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Original article

Clinical evaluation of changes in biomarkers by oral intake of NMN

Yuji Morita [1, 2], Hiromi Izawa [1, 3], Atsuyuki Hirano [1], Eriko Mayumi [1], Seigo Isozaki [2, 4], Yoshikazu Yonei [2]

1) Renais Clinic Nihonbashi, Tokyo, Japan
2) Anti-aging Medical Research Center and Glycative Stress Research Center, Faculty of Life and Medical Sciences. Doshisha University, Kyoto, Japan
3) Jingu-Gaien Woman Life Clinic, Tokyo, Japan
4) Antiaging-Bank Co., Ltd, Tokyo, Japan

Abstract

***Purpose*:** The "NAD World" (proposed by Professor Shin-Ichiro Imai of the University of Washington), in which the control of aging, lifespan, and metabolism is systemically integrated through NAD (nicotinamide adenine dinucleotide), has attracted worldwide attention and is being studied from various angles, however, there are few reports of clinical studies in humans. In this study, we investigate the changes in various biomarkers in humans after oral intake of nicotinamide mononucleotide (NMN), a precursor of NAD, and evaluate its clinical significance.

***Methods*:** Based on the approval to start the study at the ethics review, 17 postmenopausal women (mean age 55.0 years) without underlying diseases who agreed to participate in the study were subjects and received oral intake of 300 mg/day of pure NMN for eight weeks. The examination items included body measurements, basal metabolic rate, blood pressure, grip strength, glycation level (AF value), blood biochemical tests, various hormones, blood *SIRT1* mRNA expression and NMN, NAD and NAM levels, immunological tests (aging, T cell subset including exhausted cells), and skin VAS (visual analog scale). Results were statistically analyzed and compared at zero and eight weeks (blood biochemistry tests were performed at four weeks to confirm safety). In addition, we asked the patients to keep a diary during the course of the study to monitor their NMN intake, their bodily sensation, and side effects.

***Results*:** One of the 17 subjects dropped out at the end of four weeks due to persistent mild headache. The results showed significant changes in NAM (nicotinamide/vitamin B3) from 45.2 to 164.7 ($p < 0.001$), adiponectin from 13.6 to 16.2 ($p = 0.004$), and skin VAS score (6/7 items, $p < 0.001$ to $= 0.001$) before and after intake. Significant differences were also observed for BMI, AF levels, platelets, HbA1c, HDL-C, amylase, DHEA-s, NAD, and narrowly defined regulatory T cells ($p < 0.05$ above). In addition, all subjects showed a positive bodily sensation with skin, sleep, and fatigue. There were no problems with subjective symptoms or laboratory data in the 16 who completed the study.

***Conclusion*:** NMN 300 mg/day orally for eight weeks showed no safety issues and favorable changes in many biomarkers, suggesting that NMN, a member of the NAD world, may be a promising nutritional material for aging and metabolic control in humans.

***KEY WORDS*:** nicotinamide mononucleotide (NMN), nicotinamide adenine dinucleotide (NAD), sirtuin, biomarker, oral intake, clinical trial

Corresponding to: Yuji Morita, MD
Renais Clinic Nihonbashi
3-3-1 Nihonbashi-Muromachi, Chuo, Tokyo 103-0022, Japan
TEL: +81-3-6665-0888 e-mail: y-morita@renais-clinic.com
Co-authors; Izawa H, izawa@woman-life-clinic.com;
Hirano A, a-hirano@renais-clinic.com; Mayumi E, e-mayumi@renais-clinc.com;
Isozaki S, isozaki@antiaging-bank.com; Yonei Y, yyonei@mail.doshisha.ac.jp

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Introduction

Medical and physiological research using molecular genetics and molecular biology has revealed the mechanisms of the life phenomena of ageing and lifespan. Even if immortality and eternal beauty are not possible, we are beginning to see pathways to slow down ageing and maintain a youthful and healthy state. The way we age is not the same for everyone. Physiological ageing, which is the fate of all living things and unavoidable, is more or less in addition to ageing beyond the physiological level due to lifestyle disorders. The extra ageing added to such physiological ageing can be regarded as 'pathological ageing,' which can be corrected and recovered by lifestyle modification and medical intervention. In other words, ageing can also be seen as a lifestyle-related disease. Many diseases sprout from the soil of such pathological ageing. Therefore, by continuing with a lifestyle that does not allow them to grow, we can expect to achieve a youthful, healthy, and long life.

In recent years, as research on ageing has progressed, studies on sirtuin, which has become widely known as a longevity/anti-ageing gene, and on nicotinamide adenine dinucleotide (NAD) metabolism have been intensively pursued worldwide [1-8]. Some of these may be able to control pathological ageing and bring it closer to physiological ageing. Concurrently, it will contribute significantly to the prevention and treatment of various systemic diseases.

