

Research Protocol

A randomized, placebo-controlled, double-blind, parallel-group study of the effects of nicotinamide mononucleotide on body composition in elderly men

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Clinical Study

A randomized, placebo-controlled, double-blind, parallel-group study of the effect of nicotinamide mononucleotide (NMN) on body composition in elderly men.

Research Protocol

1. Background of the study

A rapidly aging population highlights the need to maintain good physical and mental health in elderly people. Nicotinamide adenine dinucleotide (NAD⁺)-dependent deacetylases sirtuins have received growing attention as longevity genes. Preclinical studies have shown that activation of sirtuins delays the inevitable decline in physiological functions that occur with aging (e.g., muscle strength and immunity to disease).^{1,2,3} It is hypothesized that preventing the age-dependent decline in NAD⁺ levels would prolong the normal physiological function in humans⁴. Given that NAD⁺ is generally not permeable to cell membranes, it is substituted by nicotinamide mononucleotide (NMN) and nicotinamide riboside (NR), two of its precursors, during intervention studies. NMN is a vitamin B3 analog, which is sold and consumed in foods and supplements. At present, there is insufficient clinical evidence supporting the efficacy of NMN ingestion on physiological markers of aging. The proposed clinical trial is a randomized, placebo-controlled, double-blind, parallel-group study of the effects of NMN intake on physiological indicators of aging including body composition changes in healthy elderly men. This study is equivalent to an exploratory study.

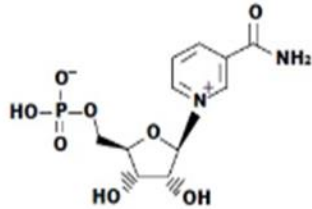
2. Objectives of the study

The purpose of this study is to determine the efficacy of NMN intake by comparing the body composition of NMN-treated and placebo-treated elderly men.

3. Outline of the food

3.1 Food Information

Food name: Nicotinamide mononucleotide (structural formula: see figure).



Dosage form: tablet.

Source of the food: Mitsubishi Corporation Life Sciences Limited, Tokyo, Japan.

Storage method: refrigeration.

3.2 Anticipated adverse reactions due to participation in the study

Allergic symptoms, gastrointestinal symptoms (e.g., diarrhea), facial flushing, liver damage.

4. Eligibility

Healthy elderly people who meet the following criteria (recruitment is entrusted to Newing NPO Corporation, Tokyo, Japan) are eligible for the study.

4.1 Selection criteria

- 1) Individuals who have received sufficient explanations regarding participation in this study and have given their free written consent with full understanding.
- 2) Males aged 65 years or older at the time of obtaining consent.
- 3) Participants with a body mass index (BMI) between 22 and 28.
- 4) Non-smokers.

4.2 Exclusion criteria

- 1) Individuals who consume healthy foods or supplements that may affect the research (e.g., NMN, NR, resveratrol, niacin, and nicotinamide).

- 2) Individuals who have been continuously exercising for at least 1 h every day for at least six months.
- 3) Individuals with a history of malignancy, heart failure or treatment for myocardial infarction.
- 4) Individuals diagnosed with any of the following chronic diseases: atrial fibrillation, arrhythmia, hepatic disorder, renal disorder, cerebrovascular disorder, rheumatism, diabetes, dyslipidemia, hypertension, and other chronic diseases.
- 5) Individuals who regularly use medicines (including herbal medicines).
- 6) Individuals with allergies to pharmaceuticals or test food-related foods.
- 7) Individuals currently participating in other clinical research/trials or have participated in other clinical research/trials within the past 3 months.
- 8) Other participants judged by the investigator as inappropriate for this study.

Rationale for setting exclusion criteria: 1–2, impact on efficacy assessment; 3–7, safety considerations.

5. Explanatory information and consent form for research participants

The explanatory and consent documents are approved by the Ethics Committee and given to research participants; their free and voluntary consent is obtained in writing after sufficient oral and written explanation is provided.

Research participants are promptly notified when information on efficacy and safety that may affect their consent emerges; their willingness to continue or withdraw from the study is confirmed. Research participants are promptly notified of any changes to the research plan that may affect their consent, and their intention to participate in the research is confirmed in advance.

The consent document should be prepared in three copies: one copy is sent to the Clinical Research Support Center for safekeeping, the other copies are for the research participants and the department.

