Acetyl-CoA

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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.

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FUNCTION

Acetyl-CoA governs the balance between cellular catabolism and anabolism as both an intermediate in metabolic pathways and as a secondary messenger, for instance by allosterically modifying enzyme activities and acetylating proteins, including histones, thereby also regulating gene expression (1). In fact, so central is acetyl-CoA to metabolism, that evolutionary reconstructions postulate its involvement in the methanotrophic reactions of the most recent common ancestor of prokaryotes (2).

N-terminal acetylation affects most human proteins, impacting their stability, localisation, and activity (3). Besides acetyltransferase activity (e.g. lysine acetyltransferase, "KAT"), N-terminal acetylation can also take place non-enzymatically, especially in alkaline environments like the mitochondrial matrix (4). In fact, Davies *et al.* (5) mention that the preponderance of non-enzymatic lysine-acetylation in the mitochondrion, as opposed to other compartments, is gaining increasing traction; they argue that the concentration of acetyl-CoA (so-called "carbon pressure") determines the extent of acetylation and that the most important mechanism for regulating this is the ability of CrAT and L-carnitine to convert acetyl-CoA to acetylcarnitine. The lacking evidence of a *bona fide* mitochondrial KAT is further evidence for this point of view. Specifically, they find that more that 80% of K-acetylations in the mitochondrion occur on matrix proteins, whereas only 40% of mitochondrial proteins are matrix-localised (5), perhaps because these proteins have more exposed sites for acetylation.

On top of this, many KAT enzymes have relatively low affinity for acetyl-CoAs (high K_d values), suggesting that the extent of acetylation really is a product of fluctuations in its concentration and that of CoA (via product inhibition). Arima *et al.* (6) confirmed that incubation of isolated mouse liver mitochondria in 1.5 mM of acetyl-CoA for 2 hours led to an uptick in the acetylation of mitochondrial proteins. Since this is only double the steady state liver acetyl-CoA concentration measured by Bachmann *et al.* (7), this suggests that acetylation in the mitochondrion is a true regulatory mechanism.

"Increased nucleo-cytosolic acetyl-CoA levels shift cellular metabolism toward anabolic reactions as they shut off catabolic circuitries. This effect is not limited to basic biochemical circuitries, but involves complex cellular (and organismal) programs. Thus, acetyl-CoA levels affect the propensity of cells to grow, progress along the cell cycle, mount autophagic responses to stress, and succumb to RCD. In addition, the abundance of acetyl-CoA in defined cell types influences the metabolic relationship between different organs, as well as behavioral cues such as appetite control," (1).

PREVIOUS MODELS AND NOMENCLATURE

Van Eunen et al. (8)

Van Eunen *et al.* (8) inserted acetyl-CoA as a terminal variable which is exported from the mitochondrion (and from the system as hepatic terminal products like ketone bodies which are exported to the blood). This process is described as simple mass-action export kinetics:

$$v_{ExAcCoA} = K_{s,ExAcCoA} \cdot ([AcetylCoA] - K_{1,ExAcCoA})/V_{mat} \quad (1)$$

While the units are not expressly given, from the structure of the equations in Modre-Osprian *et al.* (9), it seems that these export equations are mass action kinetics, which implies the given units:

Where (the units are not given in the work of Van Eunen *et al.* (8), but I take them as they were converted by Van Eunen *et al.* (8) from Modre-Osprian *et al.* (9):

K_s,AcCoA = 6 000 000 min^{-1} (8,9)

K_1,AcCoA = 70 µM (Horie *et al.*, 1986; in Horie *et al.* (1986) the control – i.e. non-fasted – mitochondrial [Acetyl-CoA] is given as 70 µM; *surely it would make more sense to use the fasted parameter of 120 µM?)*

Modre-Osprian et al. (9)

The structure of the equation in Modre-Osprian *et al.* (9) is the same as in Van Eunen *et al.* (8). The only difference is that the active mass parameter in Modre-Osprian *et al.* (9) is different and that Van Eunen *et al.* (2013) divided there equation by the mitochondrial volume, whereas Modre-Osprian *et al.* (9) multiplied by the volume term. The parameters are:

