DESCRIPTION

TITLE OF INVENTION: COMPOSITION CONTAINING SESAMIN OR LIKE AND NR AND/OR NMN

TECHNICAL FIELD

[0001]

The present invention relates to a composition containing at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and a sesamin-class compound.

BACKGROUND ART

[0002]

In recent years, countries with a high proportion of elderly people have faced the challenge of extending healthy life expectancy. One of the factors of shorter healthy life expectancy is a decline in physical function or the like caused by an age-related decline in nicotinamide adenine dinucleotide (NAD^+) production in the body.

[0003]

Nicotinamide adenine dinucleotide (NAD^+, (hereafter also referred to as NAD)) is a coenzyme that mediates many redox reactions in the body. The NAD level is known to decrease in the aging process, which causes defects in nuclear and mitochondrial function, leading to many age-related pathologies. For example, Non-Patent Literature 1 suggests that aging and age-related diseases are associated with a decline in nicotinamide adenine dinucleotide (NAD^+) level in cells and a decline in enzymatic activity of sirtuins, the longevity genes, which are NAD^+-dependent deacetylases. A recent report says that increasing NAD^+ activates sirtuins, resulting in anti-aging effects (Non-Patent Literature 2). Vigorous research is therefore being

conducted to search for ingredients that are effective in preventing or ameliorating an age-related decline in NAD^+ and are applicable to pharmaceutical products, foods for specified health uses, foods with function claims, and other products.

[0004]

For example, Non-Patent Literature 3 discloses that nicotinamide riboside (NR), which is a NAD precursor, induced mitochondrial unfolded protein response and synthesis of prohibitin proteins, thereby activating muscle stem cells (MuSCs) in aged mice, and that NR inhibited MuSC aging in a mouse model of muscular dystrophy. Non-Patent Literature 3 also discloses that NR delays neural SC (stem cell) senescence and melanocyte SC extends lifespan in mice.

[0005]

Non-Patent Literature 4 says that nicotinamide riboside (NR) is widely used as an NAD^+ precursor vitamin and shows that a single oral administration of NR dose-dependently increases NAD^+ concentration in human blood. Non-Patent Literature 5 discloses that NMN activates sirtuins. Specifically, Non-Patent Literature 5 discloses that administration of nicotinamide mononucleotide (NMN) which is a major natural NAD^+ intermediate (also called intermediate metabolite) to normal wild-type mice for 12 months effectively alleviated an age-related physiological decline in mice without apparent toxicity, suggesting a great potential of NMN as an effective anti-aging intervention in humans.

[0006]

These documents however include no suggestion or disclosure about any action of sesamin and/or episesamin to increase nicotinamide adenine dinucleotide (NAD) in vivo.

CITATION LIST

- Non-Patent Literature

[0007]

Non-Patent Literature 1: Imai, S. & Guarente, L. (2014), Trends Cell Biol., 24, 464-471. "NAD^+ and sirtuins in aging and disease"

Non-Patent Literature 2: Yoshida M, Satoh A, Lin JB, Mills KF, Sasaki Y, Rensing N, Wong M, Apte RS, Imai SI., Cell Metab. (2019), 30(2): 329-342. e5. "Extracellular Vesicle-Contained eNAMPT Delays Aging and Extends Lifespan in Mice"

Non-Patent Literature 3: Zhang, H., Ryu, D., Wu, Y., Gariani, K., Wang, X., Luan, P., D'Amico, D., Ropelle, E. R., Lutolf, M. P., Aebersold, R., Schoonjans, K., Menzies, K. J., & Auwerx, J. (2016), Science, 352, 1436-1443. "NAD^+ repletion improves mitochondrial and stem cell function and enhances life span in mice"

Non-Patent Literature 4: Samuel A. J. Trammell, Mark S. Schmidt, Benjamin J. Weidemann, Philip Redpath, Frank Jaksch, Ryan W. Dellinger, Zhonggang Li, E. Dale Abel, Marie E. Migaud & Charles Brenner, (2016) Nat. Commun. 7, 12948. "Nicotinamide riboside is uniquely and orally bioavailable in mice and humans"

Non-Patent Literature 5: Mills, K. F., Yoshida, S., Stein, L. R., Grozio, A., Kubota, S., Sasaki, Y., Redpath, P., Migaud, M. E., Apte, R. S., Uchida, K., Yoshino, J., & Imai, S. I. (2016), Cell Metab., 24, 795-806. "Long-Term Administration of Nicotinamide Mononucleotide Mitigates Age-Associated Physiological Decline in Mice"

SUMMARY OF INVENTION

- Technical Problem

[0008]

The present invention aims to provide a composition that has an effect of increasing NAD concentration.

- Solution to Problem

[0009]

The present inventor made intensive studies to solve the above problem to find out that sesamin-class compounds have an effect of increasing nicotinamide adenine dinucleotide (NAD) concentration and that a composition containing a sesamin-class compound and at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof effectively increases nicotinamide adenine dinucleotide (NAD) concentration.

[0010]

The present invention relates to the following composition.

(1) A composition containing, as active ingredients: at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof; and at least one sesamin-class compound.

(2) The composition according to (1) above, wherein the at least one sesamin-class compound includes at least one of sesamin or episesamin.

(3) The composition according to (1) or (2) above, wherein a molar ratio of the at least one selected from the group consisting of NR, NMN, and salts thereof to the at least one sesamin-class compound (at least one selected from the group consisting of NR, NMN, and salts thereof/at least one sesamin-class compound) is 1 to 100.

(4) The composition according to any one of (1) to (3) above, wherein the at least one sesamin-class compound is contained in a total amount of 0.001 to 10 wt%.

(5) The composition according to any one of (1) to (4) above, wherein the composition improves, maintains, or ameliorates nicotinamide adenine dinucleotide (NAD) concentration or reduces a decline in NAD concentration.

(6) The composition according to any one of (1) to (5) above, wherein the composition improves, maintains, or ameliorates mitochondrial function or reduces a decline in mitochondrial function.

(7) The composition according to any one of (1) to (6) above, wherein the composition improves, maintains, or ameliorates mitochondrial energy production capacity or reduces a decline in mitochondrial energy production capacity.

(8) The composition according to any one of (1) to (7) above, wherein the composition is used to inhibit and/or delay aging associated with a decline in NAD concentration.

(9) The composition according to any one of (1) to (8) above, wherein the composition is an anti-aging composition.

(10) The composition according to any one of (1) to (9) above, wherein the composition is an oral composition.

(11) The composition according to any one of (1) to (10) above, wherein the composition is a food or beverage.

(12) The composition according to any one of (1) to (11) above, wherein the composition is labeled with at least one of “inhibiting and/or delaying aging associated with a decline in NAD concentration” or “inhibiting and/or delaying cellular senescence”.

(13) A method of increasing nicotinamide adenine dinucleotide (NAD) concentration, including administering at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and at least one sesamin-class compound.