If research participants or their families wish to receive information or consultations about the research, they should contact their physician or the Clinical Research Support Center.

The following 18 items are included in the explanatory and consent documents:

1. Introduction: overview of clinical research.
2. Purpose of the study.
3. Methods used in the study.
4. Expected duration of participation in the study.
5. Expected number of participants in the study.
6. Anticipated benefits and possible disadvantages of participation in the study.
7. Available alternative treatment options if one does not participate in the study.
8. Any damage to one's health that may occur during the study.
9. Participation in the study is of anyone's own free will.
10. Information about the study will be communicated to participants as needed.
11. The research may be discontinued at any time.
12. Participation in the study implies that participants' medical records and other information may be examined during or after the study.
13. Even if the results of the study are made public, participants' identity will not be revealed.
14. What participants must do if they agree to participate in this study.
15. Participants' cost burden.
16. Intellectual property rights and conflict of interest.
17. Physician in Charge.
18. Consultation Service.

6. Research methods

6.1 Approval by the Ethics Committee and other bodies

The principal investigator shall have the research protocol, explanatory documents, and consent documents reviewed by the Ethics Committee and obtain permission for implementation by the hospital director.

6.2 Type and design of the study

Type of control: placebo-controlled.

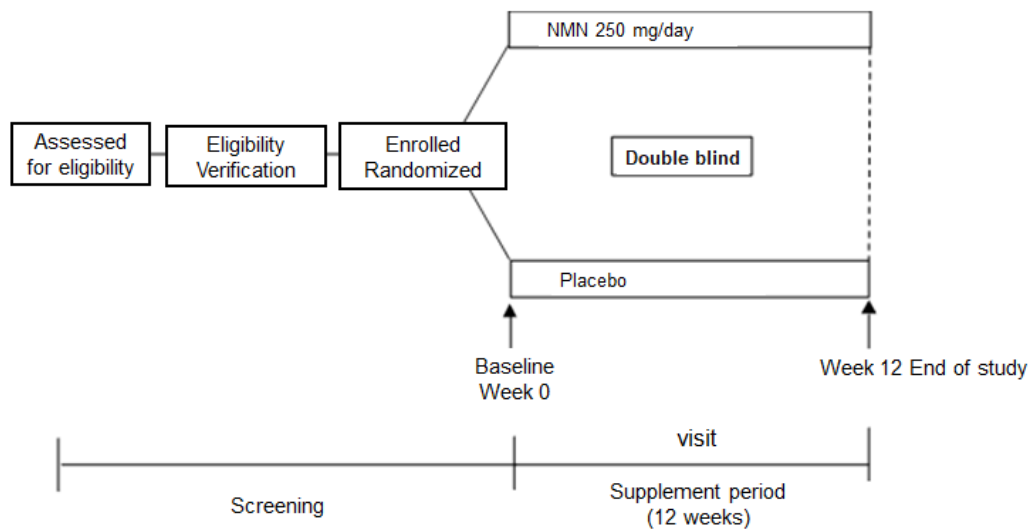
Characteristics of the design: parallel-group.

Randomization: yes.

Blinding: double-blind.

Exploratory clinical study.

6.3 Outline of the study (see the flowchart)



6.4 Expected duration of participation in the study

Minimum 13 weeks; maximum 16 weeks.

Minimum observation period: 1 week; maximum observation period: 4 weeks; duration of treatment: 12 weeks.

The follow-up period is not included in the study period.

6.5 Food dosage and administration period

Oral intake of 250 mg NMN or placebo for 12 weeks.

