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ORIGINAL

# Safety and efficacy of long-term nicotinamide mononucleotide supplementation on metabolism, sleep, and nicotinamide adenine dinucleotide biosynthesis in healthy, middle-aged Japanese men

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**Abstract.** Obesity and aging are major risk factors for several life-threatening diseases. Accumulating evidence from both rodents and humans suggests that the levels of nicotinamide adenine dinucleotide ($NAD^+$), a regulator of many biological processes, declines in multiple organs and tissues with aging and obesity. Administration of an $NAD^+$ intermediate, nicotinamide mononucleotide (NMN), replenishes intracellular $NAD^+$ levels and mitigates aging- and obesity-associated derangements in animal models. In this human clinical study, we aimed to investigate the safety and effects of 8-week oral administration of NMN on biochemical, metabolic, ophthalmologic, and sleep quality parameters as well as on chronological alterations in $NAD^+$ content in peripheral tissues. An 8-week, single-center, single-arm, open-label clinical trial was conducted. Eleven healthy, middle-aged Japanese men received two 125-mg NMN capsules once daily before breakfast. The 8-week NMN supplementation regimen was well-tolerated; $NAD^+$ levels in peripheral blood mononuclear cells increased over the course of NMN administration. In participants with insulin oversecretion after oral glucose loading, NMN modestly attenuated postprandial hyperinsulinemia, a risk factor for coronary artery disease ($n = 3$). In conclusion, NMN overall safely and effectively boosted $NAD^+$ biosynthesis in healthy, middle-aged Japanese men, showing its potential for alleviating postprandial hyperinsulinemia.

*Key words*: Nicotinamide mononucleotide, Nicotinamide adenine dinucleotide, Metabolism, Postprandial hyperinsulinemia

**OBESITY AND AGING** are two major risk factors for insulin resistance, which further increases the risk of other obesity- and age-associated diseases, including type 2 diabetes, hypertension, coronary heart disease, stroke, cancer, and Alzheimer's disease [1, 2]. Therefore, continuous efforts have been made to develop new therapeutics to ameliorate them.

Nicotinamide adenine dinucleotide ($NAD^+$) is a critical coenzyme in metabolic redox reactions, thereby regulating aging, cell growth, and inflammation [3-7]. $NAD^+$ is also an essential substrate for several $NAD^+$-consuming enzymes, including members of the sirtuin (Sirt) family, which play essential roles in metabolic homeostasis, DNA repair and stress resistance, circadian

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rhythms, and behavioral and cognitive functions [6, 8-13]. It has become a consensus that the systemic decrease in $NAD^+$ levels is a driving force for some age-associated pathophysiologies [5, 11, 14, 15]. $NAD^+$ levels decrease with aging and obesity in many tissues and organs in both rodents [16-25] and humans [22, 26-28], causing obesity- and aging-related complications, including postprandial hyperglycemia [16, 29, 30], insulin resistance [6, 31, 32], impaired energy metabolism [33], muscle atrophy [20], and impaired memory/cognition [34-36] in rodents. These findings provide a scientific rationale for augmenting $NAD^+$ biosynthesis as a preventive and therapeutic strategy for obese and/or elderly individuals.

Nicotinamide mononucleotide (NMN), an $NAD^+$ intermediate, is an endogenously biosynthesized metabolite detected in mouse plasma [29], red blood cells [37], and human breast milk [38]; it is present in small quantities in some common foods, including edamame, broccoli, cucumber, avocado, tomato, beef, and shrimp [39]. Oral or intraperitoneal NMN administration increases $NAD^+$ levels in major metabolic organs in rodent disease models, mitigating obesity- and aging-associated complications [12, 14, 40, 41]. For example, in obese or aged diabetic mice, intraperitoneal NMN supplementation restored $NAD^+$ concentrations in metabolic organs, improving insulin secretion and action [19]. We recently reported that oral NMN normalized intestinal epithelial $NAD^+$ levels in diet-induced obese mice, regulating glucagon-like peptide-1 (GLP-1) production and postprandial glucose metabolism [16]. Additionally, 12 months of oral NMN administration mitigated age-associated functional declines such as weight gain and insulin resistance, as well as reductions in physical activity, retinal function, and bone density in healthy aging mice [39].

Based on animal data, human clinical trials have been conducted to investigate the safety and efficacy of NMN. We previously conducted a first-in-human study to examine the safety of single oral NMN doses of 100, 250, or 500 mg in healthy, middle-aged men, which increased plasma levels of nicotinamide metabolites in a dose-dependent manner, including *N*-methyl-2-pyridone-5-carboxamide (2-PY) and *N*-methyl-4-pyridone-5-carboxamide (4-PY), without exerting any significant deleterious effects [42]. The levels of $NAD^+$ or $NAD^+$-related metabolites in whole blood, serum, or peripheral blood mononuclear cells (PBMCs) were increased by oral NMN administration at doses of 250–900 mg/d in obese, overweight, or healthy participants aged 20–65 years or >65 years [43-47]. A recent study demonstrated that NMN and $NAD^+$ plasma levels were elevated by 250 mg/d oral NMN supplementation [48]. No apparent side effects were noted at doses of up to 1,250 mg/d [49].

Oral NMN improves skeletal muscle insulin sensitivity in obese/overweight postmenopausal women with prediabetes [46]; alleviates arterial stiffness in healthy adults [50]; reduces the prevalence of frailty in older men with type 2 diabetes exhibiting reduced grip strength or walking speed [51]; improves gait speed, left grip, and lower limb function in healthy older men [47, 52]; increases walking distance and aerobic capacity in healthy adults [44, 53]; ameliorates sleep quality in adults with sleep disturbances [54]; and improves subjective general health and well-being assessments in healthy adults [44, 45]. In this study, we conducted a single-center, single-arm, open-label, 8-week trial to assess temporal changes in trough PBMC $NAD^+$ concentrations, safety, sleep quality, and glucose metabolism, particularly hyperinsulinemia—an early manifestation of insulin resistance [55]—in healthy, middle-aged Japanese men.

## Materials and Methods

### *Ethics approval and informed consent*

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Certified Review Board of Keio University (N20170210) on March 6, 2019. The study was registered at jRCT (jRCTs031180242) before participants were recruited (March 12, 2019). Each participant was given a full explanation of the trial and signed an informed consent form before screening. The study was conducted at the Clinical Trial Unit of Keio University School of Medicine, Japan.

### *Study design, participants, and intervention*

This was a single-center, single-arm, open-label trial. The follow-up duration was 12 weeks. Enrollment and follow-up were conducted between August 6, 2019, and October 26, 2021. Twenty-eight healthy adult Japanese male volunteers were recruited and assessed for eligibility. The inclusion criteria were healthy Japanese male volunteers 1) aged 40–60 years, 2) without a disease with an obvious medical diagnosis, and 3) who fully understood the purpose of the study, agreed to follow oral and written trial directions, and were willing to take placebo or NMN capsules.

The exclusion criteria were 1) a medical history of diagnosable disease, 2) malignancy, 3) severe infectious disease, 4) mental illness, 5) ophthalmological disease, 6) history of allergies, 7) any other medical condition that the principal investigator deemed inappropriate for study participation, and 8) abnormalities detected in screening tests.

Screening involved demographic data; medical history; and physical examinations, including systolic and diastolic blood pressure, pulse rate, respiration rate, body temperature, abdominal circumference, auscultation of heart and lungs, electrocardiography (ECG), and chest radiography. Ophthalmological examinations, including those for best-corrected visual acuity, functional visual acuity, intraocular pressure (IOP), tear function, standard tear film break-up time, central flicker frequency, corneal thickness, and fundus photography, were performed as described previously [42]. Blood and urine samples were collected for hematology, clinical chemistry, and urinalysis. Screening was performed 1–4 weeks before the study. Finally, 14 participants remained eligible after screening. They underwent examination at the 0- and 4-week visits during placebo supplementation, which was performed to mitigate both placebo and nocebo effects. After a 2-week washout period, all participants were allocated to 8-week supplementation with NMN. During this period, participants were examined before NMN supplementation and at 1, 2, 4, and 8 weeks. They received two oral capsules of placebo containing corn starch or two capsules of 125 mg NMN once daily before breakfast. Both NMN and placebo were manufactured and supplied by Oriental Yeast Company, Ltd. (Tokyo, Japan). During intervention, participants were instructed not to make lifestyle changes and to follow their habitual diets and daily living routines. They were allowed to take necessary medications for minor symptoms such as cold, back pain, and headache during the study. When any such medications were taken, the participants were instructed to inform the study group. Eventually, five participants dropped out and nine completed the study.

