Intravenous Uptake of NMN is Safely Metabolize and Increases NAD⁺ Levels in Healthy Subjects

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Abstract

Increasing nicotinamide adenine dinucleotide (NAD$^+$) levels alleviates the progression of age-related diseases and promotes healthy life expectancy. Several studies have demonstrated that NAD$^+$ levels can be efficiently replenished via intake of nicotinamide mononucleotide (NMN), and the safety of oral ingestion of NMN has been confirmed in recent clinical trials on healthy Japanese men. However, the efficacy and safety of intravenous NMN administration in humans remains unclear. Therefore, we verified the safety of NMN via venous intake in 10 healthy volunteers. Intravenous administration of NMN did not affect electrocardiograms, pulse, and blood pressure, nor did it affect metabolic markers in the liver, heart, pancreas, and kidneys. Furthermore, NMN administration not only significantly increased blood NAD$^+$ levels without damaging blood cells, but also significantly improved sleep quality. Therefore, our findings indicate that intravenous NMN administration in humans is safe and beneficial for the prevention of aging-related diseases.

1. Introduction

The risk of type II diabetes mellitus, Alzheimer-type dementia, and cardiomegaly increases with age [1–3]; therefore, the suppression of aging is sought after to prevent aging-related disorders. The use of nicotinamide adenine dinucleotide (NAD$^+$) is attracting attention in the suppression of aging [4]. NAD$^+$, which is highly soluble in water, cannot penetrate the cell membrane [5]; no dedicated transporter has been identified in mammals. Hence, niacin compounds, such as nicotinic acid (NA) and nicotinamide (NAM), have long been used as nutrients involved in the synthesis of NAD$^+$ [6]. NAM is produced in the process of NAD$^+$ utilization by NAD-dependent enzymes, such as sirtuins, poly (ADP-ribose) polymerases (PARP), and cluster of differentiation 38 (CD38) [7]; the process is enzymatically catalyzed by nicotinamide phosphoribosyltransferase (NAMPT) and NAM is converted to NMN [8], after which NAD$^+$ is synthesized from NMN and adenosine triphosphate (ATP) which is catalyzed by nicotinamide mononucleotide adenylyltransferase (NMNAT) [9]. Previous studies have demonstrated that NAMPT is the rate-limiting enzyme in NAD$^+$ synthesis and its expression level decrease with age [10]. NAD$^+$ is efficiently synthesized via NMN utilization, because NMN is rapidly incorporated into the cytoplasm via a specific transporter Slc12a8 [11].

Imai et al. orally administered NMN at a dose of 300 mg/kg to mice aged 5–17 months for 1 year, resulting in a 9% weight loss despite higher dietary intake than that in the control group; they found that not only is the aging-related increase in cholesterol level suppressed, but there are no significant changes in aging-related gene expression in the skeletal muscle, liver, and fat [12]. Recently, this group has conducted clinical trials on oral administration of NMN to healthy subjects, demonstrating that NMN is safely metabolized in the blood without affecting glucose metabolism, lipid metabolism, liver, kidneys, and leukocytes [13]. Intravenous NMN administration has not been evaluated previously; however, based on the findings of these studies, we speculated that NMN can be safely provided via intravenous administration, because it is a metabolite produced in the body [9].

NAD$^+$ has been studied as a coenzyme required for the activation of sirtuin family proteins that have anti-aging function [14]. Among the members of the sirtuin family, sirtuin 1 (SIRT1) has been studied as a factor that directly prevents aging via glucose metabolism [15], insulin secretion [16], lipid metabolism [17], angiogenesis [18], and elimination of reactive oxygen species (ROS) [19]. Furthermore, SIRT1 controls the circadian rhythm by binding to circadian locomotor output cycles kaput (CLOCK)-brain and muscle Arnt-like protein-1(BMAL1) and period circadian protein 1 (PER1) in an NAD$^+$-dependent manner, and deacetylates these proteins to promote their degradation [20]. Therefore, we evaluated NAD$^+$ and SIRT1 activities in blood cells and the degree of fatigue and sleep quality after intravenous administration of NMN.

2. Results

There were no significant changes in body weight or body mass index before and 5 h after intravenous administration of NMN. Fatigue was found to slightly improve one month after intravenous NMN administration, although there was no significant difference compared to that at before administration. Sleep quality improved significantly compared to that at one month before administration (Table 1). Analysis of body temperature, systolic blood pressure, diastolic blood pressure, pulse, and oxygen saturation at 0.5, 1, 2, 3, and 5 h after intravenous injection of NMN showed no significant differences in all parameters before and after administration (Fig. 1a-d). No significant effect on plasma protein level and glucose metabolism was observed at 0.5, 1, 2, 3, and 5 h after intravenous administration of NMN (Fig. 1e-i). Although low-density lipoprotein, high-density lipoprotein, and total cholesterol levels did not significantly differ before and 0.5, 1, 2, 3, and 5 h after intravenous administration of NMN (Fig. 1k-m), the triglyceride (TG) levels decreased significantly from 0.5 to 5 h after administration, and after 5 h, there was a slight tendency to return to the value before administration, notwithstanding a significant difference (Fig. 1j).