KsAcCoA = 6 000 000 min^{-1}

K1AcCoA = 30 µM

Volumes and rate equations

Van Eunen *et al.* (8) do not explicitly indicate units for the efflux parameters. However, the structure of their reactions are exactly that of Modre-Osprian *et al.* (9), who expressed their ODEs as changes in *moles* instead of changes in *concentrations*; Modre-Osprian *et al.* (9) also expressed their reactions with rate constants, enzyme concentrations, and metabolite concentrations. They would also not divide each concentration by its corresponding K_m, which means that – after calculation – their values were in concentration changes per time unit (I do not know whether they corrected for protein concentration, but they never indicate it).

At the end they multiplied everything with the compartment volume to convert the change in concentration per time to a change in *moles* per time.

Now, this is important, because most equations in Van Eunen *et al.* (8) were not expressed in this way. By using the generalized random order Bi-Bi reversible Michaelis-Menten equation (10), they ended up with changes in *moles* per minute per milligram protein. This was then converted to changes in concentrations by dividing by a specific volume (L.mg-protein^{-1}), which yielded values of µM.min^{-1}, which could be directly used in ODEs.

However, Van Eunen *et al.* (8) directly used the kinetics from Modre-Osprian *et al.* (9) – with one or two changes in value – for their export steps and did not indicate units in their parameter list. The reason for this might simply be that they did not regard these reactions as particularly important, just as long as they were not limiting.

However *technically* the reaction in Modre-Osprian *et al.* (9) without volume correction yields a value in terms of concentration change per time, and if further divided by volume – as Van Eunen *et al.* (8) did – gives a reaction rat in $\mu M.min^{-1}.L^{-1}.mg^{+1}$. This is probably an error in the rat model.

So, if we want this rate equation to make sense, we need to not divide by volume. Noting that we are going to calculate a steady-state acetyl-CoA export rate later, we can anticipate that that rate should also be calculated as change in concentration over time. Otherwise the milligram-mitochondrial-protein term will never enter the equation. Since the kinetics of this step are not enzymatic in nature, this all need not be a problem. They do not come from given enzyme mechanism directly and will be more bluntly defined as concentrations of acetyl-CoA that exit the mitochondrion over time.

Martines (11)

88% of hepatocytic acetyl-CoA is mitochondrial (12). Mitochondrial [acetyl-CoA] also stays reasonably constant, despite changes in flux through this pool in different metabolic states (13).

* Modelling decision: omit cytosolic [acetyl-CoA]

Initially, I will also not insert a cytosolic acetyl-CoA quantity, as this would beg the question of how acetyl-CoA and other short-chain acyl-CoAs might enter the mitochondrion by passive diffusion (14).

* Modelling decision – fix mitochondrial [acetyl-CoA]

Likely, the modulatory impact of acetyl-CoA in the mitochondrial matrix is felt in the form of protein modifications. However, since our model is only sensitive to kinetic modulation, and considering the stiffness that comes with a constant efflux parameter as in the case of Van Eunen *et al.* (8)'s model, I select a constant terminal acetyl-CoA concentration for the pathway.

PASSIVE DIFFUSION

*Modelling decision: acyl-CoAs do not diffuse across the mitochondrial membrane

* Unexplored kinetic property: can acylcarnitines passively cross the mitochondrial membrane?

Martines (11) takes as point of departure that short-chain acyl-CoAs and acylcarnitines can passively diffuse across the inner-mitochondrial membrane. Correspondingly, Davies *et al.* (5) quite clearly state that acylcarnitines can diffuse freely across the mitochondrial membrane. Pietrocola *et al.* (1), however, classify acetyl-CoA as "membrane-impermeant", suggesting that also longer acyl-CoA chains might not be able to just passively diffuse across the membrane. Acylcarnitines are much smaller molecules, however, and it might be that these pass the membrane passively. Pietrocola *et al.* (1), however, also discuss the transport of acetyl-CoA between the cytosol and the mitochondrion, noting that acetyl-CoA can exit the mitochondria in two ways:

1. Via the citrate-malate-pyruvate shuttle, whereby acetyl-CoA is condensed with oxaloacetate to citrate, which can then be released via the citrate carrier (SLC25A1)
2. The carnitine shuttle, in which acetyl-CoA is converted to acetylcarnitine by CrAT (carnitine acylcarnitine transferase) and then exported by CACT; in this scenario, acetylcarnitine shares with L-carnitine the ability to be exported into the cytosol in exchange for longer acylcarnitines by the CACT antiporter.