(14) A method of improving, maintaining, or ameliorating nicotinamide adenine dinucleotide (NAD) concentration or reducing a decline in NAD concentration, including administering at least one selected from the

group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and at least one sesamin-class compound.

(15) Use of at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and at least one sesamin-class compound, for increasing nicotinamide adenine dinucleotide (NAD) concentration.

(16) Use of at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and at least one sesamin-class compound, for improving, maintaining, or ameliorating nicotinamide adenine dinucleotide (NAD) concentration or reducing a decline in NAD concentration.

- Advantageous Effects of Invention

[0011]

The present invention can provide a composition that has an effect of increasing nicotinamide adenine dinucleotide (NAD) concentration.

BRIEF DESCRIPTION OF DRAWINGS

[0012]

FIG. 1 is a graph showing effects of a composition containing a sesamin-episesamin mixture (SE) (sesamin:episesamin (weight ratio = 1:1)) and NR at a molar ratio of 1:100, SE, or NR on the increase in intracellular NAD concentration.

FIG. 2 is a graph showing effects of a composition containing a sesamin-episesamin mixture (SE) (sesamin:episesamin (weight ratio = 1:1)) and NR at a molar ratio of 1:1, SE, or NR on the increase in intracellular NAD concentration.

FIG. 3 is a graph showing effects of a composition containing a sesamin-episesamin mixture (SE)

(sesamin:episesamin (weight ratio = 1:1)) and NR at a molar ratio of 1:3.3 or 1:10 or NR on the increase in intracellular NAD concentration.

FIG. 4 is a graph showing effects of a composition containing a sesamin-episesamin mixture (SE) (sesamin:episesamin (weight ratio = 1:1)) and NMN at a molar ratio of 1:3 or 1:10, SE, or NMN on the increase in intracellular NAD concentration.

DESCRIPTION OF EMBODIMENTS

[0013]

The composition of the present invention contains at least one sesamin-class compound.

Sesamin-class compounds have an effect of increasing nicotinamide adenine dinucleotide (NAD) concentration and an effect of improving, maintaining, or ameliorating intracellular nicotinamide adenine dinucleotide (NAD) concentration or reducing a decline in intracellular NAD concentration. Sesamin-class compounds are used to improve NAD concentration, to reduce a decline in NAD concentration, to maintain NAD concentration, or to ameliorate NAD concentration. Increasing nicotinamide adenine dinucleotide (NAD) concentration means increasing the amount of nicotinamide adenine dinucleotide (NAD). The NAD concentration refers to the intracellular NAD concentration.

[0014]

Sesamin-class compounds have an effect of increasing nicotinamide adenine dinucleotide (NAD) concentration and are expected to contribute to inhibition or amelioration of aging associated with a decline in NAD concentration by the effect of improving, maintaining, or ameliorating NAD concentration or reducing a decline in NAD concentration. Aging is understood to be a phenomenon in which physical, physiological, and mental functions decline. The physical

changes associated with aging begin at about age 40, after reaching maturity. The physical changes include skin wrinkling, loss of head hair and teeth, graying of hair, fatigue accumulation and slow recovery from fatigue, loss of vision and hearing, loss of motor function, loss of muscle strength and activity, loss of sleep quality, and loss of bone mass. Although aging itself is not a disease, the decline in physical and physiological functions increases the risk of so-called geriatric diseases such as arteriosclerosis, abnormal glucose and lipid metabolism, neurodegeneration, osteoporosis, and cataracts. Also, aging with respect to mental functions such as memory and learning occurs along with the decline in physical functions. Increasing nicotinamide adenine dinucleotide (NAD) concentration is effective in inhibition or amelioration of aging associated with a decline in NAD concentration.

[0015]

(Sesamin-class compound)

In the present invention, the term “the sesamin-class compound” is a collective term for compounds including sesamin and its analogs. Sesamin is one of main lignan compounds found in sesame. Examples of the sesamin analogs include episesamin and dioxabicyclo[3.3.0]octane derivatives described in JP H04-9331 A. The at least one sesamin-class compound may include a single compound alone or two or more of these compounds above. Specific examples of the sesamin-class compound include sesamin, episesamin, sesaminol, episesaminol, sesamol, and sesamolin. Stereoisomers or racemates of these compounds may be used alone or in mixture. In addition, metabolites of the sesamin-class compound (e.g., those described in JP 2001-139579 A) are also sesamin analogs included in the sesamin-class compound of the present invention, and can also be used in the present invention, as long as they exhibit the

effect(s) of the present invention. In the present invention, sesamin and/or episesamin can be suitably used as the at least one sesamin-class compound. Sesamin and episesamin can be more suitably used. When sesamin and episesamin are used, the ratio of these components is not limited. For example, the ratio of sesamin to episesamin by weight is preferably 1:0.1 to 1:9, more preferably 1:0.3 to 1:3, still more preferably 1:0.5 to 1:2.

[0016]

The sesamin-class compound for use in the present invention is not limited in any way by its form, production method, or the like. For example, when the sesamin-class compound is sesamin, sesamin extracted from sesame oil by a known method (e.g., the method described in JP H04-9331 A) can be used (hereinafter, such sesamin is called a sesamin extract or purified sesamin). Commercially available sesame oil (in liquid form) can also be used as is. However, when sesame oil is used, the characteristic flavor of sesame oil is sometimes evaluated as being organoleptically undesirable. Thus, a tasteless and odorless sesamin extract (or purified sesamin) from sesame oil is preferably used. In addition, since sesame oil has a low sesamin content, use of sesame oil to incorporate a desirable amount of sesamin results in excess volume per unit dose of a composition to be prescribed. This sometimes causes inconvenience in ingestion. In particular, in the case where the composition is formulated for oral administration, the preparation (e.g., tablet or capsule) becomes so bulky that it causes trouble in ingestion. Thus, use of sesamin extract (or purified sesamin) from sesame oil is preferred because it does not require a large amount of ingestion. The sesamin-class compound can also be obtained by synthesis. For example, sesamin and episesamin can be synthesized by the method of Beroza et al. (J. Am. Chem. Soc., 78, 1242 (1956)).

Metabolites of sesamin and episesamin can be synthesized by the method of Urata et al. (Chem. Pharm. Bull. (Tokyo), 56(11), 1611-2 (2008)).

[0017]

The sesamin-class compound is found in natural products and food and beverages. It is a compound with a long history of consumption and recognized high levels of safety. The sesamin-class compound with secured safety is ideal for continuous and long-term ingestion.

[0018]

(Nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof)

The composition of the present invention contains at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof.

NR and NMN are intermediate metabolites of NAD. NR is known to be converted to NMN by nicotinamide riboside kinase (NRK) and NAD^+ is synthesized. NR, NMN, and salts thereof are known compounds and can be produced by known production methods, or commercial products can be used.

