

DESCRIPTION

TITLE OF INVENTION: VASCULAR ENDOTHELIUM FUNCTION-IMPROVING
COMPOSITION

5

TECHNICAL FIELD

[0001]

The present invention relates to, for example, a
composition for improving vascular endothelial function.

10

BACKGROUND ART

[0002]

Vascular endothelial cells are cells that make up the
inner lining of blood vessels. Vascular endothelial cells
15 have a function to produce physiologically active
substances such as nitric oxide (hereinafter sometimes
described as NO) and play a vital role in maintaining
vascular health. A sustained condition of decline in
vascular endothelial cell function (vascular endothelial
20 function) may lead to progression of arteriosclerosis, for
example. Since the decline in vascular endothelial
function is reversible, reducing or preventing a decline in
vascular endothelial function is effective in preventing
arteriosclerosis, for example.

25 [0003]

Sesamin is one of the main lignan compounds found in
sesame. According to Non-Patent Literature 1, sesamin
induces nitric oxide in human umbilical vein endothelial
cells (hereinafter sometimes referred to as HUVECs).

30 [0004]

Docosahexaenoic acid (hereinafter sometimes referred
to as DHA) is a type of highly unsaturated fatty acid found
in fish oil and the like. According to Non-Patent
Literature 2, DHA increases NO production in HUVECs.

35

CITATION LIST

- Non-Patent Literature

[0005]

Non-Patent Literature 1: Lee, C.C. et al. J. Hypertens. 22,
5 2329-2338 (2004).

Non-Patent Literature 2: Jung, S.B. et al. Biochem.
Biophys. Res. Commun. 437, 114-119 (2013).

SUMMARY OF INVENTION

10 - Technical Problem

[0006]

Improvement in vascular endothelial function requires
continuous ingestion of active ingredients. In view of
ease of administration and safety for continuous ingestion,
15 there is a demand for a composition that can exhibit a
vascular endothelial function improving action even with a
low intake.

[0007]

The present invention aims to provide a composition
20 for improving vascular endothelial function, the
composition having an excellent vascular endothelial
function improving action.

- Solution to Problem

25 [0008]

As a result of extensive studies to achieve the above
aim, the present inventors found that an excellent vascular
endothelial function improving effect can be achieved by
using a docosahexaenoic acid-class compound and a sesamin-
30 class compound in combination at a specific ratio.

[0009]

The present invention relates to, but is not limited
to, the following composition for improving vascular
endothelial function.

35 (1) A composition for improving vascular endothelial

function, the composition containing: a sesamin-class compound; and a docosahexaenoic acid-class compound, wherein the docosahexaenoic acid-class compound is at least one compound selected from the group consisting of
5 docosahexaenoic acid, a compound containing docosahexaenoic acid as a constituent fatty acid, and salts thereof, and a sesamin equivalent weight of the sesamin-class compound is 2 to 150 relative to a docosahexaenoic acid equivalent weight of the docosahexaenoic acid-class compound taken as
10 100.

(2) The composition according to (1) above, wherein the sesamin equivalent weight of the sesamin-class compound is 2 to 110 relative to the docosahexaenoic acid equivalent weight of the docosahexaenoic acid-class compound taken as
15 100.

(3) The composition according to (1) or (2) above, wherein the sesamin-class compound is at least one compound selected from the group consisting of sesamin, episesamin, and metabolites thereof.

20 (4) The composition according to any one of (1) to (3) above, wherein the compound containing docosahexaenoic acid as a constituent fatty acid is at least one compound selected from the group consisting of a triglyceride in which at least one or each constituent fatty acid is
25 docosahexaenoic acid, a diglyceride in which at least one or each constituent fatty acid is docosahexaenoic acid, a monoglyceride in which a constituent fatty acid is docosahexaenoic acid, a phospholipid in which at least one or each constituent fatty acid is docosahexaenoic acid, a
30 glycolipid in which at least one or each constituent fatty acid is docosahexaenoic acid, and an alcohol ester of docosahexaenoic acid.

(5) The composition according to any one of (1) to (4) above, wherein the composition is an oral composition.

35 (6) The composition according to any one of (1) to

(5) above, wherein the composition is a food or beverage.

(7) The composition according to any one of (1) to (6) above, wherein the composition is labeled with at least one function claim selected from the group consisting of
5 "improving vascular endothelial function", "preventing a decline in vascular endothelial function", "maintaining vascular health", "maintaining vascular flexibility", "dilating blood vessels to maintain blood flow", "promoting blood circulation", "improving blood flow to maintain body
10 temperature", "improving blood flow to prevent or reduce sensitivity to the cold", "improving blood flow to prevent or improve shoulder stiffness", "preventing or reducing swelling", and "improving blood flow to improve skin conditions".

(8) Use of a composition for improving vascular
15 endothelial function, the composition containing a sesamin-class compound and a docosahexaenoic acid-class compound, wherein the docosahexaenoic acid-class compound is at least one compound selected from the group consisting of
20 docosahexaenoic acid, a compound containing docosahexaenoic acid as a constituent fatty acid, and salts thereof, and in the composition, a sesamin equivalent weight of the sesamin-class compound is 2 to 150 relative to a docosahexaenoic acid equivalent weight of the
25 docosahexaenoic acid-class compound taken as 100.