Table 1
Characteristics of subjects including physical findings, fatigue, and sleep after nicotinamide mononucleotide administration

Variable	Pretreatment		Post-treatment	
Age (years)	43.4	(±12.6)	43.4	(±12.6)
Height (cm)	164.7	(±7.4)	164.6	(±7.5)
Weight (kg)	66.1	(±9.6)	65.7	(±9.6)
Body mass index (kg/m^2)	24.4	(±3.5)	24.3	(±3.4)
Visual Analogue Scale	5.7	(±2.0)	3.9	(±2.1)
Pittsburgh Sleep Quality Index	8.1	(±2.3)	4.6	(±2.5) **
The results were analyzed using Welch's t-test and are expressed as the mean ± standard deviation (n = 10, ** p < 0.01).				

We investigated the pharmacokinetics specific to metabolic markers of the liver, heart, pancreas, and kidneys after intravenous administration of NMN, because drugs administered intravenously can rapidly reach organs throughout the body, and may cause burden on specific organs even if the substances are safe for oral administration. Liver-, pancreas-, heart-, and kidney-related metabolism marker levels in the plasma measured at 0.5, 1, 2, 3, and 5 h after intravenous injection of NMN showed no significant differences compared to those before administration (Fig. 2). Similarly, 0.5, 1, 2, 3, and 5 h after intravenous injection of NMN, there was no significant effect on plasma electrolytes (Supplemental Fig. 1). Furthermore, analysis of red blood cells, white blood cells, platelets, and related markers in the blood showed no significant change before and at 0.5, 1, 2, 3, and 5 h after intravenous administration of NMN (Fig. 3).

The total amount of NAD^+/NADH and the ratio of NAD^+ to NADH in the blood was measured to determine whether intravenously administered NMN could efficiently increase the amount of NAD^+ in blood cells. NAD^+/NADH levels in blood measured at 0.5, 1, 2, 3, and 5 h after intravenous NMN administration showed a significant increase in NAD^+ levels from 0.5 to 3 h compared to that at before administration (Fig. 4a), and a significant increase in total NAD^+ level, except at 4 h after administration (Fig. 4b). In contrast, NADH levels could not be measured accurately because the measured values varied considerably (Fig. 4c). To suppress aging, NAD^+ should be utilized by sirtuin family proteins as a coenzyme during its increase in level [14]; therefore, we sought to determine whether SIRT1, which is considered to be particularly important for aging, is activated via intravenous administration of NMN. The activation of nuclear SIRT1 also increased, similar to the trend of NAD^+ synthesis, after intravenous NMN administration (Fig. 4e); however, no significant difference was observed because of the large variation in the measured values. Cytosolic SIRT1 activation showed almost no change before and after intravenous administration of NMN (Fig. 4f).

Analysis performed by a physician indicated that there were no abnormalities or general disorders in administration site conditions, urinalysis, electrocardiograms, and chest radiographs before and after intravenous administration of NMN (Supplemental Tables 1, 2, 3).

3. Discussion

Accumulating evidence has demonstrated that the levels of NAD^+, which is essential for vital activity, declines with age in various organs and tissues throughout the body [10]; thus, intracellular enhancement of NAD^+ synthesis helps prevent aging-related diseases such as diabetes, Alzheimer's disease, and cardiopathy [21]. NAD^+ is intracellularly generated using tryptophan, niacin, nicotinamide riboside (NR), and NMN [8]. However, these molecules are not effective in increasing NAD^+ levels because tryptophan is also used in neurotransmission and protein synthesis [22], and niacin supplementation is associated with side effects, such as flushing and liver damage due to overconsumption [23]. Consequently, research has mainly focused on using NR and NMN as substrates for NAD^+ synthesis. Recent studies have demonstrated that NR is mostly decomposed by intestinal bacteria when orally administered to humans

[24]; however, NMN is rapidly released into the blood via uptake by intestinal epithelial cells via Scl12a8 [11]. We hypothesized that NMN supplementation increased NAD^+ levels more efficiently than that obtained using NR, even when administered intravenously, because Scl12a8 is expressed in almost every cell as well as intestinal epithelial cells.