In this study, we investigated the changes in biomarkers after oral intake of the NAD precursor NMN (nicotinamide mononucleotide) and examined the possibility that NMN could be an effective nutritional material for anti-ageing life and healthy life extension.

Method

Subjects

The subjects were 17 postmenopausal women aged between 50 and 80 years (mean age 55.0 years). Exclusion criteria were as follows: currently undergoing drug treatment/follow-up for any disease, history of serious illness, history of gastrointestinal surgery, drug allergy, possible change in lifestyle during the study period, and those deemed unsuitable as subjects for the study by the supervising physician. Subjects were fully informed in advance and consent was obtained in writing before the study was conducted. Of the 17 subjects at the start of the study, one subject dropped out due to persistent mild headache, and 16 subjects were finally included in the final analysis.

Test product

Hard capsules containing 150 mg of NMN produced by a yeast-based fermentation process (CNB Medical Research Institute, Tokyo, Japan) were used.

Study protocol.

Two capsules of the test product (containing a total of 300 mg NMN) were taken orally in one batch after breakfast and tested at three points in the morning before (week zero), four weeks after, and eight weeks after ingestion. During the study period, participants were prohibited from changing their lifestyle, including drinking and smoking habits, starting new health food intake, and any other items that might affect the study. They were also asked to refrain from excessive exercise on the day before the test, to refrain from eating or drinking anything other than water 12 hours before the test, to avoid staying up late, and not to eat breakfast on the day of the test as test products only.

At zero-week, height, weight, body mass index (BMI), body fat percentage, basal metabolic rate, blood pressure, grip strength, and glycaemia were measured and skin condition was assessed using a visual analog scale (VAS). Blood tests included white blood cell (WBC), WBC fraction, red blood cell (RBC), hemoglobin (Hb), Ht, MCV, MCH, MCHC, platelet (PLT), total protein (TP), albumin (Alb), A/G ratio, T-Bil, D-Bil, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), alkaline phosphatase (ALP), leucine dehydrogenase (LDH), leucine aminopeptidase (LAP), cholinesterase (ChE), creatine phosphokinase (CPK), total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C), amylase, blood urea nitrogen (BUN), creatinine (Crea), uric acid (UA), Na, K, Cl, Ca, fasting plasma glucose (FPG), HbA1c, C-reactive protein (CRP), somatomedin C, dehydroepiandrosterone-sulfate (DHEA-s), cortisol, thyroid stimulating hormone (TSH), free T3 (FT3), free T4 (FT4), estradiol (E2), free testosterone, insulin, adiponectin, cross-linked N-telopeptide of type 1 collagen (NTX), osteocalcin, immunological tests (T-cell sub Sets; T cells, killer T cells, natural killer cells, regulatory T cells, natural killer (NK) T cells, ageing T cells, exhausted T cells), *SIRT1* mRNA expression, NMN, NAD, NAM (nicotinamide = a type of niacin (vitamin B3) were measured.

At four weeks, weight, BMI, body fat percentage and blood pressure were measured, as well as blood counts and biochemical tests and various hormones; at eight weeks, the same tests as at week zero were performed

The expression level of *SIRT1* mRNA in blood was measured from leukemic cells by quantitative RT-PCR and evaluated using the criteria set by the Analysis Center. Blood NMN, NAD, and NAM levels were measured by mass spectrometry. The degree of glycation was assessed for fluorescent advanced glycation end products (AGEs) deposited in the epidermis to dermis layer of the skin, using the autofluorescence (AF). The subjects were asked to keep a diary during the course of the study in order to monitor NMN intake, their bodily sensation, and side-effects.

The above clinical trial was conducted between September and November 2021.

Statistical analysis

Excel Statistics (Social Information Service, Tokyo, Japan) was used for statistical analysis and a corresponding t-test was conducted. A two-tailed test with a risk rate of less than 5% was considered significantly different.

Ethical standards

The study complied with the ethical principles based

on the Declaration of Helsinki and the Personal Data Protection Act, and referred to the Ministerial Ordinance on the Conduct of Clinical Trials of Medicinal Products and the Ethical Guidelines for Epidemiological Research. The study was conducted after approval of the ethics and validity of the study by the Ethical Review Committee on Research Involving Human Subjects of Doshisha University (Approval No.: GSE #2021009) and pre-registration for the clinical trial was conducted (Registration No.: UMIN #000045347).

Result

Physical measurements

No significant changes in body fat percentage, basal metabolic rate, blood pressure, or grip strength were observed over the eight-week course, but BMI increased significantly from 23.2 to 23.4, $p < 0.05$.

