[NAD(H)] in the plasma and cerebrospinal fluid of healthy humans

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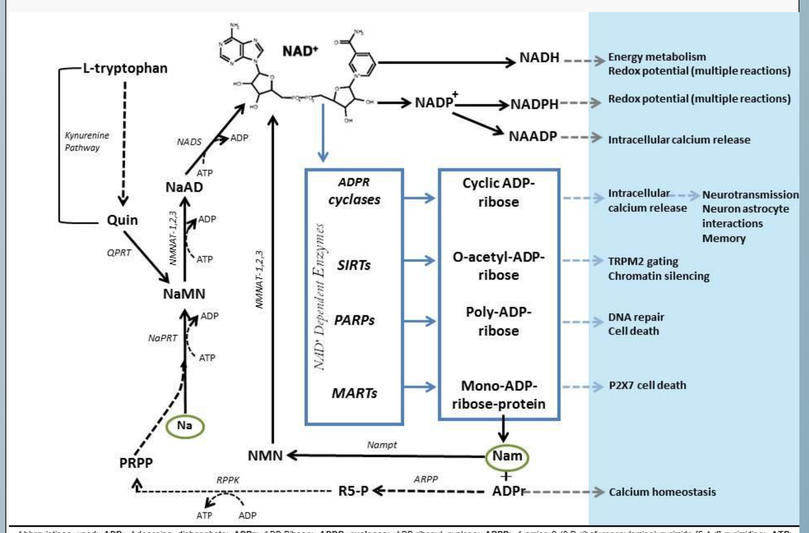
Introduction

Nicotinamide adenine dinucleotide (NAD^+) is a ubiquitous molecule that participates in many biological processes as either a coenzyme, redox couple or as a donor of ADP-ribose units. Though a large body of work has focused on the role of peripheral NAD^+, comparatively limited research has been conducted on the potential importance of NAD^+ within the central nervous system (CNS). Despite this, evidence exists that NAD^+ can play a direct role in neurotransmission as well as CNS biochemical homeostasis (Figure 1). In addition to acting as a modulator of cellular energy, a number of NAD^+ metabolites, including NAADP and cyclic ADP-ribose, are potent mobilisers of neuron and glial intracellular Ca^{2+} stores.[1,2] NAADP mediated Ca^{2+} release has been implicated in important neuronal functions, such as neurosecretion and extracellular communication.[3,4] Through cyclic ADP-ribose mediated release of intracellular Ca^{2+} a novel role for NAD^+ as a neurotransmitter has also been suggested.[5] Cyclic ADP-ribose has further been shown to mediate signalling pathways that are essential for inducing long-term depression of synaptic strength, an important method of memory storage.[6]

Poly (ADP-ribose) polymerase-1 (PARP-1), a nuclear enzyme central for the repair of DNA damage, likewise requires NAD^+ as a substrate. Apart from its role in repairing DNA damage, within the brain PARP also plays important roles in cell death,[7] transcription,[8,9] regulation of astrocyte and microglial function,[9,10] aging and perhaps even long term memory.[11,12] Another NAD^+ dependent enzyme, mono-ADP-ribosyltransferase, has recently been shown to be expressed by astrocytes and to be involved in astrocyte death by an NAD^+ induced, P2X7-mediated mechanism.[13]

Figure 1: The Role of NAD^+ in the CNS



Abbreviations used: **ADP**: Adenosine diphosphate; **ADPr**: ADP-Ribose; **ADPR cyclases**: ADP-ribosyl cyclase; **ARPP**: 4-amino-8-(β-D-ribofuranosylamino)-pyrimido-[5,4-d]-pyrimidine; **ATP**: Adenosine-5'-triphosphate; **MARTs**: Mono-ADP-ribosyltransferases; **Na**: Nicotinic acid; **NaAD**: Nicotinic acid adenine dinucleotide; **NAADP**: Nicotinic Acid Adenine Dinucleotide Phosphate; **NAD^+**: Nicotinamide adenine dinucleotide; **NADH**: Nicotinamide adenine dinucleotide (reduced form); **$NADP^+$**: Nicotinamide adenine dinucleotide phosphate; **NADPH**: Nicotinamide adenine dinucleotide phosphate (reduced form); **NADS**: Nicotinamide Adenine Dinucleotide Synthetase; **Nam**: Nicotinamide; **NaMN**: Nicotinic acid mononucleotide; **Nampt**: Nicotinamide phosphoribosyltransferase; **NaPRT**: Nicotinic acid phosphoribosyltransferase; **NMN**: Nicotinamide mononucleotide; **NMNAT-1,2,3**: Nicotinamide mononucleotide adenylyltransferase; **PARPs**: Poly-ADPribose polymerase; **PRPP**: 5-Phosphoribosyl-1-Pyrophosphate; **QPRT**: Quinolinic acid phosphoribosyltransferase; **Quin**: Quinolinic acid; **R5-P**: Ribose 5-phosphate; **RPPK**: Ribose phosphate pyrophosphokinase; **SIRTs**: Sirtuins.

