Endocrine Journal

ORIGINAL

Advance Publication
doi:10.1507/endocrj.EJ19-0313

Effect of oral administration of nicotinamide mononucleotide on clinical parameters and nicotinamide metabolite levels in healthy Japanese men

Junichiro Irie[1)], Emi Inagaki[2),3),4)], Masataka Fujita[1)], Hideaki Nakaya[1)], Masanori Mitsuishi[1)], Shintaro Yamaguchi[1)], Kazuya Yamashita[2)], Shuhei Shigaki[5)], Takashi Ono[6)], Hideo Yukioka[6)], Hideyuki Okano[4),7)], Yo-ichi Nabeshima[8)], Shin-ichiro Imai[8),9)], Masato Yasui[7),10)], Kazuo Tsubota[2),7)] and Hiroshi Itoh[1),7)]

[1)] *Department of Endocrinology, Metabolism and Nephrology, Keio University School of Medicine, Shinjuku, Tokyo 160-8582, Japan*
[2)] *Department of Ophthalmology, Keio University School of Medicine, Shinjuku, Tokyo 160-8582, Japan*
[3)] *Japan Society for the Promotion of Science, Chiyoda, Tokyo 102- 0083, Japan*
[4)] *Department of Physiology, Keio University School of Medicine, Shinjuku, Tokyo 160-8582, Japan*
[5)] *Biomarker Research and Development Department, Shionogi & Co., Ltd., Toyonaka, Osaka 561-0825, Japan*
[6)] *Drug Discovery and Disease Research Laboratory, Shionogi & Co., Ltd, Toyonaka, Osaka 561-0825, Japan*
[7)] *Keio University Global Research Institute (KGRI), Minato, Tokyo 108-8345, Japan*
[8)] *Laboratory of Molecular Life Science, Department of Gerontology, Institute of Biomedical Research and Innovation, Kobe, Hyogo 650-0047, Japan*
[9)] *Department of Developmental Biology, Washington University School of Medicine, St. Louis, MO 63110, USA*
[10)] *Department of Pharmacology, Keio University School of Medicine, Shinjuku, Tokyo 160-8582, Japan*

Abstract. Recent studies have revealed that decline in cellular nicotinamide adenine dinucleotide (NAD^+) levels causes aging-related disorders and therapeutic approaches increasing cellular NAD^+ prevent these disorders in animal models. The administration of nicotinamide mononucleotide (NMN) has been shown to mitigate aging-related dysfunctions. However, the safety of NMN in humans have remained unclear. We, therefore, conducted a clinical trial to investigate the safety of single NMN administration in 10 healthy men. A single-arm non-randomized intervention was conducted by single oral administration of 100, 250, and 500 mg NMN. Clinical findings and parameters, and the pharmacokinetics of NMN metabolites were investigated for 5 h after each intervention. Ophthalmic examination and sleep quality assessment were also conducted before and after the intervention. The single oral administrations of NMN did not cause any significant clinical symptoms or changes in heart rate, blood pressure, oxygen saturation, and body temperature. Laboratory analysis results did not show significant changes, except for increases in serum bilirubin levels and decreases in serum creatinine, chloride, and blood glucose levels within the normal ranges, independent of the dose of NMN. Results of ophthalmic examination and sleep quality score showed no differences before and after the intervention. Plasma concentrations of N-methyl-2-pyridone-5-carboxamide and N-methyl-4-pyridone-5-carboxamide were significantly increased dose-dependently by NMN administration. The single oral administration of NMN was safe and effectively metabolized in healthy men without causing any significant deleterious effects. Thus, the oral administration of NMN was found to be feasible, implicating a potential therapeutic strategy to mitigate aging-related disorders in humans.

Key words: Nicotinamide mononucleotide, N-methyl-2-pyridone-5-carboxamide, N-methyl-4-pyridone-5-carboxamide, Pharmacokinetics

AGING increases incidences of age-associated diseases, such as cancer, cardiovascular diseases, type 2 diabetes, obesity, hypertension, macular degeneration, and many others. Prevention of age-associated diseases has increasingly become important because the average human lifespan has been extended worldwide. Recent studies have shown that decreases in nicotinamide adenine dinucleotide (NAD^+) levels in multiple tissues contribute to the pathogenesis of age-associated functional disorders [1]. Thus, preventive and therapeutic interventions with key NAD^+ intermediates, such as nicotinamide mononucleotide (NMN) and nicotinamide riboside (NR), which can

Submitted Jul. 18, 2019; Accepted Oct. 5, 2019 as EJ19-0313
Released online in J-STAGE as advance publication Nov. 2, 2019
Correspondence to: Hiroshi Itoh, Department of Endocrinology, Metabolism and Nephrology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku, Tokyo 160-8582, Japan.
E-mail: hiito@keio.jp

replenish cellular NAD^+ levels, have drawn significant attention in the field of aging research [2].

