The NAD^+ precursors, nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN), regulate exercise capacity in aged mice in a SIRT1-dependent manner

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Improving mitochondrial oxidative metabolism is thought to be an effective approach for treating diseases such as obesity, type 2 diabetes, cardiovascular diseases and muscular disorders. A number of recent studies have demonstrated that raising NAD^+ levels by either facilitating its biosynthesis or by deleting NAD^+ consuming enzymes (*e.g.* PARP or CD38) results in improved mitochondrial function and protection against metabolic disease. These effects have been proposed to be mediated by NAD^+-dependent activation of the sirtuin family of deacylase enzymes, however the precise role of specific sirtuins (*i.e.* SIRT1-7) in these processes is not completely understood. SIRT1 is thought to have a predominant role in this process, as it has been shown to deacylate and activate PGC-1α – a master regulator of mitochondrial biogenesis, fatty acid oxidation, glucose utilization as well as fibre-type determination.

In this study, our aim was to investigate the role of SIRT1 in driving the beneficial effects of increasing cellular NAD^+ levels using *in vitro* and *in vivo* models. Firstly, we treated HEK293 cells with NR or NMN (1 mM) for 24 hours and then measured NAD^+ levels. Both NR and NMN increased NAD^+ levels significantly (*e.g.* 2.65 ± 0.58, 4.78 ± 0.72, 5.16 ± 0.4 ρmoles NAD^+/μg of protein for untreated, NR-treated and NMN-treated cells, respectively, n=3). The expression of proteins in the electron-transport chain complexes were also analysed and there was a marked increase in the content of subunits of complexes I, II, III and V with both NR and NMN treatment. Interestingly, co-treatment with EX527 (1 μM), a potent SIRT1 inhibitor, largely abrogated these effects, suggesting that increase in cellular NAD^+ levels and consequent activation of SIRT1 is critical for the improvement of mitochondrial oxidative metabolism. To further investigate these findings *in vivo*, we used a tamoxifen-inducible system in 18-months old animals to generate whole-body SIRT1 knockout mice (iT1KO). Mice were then administered with either vehicle or NR or NMN (500 mg/kg body weight) in drinking water for 10 weeks. At this stage, deletion of SIRT1 and treatment of mice with NAD^+ precursors had only minor impact on body composition, glucose tolerance and insulin tolerance. However, in a motorized treadmill test to exhaustion (incremental variable speed ranging from at 5 to 20 meter/minute), both NR and NMN substantially improved endurance capacity (1019.9 ± 195.8 metres or 817.2 ± 177.6 metres distance run in NMN or NR-treated WT mice, respectively *vs* 463.7 ± 89.3 meters in untreated WT mice, n=4-7). Surprisingly, under vehicle conditions and in the presence of NR and NMN, SIRT-1 KO mice displayed approximately 1.5 to 3-fold increase in distance run. We are currently carrying out physiological, morphological and biochemical analysis of heart, muscle and muscle microvasculature that will provide better understanding of these observations. Overall, our results indicate that increasing NAD^+ biosynthesis in old mice enhances exercise capacity, but it remains to be investigated how the absence of SIRT1-PGC-1α axis leads to enhanced endurance in these aged animals.