This discussion makes it seem like acetylcarnitine – the shortest and therefore least hydrophobic of all the acylcarnitines, is dependent on transporter proteins to exit the mitochondria, perhaps suggesting that there is not much passive movement of acylcarnitines or -CoAs across the mitochondrial bilayer membrane. This is in keeping with the finding of Violante *et al.* (15) that knocking out the acylcarnitine transporter (CACT) masks the accumulation of octanoylcarnitine in MCAD-KO fibroblasts – this would of course mean that the acylcarnitine are not reaching the extracellular medium, suggesting that these acylcarnitines do not just diffuse over the membrane willy-nilly.

Finally, Violante *et al.* (15) also mentions that they assume that medium-chain free fatty acids diffuse over the mitochondrial membrane whereas long chains have to be activated to acyl-CoAs first and pass via the carnitine shuttle.

*Modelling decision: we do not explicitly include diffusion of any metabolite across the membrane

In case there is some passive diffusion across the membrane of acylcarnitine species, which are not classified as categorically membrane-impermeant as acetyl-CoA (1), we consider that transport as accounted for by the assays done with CACT activity: presumably, an assay measuring the rate of acylcarnitine transport across the membrane would also classify short-chain acylcarnitine movement across the membrane – even if a product of diffusion – as successful transport events.

Martines (11) states that she assumes that her modelled equation also captures the effect of acyl-CoA being directly transported across the membrane – I make the same assumption.

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

A NOTE ON THE BOUNDARY CONDITIONS

The boundary conditions, conserved moieties, and compartment volumes are not varied. If I am interested in the contributions of these parameters, I might vary them systematically later on.

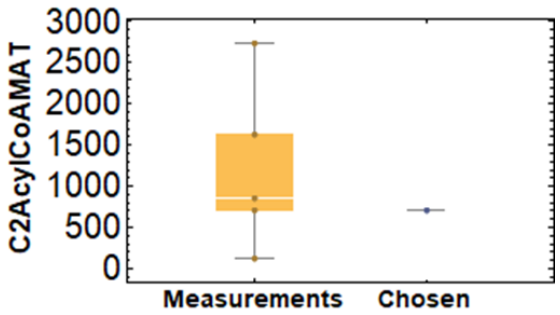
I will rather use a constant terminal acetyl-CoA concentration for simplicity and since it appears to make the model let stiff (not proved, but suspected from experience.

AcetylCoAMAT

Semi-satisfactory value found: no explicitly measured human hepatic, mitochondrial [acetyl-CoA]

	Parameter	Chosen value [range]	Alternatives				Comments
AcetylCoAMAT		Bachmann *et al.* (1982, (7)) Human liver intramitochondrial [acetyl-CoA]; not directly give a source for their data Take average of their given range of 0.6 – 0.8 mM	Horie *et al.* (1986, (16)): fasted rat liver mitochondrial [Acetyl-CoA]	Siess *et al.* (1976, (17)) fasted rat hepatocytic homogenates: incubated *in oleate and lactate*	Siess *et al.* (1976, (17)) fasted rat hepatocytic homogenates: incubated *in only oleate*	Siess *et al.* (1976, (17)) fasted rat hepatocytic homogenates: incubated *without substrate*	**1)** Despite the considerable variation in the values from literature, I will choose the human liver concentrations as my parameters.
	AcetylCoAMAT	700 µM *{0.5, direct source unclear}* [120 – 2740]	120 µM *{0.1, fasted rat}*	1630 µM *{0.1, rat homogenates + appropriateness of conditions unclear}*	2740 µM *{0.1, rat homogenates + appropriateness of conditions unclear}*	860 µM *{0.1, rat homogenates + appropriateness of conditions unclear}*	

Unique	
Values	700



Comments: No variation allowed.

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