In various animal models, the administration of NMN has been shown to mitigate age-associated physiological declines in the liver, adipose tissue, muscle, pancreas, kidney, retina, and central nervous system. Several studies have already reported that NMN restores tissue NAD^+ levels and ameliorates obesity, insulin resistance, muscle mitochondrial dysfunction, renal failure, and retinal degeneration in rodent models [3-6]. Moreover, it was recently reported that NMN was rapidly absorbed through a transporter in the small intestine [7]. Although preclinical results provide a hope to develop NMN as an evidence-based anti-aging intervention, the safety and efficacy of NMN in humans remain unclear. Therefore, we conducted the first human study to examine the safety of a single NMN administration by assessing clinical parameters and its metabolite levels in healthy Japanese men.

Materials and Methods

Patients and methods

We performed a nonblinded clinical trial in 10 healthy men to investigate the pharmacokinetics of nicotinamide metabolites and evaluate the safety of orally administered NMN at the Clinical Trial Unit, Keio University School of Medicine, Japan. The healthy male subjects, who were 40 to 60 years of age and agreed to participate in the trial, were recruited. Subjects having 1) a previous history of diseases, 2) malignant neoplasms, 3) serious infections, 4) psychiatric disorders, 5) ophthalmic disorders, 6) allergic disorders, or 7) metabolic diseases were excluded.

The subjects orally received 100, 250, or 500 mg of NMN in capsules at 9 AM after an overnight fasting at each visit and followed for 5 h at rest with drinking only water freely. The interval of each visit was longer than one week. The measurement of height and body weight, a physical examination by registered physicians, and chest radiography and electrocardiography were conducted before and after the intervention. Heart rate, blood pressure, oxygen saturation, and body temperature were monitored for 5 h after the administration of NMN. Urine collections were conducted every 30 to 60 min for the first 2 h and at the end of the study, and urine samples were analyzed as previously reported [8]. Blood samples were obtained from the forearm vein *via* an inserted catheter every 5 to 20 min for the first 1 h, followed by every 30 to 120 min for the next 4 h on the basis of the findings that plasma NMN levels decreased to baseline in 30 minutes after oral administration of NMN in mice and that serum nicotinamide levels decreased to baseline in 3 h after oral intake of nicotinamide in healthy subjects [4, 9]. Blood analyses including complete blood count, liver and kidney function, glucose level, lipid profile, and amylase level were conducted using routine automated laboratory methods as previously described [10]. Blood samples with hemolysis were excluded from the analysis. Ophthalmic examinations and measurements of ophthalmic parameters, including visual acuity, functional visual acuity, intraocular pressure measurement, and meniscometry were conducted by registered ophthalmologists on the day of intervention with 100 and 500 mg of NMN before and after the administration. The best-corrected visual acuities were 20/16 in all subjects, as measured by Landolt C optotypes. Functional visual acuity (FVA) was measured by an FVA measurement system (AS-28; Kowa Company, Japan) to examine time-wise changes in the continuous visual acuity for 1 minute. Tear function was evaluated by meniscometry test (Echo Electricity, Japan). Standard tear film break-up time was measured. Central flicker frequency (CFF) was measured by a CFF test apparatus (Yagami Scientific Instruments, Japan). Corneal endothelial cell density was measured by an EM-3000 specular microscope (Tomey Corp., Japan), and corneal thickness was measured by CASIA optical coherence tomography system (Tomey Corp.). The left eyes were chosen for the analysis. Fundus photo was captured by an AFC-330 non-mydriatic auto fundus camera (NIDEK Co., Japan) [11]. The quality of sleep was evaluated using the Pittsburgh Sleep Quality Index (PSQI) before and after the day of intervention [12].

NMN was provided in a capsule of 100 mg or 250 mg by Oriental Yeast Co., Ltd. (Tokyo, Japan). The purity of NMN was 96–97% according to a high-performance liquid chromatography (HPLC) analysis result, and its endotoxin level was 0.1–0.2 EU/mg as previously described [4].

This study was conducted in accordance with the 1964 Declaration of Helsinki and approved by the Keio University School of Medicine Ethics Committee (registration number 20150414). This study was also registered in the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (http://www.umin.ac.jp/ctr/) as UMIN000021309. A written informed consent was obtained from all participants in the study. The safety monitoring was conducted at each dose by independent researchers.

Analysis of nicotinamide metabolites

Blood samples were stored in a cooled heparinized tube and centrifuged at 4°C for 5 min. The obtained plasma was frozen under –80°C until analysis. Levels of nicotinamide metabolites including methylnicotinamide

