Carnitine

L-carnitine

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Last Edited: 2024-02-27

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

Contents

BACKGROUND

Two types of acylcarnitines: 1) acid-insoluble acylcarnitines, which consist of mainly long-chain acylcarnitines and represent the intermediates of the carnitine shuttling of fatty acids into the mitochondria, and 2) acid-soluble acylcarnitines, for which no specific role is defined (1). *Acid-soluble acylcarnitines* are present in higher concentrations than *acid-insoluble acylcarnitines* in almost all tissues (1).

CHANGES IN CARNITINE CONCENTRATION

Two adaptive changes in carnitine physiology have been described: 1) increase or decrease in the total carnitine of a tissue or fluid compartment and 2) a change in the carnitine:acylcarnitine proportion (1).

The changes can either be over the short to medium term, such a after glucagon secretion (2) or over the longer term, such as during ageing (3).

* Arbitrary modelling decision: carnitine in both the mitochondrial and the cytosolic compartments varies slowly enough so we can set it as a conserved moiety in our model

Mechanisms

Uniporter-mediated efflux of carnitine from mitochondria to cytosol might be physiologically relevant for the equilibration of carnitine concentrations across the mitochondrial membrane (4).

Age-dependent [carnitine]

In terms of long term carnitine metabolism changes, it should be noted that neonates generally lack a strong carnitine synthesis function (3) and are largely dependent on nutritional carnitine to fulfil the functions of carnitine-dependent reactions. In fact, if carnitine is not available to neonates nutritionally, their carnitine pools tend to become depleted. Mother's milk, for instance, contains about 50 mM of carnitine (5). ***This is very important for us, as it shows that neonates are particularly vulnerable to the dysregulation in the carnitine pool as well, which fits with the symptomatology of MCADD metabolic crises.***

Nakano *et al.* (3) looked at age-dependent changes in carnitine content of various tissues. They found that *skeletal muscle* (which has the highest carnitine content in adult humans) increases its carnitine concentration during gestation ([carnitine]:[acylcarnitine] = 1:1) and reaches adult levels ([carnitine]:[acylcarnitine] = 4:1) around about birth; *liver* carnitines are constant during gestation and then increase in the postnatal period, reaching adult levels after about 1 year of age ([carnitine]:[acylcarnitine] = 4:1 throughout life); finally, *brain* carnitine concentrations increase significantly from the preterm ([carnitine]:[acylcarnitine] = 1:2) to the postnatal period ([carnitine]:[acylcarnitine] = 1:1), though the change is small compared to the that of skeletal muscle and liver.

Basically, there is a trend on the body of increasing the relative size of the carnitine pool from gestation to about 1 year of age, at which point free carnitine is more than acylcarnitine in liver and skeletal muscle, and roughly equal in the brain. This makes some sense, since the brain does not really burn fat for energy, and the function of CPT1c is likely more one of signalling or the production of necessary metabolites. In the liver, which is the main organ responsible for metabolic homeostasis in the body, is always at its adult [carnitine]:[acylcarnitine] = 4:1, which might be due to its importance.

* Unexplored physiological implication: low carnitine in infants

For our modelling purposes, therefore, it might be useful to look into decreasing the [carnitine] to simulate fasting conditions for infants, where nutritional carnitine is the only carnitine. Seeing what the ratio [carnitine]:[acylcarnitine] is in MCADD crisis samples might also shed some light on the onset of pathology**.**

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 (1). 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 (6).

Brass & Hoppel (1) 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 (2). 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 (1). The data provided by Brass & Hoppel (1) 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 (1).

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.* (2): "[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.* (4) 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 (13) suggests that an increased carnitine concentration in the liver is an important stimulant of mFAO. This contradicts the findings of Brass & Hoppel (1) 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.**

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.

Both the cytosolic and mitochondrial pools of carnitine will be changed to separate, conserved moieties. In the rat model (14), free carnitine on both sides of the membrane was a constant value.

