		Estimated using eQuilibrator (26) Ionic strength = 0.125 mM, pH = 8.0, temperature correction estimate using Van 't Hoff relation	Kohn & Garfinkel (1983, (27)) *Conditions unknown*	**1)** Similar to the value in Kohn and Garfinkel (27).
	KeqmschadC16	0.00388 *{1.0}* [0.00388 – 0.000217]	0.000217 *{0.1, conditions unknown}*	
	KeqmschadC14	0.00388 *{1.0}*		
	KeqmschadC12	0.00388 *{1.0}*		
	KeqmschadC10	0.00388 *{1.0}*		
	KeqmschadC8	0.00388 *{1.0}*		
	KeqmschadC6	0.000825 *{1.0}*		
	KeqmschadC4	0.00767 *{1.0}*		

Unique	
Values	{{4, 0.00767}, {6, 0.000825}, {8, 0.00388}, {10, 0.00388}, {12, 0.00388}, {14, 0.00388}, {16, 0.00388}}



Comments: No variation allowed, unique values from eQuilibrator.

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