- Advantageous Effects of Invention

[0010]

The present invention can provide a composition for
30 improving vascular endothelial function, the composition having an excellent vascular endothelial function improving action.

BRIEF DESCRIPTION OF DRAWINGS

35 [0011]

FIG. 1 is a graph showing the results obtained by examining the intracellular reactive oxygen species level in human umbilical vein endothelial cells (HUVECs) by varying the weight ratio of a metabolite of sesamin (SC1) to docosahexaenoic acid (DHA) (means $\pm$ standard error, N = 6).

FIG. 2 is a graph showing the effect of a combination of DHA and SC1 on NO production in HUVECs (means $\pm$ standard error, N = 5, Dunnett's test, *p < 0.05, **p < 0.01).

DESCRIPTION OF EMBODIMENTS

[0012]

The composition for improving vascular endothelial function of the present invention contains a sesamin-class compound and a docosahexaenoic acid-class compound, wherein the docosahexaenoic acid-class compound is at least one compound selected from the group consisting of docosahexaenoic acid, a compound containing docosahexaenoic acid as a constituent fatty acid, and salts thereof, and the sesamin equivalent weight of the sesamin-class compound is 2 to 150 relative to the docosahexaenoic acid equivalent weight of the docosahexaenoic acid-class compound taken as 100.

Hereinafter, the composition for improving vascular endothelial function of the present invention is sometimes simply referred to as the composition of the present invention. The composition of the present invention typically contains a sesamin-class compound and a docosahexaenoic acid-class compound as active ingredients.

[0013]

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 the 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. In the present invention, the sesamin-class compound may include one compound or two or more compounds. Specific examples of the sesamin-class compound include sesamin, episesamin, 5 sesaminol, episesaminol, sesamol, sesamolin, and metabolites thereof. Stereoisomers or racemates of these compounds may be used alone or in mixture.

Examples of a metabolite that can be used as the sesamin-class compound include a compound disclosed in JP 10 2009-143884 A. Preferred metabolites of sesamin are SC1 ((7 α ,7' α ,8 α ,8' α)-3',4'-methylenedioxy-7,9':7',9-diepoxy lignane-3,4-diol) and SC2((7 α ,7' α ,8 α ,8' α)-7,9':7',9-diepoxy lignane-3,3',4,4'-tetraol). A preferred metabolite is a metabolite of sesamin. SC1 is more preferred.

15 [0014]

In the present invention, the sesamin-class compound is preferably at least one compound selected from the group consisting of sesamin, episesamin, and metabolites thereof. Preferred metabolites of sesamin or episesamin are SC1 and 20 SC2. SC1 is more preferred. In one embodiment, the sesamin-class compound is further preferably at least one compound selected from the group consisting of sesamin, episesamin, and SC1.

[0015]

25 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) 30 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 directly. However, when sesame oil is used, the characteristic flavor of sesame oil is sometimes evaluated as being

35 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 add a desirable amount of sesamin results in excess volume per unit dose of a composition to be formulated. This sometimes causes inconvenience in ingestion. In particular, in the case where the composition is provided as a preparation for oral ingestion, the preparation (tablet, capsule, etc.) becomes so bulky that it causes problems in ingestion. Thus, use of a sesamin extract (or purified sesamin) from sesame oil is preferred in terms of low intake. 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)). The sesamin-class compound can also be produced by the method disclosed in JP 2009-143884 A, for example.

[0016]

In the present invention, the docosaheptaenoic acid-class compound is at least one selected from the group consisting of docosaheptaenoic acid (free docosaheptaenoic acid), a compound containing docosaheptaenoic acid as a constituent fatty acid, and salts thereof. The docosaheptaenoic acid-class compound may include one compound or two or more compounds. The compound containing docosaheptaenoic acid as a constituent fatty acid releases docosaheptaenoic acid when hydrolyzed.

[0017]

Examples of the salts of docosaheptaenoic acid and the compound containing docosaheptaenoic acid as a constituent fatty acid include calcium salt and sodium salt.

Preferably, the compound containing docosaheptaenoic acid as a constituent fatty acid is at least one compound selected from the group consisting of a triglyceride in which at least one or each constituent fatty acid is docosaheptaenoic acid, a diglyceride in which at least one

or each constituent fatty acid is docosahexaenoic acid, a monoglyceride in which a constituent fatty acid is docosahexaenoic acid, a phospholipid in which at least one or each constituent fatty acid is docosahexaenoic acid, a glycolipid in which at least one or each constituent fatty acid is docosahexaenoic acid, and an alcohol ester of docosahexaenoic acid.

In particular, the docosahexaenoic acid-class compound is preferably docosahexaenoic acid, a triglyceride in which at least one or each constituent fatty acid is docosahexaenoic acid, a diglyceride in which at least one or each constituent fatty acid is docosahexaenoic acid, or a phospholipid in which at least one or each constituent fatty acid is docosahexaenoic acid; more preferably docosahexaenoic acid, triglyceride in which at least one or each constituent fatty acid is docosahexaenoic acid, or diglyceride in which at least one or each constituent fatty acid is docosahexaenoic acid; particularly preferably docosahexaenoic acid or triglyceride in which at least one or each constituent fatty acid is docosahexaenoic acid.