There have been several reports of NMN administration to mice via intraperitoneal or oral routes at doses of 100–500 mg/kg/day¹. In particular, in long-term experiments, whereby NMN was administered at doses of 100 or 300 mg/kg/day for 1 year, no significant adverse side effects were observed². Taking into account the absorption area of the small intestine, 100 mg/kg/day of NMN in mice is equivalent to 8 mg/kg/day of NMN in humans². In an oral acute toxicity test on rats conducted by Mitsubishi Corporation Life Sciences Limited. , no particular problems were observed at a dose of 2000 mg/kg/day. Even though there are no reports of NMN administration in humans, three clinical studies have been registered in Japan and one in the United States. The latter (ClinicalTrials.gov Identifier: NCT03151239) foresees a dose of 250 mg/day for 8 weeks, and one of the Japanese studies (UMIN Study ID UMIN000025739) envisions a dose of 100 or 200 mg/day for 24 weeks. In the case of NR, several clinical studies in humans have been reported, with doses of 100–2000 mg/day administered for up to 12 weeks causing no serious side effects⁴. In long-term NR administration experiments in mice, a dose of 400 mg/kg/day was administered for 12 weeks³. Based on the above experimental evidence, there is no significant difference between the effective dosage of NMN (100–300 mg/kg/day) and NR (400 mg/kg/day) in mice. The dose chosen in the present study was set in consideration of the doses used in the abovementioned clinical trials (100, 200, and 250 mg/day).

6.6 Dosage form and food ingredients

Dosage form: tablet (250 mg NMN in 6 tablets).

NMN food ingredients: NMN, maltitol, crystal cellulose, silicon dioxide, magnesium stearate, flavor (food additive).

Placebo component: maltitol, crystal cellulose, silicon dioxide, magnesium stearate, flavor (food additive).

6.7 Provisions for concomitant medication

Individuals who regularly use pharmaceutical products are excluded from the study.

6.8 Procedures for the management and delivery of food

The person in charge of assigning the study foods will enter placebo or NMN food numbers on the study food assignment sheet at Mitsubishi Corporation Life Sciences Limited., and store the food without disclosing its identity to others until the end of the study. The food will be delivered by Mitsubishi Corporation Life Sciences Limited. to the principal investigator, who will keep it refrigerated. The food will be provided to the participants at the time of the interview.

6.9 Instructions regarding food intake

Time of intake: after breakfast.

Method of intake: oral intake.

Administration in case of forgotten intake: daily intake after breakfast is preferable, but in case of forgotten intake, it can be taken at any time during the day.

Storage and return of leftover medication and containers: food is to be stored in a cool place away from direct sunlight, high temperature, and humidity. Any leftover food will be returned at the end of the study.

Research participants will be instructed not to change their exercise habits before and after the start of the study.

6.10 Case enrollment and allocation procedure

Research participants who meet the selection criteria (4.1) but do not fall under the exclusion criteria (4.2) are confirmed as eligible for the study. The principal investigator or study collaborator will fill in the necessary information on the registration form and send it to the third-party company responsible for allocation (C&C QUALITAVE RESEARCH INSTITUTE INC. Tokyo, Japan.; Manager: Akira Sato). The company responsible for the allocation will confirm the contents of the registration form and assign a food number. Confirmation of the registration form with the food number will be sent to the University of Tokyo Hospital.

1) Among research participants who have expressed their willingness to participate in this study, those who have given written informed consent will be selected based on the results of the medical interview and blood test, and will be listed on the registration form as study participants and assigned a registration number.

2) The randomization of food intake will be conducted by a third-party company responsible for allocation (C&C QUALITAVE RESEARCH INSTITUTE INC.) using the registration number on the registration form. The randomization procedure will not involve the University's researchers, the principal investigator, or Mitsubishi Corporation Life Sciences Limited., and will not be disclosed until the end of the study.

Allocation criteria:

- a. Age should not differ significantly between groups.
- b. Skeletal muscle mass index (SMI) and BMI should not differ significantly between groups.

6.11 Actions taken after completion of the study

After completion of the study, research participants will return to their habitual dietary habits and no special measures will be taken.

6.12 Preservation of samples obtained from human participants and information used in the research

1) Anonymization of samples and storage method

After samples are obtained, they will be promptly anonymized by deleting any personal identifiers, such as names and registration IDs; instead, a research identification number will be attached. The anonymized samples will be measured immediately or stored at the P1 Unit, University of Tokyo Hospital, where access privileges are restricted by a locked door, at -80°C until the end of the study. After the measurement is completed or the storage period has elapsed, the identification number will be deleted by cutting the identification label, and the samples will be disposed of.

2) Anonymization of information and storage method

After the information is obtained, it will be anonymized by immediately deleting any personal identifiers, such as names and medical record IDs, and will be replaced by a research identification number. A correspondence table that can be linked to personal information will be created. The anonymized information and the correspondence table will be held in the laboratory of the person in charge of management (A501 and 618, Clinical Research Building, University of Tokyo Hospital) in separate storage rooms with locked and restricted access.

