CoA

Coenzyme A

Author: Johannes C.W. Odendaal

Reviewed: Ligia Kiyuna

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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

BACKGROUND

“CoA levels differ among cell compartments. Mitochondria are the most striking example. Mitochondria have the highest CoA levels in cells, with total acyl-CoA concentrations>10-fold greater than those of cytoplasm. In rat heart mitochondria, total CoA concentration is estimated to be about 2.3 mmol/L, accounting for over 95% of total cellular CoA. In rat liver, about 75% of CoA is mitochondrial. CoA is transported into mitochondria by the inner mitochondrial membrane carrier encoded by SLC25A42.” (1)

“In heart, over 95% of carnitine is cytoplasmic, and cytoplasmic levels of total L-carnitine, ~2 mmol/L, are over 10-fold higher than those of cytoplasmic CoA [30].” (1)

Biosynthesis

Pantothenate kinase (PanK) catalyzes the first and rate-controlling step in the biosynthesis of CoA (2). The mitochondrial isoform of this enzyme is produced by the gene PANK2, and it results in a long-lived 48 -kDA mature protein (2). A common characteristic of these enzymes is that they are inhibited by thioesters (so, fatty acyl-CoAs), which constitutes a form of feedback inhibition (2).

“This work describes the reversal of acetyl-CoA inhibition of PanK2 by palmitoylcarnitine. This new biochemical property of PanK2 provides an explanation for how PanK2 is activated in vivo and suggests that its mitochondrial location is important for its function as an acylcarnitine sensor that up-regulates CoA biosynthesis in response to accelerated demand for mitochondrial β-oxidation” (2).

* Unexplored kinetic implication: acyl-CoAs inhibit CoA synthesis

Castor diseases

CoA sequestration, toxicity and redistribution, a phenomenon termed ‘‘CASTOR”, is the pathological situation that occurs when one or more acyl-CoA species accumulates to high levels,” according to Mitchell *et al.* (3): “...primary events initiate a cascade that culminates in chronic illness and acute decompensations.” These diseases, however, are not usually regarded as belonging to the same grouping. “(F)or instance branched chain amino acids (BCAA) or long chain (LC) fatty acids (FA), and are often discussed with non-CASTOR diseases treated with similar diets. Hence they are rarely considered together as a group.”

CARNITINE AND CoA: A CRUCIAL RELATIONSHIP

Since acylcarnitines are produced from acyl-CoAs, there is reason to believe that acylcarnitines should mirror, to some extent, acyl-CoA concentrations (4). In fact, some studies have been predicated on the fact that acylcarnitines – in the presence of ample carnitine – should be representative of the CoA ester profile of the mitochondrion (5).

Brass & Hoppel (4) saw the production of large amounts of acylcarnitines in response to carnitine administration to both fed and fasted, but in both cases the hepatic acyl-CoA and CoA pools remained rather constant: the suggestion is that the flux through the CoA pool is fast enough to prevent acyl-CoA accumulation. Interestingly, 24h fasted rats showed a larger initial increase in liver acylcarnitine levels in response to increased carnitine, perhaps because they have a larger acyl-CoA pool to begin with.

* Unexplored kinetics implication: *total CoA changes slowly*

This suggests, perhaps, that CoA controls the mitochondrial acyl-CoA pool and flux through mFAO, and that [CoA] changes slowly over time in reaction to changing nutritional conditions. Carnitine, on the other hand, might buffer the mitochondrial acyl-CoA pool against sudden changes by the rapid formation of acylcarnitines which can be adsorbed to an abundance of binding proteins and urinated out as needed.

Evidence for this might stem from the fact that carnitine concentrations in the liver are observed to increase in conditions of starvation and glucagon release (6). Additionally, Seiler and colleagues (7) observed the carnitine pool in the mitochondrion to be readily depleted in comparison to the other pools, for instance, the cytosolic.

McGarry *et al.* (8) and Long *et al.* (9) found CPT1's "K_m for carnitine and total carnitine content" to correlate (rat liver, rat skeletal muscle, human skeletal muscle have K_m values for carnitine of 10-15 µM, 40-50 µM, and 200-400 µM and [carnitine] of 0.12, 0.5, 3.0 µmol.g-wet-weight^{-1}) but that the content is always "markedly greater than" the K_m, which suggests carnitine is normally not limiting. Dysregulation in the ability of the cytosolic carnitine pool to buffer the mitochondrial CoA pool against sudden influxes fatty acids, however, is not a normal situation: it is predicated on the failure of the cell to respond to changes.