[0019]

Pharmacologically acceptable salts of NR and/or NMN can be used. Examples thereof include acid-added salts and base-added salts. Specific examples include metal salts (e.g., alkali metal salts such as sodium salt and potassium salt; alkaline earth metal salts such as calcium salt, magnesium salt, and barium salt), ammonium salts, salts with inorganic acids (e.g., salts with inorganic acids such as hydrochloric acid, hydrobromic acid, nitric acid, sulfuric acid, and phosphoric acid), and salts with organic acids (e.g., salts with organic acids such as acetic acid, phthalic acid, fumaric acid, oxalic acid, tartaric acid, maleic acid, citric acid, succinic acid, methanesulfonic acid, and p-toluenesulfonic acid).

[0020]

The salts of NR and/or NMN are preferably salts that can be used in food or beverages, pharmaceutical products, and the like. Examples thereof include chloride; alkali metal salts such as sodium salt and potassium salt; and alkaline earth metal salts such as calcium salt and magnesium salt. The salt of NR is preferably nicotinamide riboside chloride.

[0021]

(Composition containing at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and at least one sesamin-class compound)

The composition of the present invention contains at least one selected from the group consisting of the nicotinamide riboside (NR), the nicotinamide mononucleotide (NMN), and the salts thereof and at least one of the sesamin-class compounds. The composition of the present invention contains as active ingredients at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and at least one of the sesamin-class compounds. The composition of the present invention may be a composition for increasing nicotinamide adenine dinucleotide (NAD) concentration.

In the composition of the present invention, the molar ratio of the at least one selected from the group consisting of NR, NMN, and salts thereof to the at least one sesamin-class compound (at least one selected from the group consisting of NR, NMN, and salts thereof/at least one sesamin-class compound) is preferably 1 to 100 from the standpoint of effectively enhancing the effect of increasing nicotinamide adenine dinucleotide (NAD) concentration, i.e. from the standpoint of effectively enhancing the effect of improving, maintaining, or

ameliorating NAD concentration or reducing a decline in NAD concentration. The molar ratio of the at least one selected from the group consisting of NR, NMN, and salts thereof to the at least one sesamin-class compound (at least one selected from the group consisting of NR, NMN, and salts thereof/at least one sesamin-class compound) is more preferably 3 to 80, still more preferably 5 to 50. An anti-aging effect is produced by increasing the nicotinamide adenine dinucleotide (NAD) concentration, i.e., by enhancing the effect of improving, maintaining, or ameliorating NAD concentration or reducing a decline in NAD concentration. The molar ratio of NR, NMN, or salts thereof to the sesamin-class compound(s) in the composition of the present invention is therefore preferably set within the above specific range also from the standpoint of the anti-aging effect.

The amount of NR, NMN, or salts thereof herein is indicated as the amount in terms of nicotinamide riboside (NR). The weight in terms of NR of the at least one selected from the group consisting of NR, NMN, and salts thereof is the sum of the weight(s) in terms of NR of the at least one selected from the group consisting of NR, NMN, and salts thereof.

[0022]

The total amount of the at least one sesamin-class compound in the composition of the present invention is not limited. The total amount can be set depending on the form of the composition or the like.

The total amount of the at least one sesamin-class compound in the composition of the present invention is, for example, preferably 0.001 wt% or more, more preferably 0.01 wt% or more, still more preferably 0.05 wt% or more, and is preferably 10 wt% or less, more preferably 5 wt% or less. In one embodiment, the total amount of the at least one sesamin-class compound in the composition is preferably

0.001 to 10 wt%, more preferably 0.01 to 5 wt%, still more preferably 0.05 to 5 wt%. When the composition contains two or more sesamin-class compounds, the total amount is the sum of the amounts of these compounds.

[0023]

The total amount of the at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof in the composition of the present invention is not limited. The total amount can be set depending on the form of the composition or the like.

The total amount of the at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof in the composition of the present invention is preferably 0.001 wt% or more, more preferably 0.01 wt% or more, still more preferably 0.05 wt% or more, and is preferably 95 wt% or less, more preferably 80 wt% or less, for example. In one embodiment, the total amount of the at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof in the composition is preferably 0.001 to 95 wt%, more preferably 0.01 to 80 wt%, still more preferably 0.05 to 70 wt%. When the composition contains two or more compounds selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof, the total amount is the sum of the amounts of these compounds.

[0024]

The composition of the present invention is capable of increasing nicotinamide adenine dinucleotide (NAD) concentration, capable of improving, maintaining, or ameliorating nicotinamide adenine dinucleotide (NAD) concentration or reducing a decline in nicotinamide adenine dinucleotide (NAD) concentration, and capable of improving, maintaining, or ameliorating nicotinamide adenine

dinucleotide (NAD) concentration that has declined with age or reducing a decline in nicotinamide adenine dinucleotide (NAD) concentration associated with aging.

[0025]

NAD in vivo plays an important role in activating sirtuins to deacetylate downstream enzymes or transcription factors, thereby promoting the expression and synthesis of molecules involved in mitochondrial function and energy production. Therefore, improving, maintaining, or ameliorating NAD concentration or reducing a decline in NAD concentration has an effect of improving, maintaining, or ameliorating mitochondrial function or reducing a decline in mitochondrial function. The composition of the present invention can improve, maintain, or ameliorate mitochondrial function or reduce a decline in mitochondrial function. The composition of the present invention can also improve, maintain, or ameliorate mitochondrial energy production capacity or reduce a decline in mitochondrial energy production capacity. Thus, the composition of the present invention achieves an anti-cellular senescence effect.

[0026]

Herein, the mitochondrial function and mitochondrial energy production capacity only need to be evaluated based on common knowledge in the technical field to which the present invention belongs. Any method may be used for evaluation. For example, the mitochondrial function can be evaluated by continuously evaluating the oxygen consumption rate (hereinafter also described as “OCR”) of mitochondria during ATP synthesis in cells as the measurement target, using an extracellular flux analyzer, while adding ATP synthase inhibitors (e.g., oligomycin and rotenone) and an uncoupler (e.g., carbonyl cyanide-p-trifluoromethoxy phenylhydrazone (FCCP)) thereto. Evaluation items may be, for example, basal respiration, ATP production capacity,

and maximal respiration, which are considered to be major indices for evaluation of mitochondrial function. The mitochondrial function can be evaluated by analyzing these evaluation items.

[0027]

The composition of the present invention can be used to inhibit and/or delay aging, and is preferably used to inhibit and/or delay aging associated with a decline in NAD concentration.

[0028]

In one embodiment, the composition of the present invention improves, maintains, or ameliorates NAD concentration or reduces a decline in NAD concentration, and is suitably used to improve, maintain, or ameliorates NAD concentration that has declined with age or to reduce a decline in NAD concentration associated with aging.