Bloodchemistry

Platelets increased from 25.9 to 27.6 x 10^4/μL and amylase decreased from 75.6to69.8 U/L, showing a significant difference (both $p < 0.05$). In terms of metabolism-related markers, HbA1c in glucose metabolism decreased from 5.34 to 5.23 % and HDL-C in lipid metabolism increased from 67.5 to 72.3 mg/dL, both significantly different at $p < 0.05$ (***Fig. 1-a, b***). No significant changes were observed in the bone metabolism markers osteocalcin, and type I collagen cross-linked-N telopeptide (NTX).

Hormone

Adiponectin, which is secreted by fat cells and acts in an inhibitory manner against lifestyle-related diseases, showed an increase from 13.6 to 16.2 μg/mL, with a significant difference at $p < 0.01$ ($p = 0.004$). The adrenal hormone DHEA-s, considered the mother hormone, also showed a significant increase from 1,108 to 1,275 ng/mL, $p < 0.05$ (***Fig. 2-a, b***). No significant differences were found for cortisol, estradiol (E2), free testosterone, thyroid-stimulating hormone (TSH), thyroid hormones (free T3 and T4), somatomedin C, and insulin.

Immune function

In T cell subsets (T cells, killer T cells, natural killer cells, NKT cells, regulatory T cells, killer/helper-aging T cells, killer/helper-exhausted T cells), narrowly defined regulatory

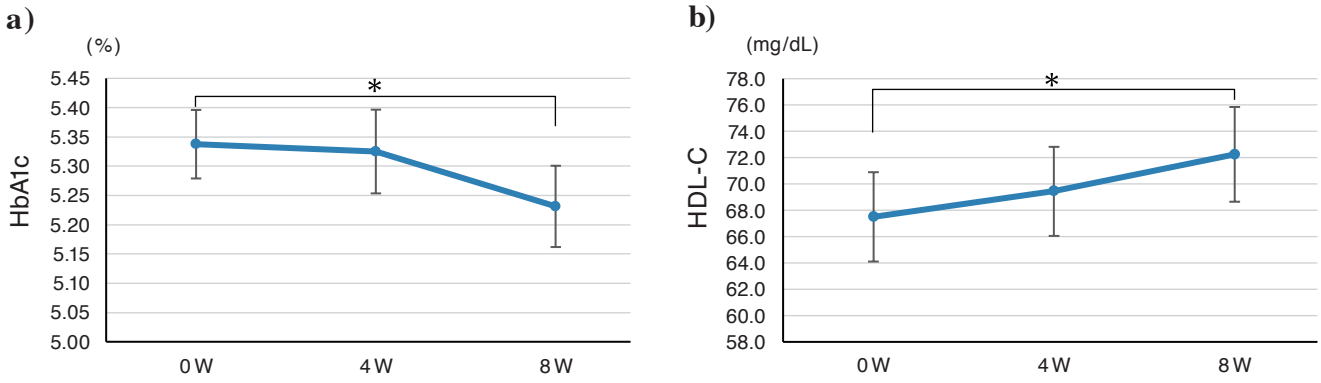

Fig. 1. **Change in serum HbA1c and HDL-C.**

a) HbA1c, **b)** HDL-C. Results are expressed as mean ± SEM, n = 16, * $p < 0.05$, paired t test. HbA1c, hemoglobin A1c; HDL-C, high density lipoprotein-cholesterol; SEM, standard error mean.

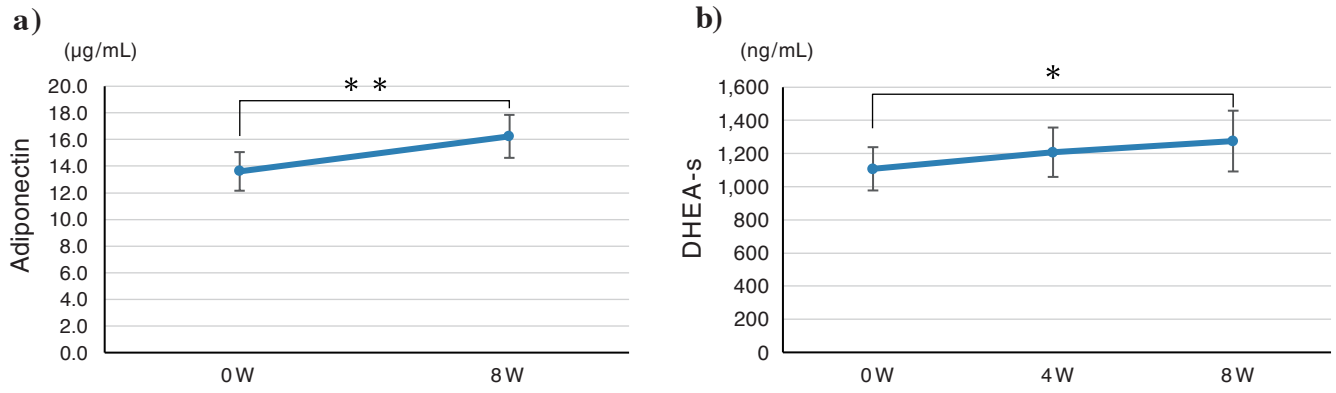

Fig. 2. **Change in serum adiponectin and DHEA-s.**

a) Adiponectin, **b)** DHEA-s. Results are expressed as mean ± SEM, n = 16, * $p < 0.05$, ** $p < 0.01$, paired t test. DHEA-s, dehydroepiandrosterone-sulfate; SEM, standard error mean.