NMN, which is a precursor of NAD^+, has been proven to increase NAD^+ levels via uptake into the body in both rodents and humans [13, 25]. Although NMN has been studied using various administration methods such as oral, intraperitoneal injection, and intravenous injection to enhance NAD^+ synthesis [8], the efficacy and safety of intravenous administration in humans have not yet been verified. Our study showed that a single intravenous dose of 300 mg of NMN enhanced NAD^+ activity in blood cells without affecting these cells, including erythrocytes, leukocytes, and platelets, and major markers in the liver, heart, pancreas, and kidneys.

We hypothesized that NMN-mediated activation of mitochondria relieves fatigue because NAD^+ plays an important role in oxidative phosphorylation involved in ATP synthesis in mitochondria [26]. In this study, intravenous NMN administration to healthy subjects showed a tendency for fatigue recovery, although the difference was not significant (Table 1). We expect that a considerable recovery from fatigue will be obtained in studies on intravenous NMN administration in relatively larger cohorts.

NAD^+ is involved in the regulation of circadian rhythms, and an increase in intracellular NAD^+ levels via intravenous NMN administration is expected to improve sleep quality [20]. Based on the Pittsburgh Sleep Quality Index (PSQI) results, we found that sleep quality improved significantly at one month after intravenous NMN administration (Table 1). We speculate that oral administration of NMN did not significantly improve sleep quality in a previous study [13], whereas intravenous administration improved sleep quality in the present study by increasing NAD^+ levels in the suprachiasmatic nucleus of the hypothalamus and spreading throughout the body before NMN could be metabolized in the liver.

Body temperature, blood pressure, pulse, and oxygen saturation were not significantly affected via intravenous administration of NMN (Fig. 1a-d). There was no change in the levels of metabolic markers of sugars and proteins after intravenous NMN administration (Fig. 1e-i); however, there was a significant decrease in TG level even though there was no change in cholesterol level (Fig. 1j-m). Several studies have demonstrated that NAM is produced when NAD^+ is used as a coenzyme by sirtuin family proteins after NAD^+ is synthesized from NMN [14]. Previous studies have suggested that NAM activates the NA-specific receptor GPR109A when hydrolyzed to yield NA [27]. GPR109A is a predominantly expressed G protein symbiotic receptor in fat cells, and GPR109A is activated in adipocytes via NA to suppress the decomposition of TG and reduce the amount of free fatty acids (FFAs) released into the blood; subsequently, FFA in blood is taken up by the liver and TG is synthesized. The activation of GPR109A by NA leads to the suppression of TG synthesis in blood [28]. Therefore, we speculate that NAM produced as a by-product of intravenously administered NMN metabolism mediated the suppression of TG synthesis. No reduction in TG levels was observed in previous clinical trials on oral administration of NMN [13],

suggesting that NMN may be metabolized via different pathways between oral and intravenous administration. This is thought to be caused by NMN being metabolized in cells throughout the body by avoiding the first passage through the liver via intravenous administration; the proportion of the produced GPR109A-bound NA on the membrane of adipocytes is increased. A previous study reported that the increase in TG levels in the plasma and liver of mice deficient in adipocyte-specific NAMPT1, an enzyme that synthesizes NMN from NAM, indicates that increased intracellular NMN synthesis plays a critical role in decreasing TG levels. Therefore, NMN is involved in the reduction of TG levels [29].

There was no noticeable change in levels of metabolic markers of any of the organs after intravenous NMN administration (Fig. 2–3). The amount of NAD^+ was significantly increased at NMN administration to 2 h after administration compared to that at before administration, and the total NAD^+ levels showed a significant increase except at 3 h after administration (Fig. 4a-b). In contrast, since the amount of NADH had a large experimental error and an accurate value could not be detected (Fig. 4c), the ratio of NAD^+ to NADH could not be determined accurately (Fig. 4d). The current clinical trial on intravenous NMN administration demonstrated that NMN helped increase the amount of NAD^+ in blood cells; however, the amount of NADH should be reevaluated in a relatively larger cohort. Although the experimental error was large and no significant increase was observed (Fig. 4e-f), nuclear SIRT1 tended to be activated 30 min to 1 h after NMN administration, similar to the increase in NAD^+ level (Fig. 4e).

In conclusion, our clinical study demonstrated that NMN administration at 300 mg is tolerated by humans because it does not cause significant damage to blood cells, liver, pancreas, heart, and kidneys when injected intravenously, and effectively increases the amount of NAD^+ in blood cells. In addition, intravenous administration of 300 mg of NMN may help prevent aging-related diseases such as diabetes, Alzheimer's disease, and heart disease, because our findings suggest that SIRT1 may be activated. Future studies should establish activation conditions for sirtuin family proteins, including SIRT1, following intravenous administration of NMN, and verify the safety of intravenous administration of NMN several times over a long period of time.