In one embodiment, the composition of the present invention is suitably used to improve, maintain, or ameliorate NAD concentration that has declined with age in middle-aged and older people, or to reduce a decline in NAD concentration associated with aging in middle-aged and older people. The middle-aged and older people include the elderly. The middle-aged and older people may be those who are 40 years old and older, for example. The elderly may be those who are 60 years old and older or 65 years old or older, for example.

The NAD concentration in humans and other animals can be measured by a common method in the technical field to which the present invention belongs, such as measurement of the intracellular NAD concentration using Amplite® Colorimetric NAD/NADH Ratio Assay Kit (AAT Bioquest) or NAD^+/NADH Quantification Colorimetric Kit (BioVision Inc.).

[0029]

The composition of the present invention can be used for the treatment of conditions or diseases that are

expected to be prevented or alleviated by improving, maintaining, or ameliorating NAD concentration or reducing a decline in NAD concentration. Examples of such conditions or diseases include age-related declines in physical, physiological, and mental functions. Specific examples thereof include the occurrence of skin aging symptoms (wrinkles, sagging, loss of skin firmness, etc.), dry skin due to aging (reduced skin moisture retention), skin blotches, freckles, rough skin, decreased or increased secretion of hormones (growth hormone, thyroid hormone, adrenal cortical hormone, sex hormone, prolactin, antidiuretic hormone, parathyroid hormone, melatonin, etc.), damage to cells (brain cells, cardiac muscle cells, etc.) due to active oxygen, loss of head hair and teeth, loss of vision and hearing, loss of motor function, loss of bone mass, loss of physical fitness, memory decline, loss of learning ability, loss of immune function, and onset of geriatric diseases.

The term “preventing” as used herein encompasses preventing disease or symptom onset, delaying disease or symptom onset, decreasing disease or symptom incidence, decreasing risk of disease or symptom onset, and the like. The phrase “alleviating conditions or diseases” encompasses helping the subject recover from conditions or diseases, alleviating conditions or disease symptoms, making positive changes in conditions or disease symptoms, delaying or preventing progression of conditions or diseases, and the like.

[0030]

The composition of the present invention is applicable for therapeutic use (medical use) and non-therapeutic use (non-medical use). The “non-therapeutic” is a concept that does not include medical activities, i.e., a concept that does not include methods of surgery, therapy, or diagnosis of humans.

The composition of the present invention can be provided, for example, in the form of a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like. The composition of the present invention may be a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like to be used for improving, maintaining, or ameliorating nicotinamide adenine dinucleotide (NAD) concentration, or reducing a decline in NAD concentration by itself; or the composition may be a material, a formulation, or the like to be added to these products.

The composition of the present invention may be a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like for improving, maintaining, or ameliorating mitochondrial function or reducing a decline in mitochondrial function or for improving, maintaining, or ameliorating mitochondrial energy production capacity or reducing a decline in mitochondrial energy production capacity by itself; or the composition may be a material, a formulation, or the like to be added to these products.

The composition of the present invention can be provided in the form of an agent, for example, but the form is not limited thereto. Such an agent may be provided as a composition directly, or as a composition containing the agent.

The composition of the present invention may be an oral composition or a parenteral composition, and is preferably an oral composition. The oral composition may be as is a food or beverage, an oral pharmaceutical product, an oral quasi-pharmaceutical product, oral feed, or the like, or may be contained in these products. The composition of the present invention is preferably a food or beverage or an oral pharmaceutical product, more preferably a food or beverage.

[0031]

The composition of the present invention may contain optional additives and optional components, in addition to the at least one selected from the group consisting of NR, NMN, and salts thereof and the at least one sesamin-class compound, as long as the effect(s) of the present invention is/are not impaired. Such additives and components can be selected according to the form of the composition or the like. Those that can be used generally in foods, beverages, pharmaceutical products, quasi-pharmaceutical products, feed, and the like can be used. When the composition of the present invention is provided as a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like, any common method can be used for production.

[0032]

For example, when the composition of the present invention is provided as a food or beverage, a component usable in food or beverages (e.g., a food material or an optional food additive) can be added to the at least one selected from the group consisting of NR, NMN, and salts thereof and the at least one sesamin-class compound to provide various types of foods or beverages. Non-limiting examples of the foods or beverages include general foods and beverages, health foods, health beverages, foods with function claims, foods for specified health uses, and foods and beverages for the sick. The health foods, the foods with function claims, the foods for specified health uses, and the like can be used, for example, in various forms of preparations such as fine granules, tablets, granules, powders, capsules, chewable tablets, dry syrups, syrups, liquids, beverages, and liquid foods.

[0033]

When the composition of the present invention is provided as a pharmaceutical product or a quasi-

pharmaceutical product, for example, a pharmacologically acceptable carrier, an optional additive, or the like can be added to the at least one selected from the group consisting of NR, NMN, and salts thereof and the at least one sesamin-class compound to provide various dosage forms of pharmaceutical products or quasi-pharmaceutical products. Such a carrier, additive, or the like may be any pharmacologically acceptable one that can be used in pharmaceutical products or quasi-pharmaceutical products. Examples thereof include excipients, binders, disintegrants, lubricants, antioxidants, and colorants. One or more of these can be used. The form of administration (ingestion) of the pharmaceutical product or the quasi-pharmaceutical product may be oral or parenteral (e.g., transdermal, transmucosal, enteral, or injection) administration. When the composition of the present invention is provided as a pharmaceutical product or a quasi-pharmaceutical product, it is preferably an oral pharmaceutical product or an oral quasi-pharmaceutical product. Examples of the dosage form for oral administration include liquids, tablets, powders, fine granules, granules, sugar-coated tablets, capsules, suspensions, emulsions, and chewable tablets. The pharmaceutical product may be for non-human animals.

[0034]

When the composition of the present invention is provided as feed, at least one selected from the group consisting of NR, NMN, and salts thereof and at least one sesamin-class compound are simply added to feed. The feed includes feed additives. Examples of the feed include feed for farm animals such as cows, pigs, chickens, sheep, and horses; feed for small animals such as rabbits, rats, and mice; and pet food for animals such as dogs, cats, and birds.

[0035]

The composition of the present invention is preferably an oral composition (a composition to be ingested orally (orally administered)). The dosage (which can also be referred to as "intake") of the composition of the present invention is not limited. The dosage of the composition of the present invention may be any amount that achieves an effect of improving, maintaining, or ameliorating nicotinamide adenine dinucleotide (NAD) concentration or reducing a decline in NAD concentration. The dosage may be appropriately set according to the administration form, administration method, body weight of a subject, and the like.