T cells ($CD4^+CD25^{int/high}CD127^{low}$) increased from 4.66 to 5.10 % ($CD4^+$ cell area within ratio), showing a significant increase at $p < 0.05$ (***Fig. 3***). No other significant differences were observed.

NAD synthesis system / sirtuin

Regarding the amount of NAM, NMN, and NAD in the blood, the main pathway components that synthesize NAD, NAM, the starting point of the pathway, increased markedly from 45.2 to 164.7 ng/mL, with a significant difference at $p < 0.01$ ($p = 0.000$). NAD also showed a significant decrease from 17.4 to 13.7 ng/mL, $p < 0.05$, while NMN showed no significant change from 1.32 to 1.28 ng/mL. *SIRT1* mRNA expression, the blueprint for the sirtuin protein that acts as an NAD-dependent protein deacetylase (For leukaemia-derived leukaemic cells, a marked increase was observed in one case, from 6.7 to 33.5. However, the average for all subjects was 5.75Æ6.19, with no significant change (***Fig. 4-a, b, c, d***).

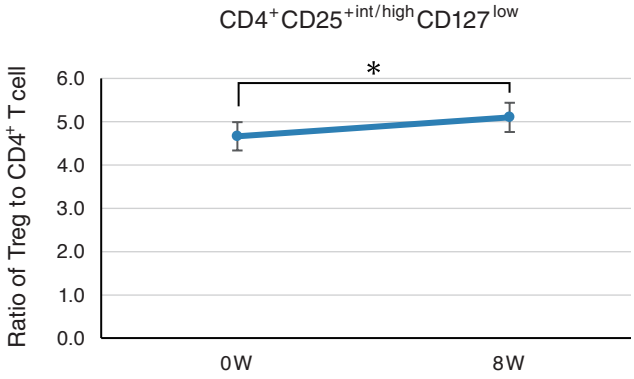

Fig. 3. **Ratio of Treg to $CD4^+$ T cell.**

Results are expressed as mean ± SEM, n = 16, * $p < 0.05$, paired t test. Treg: regulatory T cell, $CD4^+CD25^{int/high}CD127^{low}$; SEM, standard error mean.