[0018]

The docosahexaenoic acid-class compound for use in the present invention is not limited in any way by its form, production method, or the like. A chemically synthesized product, a product derived from a natural product, or a product derived from a genetically modified organism (e.g., *Escherichia coli*, yeast, plants, etc.) may be used. Examples of the product derived from a natural product include those derived from fish or shellfish, algae, fungi (*Colwellia* bacteria, *Labyrinthulomycetes* etc.), and plants (*Marchantia polymorpha*, etc.). In particular, the docosahexaenoic acid-class compound is preferably derived from fish or shellfish.

A docosahexaenoic acid-class compound extracted and/or purified from a raw material such as a natural

product containing a docosahexaenoic acid-class compound may also be used, or such a raw material may be directly used to add a docosahexaenoic acid-class compound to a composition. Examples of the raw material containing a docosahexaenoic acid-class compound include edible fats and oils such as fat and oil extracts from fish oil, meat, and milk. The fish oil containing a docosahexaenoic acid-class compound is preferably fish oil in which the docosahexaenoic acid-class compound content (docosahexaenoic acid equivalent content) is concentrated to 5 wt% or more, preferably 10 wt% or more, more preferably 50 wt% or more. For example, a docosahexaenoic acid-class compound extracted and/or purified from fish oil containing a docosahexaenoic acid-class compound may be used, or such fish oil may be used directly.

[0019]

In the composition of the present invention, the sesamin equivalent weight of the sesamin-class compound is 2 to 150 relative to the docosahexaenoic acid equivalent weight of the docosahexaenoic acid-class compound taken as 100.

Use of the docosahexaenoic acid-class compound and the sesamin-class compound at the above weight ratio can achieve an excellent vascular endothelial function improving effect. In the present invention, the weight ratio of the sesamin-class compound to the docosahexaenoic acid-class compound, specifically, the sesamin equivalent weight of the sesamin-class compound relative to the docosahexaenoic acid equivalent weight of the docosahexaenoic acid-class compound taken as 100, is 2 or more, preferably 2.5 or more, more preferably 3 or more, still more preferably 3.1 or more, while it is preferably 150 or less, preferably 110 or less, more preferably 109 or less, still more preferably 108 or less. In one embodiment of the present invention, the sesamin equivalent weight of

the sesamin-class compound is preferably 2 to 110, more preferably 2.5 to 110, still more preferably 3 to 109, particularly preferably 3.1 to 108 relative to the docosahexaenoic acid equivalent weight of the docosahexaenoic acid-class compound taken as 100. When the weight ratio of the sesamin-class compound to the docosahexaenoic acid-class compound is in the above range, a higher vascular endothelial function improving effect can be achieved.

10 [0020]

In the present invention, when the composition contains two or more sesamin-class compounds, the term "the amount of the sesamin-class compound" refers to the total amount of these compounds. When the sesamin-class compound is sesamin, the term "the sesamin equivalent amount of the sesamin-class compound" or a similar term refers to the amount of sesamin. When the sesamin-class compound is a compound other than sesamin, the term refers to a value obtained by multiplying the molar number of the compound by the molecular weight of sesamin.

20 [0021]

In the present invention, when the composition contains two or more docosahexaenoic acid-class compounds, the term "the amount of the docosahexaenoic acid-class compound" refers to the total amount of these compounds. When the docosahexaenoic acid-class compound is docosahexaenoic acid, the term "the docosahexaenoic acid equivalent amount of the docosahexaenoic acid-class compound" or a similar term refers to the amount of docosahexaenoic acid. When the docosahexaenoic acid-class compound is not free docosahexaenoic acid, the amount is calculated in terms of free docosahexaenoic acid. When the docosahexaenoic acid-class compound is not free docosahexaenoic acid, the docosahexaenoic acid equivalent amount can be determined by isolating docosahexaenoic acid

by saponification or enzymatic treatment and measuring the isolated docosahexaenoic acid.

[0022]

Vascular endothelial function is an action of
5 vascular endothelial cells. The action of vascular
endothelial cells is an important action for maintaining
vascular health and includes a nitric oxide (NO) producing
action by vascular endothelial cells. Nitric oxide is one
of vascular endothelium-derived relaxing factors, and it is
10 produced in vivo by nitric oxide synthase (NOS).

In the present invention, the expression "improving
vascular endothelial function" or a similar expression
encompasses "reducing a decline in vascular endothelial
function", "maintaining vascular endothelial function", and
15 "recovering a decline in vascular endothelial function".

[0023]

As shown in Examples (described later), when the
docosahexaenoic acid-class compound and the sesamin-class
compound were used in combination such that the weight
20 (sesamin equivalent) of the sesamin-class compound was 2 to
150 relative to the weight (docosahexaenoic acid
equivalent) of the docosahexaenoic acid-class compound
taken as 100, the use effectively reduced the intracellular
reactive oxygen species level in vascular endothelial
25 cells, as compared to when neither compound was used. In
other words, intracellular oxidative stress was effectively
reduced in vascular endothelial cells.