[0036]

In one embodiment, when the composition of the present invention is orally ingested by or administered to a human (adult), the total intake of the sesamin-class compound is preferably 0.5 mg or more, more preferably 1 mg or more, still more preferably 3 mg or more, and is preferably 200 mg or less, more preferably 100 mg or less, still more preferably 80 mg or less per 60 kg body weight per day. In one embodiment, the total intake of the sesamin-class compound is preferably 0.5 to 200 mg, more preferably 1 to 100 mg, still more preferably 3 to 80 mg per 60 kg body weight per day for a human (adult). Preferably, the sesamin-class compound in the above amount is ingested or administered in one or more portions per day, for example, in one to several portions (e.g., two or three portions) per day. In one embodiment, preferably, the sesamin-class compound in the above amount is orally ingested by or administered to a human. In one embodiment, the composition of the present invention can be used to allow the sesamin-class compound in the above amount to be ingested by or administered to a human per 60 kg body weight per day. When the composition contains two or more sesamin-class compounds, the total intake is the sum of

these compounds. In one embodiment, sesamin and/or episesamin is orally ingested by or administered to a human (adult) in an amount of preferably 0.5 to 200 mg, more preferably 1 to 100 mg, still more preferably 3 to 80 mg per 60 kg body weight per day as the total intake of sesamin and episesamin.

[0037]

In one embodiment, when the composition of the present invention is orally ingested by or administered to a human (adult), the total intake in terms of NR of the at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof is preferably 0.5 mg or more, more preferably 1 mg or more, still more preferably 3 mg or more, and is preferably 20000 mg or less, more preferably 10000 mg or less, still more preferably 8000 mg or less per 60 kg body weight per day. In one embodiment, the total intake in terms of NR of the at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof is preferably 0.5 to 20000 mg, more preferably 1 to 10000 mg, still more preferably 3 to 8000 mg per 60 kg body weight per day for a human (adult). Preferably, the at least one selected from the group consisting of NR, NMN, and salts thereof in the above amount is ingested or administered in one or more portions per day, for example, in one to several portions (e.g., two or three portions) per day. In one embodiment, preferably the at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof in the above amount is orally ingested by or administered to a human. In one embodiment, the composition of the present invention can be used to allow the at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof in the

above amount to be ingested by or administered to a human per 60 kg body weight per day. When the composition contains two or more compounds selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof, the total intake is the sum of these compounds.

[0038]

Preferably, the composition of the present invention is continuously ingested or administered. Continuous ingestion or administration of the sesamin-class compound is likely to enhance the above effects. Also, continuous ingestion or administration of the compound selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof is likely to enhance the above effects. In one embodiment, the composition of the present invention is continuously ingested or administered for preferably one week or more, more preferably four weeks or more, still more preferably eight weeks or more, particularly preferably 12 weeks or more.

[0039]

The composition of the present invention may be labeled with a function claim of a function exerted by improving, maintaining, or ameliorating nicotinamide adenine dinucleotide (NAD) concentration or reducing a decline in NAD concentration. The composition of the present invention may be labeled with one or more function claims selected from the group consisting of “improving or maintaining mitochondrial function or reducing a decline in mitochondrial function”, “improving or maintaining mitochondrial energy production capacity or reducing a decline in mitochondrial energy production capacity”, “inhibiting and/or delaying aging associated with a decline in NAD concentration”, “inhibiting and/or delaying cellular senescence”, and “inhibiting aging”.

In one embodiment of the present invention, the composition of the present invention is preferably a food or beverage labeled with one or more of the above function claims. The label may be one indicating use for obtaining one or more of these functions.

[0040]

The subject (which can also be referred to as "administration subject") subjected to ingestion or administration of the composition of the present invention is not limited. Humans and non-human animals can be subjected to ingestion. Examples of the non-human animals include industrial animals, pets, and laboratory animals. Specifically, the term "industrial animals" refers to animals that are bred for industrial purposes. Examples include farm animals such as cows, horses, pigs, goats, and sheep; poultry such as chickens, ducks, quals, turkeys, and ostriches; and fish such as yellowtail, young yellowtail, red seabream, Japanese horse mackerel, carp, rainbow trout, and eel. The term "pets" refers to pet animals or companion animals such as dogs, cats, common marmosets, birds, and hamsters. The term "laboratory animals" refers to mice, rats, guinea pigs, beagles, miniature pigs, rhesus monkeys, crab-eating monkeys, and other animals that are used in research in fields of medicine, biology, agronomy, pharmacy, and the like.

[0041]

The subject (which can be also referred to as "administration subject") subjected to ingestion or administration of the composition of the present invention is preferably a human or non-human mammal, more preferably a human.

In one embodiment, the administration subject may be one needing or wanting to improve, maintain, or ameliorate nicotinamide adenine dinucleotide (NAD) concentration or reduce a decline in NAD concentration, and the like.

Examples of the subjects include middle-aged and older people, one needing or wanting to improve, maintain, or ameliorate mitochondrial function or reducing a decline in mitochondrial function, one needing or wanting to improve, maintain, or ameliorate mitochondrial energy production capacity or reducing a decline in mitochondrial energy production capacity, one needing or wanting to inhibit and/or delay aging associated with a decline in NAD concentration, and the like. The middle-aged and older people may be those who are 40 years old and older, for example. In one embodiment, the elderly are preferred subjects among the middle-aged and older people. The elderly may be people who are 60 years old and older or 65 years old and older, for example. The composition of the present invention can be used by healthy people for purposes such as preventing conditions or diseases that are likely to be prevented or alleviated by improving, maintaining, or ameliorating nicotinamide adenine dinucleotide (NAD) concentration or reducing a decline in NAD concentration.

[0042]

The present invention also encompasses the following methods.

A method of increasing nicotinamide adenine dinucleotide (NAD) concentration, including administering at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and at least one sesamin-class compound.

A method of improving, maintaining, or ameliorating nicotinamide adenine dinucleotide (NAD) concentration or reducing a decline in NAD concentration, including administering at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide

mononucleotide (NMN), and salts thereof and at least one sesamin-class compound.

A method of improving, maintaining, or ameliorating nicotinamide adenine dinucleotide (NAD) concentration that has declined with age or reducing a decline in NAD concentration associated with aging, including administering at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and at least one sesamin-class compound.

[0043]

The invention also encompasses the following uses.

Use of at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and at least one sesamin-class compound, for increasing nicotinamide adenine dinucleotide (NAD) concentration.

Use of at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and at least one sesamin-class compound, for improving, maintaining, or ameliorating nicotinamide adenine dinucleotide (NAD) concentration or reducing a decline in NAD concentration.

Use of at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and at least one sesamin-class compound, for improving, maintaining, or ameliorating nicotinamide adenine dinucleotide (NAD) concentration that has declined with age or reducing a decline in NAD concentration associated with aging. The above methods and uses may be therapeutic or non-therapeutic.