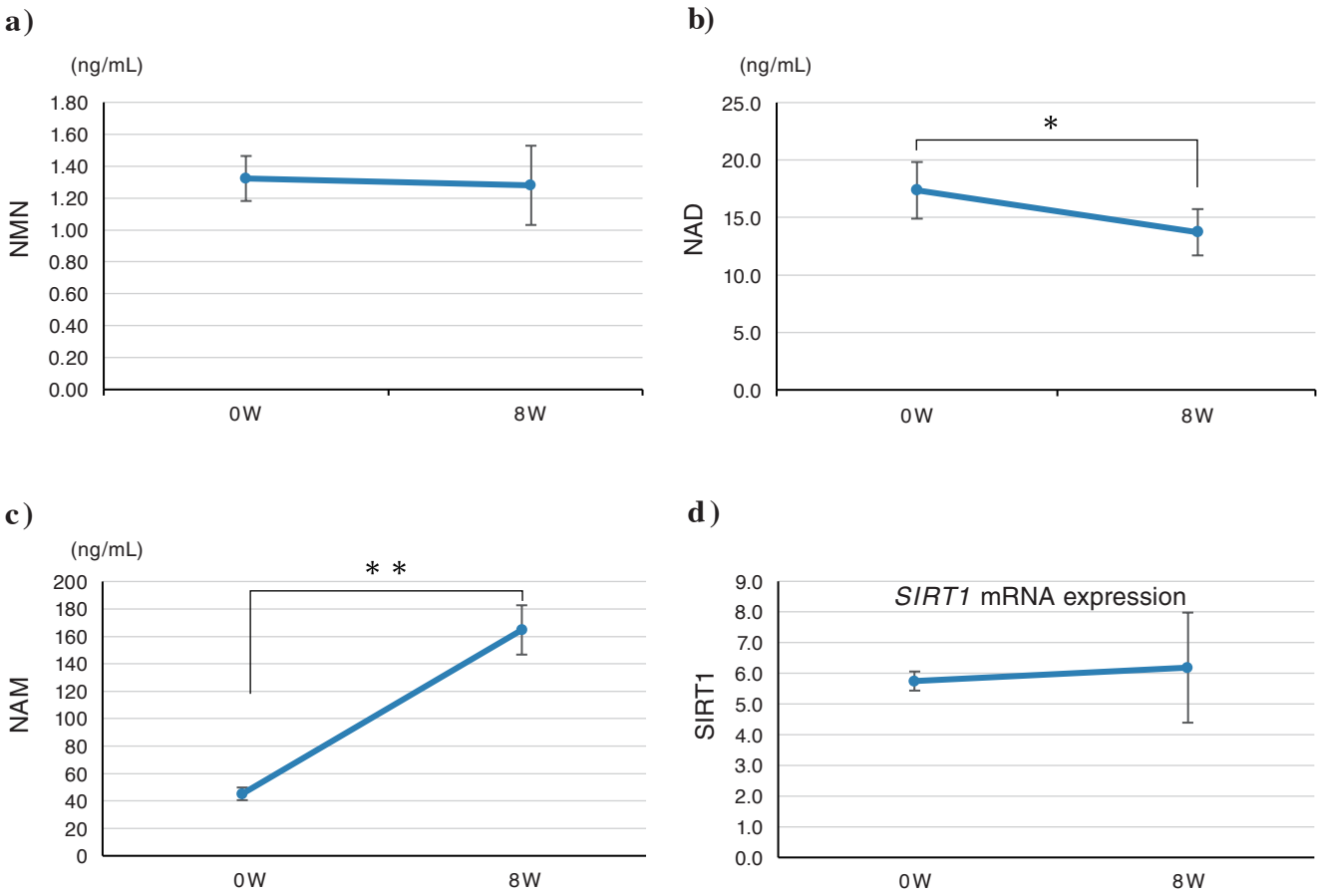

Fig. 4. **Change in serum NMN/NAD/NAM/sirt1mRNA expression.**

a) NMN, **b)** NAD, **c)** NAM, **d)** sirt1 mRNA expression. Results are expressed as mean ± SEM, n = 16, * $p < 0.05$, ** $p < 0.01$, paired t test. mRNA expression of sirtuin 1 was evaluated in isolated peripheral mononuclear cell. NMN, nicotinamide mononucleotide; NAD, nicotinamide adenine dinucleotide; NAM, nicotinamide; SEM, standard error mean.

Glycative stress

The AF value, which reflects the amount of fluorescent end-glycation products (AGEs) in the skin, decreased from 2.43 to 2.35, with a significant difference at $p < 0.05$ (***Fig. 5***).

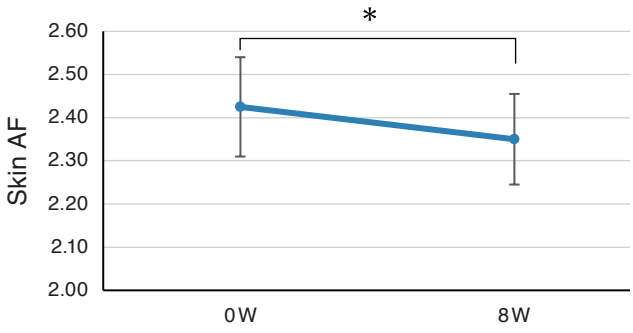

Fig. 5. **Change of skin AF.**

Results are expressed as mean ± SEM, n = 16, * $p < 0.05$, paired t test. Skin AF value was measured by AGE reader mu. AF, autofluorescence; AGE, advanced glycation endproduct; SEM, standard error mean.

Skin

Of the seven VAS items as subjective evaluation (1. moisture 2. flakiness (dry skin) 3. blemishes 4. tension 5. make-up application 6. rough skin 7. eruptions), six items except eruptions decreased, showing a highly significant change with $p < 0.01$ ($p = 0.000$ to 0.001) (***Table 1***).

Subjective changes (bodily sensation)

The following effects were observed in bodily sensation of the subjects after eight weeks of oral intake: improvement of skin (reduction of spots, fine lines and freckles, skin moisturizing, tautness, less noticeable lines, whitening), improvement of sleep (better sleep, less difficulty staying asleep, better wakefulness in the morning), fatigue and physical fitness (less fatigue, faster recovery from fatigue, better mobility, faster running laps, less muscle pain after exercise).

Other positive results included warmer body (improvement of sensitivity to cold), less eye strain, better night vision, less allergic eye symptoms, less weight gain, less drunkenness, improved constipation, shinier hair, faster hair growth, increased appetite, better mood, less joint pain, and improved quick-thinking.

On the contrary, headache, feeling tired, diarrhea, dizziness, and drowsiness, all very mild but transient in two patients (***Table 2***).

Table 1. Skin changes evaluated by visual analog scale.