Silent information regulator proteins (SIRT) are a group of seven histone deacetylases whose activities are dependent on and regulated by NAD^+. They suppress genome-wide transcription, yet upregulate various proteins related to energy metabolism and prosurvival mechanisms, and therefore play a key role in longevity. Within the brain SIRT1 is expressed in the hypothalamus, hippocampus, striatum and cortex where it has a number of direct neuroprotective roles.[14] SIRT1 mediated deacetylation results in the generation of O-acetyl-ADP-ribose, a metabolite involved in the formation of silent chromatin, important for maintenance of gene expression patterns and genomic stability.[15]

Taking into account the central role of NAD^+ in key CNS processes, it is not surprising that alteration in NAD^+/NADH dependant biochemistry has been associated with the pathogenesis of a number of neurodegenerative diseases, including Alzheimer's and Parkinson's disease.[16,17] Treatment with intranasal NAD^+ has also been shown to prevent neuronal death induced by traumatic brain injury and stabilised oral NADH administration has been shown to have a beneficial effect on cognitive function in Alzheimer's disease.[18,19] While the physiologic and pathologic importance of NAD^+/NADH dependant mechanisms' in both the CNS and periphery has become increasingly apparent, it is not yet known whether a relationship exists between peripheral and central stores of NAD^+.

Aim

The aim of this current study was to assess the relationship between plasma and cerebrospinal fluid (CSF) levels of total NAD(H) in matched samples from healthy participants across a wide age range.

Methods

Participants

Male and female participants scheduled for either a caesarean section or total hip/knee replacement and who required a spinal for the administration of anaesthetic, were recruited at the Sydney Adventist Hospital.

Exclusion Criteria

Participants were excluded from the cohort if they had a confirmed diagnosis of a) cancer b) cardiovascular disease c) diabetes d) systemic inflammatory condition e) viral infection or f) neurological/neurodegenerative disorder.

Of 62 participants recruited into the study, 12 were excluded on the basis of adverse pathology findings. In total 50 CSF samples and 28 matched blood samples were collected from consenting participants considered in general good health.

Sample Collection

Fasting blood and CSF samples were collected by an accredited anaesthetist no longer than 30 minutes apart and, following centrifugation, stored within 1 hour at -196ºC until analysis (figure 2).

Figure 2: CSF collection.

CSF was collected by anaesthetists from generally healthy participants who required a spinal as part of routine care.

NAD(H) Measurement

Total NAD(H) concentrations in plasma and CSF samples were measured spectrophotometrically using a thiazolyl blue microcycling assay established by Bernofsky and Swan,[20] and adapted to a 96 well plate format by Grant and Kapoor .[21] In brief, 125µl of the reaction mixture containing 120mM bicine (pH 7.8), 0.5mM MTT, 2mM PMS, 0.6M ethanol and alcohol dehydrogenase (300 units/ml) was added to either 6µl of plasma or 20 µl of CSF. Following a 10 minute incubation at 37˚C, the concentration of total NAD(H) was measured, using a Model 680XR microplate reader (BioRad, Hercules), as the change in absorbance at 570 nm.

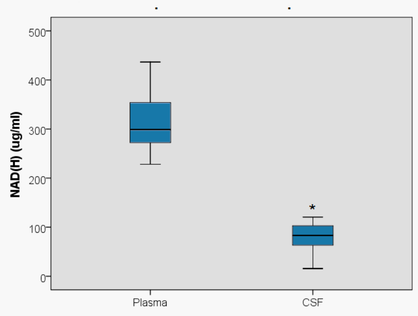
Statistical Analysis

Normality tests were performed for all continuous variables. When the normality of the variables was not confirmed the Spearman's correlation coefficient, Wilcoxon signed ranks test or Mann-Whitney U test was used. Means are presented with 95% confidence intervals. The significance limit was set at 0.05. Statistical analyses were performed using SPSS 16.0 for Windows.

Results

The mean level of total NAD(H) was significantly lower in CSF (81.3 mg/ml, 95% CI: 74.4 to 88.1, n=50) compared to plasma (314.1 mg/ml, 95% CI: 293.5 to 334.6, n=28) (p=0.000) (figure 3).

Figure 3: Mean NAD(H) in matched plasma & CSF samples

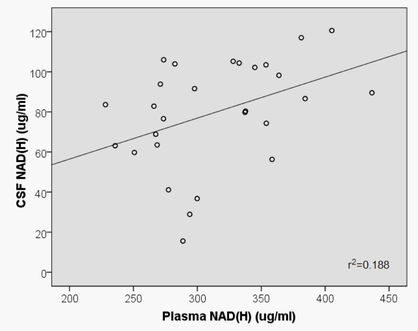


*p=0.000

Results Cont.

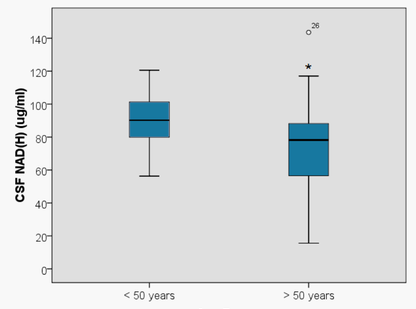
The concentration of total NAD(H) in plasma positively correlated with the concentration of total NAD(H) in CSF (r^2=0.188, p=0.05, n=29) (figure 4)

Figure 4: NAD(H) in plasma vs. CSF



As previously demonstrated by our lab in both blood and tissue samples,[22] CSF total NAD(H) was significantly lower in the older population (>50 years) compared to those is the younger population, less than 50 years (n=50, p=0.04) (figure 5).

Figure 5: CSF NAD(H) decreases with age



*p=0.05

Conclusion

While the physiologic and pathologic importance of NAD^+/NADH dependant mechanisms' in both the CNS and periphery has become increasingly apparent, it was not previously known whether a relationship exists between peripheral and central stores of NAD(H). This study is the first to show a positive correlation between matched CSF and serum NAD(H) levels in a healthy human population. Interestingly, NAD(H) levels were markedly lower in the CSF compared to the blood. This study also observed for the first time that, on average, total NAD(H) levels decline significantly in the CSF with age.

Acknowledgments

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