### *Primary and secondary outcomes*

The primary outcome was the safety of NMN at 250 mg/d for 8 weeks, evaluated with the following physical measurements: vital signs, ECG, chest radiography, Pittsburgh Sleep Quality Index (PSQI) [56], ophthalmic examinations, blood and urine tests, and subjective symptoms. The secondary outcome was chronological changes in PBMC $NAD^+$ trough levels during the intervention period. Glucose tolerance was assessed using 75-g oral glucose tolerance tests (OGTTs). Insulin resistance was measured using homeostatic model assessment of insulin resistance (HOMA-IR).

### *Evaluation of safety, tolerability, and adherence*

During each visit, participants were asked about any subjective adverse events, difficulties, or problems they had experienced since the previous visit. We requested that they immediately report any serious adverse events during the intervention period.

Safety assessments (Fig. 1) were performed at each visit. Objective side effects were recognized through abnormal laboratory values in blood and urine tests; changes in vital signs, body weight, and waist circumference; ECG; chest X-ray; ophthalmic examinations; glucose metabolism assessed using OGTT; and PSQI. In case of an adverse event, we implemented appropriate

| | Placebo period | | NMN period | | | | |
|---|---|---|---|---|---|---|---|
| | Week 0 (*n* = 13) | Week 4 (*n* = 13) | Week 0 (*n* = 12) | Week 1 (*n* = 11) | Week 2 (*n* = 11) | Week 4 (*n* = 11) | Week 8 (*n* = 9) |
| Physical measurement | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| Hematological test | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| Urine test | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| Electrocardiogram | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| Chest X-ray | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| PBMCs $NAD^+$ measurement | ■ | ■ | ■ | ■ | ■ | ■ | ■ |
| 75-g OGTT | ■ | ■ | ■ | | | | ■ |
| Ophthalmic examination | | | ■ | | | ■ | ■ |
| Sleep Questionnaire | ■ | ■ | ■ | | | ■ | ■ |

**Fig. 1** Study design

Physical measurements, hematological tests, urine tests, electrocardiogram, chest X-ray, and $NAD^+$ measurements in PBMCs were performed at 0 and 4 weeks of the placebo period and at 0, 1, 2, 4, and 8 weeks of the NMN period. The 75-g OGTTs were conducted at 0 and 4 weeks of the placebo period as well as at 0 and 8 weeks of the NMN period. Ophthalmic examinations were performed only during the NMN period (0, 4, and 8 weeks). Sleep questionnaires were administered before placebo intake and at 4 weeks of the placebo period, as well as before NMN intake and again at 4 and 8 weeks of the NMN period.

$NAD^+$, nicotinamide adenine dinucleotide; NMN, nicotinamide mononucleotide; PBMC, peripheral blood mononuclear cell; OGTT, oral glucose tolerance test

measures immediately and decided whether the participant should withdraw from the trial. We assessed possible causal relationship(s) between adverse events and NMN test capsule administration. Evaluations of adverse events, including causality and reasons why participants discontinued the trial, were reported in the Case Report Form. Participants were required to record both time and amount of capsule intake in a diary. Adherence was confirmed by pill counts at each visit.

### *Clinical tests*

Clinical laboratory tests were conducted at initial screening and at each visit during the intervention period (Fig. 1). Blood samples were collected from the forearm of each participant. Urine samples were collected at each visit. Hematological and biochemical parameters, including triglyceride, total cholesterol, HDL-cholesterol, glucose, insulin, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), amylase, cholinesterase, g-GTP, total protein, albumin, uric acid, urea nitrogen, creatinine, sodium, potassium, and chloride levels, were measured. Qualitative and quantitative urine tests for sugar, protein, urobilinogen, ketone bodies, pH, specific gravity, occult blood reaction, bilirubin, creatine, and microalbumin were performed.

An OGTT using 75 g of glucose was performed at the 0- and 4-week visits for placebo and the 0- and 8-week visits for NMN supplementation. Blood glucose and insulin levels were measured 0, 30, 60, and 120 min after oral glucose loading. Areas under the curve (AUCs) for glucose and insulin levels were calculated using a trapezoidal formula. Insulin resistance, as determined based on HOMA-IR, was calculated using the following formula: fasting glucose (mg/dL) × fasting insulin (μU/mL)/405; HOMA-β was calculated using the following formula: 360 × fasting insulin (μU/mL)/(fasting glucose [mg/dL] – 63); and the insulinogenic index, which represents β-cell function, was calculated using the following formula: (serum insulin [μU/mL] at 30 min following oral glucose load – fasting insulin [μU/mL])/(blood glucose [mg/dL] at 30 min following oral glucose load – fasting glucose [mg/dL]) [57]. The oral disposition index, which also describes β-cell function, was calculated using the formula: (serum insulin [μU/mL] at 30 min following glucose load – fasting insulin [μU/mL])/(blood glucose [mg/dL] at 30 min following glucose load – fasting glucose [mg/dL]) × 1/fasting insulin (μU/mL) [58].

### *$NAD^+$ assays*

PBMCs were isolated from blood samples obtained from the participants at each visit by density gradient centrifugation using Histopaque-1077 (No. 10771; Sigma-Aldrich, St. Louis, MO, USA) [59]. $NAD^+$ was extracted from frozen PBMCs using ice-cold perchloric acid and neutralized with potassium carbonate. $NAD^+$ concentrations were determined using an HPLC system (Prominence; Shimadzu Scientific Instruments, Kyoto, Japan) fitted with a Supelco LC-18-T column (#58970-U; Sigma-Aldrich) at Keio University School of Medicine. HPLC was run at a flow rate of 1 mL/min with 100% buffer A (0.05 M phosphate buffer) from 0 to 5 min, a linear gradient to 95% buffer A/5% buffer B (100% methanol) from 5 to 6 min, 95% buffer A/5% buffer B from 6 to 11 min, a linear gradient to 85% buffer A/15% buffer B from 11 to 13 min, 85% buffer A/15% buffer B from 13 to 23 min, a linear gradient to 100% buffer A from 23 to 24 min, and 100% buffer A from 24 to 30 min [3]. $NAD^+$ concentrations were normalized to wet-cell PBMC weights [16], calculated as the difference between the weight of the Eppendorf tube before and after PBMC pellets were added.

### *Statistical analysis*

Statistical analyses included data from individuals who completed the 8-week NMN supplementation regimen ($n = 9$). Comparisons between pre- and post-intake values for placebo or NMN were conducted using Student's paired *t*-test. One-way repeated-measures analysis of variance (ANOVA) followed by the Bonferroni *post hoc* test were applied for multiple time points during the NMN administration period. All statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). Confidence intervals (CIs) were calculated using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA). Results at $p < 0.05$ were considered statistically significant.

## Results

### *Participant characteristics*

This study engaged a cohort of 28 male participants aged 40–60 years. Following rigorous screening based on predetermined exclusion criteria, 14 candidates were excluded; 13 of them met one or more of the multiple exclusion criteria, while 1 voluntarily withdrew due to scheduling conflicts. Consequently, the study was carried out with a final sample size of 14 participants, whose baseline characteristics have been collated and are presented in Table 1. One participant dropped out because of fever before initiating the study medication in the placebo period. Of the 13 participants who started taking placebo capsules, 1 dropped out at the 4-week visit due to elevated transaminase levels. Another discontinued participation after the washout period because

**Table 1** Baseline clinical characteristics of study participants

| Number | 14 |
|---|---|
| Age (years) | 47.8 ± 5.6 |
| Body weight (kg) | 69.76 ± 6.14 |
| Height (cm) | 171.7 ± 3.21 |
| Body mass index (kg/m$^2$) | 23.71 ± 2.44 |
| Waist circumference (cm) | 85.1 ± 7.6 |
| Systolic blood pressure (mmHg) | 114.9 ± 16 |
| Diastolic blood pressure (mmHg) | 69.4 ± 10.1 |
| Blood Glucose (mg/dL) | 99.14 ± 6.46 |
| Total Cholesterol (mg/dL) | 191.6 ± 28.8 |
| Triglyceride (mg/dL) | 88.2 ± 32.5 |
| Aspartate aminotransferase (IU/L) | 22.28 ± 6.29 |
| Alanine aminotransferase (IU/L) | 20.92 ± 8.88 |

Values are expressed as mean ± standard deviation

of elevated transaminase levels and anemia. Two withdrew, one each because of elevated transaminase levels and IOP, at the 4-week visit during the NMN period. Nine participants completed all planned examinations and evaluations (Fig. 2).