Questions	Before intake (0 week)	After intake (8 weeks)	p value
How is your skin moisturized ?	52.1 ± 5.1	31.6 ± 4.3	<0.001
Is your skin dry ?	46.5 ± 5.3	25.1 ± 4.7	<0.001
Do you care about age spot ?	74.5 ± 4.6	52.8 ± 6.0	0.001
How is your skin firm & elastic ?	58.1 ± 4.3	31.8 ± 4.1	<0.001
How about a make-up routine ?	53.9 ± 5.0	30.1 ± 4.4	<0.001
Do you care about pimples ?	69.1 ± 5.2	70.1 ± 7.1	0.865
How about rough skin ?	46.5 ± 5.5	23.0 ± 4.1	<0.001

Table 2. Subjective change (Bodily sensation)

☐ SKIN
- Age spots, wrinkles, and freckles have decreased/Nasolabial fold becomes inconspicuous
- Skin becomes moisturized/Skin is getting whiter

☐ SLEEP
- Better sleep quality/Not easy to wake up in the middle of the night
- Awaking is getting better

☐ PHYSICAL PERFORMANCE
- Not easy to get tired/Fatigue recovery has become faster/Body becomes flexible
- Run faster/Less muscle pain after exercise

☐ OTHER
- Reduced eye strain/Better night vision/Reduced allergies around the eyes
- Body becomes warm/Not easy to get fat/Not easy to get a hangover
- Constipation improved/Hair grows faster/Hair becomes smooth/Appetite improved
- Reduced joint pain/Feel better/Mind becomes clear
- Headache, malaise, diarrhea, dizzy, sleepiness (transient symptoms 1-2 participants)

Discussion

A paper published in Nature in 2000 by Imai S et al., who were part of Professor Guarente's research team at Massachusetts Institute of Technology, opened the door wide for the 21st century in aging research [9]. Their research using yeast revealed that sirtuin protein, which is activated by NAD, acts as a protein deacetylase that regulates the expression of various genes and is deeply involved in aging and metabolism. Since then, research on the involvement of sirtuin genes and the NAD synthesis and consumption systems in various biological functions has progressed dramatically.

While many basic studies and animal studies have been reported, clinical studies in humans using NR (nicotinamide riboside), a type of vitamin B3 that is converted to NAD via NMN, have also been reported [10-12]. However, none of these studies has demonstrated any clear anti-aging effect, and it is possible that the reduced absorption of NR may have led to its weakened effect, since much of it is broken down by intestinal bacteria when taken orally. Against this backdrop, in April 2021, the Imai group at the University of Washington published the world's first clinical research paper using NMN, an NAD precursor, in Science [13]. This was a randomized, placebo-controlled, double-blind study of 25 obese postmenopausal women with borderline diabetes. Subjects were randomized to receive placebo or NMN (250 mg/day) for ten weeks. No adverse events were observed, and the NMN group showed increased skeletal muscle insulin sensitivity, insulin signaling, and muscle remodeling. However, these changes were seen only in muscle, not in liver reserves or adipose tissue, and the effects were not as dramatic as those seen in the mice. The results of a second clinical study involving an increased number of men are awaited.

In June of the same year, another clinical study of 48 amateur runners was reported from the Guanghou Sports University in China [14]. The study compared NMN at baseline and after six weeks of intervention between a placebo group and four groups divided into three doses of 300, 600, and 1200 mg/day of NMN. There were no changes in body composition or cardiac function indices such as body fat mass, body fat percentage, slow fat weight, or BMI regardless of NMN dose, but there was a dose-dependent increase in skeletal muscle oxygen utilization in the medium- and high-dose groups. No adverse events were observed in the high-dose group. These results suggest that muscle is one of the tissues most sensitive to NMN and that exercise training with NMN supplementation may be an exercise strategy to improve endurance in athletes.

In a clinical study at the University of Tsukuba published in January 2022, 108 elderly subjects were divided into four groups (NMN morning intake, afternoon intake, placebo morning intake, and afternoon intake) and examined the effects of NMN 250 mg/day for 12 weeks on sleep quality, fatigue, and physical performance [15]. In particular, the NMN afternoon intake group showed the greatest improvement in lower limb motor function and sleepiness. These findings suggest that afternoon NMN intake may be more effective in improving lower limb function and reducing sleepiness in the elderly, and may be beneficial for mental and physical health.

These clinical studies showed differences in organ sensitivity to NMN in humans, effects on exercise capacity, sleep, and fatigue, timing of intake, and safety of intake, and long-term continuous use. This study provided findings that may be useful in daily medical practice, such as changes in various biomarkers (i.e., blood biochemistry, glucose metabolism, lipid metabolism, hormones, and immunity) measured as clinical tests as well as safety, and bodily sensation.