4. Methods

4.1. Study population

NMN was purchased from Nordeste (Tokyo, Japan). We conducted an open-label, single-arm exploratory study on 10 healthy subjects. This study was approved by Japanese Organization for Safety Assessment of Clinical Research (#20210623-02). The Tokyo Tsukishima Clinic recruited 10 healthy male and female subjects (age, 20–70) and obtained informed consent before the clinical trial which conducted in accordance with Ethical Guidelines for Medical and Health Research Involving Human Subjects s set by Ministry of Health, Labor and Welfare in Japan.

Individuals with history of disease diagnosis, malignant neoplasms, serious infections, psychiatric disorders, ophthalmic disorders, allergic disorders, and metabolic disorders were excluded from the study.

Subjects fasted for 12 h before intravenous administration of NMN until the end of the clinical trial, but were provided free hydration. Intravenous drip infusion was performed at 5 mL/min by dissolving 300 mg of NMN in 100 mL of saline and inserting an extension tube through a vein in the middle of the arm. The height and weight of the subjects were measured, and chest radiographs were obtained before and after intravenous administration of NMN. General disorders and administration site conditions were diagnosed by a physician according to CTCAE v5.0-JCOG, before and after intravenous administration of NMN. Body temperature, blood pressure, pulse, and oxygen saturation were measured at 0.5, 1, 2, 3, and 5 h before and after intravenous administration of NMN, and blood was collected at the same time. Blood collection tubes containing EDTA-2NA (Falco Biosystems, Kyoto, Japan) were used to obtain 17 mL of blood samples, and 12 mL of the collected blood was provided to FALCO Biosystems for hematological analysis. The remaining blood was stored at -80°C and used to measure blood NAD$^+$ levels and SIRT1 activation. Urine was collected at 1, 3, and 5 h before and after intravenous administration of NMN to the subjects, and urinary urobilinogen, protein, glucose, pH, and occult blood were measured at the Tokyo Tsukishima Clinic. The subjects were asked to complete a visual analog scale (VAS) survey to assess their fatigue and PSQI survey to assess their sleep quality before and one month after intravenous administration of NMN.

4.2. NAD$^+$/NAD assay

The amounts of NAD$^+$ and NADH in blood cells were assessed using the NAD/NADH Assay Kit-WST (Dojindo Laboratories, Kumamoto, Japan). Briefly, a 20 μL blood sample was dissolved in 300 μL extraction buffer, and a 250 μL of extract sample was transferred to an MWCO 10 K filtration tube for ultrafiltration. The obtained filtrate was transferred to two new Eppendorf tubes (100 μL each), and one of them was incubated at 60°C for 1 h. The unheated sample was used as the total NAD sample and the heated sample was used as the NADH sample. The absorbance of these samples was measured at 450 nm using a Varioskan plate reader (Thermo Fisher Scientific, Yokohama, Japan). The amount of NAD$^+$ was calculated by subtracting the amount of NADH from the total NAD amount.

4.3. SIRT assay

Nuclear proteins were extracted from blood cells using the LysoPureTM Nuclear and Cytoplasmic Extractor Kit (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan). Briefly, 500 μL of blood was added to 500 μL of initial buffer, incubated on ice for 10 min, vortexed, and centrifuged at 500 × g at 4°C for 10 min. The supernatant was transferred to a new Eppendorf tube as a cytosolic fraction; buffer 1 was added to the pellet again, and the pellet was vortexed and centrifuged at 500 g at 4°C for 10 min. The supernatant was discarded, and 250 μL of buffer 2 was added after which the mixture was vortexed and then incubated on ice for 30 min. The supernatant was collected as a nuclear extraction fraction after centrifugation at 2,000 × g at 4°C for 10 min.

The cytoplasmic and nuclear fractions were evaluated for SIRT1 activation using the CycLex® SIRT1/Sir2 Deacetylase Fluorometric Assay Kit Ver.2 (Medical & Biological Laboratories, Aichi, Japan). Briefly, 25 μL distilled water, 5 μL SIRT1 Assay Buffer, 5 μL Fluoro-Substrate Peptide, and 5 μL Developer

were added to a 50 μL nuclear extraction fraction sample and cytosol fraction sample in this order and mixed well, and the reaction was initiated by adding 50 μL enzyme sample following incubation at 37°C for 30 min before quenching the reaction using 20 μL of stop solution. The prepared samples were analyzed using a Fluoroskan microplate fluorometer (Thermo Fisher Scientific) at an excitation wavelength of 350 nm and an emission wavelength of 450 nm to detect SIRT1 activity.