### *Temporal PBMC NAD$^+$ level increases during NMN supplementation*

Based on capsule counts and diary records, the consumption rates of both placebo and NMN capsules were 100%. Basal NAD$^+$ content in PBMCs was measured at 0- and 4-week visits in the placebo group and at 0-, 1-, 2-, 4-, and 8-week visits in the NMN group (Table 2 and Fig. 1). PBMCs were isolated from whole-blood samples collected before daily placebo or NMN intake to ensure that trough levels of NAD$^+$ were measured. NAD$^+$ concentrations increased as a function of the duration of NMN administration (week 0 *vs.* week 8, $p$ = 0.0046,

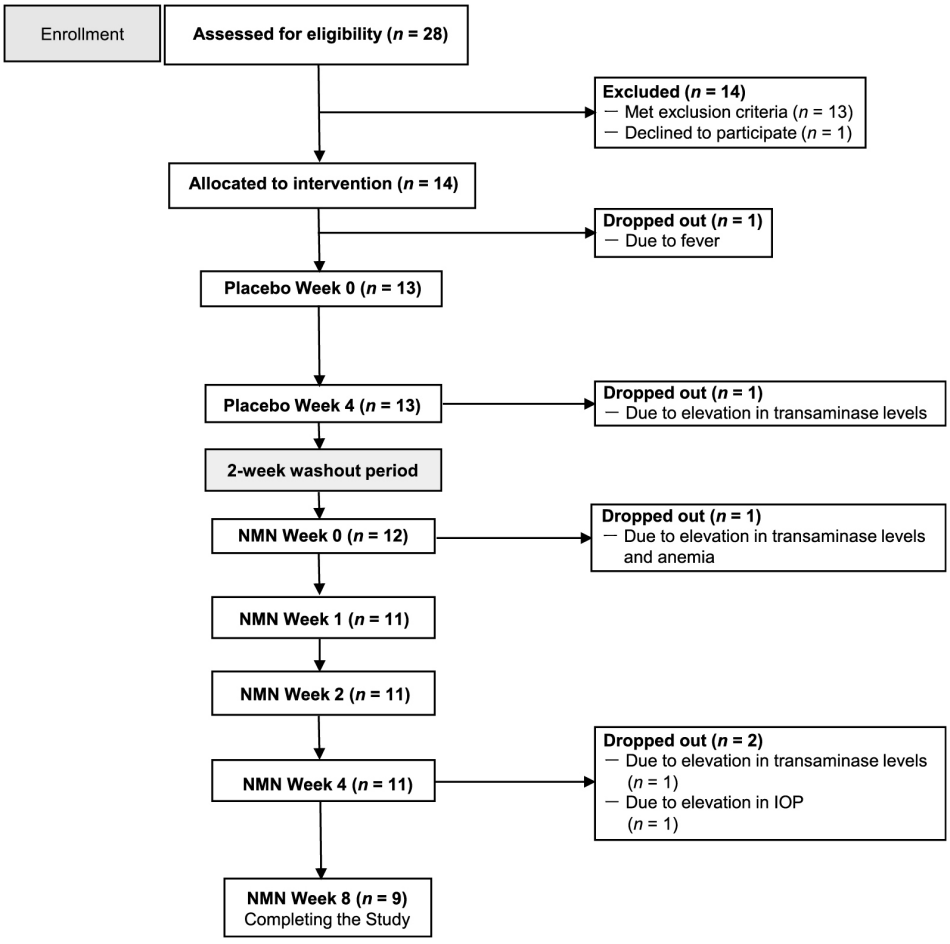


**Fig. 2** Trial flowchart

A total of 28 potential participants were screened. Of these, 14 were eligible and were allocated to the intervention group. After one participant dropped out owing to unexpected fever prior to study initiation, 13 participants started taking placebo pills. Clinical examinations were performed at 0 and 4 weeks of the placebo period as well as at 0, 1, 2, 4, and 8 weeks of the NMN period. Four participants dropped out of the study, and nine participants completed it.

NMN, nicotinamide mononucleotide; IOP, intraocular pressure

**Table 2** PBMC $NAD^+$ levels

| Test items | Placebo period | | | NMN period | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Week 0 ($n$ = 9) | Week 4 ($n$ = 9) | $p$-Value | Week 0 ($n$ = 9) | Week 1 ($n$ = 9) | Week 2 ($n$ = 9) | Week 4 ($n$ = 9) | Week 8 ($n$ = 9) | $p$-Value (Week 0 *vs.* Week 8) |
| PBMC $NAD^+$ (pmole/mg wet cells) | 23.84 (15.96 to 31.72) | 26.83 (19.42 to 34.25) | 0.22 | 25.48 (17.16 to 33.80) | 29.72 (13.26 to 46.18) | 34.35 (21.98 to 46.71) | 37.53 (23.41 to 51.64) | 41.43 (32.11 to 50.75) | 0.0046 |

Data are presented as mean (95% confidence interval). Data from the placebo period were analyzed using Student's paired $t$-test ($n$ = 9). NMN data were analyzed using one-way repeated-measures ANOVA with $p$ = 0.04 ($n$ = 9). Data from the NMN period were further analyzed using the Bonferroni *post hoc* test ($n$ = 9).

one-way repeated-measures ANOVA followed by the Bonferroni *post hoc* test, $n$ = 9), but did not change during the placebo period ($p$ = 0.22, Student's paired $t$-test, $n$ = 9) (Table 2 and Fig. 3). These findings indicate that oral NMN was effectively absorbed, chronologically augmenting $NAD^+$ metabolism in peripheral tissues.

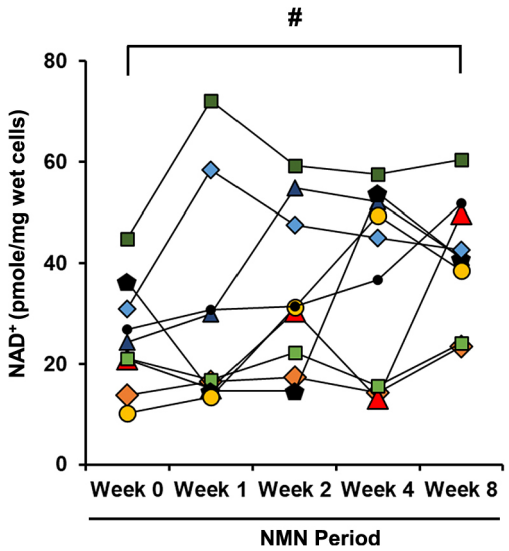


**Fig. 3** Chronic oral NMN increases PBMC $NAD^+$ levels

Chronological changes in basal PBMC $NAD^+$ content during NMN treatment ($n$ = 9). # $p$ < 0.01, Week 0 *vs.* Week 8, one-way repeated-measures ANOVA followed by the Bonferroni *post hoc* test. PBMCs were isolated from blood samples obtained at each visit at the trough level. $NAD^+$ concentrations in PBMCs significantly increased over time with increasing duration of NMN administration.