Administration of at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and at

least one sesamin-class compound can improve, maintain, or ameliorate nicotinamide adenine dinucleotide (NAD) concentration or reduce a decline in NAD concentration. Thus, administration of at least one selected from the group consisting of NR, NMN, and salts thereof and at least one sesamin-class compound enables: improvement, maintenance, or amelioration of mitochondrial function or reduction of a decline in mitochondrial function; improvement, maintenance, or amelioration of mitochondrial energy production capacity or reduction of a decline in mitochondrial energy production capacity; and inhibition and/or delay of aging associated with a decline in NAD concentration.

[0044]

In the methods and uses, the compound selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof, the sesamin-class compound, and preferred embodiments thereof are the same as those of the composition of the present invention. The at least one sesamin-class compound may include one sesamin-class compound or two or more sesamin-class compounds. The compound selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof include one compound or two or more compounds. In the above methods and uses, preferably, the at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and the at least one sesamin-class compound are administered to (injected by) a subject at least once per day, for example, one to several times (e.g., two or three times) per day. In the above methods and uses, the composition of the present invention is preferably orally administered (orally ingested). The above uses are preferably for humans or non-human mammals, more preferably for humans.

[0045]

The above methods and uses only require use of the at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and the at least one sesamin-class compound each in an amount (which can also be referred to as "effective amount") that achieves a desired effect (the effect of increasing nicotinamide adenine dinucleotide (NAD) concentration, i.e., the effect of improving, maintaining, or ameliorating NAD concentration or reducing a decline in NAD concentration, and the effect(s) obtained by the above effect, including: the effect of improving, maintaining, or ameliorating mitochondrial function or reducing a decline in mitochondrial function; the effect of improving, maintaining, or ameliorating mitochondrial energy production capacity or reducing a decline in mitochondrial energy production capacity; and/or the effect of inhibiting and/or delaying aging associated with a decline in NAD concentration). Preferred dosage, preferred administration subjects, and the like of the at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and the at least one sesamin-class compound are as described above for the composition of the present invention. The at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and the at least one sesamin-class compound may be administered directly or may be administered in the form of a composition containing these. For example, the composition of the present invention described above may be used.

[0046]

In the above uses, a composition containing at least one sesamin-class compound and a composition containing at least one selected from the group consisting of

nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof may be separately prepared, and the compositions may be taken substantially at the same time or one of the compositions may be taken first, followed by taking of the other while the effect of the first composition lasts. This enhances the effect intended by the present invention, i.e., the effect of combining at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and at least one sesamin-class compound (the effect of increasing NAD concentration, i.e., the effect of improving, maintaining, or ameliorating NAD concentration or reducing a decline in NAD concentration, and the effect(s) obtained by the above effect, including: the effect of improving, maintaining, or ameliorating mitochondrial function or reducing a decline in mitochondrial function; the effect of improving, maintaining, or ameliorating mitochondrial energy production capacity or reducing a decline in mitochondrial energy production capacity; and/or the effect of inhibiting and/or delaying aging associated with a decline in NAD concentration). Thus, a kit or the like including a composition containing at least one sesamin-class compound and a composition containing at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof falls within the scope of the composition of the present invention.

[0047]

The present invention also encompasses use of at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and at least one sesamin-class compound, for producing the composition of the present invention.

EXAMPLES

[0048]

The present invention is described in more detail with reference to examples. The present invention is not limited to these examples.

[0049]

In examples and comparative examples, a mixture of sesamin and episesamin (sesamin:episesamin (weight ratio) = 1:1) was used as a sesamin-episesamin mixture (SE).

[0050]

<Comparative Examples 1 to 3, Example 1: Evaluation test of effect of sesamin-episesamin mixture (SE) and nicotinamide riboside (NR)on intracellular NAD^+ concentration>

To examine the effect on intracellular NAD^+ concentration, Hepa 1-6 cells were seeded in 96-well plates at 0.5×10^4 cells/well, and cultured in a DMEM medium (Nacalai Tesque, Inc., containing 10% FBS) at 37°C with CO_2 (5%) for 96 hours. After 96 hours of culturing, the medium in each well was replaced with a DMEM medium (containing 1% albumin and no FBS (Comparative Example 1)) containing no sesamin-episesamin mixture (SE) or nicotinamide riboside (NR chloride (available from Carbosynth Limited)), a DMEM medium (containing 1% albumin and no FBS (Comparative Example 2)) containing 0.3 μM of SE, a DMEM medium (containing 1% albumin and no FBS (Comparative Example 3)) containing 30 μM of NR, or a DMEM medium (containing 1% albumin and no FBS (Example 1)) containing 0.3 μM of SE and 30 μM of NR, followed by culturing for additional 24 hours. The added media were removed, and the cells in the wells were washed with D-PBS (Nacalai Tesque Inc.), followed by collection of cell extracts by adding Lysis Buffer (Amplite® Colorimetric NAD/NADH Ratio Assay Kit, available from AAT Bioquest). The intracellular NAD^+ concentrations were then analyzed according to the manual of the Kit. The

intracellular NAD^+ concentration was determined by measuring the protein concentration of the cell extract (Pierce® BCA Protein Assay Kit, available from Thermo Scientific, Inc.), and calculated as a value per protein concentration. The significant test performed was an unpaired t-test (significance level: $p < 0.05$, relative to the control group).

FIG. 1 shows the results (average of N = 3) (*: $p < 0.05$).

[0051]

FIG. 1 shows that the intracellular NAD^+ concentration was increased by 2.3% in the 0.3 μM SE group of Comparative Example 2 and by 3.1% in the 30 μM NR group of Comparative Example 3, relative to Comparative Example 1 (medium containing no SE or no NR). In contrast, the intracellular NAD^+ concentration was increased by 14.6% in the 0.3 μM SE + 30 μM NR group of Example 1, which was significantly greater than the value (5.4%) obtained by simply summing up the percentage increase by the treatment with 0.3 μM of SE in Comparative Example 2 and the percentage increase by the treatment with 30 μM of NR in Comparative Example 3. From the above, it was confirmed that a combination of SE and NR at a molar ratio (SE:NR) of 1:100 in the treatment synergistically increased the intracellular NAD^+ concentration.

[0052]

<Comparative Examples 4 to 6, Example 2: Evaluation test of effect of sesamin-episesamin mixture (SE) and nicotinamide riboside (NR) on intracellular NAD^+ concentration>

To examine the effect on intracellular NAD^+ concentration, Hepa 1-6 cells were seeded in 96-well plates at 1.0×10^4 cells/well, and cultured in a DMEM medium (Nacalai Tesque, Inc., containing 10% FBS) at 37°C with CO_2 (5%) for 48 hours. After 48 hours of culturing, the medium in each well was replaced with a DMEM medium (containing 1%

albumin and no FBS (Comparative Example 4)) containing no sesamin-episesamin mixture (SE) or nicotinamide riboside (NR, chloride), a DMEM medium (containing 1% albumin and no FBS (Comparative Example 5)) containing 10 μM of SE, a DMEM medium (containing 1% albumin and no FBS (Comparative Example 6)) containing 10 μM of NR, or a DMEM medium (containing 1% albumin and no FBS (Example 2)) containing 10 μM of SE and 10 μM of NR, followed by culturing for additional 24 hours. The added media were removed, and the cells in the wells were washed with D-PBS (Nacalai Tesque Inc.), followed by collection of cell extracts by adding Lysis Buffer (Amplite® Colorimetric NAD/NADH Ratio Assay Kit, available from AAT Bioquest). The intracellular NAD^+ concentrations were then analyzed according to the manual of the Kit. The intracellular NAD^+ concentration was determined by measuring the protein concentration of the cell extract (Pierce® BCA Protein Assay Kit, available from Thermo Scientific, Inc.), and calculated as a value per protein concentration. FIG. 2 shows the results (average of N = 3) (*: $p < 0.05$).