More evidence for the buffering role of carnitine lies in the fact that an increase in carnitine did not substantially increase the rate of ketogenesis, mFAO or decrease the amount of non-esterified fatty acids (NEFA), while carnitine was cleared from the plasma at the same rate in the fed and fasted animals (4). The data provided by Brass & Hoppel (4) suggest that the acylcarnitines formed by a sudden influx of carnitine do not enter and stimulate mFAO, and are actually quite metabolically inert: you see massive acylcarnitine production, but not much downstream effect over the short term; in other words, [acylcarnitine] can change quite a bit without necessarily doing much to the cellular metabolism.

Zhang and colleagues (10), by inhibiting pantothenate kinase – the first enzyme of CoA synthesis – observed a sharp decline in liver CoA. This was accompanied by a severe hypoglycaemia and a large increase in acylcarnitines, alluding to the carnitine pool's attempt at buffering acyl groups to keep the non-esterified CoASH levels high.

This would also lend some credibility to the current clinical practice of administering carnitines to patients with mFAO deficiencies – a practice which is controversial, but common among metabolic disease specialists (11,12).

To understand this point more clearly, carnitine seems not to be the limiting factor in ketogenesis and also does not seem to clear NEFAs from the bloodstream, while it is taken up into the tissues or excreted in the urine so as to lower its level to basal conditions quite quickly (carnitine). The thing that does change, is the [acylcarnitine]:[carnitine] ratio, and this is characteristic the metabolic state (fed or fasted). The body appears to not “want” to have excess carnitine skew that ratio for very long (within two hours, the ratio is all but restored) – this suggests a sort of sensing mechanism, which reflects the metabolic state of the liver (4).

This could be very important for us, as it suggests that the metabolically balanced state is one where flux through mFAO is quick and where CoA intermediates do not accumulate, thanks to carnitines’ buffering capacity. If there is a sudden and unexpected accumulation, will the carnitine pool be able to respond? And what would be the consequences if it cannot and we see a lowered ability of the acylcarnitine pool to accept more acyl? Perhaps this can – at the wrong place and time – trap acyl-CoAs that would otherwise be released simply as acylcarnitines?

Perhaps the best summary of this principle, comes from Ramsay *et al.* (6): "[The] carnitine system both connects the various acyl-CoA pools and damps fluctuations in their acylation state that would be detrimental to cell homeostasis."

Indiveri *et al.* (13) mentions that this is lower than the K_m of CACT for carnitine intramitochondrially. In this way, carnitine is limiting for the importation of acylcarnitine, and can act as a regulatory step.

Counterpoint

However, Foster (14) suggests that an increased carnitine concentration in the liver is an important stimulant of mFAO. This contradicts the findings of Brass & Hoppel (4) that carnitine leads to very little in terms of changing metabolism beyond leading to an increase in acylcarnitines. It may be that there is some third factor which modulates the responsivity of mitochondrial metabolism to changes in [carnitine]. Perhaps an increase in mitochondrial CoA might explain allow the cells to tolerate a stronger upwards regulation of CPT1 activity by carnitine by providing more substrate for the uptake and clearance of mFAO intermediates. **This is an idea worth exploring: carnitine and CoA in concert might be important regulators of a cell’s ability to take up and catabolise fatty acids.**

Non-mFAO CoA SEQUESTRATION

Succinyl-CoA

Siess *et al.* (15) estimate control rat hepatocyte mitochondrial succinyl-CoA to be **200 µM**.

Hansford and Johnson (16) measured 0.4 nmol.mg-mitochondrial-protein^{-1} succinyl-CoA in rabbit heart mitochondria. They also measured a mitochondrial acetyl-CoA concentration content of 0.15 nmol.mg-mitochondrial-protein^{-1}. If this ratio is preserved in human hepatocytes, and we assume a human hepatocytic mitochondrial acetyl-CoA concentration of 0.7 mM (average of 0.6 – 0.8 mM range in Bachmann *et al.* (17)), then we can estimate a concentration of **1867 µM succinyl-CoA.**

Barrit *et al.* (18) also report normal rat liver succinyl-CoA concentration to be **about 200 µM**.