$NAD^+$, nicotinamide adenine dinucleotide; NMN, nicotinamide mononucleotide; PBMC, peripheral blood mononuclear cell; ANOVA, analysis of variance

### *Clinical parameters, biochemistry, ophthalmic function, and urinalysis*

Mean values and their 95% CIs for clinical parameters (Supplementary Table 1), blood laboratory data (Supplementary Table 2), ophthalmic function (Supplementary Table 3), and urinary parameters (Supplementary Table 4) were comparable throughout the study period. Qualitative urine tests were outside the clinical laboratory reference ranges during both placebo and NMN periods for some participants (Supplementary Table 5). Specifically, urinary protein at 0 weeks of placebo and 4 weeks of NMN supplementation, white blood cells at baseline of placebo, and ketone bodies at 4 weeks of NMN supplementation were positive. In addition, electrocardiographic and chest X-ray findings were within normal ranges and showed no signs of alteration (Supplementary Table 6).

### *Sleep quality*

Quality was preliminarily assessed using the PSQI [56] at 0- and 4-week visits of placebo, as well as at 0, 4, and 8 weeks of NMN. The PSQI consists of sleep duration, sleep latency, sleeping medications, sleep disturbance, daytime dysfunction, sleep quality, and sleep efficiency. PSQI scores range from 0 to 21, with higher scores indicating decreased subjective sleep quality. The mean PSQI scores during NMN supplementation were 3.33 at 0 weeks, 2.78 at 4 weeks, and 2.67 at 8 weeks, which were not significant differences (week 0 *vs.* week 8, $p$ > 0.99, one-way repeated-measures ANOVA followed by the Bonferroni *post hoc* test, $n$ = 9) (Table 3). These findings suggest that no improvement in the scores was observed during NMN supplementation.

### *Glucose and insulin tolerance*

Consistent with body composition not being altered by NMN administration (Supplementary Table 1), the mean and 95% CI values for blood glucose, insulin,

HOMA-IR, HOMA-β, and AUC values for glucose and insulin as well as insulinogenic and oral deposition indices in OGTT, were comparable between 0 and 8 weeks of NMN supplementation (Tables 4 and 5).

Skeletal muscle insulin sensitivity has been shown to be improved in prediabetic postmenopausal obese/overweight women by oral NMN administration at 250 mg/d for 10 weeks [46]; moreover, 300 mg/d NMN for 60 d alleviates the exacerbation of insulin resistance in healthy adults [45]. Thus, we hypothesized that NMN could ameliorate hyperinsulinemia. We therefore performed subgroup analyses based on the AUC value for insulin during OGTT performed at the 0-week NMN visit. For participants with below-mean AUC value for insulin during OGTT, the mean AUC value for insulin during NMN supplementation was 3,631.5 $\mu U \cdot mL^{-1}min^{-1}$ at 0-week and 4,039.75 $\mu U \cdot mL^{-1}min^{-1}$ at 8-week NMN supplementation ($p = 0.47$, Student's paired *t*-test, $n = 6$), suggesting that there was no significant change throughout the NMN intervention (Fig. 4A). In contrast, three participants exhibited above-mean AUC values for insulin during OGTT (the mean AUC value at 0-week of NMN supplementation: 5,593.5 $\mu U \cdot mL^{-1}min^{-1}$), which decreased after 8 weeks in all three (the mean AUC value at 8-week of NMN supplementation: 4,046.5 $\mu U \cdot mL^{-1}min^{-1}$), although not statistically significant ($p = 0.11$, Student's paired *t*-test, $n = 3$) (Fig. 4B).

### *Adverse events*

Reports of all adverse events during the study period are summarized in Table 6. No serious adverse events occurred during the placebo, washout, or NMN treatment periods. However, one participant receiving placebo discontinued the study at the 4-week visit because of

**Table 3** Sleep quality

| Test items | Placebo period | | | NMN period | | | |
|---|---|---|---|---|---|---|---|
| | Week 0 ($n = 9$) | Week 4 ($n = 9$) | *p*-Value | Week 0 ($n = 9$) | Week 4 ($n = 9$) | Week 8 ($n = 9$) | *p*-Value (Week 0 *vs.* Week 8) |
| PSQI Scores | 3.78 (2.02 to 5.53) | 3.44 (2.11 to 4.78) | 0.47 | 3.33 (1.53 to 5.13) | 2.78 (1.64 to 3.92) | 2.67 (2.00 to 3.33) | >0.99 |

Data are presented as mean (95% confidence interval). Data from the placebo period were analyzed using Student's paired *t*-test ($n = 9$). NMN data were analyzed using one-way repeated-measures ANOVA with $p = 0.38$ ($n = 9$). Data from the NMN period were further analyzed using the Bonferroni *post hoc* test ($n = 9$).

**Table 4** Blood glucose and insulin levels during the 75 g oral glucose tolerance tests

| Oral glucose tolerance test | | Blood Glucose (mg/dL) | | | | Insulin (μU/mL) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 min | 30 min | 60 min | 120 min | 0 min | 30 min | 60 min | 120 min |
| Placebo period | Week 0 ($n = 9$) Mean (95% CI) | 98.8 (93.8 to 103.8) | 151.3 (143.7 to 159.0) | 137.3 (98.7 to 176.0) | 110.2 (84.9 to 135.5) | 5.2 (3.0 to 7.3) | 53.0 (33.0 to 73.1) | 49.6 (33.7 to 65.5) | 40.7 (8.2 to 73.3) |
| | Week 4 ($n = 9$) Mean (95% CI) | 97.7 (94.1 to 101.2) | 145.1 (126.5 to 163.8) | 128.8 (101.4 to 156.2) | 113.9 (99.5 to 128.2) | 4.8 (3.8 to 5.8) | 54.2 (34.5 to 73.9) | 43.8 (34.3 to 53.2) | 33.7 (24.4 to 43.1) |
| | *p*-Value | 0.64 | 0.42 | 0.37 | 0.7 | 0.7 | 0.68 | 0.47 | 0.58 |
| NMN period | Week 0 ($n = 9$) Mean (95% CI) | 99.3 (94.9 to 103.8) | 137.7 (121.1 to 154.2) | 140.8 (111.3 to 170.3) | 114.4 (97.3 to 131.6) | 4.3 (3.9 to 5.6) | 40.3 (19.3 to 61.3) | 43.5 (35.9 to 51.2) | 35.1 (22.0 to 48.3) |
| | Week 8 ($n = 9$) Mean (95% CI) | 98.0 (93.3 to 102.7) | 132.4 (121.9 to 143.0) | 141.7 (105.5 to 177.8) | 115.8 (98.6 to 132.9) | 4.6 (3.3 to 6.0) | 35.6 (15.5 to 55.7) | 42.2 (28.4 to 56.0) | 33.5 (17.6 to 49.5) |
| | *p*-Value | 0.24 | 0.43 | 0.94 | 0.87 | 0.43 | 0.42 | 0.86 | 0.79 |

Data at each time point within placebo and NMN period were analyzed using Student's paired *t*-test ($n = 9$ for each period). CI, confidence interval

**Table 5** Glucose, insulin, and pancreatic response indices during the 75 g oral glucose tolerance tests

| OGTT | Placebo period | | | NMN period | | |
|---|---|---|---|---|---|---|
| | Week 0 ($n$ = 9) | Week 4 ($n$ = 9) | $p$-Value | Week 0 ($n$ = 9) | Week 8 ($n$ = 9) | $p$-Value |
| HOMA-IR | 1.28 (0.68 to 1.87) | 1.16 (0.90 to 1.42) | 0.59 | 1.06 (0.71 to 1.40) | 1.12 (0.77 to 1.46) | 0.56 |
| $AUC_{Glucose\ (0–120)}$ (mg/dL*min) | 15,508.33 (12,974.43 to 18,042.23) | 15,030 (13,195.87 to 16,864.13) | 0.34 | 15,388.3 (13,317.74 to 17,458.92) | 15,291.67 (13,301.37 to 17,281.97) | 0.89 |
| HOMA-β | 51.76 (34.75 to 68.79) | 50.38 (38.80 to 61.97) | 0.74 | 42.88 (30.84 to 54.92) | 48.88 (34.18 to 63.58) | 0.15 |
| $AUC_{Insulin\ (0–120)}$ (μU/mL*min) | 5,122.67 (3,239.06 to 7,006.27) | 4,679.67 (3,739.19 to 5,620.15) | 0.51 | 4,285.5 (3,392.97 to 5,178.03) | 4,042.0 (2,396.25 to 5,147.75) | 0.64 |
| Insulinogenic index | 0.92 (0.57 to 1.27) | 3.60 (–2.46 to 9.66) | 0.33 | 1.12 (0.38 to 1.85) | 1.3 (0.31 to 2.29) | 0.45 |
| Oral disposition index | 0.20 (0.13 to 0.27) | 0.85 (–0.64 to 2.34) | 0.33 | 0.30 (0.08 to 0.52) | 0.4 (–0.10 to 0.90) | 0.53 |