An increase in the intracellular reactive oxygen
species level in vascular endothelial cells leads to a
30 decrease in the amount of NO production. The NO production
can be promoted by reducing intracellular reactive oxygen
species in vascular endothelial cells. The vascular
endothelial function can be improved by reducing
intracellular reactive oxygen species in vascular
35 endothelial cells. Thus, the vascular endothelial function

can be effectively improved by using a docosahexaenoic acid-class compound and a sesamin-class compound in combination at the above weight ratio.

As shown in Examples, use of a docosahexaenoic acid-class compound and a sesamin-class compound in combination at the above weight ratio significantly increased the relative amount of NO production in HUVECs as compared to control. In contrast, in the case of HUVECs cultured in the medium supplemented with only the docosahexaenoic acid-class compound or the sesamin-class compound, the relative amount of NO production was not significantly increased as compared to control.

No reports have been made on achievement of an excellent vascular endothelial function improving effect by use of the docosahexaenoic acid-class compound and the sesamin-class compound in combination at the above weight ratio.

In one embodiment, the composition of the present invention can be suitably used to improve vascular endothelial function by reducing intracellular reactive oxygen species in vascular endothelial cells.

[0024]

In one embodiment, improving vascular endothelial function may be promoting NO production in vascular endothelial cells. In one embodiment, the composition of the present invention can be used to promote nitric oxide production in vascular endothelial cells. An increase in the amount of NO production in vascular endothelial cells can dilate blood vessels and increase the blood flow (promote blood circulation). Thus, promoting nitric oxide production in vascular endothelial cells can improve the blood flow.

[0025]

In one embodiment, the composition of the present invention can be used to dilate blood vessels and increase

the blood flow by the vascular endothelial function improving action. The vascular endothelial function improving action can also maintain vascular health (functionality), vascular flexibility, vascular elasticity, and the like. Thus, the composition of the present invention is useful, for example, in maintaining or improving vascular flexibility, vascular elasticity, and the like. In one embodiment, the composition of the present invention can be used to maintain or improve vascular flexibility and/or vascular elasticity.

[0026]

The composition for improving vascular endothelial function of the present invention can be used to prevent or ameliorate a condition or disease that can be expected to be prevented or ameliorated by improving vascular endothelial function. Such a condition or disease may be, for example, a condition or disease caused by arteriosclerosis or decreased blood flow.

The expression "to prevent a condition or disease" encompasses "to prevent the onset of a condition or disease", "to delay the onset of a condition or disease", "to reduce the incidence of a condition or disease", "to reduce the onset risk of a condition or disease", and the like. The expression "to ameliorate a condition or disease" encompasses "to help a subject recover from a condition or disease", "to change symptoms of a condition or disease for better", "to alleviate symptoms of a condition or disease", "to delay or prevent the progression of a condition or disease", and the like. The expression "to recovery" encompasses "to partially recovery".

[0027]

Sesamin-class compounds and docosahexaenoic acid-class compounds are compounds that are found in natural products and food or beverages and that have been consumed. Thus, for example, daily ingestion of a sesamin-class

compound and a docosaheptaenoic acid-class compound is less likely to cause problems in terms of safety. The present invention can provide a composition containing highly safe substances as active ingredients and having an excellent
5 vascular endothelial function improving action.

[0028]

 The composition for improving vascular endothelial function of the present invention is applicable for therapeutic use (medical use) and non-therapeutic use (non-
10 medical use). The term "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 for improving vascular endothelial
15 function of the present invention can be provided in the form of a food or beverage, a cosmetic, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like. The composition of the present invention may be a food or beverage, a cosmetic, a pharmaceutical product, a quasi-
20 pharmaceutical product, feed, or the like by itself for use in improving vascular endothelial function; or the composition may be a preparation, material, or the like to be added to these products.

 For example, the composition for improving vascular
25 endothelial function of the present invention may be provided as an agent or the like, but it is not limited thereto. The agent can be directly provided as a composition, or can be provided as a composition containing the agent. In one embodiment, the composition of the
30 present invention can also be referred to as a vascular endothelial function improving agent.

[0029]

 The composition of the present invention may be either an oral composition or a parenteral composition. In
35 one embodiment, the composition of the present invention is

preferably an oral composition. The oral composition may be a food or beverage, an oral pharmaceutical product, an oral quasi-pharmaceutical product, or feed, preferably a food or beverage or an oral pharmaceutical product, more preferably a food or beverage.

5 [0030]

The composition of the present invention can contain optional additives and optional components in addition to the sesamin-class compound and the docosahexaenoic acid-class compound, as long as the effect of the present invention is not impaired. Such additives and components may be selected depending on the form of the composition, for example, and those generally usable in food or beverages, cosmetics, pharmaceutical products, quasi-pharmaceutical products, feed, and the like can be used.

10 15

When the composition of the present invention is provided as a food or beverage, a cosmetic, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like, any common method can be used for production.