3) Storage period and disposal method

Information related to this research will be stored for 5 years from completion of the study or three years from the final publication of the study results, whichever is later, and then disposed of in an unrecoverable form by shredding the printed media or deleting the electronic files.

7. Evaluation items

Primary endpoint: change in SMI measured by bioelectrical impedance analysis (BIA).

Secondary endpoints: changes in markers of physiological aging determined by: (1) physical measurements, (2) blood tests, (3) physiological tests, (4) radiological examination, (5) questionnaires, and (6) diary.

Correlations between blood concentrations of NAD-related metabolites, including NAD, nicotinamide, nicotinic acid (NA), NR, NAR, nicotinic acid mononucleotide (NAMN), nicotinic acid adenine dinucleotide (NAAD), tryptophan, methyl nicotinamide, quinolinic acid, glutathione, adenosine monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP), before intake and measured aging markers will be evaluated.

7.1 Body measurements

Height, weight, body composition (BIA method), waist circumference, lower leg circumference, blood pressure, heart rate, grip strength, visual test, hearing test, 30-s chair stand test, and gait speed.

*Height is measured only during pre-treatment screening.

7.2 Blood tests

1) Hematological examination

White blood cell count, red blood cell count, hemoglobin, hematocrit level, platelet count, average red blood cell pigment content, average red blood cell volume, and average red blood cell pigment concentration.

2) Blood biochemical tests (including safety endpoints)

Triglyceride, total cholesterol, LDL-cholesterol, HDL-cholesterol, glucose, HbA1c, insulin, serum c-peptide (CPR), aspartate transaminase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (γ -GTP), creatine kinase (CK), total protein, albumin, uric acid, uric acid nitrogen, creatinine, sodium, potassium, hormone tests (free testosterone, testosterone, dehydroepiandrosterone sulfate [DHEA-S], osteocalcin, pentosidine, and adiponectin), interleukin-6 (IL6), and NAD metabolism-related metabolites (NAD, NMN, nicotinamide, NA, NR, NAR, NAMN, NAAD, tryptophan, methyl nicotinamide, quinolinic acid, glutathione, AMP, ADP, and ATP).

3) 75 g oral glucose tolerance test (75g OGTT)

Performed at 0 min, 30 min, 60 min, and 120 min; it will measure blood glucose, insulin, and serum-CPR.

7.3 Physiological tests

Assessment of vascular endothelial function by the flow-mediated dilation (FMD) test and bone density test.

7.4 Radiological examination

Evaluation of visceral fat and fatty liver by computed tomography (CT).

7.5 Questionnaires

Common questionnaire about dietary behavior and anti-aging QOL, Mini-Mental State Examination-Japanese (MMSE-J), and Japanese version of Montreal Cognitive Assessment (MoCA-J). *MMSE-J and MoCA-J are administered only at week 0 and week 12.

7.6 Daily diary

During the intake period, the presence/absence of test food intake, physical condition, and dietary survey will be recorded daily in a designated diary.

8. Observation and test items, methods of data collection

Schedule				
items	Screening	Baseline	Supplement period	
Period	1-4weeks before	Week 0	Week 6	Week 12
Visit	1	2	3	4
Obtaining consent	○			
Background	○			
Supplement intake		←—————→		
Subjective symptoms	○	○	●	●
Confirmation of adverse events	←—————→			
Vital signs	○			●
Body measurements	○			●
Blood test 1	○			●
Blood test 2		○		●
75g OGTT		○		●
FMD test		○	●	●
Bone density test		○		●
Radiological examination		○		●
Questionnaires		○	●	●
Diary		←—————→		

Items marked with ○ are to be assessed before the start of supplements intake.

Items marked with ● are to be assessed after the start of supplements intake.

- 1) Background: gender, date of birth, height, weight, and medical history.
- 2) Supplements intake: record of start and end dates.
- 3) Subjective symptoms and other findings: to be confirmed by interview or other means.
- 4) Confirmation of adverse events and side effects: adverse events include various laboratory abnormalities.

