Response to Comment on “Nicotinamide mononucleotide increases muscle insulin sensitivity in prediabetic women”

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In evaluating any randomized clinical trial, it is important to determine whether baseline differences between groups could have affected the primary outcome. In our study, muscle insulin sensitivity, which was identical in both groups at baseline, improved after nicotinamide mononucleotide (NMN), not placebo, therapy. Differences in baseline intrahepatic triglyceride content between groups do not negate the effects of NMN observed in muscle.

We appreciate Brenner's Comment (1) and the opportunity to respond. Brenner suggests that the difference in baseline intrahepatic triglyceride (IHTG) content between the placebo and nicotinamide mononucleotide (NMN) treatment groups in our study (2) invalidates our conclusion that NMN administration increased skeletal muscle insulin sensitivity. In randomized clinical trials (RCTs) conducted in people, it is not unusual that there will be differences in some baseline variables between study groups. Therefore, it is important to identify the primary outcome of a study that was established a priori and whether post hoc analyses of other factors should affect the predefined assessment of the data.

As we reported in ClinicalTrials.gov (NCT 03151239) and in the supplementary materials, the primary predefined outcome of our study was the change in skeletal muscle insulin sensitivity, assessed as the rate of glucose disposal [expressed as µmol (kg fat-free mass)−1 min−1] measured by using the hyperinsulinemic-euglycemic clamp procedure in conjunction with stable isotopically labeled glucose tracer infusion. A reduction in IHTG content was a secondary outcome of our study. As is typical in RCTs, secondary outcomes are hypothesis-generating, not hypothesis-confirming. Our primary outcome was also used to determine the number of subjects needed to detect statistically significant (and physiologically significant) differences between groups with a two-tailed α value of 0.05 and a β value of 0.9. The baseline (before placebo or NMN treatment) measure of skeletal muscle insulin sensitivity, assessed as either the absolute rate of glucose disposal during insulin infusion or the percentage increase from basal in the rate of glucose disposal during insulin infusion, was nearly identical in the placebo and NMN groups.

We did not randomize participants on the basis of body mass index (BMI), as stated in Brenner's Comment. Participants were randomized to treatment with either placebo or NMN after determining their eligibility, based on a comprehensive medical evaluation that included a history and physical examination, blood tests, and an oral glucose tolerance test. The inclusion criteria were as follows: (i) postmenopausal women; (ii) age 55 to 75 years; (iii) BMI 25.0 to 44.9 kg/m²; and (iv) prediabetes based on criteria proposed by the American Diabetes Association (3). A measurement of IHTG content was not used to determine eligibility.

The difference in baseline IHTG content between groups should not have influenced the effect of NMN supplementation on skeletal muscle insulin sensitivity for several reasons. First, the absence of an effect of treatment with placebo, which was expected, does not negate the effects observed after treatment with NMN. Second, there is no physiological mechanism to support Brenner's belief that differences in IHTG content between groups would influence our assessments of skeletal muscle metabolism. Brenner misinterpreted the findings from one of our previous studies (4) and concluded that the data from that study “established that fatty liver depresses muscle insulin sensitivity in people.” That study and studies from other groups (5) show that hepatic steatosis is associated with muscle insulin resistance, but should not be misconstrued to mean that hepatic steatosis causes insulin resistance. It is more likely that systemic insulin resistance and its associated increases in plasma free fatty acid, glucose, and insulin concentrations are involved in the pathogenesis of hepatic steatosis in people with obesity, rather than that hepatic steatosis causes muscle insulin resistance (6, 7).
fact, people with hepatic steatosis caused by hypobetalipoproteinemia, a genetic defect in the ability to export very-low-density lipoproteins out of the liver, are not insulin-resistant (8, 9). Third, we reevaluated our data by matching subsets of participants in the NMN and placebo groups who had high IHTG content and found that this did not change the differences we observed in muscle insulin sensitivity between groups. As shown in Fig. 1, there is an increase in the glucose disposal rate during insulin infusion after treatment with NMN but not placebo, even when the participants in the two groups are matched on high IHTG content (baseline IHTG content = 12.3 ± 0.3% in the placebo group and 12.1 ± 0.6% in the NMN group). In addition, we did not detect a significant correlation between baseline IHTG content and insulin sensitivity [assessed as glucose disposal rate during insulin infusion (R² = 0.092, P = 0.15)], which suggests that IHTG was not directly associated with the primary outcome measure. Although NMN treatment improved skeletal muscle insulin sensitivity in our participants, it did not affect insulin sensitivity in the liver, nor did it alter basal plasma free fatty acid, glucose, and insulin concentrations. These findings suggest that an improvement in insulin sensitivity in skeletal muscle alone, without an improvement in systemic insulin action that affects plasma substrate and hormone concentrations, does not induce a decrease in IHTG content.

We believe the interpretation of the data from our study is appropriate and represents a valid assessment of the findings. Moreover, the effect of NMN that we observed on in vivo skeletal muscle insulin sensitivity is supported by the increase in skeletal muscle cellular markers of insulin signaling during insulin infusion in the NMN, but not the placebo, group. Nonetheless, it is important that the findings from any one RCT are confirmed by additional studies. We hope Brenner and others will conduct additional clinical studies to further evaluate the effect of NMN on metabolic function. These studies are critically needed to advance our understanding of the effects of NMN supplementation in people.

REFERENCES


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