20 [0031]

For example, when the composition of the present invention is provided as a food or beverage, various types of food or beverages can be provided by adding a component (e.g., a food material, an optional food additive, etc.) that can be used in food or beverages to the sesamin-class compound and the docosahexaenoic acid-class compound. The food or beverage is not limited. Examples thereof include general food or beverages, health foods, health drinks, foods with function claims, foods for specified health uses, health supplements, and foods for the sick. The health foods, foods with function claims, foods for specified health uses, health supplements, and the like can be used in various forms of formulations such as fine granules, tablets, granules, powders, capsules, chewable tablets, dry syrups, syrups, liquid agents, beverages,

25 30 35

energy drinks, and liquid foods.

[0032]

When the composition of the present invention is provided as a cosmetic, various types of cosmetics can be provided by adding a component (e.g., a cosmetically acceptable carrier, additive, etc.) that can be used in cosmetics to the sesamin-class compound and the docosahexaenoic acid-class compound. The cosmetic may be provided in any product form. Examples thereof include skin care cosmetics such as skin lotions, serums, face packs, emulsions, creams, and sunscreen lotions.

[0033]

When the composition of the present invention is provided as a pharmaceutical product or a quasi-pharmaceutical product, various dosage forms of pharmaceutical products or quasi-pharmaceutical products can be provided by, for example, adding a pharmacologically acceptable carrier, an optional additive, or the like to the sesamin-class compound and the docosahexaenoic acid-class compound. Such a carrier, additive, or the like may be any pharmacologically acceptable one that can be used in pharmaceutical 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 or quasi-pharmaceutical product may be oral or parenteral (e.g., transdermal, transmucosal, enteral, or injection) administration. In one embodiment, 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
5 provided as feed, the sesamin-class compound and the
docosahexaenoic acid-class compound are simply added to
feed. The feed includes feed additives. Examples of the
feed include livestock feed for animals such as cows, pigs,
chickens, sheep, and horses; feed for small animals such as
10 rabbits, rats, and mice; and pet food for animals such as
dogs, cats, and birds.

[0035]

The amount of the sesamin-class compound in the
composition of the present invention is not limited and can
15 be set according to the form of the composition or the
like. The sesamin equivalent amount of the sesamin-class
compound in the composition of the present invention is,
for example, preferably 0.01 wt% or more, more preferably
0.1 wt% or more, still more preferably 0.3 wt% or more,
20 while it is preferably 50 wt% or less. In one embodiment,
the sesamin equivalent amount of the sesamin-class compound
in the composition is preferably 0.01 to 50 wt%, more
preferably 0.1 to 50 wt%, still more preferably 0.3 to 50
wt%.

25 The amount of the sesamin-class compound can be
measured by high-performance liquid chromatography (HPLC).
The amount of the sesamin-class compound is the total
amount when two or more sesamin-class compounds are
contained.

30 [0036]

The amount of the docosahexaenoic acid-class compound
in the composition of the present invention is not limited
and can be set according to the form of the composition and
the like. The docosahexaenoic acid equivalent amount of
35 the docosahexaenoic acid-class compound in the composition

of the present invention is, for example, preferably 0.3 wt% or more, more preferably 5 wt% or more, still more preferably 10 wt% or more, particularly preferably 20 wt% or more, while it is preferably 80 wt% or less. In one
5 embodiment, the docosahexaenoic acid equivalent amount of the docosahexaenoic acid-class compound in the composition is preferably 0.3 to 80 wt%, more preferably 5 to 80 wt%, still more preferably 10 to 80 wt%, particularly preferably 20 to 80 wt%.

10 The amount of the docosahexaenoic acid can be measured by gas chromatography (GC).
[0037]

 The composition of the present invention can be ingested or administered using various methods known per
15 se, depending on the form or dosage form thereof. In one embodiment, preferably, the composition of the present invention is orally ingested (orally administered).

 The administration amount (intake) of the composition of the present invention is not limited. The
20 administration amount of the composition of the present invention is not limited as long as it is an amount with which the vascular endothelial function improving effect can be achieved. The administration amount may be suitably set according to the dosage form, administration method,
25 body weight of the subject, and the like.
[0038]

 In one embodiment, when the composition of the present invention is ingested by or administered to a human (adult), the sesamin-equivalent administration amount of
30 the sesamin-class compound per day is preferably 0.1 mg or more, more preferably 1 mg or more, still more preferably 3 mg or more, while it is preferably 500 mg or less. In one embodiment, when the sesamin-class compound is administered to a human (adult), the administration amount per day is
35 preferably 0.1 to 500 mg, more preferably 1 to 500 mg,

still more preferably 3 to 500 mg. The administration amount may be the administration amount per 60 kg body weight per day.

[0039]

5 When the composition of the present invention is ingested by or administered to a human (adult), the docosahexaenoic acid equivalent administration amount of the docosahexaenoic acid-class compound per day is preferably 3 mg or more, more preferably 50 mg or more,
10 still more preferably 100 mg or more, particularly preferably 200 mg or more, while it is preferably 800 mg or less. In one embodiment, when the docosahexaenoic acid-class compound is administered to a human (adult), the administration amount of the docosahexaenoic acid-class
15 compound (docosahexaenoic acid equivalent) per day is preferably 3 to 800 mg, more preferably 50 to 800 mg, still more preferably 100 to 800 mg, particularly preferably 200 to 800 mg. The administration amount may be the administration amount per 60 kg body weight per day.

