High dose NAD+ boosting as pan-sirtuin activation increases atherosclerotic plaques and systemic inflammation in Apoe knockout mice

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Background: NAD+ (nicotinamide adenine diphosphate) is a cosubstrate of sirtuins (SIRT) that are activated upon caloric restriction. The nuclear SIRT1 and SIRT6 exert protective effects on atherothrombosis through immunometabolic pathways. By supplementing its precursors, an increase in the NAD+ pool has been reported to extend life span in mice. However, supplementing NAD+ precursors may also stimulate inflammation depending on the context. Nicotinamide riboside (NR) is a precursor of NAD+, whose supplementation has been used to combat metabolic syndromes and liver steatosis. Notably, the effects of pan-sirtuin activation using NR on atherosclerosis remain unknown.

Purpose: We hypothesized that NR supplementation confers atheroprotection by activating the NAD+ sirtuin axis. We aimed to test this hypothesis by investigating the effects of NR supplementation on plaque formation using an atherosclerosis-prone mouse model.

Methods: 8-week-old male apolipoprotein E (ApoE) knockout (KO) mice were fed for 12 weeks a high-cholesterol diet (HCD, 1.25 wt%) supplemented with three NR doses: 0, 1.2, and 2.4 g/kg diet. The groups were designated as NR-, NR+, and NR++, respectively (n=14/group). After overnight fasting, aortae, liver, and blood were collected. Cytokines were analyzed using a multiplex ELISA. Triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C) levels were determined by colorimetry and the Friedewald equation. Cross sections of the aortic root were stained with Oil-Red O.

Results: High-dose (HD) NR supplementation increased plaque thickness in NR++ ApoE KO mice, compared with NR+ mice (Figure 1A and B). In parallel, plasma expression levels of tumor necrosis factor (TNF)-α and interleukin (IL)-6 as well as TG and LDL-C levels were increased in NR++ mice (Figure 1C-F) suggesting that NR supplementation induced systemic inflammation and disturbed lipid metabolism. Analyses of liver lysates showed that expression levels of SIRT1 were decreased and CD38 increased in NR++ mice. Full length PARP1 remained unchanged, while both cleaved PARP1 and total PARylation in liver decreased. NAD+ concentrations in liver and plasma remained unchanged. These data suggest that an increase in hepatic CD38 might consume more NAD+ in the atherosclerotic environment and could be the culprits for worsening atherosclerotic progression and systemic inflammation (Figure 2).

Conclusions: HD NR supplementation increased plaque thickness, as well as IL-6 and LDL-C plasma levels, moderate NR dose had neutral effects. Elevated hepatic expression levels of CD38 and decreased SIRT1 may at least in part explain the atherosclerotic phenotype. Thus, HD NR supplementation in mice does not decrease but worsen atherosclerosis and induce systemic inflammation. A cautious approach should be considered when applying NAD+ boosting in patients with atherosclerotic disease.
Figure 1. HD NR supplementation increased plaque thickness and plasma levels of pro-inflammatory cytokines and lipids. (A) Cross sections of aortic roots and (B) plaque quantification. Scale bar= 200 μm. (C) TNF-α and (D) IL-6 concentration (E) TG and (F) LDL-C concentration in plasma. Data for each mouse are shown as mean ± SD. Significance is determined by one-way ANOVA with Tukey's test or non-parametric Kruskal-Wallis test with Dunn's test.
Figure 2. Graphical summary of the potential mechanism by which HD NR supplementation enhances systemic inflammation and atherosclerosis.