The nature, timing of onset and disappearance, degree, treatment, outcome, evaluation of severity, and relationship to the study drug should be described in the medical record and case report. Follow-up studies should be conducted if necessary. The severity of the disease will be defined as 1) mild: the patient can continue the trial without treatment, 2) moderate: the patient can continue the trial with some treatment, and 3) severe: the patient should stop or discontinue the trial. Serious adverse events are defined on a scale of 10 and should be reported promptly if applicable.

5) Vital signs: blood pressure, pulse rate, respiratory rate, body temperature, and oxygen saturation.

6) Body measurements: height, weight, body composition (BIA method), waist circumference, leg circumference, grip strength, visual test, hearing test, 30-s chair stand test, and gait speed.

*Height is measured only during pre-intake screening.

7) Blood test 1: CK, total protein, albumin, uric acid, blood urea nitrogen (BUN), creatinine, sodium, potassium, as well as immunobiological tests for HBs antigen, HCV antibody, HIV antibody, and syphilis (STS and TPHA).

8) Blood test 2: CRP, hormone tests (free testosterone, testosterone, DHEA-S, osteocalcin, pentosidine, and adiponectin), IL6, NAD metabolism-related metabolites (NAD, NMN, nicotinamide, NA, NR, NAR, NAMN, NAAD tryptophan, methyl nicotinamide, quinolinic acid, glutathione, AMP, ADP, and ATP). About 5 mL of whole blood is sent frozen to the Department of Metabolism and Nutrition, Graduate School of Medicine and Pharmaceutical Science for Research, Toyama University, for measurement using a mass spectrometer. After measurements, the sample will be destroyed and the data will be sent to the principal investigator.

9) 75g OGTT at 0 min, 30 min, 60 min, and 120 min; measuring blood glucose, insulin, serum-CPR.

10) FMD test.

11) Bone density test: performed using a bone densitometer.

12) Radiological examination: abdominal CT scan.

13) Questionnaire: common questionnaires about food attitude and anti-aging QOL, MMSE-J, and MoCA-J. *MMSE-J and MoCA-J are administered only at week 0 and week 12.

14) Diary: during the intake period, the participants' physical condition and dietary survey will be recorded daily in a designated diary.

On each visit, the patient will be asked to come to the hospital with a fasting breakfast and no test food intake to perform the test properly.

9. Discontinuation criteria

The following criteria will be considered grounds for discontinuing the research.

- 1) If the research participant declines to participate in the research or withdraws consent.
- 2) If the eligibility of the participant is found to be no longer valid after enrollment.
- 3) If it is difficult to continue the research due to adverse events.
- 4) If the entire study is discontinued.
- 5) If the physician deems it appropriate to discontinue the research for any other reason.

If the principal investigator and sub-investigators determine that it is impossible to continue the study for any reason, the date, reason, and course of discontinuation/withdrawal will be clearly stated in the medical record and case report, and necessary tests will be conducted at the time of discontinuation/withdrawal to evaluate efficacy and safety.

In the case of discontinuation due to an adverse event, treatment or follow-up observation should be conducted until the patient recovers as much as possible to the original state.

10. Handling in the case of an adverse event

10.1 Actions to be taken in the case of an adverse event

When an adverse event is recognized, the principal investigator or sub-investigator shall immediately take appropriate measures and record the event in the medical record and case report form without any discrepancy. In addition, when food intake is discontinued or treatment for an adverse event becomes necessary, research participants should be informed.

To identify the food during an emergency evaluation, the principal investigator will ask the person in charge of allocation to disclose the results by opening the package for the case in question.

10.2 Reporting of serious adverse events

If the principal investigator recognizes the occurrence of a serious adverse event, it should be promptly reported to the hospital through the Clinical Research Support Center, and the hospital director shall request the Ethics Committee to deliberate on whether or not to continue the trial. The principal investigator shall share information on the occurrence of the adverse event with the sub-investigators involved in the research.

A first report (emergency report) and a second report (detailed report) shall be made.

10.3 Other adverse events

Other adverse events are recorded in case report forms, according to the procedures described in 8 (Confirmation of adverse events and side effects).

11. Compliance with the research protocol and handling of deviations from it

11.1 Compliance with the research protocol

The principal investigator and sub-investigators will conduct the trial in compliance with the research protocol.

11.2 Handling of deviations from the research protocol

The principal investigator or sub-investigator shall not deviate from or change the research protocol before obtaining approval by the hospital director and prior review by the Ethics Committee.