20 Preferably, the above amounts of the sesamin-class compound and the docosahexaenoic acid-class compound are ingested or administered once a day or in several portions (e.g., 2 to 3 times) a day. In one embodiment, preferably, the above amounts of the sesamin-class compound and the
25 docosahexaenoic acid-class compound are orally ingested by or administered to a human.

 In one embodiment, the composition for improving vascular endothelial function of the present invention can be used to allow the above amounts of the sesamin-class
30 compound and the docosahexaenoic acid-class compound per 60 kg body weight per day to be ingested by or administered to a human.

[0040]

 Preferably, the composition of the present invention
35 is continuously ingested or administered. Continuous

ingestion or administration of the sesamin-class compound and the docosahexaenoic acid-class compound is expected to result in a higher vascular endothelial function improving effect. 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.

[0041]

10 The composition of the present invention may be ingested by or administered to any subject (administration subject). The administration subject is preferably a human or non-human mammal, more preferably a human.

15 In one embodiment, the administration subject may be, for example, one needing or wanting to improve vascular endothelial function. Since the vascular endothelial function is known to decline with aging, examples of such a subject include middle-aged and older people. In one embodiment, the composition of the present invention is suitably used as a composition for improving vascular endothelial function for middle-aged and older people. The term "middle-aged and older people" includes the elderly. 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 administration subject may be a healthy person. For example, the composition of the present invention can be used on healthy people for purposes such as preventing conditions or diseases that can be expected to be prevented or ameliorated by improving vascular endothelial function.

[0042]

35 The composition for improving vascular endothelial function of the present invention may be labeled with a

function claim such as reducing or preventing a decline in vascular function or a decline in vascular endothelial function. For example, the composition for improving vascular endothelial function of the present invention may be labeled with at least one function claim selected from the group consisting of "improving vascular endothelial function", "preventing a decline in vascular endothelial function", "maintaining vascular health", "maintaining vascular flexibility", "dilating blood vessels to maintain blood flow", "promoting blood circulation", "improving blood flow to maintain body temperature", "improving blood flow to prevent or reduce sensitivity to the cold", "improving blood flow to prevent or improve shoulder stiffness", "preventing or reducing swelling", and "improving blood flow to improve skin conditions".

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

[0043]

The present invention also encompasses the following method:

a method for improving vascular endothelial function, the method including administering a composition containing a sesamin-class compound and a docosahexaenoic acid-class compound to a subject, wherein the docosahexaenoic acid-class compound is at least one compound selected from the group consisting of docosahexaenoic acid, a compound containing docosahexaenoic acid as a constituent fatty acid, and salts thereof, and in the composition, a sesamin equivalent weight of the sesamin-class compound is 2 to 150 relative to a docosahexaenoic acid equivalent weight of the

docosahexaenoic acid-class compound taken as 100.

The method may be a therapeutic or non-therapeutic method.

Administration of the composition to a subject can
5 improve vascular endothelial function.

[0044]

The present invention also encompasses the following use:

10 use of a composition containing a sesamin-class compound and a docosahexaenoic acid-class compound for improving vascular endothelial function, wherein the docosahexaenoic acid-class compound is at least one compound selected from the group consisting of docosahexaenoic acid, a compound containing docosahexaenoic
15 acid as a constituent fatty acid, and salts thereof, and in the composition, a sesamin equivalent weight of the sesamin-class compound is 2 to 150 relative to a docosahexaenoic acid equivalent weight of the docosahexaenoic acid-class compound taken as 100.

20 The above use is preferably for humans or non-human mammals, more preferably for humans. The use may be a therapeutic or non-therapeutic use.

[0045]

In the above method and use, the sesamin-class
25 compound, the docosahexaenoic acid-class compound, and preferred embodiments thereof are as described above for the composition for improving vascular endothelial function of the present invention. Preferred embodiments of the weight ratio of the sesamin-class compound to the
30 docosahexaenoic acid-class compound are also as described above for the composition for improving vascular endothelial function of the present invention. The sesamin-class compound may include one sesamin-class compound or two or more sesamin-class compounds. The
35 docosahexaenoic acid-class compound may include one

docosahexaenoic acid-class compound or two or more docosahexaenoic acid-class compounds. In the above method and use, preferably, the composition is administered to (ingested by) a subject at least once a day, for example, one to several times (e.g., two to three times) a day. In one embodiment, in the above method and use, preferably, the compound is orally administered (ingested).

[0046]

In the above method and use, the sesamin-class compound and the docosahexaenoic acid-class compound may be used in amounts (effective amounts) that achieve a desired effect. Preferred administration amounts of the sesamin-class compound and the docosahexaenoic acid-class compound, preferred administration subject, and the like are as described above for the composition of the present invention. In one embodiment, the composition can be used to prevent or ameliorate a condition or disease such as arteriosclerosis. In one embodiment, the composition can be used to prevent or reduce an aging-induced decline in vascular endothelial function.

