Increasing NAD⁺ availability in skeletal muscle to augment energy metabolism

Rachel Fletcher, Craig Doig, Agnieszka Zielinska, Aliesha Griffin, Andrew Philp & Gareth Lavery.
Centre for Endocrinology, Diabetes and Metabolism, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

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 patho-physiological 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.

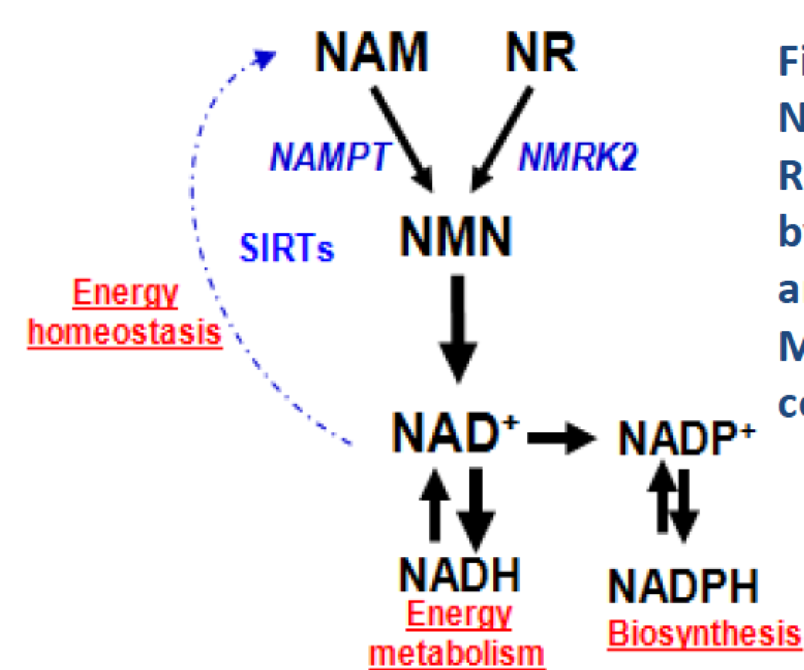


Figure 1. The skeletal muscle pathways to NAD+. Nicotinamide (NAM) and Nicotinamide Riboside (NR) are NAD+ precursors salvaged by enzymes NAMPT and NMRK2 respectively and metabolised to Nicotinamide Mononucleotide (NMN), which is then converted to NAD+ via NMNAT.

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 Nmrk2 KO muscle to characterise phenotype, harvest muscle tissue and isolate satellite cells for culture of primary myotubes (Fig.2).

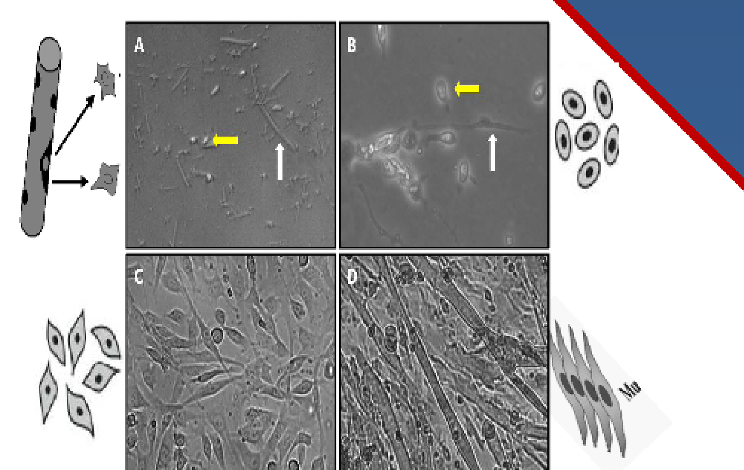


Figure 2. Primary myotubes growth from isolated satellite cells (yellow arrow). Satellite cells migrate from myofiber (white arrow) (A,B). Satellite cell derived myoblasts proliferate(C) and differentiate into fused myotubes (D).