Data are presented as mean (95% confidence interval). Data within placebo and NMN periods were analyzed using Student's paired $t$-test ($n$ = 9 for each period).
HOMA-IR, homeostatic model assessment of insulin resistance

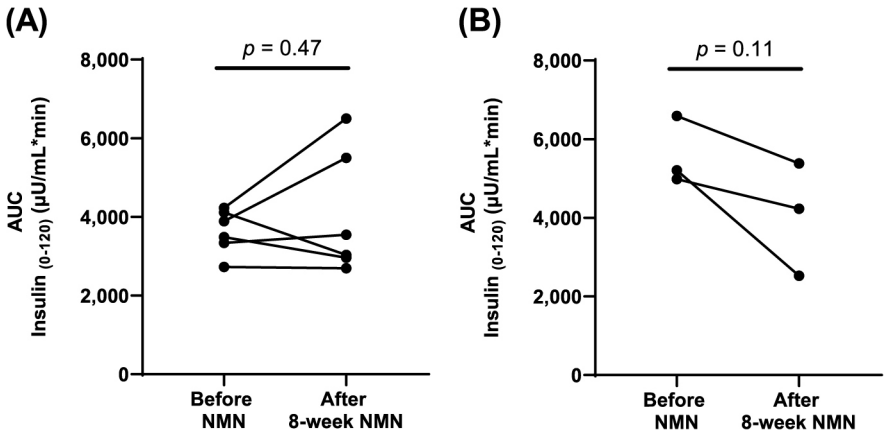


**Fig. 4** Subgroup analyses of AUC for insulin responses to OGTT
(A) Participants with below-mean ($n$ = 6) and (B) above-mean ($n$ = 3) AUC values for insulin.
Data were analyzed using Student's paired $t$-test.
AUC, area under the curve; OGTT, oral glucose tolerance test

transient mild elevations in AST (69 U/L), ALT (140 U/L), and g-GTP (281 U/L) levels. Another participant who finished the washout period did not progress to NMN administration because of a mild transient elevation in AST level (58 U/L) and anemia (hemoglobin 11.2 g/dL). Two participants withdrew from the study during the NMN period at the 4-week visit: one due to mild transient elevations in AST (69 U/L), ALT (56 U/L), and g-GTP (63 U/L) levels and the other due to a right IOP increase of 29.0 mmHg. Thus, the side-effect incidences were as follows: before NMN initiation, 15.3% (placebo period, 7.6%; washout period, 7.6%), and after NMN initiation, 16.7%. These data suggest that the total incidence of side effects before NMN initiation is comparable to that after. Notably, we observed one case of elevated transaminase level during each study period. The participant who exhibited an elevated right IOP 4 weeks of NMN supplementation had a relatively higher IOP of 21 mmHg in the initial screening. Another participant had high IOP values (right and left, 19.0 and 19.7 mmHg, respectively) at the baseline of NMN intervention. After 4 weeks of NMN supplementation, neither right nor left IOP values were substantially altered (20.0 and 18.0 mmHg, respectively).

**Table 6** Adverse events

| Adverse events | Placebo period ($n$ = 13) | Washout period ($n$ = 13) | NMN period ($n$ = 12) |
|---|---|---|---|
| Participants with any adverse events | 1 (7.6%) | 1 (7.6%) | 2 (16.7%) |
| Participants with serious adverse event | 0 | 0 | 0 |
| Discontinuation of study | 1 (7.6%) | 1 (7.6%) | 2 (16.7%) |
| Face flushing | 0 | 0 | 0 |
| Subjective symptoms | 0 | 0 | 0 |
| Fluctuations in | | | |
| body temperature | 0 | 0 | 0 |
| Heart rate | 0 | 0 | 0 |
| Blood pressure | 0 | 0 | 0 |
| Elevations in transaminase levels | 1 | 1 | 1 |
| Renal dysfunction | 0 | 0 | 0 |
| Cardio-pulmonary dysfunction | 0 | 0 | 0 |
| Glucose intolerance | 0 | 0 | 0 |
| Vision impairment | 0 | 0 | 0 |
| Elevations in IOP | 0 | 0 | 1 |
| Anemia | 0 | 1 | 0 |
| Back pain | 0 | 0 | 1 |
| Common cold symptoms | 0 | 0 | 1 |

Data are presented as number of participants (%) and number of events.

## Discussion

Our study provides empirical evidence to suggest that an 8-week course of 250 mg/d NMN before breakfast is overall safe, tolerable, and effective in augmenting PBMC $NAD^+$ biosynthesis in healthy Japanese males aged 40–60 years. These findings support the clinical implications of NMN supplementation. Its potential for mitigating postprandial hyperinsulinemia warrants further comprehensive exploration.

In terms of the safety assessment of oral NMN administration, of the 14 participants, 3 had mildly elevated transaminase levels, one during the placebo period, one during the washout period, and one during the NMN period. It was recently reported that NMN administration at a dose of 1,250 mg/d produced no significant hepatotoxicity for up to 4 weeks [49]. Although oral intake of nicotinamide (NAM), a metabolite of NMN, improves lipid metabolism, exceeding a dose of 3 g/d NAM increases the risk of hepatotoxicity [60, 61]. Therefore, a maximum daily dose of 900 mg of NAM has been recommended [62]. A 12-week course of NMN at 250 mg/d did not increase whole-blood NAM levels [43, 47]. While we instructed the participants not to change their lifestyles, including habitual diets, the transient elevations in transaminase levels we observed might still be related to diet, alcohol consumption, or merely coincidence.

NMN administered to beagle dogs weighing approximately 10 kg at 1,340 mg/d for 14 d has been shown to cause renal dysfunction [63]. In contrast, transient NMN administration in a murine model of obesity- and diabetes-induced nephropathy boosted $NAD^+$ biosynthesis and Sirt1 activity; this in turn should exert renoprotective effects, such as decreasing urinary albumin excretion [64]. In the present study, NMN administered to healthy men weighing approximately 70 kg at 250 mg/d for 8 weeks did not affect renal function, including albuminuria. One patient had right and left IOP values of 21.0 and 21.3 mmHg, respectively, at the initial screening. After 4 weeks of NMN supplementation, the right IOP increased to 29 mmHg. Although NMN administration was halted, the right IOP further increased to 32.7 mmHg in the following 4 weeks. However, the left IOP was 17.7 mmHg at 4 weeks of NMN supplementation and 19.7 mmHg at 4 weeks after discontinuation. In rodents, NMN did not elevate IOP in a unilateral common carotid artery occlusion model [65]. Additionally, we found that IOP tended to decrease after a single oral dose of 100 mg NMN in humans [42]. A daily dose of 250 mg for 24 weeks in older men with type 2 diabetes did not elevate IOP [51]. In our trial, in another participant with relatively high initial right and left IOP values of 19.0 and 19.7 mmHg, respectively, 4 weeks of consistent NMN administration did not increase them (20.0 and 18.0 mmHg, respectively). Thus, the elevation

of IOP observed in this case is unlikely associated with NMN administration. Therefore, we did not infer any direct causal relationships between NMN and any adverse events. These results indicate that 8-week NMN supplementation at 250 mg/d is overall safe and tolerable in healthy middle-aged Japanese men.