4.4. Statistical analysis

All results are expressed as the mean ± standard deviation. Statistically significant differences between PSQI and VAS were analyzed using Welch's t-test, and other statistically significant differences were analyzed via one-way ANOVA with Bonferroni's post-test. The significance levels were set at $*p < 0.05$ and $**p < 0.01$.

Data Availability

All the data necessary to evaluate the conclusion of a paper is present in the paper and/or in the supplementary information. Additional data is available from the corresponding author [S.K.] on request.

Declarations

Acknowledgments

Author contributions

S.K. and M.I. designed and executed the experiments and wrote the manuscript. S.S. worked as a nurse at the study site. T.K. and Y.H. performed the NAD$^+$/NAD and SIRT assays. T.I., W.M., and A.G. supervised the project. All authors have reviewed and approved the final manuscript.

Additional information

Conflict of interest: S.K., M.I, S.S., T.K., Y.H., and T.I. are employed as researchers at StateArt Inc. W.M. and A.G. have no conflict of interest.

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Figures
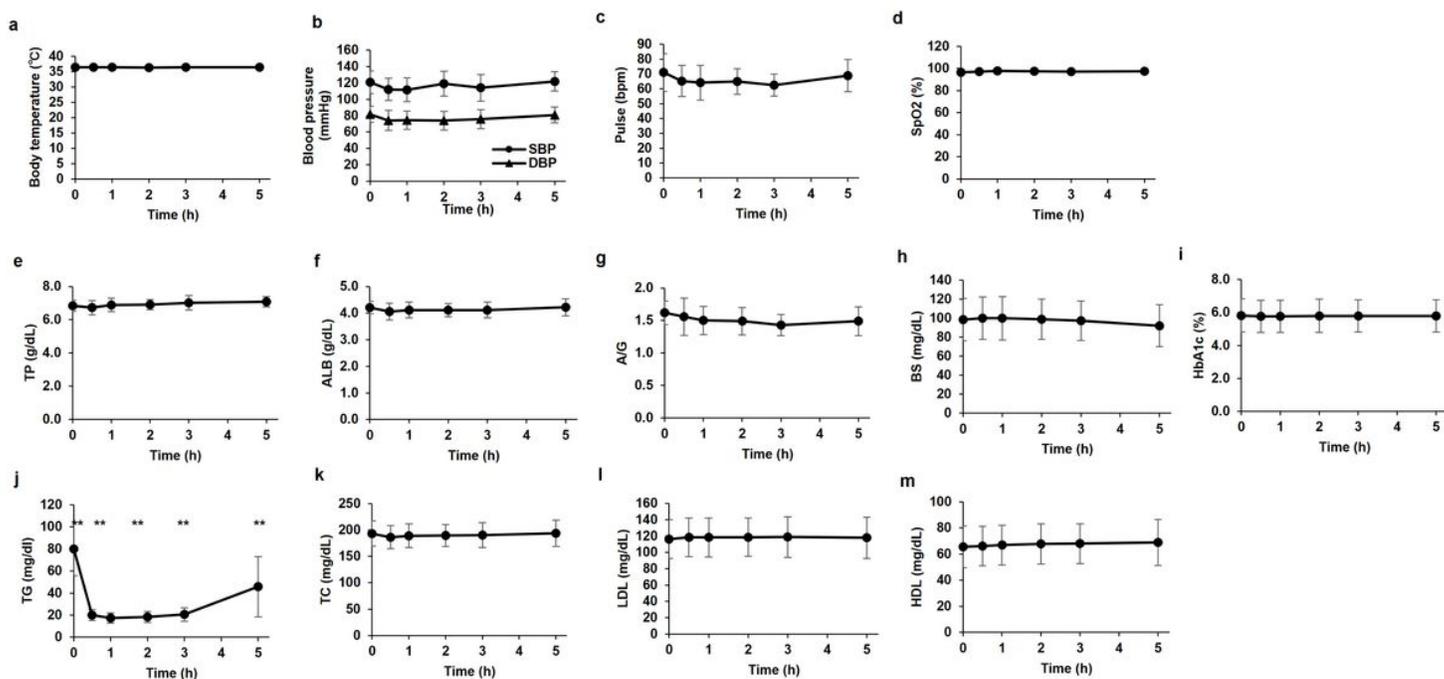
Figure 1

Changes in clinical parameters and sugar, lipid, and protein metabolism markers due to intravenous administration of NMN. (a) Body temperature, (b) blood pressure, (c) pulse, and (d) SpO2 were measured before and 0.5, 1, 2, 3, and 5 h after intravenous administration of NMN. Protein metabolism was evaluated in the plasma before and after intravenous NMN administration based on (e) TP, (f) ALB, and