(MNA), N-methyl-2-pyridone-5-carboxamide (2Py), and N-methyl-4-pyridone-5-carboxamide (4Py), were measured using LC/MS/MS as described previously [13] with minor modifications. Briefly, plasma was deproteinized using methanol containing a deuterated internal standard. The mixture was vortexed and centrifuged. The supernatant was diluted with water and analyzed using a Shimadzu Nexera UHPLC system (Shimadzu, Kyoto, Japan) coupled with an API5000 triple quadrupole mass spectrometer (SCIEX, Framingham, MA, USA) with electrospray ionization operated in positive ion mode. The separation was performed on a Triart C18 column (3.0 × 150 mm, 5 μm, YMC, Kyoto, Japan) at 50°C. Mobile phase A and B were water/formic acid/undecafluorohexanoic acid (1,000/0.1/0.2, v/v/v) and methanol, respectively. The chromatographic conditions were: 0–4 min (5–80% B, 0.5 mL/min), 4–4.01 min (80–95% B, 0.5–1.0 mL/min), 4.01–7 min (95% B, 1.0 mL/min), 7–7.01 min (95–5% B, 1.0–0.5 mL/min), and 7.01–13 min (5% B, 0.5 mL/min). Quantitations were performed using multiple reaction monitoring with the following transitions: m/z 137 → 94 for MNA, m/z 140 → 97 for MNA-d_3, m/z 153 → 110 for 2Py, m/z 156 → 113 for 2Py-d_3, m/z 153 → 136 for 4Py, m/z 156 → 139 for 4Py-d_3. 1-methylnicotinamide chloride was obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). N-methyl-2-pyridone-5-carboxamide, N-(methyl-d_3)-2-pyridone-5-carboxamide, N-methyl-4-pyridone-5-carboxamide, N-methyl-4-pyridone-5-carboxamide-d_3 were obtained from Toronto Research Chemicals Inc., (Toronto, ON, Canada). 1-methylnicotinamide-d_3 was synthesized at Shionogi laboratory (Osaka, Japan). Formic acid was obtained from Nacalai Tesque, Inc. (Kyoto, Japan), undecafluorohexanoic acid was obtained from Tokyo Chemical Industry Co., Ltd., (Tokyo, Japan), and methanol was obtained from Kanto Chemical Co. Inc., (Tokyo, Japan). Water was purified before use with an ultra-pure water generating equipment.

The incremental area under the curve was calculated by multiplying the interval time between two points by the difference between the value and baseline.

Statistical analyses

Differences between two groups were examined by the Wilcoxon signed-rank test. The changes from the baseline in clinical parameters and laboratory data were estimated by using the mixed-effects model for repeated measures model with a compound symmetry covariance structure. Bonferroni's multiple comparison was conducted to identify the *time* and *group* differences.

Pearson's correlation analysis was used to analyze statistical correlation between two parameters. A p value of <0.05 was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics version 23 (IBM Japan, Tokyo, Japan).

Results

Ten healthy male subjects were enrolled, and their characteristics are shown in Table 1. They received 100, 250, or 500 mg NMN, and these doses were well-tolerated without causing severe adverse events, including flush and gastrointestinal symptoms (Fig. 1). The average interval between each visit was 63 ± 23 days (mean ± SD). The NMN administration at all doses did not affect the heart rate, blood pressure, oxygen saturation, nor body temperature (Fig. 2 and Supplementary Fig. 1). The examination results of the neurological system, ocular fundus, and ophthalmic parameters did not show any significant changes after NMN administration at all doses (Table 2). The sleep quality score by PSQI did not differ before and after the intervention (3.1 ± 1.5 *versus* 2.8 ± 2.5, 2.0 ± 2.3 *versus* 2.1 ± 2.0, and 1.8 ± 2.3 *versus* 1.8 ± 2.3 in the 100 mg, 250 mg, and 500 mg groups, respectively).

The results of laboratory analysis of blood and urine did not show any significant changes before and after NMN administration, except for serum bilirubin, creatinine, chloride, and glucose levels (Fig. 3 and Supplementary Table 1). Whereas the serum levels of bilirubin significantly increased by 51.3%, the levels of glucose, creatinine, and chloride significantly decreased by 11.7%, 5.1%, and 2.3%, respectively, at 300 min at all doses from the baseline. These changes stayed within normal ranges, independent of the dose of NMN.

The major final metabolites of NMN, such as MNA, 2Py, and 4Py, were detected at the baseline (Fig. 4A). The mean plasma concentrations of 2Py and 4Py were 1,423 nM and 237 nM at baseline, respectively, and they both showed significant increases at all doses throughout the time course after the NMN administration (Fig. 4B, C). The plasma concentration of MNA tended to moderately increase (Fig. 4D and Supplementary Fig. 2). The peak concentrations of 2Py and 4Py were observed at 300 min after the administration, and the mean peak concentrations of MNA were 253 ± 70 nM, 292 ± 131 nM, and 316 ± 58 nM in the 100 mg, 250 mg, and 500 mg groups, respectively. The incremental area under the curve of 2Py and 4Py over the baseline value was significantly greater in the group with 500 mg NMN than those in the groups with 100 mg and 250 mg NMN (Fig. 4E). The incremental areas under the curve of 2Py were positively correlated with those of 4Py, and those of MNA were also correlated with those of 2Py and 4Py (Fig. 4F, 4G, 4H).