One of our secondary outcomes was the efficacy of NMN in boosting $NAD^+$ in peripheral tissues. Previously, we found that NMN increased plasma levels of NAM metabolites, including 2-PY and 4-PY, in a dose-dependent manner, but we did not assess changes in $NAD^+$ levels in tissues following NMN administration [42]. Thereafter, it was confirmed that NMN administration increases $NAD^+$ levels in PBMCs [46] and whole blood [43, 47] and that blood $NAD^+$ concentrations increase with NMN intake in a dose-dependent manner [44]. Furthermore, serum $NAD^+$/NADH levels were increased by 11.3% on day 30 and 38% on day 60 with a daily NMN dose of 300 mg/d, demonstrating that $NAD^+$ levels increased with the duration of NMN administration [45]. Recent studies have also demonstrated that NMN levels in whole blood [47] and plasma [48] were elevated after 12 weeks of NMN supplementation at a dose of 250 mg/d. This study further confirms that $NAD^+$ concentration in PBMCs increases over time with NMN administration.

Another secondary outcome of this study was the effect of NMN on glucose metabolism. NMN administered to healthy middle-aged Japanese men at 250 mg/d for 8 weeks did not affect multiple parameters, including HOMA-IR and HOMA-β. Therefore, we focused on postprandial insulinemia, as it is related to coronary artery disease (CAD) in obesity [66, 67]. Previous data obtained from nondiabetic women demonstrated that postprandial hyperinsulinemia is associated with CAD, irrespective of fasting glucose, insulin, and postprandial glucose levels [68]. Similarly, postprandial insulin levels and the AUC value for insulin in the OGTT, but not fasting plasma insulin levels, predicted CAD risk in men without diabetes between 34 and 64 years of age [69]. In our subgroup analyses, limited to participants with higher-than-mean AUC values for insulin in the 75-g OGTT, three participants exhibited above-mean AUC value and 8-week NMN intake decreased the AUC values in all three, indicating a potential effect of NMN to modestly attenuate postprandial hyperinsulinemia. These findings are consistent with those of a recent clinical trial conducted by Igarashi *et al.*, which demonstrated that daily oral intake of 250 mg NMN in men over 65 years old for 12 weeks changed the trend of the AUC for insulin after NMN supplementation [47]. Taken together, our data raise the possibility that NMN may modestly alleviate hyperinsulinemia in participants with postprandial insulin resistance.

The other secondary outcome of this study was the effect of NMN administration on sleep quality. Our study did not show improvements, which is consistent with our previous findings in a clinical trial demonstrating that a single dose of NMN of 100, 250, or 500 mg did not affect sleep quality [42]. However, oral NMN at 300 mg/d for 10 weeks in 45–75-year-old men and women with sleep disturbances improved sleep quality assessed using the PSQI [54]; moreover, NMN intake after 18:00 at 250 mg/d for 12 weeks increased sleep quality in participants over 65 years of age [52]. Since our assessments of sleep quality using a subjective questionnaire are relatively weak, we cannot exclude the possibility that NMN improves sleep quality.

This study has several limitations. First, it was neither placebo-controlled nor double-blinded. In addition, its sample size was small. Therefore, the assessments need to be repeated with a larger number of participants. Second, our data indicate that NMN may modestly alleviate hyperinsulinemia in middle-aged men. However, we neither performed hyperinsulinemic-euglycemic clamping, which will be necessary to more accurately assess the kinetics of glucose metabolism, nor did we measure GLP-1 levels, particularly postprandially. Given that the sample size of this trial was small, a more mechanistic and comprehensive investigation of the effect of NMN treatment on hyperinsulinemia is warranted in a larger cohort with postprandial hyperinsulinemia, such as those with obesity. Finally, to further evaluate the effect of NMN on sleep, it would be necessary to perform electroencephalography and analyze each stage of sleep.

In conclusion, we found that NMN at 250 mg/d for 8 weeks was overall safe and increased PBMC $NAD^+$ levels. In addition, we observed a possible trend suggesting the suppression of hyperinsulinemia, which could potentially benefit people with increased CAD risk as well as those with irregular lifestyles.

## Acknowledgments

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## Disclosure

E.I., H.O., and K.T. have been awarded a patent (Japanese Patent No. 7090336) titled "Utilization of NAD-related metabolites." J.Y. is listed as an inventor on patent applications related to NMN (US20180228824, JP2018131418A). H.I. received research funding from Oriental Yeast Company, Ltd. The potential conflict of interest was adequately managed, following the recommendations of the Institutional Review Board at Keio University School of Medicine.

**Supplementary Table 1** Body composition and clinical parameters

| Test items | Placebo period | | | NMN period | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Week 0 ($n$ = 9) | Week 4 ($n$ = 9) | $p$-Value | Week 0 ($n$ = 9) | Week 1 ($n$ = 9) | Week 2 ($n$ = 9) | Week 4 ($n$ = 9) | Week 8 ($n$ = 9) | $p$-Value |
| Body composition | | | | | | | | | |
| Height (cm) | 172.17 (170.02 to 174.32) | 172.12 (169.89 to 174.35) | 0.74 | 172.06 (169.93 to 174.18) | 172.17 (169.98 to 174.35) | 172.20 (169.86 to 174.54) | 172.02 (169.71 to 173.34) | 171.91 (169.89 to 173.94) | 0.49 |
| Weight (kg) | 71.12 (66.25 to 76.0) | 71.11 (66.47 to 75.75) | 0.96 | 71.04 (66.41 to 75.67) | 71.18 (66.58 to 75.78) | 71.29 (66.54 to 76.04) | 71.17 (66.58 to 75.75) | 71 (66.22 to 75.78) | 0.68 |
| Waist (cm) | 85.9 (80.6 to 91.2) | 85.8 (80.8 to 90.8) | 0.84 | 86.7 (81.5 to 91.9) | 86.1 (81.4 to 90.8) | 86.2 (81.3 to 91.1) | 86.7 (81.3 to 92.0) | 86.3 (81.9 to 90.8) | 0.82 |
| Clinical parameter | | | | | | | | | |
| Systolic blood pressure (mmHg) | 113.2 (103.3 to 123.1) | 110.6 (101.5 to 119.6) | 0.15 | 111.7 (99.5 to 123.8) | 114.2 (102.9 to 125.6) | 113.8 (102.4 to 125.2) | 114.7 (104.5 to 124.8) | 112.3 (101.4 to 123.2) | 0.53 |
| Diastolic blood pressure (mmHg) | 68.4 (59.5 to 77.3) | 68.8 (59.6 to 77.9) | 0.85 | 69.7 (60.9 to 78.5) | 68.9 (59.4 to 78.3) | 69.1 (59.0 to 79.2) | 70.3 (62.2 to 78.5) | 71.9 (62.6 to 81.2) | 0.24 |
| Heat rate (bpm) | 58 (55.1 to 60.9) | 56.6 (52.4 to 60.7) | 0.45 | 58.3 (51.3 to 65.4) | 62.3 (54.3 to 70.3) | 66.1 (55.1 to 77.1) | 60.1 (53.5 to 66.8) | 59 (52.7 to 65.3) | 0.25 |

Data are presented as mean (95% confidence interval). Data from the placebo period were analyzed using Student's paired $t$-test ($n$ = 9). Data from the NMN period were analyzed using one-way repeated-measures ANOVA ($n$ = 9).

