Increasing NAD+ availability in skeletal muscle to augment energy metabolism

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Background

NAD+, an essential coenzyme in energy production, has recently risen to prominence as a signalling molecule central in mediating cellular metabolism and mitochondrial function. NAD+ dependent protein deacetylase sirtuin (SIRT) proteins regulate key metabolic transcription factors, including FOXOs and PGC-1α in muscle in response to cellular energy demands and metabolic stress (1). Declining NAD+, metabolic and mitochondrial function are hallmark features of many pathophysiological processes such as ageing and type 2 diabetes (2). Thus, boosting NAD+ availability may have beneficial and therapeutic potential. NAD+ consumption (e.g. SIRTs) requires its re-synthesis through precursor salvage to maintain appropriate levels.

Here we identify important NAD+ salvage pathways in skeletal muscle that could be utilised to ‘boost’ NAD+ levels to support energy homeostasis during metabolic decline and stress (Fig. 1). Furthermore, we test the potential of salvageable NAD+ precursors to modulate skeletal muscle mitochondrial function.

Results

NMRR2 as a regulator of muscle NAD+ salvage and energy metabolism

Figure 1. The skeletal muscle pathways to NAD+. Nicotinamide (NAM) and Nicotinamide Riboside (NR) are NAD+ precursors salvaged by enzymes NAMPT and NMRR2 respectively and metabolised to Nicotinamide Mononucleotide (NNM), which is then converted to NAD+ via NMMAT.

Figure 4 A. 24h NR treatment of C2C12 myotubes significantly increases cellular NAD+ levels. B. NAD+ levels are lower in Ninm2 KD mouse primary myotubes compared to WT. NR treatment increases NAD+ content in both WT and KO myotubes. NAMPT inhibitor FR866 significantly decreases NAD+ levels by >50%. NAD+ can be recovered with NR and to a greater degree than NAM (C). D. The NAD+/NADH ratio appears to shift towards NAD+ in Ninm2 KO mice. E. Immunoblot shows even expression of key mitochondrial complexes in myotubes from WT and Ninm2 KO +/- NR supplementation. F. Using the Seahorse XF analyser mito stress kit, the maximal mitochondrial oxygen consumption rate (OCR) is unchanged in WT and Ninm2 KO primary myotubes but enhanced in both following NR treatment (0.5mM).

Experimental design

- Identify the NAD+ biosynthesis genes in skeletal muscle
- Use C2C12 muscle cells as an in vitro model for manipulation of cellular NAD+ content (e.g. Precursor supplementation, enzyme inhibition)
- Use Ninm2 KO muscle to characterise phenotype, harvest muscle tissue and isolate satellite cells for culture of primary myotubes (Fig.2).

Figure 2. Primary myotubes growth from isolated satellite cells (yellow arrow). Satellite cells migrate from myofiber (white arrow) (A). Satellite cell derived myoblasts proliferate (C) and differentiate into fused myotubes (D).

Conclusions

NAD+ is modulated by NMRR2 and NR

- Skeletal muscle relies on a limited set of salvage enzymes for NAD+ biosynthesis of which Ninm2 is the most highly expressed - in a fibre type enriched manner.
- Across species Ninm2 expression is switched on during skeletal muscle differentiation/development.
- Ninm2 can mediate NAD+ availability (lower NAD+ in Ninm2 KO mice) and NR can rescue NAD+ depletion in skeletal muscle when both NAMPT and Ninm2 mediated salvage is blocked suggesting an alternative route to NAD+.
- NR supplementation to increase NAD+ content may provide a new therapeutic approach to treat age-related sarcopenia.

Figure 3 (A) Microarray of all NAD+ biosynthesis genes identifies Ninm2, NAMPT and NMMAT1 as the only genes expressed in skeletal muscle with Ninm2 most predominant. (B) mRNA and (C) protein analysis of Ninm2 and NAMPT in metabolic mouse tissue shows NAMPT is ubiquitously expressed and Ninm2 is muscle specific. Ninm2 expression in slow-twitch soleus and fast-twitch tibial anterior muscle demonstrates fast-twitch fibre type enrichment. (D) During C2C12 differentiation and (E) zebrafish embryo development Ninm2 expression is switched on at time of muscle development.

Figure 5. Skeletal muscle specific pathways to NAD+ and metabolic targets of our research. Our research interventions (RED) shows NR supplementation can increase cellular NAD+ and, although basal NAD+ levels are lower in Ninm2 KO, NR can still increase NAD+ independent of Ninm2. Future areas of interest for interventions and research are indicated by dashed lines.

References


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Diabetes (to include obesity, pathophysiology & epidemiology)

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