The principal investigator or sub-investigator may deviate from or change the research protocol for unavoidable reasons such as emergency avoidance, prior to approval by the Ethics Committee. In such cases, the principal investigator or sub-investigator shall promptly submit to the Ethics Committee the details of the deviation or change, the reasons for the deviation or change, and a draft of the revised research protocol, if necessary, for approval by the Ethics Committee and the hospital director.

If there is any deviation from the research protocol, the principal investigator or sub-investigator shall record all deviations, together with the underlying reasons.

If the principal investigator or sub-investigator realizes that this clinical research does not comply with ethical guidelines for medical research on human participants (only in cases where the degree of non-compliance is serious), approval from the hospital

director will be requested. The hospital director shall request the Ethics Committee to deliberate on whether or not to continue the trial, take the necessary measures, and then cooperate with the hospital director in reporting and publicizing the status and results of the measures to the Minister of Health, Labor, and Welfare.

12. Renewal, termination, discontinuation, and suspension of research

12.1 Renewal and termination of research

The principal investigator shall report the status of the research to the hospital director once a year (in January), and the hospital director shall request the Ethics Committee to discuss the status report.

12.2 Discontinuation or suspension of research

The principal investigator will consider whether or not to continue the trial if any of the following items applies:

- 1) Critical information on the quality, safety, or efficacy of the food intake is obtained.
- 2) The Ethics Committee instructs changes to the implementation plan, and it is judged to be difficult to accept these changes.
- 3) The hospital director recommends or instructs discontinuation based on the opinion of the Ethics Committee or the Effectiveness and Safety Evaluation Committee.

When the decision to discontinue or suspend the research is made, the decision shall be promptly reported to the hospital director in writing, together with the reasons for the decision.

13. Research implementation period

From approval by the Ethics Committee until December 31, 2023.

Registration period: from approval by the Ethics Committee until December 31, 2019.

14. Statistical matters

14.1 Target number of cases and rationale for setting the target

The null hypothesis posits that there is no change in SMI between the NMN intake group and the placebo intake group. Based on existing published evidence⁵, we assumed that the standard deviation for SMI in healthy participants was 0.60 kg/m² and that the expected change in SMI due to food intake was 0.45 kg/m²*. In this case, the sample size that would allow us to reject the above null hypothesis with a 95% confidence interval would be 20 samples for each group (40 samples in total) using calculations based on Cancer Research and Biostatistics. The predicted dropout rate was 5%, and the study was started with 42 participants (21 participants in each group).

*We expect a change of 6–7% based on pre-studies^{6,7}. Given a baseline value for muscle mass loss in the elderly of 6.75 kg/m² for men⁸, the cutoff value for the amount of NMN that could improve muscle mass loss associated with aging was calculated to be $6.75 \text{ kg/m}^2 \times 0.06 = 0.405 \text{ kg/m}^2$ and $6.75 \text{ kg/m}^2 \times 0.07 = 0.4725 \text{ kg/m}^2$. The cutoff value was 0.45 kg/m². Considering that SMI decreases with age, an increase in SMI of 0.45 kg/m² in men aged 70–74 years is equivalent to a rejuvenation of 10–20 years⁹.

14.2 Analysis items and methods

Primary endpoint: change in SMI measured by BIA.

Change in SMI defined as SMI at week 12 - SMI at baseline, where SMI is calculated as limb muscle mass (kg) / height² (m²).

Each endpoint is a continuous quantity, and is based on data from a single time point.

The results of the analysis will be presented as the adjusted least square means ± standard error.

Subgroup analysis is performed with age and BMI as subgroups.

The incidence rate and 95% confidence interval will be presented for each adverse event.

1) Within-group comparison

Depending on the variability of the population, the corresponding *t*-test (paired *t*-test) or Wilcoxon signed-rank test will be used to compare the measurements before, 6 weeks after, and 12 weeks after intake. In cases where the aforementioned analysis methods do not apply, a statistical method more appropriate for aggregated data will be employed.