[0053]

FIG. 2 shows that the intracellular NAD^+ concentration was increased by 1.5% in the 10 μM SE group of Comparative Example 5 and by 11.6% in the 10 μM NR group of Comparative Example 6, relative to Comparative Example 4 (medium containing no SE or no NR). In contrast, the intracellular NAD^+ concentration was increased by 15.7% in the 10 μM SE + 10 μM NR group of Example 2, which was significantly greater than the value (13.1%) obtained by simply summing up the percentage increase by the treatment with 10 μM of SE in Comparative Example 5 and the percentage increase by the treatment with 10 μM of NR in Comparative Example 6. From the above, it was confirmed that a combination of SE and NR at a molar ratio (SE:NR) of

1:1 in the treatment synergistically increased the intracellular NAD^+ concentration.

[0054]

<Comparative Examples 7 to 9, Examples 3 and 4: Evaluation test of effect of sesamin-episesamin mixture (SE) and nicotinamide riboside (NR) on intracellular NAD^+ concentration>

To examine the effect on intracellular NAD^+ concentration, Hepa 1-6 cells were seeded in 96-well plates at 1.0×10^4 cells/well, and cultured in a DMEM medium (Nacalai Tesque, Inc., containing 10% FBS) at 37°C with CO_2 (5%) for 48 hours. After 48 hours of culturing, the medium in each well was replaced with a DMEM medium (containing 1% albumin and no FBS (Comparative Example 7)) containing no sesamin-episesamin mixture (SE) or nicotinamide riboside (NR, chloride), a DMEM medium (containing 1% albumin and no FBS (Comparative Example 8)) containing 10 μM of NR, a DMEM medium (containing 1% albumin and no FBS (Comparative Example 9)) containing 30 μM of NR, a DMEM medium (containing 1% albumin and no FBS (Example 3)) containing 3 μM of SE and 10 μM of NR, or a DMEM medium (containing 1% albumin and no FBS (Example 4)) containing 3 μM of SE and 30 μM of NR, followed by culturing for additional 24 hours. The added media were removed, and the cells in the wells were washed with D-PBS (Nacalai Tesque Inc.), followed by collection of cell extracts by adding Lysis Buffer (Amplite® Colorimetric NAD/NADH Ratio Assay Kit, available from AAT Bioquest). The intracellular NAD^+ concentrations were then analyzed according to the manual of the Kit. The intracellular NAD^+ concentration was determined by measuring the protein concentration of the cell extract (Pierce® BCA Protein Assay Kit, available from Thermo Scientific, Inc.), and calculated as a value per protein concentration. FIG. 3 shows the results (average of N = 3) (*: $p < 0.05$).

[0055]

FIG. 3 shows that the intracellular NAD^+ concentration was increased by 11.6% in the 10 μM NR group of Comparative Example 8 and by 15.5% in the 30 μM NR group of Comparative Example 9, relative to Comparative Example 7 (medium containing no SE or no NR). In contrast, the intracellular NAD^+ concentration was increased by 17.5% in the 3 μM SE + 10 μM NR group of Example 3 and by 21.8% in the 3 μM SE + 30 μM NR group of Example 4. The evaluation test did not include a case using a DMEM medium containing 3 μM of SE. However, based on the data that the NAD concentration was increased by 2.3% in the 0.3 μM SE group of Comparative Example 2 and by 1.5% in the 10 μM SE group of Comparative Example 5, the percentage increase in NAD concentration in the 3 μM SE group is presumably similar to these percentage increases. When the value obtainable by simply summing up the percentage increase in NAD concentration in Comparative Example 8 or Comparative Example 9 and the percentage increase in NAD concentration that is expected to be obtained by using 3 μM of SE (2.3 to 1.5%) is compared with the percentage increases in NAD concentration in Example 3 and Example 4, the percentage increases in NAD concentration in Examples 3 and 4 are clearly greater. From the above, it was confirmed that a combination of SE and NR at a molar ratio (SE:NR) of 1:3.3 or 1:10 in the treatment synergistically increased the intracellular NAD^+ concentration.

[0056]

<Comparative Examples 10 to 12, Example 5: Evaluation test of effect of sesamin-episesamin mixture (SE) and nicotinamide riboside (NR) on intracellular NAD^+ concentration (intracellular NAD concentration)>

To examine the effect on intracellular NAD^+ concentration, HepG2 cells were seeded in 96-well plates at 1.5×10^4 cells/well, and cultured in a DMEM medium

(Nacalai Tesque, Inc., containing 10% FBS) at 37°C with CO_2 (5%) for 96 hours. After 96 hours of culturing, the medium in each well was replaced with a DMEM medium (containing 1% albumin and no FBS (Comparative Example 10)) containing no sesamin-episesamin mixture (SE) or nicotinamide riboside (NR, chloride), a DMEM medium (containing 1% albumin and no FBS (Comparative Example 11)) containing 0.3 μM of SE, a DMEM medium (containing 1% albumin and no FBS (Comparative Example 12)) containing 10 μM of NR, or a DMEM medium (containing 1% albumin and no FBS (Example 5)) containing 0.3 μM of SE and 10 μM of NR, followed by culturing for additional 24 hours. The added media were removed, and the cells in the wells were washed with D-PBS (Nacalai Tesque Inc.), followed by collection of cell extracts by adding Lysis Buffer (Amplite® Colorimetric NAD/NADH Ratio Assay Kit, available from AAT Bioquest). The intracellular NAD^+ concentrations were then analyzed according to the manual of the Kit. The intracellular NAD^+ concentration was determined by measuring the protein concentration of the cell extract (Pierce® BCA Protein Assay Kit, available from Thermo Scientific, Inc.), and calculated as a value per protein concentration.