(g) A/G; glucose metabolism was evaluated based on (h) BS and (i) HbA1c; lipid metabolism was evaluated based on (j) TG, (k) TC, (l) LDL, and (m) HDL. Data were analyzed via one-way analysis of variance with Bonferroni's post-test and are expressed as the mean ± standard deviation (n = 10, * p < 0.05, ** p < 0.01). NMN, nicotinamide mononucleotide; TP, total protein; ALB, albumin; BS, blood sugar; HbA1c, hemoglobin A1C; TG, triglyceride; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein, SpO2, oxygen saturation.
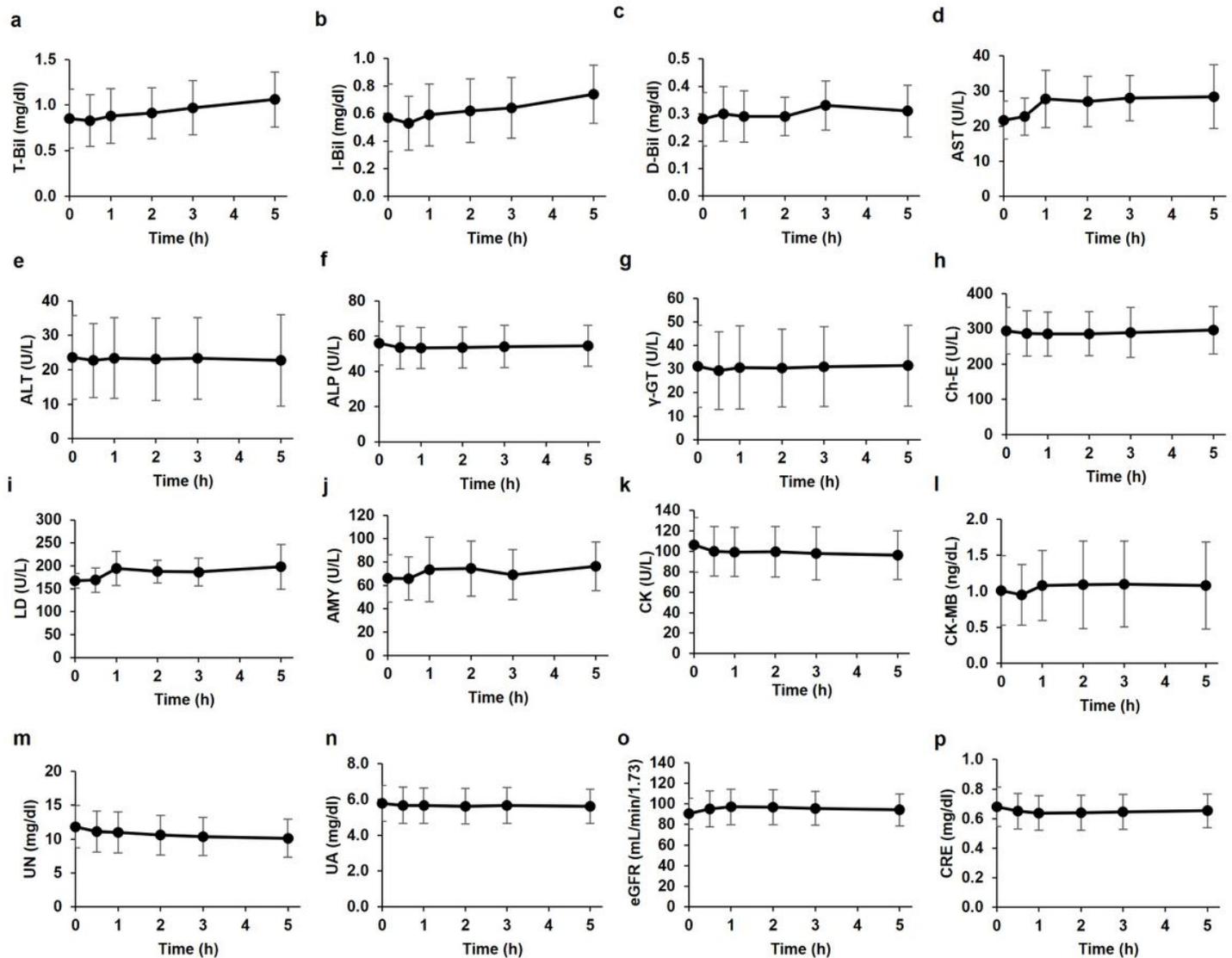
Figure 2