2) Between-group comparison

Depending on the variability of the population, the unpaired *t*-test or Mann–Whitney U test will be used to compare the measured values before, 6 weeks after intake, and 12 weeks after intake, and calculate the corresponding changes. The difference between pre-and post-intake values will be compared between groups. Analysis of covariance will be performed if the pre-intake values cannot be ignored as confounding. In addition, treatment comparison is performed using mixed model analysis. When endpoints cannot be calculated using mixed model analysis, endpoints follow mixed-effect model for repeated measures (MMRM). In other cases where the aforementioned analysis methods do not apply, a statistical method more appropriate for aggregated data will be employed.

3) Safety evaluation

Analysis will confirm the absence of any changes associated with the intake of test foods that would require medical attention.

4) Standards and software

Statistical analysis shall be conducted using a two-tailed test, and the significance level shall be set at 5%. Participants who do not comply with the study requirements, such as an intake rate of less than 90%, will be excluded from the analysis.

Secondary endpoints: changes to physiological markers of aging determined by (1) physical measurements, (2) blood tests, (3) physiological tests, (4) radiological examination, (5) questionnaires, and (6) diary (see 7 “Evaluation items”) will be assessed in the same way as the main evaluation items above. The correlation between blood levels of NAD-related metabolites (NAD, nicotinamide, NA, NR, NAR, NAMN, NAAD, tryptophan, methyl nicotinamide, quinolinic acid, glutathione, AMP, ADP, and ATP) before oral administration and measured aging markers will be calculated using Pearson's product rate correlation coefficient (normal distribution) or Spearman's rank correlation coefficient (non-normal distribution). The effect of various endpoints on the different parameters will be analyzed. In cases where the aforementioned analysis methods are not applicable, statistical methods more appropriate for aggregated data will be employed.

14.3 Interim analysis

No interim analysis will be conducted.

15. Quality control and quality assurance

15.1 Monitoring

To ensure that the trial is conducted properly, the principal investigator shall regularly monitor how research is progressing and whether it is being conducted according to the relevant guidelines, regulations, and research protocol to ensure its reliability. A person shall be designated for this purpose by the principal investigator.

The person in charge of monitoring will refer to a checklist prepared in advance. After confirming matters related to the implementation status and reliability of the research by telephone, fax, e-mail, or direct inspection of the original materials at the medical institution, the results will be compiled into a monitoring report and communicated to the principal investigator.

The following items shall be checked during the monitoring process

1) Cases

Records concerning consent acquisition, eligibility, and adverse events of the participants, as well as consistency between the data in the case report form and the records in the source documents will be confirmed. The trial will be conducted in compliance with the research protocol and ethical guidelines.

2) Procedures

The monitoring personnel will confirm that all procedures comply with the requirements set by Ethics Committee, that reports on safety are appropriately filled in and adhered to, that the review status of these procedures, the drugs, and equipment used in the trial are appropriately managed and recorded, and that procedures are properly conducted in compliance with the research protocol.

The person in charge of monitoring will not be involved in the evaluation of this research or the preparation of case report forms. In addition, this person must not divulge any information obtained during monitoring, as well as the confidentiality, identity, and other personal identifiers pertaining to research participants.

15.2 Data management

The principal investigator will designate a data management officer, who shall lay out in advance a data management plan following the Standard Operating Procedures of the Clinical Research Support Center. During the trial, this person shall perform data management operations on the applied procedures. After completion of the study, a report on the status of data management operations is prepared and submitted to the responsible physician together with fixed research data. Original materials will be stored as described in detail in **20** (Preservation of records). The person in charge of data management will not be involved in the clinical evaluation of the study.

15.3 Auditing

No audit will be conducted for this research.

16. Consideration about human rights and safety/disadvantage of research participants

16.1 Consideration about human rights (protection of personal information)

When handling raw data or consent documents related to the implementation of research, sufficient consideration shall be given to protection of research participants' confidentiality. For case reports and other documents presented outside the hospital, identification codes should be used. When publishing the results of the research, information that can identify research participants will not be included. The information and data collected during this study will be anonymized by removing personal identifiers, such as names and initials, and replacing them with a new code so that individuals cannot be identified. Data will be stored in the laboratory of the principal investigator (A501, 618, Clinical Research Building, University of Tokyo Hospital) on a stand-alone computer with a password lock. Any printed materials should also be stored in a lockable locker in the principal investigator's laboratory. The anonymization correspondence table will not be used for the analysis itself but to change the code back to personal information and dispose of it, such as when a research participant withdraws consent.

Data on research participants obtained in the course of the present study will not be used for any purpose other than the purpose of this research.