[0047]

The present invention also encompasses use of a sesamin-class compound and a docosahexaenoic acid-class compound to produce a composition for improving vascular endothelial function. The docosahexaenoic acid-class compound is at least one compound selected from the group consisting of docosahexaenoic acid, a compound containing docosahexaenoic acid as a constituent fatty acid, and salts thereof. Preferably, the sesamin-class compound and the docosahexaenoic acid-class compound are used such that in the composition, the sesamin equivalent weight of the sesamin-class compound is 2 to 150 relative to the docosahexaenoic acid equivalent weight of the docosahexaenoic acid-class compound taken as 100. Preferred embodiments and the like of the composition for

improving vascular endothelial function are as described above.

[0048]

The numerical range defined by the lower limit and
5 the upper limit herein, i.e., "the lower limit to the upper
limit", includes the lower limit and the upper limit. For
example, the range defined by "1 to 2" means 1 or more and
2 or less, with 1 and 2 being inclusive. Herein, the range
10 may be any combination of any upper limit and any lower
limit.

EXAMPLES

[0049]

The present invention is described in further detail
15 below with reference to Examples. The present invention is
not limited to these Examples.

[0050]

<Example 1>

1-1. Test Method

20 To study whether a combination of a docosahexaenoic
acid-class compound and a sesamin-class compound acts on
vascular function, the usefulness of the combination was
studied using human umbilical vein endothelial cells
(HUVECs).

25 Docosahexaenoic acid (DHA) was used as the
docosahexaenoic acid-class compound.

DHA (Sigma-Aldrich) dissolved in dimethyl sulfoxide
(DMSO) (Nacalai Tesque, Inc.) was used. Since sesamin is
metabolized in the body, SC1, which is a major metabolite
30 detected in human blood after ingestion of sesamin, was
used in this experiment. SC1 dissolved in DMSO was used.

[0051]

HUVECs (LONZA) were seeded onto a 96-well plate,
thawed in EGM-2 (LONZA), and cultured in a CO₂ incubator
35 (37°C, 5% CO₂, ambient O₂, wet). On the day after the

seeding onto a 96-well plate, the medium was replaced with a medium (EGM-2) supplemented with DMSO or the relevant test substances (DHA and SC1), and the cells were cultured in a CO₂ incubator for 24 hours. The cells cultured in the medium supplemented with DMSO were used as control.

[0052]

Table 1 shows the concentrations of the test substances (DHA and SC1) in the medium and the weight ratio of SC1 to DHA. The weight ratio of SC1 to DHA is the weight ratio of SC1 to DHA, taking the weight of DHA as 100. The value in each parenthesis indicates the sesamin (SE) equivalent weight ratio of SC1 to DHA, taking the weight of DHA as 100.

[0053]

[Table 1]

	Concentration (μ M)		Weight ratio (DHA taken as 100)	
	DHA	SC1	DHA	SC1 (SE equivalent)
Ratio 1	1	0.00096	100	0.1 (0.1035)
Ratio 2	1	0.0096	100	1 (1.035)
Ratio 3	1	0.0288	100	3 (3.105)
Ratio 4	1	0.096	100	10 (10.35)
Ratio 5	1	0.96	100	100 (103.5)
Ratio 6	1	9.6	100	1000 (1035)

[0054]

Then, the medium was replaced with a medium supplemented with CellROX Deep Red (Thermo Fisher) and Hoechst 33342 (Thermo Fisher), and the cells were incubated in a CO₂ incubator for 30 minutes. Subsequently, using a plate reader, the fluorescence intensity (excitation wavelength: 635 nm; detection wavelength: 675 nm) of CellROX Deep Red and the fluorescence intensity (excitation wavelength: 350 nm; detection wavelength: 460 nm) of Hoechst 33342 were measured. The fluorescence intensity of CellROX Deep Red was corrected based on the fluorescence intensity of Hoechst 33342, and the relative fluorescence

intensity was determined as the level of relative intracellular oxidative stress. A higher relative fluorescence intensity indicates a higher intracellular reactive oxygen species level.

5 [0055]

1-2. Results

FIG. 1 shows the results. FIG. 1 is a graph showing the results obtained by examining the intracellular reactive oxygen species level in human umbilical vein endothelial cells (HUVECs) by varying the weight ratio of a metabolite of sesamin (SC1) to docosahexaenoic acid (DHA) (means $\pm$ standard error, N = 6). The results shown in FIG. 1 are values relative to the intracellular reactive oxygen species level in the control (DMSO) taken as 1. In FIG. 1, 1 to 6 respectively correspond to ratios 1 to 6 in Table 1.

At ratio 3, ratio 4, and ratio 5, the combination of DHA and the metabolite of sesamin reduced the reactive oxygen species level in vascular endothelial cells. In contrast, at ratio 1, ratio 2, and ratio 6, no changes were observed in the intracellular reactive oxygen species level. The above indicates that the intracellular reactive oxygen species level in vascular endothelial cells was effectively reduced by combining DHA and the metabolite of sesamin at the weight ratio of ratio 3, ratio 4, or ratio 5.