Results

Characterisation of mouse muscle NAD+ salvage pathways

Nmrk2 and Nampt are the major NAD+ skeletal muscle biosynthesis pathways

3.A. B.

Nmrk2 is switched on during muscle development

C. D. E.

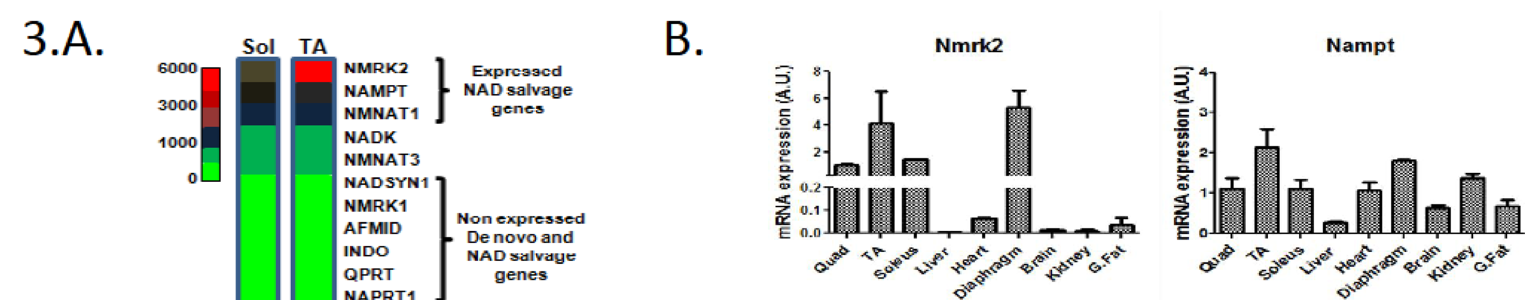
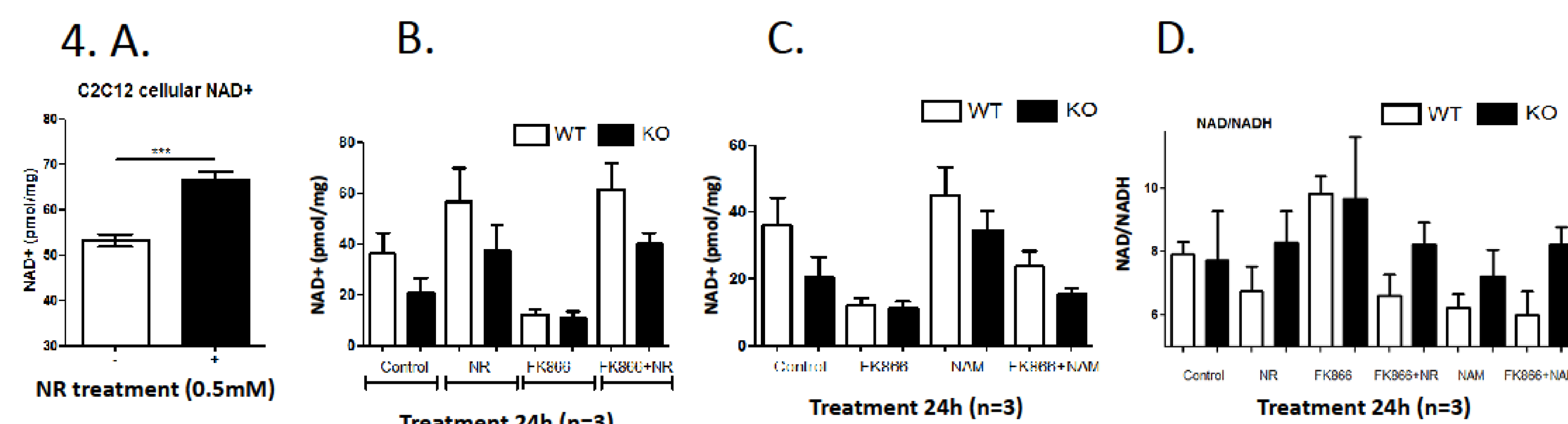