16.2 Consideration of safety and disadvantage, and balance with benefit

Research participants will consume food and undergo a total of four medical examinations and tests. Although the direct benefits of the research participants' participation in this study are unknown, they will contribute to the generation of scientific evidence on the efficacy of NMN in humans. Given that the present trial involves intervention, the possibility of adverse health effects, although not highly likely, cannot be excluded. Therefore, a consultation service will be set up in case any abnormality is detected, and the conditions for discontinuation will be strictly defined to minimize health hazards caused by ingestion. In addition, personal information will be anonymized and protected with locks and passwords to prevent leakage.

17. Cost burden for research participants

All tests and other procedures carried out as part of this study will be performed as uninsured medical treatment and will be covered by research expenses. There is no cost burden for research participants.

18. Compensation for health damage and insurance coverage

18.1 Compensation for health damage

If a study participant suffers health hazards due to this study during the study period, or if a study participant sues for damages caused by this study, and if the study investigator determines that there is a causal relationship between the adverse event and the test food, compensation will be provided within the scope of PL insurance coverage for food testing.

18.2 Participation in compensation insurance

The principal investigator and sub-investigators will be covered by liability insurance.

19. Compliance with the Declaration of Helsinki, ICH-GCP, and ethical guidelines

This research will be conducted in compliance with the latest version of the Declaration of Helsinki and the Ethical Guidelines for Medical Research Involving Human participants.

20. Preservation of records

The information and data collected during this study will be stored in a stand-alone computer with a password lock in the principal investigator's laboratory (A501 and 618, Clinical Research Building, University of Tokyo Hospital) after removing personal identifiers, such as names, initials, and patient IDs, and creating an anonymized correspondence table with a new code so that individuals cannot be identified. Any printed materials should also be stored in a lockable locker in the principal investigator's laboratory. The anonymization correspondence table will not be used for the analysis itself, but to change the code back to personal information when necessary, such as when a research participant withdraws consent, and to dispose of the relevant person's information and data. Information pertaining to this trial will be kept for 5 years from completion of the study or 3 years from the final publication of the study results. The information will be then disposed of in an unrecoverable form by shredding the paper media or deleting the electronic files. After completion of the study, the company responsible for allocation should send the registration form to the principal investigator, and the registration form will be kept.

21. Registration of the research plan and publication of research results

The content of the study plan is registered in UMIN-CTR before the start of the study. The principal investigator and cooperating physician will publish the results in a scientific article as soon as possible after completion of the study.

22. Research Organization

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23. Research funding, conflict of interest, intellectual property rights, and use of research results

This research will be conducted under a joint research agreement with Mitsubishi Corporation Life Sciences Limited., with the provision of test foods and funding. Researchers will report to the principal investigator on the status of conflicts of interest related to the research, including personal earnings, for every fiscal year while the research is conducted and when new items are reported. The principal investigator shall submit a self-report on conflicts of interest after confirming that the status of such conflicts is appropriately described, and shall follow the instructions of the hospital director (based on the opinion of the Conflict of Interest Management Committee).

The ownership of intellectual property rights shall be determined in accordance with the agreement with Mitsubishi Corporation Life Sciences Limited. When Mitsubishi Corporation Life Sciences Limited. uses the name of the implementing medical institution or the researcher for publicity or advertisement of the product, the content and method of such use shall be discussed with the principal investigator in advance.

1) Conflict of Interest in Research Funding and Research Organization

This research will be conducted under a contract research agreement with Mitsubishi Corporation Life Sciences Limited., which will provide funding and test food supplements. Mitsubishi Corporation Life Sciences Limited. will provide information on the test food supplements, but will not be involved in the conduct, analysis, or reporting of the study.

2) Conflict of interest of the researcher

The principal investigator of this study has no conflicts of interest to disclose.

The principal investigator will continuously check (once a year) with the investigators to determine if any new conflicts of interest have arisen in the planning, implementation, or reporting of the research, and which may affect the results of the study or the interpretation of the results. The principal investigator will confirm that implementation of the research does not compromise the rights and interests of participants.

24. Changes to the research protocol

Any changes (revisions) to the research protocol, explanatory documents, or consent forms must be reported in advance to the hospital director and approved by the Ethics Committee.

25. References

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