NAD acts as a coenzyme that catalyzes redox reactions during energy production and is also important for post-translational modifications such as NAD-dependent protein deacetylation by sirtuin and induction of DNA damage repair reactions by ADP-ribosylation catalyzed by PARPs. Since NAD cannot be taken in directly as a nutrient, it is synthesized in vivo by two pathways: from NAM and from tryptophan or NA (nicotinic acid, a type of vitamin B3 like NAM and NR). The former, the major pathway, is the salvage pathway through the recycling of NAM, a reaction product after NAD acts as a coenzyme or substrate, which is resynthesized via the intermediate NMN. There is also a known pathway from NR via NMN in the salvage pathway; NMN is also found in broccoli, edamame, and cucumbers, and dietary NMN is also used. The latter is a newly synthesized de novo pathway (***Fig. 6***). The salvage pathway consists of the conversion of NAM to NMN by NAMPT (nicotinamide phosphoribosyltransferase) and NMNAT (nicotinamide mononucleotide adenyltransferase) is a two-step enzymatic reaction from NMN to NAD.

It is known that NAMPT, which acts in the initial stage, is the rate-limiting enzyme for this reaction, which is regulated in response to environmental and nutritional conditions, and declines with age. The amount of NAD in the body declines accordingly, and after middle age, the amount of NAD in the body is less than half of its peak level.

The clinical studies reported so far have not examined NAD and sirtuin in blood. In this study, we measured NMN, NAD, NAM, and *SIRT1* mRNA expression in blood with one major objective of exploring the metabolic status of these in humans.

There were no changes in leukocyte *SIRT1* mRNA expression and blood NMN levels before and after oral intake of 300 mg/day of NMN over an eight week period. However, there was a marked increase in the amount of NAM and a decrease in the amount of NAD (***Fig. 4-a, b, c, d***). NMN is known to have an extremely short residence time in the blood and is rapidly absorbed into tissues and converted to NAD in minutes [16]. Therefore, the amount of NMN in the blood decreases dramatically over time, making its measurement difficult. Intracellularly increased NAD activates redox reactions and enzymatic reactions by sirtuin, PARPs, and is itself metabolized to NAM. The rate-limiting enzyme NAMPT, which acts in the metabolism of NAM to NMN, is not increased, so NAM levels remain high. This sequence of events was formed during the course of NMN ingestion.

NAD consu mption is known to be as rapid as that of NMN on a minute-by-minute basis [17]. Since *SIRT1* mRNA is also rapidly degraded after its expression to replenish the rapidly diminishing sirtuin working in concert with NAD, its intracellular localization time is likely to be short. In this

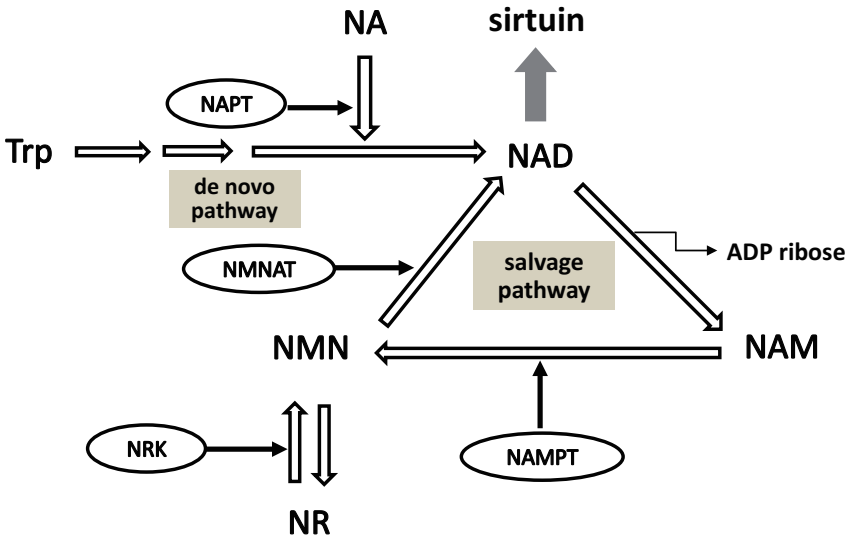

Fig. 6. **NAD synthetic pathway.**

Trp, tryptophan; NA, nicotinic acid; NAD, nicotinamide adenine dinucleotide; NMN, nicotinamide mononucleotide; NAM, nicotinamide; NR, nicotinamide riboside; NAPT, nicotinic acid phosphoribosyltransferase; NMNAT, nicotinamide mononucleotide adenylyltransferase; NMNPT, nicotinamide phosphoribosyltransferase; NRK, nicotinamide riboside kinase; ADP, adenosine diphosphate.

study, NMN was ingested orally only once, after breakfast, and since it would be rapidly expressed and degraded thereafter, the timing of the measurement would cause significant differences in expression levels. Therefore, the *SIRT1* mRNA expression in blood is not suitable for a one-point comparison before and after long-term intake, as in this study, because it only looks at the time when the blood sample was taken. The purpose of this study should be to examine short-term changes over time.