Figure 3
(A) Microarray of all NAD+ biosynthesis genes identifies NMRK2, NAMPT and NMNAT1 as the only genes expressed in skeletal muscle with NMRK2 most predominant.
(B) mRNA and (C) protein analysis of NMRK2 and NAMPT in metabolic mouse tissue shows NAMPT is ubiquitously expressed and NMRK2 is muscle specific. NMRK2 expression in slow-twitch soleus and fast-twitch tibialis anterior muscle demonstrates fast –twitch fibre type enrichment.
(D) During C2C12 differentiation and (E) zebrafish embryo development NMRK2 expression is switched on at time of muscle development.

Results

NMRK2 as a regulator of muscle NAD+ salvage and energy metabolism

Nmrk2 can mediate basal NAD+ levels in skeletal muscle



NR can enhance maximal mitochondrial respiration independent of mitochondrial content

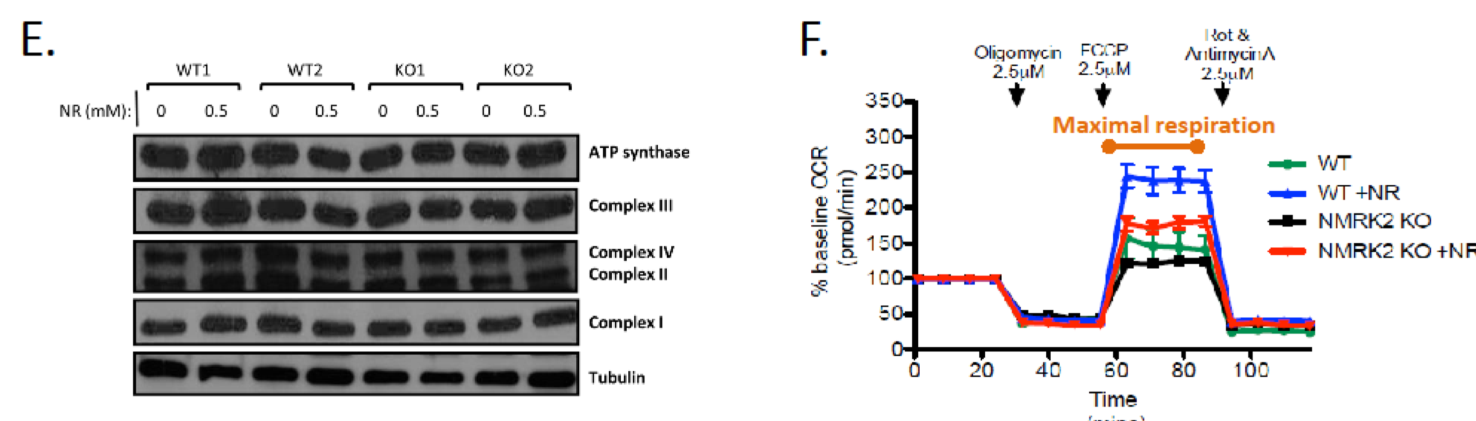


Figure 4
A. 24h NR treatment of C2C12 myotubes significantly increases cellular NAD+ levels.
B. NAD+ levels are lower in Nmrk2 KO mouse primary myotubes compared to WT. NR treatment increases NAD content in both WT and KO myotubes. NAMPT inhibitor FK866 significantly decreases NAD+ levels by >50%. NAD+ can be recovered with NR and to a greater degree than NAM **[C]**.
D. The NAD/NADH ratio appears to shift towards NAD+ in Nmrk2 KO mice.
E. Immunoblot shows even expression of key mitochondrial complexes in myotubes from WT and NMRK2 KO +/- NR supplementation.
F. Using the Seahorse XF analyser mito stress kit, the maximal mitochondrial oxygen consumption rate (OCR) is unchanged in WT and NMRK2 KO primary myotubes but enhanced in both following NR treatment (0.5mM).