Table 1 Baseline characteristics of the participants

Number	10
Age (year)	47.9 ± 6.0
Body weight (kg)	71.2 ± 5.7
Body mass index (kg/m^2)	23.7 ± 2.1
Waist circumference (cm)	88.0 ± 6.8
Glucose (mg/dL)	104.2 ± 7.9
Total cholesterol (mg/dL)	178.5 ± 35.2
Triglyceride (mg/dL)	101.8 ± 29.4
Aspartate aminotransferase (IU/L)	19.8 ± 4.7
Alanine aminotransferase (IU/L)	19.4 ± 6.9

Values are expressed as mean ± standard deviation

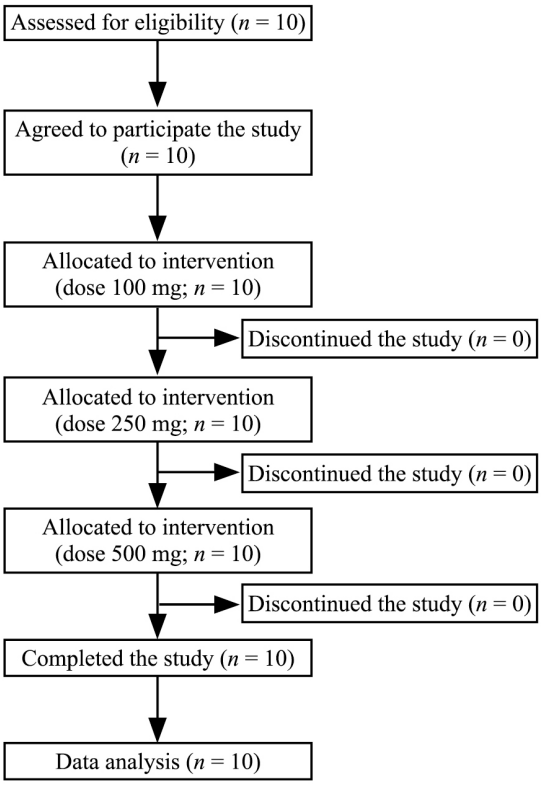

Fig. 1 Flowchart of the study

Discussion

In this study, we reported the safety of the single oral administration of NMN and the kinetics of NMN metabolites in healthy men. The recent studies have shown that the parenteral and enteral supplementation of NMN increased NAD^+ levels in various organs and improved age-associated disease conditions such as obesity, insulin resistance, and retinal degeneration in aged mice [4-6]. However, the safety and efficacy of NMN in humans have remained unclear.

The single oral administration of NMN did not cause any specific deleterious effects in healthy men, whereas nicotinamide has been known to induce nausea and flushing, thereby causing difficulty in using nicotinamide at high amounts to increase cellular NAD^+ [14]. Because we did not observe adverse events such as nausea, NMN would not increase nicotinamide blood levels enough to cause any adverse events in this study. Moreover, the administration of nicotinamide at a very high dose has been reported to induce hepatoxicity, but we did not observe any adverse effects of NMN on liver enzymes [14]. Whereas the plasma bilirubin levels increased, the glucose levels decreased significantly within the reference range. It is likely that these changes were due to fasting for 5 h [15]. All other parameters did not show any clinically significant changes at any single dose of NMN. Overall, NMN was well tolerated up to a single dose of 500 mg.

We previously reported that the light-dark cycle regulated the retinal expression of nicotinamide phosphoribosyltransferase (*Nampt*), which converts nicotinamide to NMN, and that photoreceptor-specific deletion of *Nampt* caused retinal NAD^+ deficiency and degeneration in mice [5, 16]. Moreover, the supplementation of NMN prevented the age-associated changes in the fundus as well as decline in retinal response and lacrimal gland functioning in 17-month-old C57BL/6N mice [4]. In this study, no single dose of NMN changed eye functions. The intraocular pressure tended to decrease after the administration of 100 mg NMN. However, it was reported to decrease in the postmeridian period so that the change could simply be due to diurnal variations [17].

In this study, we also performed preliminary evaluation of the sleep quality by PSQI score before and after the intervention with NMN. In mice, Sirt1, a mammalian NAD^+-dependent protein deacetylase, has been reported to be involved in the homeostatic regulation of sleep in the hypothalamus [18]. In our study, the indices did not show any significant differences by the intervention.

We showed that 2Py and 4Py levels significantly increased and their incremental areas above the baseline were increased in a dose-dependent manner. A previous study reported that the incremental changes in NAD^+ level were correlated with those in MNA and 2Py levels in peripheral blood mononuclear cells in healthy subject after the administration of NR [19]. The finding supports the speculation that the administration of NMN increased tissue NAD^+ and plasma metabolites such as MNA, 2Py, and 4Py in this study. The levels of 2Py and 4Py at baseline in our study were consistent with those in a previous study reporting that the mean serum concentrations of 2Py and 4Py were 830 nM and 260 nM, respectively, in healthy subjects [20]. In addition, we found that the