In experiments with BRASTO mice, in which SIRT1 was genetically engineered to be overexpressed only in the hypothalamus, the appearance of symptoms and diseases associated with aging was delayed, and healthy life expectancy was increased by 16.4 % in females and 9.1 % in males compared to the target group[18]. This is 13 to 14 years for females and 7 to 8 years for males in human terms, indicating the possibility of changing the current situation in which healthy life expectancy has not kept pace with the increase in life expectancy. For the purpose to extend healthy life span, it is desirable not only to reduce oxidative and glycative stress, which accelerate aging and induce/promote diseases, but also to keep the various metabolisms such as sugar, lipid, and bone, as well as hormones and immune system, in balance in cooperation without any of them becoming weak points.

In this study, there was a decrease in HbA1c, which indicates the status of blood glucose levels for approximately the past two months (***Fig. 1-a***). Glycation, a reaction between reducing sugars such as glucose and fructose and proteins under non-enzymatic conditions, produces terminal glycation products (AGEs) via HbA1c and other intermediates, resulting in qualitative and functional degradation of proteins in the body[19]. As an evaluation of the degree of glycation, AF values, which reflect the amount of fluorescent AGE accumulated in the skin, also showed a significant decrease (***Fig. 5***)[20]. While LDL-c values were unchanged, HDL-C values increased, indicating a positive effect on lipid metabolism, which is consistent with previous reports of improved glucose and lipid metabolism (***Fig. 1-b***)[6,18].

It is known that SIRT1 promotes the expression of adiponectin, which has a suppressive effect on lifestyle-related diseases[21]. The increase in adiponectin secretion from adipose tissue (especially visceral fat), which is highly sensitive to NMN, and the increase in DHEA-s, the mother hormone secreted by the adrenal gland, may indicate that NMN acts on the hormonal system (***Fig. 2-a, b***).

The lymphocytes, the mainstay of immune cells, also become exhausted and undergo functional aging due to lifestyle disorders and aging[22,23]. Regulatory T cells (Treg), one type of Tregs, control excessive immune responses and have attracted attention as a countermeasure for autoimmune diseases such as ulcerative colitis and Crohn's disease, allergic rhinitis, and conjunctivitis[24]. In the present study, we examined T lymphocyte subsets, including Tregs, exhausted, and senescent cells, which were not found in previous clinical studies. Narrowly defined Tregs were significantly increased, but neither exhausted nor senescent T cells showed significant changes (***Fig. 3***). Since the immune system takes time to adjust, eight weeks may not be sufficient time. In recent years, while research has been conducted on anti-aging therapies by eliminating senescent cells, a vaccine targeting senescent cells has been developed[25]. It has been shown to reduce senescent T cells and improve glucose tolerance in mice. Future validation in humans is awaited.

For the skin, which is easily recognized as an aging phenomenon, there was a clear improvement in all subjects (***Table 1***)[26,27]. In addition to skin changes, many examinees also commented on the improvement effects of various

discomforts and modifications, such as poor sleep quality and fatigue (***Table 2***). It can be said that NMN intake is a nutritional material that is likely to cause not only changes in various biomarkers but also subjective changes of bodily sensation.

It is also known that in conditions of high glycation stress, such as in diabetics, the TCA cycle in mitochondria is disrupted and various disorders are induced [28-30], presumably due to NAD shortage. NMN supplementation appears to be an effective treatment for NAD deficiency.

There is hope for supplementation of NMN, an important component of the NAD world [ageing, lifespan, and metabolic control are systemically integrated via NAD[31]] proposed by Dr Shinichiro Imai, University of Washington, but it is known that increased NAD activates sirtuin even with calorie restriction[32]. Resveratrol, which is found in red wine, was briefly discussed as an activator of the sirtuin gene, but today the majority of opinions are negative. However, a clinical study was reported in 2020[33] that showed that oral intake of red wine extract containing resveratrol (resveratrol 19.2 mg/day) for eight weeks increased *SIRT1* mRNA expression extracted from blood mononuclear cells and increased insulin sensitivity. This is an interesting result and a follow-up study is expected. In addition, lifestyle modification should be started with daily habit, which is expected to have no small effect, as resistance training and aerobic exercise increase NAMPT, the rate-limiting enzyme in the NAD synthesis pathway.

Conclusion

The positive changes in various biomarkers observed with oral intake of NMN suggest that NMN may be a promising nutritional material with the potential to control ageing and extend healthy life expectancy.

Acknowledgments

Authors of this study would like to thank the participants for their cooperation. This study was presented at the 22nd Meeting of Japan Society of Anti-Aging Medicine on Jun, 2022, Osaka, Japan.

Conflict of Interest Statement

Authors have no conflict of interest in this study.

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