Quant *et al.* (19) assayed succinyl-CoA concentrations in isolated rat and ox liver mitochondria at varying oxoglutarate concentrations. They assumed 1µL of matrix water per mg mitochondrial protein, and calculated **succinyl-CoA mitochondrial concentrations ranging from 50 – 700 µM.**

Table 4 in Krahlenbuhl and Brass (20) measured rat liver hepatocyte mitochondrial CoA pools; they measure the pool sizes in units of nmol/10^6 cells, which are not readily convertible to molar concentrations in the mitochondria. However, we might derive some information from the ratios that they measured: at combined high propionate, high octanoate, and high pyruvate conditions they saw a **ratio of acetyl-CoA:succinyl-CoA:propionyl-CoA:methlmalonyl-CoA of about 3:1:1:6.** This ratio shifted depending on the conditions to which the mitochondria were exposed, but not by orders of magnitude. There, we might infer from an acetyl-CoA concentration of 700 µM (17) the following estimated concentrations:

[succinyl-CoA] ≈ 200 µM

[propionyl-CoA] ≈ 200 µM

Propionyl-CoA

Coude *et al.* (21) measured a K_i of about 0.7 mM for the mitochondrial enzyme N-acetylglutamate synthetase in rat liver mitochondria for inhibition by propionyl-CoA. **This suggests that propionyl-CoA might be present in the mitochondria in the high hundreds of micromolars.** A similar K_m is seen for acetyl-CoA, its substrate, which – as note previously – has been measured at a concentration of about 0.7 mM in liver mitochondria.

Estimated **[propionyl-CoA] ≈ 200 µM** (20)

Methylmalonyl-CoA

Estimate **[methylmalonyl-CoA] ≈ 1400 µM** (20)

* Modelling decision: include 1.8 mM non-mFAO sequestration as fixed value in model that can be varied

The CoA pool is quite dynamic, and it would be a mistake to think that an absolute non-mFAO CoA ester pool can be estimated for the metabolic stresses that we are trying to simulate in the model. However, seeing that estimates of three significant contributors to CoA sequestration in the mitochondria (at least in rats and oxen) can have concentrations in the high hundreds of micromolars, **together as much as 2 mM**, and that we haven't even looked at all of the CoA esters outside of mFAO yet (HMG-CoA, tiglyl-CoA, methylacrylyl-CoA, acryloyl-CoA, etc.), **it is perfectly reasonable to sequester as much as 2 mM (even more) of mitochondrial CoASH as a model input.**

KINETICS

* Modelling decision: CoAMAT is a conserved moiety

* Modelling decision: CoACYT is a conserved moeity

It was decided that this should fluctuate, even though the fluctuating liver cytosolic [CoA] would be a product of various processes that would complicate our simulations somewhat; the alternative, a constant coenzyme A concentration in the cytosol, would allow for a functionally infinite movement of carbon chains to the cytosol, which is not realistic. The differences between the fed, fasted, and diabetic liver cytosolic [CoA] is not big (140 µM, 60 µM, and 210 µM, respectively) with only high-fat diet mice showing a much higher [CoA] in the cytosol of the liver: 470 µM). 2) Encouraging should be that the value suggested for cytosolic [CoA] in the liver falls within the range suggested by Leonardi *et al.* (2): 20 - 140 µM; it also explains why Siess *et al.* (15) could measure almost no [CoASH] in the liver of fasted male Sprague-Dawley rats.

Since MCADD is known to lead to an extended accumulation of fats in the liver, we will assume a [CoASH] of 500 µM, also so that it isn't limiting.

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

A NOTE ON THE BOUNDARY CONDITIONS

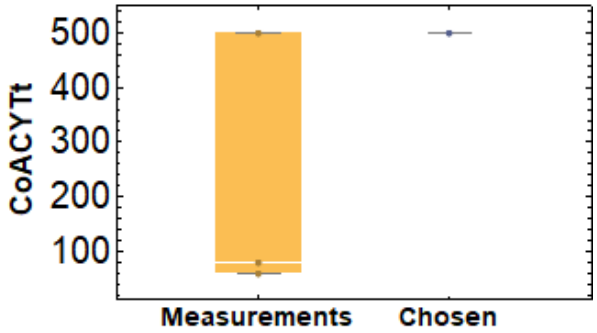
The boundary conditions, conserved moieties, and compartment volumes are not varied. If I am interested in the contributions of these parameters, I might vary them systematically later on.