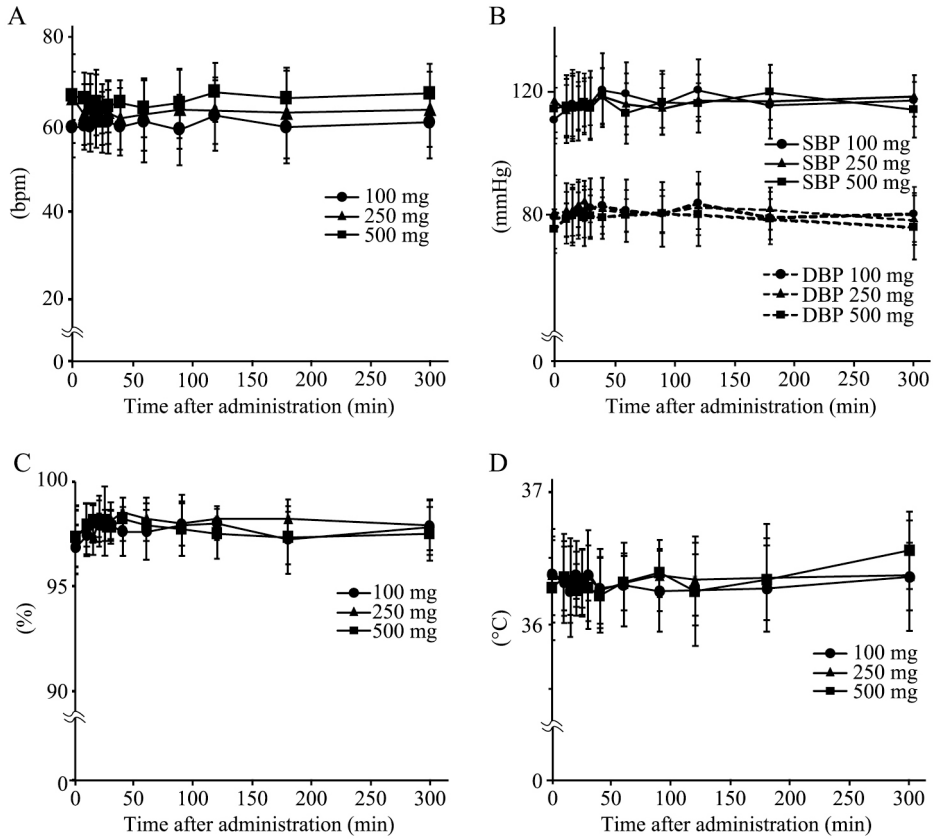

Fig. 2 Changes in clinical parameters after the oral administration of NMN

Nicotinamide mononucleotide (NMN) was orally administered to 10 healthy men and the clinical parameters were measured for 5 h. Heart rate (A), blood pressures (B), oxygen saturation (C), and body temperature (D) are indicated. $N = 10$. Data are expressed as mean ± standard deviation. Circle, triangle, and square indicate 100, 250, and 500 mg of NMN, respectively. Solid lines and dot lines represent systolic and diastolic blood pressures, respectively. SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table 2 Ophthalmic parameters before and after NMN administration

NMN dose (mg)	100		500	
Time point	Before	After	Before	After
BCVA (logMAR)	–0.079	–0.079	–0.079	–0.079
Functional VA (logMAR)	0.043 ± 0.14	0.065 ± 0.11	0.052 ± 0.11	0.078 ± 0.09
Intraocular pressure (mmHg)	12.63 ± 1.63	11.63 ± 2.09	12.87 ± 2.28	12.43 ± 1.68
Tear break-up time (sec)	6.4 ± 3.8	6.5 ± 3.8	6.8 ± 3.5	6.6 ± 3.2
Tear function test (mm)	6.4 ± 4.0	5.5 ± 3.5	6.1 ± 4.7	5.8 ± 4.7
Critical flicker frequency (Hz)	49.6 ± 4.0	49.8 ± 3.3	49.5 ± 4.9	48.9 ± 5.6
Corneal endothelial density (cells/mm^2)	2,754 ± 228	2,713 ± 235	2,748 ± 231	2,718 ± 236
Corneal thickness (μm)	541 ± 18	540 ± 19	542 ± 18	541 ± 19

Values are expressed as mean ± standard deviation. BCVA, best-corrected visual acuity; MAR, minimum angle of resolution; Functional VA, functional visual acuity.

incremental areas above the baseline for 300 min in 2Py were strongly correlated with those in 4Py and the ratio of 2Py to 4Py was constant among the subjects. These findings indicated that administered NMN was degraded and converted to 2Py and 4Py proportionally in the healthy subjects. However, the incremental areas in 2Py and 4Py varied largely by the subjects in this study. The difference may be explained by an efficiency of absorption and metabolism of NMN, although the precise kinetics of NMN were not evaluated in this study.