[0057]

The intracellular NAD^+ concentration was increased by 5.0% in the 0.3 μM SE group of Comparative Example 11 and was not increased in the 10 μM NR group of Comparative Example 12, relative to Comparative Example 10 (Control, medium containing no SE or no NR). In contrast, the intracellular NAD^+ concentration was increased by 8.0% in the 0.3 μM SE + 10 μM NR group of Example 5, which was significantly greater than the value (5.0%) obtained by simply summing up the percentage increase by the treatment with 0.3 μM of SE and the percentage increase by the treatment with 10 μM of NR. From the above, it was confirmed that a combination of SE and NR at a molar ratio

(SE:NR) of 1:33 in the treatment synergistically increased the intracellular NAD^+ concentration.

[0058]

These results clearly indicate that compositions containing a sesamin-episesamin mixture and nicotinamide riboside (NR) have a synergistic effect on increasing the intracellular NAD^+ concentration.

[0059]

<Comparative Examples 13 to 16, Examples 6 and 7: Evaluation test of effect of sesamin-episesamin mixture (SE) and nicotinamide mononucleotide (NMN) on intracellular NAD^+ concentration>

To examine the effect on intracellular NAD^+ concentration, HepG2 cells were seeded in 96-well plates at 2.5×10^4 cells/well, and cultured in a DMEM medium (Nacalai Tesque, Inc., containing 10% FBS) at 37°C with CO_2 (5%) for 48 hours. After 48 hours of culturing, the medium in each well was replaced with a DMEM medium (containing 1% albumin and no FBS (Comparative Example 13)) containing no sesamin-episesamin mixture (SE) or nicotinamide mononucleotide (NMN), a DMEM medium (containing 1% albumin and no FBS (Comparative Example 14)) containing 10 μM of SE, a DMEM medium (containing 1% albumin and no FBS (Comparative Example 15)) containing 30 μM of NMN, a DMEM medium (containing 1% albumin and no FBS (Comparative Example 16)) containing 100 μM of NMN, a DMEM medium (containing 1% albumin and no FBS (Example 6)) containing 10 μM of SE and 30 μM of NMN, or a DMEM medium (containing 1% albumin and no FBS (Example 7)) containing 10 μM of SE and 100 μM of NMN, followed by culturing for additional 24 hours. The added media were removed, and the cells in the wells were washed with D-PBS (Nacalai Tesque Inc.), followed by collection of cell extracts by adding Lysis Buffer (Amplite® Colorimetric NAD/NADH Ratio Assay Kit, available from AAT Bioquest). The intracellular NAD^+

concentrations were then analyzed according to the manual of the Kit. The intracellular NAD^+ concentration was determined by measuring the protein concentration of the cell extract (Pierce® BCA Protein Assay Kit, available from Thermo Scientific, Inc.), and calculated as a value per protein concentration. FIG. 4 shows the results (average of N = 3) (*: $p < 0.05$).

[0060]

FIG. 4 shows that the intracellular NAD^+ concentration was increased by 14.2% in the 10 μM SE group of Comparative Example 14, by 18.9% in the 30 μM NMN group of Comparative Example 15, and by 21.3% in the 100 μM NMN group of Comparative Example 16, relative to Comparative Example 13 (medium containing no SE or no NMN). In contrast, the intracellular NAD^+ concentration was increased by 38.0% in the 10 μM SE + 30 μM NMN group of Example 6, which was significantly greater than the value (33.1%) obtained by simply summing up the percentage increase by the treatment with 10 μM of SE in Comparative Example 1 and the percentage increase by the treatment with 30 μM of NMN in Comparative Example 15.

The intracellular NAD^+ concentration was increased by 74.6% in the 10 μM SE + 100 μM NMN group of Example 7, which was significantly greater than the value (35.5%) obtained by simply summing up the percentage increase by the treatment with 10 μM of SE in Comparative Example 14 and the percentage increase by the treatment with 100 μM of NMN in Comparative Example 16.

From the above, it was confirmed that a combination of SE and NMN at a molar ratio (SE:NMN) of 1:3 or 1:10 in the treatment synergistically increased the intracellular NAD^+ concentration.

[0061]

The above results clearly shows that a composition containing a combination of NMN which is known as a NAD

intermediate metabolite and/or a salt thereof with at least one sesamin-class compound (preferably a sesamin-episesamin mixture) has the same synergistic effect of increasing the intracellular NAD^+ concentration as the composition containing a sesamin-episesamin mixture and NR.

CLAIMS

Claim 1. A composition comprising, as active ingredients:

at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof; and

at least one sesamin-class compound.

Claim 2. The composition according to claim 1,

wherein the at least one sesamin-class compound includes at least one of sesamin or episesamin.

Claim 3. The composition according to claim 1 or 2,

wherein a molar ratio of the at least one selected from the group consisting of NR, NMN, and salts thereof to the at least one sesamin-class compound (at least one selected from the group consisting of NR, NMN, and salts thereof/at least one sesamin-class compound) is 1 to 100.

Claim 4. The composition according to any one of claims 1 to 3,

wherein the at least one sesamin-class compound is contained in a total amount of 0.001 to 10 wt%.

Claim 5. The composition according to any one of claims 1 to 4,

wherein the composition improves, maintains, or ameliorates nicotinamide adenine dinucleotide (NAD) concentration or reduces a decline in NAD concentration.

Claim 6. The composition according to any one of claims 1 to 5,

wherein the composition improves, maintains, or ameliorates mitochondrial function or reduces a decline in mitochondrial function.

Claim 7. The composition according to any one of claims 1 to 6,

wherein the composition improves, maintains, or ameliorates mitochondrial energy production capacity or reduces a decline in mitochondrial energy production capacity.

Claim 8. The composition according to any one of claims 1 to 7,

wherein the composition is used to inhibit and/or delay aging associated with a decline in NAD concentration.

Claim 9. The composition according to any one of claims 1 to 8,

wherein the composition is an anti-aging composition.

Claim 10. The composition according to any one of claims 1 to 9,

wherein the composition is an oral composition.

Claim 11. The composition according to any one of claims 1 to 10,

wherein the composition is a food or beverage.

Claim 12. The composition according to any one of claims 1 to 11,

wherein the composition is labeled with at least one of “inhibiting and/or delaying aging associated with a decline in NAD concentration” or “inhibiting and/or delaying cellular senescence”.

Claim 13. A method of increasing nicotinamide adenine dinucleotide (NAD) concentration, comprising

administering at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and at least one sesamin-class compound.

Claim 14. A method of improving, maintaining, or ameliorating nicotinamide adenine dinucleotide (NAD) concentration or reducing a decline in NAD concentration, comprising

administering at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and at least one sesamin-class compound.

Claim 15. Use of at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and at least one sesamin-class compound, for increasing nicotinamide adenine dinucleotide (NAD) concentration.

Claim 16. Use of at least one selected from the group consisting of nicotinamide riboside (NR), nicotinamide mononucleotide (NMN), and salts thereof and at least one sesamin-class compound, for improving, maintaining, or ameliorating nicotinamide adenine dinucleotide (NAD) concentration or reducing a decline in NAD concentration.