Effects of intravenous NMN administration on metabolic markers of liver, pancreas, heart and kidney. Levels of metabolic markers of the liver, pancreas, heart, and kidney were measured in plasma obtained from subjects before and 0.5, 1, 2, 3, and 5 h after intravenous NMN administration. The effects of intravenous NMN administration on liver metabolism were evaluated via (a) T-Bill, (b) I-Bill, (c) D-Bill, (d) AST, (e) ALT, (f) ALP, (g) γ-GT, (h) Ch-E, and (i) LD; pancreatic metabolism was evaluated based on (j) AMY; cardiac metabolism was observed based on (k) CK and (l) CK-MB; renal metabolism was evaluated

based on (m) UN, (n) UA, (o) eGFR, and (p) CRE. The results are expressed as the mean ± standard deviation (n = 10). NMN, nicotinamide mononucleotide; T-Bill, total bilirubin; I-Bill, indirect bilirubin; D-Bill, direct bilirubin; AST, aspartate transaminase; ALT, alanine aminotransferase; ALP, alanine phosphotransferase; γ-GT, gamma-glutamyl transferase; LD, lactate dehydrogenase; Ch-E, cholinesterase; AMY, amylase; CK, creatine kinase; CK-MB, CK myocardial band; UN, urea nitrogen; CRE, creatinine; UA, urinanalysis; eGFR, estimated glomerular filtration rate.
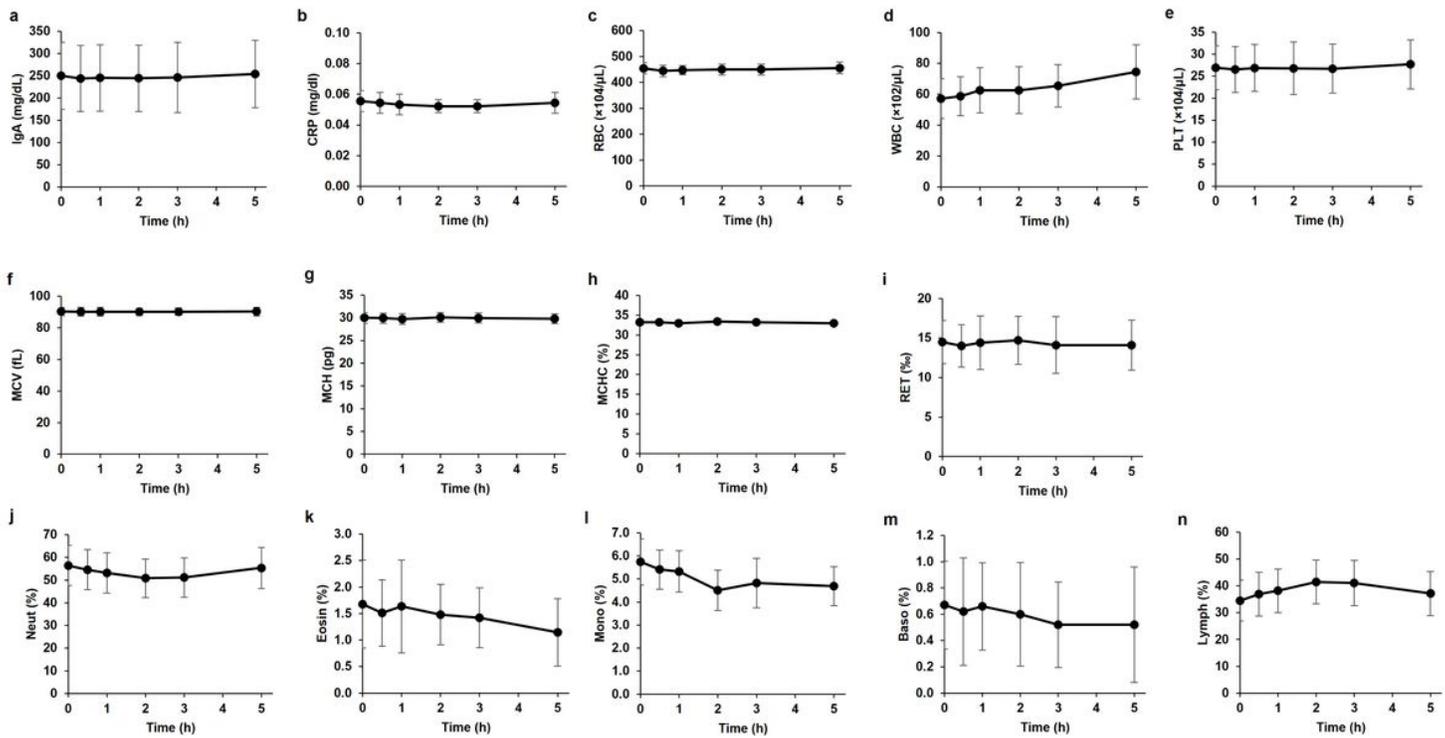
Figure 3

Effects of intravenous NMN administration on immune markers and blood cells.