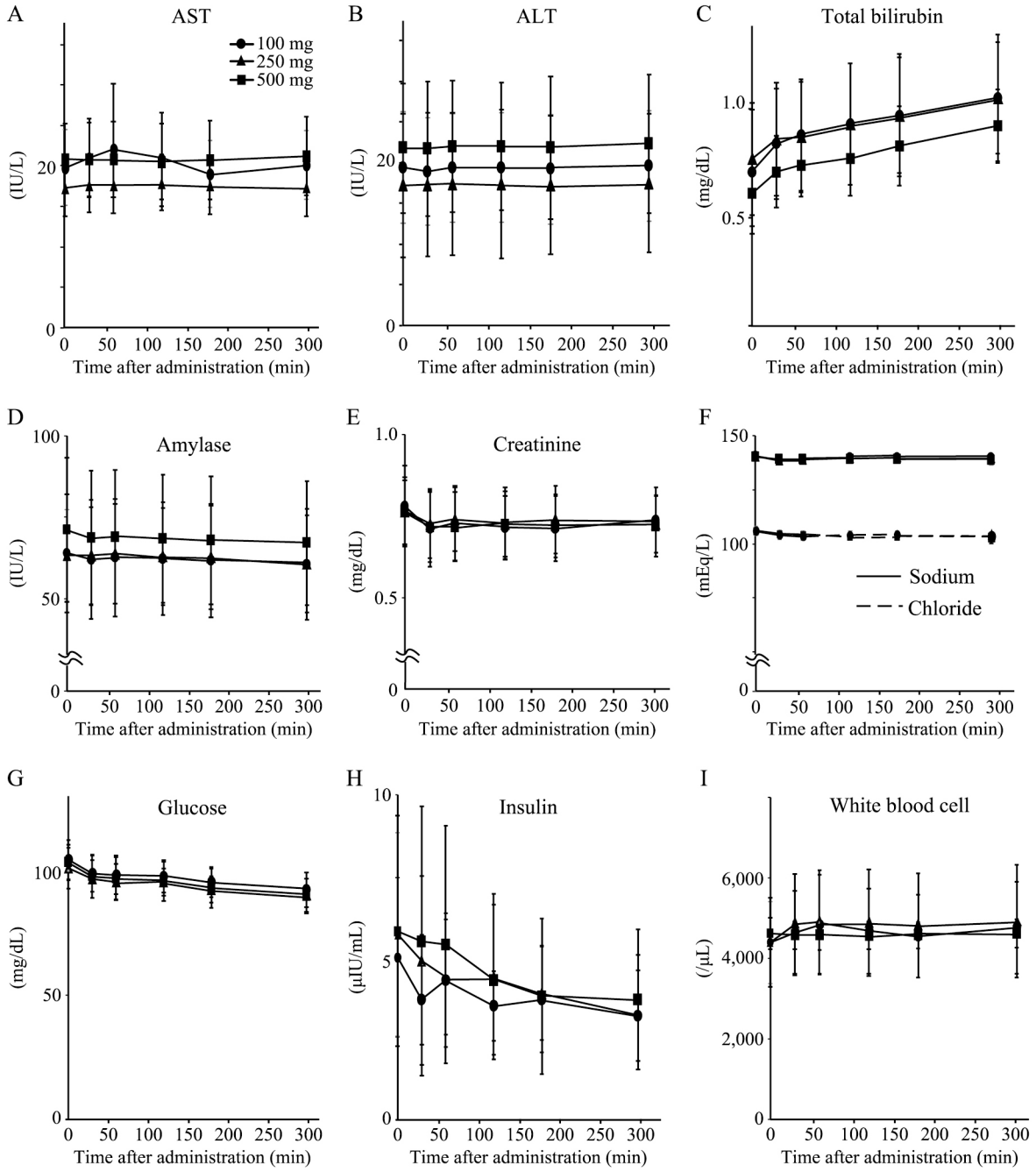

Fig. 3 Changes in serum parameters after the oral administration of NMN

NMN was orally administered to 10 healthy men and the serum parameters were measured for 5 h. Serum parameters included aspartate transaminase (AST) (A), alanine transaminase (ALT) (B), total bilirubin (C), amylase (D), creatinine (E), sodium and chloride (F), glucose (G), and insulin (H) levels, and white blood cell count (I). $N = 8–10$. Data are presented as mean ± standard deviation. Circle, triangle, and square indicate 100, 250, and 500 mg of NMN, respectively.

Unfortunately, we failed to detect NMN in plasma samples in this study, most likely because freezing plasma samples before extraction might have caused the degradation of NMN. The procedure of blood sampling could have also contributed to the failure of the detection of NMN, because the recent reports showed that NMN was degraded very rapidly in blood that was handled above –80°C [7, 21].

There are several limitations to this study. First, the study was not a placebo-controlled study. Therefore, it was not clear if the alteration in clinical and laboratory data was due to the administration of NMN or fasting for 5 h. In fact, the decrease in blood glucose level and the increase in serum bilirubin level were consistent with the

