NADH and NAD^+

Nicotinamide adinine dinucleotide

Author: Christoff Odendaal

Reviewed: Ligia A. Kiyuna

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

Nicotinamide adenine dinucleotide (NAD) in its reduced (NADH) and oxidized (NAD^+) form is an essential cofactor to produce ATP through the TCA cycle and from β-oxidation (1). NAD^+ gains two electrons and one proton during reduction as cofactor and then donates these electrons to Electron Transfer Chain Complex I, where they are used for the creation of an electrochemical gradient which can be used to produce ATP. Beyond its role in energy metabolism, NAD is also important as a participant in DNA repair and transcription (2).

As NADH is constantly being produced by, mostly, catabolic processes in the cell, concurrent processes need to reoxidise NADH to NAD^+ to maintain redox balance (2).

Essential for regulation

Since NAD is such a universal cofactor, it is used by the cell as a regulatory signal (2) in which the ratios of NAD^+/NADH are very important within each compartment, but also the ratios between these ratios: for instance, an increased NAD/NADH in the mitochondrion leads to the production of acetaldehyde by mitochondrial alcohol dehydrogenase (ADH), which can then diffuse to the cytosol and be reduced to ethanol, and lead to an increased NAD in the cytosol (2); similarly, transcriptional regulation can be effected by the differences in these ratios between the cytoplasm and the nucleus. The crosstalk between the pools is facilitated by NAD/NADH redox shuttle (most frequently the malate/aspartate and glycerol-3-phosphate shuttles) and NAD biosynthetic pathways (Stein and Imai (1); Fig. 1).

The requirement of NAD^+ for GAPDH's step in glycolysis – as well as other regulators – cytoplasmic NAD availability is a strong regulator of pyruvate flux into the mitochondrion. Indeed, when glucose is the predominant exogenous substrate, depletion of cytoplasmic NAD to 50% or less can block glycolysis and mitochondrial substrate flux (1).

The NAD pool is about 300-800µM in concentration in the cell, depending on the organ (1).

Synthesis

It is suggested that NAD can be synthesised by mitochondria themselves in response to environmental or nutritional stresses (3). However, the canonical NAD-synthetic pathways are typically cytosolic:

1) *De novo* synthesis from tryptophan
2) Salvage pathways (which seem to be more important under normal conditions – Lin *et al.* (2)):
    a. From nicotinic acid (NA)
    b. From nicotinamide (NAM, Vit B_3) – major precursor in mammals
    c. Or by conversion of nicotinamide riboside (NR)

Degradation

Three families of enzymes – poly-ADP-ribose polymerases (PARPs), CD38/CD157 ectozymes, and sirtuins consume NAD: generally, they break the glycosydic bond between the NAM and ribose moieties of NAD, which leads to the short half-life of NAD of 1-10 hours (4).

Transport

While the outer mitochondrial membrane is permeable to small and charged molecules, the inner mitochondrial membrane is not. In mammals at the time of Stein and Imai (1) no mitochondrial NAD transporter had been found, though NMN – a precursor (Box I) – can be transported.

The mitochondrial pool

Mitochondrion:Cytosol ratio

The differences between the size of the NAD pool in the mitochondrion *versus* the cytosol is dependent on the cell types: in cardiac myocytes, it's 70% mitochondrial, in neurons 50%, and 30-40% in hepatocytes (1).

Robustness

The mitochondrial NAD pool is also very resilient to changes in energy demand, meaning that it can be maintained for at least 24h whereas the cytosolic NAD pool would be severely depleted (1). Yang *et al.* (3) propose that this is due to the active presence of NAMPT in the mitochondria.

Nutrition

Studies have shown that caloric restriction and fasting lead to an increase in NAMPT expression (which synthesises NAD from NAM) and, subsequently, [NAD], and have even localized this to the mitochondrion (3).

Similarly, a high-fat diet seems to reduce levels of NAD in the liver, mimicking the effect of low energy intake (5).