Conclusions

NAD+ is modulated by NMRK2 and NR

- Skeletal muscle relies on a limited set of salvage enzymes for NAD⁺ biosynthesis of which Nmrk2 is the most highly expressed - in a fibre type enriched manner.
- Across species Nmrk2 expression is switched on during skeletal muscle differentiation/development .
- NMRK2 can mediate NAD+ availability (lower NAD+ in NMRK2 KO mice) and NR can rescue NAD+ depletion in skeletal muscle when both NAMPT and NMRK2 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.

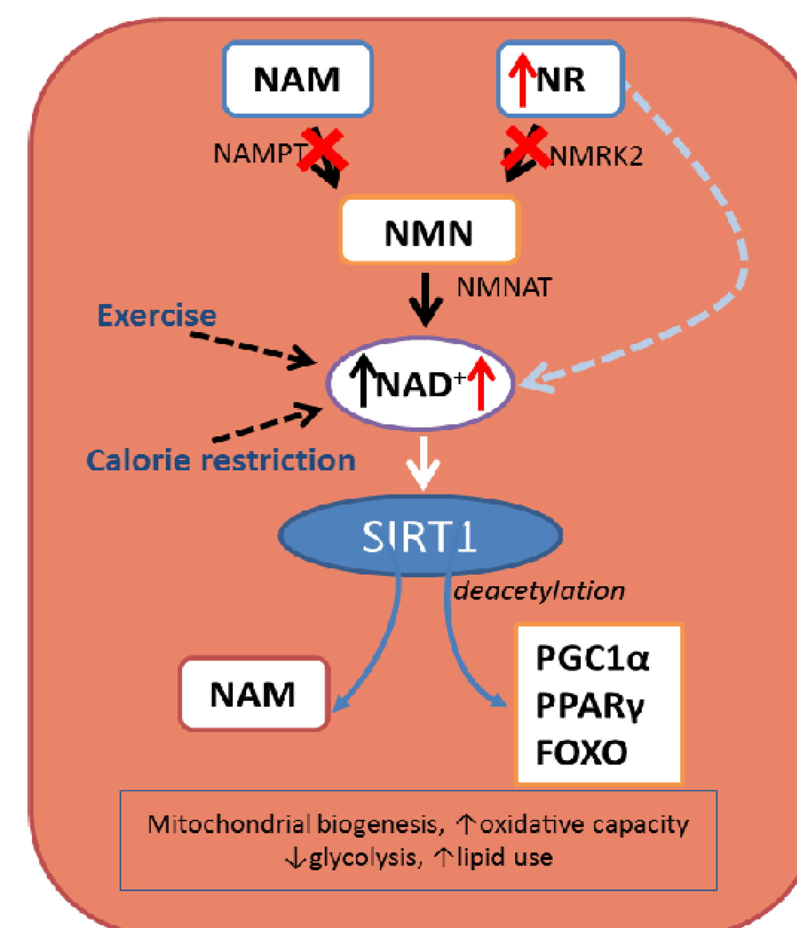


Figure 6. Skeletal muscle specific pathways to NAD+ and metabolic targets of our research. Our research interventions (RED) shows NR supplementation can increase cellular NAD+ and, although basal NAD+ levels are lower in NMRK2 KO, NR can still increase NAD+ independent of NMRK2. Future areas of interest for interventions and research are indicated by dashed lines.

References 1.White, A.T. and S. Schenk, *NAD+/NADH and skeletal muscle mitochondrial adaptations to exercise.* American Journal of Physiology - Endocrinology and Metabolism, 2012. 303(3): p. E308-E321. 2.Laurent Mouchiroud, et al., *The NAD+/Sirtuin Pathway Modulates Longevity through Activation of Mitochondrial UPR and FOXO Signaling.* Cell, 2013. 154(2): p. 430-441.

17 ECE 389--EP
Diabetes (to include obesity, pathophysiology & epidemiology)
Rachel Fletcher
DOI: 10.3252/pso.eu.17ece.2015
Poster presented at: ECE2015 16-20 May 2015 Dublin, Ireland

PosterSessionOnline.com