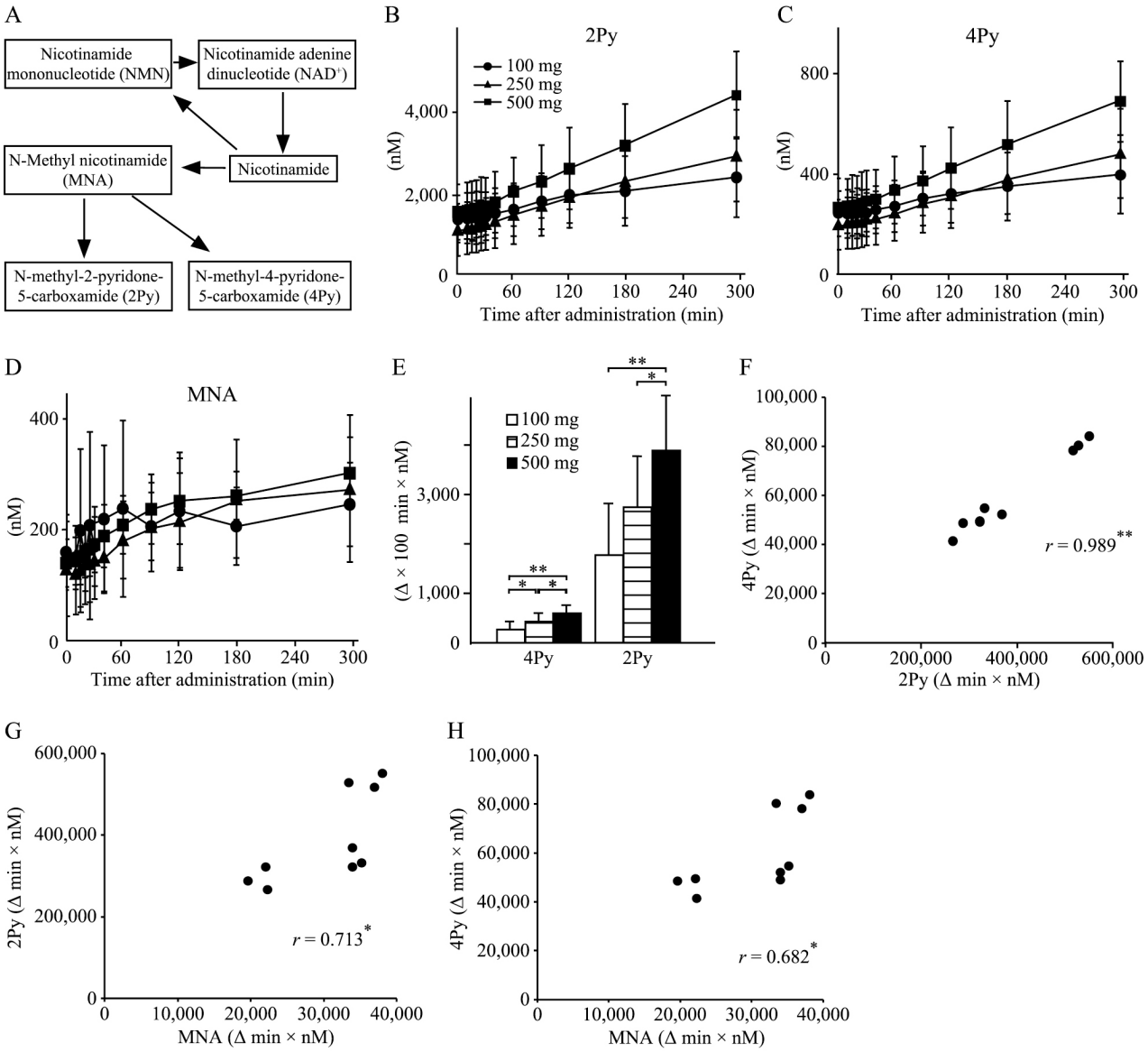

Fig. 4 Changes in plasma levels of NMN metabolites after the oral administration of NMN

NMN was orally administered to 10 healthy men and the plasma concentration of N-methyl-2-pyridone-5-carboxamide (2Py), N-methyl-4-pyridone-5-carboxamide (4Py), and N-methyl nicotinamide (MNA) were measured for 300 min. The metabolic pathway of NMN is shown (A). The plasma concentrations of 2Py (B), 4Py (C), and MNA (D) are shown. $N = 7–10$. Data are presented as mean ± standard deviation. Circle, triangle, and square indicate 100, 250, and 500 mg of NMN, respectively. The incremental area under the curve was calculated by multiplying the interval time between two points by the difference between the value and the baseline. The areas for 300 min are shown for 2Py and 4Py (E). White boxes, white boxes with lines, and filled boxes represent 100, 250, and 500 mg of NMN, respectively. Data are presented as mean ± standard deviation. $*p < 0.05$, $**p < 0.01$. The correlation of the incremental area under the curve for 300 min between 2Py and 4Py (F), MNA and 2Py (G), and MNA and 4Py (H) in the group with 500 mg NMN are shown, $*p < 0.05$, $**p < 0.01$

findings frequently observed in healthy men after fasting [15]. Nonetheless, the dose-dependent increases in 2Py and 4Py levels in plasma samples supported the dose-dependent uptake of NMN in the subjects. Second, the long-term administration of NMN should be conducted to further investigate the safety and the efficacy of NMN as a new treatment for age-associated disorders. Third, tissue NAD^+ and plasma NMN levels were not measured in this study. Our next clinical study to evaluate plasma NMN levels and NAD^+ levels in peripheral blood mononuclear cells is now in progress. If possible, biopsy samples from tissues such as the adipose tissue and skeletal muscle need to be analyzed for tissue NMN and NAD^+ levels.

In conclusion, the single oral administration of NMN up to 500 mg was safe and well-tolerated in healthy men

without causing any significant deleterious effects. Thus, oral administration of NMN is feasible and could be a therapeutic strategy to replenish cellular NAD^+ levels to mitigate aging-related functional disorders in humans. The pharmacokinetics of NMN and NAD^+ in the plasma and tissues need to be investigated in future studies.

Acknowledgments

This research was also supported by internal budgets from the Institutes, including Program for the Advancement of Research in Core Projects on Longevity of Keio University Global Research Institute from Keio University to H.I., H.O., K.T., and M.Y.

The authors thank Professor Samuel Klein and Professor Rajendra Apte in Washington University School of Medicine for their supports for the study.