[NAD] during fasting

Yang and colleagues (3) found that rat liver cells that were fasted for 48 hours had a two-fold induction of NAMPT expression. In immortalised kidney cells (HEK293) it was also measured that NAMPT overexpression to a factor of 2 led to an increase in mitochondrial [NAD^+] to a factor of two (Yang *et al.* (3), Fig. 4F). This correlates with the two and a half-fold increase in mitochondrial [NAD] in isolated rat hepatocytes from 48-fasted fasted *versus* fed rats (Fig. 6C, (3)).

In HEK293 cells (immortalised human kidney cells), the total [NAD^+] was 365 µM (Fig. 4F, (3)). This was similar in mouse erythrocytes (3) in earlier assays, which is more or less the cytosolic concentration, as **HEK293** cells have few mitochondria and contribute very little to the cellular pool (3). Assaying the [NAD^+] in mitochondria, they found 245.6 µM, which is equal to 2053 pmol NAD^+/mg-mitochondrial potein (3).

Yang *et al.* (3) also measured the mitochondrial [NAD^+] in isolated rat liver cells and found it to be about 500 pmol.mg-mito-$prot^{-1}$. It is not clearly stated whether these rats were fed or fasted, but the introduction of a genotoxic stressor (MMS and FK866) indicates that these are probably fed rats, since you wouldn't want two variables here. So, the mitochondrial [NAD^+] is 500 pmol/mg-protein, which is about a quarter of what they found in HEK293 cells, which means (assuming equal protein concentrations) that it is **equal to about 62.5 µM** (250 µM (rounded HEK239 value) divided by 4).

* Unexplored kinetic implication: changes in NAD production

Changes in nicotinamide concentrations because of nutritional state might suggest a higher-level form of regulating the fed/fasting response. This is likely very difficult to disentangle, so I will disregard it for the time being.

Ageing

Beyond nutrition, there also seems to be an age-dependent effect: NAD seems to decrease with age (6). This is – again – related to a decreased expression of NAMPT.

[NAD^+]/[NADH] ratio

The mitochondrial NAD pool is relatively unique within the cell, as it has a relatively constant **NAD/NADH ratio of 7 or 8 in typical eukaryotic cells**, while eukaryotic cytosolic ratios between free NAD^+ and free NADH, which may vary between 60 and 700 (7,8). **From Williamson *et al.*** (8)**, the ratio in starved rats is closer to 5.**

Lin and Guarante (2) report an intracellular total [NAD^+]/[NADH] of 3 to 10 in mammals, which is probably the effect of disassociating NADH from proteins.

In her PhD thesis, Martines (9) modelled NADH and NAD as members of a conserved moiety cycle, whereas Van Eunen *et al.* (10), adopting the kinetics used by Modre-Osprian *et al.* (11) used a constant efflux of NADH do describe the relationship. An efflux reaction seems – at first glance – a little weird, since NADH is more or less sequestered in the mitochondrion, changing only back and forth from NADH and NAD or by being degraded of synthesized

* Modelling decision: constant NADH and NAD^+ concentrations

To avoid stiffness in the model and to keep the things as simple as possible, I will follow the example of Martines (9) in modelling NAD and NADH as constant concentrations instead of as efflux reactions. This is justified by the finding by Yang *et al.* (3) that the [NAD^+]:[NADH] ratio in mitochondria doesn't change very readily.

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

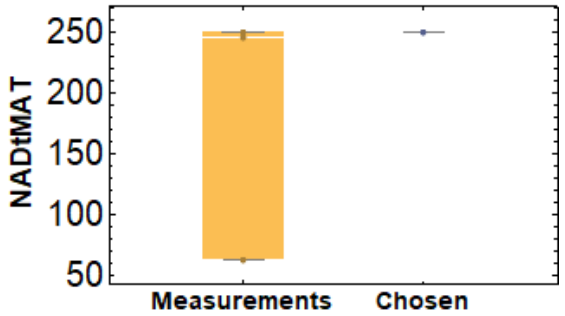
The boundary conditions, conserved moieties, and compartment volumes are not varied. If I am interested in the contributions of these parameters, I might vary them systematically later on.