**Supplementary Table 2** Hematologic and biochemical parameters

| Test items | Reference values | Placebo period | | | NMN period | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 ($n$ = 9) | Week 4 ($n$ = 9) | $p$-Value | Week 0 ($n$ = 9) | Week 1 ($n$ = 9) | Week 2 ($n$ = 9) | Week 4 ($n$ = 9) | Week 8 ($n$ = 9) | $p$-Value |
| Hematologic tests | | | | | | | | | | |
| White blood cell count ($\times 10^3/\mu L$) | 3.3–8.6 | 4.9 (3.86 to 5.94) | 5.2 (3.59 to 6.81) | 0.36 | 5.0 (3.60 to 6.40) | 4.74 (3.63 to 5.86) | 4.48 (3.46 to 5.49) | 5.0 (3.25 to 6.75) | 4.64 (3.67 to 5.61) | 0.56 |
| Hemoglobin (g/dL) | 13.7–16.8 | 14.63 (14.02 to 15.23) | 14.4 (13.77 to 15.03) | 0.22 | 14.3 (13.49 to 15.11) | 14.47 (13.84 to 15.09) | 14.31 (13.62 to 15.00) | 14.5 (14.06 to 14.94) | 14.21 (13.60 to 14.83) | 0.29 |
| Platelet count ($\times 10^3/\mu L$) | 158–348 | 235.67 (201.54 to 269.79) | 225.67 (188.17 to 263.16) | 0.16 | 234.56 (200.42 to 268.70) | 233.56 (200.90 to 266.21) | 232.22 (191.14 to 273.30) | 240.33 (205.29 to 275.38) | 238.11 (209.13 to 267.10) | 0.63 |
| Proteins | | | | | | | | | | |
| Total protein (g/dL) | 6.6–8.1 | 6.7 (6.41 to 6.99) | 6.61 (6.31 to 6.91) | 0.21 | 6.64 (6.36 to 6.93) | 6.76 (6.42 to 7.09) | 6.60 (6.35 to 6.85) | 6.77 (6.50 to 7.03) | 6.68 (6.43 to 6.92) | 0.27 |
| Albumin (g/dL) | 4.1–5.1 | 4.16 (4.02 to 4.29) | 4.08 (3.93 to 4.23) | <0.05 | 4.08 (3.93 to 4.23) | 4.13 (3.98 to 4.28) | 4.01 (3.92 to 4.10) | 4.14 (3.99 to 4.30) | 4.13 (3.98 to 4.28) | 0.26 |
| Nitrogen compound | | | | | | | | | | |
| Urea Nitrogen (mg/dL) | 8.0–20.0 | 14.13 (12.40 to 15.87) | 14.28 (12.26 to 16.30) | 0.87 | 14.04 (11.13 to 16.96) | 13.51 (11.11 to 15.91) | 13.93 (10.87 to 16.99) | 15.22 (12.62 to 17.83) | 14.29 (12.12 to 16.46) | 0.55 |
| Creatinine (mg/dL) | 0.65–1.07 | 0.85 (0.80 to 0.89) | 0.85 (0.77 to 0.93) | 0.82 | 0.85 (0.81 to 0.90) | 0.87 (0.80 to 0.93) | 0.85 (0.78 to 0.92) | 0.85 (0.79 to 0.92) | 0.85 (0.79 to 0.91) | 0.83 |
| Uric acid (mg/dL) | 3.7–7.0 | 5.49 (4.77 to 6.21) | 5.59 (4.91 to 6.27) | 0.65 | 5.74 (5.04 to 6.45) | 5.67 (5.18 to 6.15) | 5.58 (5.01 to 6.15) | 5.7 (4.97 to 6.43) | 5.68 (5.08 to 6.28) | 0.87 |
| Enzymatic activity | | | | | | | | | | |
| Total bilirubin (mg/dL) | 0.4–1.5 | 0.71 (0.56 to 0.86) | 0.66 (0.62 to 0.70) | 0.44 | 0.78 (0.62 to 0.94) | 0.66 (0.53 to 0.78) | 0.68 (0.55 to 0.81) | 0.73 (0.68 to 0.79) | 0.72 (0.60 to 0.85) | 0.25 |
| AST (U/L) | 13–30 | 22.89 (16.16 to 29.62) | 21.89 (14.80 to 28.98) | 0.13 | 19.89 (15.80 to 21.98) | 21.22 (17.70 to 24.74) | 20.67 (16.62 to 24.72) | 21.0 (18.07 to 23.93) | 19.67 (16.95 to 22.38) | 0.29 |
| ALT (U/L) | 10–42 | 24.22 (11.26 to 37.18) | 22.44 (10.45 to 34.44) | 0.09 | 19.33 (14.44 to 24.23) | 20.0 (16.42 to 23.58) | 19.11 (15.38 to 22.85) | 19.44 (14.67 to 24.22) | 17.78 (14.52 to 21.03) | 0.49 |
| g-GTP (U/L) | 13–64 | 19 (14.36 to 23.64) | 19.56 (14.05 to 25.06) | 0.6 | 19.0 (14.08 to 23.92) | 19.0 (14.11 to 23.89) | 18.89 (14.47 to 23.31) | 19.78 (14.90 to 24.65) | 18.11 (13.57 to 22.65) | 0.09 |
| LDH (U/L) | 124–222 | 168 (154.80 to 181.20) | 165.33 (146.87 to 183.80) | 0.43 | 160.78 (147.88 to 173.68) | 166.33 (150.20 to 182.47) | 159.22 (145.38 to 173.07) | 170.11 (156.45 to 183.77) | 163.11 (149.62 to 176.60) | 0.13 |
| Amylase (U/L) | 44–132 | 81.22 (58.84 to 103.60) | 80.67 (62.53 to 98.80) | 0.87 | 74.56 (54.57 to 94.54) | 80.44 (60.68 to 100.21) | 73.78 (57.91 to 89.65) | 78.33 (60.19 to 96.47) | 82.44 (62.67 to 102.21) | 0.03 |
| ChE (U/L) | 240–486 | 319.22 (280.67 to 355.77) | 317.67 (279.36 to 355.97) | 0.78 | 317 (278.34 to 338.43) | 325.78 (286.17 to 365.38) | 319.11 (278.19 to 360.03) | 336.44 (292.09 to 380.80) | 327.56 (285.85 to 369.26) | 0.05 |
| Electrolytes | | | | | | | | | | |
| Sodium (mmol/L) | 138–145 | 140.79 (140.22 to 141.36) | 140.36 (139.44 to 141.27) | 0.37 | 140.06 (139.06 to 141.05) | 140.19 (139.57 to 140.81) | 140.74 (139.85 to 141.64) | 140.41 (139.53 to 141.30) | 140.74 (139.92 to 141.57) | 0.41 |
| Potassium (mmol/L) | 3.6–4.8 | 4.13 (4.00 to 4.27) | 4.14 (3.97 to 4.32) | 0.89 | 4.07 (3.90 to 4.23) | 4.12 (3.94 to 4.30) | 4.07 (3.93 to 4.21) | 4.31 (4.13 to 4.49) | 4.06 (3.85 to 4.26) | 0.05 |
| Chloride (mmol/L) | 101–108 | 105.33 (104.32 to 106.35) | 104.78 (103.71 to 105.85) | 0.36 | 104.67 (103.81 to 105.53) | 104.22 (103.15 to 105.29) | 104.78 (103.78 to 105.78) | 104.89 (103.91 to 105.87) | 104.44 (104.04 to 104.85) | 0.63 |
| Lipid metabolism | | | | | | | | | | |
| Total Cholesterol (mg/dL) | 142–219 | 207.11 (188.29 to 225.93) | 200.44 (187.43 to 213.46 | 0.37 | 203.78 (186.48 to 221.08) | 208 (192.56 to 223.44) | 197.78 (177.14 to 218.41) | 209 (186.81 to 231.20) | 202.78 (180.84 to 224.71) | 0.3 |
| Triglyceride (mg/dL) | 40–149 | 100.78 (62.73 to 138.83) | 90.22 (65.73 to 114.71) | 0.23 | 87 (60.95 to 113.05) | 93.56 (54.17 to 132.94) | 90.22 (56.27 to 124.17) | 87 (63.86 to 110.14) | 79.33 (56.00 to 102.67) | 0.75 |
| HDL Cholesterol (mg/dL) | 40–90 | 51.67 (46.22 to 57.11) | 51.11 (46.26 to 55.96) | 0.64 | 50.67 (46.84 to 54.49) | 52.22 (47.36 to 57.08) | 50.78 (46.42 to 55.14) | 52.22 (48.07 to 56.38) | 51.22 (46.58 to 55.86) | 0.41 |
| Carbohydrate metabolism | | | | | | | | | | |
| Blood Glucose (mg/dL) | 73–109 | 100.56 (96.99 to 104.12) | 99.56 (95.56 to 103.55) | 0.62 | 100 (94.72 to 105.28) | 99.33 (95.21 to 103.46) | 100.11 (94.43 to 105.79) | 100.44 (94.53 to 106.36) | 100.56 (95.13 to 105.98) | 0.79 |
| Insulin (μU/mL) | 2.1–19 | 5.56 (2.46 to 8.67) | 5.19 (3.30 to 7.08) | 0.81 | 4.7 (3.09 to 6.31) | 5.09 (3.32 to 6.85) | 4.63 (3.16 to 6.11) | 5.37 (3.44 to 7.29) | 5.02 (3.46 to 6.58) | 0.61 |
| HOMA-IR | | 1.40 (0.57 to 2.22) | 1.29 (0.79 to 1.79) | 0.8 | 1.17 (0.75 to 1.59) | 1.26 (0.81 to 1.71) | 1.15 (0.77 to 1.53) | 1.34 (0.84 to 1.84) | 1.25 (0.85 to 1.64) | 0.63 |
| HOMA-β | | 52.49 (26.62 to 78.36) | 50.55 (34.29 to 66.80) | 0.86 | 46.41 (31.43 to 61.40) | 50.38 (34.19 to 66.58) | 45.92 (31.17 to 60.67) | 52.95 (34.37 to 71.52) | 49.7 (32.70 to 66.69) | 0.6 |

Data are presented as mean (95% confidence interval). Data from the placebo period were analyzed using Student's paired $t$-test ($n$ = 9).