Disclosure

S.I. is an inventor on patents about the use of NMN (PCT/US2014/30920) and about the Slc12a8 NMN transporter (PCT/US2018/46233), whose applicant is Washington University and which have been licensed by MetroBiotech (U.S.A.) and Teijin Limited (Japan), respectively. S.S., T.O., and H.Y. are employed by Shionogi & Co., Ltd. H.I. received research funding from Oriental Yeast Co., Ltd.

References

1. Imai S, Guarente L (2014) NAD^+ and sirtuins in aging and disease. *Trends Cell Biol* 24: 464–471.
2. Yoshino J, Baur JA, Imai S (2018) NAD^+ intermediates: the biology and therapeutic potential of NMN and NR. *Cell Metab* 27: 513–528.
3. Hasegawa K, Wakino S, Simic P, Sakamaki Y, Minakuchi H, *et al.* (2013) Renal tubular Sirt1 attenuates diabetic albuminuria by epigenetically suppressing Claudin-1 overexpression in podocytes. *Nat Med* 19: 1496–1504.
4. Mills KF, Yoshida S, Stein LR, Grozio A, Kubota S, *et al.* (2016) Long-term administration of nicotinamide mononucleotide mitigates age-associated physiological decline in mice. *Cell Metab* 24: 795–806.
5. Lin JB, Kubota S, Ban N, Yoshida M, Santeford A, *et al.* (2016) NAMPT-mediated NAD^+ biosynthesis is essential for vision in mice. *Cell Rep* 17: 69–85.
6. Yoshino J, Mills KF, Yoon MJ, Imai S (2011) Nicotinamide mononucleotide, a key NAD^+ intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice. *Cell Metab* 14: 528–536.
7. Grozio A, Mills KF, Yoshino J, Bruzzone S, Sociali G, *et al.* (2019) Slc12a8 is a nicotinamide mononucleotide transporter. *Nat Metab* 1: 47–57.
8. Masuda H, Sakamoto M, Irie J, Kitaoka A, Shiono K, *et al.* (2008) Comparison of twice-daily injections of biphasic insulin lispro and basal-bolus therapy: glycaemic control and quality-of-life of insulin-naive type 2 diabetic patients. *Diabetes Obes Metab* 10: 1261–1265.
9. Petley A, Macklin B, Renwick AG, Wilkin TJ (1995) The pharmacokinetics of nicotinamide in humans and rodents. *Diabetes* 44: 152–155.
10. Tanaka K, Saisho Y, Manesso E, Tanaka M, Meguro S, *et al.* (2015) Effects of liraglutide monotherapy on beta cell function and pancreatic enzymes compared with metformin in Japanese overweight/obese patients with type 2 diabetes mellitus: a subpopulation analysis of the KIND-LM randomized trial. *Clin Drug Investig* 35: 675–684.
11. Dogru M, Ishida K, Matsumoto Y, Goto E, Ishioka M, *et al.* (2006) Strip meniscometry: a new and simple method of tear meniscus evaluation. *Invest Ophthalmol Vis Sci* 47: 1895–1901.
12. Buysse DJ, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ (1989) The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res* 28: 193–213.
13. Komatsu M, Kanda T, Urai H, Kurokochi A, Kitahama R, *et al.* (2018) NNMT activation can contribute to the development of fatty liver disease by modulating the NAD^+ metabolism. *Sci Rep* 8: 8637.
14. Knip M, Douek IF, Moore WP, Gillmor HA, McLean AE, *et al.* (2000) Safety of high-dose nicotinamide: a review. *Diabetologia* 43: 1337–1345.
15. Griffin PM, Elliott SL, Manton KJ (2014) Fasting increases serum bilirubin levels in clinically normal, healthy males but not females: a retrospective study from phase I clinical trial participants. *J Clin Pathol* 67: 529–534.
16. Ban N, Ozawa Y, Inaba T, Miyake S, Watanabe M, *et al.* (2013) Light-dark condition regulates sirtuin mRNA levels in the retina. *Exp Gerontol* 48: 1212–1217.
17. Liu JH, Kripke DF, Twa MD, Hoffman RE, Mansberger SL, *et al.* (1999) Twenty-four-hour pattern of intraocular pressure in the aging population. *Invest Ophthalmol Vis Sci* 40: 2912–2917.
18. Satoh A, Brace CS, Rensing N, Cliften P, Wozniak DF, *et al.* (2013) Sirt1 extends life span and delays aging in mice through the regulation of Nk2 homeobox 1 in the DMH and LH. *Cell Metab* 18: 416–430.
19. Trammell SA, Schmidt MS, Weidemann BJ, Redpath P, Jaksch F, *et al.* (2016) Nicotinamide riboside is uniquely and orally bioavailable in mice and humans. *Nat Commun* 7: 12948.
20. Rutkowski B, Slominska E, Szolkiewicz M, Smolenski RT, Striley C, *et al.* (2003) N-methyl-2-pyridone-5-carboxamide: a novel uremic toxin? *Kidney Int* Suppl 84: S19–S21.
21. Liu L, Su X, Quinn WJ 3rd, Hui S, Krukenberg K, *et al.* (2018) Quantitative analysis of NAD synthesis-breakdown fluxes. *Cell Metab* 27: 1067–1080.