CoACYTt

Semi-satisfactory value found: no explicitly human hepatic data

	Parameter		Chosen value [range]	Alternatives	Comments
CoACYT		Leonardi et al. (2007, (2)): Since MCADD is known to lead to an extended accumulation of fats in the liver, we will assume a [CoASH] of 500 µM, also so that it isn't limiting. This is near high-fat diet mice showing a much higher [CoA] in the cytosol of the liver: 470 µM	Average of range in Leonardi et al. (2007, (2)): 20 – 140 µM this is mentioned in the context of cytosol in human tissues, though it not indicated that these values were measured in human cells (not liver *per se*)	Horie *et al.* (1986, (22)), fasting rat liver cytosol	**1)** The low cytosolic CoASH concentration explains why Siess *et al.* (15) could measure almost no [CoASH] in the liver of fasted male Sprague-Dawley rats. **2)** Almost all CoA is mitochondrial, so the high values for the mitochondria and the low values for the cytosol make sense (22) **3)** It was decided that this should fluctuate, even though the fluctuating liver cytosolic [CoA] would be a product of various processes that would complicate our simulations somewhat; the alternative, a constant coenzyme A concentration in the cytosol, would allow for a functionally infinite movement of carbon chains to the cytosol, which is not realistic.
	CoACYTt	500 µM *{0.1, mice + appropriateness of conditions uncertain}* [20 – 500]	80 µM *{0.1, source uncertain, probably human}* [20 – 140]	60 µM *{0.1, rat}*	

Unique	
Values	500



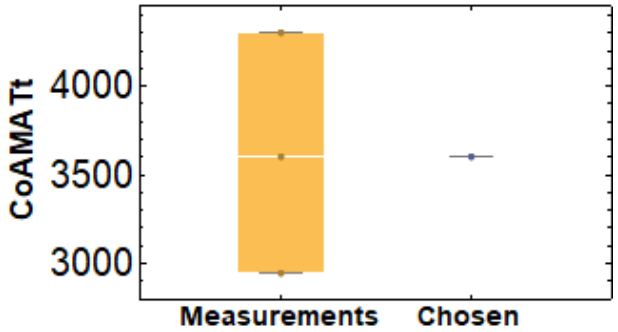
Comments: No variation allowed.

CoAMATt

Satisfactory value found.

	Parameter	Chosen value [range]	Alternatives		Comments
CoAMATt		Average of range in Leonardi *et al.* (2007, (2)): 2200 – 5000 µM this is mentioned in the context of mitochondria in human tissues, though it not indicated that these values were measured in human cells (not liver *per se*)	Horie *et al.* (1986, (22)) fasting rat liver mitochondria	Average of range in Siess *et al.* (1976, (15)) 2.3 - 3.4 mM 24-48h fasted male Sprague-Dawley rat liver cells	**1)** Good agreement in the considered values. **2)** Almost all CoA is mitochondrial, so the high values for the mitochondria and the low values for the cytosol make sense (22)
	CoAMATt	3600 µM *{0.1, source uncertain, probably human}* [2200 – 5000]	4300 µM *{0.1, rat}*	2950 µM *{0.1, rat}*	

	Unique
Values	3600



Comments: No variation allowed.

CoASHseq – non-mFAO CoA sequestration

Satisfactory values found

	Parameter	Chosen value [range]	Alternatives				Comments
CoASHseq		Siess *et al.* (1976, (15)) Estimated rat hepatocyte	*Hansford and Johnson (1975,* (16)*)* *succinyl-CoA in rabbit heart mitochondria =* (16) *0.4 nmol.mg-mitochondrial-protein^{-1}* *mitochondrial acetyl-CoA concentration = 0.15 nmol.mg-mitochondrial-protein^{-1}* *Assume preserved ratio and 0.7 mM acetyl-CoA* (17)	Barrit *et al.* (1976, (18)) Rat liver	Quant *et al.* (1989, (19)) Isolated rat and ox liver mitochondria	Krahenbuhl and Brass (1991, (20)) Rat liver mitochondria, combined; high propionate, high octanoate, and high pyruvate <u>Ratio:</u> acetyl-CoA:succinyl-CoA:propionyl-CoA:methlmalonyl-CoA of about 3:1:1:6 *Assume preserved ratio and 0.7 mM acetyl-CoA* (17)	**1)** Coude *et al.* (21) measured a K_i of about 0.7 mM for the mitochondrial enzyme N-acetylglutamate synthetase in rat liver mitochondria for inhibition by propionyl-CoA. **This suggests that propionyl-CoA might be present in the mitochondria in the high hundreds of micromolars.** A similar K_m is seen for acetyl-CoA, its substrate, which – as note previously – has been measured at a concentration of about 0.7 mM in liver mitochondria
	succinyl-CoA	200 µM	1867 µM	200 µM	50 - 700 µM	200 µM	
	propionyl-CoA					200 µM	
	Methylmalonyl-CoA					1400 µM	
	Total					1800 µM	

Unique	
Values	2300

Comments: No variation allowed.

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