Data from the NMN period were analyzed using one-way repeated-measures ANOVA ($n$ = 9).

AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; ChE, cholinesterase; HOMA-IR, homeostatic model assessment of insulin resistance

**Supplementary Table 3** Ophthalmic parameters during NMN supplementation

| Test items | NMN period | | | | | |
|---|---|---|---|---|---|---|
| | Week 0 | | Week 4 | | Week 8 | |
| | Left Mean (95% CI) | Right Mean (95% CI) | Left Mean (95% CI) | Right Mean (95% CI) | Left Mean (95% CI) | Right Mean (95% CI) |
| Functional visual acuity (VA) (logMAR) | 0.1 (–0.03 to 0.24) | 0.1 (–0.01 to 0.22) | 0.03 (–0.03 to 0.09) | 0.05 (–0.02 to 0.12) | 0.06 (0.00 to 0.12) | 0.08 (0.03 to 0.13) |
| Intraocular pressure (IOP) (mmHg) | 12.86 (10.64 to 15.08) | 13.52 (11.44 to 15.60) | 14.27 (11.82 to 16.73) | 13.98 (10.22 to 17.74) | 12.26 (9.96 to 14.57) | 12.29 (10.22 to 14.35) |
| Tear break-up time (sec) | 5.53 (2.88 to 8.17) | 5.55 (2.91 to 8.19) | 5.95 (3.05 to 8.84) | 5.91 (2.98 to 8.83) | 6.14 (2.92 to 9.37) | 6.04 (2.85 to 9.24) |
| Tear function test (mm) | 6.42 (2.81 to 10.03) | 9.75 (3.49 to 16.01) | 8.45 (4.58 to 12.33) | 9.09 (2.89 to 15.29) | 9.56 (2.16 to 16.95) | 9.11 (2.25 to 15.97) |
| Critical flicker frequency (Hz) | 47.95 (44.98 to 50.93) | 47.85 (45.02 to 50.69) | 47.75 (45.06 to 50.43) | 47.91 (45.47 to 50.35) | 48.63 (44.69 to 52.56) | 48.58 (45.48 to 51.67) |
| Corneal endothelial density (cells/mm$^2$) | 2,773.17 (2,623.18 to 2,923.15) | 2,742.17 (2,587.26 to 2,897.07) | 2,729.91 (2,552.09 to 2,907.73) | 2,693.91 (2,531.40 to 2,856.42) | 2,754.89 (2,553.98 to 2,955.80) | 2,764.33 (2,585.30 to 2,943.36) |
| Corneal thickness (μm) | 536.6 (504.09 to 569.11) | 540 (510.65 to 569.35) | 541.3 (508.77 to 573.83) | 543.0 (513.15 to 572.85) | 535.5 (498.52 to 572.48) | 536.11 (501.98 to 570.24) |

CI, confidence interval

**Supplementary Table 4** Urinalysis for microalbumin: creatine ratio

| Test items | Reference values | Placebo period | | | NMN period | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 ($n$ = 9) | Week 4 ($n$ = 9) | $p$-Value | Week 0 ($n$ = 9) | Week 1 ($n$ = 9) | Week 2 ($n$ = 9) | Week 4 ($n$ = 9) | Week 8 ($n$ = 9) | $p$-Value |
| Urinary creatine (mg/dL) | | 107.78 (61.83 to 153.73) | 122.81 (57.85 to 187.78) | 0.57 | 141.39 (102.11 to 180.67) | 115.31 (76.45 to 154.15) | 150.27 (106.81 to 193.73) | 137.89 (79.33 to 196.45) | 141.41 (54.55 to 228.27) | 0.73 |
| Urinary microalbuminuria (mg/mL) | | 4.03 (2.52 to 5.54) | 5.2 (2.17 to 8.23) | 0.43 | 6.41 (3.64 to 9.18) | 5.2 (3.53 to 6.87) | 6.12 (4.79 to 7.46) | 5.1 (3.02 to 7.18) | 5.57 (3.07 to 8.06) | 0.76 |
| Microalbumin: creatinine ratio (mg/g·Cr) | 0–29.9 | 4.08 (2.96 to 5.19) | 4.26 (3.08 to 5.45) | 0.76 | 4.56 (3.14 to 5.97) | 5.29 (2.50 to 8.08) | 4.54 (3.16 to 5.90) | 4.43 (2.78 to 6.07) | 5.19 (2.42 to 7.96) | 0.76 |

Data are presented as mean (95% confidence interval). Data from the Placebo period were analyzed using Student's paired $t$-test ($n$ = 9). Data from the NMN period were analyzed using one-way repeated-measures ANOVA ($n$ = 9).

**Supplementary Table 5** Urinary parameters

| Test items | Clinical criteria | Placebo period | | | | | | NMN period | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 ($n$ = 13) | | | Week 4 ($n$ = 13) | | | Week 0 ($n$ = 12) | | | Week 1 ($n$ = 11) | | | Week 2 ($n$ = 11) | | | Week 4 ($n$ = 11) | | | Week 8 ($n$ = 9) | | |
| | | – | ± | + | – | ± | + | – | ± | + | – | ± | + | – | ± | + | – | ± | + | – | ± | + |
| Urine protein | – | 11 | 1 | 1 | 12 | 1 | 0 | 9 | 3 | 0 | 10 | 1 | 0 | 11 | 0 | 0 | 10 | 0 | 1 | 8 | 1 | 0 |
| Urinary glucose | – | 13 | 0 | 0 | 13 | 0 | 0 | 12 | 0 | 0 | 11 | 0 | 0 | 11 | 0 | 0 | 11 | 0 | 0 | 9 | 0 | 0 |
| Urinary white blood cells | – | 12 | 0 | 1 | 13 | 0 | 0 | 12 | 0 | 0 | 11 | 0 | 0 | 11 | 0 | 0 | 11 | 0 | 0 | 9 | 0 | 0 |
| Urobilinogen | ± | 0 | 13 | 0 | 0 | 13 | 0 | 0 | 12 | 0 | 0 | 11 | 0 | 0 | 11 | 0 | 0 | 11 | 0 | 0 | 9 | 0 |
| Bilirubin | – | 13 | 0 | 0 | 13 | 0 | 0 | 12 | 0 | 0 | 11 | 0 | 0 | 11 | 0 | 0 | 11 | 0 | 0 | 9 | 0 | 0 |
| Urine ketone body | – | 13 | 0 | 0 | 13 | 0 | 0 | 12 | 0 | 0 | 11 | 0 | 0 | 11 | 0 | 0 | 10 | 0 | 1 | 9 | 0 | 0 |

Values represent numbers of participants who met the criteria.

**Supplementary Table 6** Physiological functions

| Test items | Placebo period | | NMN period | | | | |
|---|---|---|---|---|---|---|---|
| | Week 0 ($n$ = 13) | Week 4 ($n$ = 13) | Week 0 ($n$ = 12) | Week 1 ($n$ = 11) | Week 2 ($n$ = 11) | Week 4 ($n$ = 11) | Week 8 ($n$ = 9) |
| Electrocardiogram | | | | | | | |
| Any abnormal findings | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chest X-ray | | | | | | | |
| Any abnormal findings | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Values represent numbers of participants who met the criteria.

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