Immune markers (a) IgA and (b) CRP were measured in plasma obtained from patients before and 0.5, 1, 2, 3, and 5 h after intravenous NMN administration. Blood cells, red blood cell markers, and leukocyte fractions were analyzed in blood obtained from patients before and 0.5, 1, 2, 3, and 5 h after intravenous NMN administration. The damage to blood cells was measured based on the number of (c) RBC, (d) WBC, and (e) PLT; the effect of NMN administration on erythrocytes was observed based on (f) MCV, (g) MCH, (h) MCHC, and (i) RET; the effect of NMN administration on the leukocyte fraction was observed in (j) Neut, (k) Eosin, (l) Mono, (m) Basso, and (n) Lymph. The results are expressed as the mean ± standard deviation (n = 10). NMN, nicotinamide mononucleotide; IgA, immunoglobulin A; CRP, C-reactive protein; RBC, red blood cell; WBC, white blood cell; PLT, platelet; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RET, reticulocyte; Neut, neutrophil; Eosin, eosinophil; Mono, monocyte; Basso, basophil; Lymph, lymphocyte.
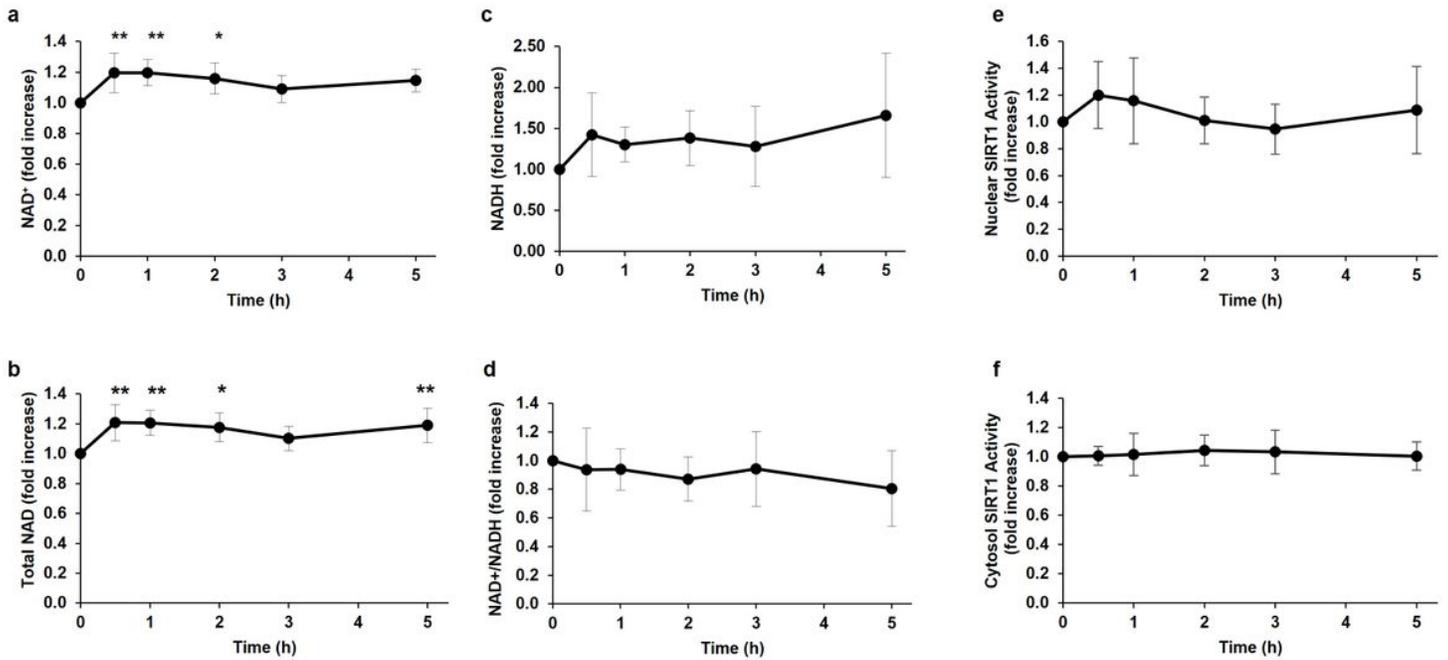
Figure 4

Effect of NMN administration on increased NAD level and SIRT1 activation. The amount of NAD and the activity of SIRT were measured in blood obtained from patients before and 0.5, 1, 2, 3, and 5 h after intravenous administration of NMN. The results were analyzed via one-way analysis of variance with Bonferroni's post-test and are expressed as the mean ± standard deviation (n = 10, * $p < 0.05$, ** $p < 0.01$). NMN, nicotinamide mononucleotide; NAD, nicotinamide adenine dinucleotide; NADH, reduced nicotinamide adenine dinucleotide; SIRT1, sirtuin 1.

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