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Comment on "Nicotinamide mononucleotide increases muscle insulin sensitivity in prediabetic women"

Charles Brenner

Department of Diabetes and Cancer Metabolism, City of Hope National Medical Center, Duarte, CA 91010, USA. Email: cbrenner@coh.org

Yoshino *et al.* (Reports, 11 June 2021, p. 1224) have reported that nicotinamide mononucleotide (NMN) increases muscle insulin sensitivity in prediabetic women. However, the 13 women who received NMN had hepatic lipid content of 6.3 ± 1.2%, whereas the 12 in the placebo group had 14.8 ± 2.0% (P = 0.003). Given that a target of NMN is liver fat clearance, this was not an effectively randomized trial.

Four nicotinamide adenine dinucleotide (NAD^+) coenzymes are the central catalysts of metabolism (*1*). In a mouse model of diabesity, hepatic liver NAD phosphate ($NADP^+$) and reduced $NADP^+$ (NADPH) were strikingly depressed and the hepatic NAD^+ metabolome was rescued by oral provision of nicotinamide riboside (NR) in a manner that lowered hepatic steatosis and circulation of liver enzymes, protected against weight gain, improved hyperglycemia, and limited diabetic polyneuropathy (*2*). Because all of these metabolic benefits are linked, it was not obvious from this mouse study what was the primary driver of improved metabolic health, and what were the benefits downstream of the primary driver. However, the safety and availability of NR as a nutritional supplement (*3*, *4*) have driven dozens of groups worldwide to test potential activities of NR in diseases and conditions of metabolic stress.

Dollerup and co-workers tested the hypothesis that NR would promote weight loss and insulin sensitivity in a 12-week trial of older obese men. They found excellent safety in this population but failed to show benefit with respect to primary outcomes (*5*). In clinical trials, specification of primary outcomes is essential because if one randomizes with respect to a single criterion, one cannot assure random assortment of other parameters (*6*). This was unfortunate in the men's trial because NR appeared potentially to have activity at lowering hepatic lipids, which were decreased from 11.3% to 9.3% in the supplemented group, versus a decrease from 14.1% to 13.9% on placebo (n = 20). Despite the factor of 10 effect size, the P value was 0.13, potentially because the groups were not randomized for hepatic lipids.

Review literature (*7*) has highlighted the need to randomize for the factors to which people appear to respond in NAD^+-boosting trials—namely hepatic lipids (*5*), body composition (*8*), and inflammatory cytokines (*9*)—and to use increased physical activity as the standard of care. According to this view, the primary site of action of NR is lipid mobilization in the liver; once this occurs, and potentially aided by exercise and the anti-inflammatory activities of NR, other effects that have been observed in rodents (such as insulin sensitization) may follow (*7*).

Nicotinamide mononucleotide (NMN) is a biosynthetic intermediate of the four NAD coenzymes. Just as pantothenate is a vitamin precursor of coenzyme A, and coenzyme A is broken down to pantothenate to be salvaged by cells to make more coenzyme A, the source of compounds such as NMN and NR in the diet is not principally NMN or NR, as Yoshino *et al.* (*10*) recently claimed. Rather, the four NAD^+ coenzymes are found in all cellular stuff and are broken down to NMN, NR, nicotinamide, and nicotinic acid (*1*). NR is the largest fragment of NAD^+ that can enter cells to be salvaged; NMN is dephosphorylated extracellularly to NR and depends on the NR kinase pathway for its biosynthesis into NAD coenzymes (*11*). However, it is possible that NMN delivers NR or nicotinamide to particular tissues better than NR, and NMN has been investigated as a NAD^+-boosting supplement in mice and humans.

In mice on a high-fat diet (HFD), the Imai group reported no significant change in the skeletal muscle NAD system. However, they showed that liver NAD^+ is depressed by HFD and restored by intraperitoneal NMN administration. This was accompanied by improved glucose tolerance in female mice (*12*).

In their clinical trial (NCT 03151239) of prediabetic women, Yoshino *et al.* defined change in muscle insulin sensitivity as the primary outcome, and change in liver insulin sensitivity and change in hepatic lipid content as the first and sixth of 12 secondary outcomes. They randomized on body mass index and reported the initial and ending values of 18 metabolic parameters. As shown in table 1 of (*10*), the groups were profoundly different with respect to hepatic lipids: Those that received placebo had 2.35 times

the level of liver fat as those assigned to NMN (P = 0.003). This between-group, treatment-unrelated difference was greater in effect size and in statistical significance than any physiological effect of NMN in the study.

After 10 weeks of 0.25 g of NMN per day, insulin-stimulated glucose disposal was improved by 25 ± 7% (P < 0.01). Hepatic insulin sensitivity was not improved by NMN. Because the improvement in muscle insulin sensitivity was seen in the NMN group but not the placebo group, the authors scored the study as positive for the primary endpoint (*10*). However, it is foundational for clinical conclusions that randomization compares an intervention to placebo in people of equal metabolic health.

Given that the Imai group established that the primary site of NMN action is the liver (*12*)—preclinical work that is consistent with mouse (*2*) and human (*5*) work on NR—and the Klein group established that fatty liver depresses muscle insulin sensitivity in people (*13*), the unfortunate assortment of much more fatty liver to the placebo group suggests that one cannot reasonably expect that the results reported (*10*) would be reproduced in an effectively randomized trial.

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