NADH:NAD$^+$

Semi-satisfactory value found: no explicitly human hepatic data

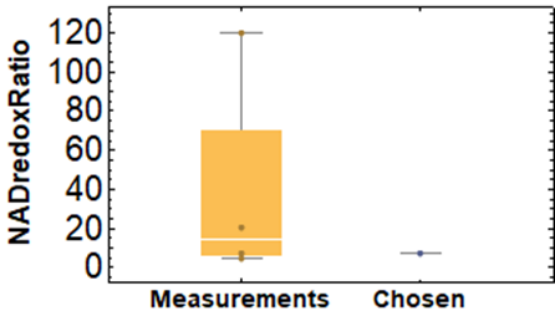
	Parameter	Chosen value [range]	Alternatives					Comments
NADH:NAD		Yang *et al.* (2007, (3)) HEK293 mitochondria (immortalised human kidney)	Stein and Imai (2012, (1)) eukaryotic cells, [NADH]:[NAD] = 1:7.5 based on Veech *et al.* (1972) and Williamson *et al.* (1967).	Williamson *et al.* (1967, (8)). starved rat mitochondria; NADH:NAD = 1:5	Seiss *et al.* (1976, (12)) fasted rat liver mitochondria NAD:NADH ratio = 120 for cells supplied with lactate, 21 for cells supplied with lactate and oleate.	Modre-Osprian *et al.* (2009, (11)) Original source unknown	Yang et al. (2007, (3)) isolated fasted rat liver cells (this value assumes that HEK293 and isolated rat liver cells have similar mitochondrial protein concentrations)	
	NADtMAT	245.6 µM (round to 250 µM) *{0.5, kidney}* [62.5 – 250]				250 µM *{0.1, unknown conditions/source}*	62.5 µM *{0.5, rat liver cells}*	
	NADredoxRation (red vs. ox)		1/7.5 *{0.1, unclear source/eukaryotic cells}*	1/5 *{0.1, starved rat}*	1/120 (only lactate provided) *{0.1, fasted rat liver + appropriateness of condition uncertain}* 1/21 (lactate and oleate provided *{0.1, fasted rat liver + appropriateness of condition uncertain}*			

Unique	
Values	250



Comments: No variation allowed.

Unique	
Values	7.5



Comments: No variation allowed.

REFERENCES

1. Stein LR, Imai SI. The dynamic regulation of NAD metabolism in mitochondria. Trends in Endocrinology and Metabolism. 2012;23(9):420–8.

2. Lin SJ, Guarente L. Nicotinamide adenine dinucleotide, a metabolic regulator of transcription, longevity and disease. Curr Opin Cell Biol. 2003;15(2):241–6.

3. Yang H, Yang T, Baur JA, Perez E, Matsui T, Carmona JJ, et al. Nutrient-Sensitive Mitochondrial NAD+ Levels Dictate Cell Survival. Cell. 2007;130(6):1095–107.

4. Houtkooper RH, Cantó C, Wanders RJ, Auwerx J. The secret life of NAD+: An old metabolite controlling new metabolic signaling pathways. Vol. 31, Endocrine Reviews. 2010. p. 194–223.

5. Yoshino J, Mills KF, Yoon MJ, Imai SI. Nicotinamide mononucleotide, a key NAD + intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. Cell Metab. 2011 Oct 5;14(4):528–36.

6. Borradaile NM, Pickering GJ. Nicotinamide phosphoribosyltransferase imparts human endothelial cells with extended replicative lifespan and enhanced angiogenic capacity in a high glucose environment. Aging Cell. 2009;8(2):100–12.

7. Veech RL, Guynn R, Veloso D. The time-course of the effects of ethanol on the redox and phosphorylation states of rat liver. Biochemical Journal. 1972;127(2):387–97.

8. Williamson DH, Lund P, Krebs HA. The redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochondria of rat liver. Biochemical journal. 1967;103(2):514.

9. Martines ACMF. Exploring the mechanisms underlying the phenotype of MCAD deficiency with Systems Medicine: from computational model to mice to man. Rijksuniversiteit Groningen; 2019.

10. 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.

11. 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.

12. Siess EA, Brocks DG, Wieland OH. Subcellular distribution of key metabolites in isolated liver cells from fasted rats. FEBS Lett. 1976;69(1–1):265–71.