* Unexplored kinetic implication: [Carnitine] in cytosol *versus* mitochondria

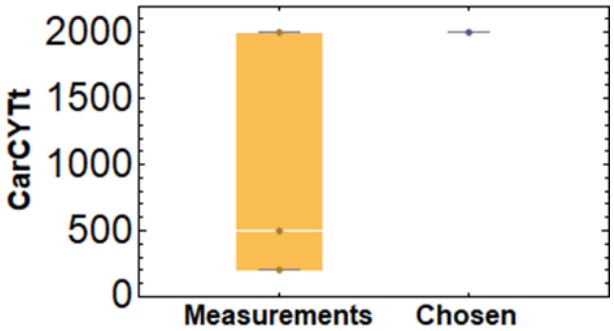
"90% of carnitine is cytosolic" (15). Scaled to the sizes of the compartments - if the mitochondrion is taken as having roughly a quarter of the volume of a hepatocyte - then the values we end up selecting in the tables below are not really accurate. The different sources contradict each other, therefore. We will disregard this foor now.

CarCYTt

No satisfactory value: though this parameter is not human nor hepatic.

	Parameter	Chosen value [range]	Alternatives		Comments
CarCYTt		Leonardi *et al.* (2007, (16))	Ramsay *et al.* (2) liver, species not specified, tough human liver discussed throughout the article; a measurement of the value is never given, though that might have something to do with the fact that it is regarded as variable.	Modre-Osprian *et al.* (2009, (17)) measuring conditions unknown	**1)** Altamimi *et al.* (18) postulate that uniporter-mediated carnitine transport should be lead to equal concentrations of carnitine on either sides of the mitochondrial membrane. I assume this for my parameter choice. Based on this, I choose a cytosolic carnitine concentration that overlaps with what I found for the mitochondrial pool **2)** "90% of carnitine is cytosolic" (15). Scaled to the sizes of the compartments - if the mitochondrion is taken as having roughly a quarter of the volume of a hepatocyte - then my values for [carnitine] are not accurate. **3)** 10% of the carnitine pool is long-chain acylcarnitine, amounting to about 200 or 400 µM (16). This might also be a way of confirming the carnitine concentrations. **4)** The carnitine pool is probably dynamic
	CarCYT	2000 µM *{0.1, source uncertain}* [200 – 2000]	500 µM *{0.1, source uncertain, probably human}*	200 µM *{0.1, source uncertain}*	

Unique	
Values	2000



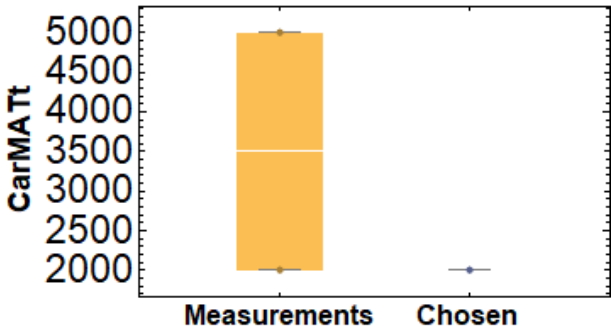
Comments: No variation allowed.

CarMATt

No satisfactory value: though this parameter is not human nor hepatic.

	Parameter	Chosen value [range]	Alternatives	Comments
CarMATt		Range: 2 mM - 5 mM Indiveri *et al.* (2011, (4)) They report to have extracted this range from: Foster (2004, (13)) and Idell-Wenger et al. (1978, (19)) neither the organism nor the tissue is mentioned, though human metabolism is discussed throughout.		**1)** Altamimi *et al.* (2018, (18)) postulate that uniporter-mediated carnitine transport should be lead to equal concentrations of carnitine on either sides of the mitochondrial membrane. I assume this for my parameter choice. Based on this, I choose a mitochondrial carnitine concentration that overlaps with what I found for the cytosolic pool **2)** "90% of carnitine is cytosolic" (Hütter et al., 1990). Scaled to the sizes of the compartments - if the mitochondrion is taken as having roughly a quarter of the volume of a hepatocyte - then my values for [carnitine] are not accurate. **3)** 10% of the carnitine pool is long-chain acylcarnitine, amounting to about 200 or 400 µM (Leonardi et al., 2007, (16)). This might also be a way of confirming the carnitine concentrations. **4)** The carnitine pool is probably dynamic
	CarMAT	2000 µM *{0.1 source uncertain, probably human}* [2000 – 5000]		

Unique	
Values	2000



Comments: No variation allowed.

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