25 [0056]

The above results suggest that the NO production can be effectively increased in vascular endothelial cells when the docosahexaenoic acid-class compound and the sesamin-class compound are used in combination such that the sesamin equivalent weight of the sesamin-class compound is 2 to 150 relative to the docosahexaenoic acid equivalent weight of the docosahexaenoic acid-class compound taken as 100. SC1 is a metabolite of sesamin. When a compound containing DHA as a constituent fatty acid is ingested, the

compound is hydrolyzed in the body to produce docosahexaenoic acid. Thus, when a sesamin-class compound other than SC1, such as sesamin, and a docosahexaenoic acid-class compound other than DHA are combined at the
5 above weight ratio and ingested, the combination can also achieve the same effect that can be achieved when DHA and SC1 are used.

[0057]

<Example 2>

10 2-1. Test method

HUVECs were cultured as in Example 1. On the day after the seeding onto a 96-well plate, the cells were cultured in a medium (EGM-2) supplemented with test substances or positive control using a CO₂ incubator for 24
15 hours. DHA and SC1 were used as the test substances. Lovastatin (FUJIFILM Wako Pure Chemical Corporation) known to increase the NO production was used as positive control. The test substances were added such that the concentration of DHA was 1 μ M and the concentration of SC1 was 1 μ M in
20 the medium (the weight of SC1 was 104.2 (sesamin equivalent weight: 107.9) relative to the weight of DHA taken as 100). Lovastatin was added such that the concentration was 10 μ M in the medium. The cells cultured in the medium supplemented with DMSO were used as control. Additionally,
25 instead of the medium supplement with the test substances (DHA and SC1), a medium supplemented with only DHA (DHA: 1 μ M) and a medium supplemented with only SC1 (SC1: 1 μ M) were prepared, and HUVECs were cultured in the same manner as described above. DHA, SC1, and lovastatin were first
30 dissolved in DMSO and then added to the respective media.

[0058]

Subsequently, each medium was replaced with a medium supplemented with DAF-2 (4,5-diaminofluorescein, Goryo Chemical, Inc.) at a final concentration of 10 μ M, and the
35 cells were incubated in a CO₂ incubator for two hours.

Then, the culture supernatant was transferred to a 96-well black plate, and the fluorescence intensity (ex. 485 nm, em. 515 nm) was measured using a plate reader (N = 5). The same measurement was performed using a cell-free medium as the background. The background average (N = 5) was subtracted from the fluorescence intensity of each sample, and the relative fluorescence intensity was determined as the relative amount of NO production.

[0059]

2-2. Results

FIG. 2 shows the results. FIG. 2 is a graph showing the effect of the combination of DHA and the metabolite of sesamin (SC1) on NO production in HUVECs (means $\pm$ standard error, N = 5, Dunnett's test, *p < 0.05, **p < 0.01). The results shown in FIG. 2 are values, which are relative to the relative amount of NO production of control (DMSO 0.1 v/v%) taken as 1, of the relative amount of NO production in the cells cultured in the medium supplemented with the test substances (or positive control). The combination of DHA and the metabolite of sesamin significantly increased the amount of NO production as compared to control. In contrast, the HUVECs cultured in the medium supplemented with only DHA or SC1 showed no significant increase in the relative amount of NO production as compared to control. The combination of the docosahexaenoic acid-class compound and the sesamin-class compound effectively increased the NO production in vascular endothelial cells and enhanced vascular endothelial function.

CLAIMS

Claim 1. A composition for improving vascular endothelial function, the composition comprising:

5 a sesamin-class compound; and

a docosahexaenoic acid-class compound,

wherein the docosahexaenoic acid-class compound is at least one compound selected from the group consisting of docosahexaenoic acid, a compound containing docosahexaenoic acid as a constituent fatty acid, and salts thereof, and

10 a sesamin equivalent weight of the sesamin-class compound is 2 to 150 relative to a docosahexaenoic acid equivalent weight of the docosahexaenoic acid-class compound taken as 100.

15

Claim 2. The composition according to claim 1,

wherein the sesamin equivalent weight of the sesamin-class compound is 2 to 110 relative to the docosahexaenoic acid equivalent weight of the docosahexaenoic acid-class

20 compound taken as 100.

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

wherein the sesamin-class compound is at least one compound selected from the group consisting of sesamin, episesamin, and metabolites thereof.

25

Claim 4. The composition according to claim 1 or 2,

wherein the compound containing docosahexaenoic acid as a constituent fatty acid is at least one compound

30 selected from the group consisting of a triglyceride in

which at least one or each constituent fatty acid is docosahexaenoic acid, a diglyceride in which at least one or each constituent fatty acid is docosahexaenoic acid, a monoglyceride in which a constituent fatty acid is

35 docosahexaenoic acid, a phospholipid in which at least one

or each constituent fatty acid is docosahexaenoic acid, a glycolipid in which at least one or each constituent fatty acid is docosahexaenoic acid, and an alcohol ester of docosahexaenoic acid.

5

Claim 5. The composition according to claim 1 or 2, wherein the composition is an oral composition.

10

Claim 6. The composition according to claim 1 or 2, wherein the composition is a food or beverage.

15

Claim 7. The composition according to claim 1 or 2, wherein the composition is labeled with at least one function claim selected from the group consisting of "improving vascular endothelial function", "preventing a decline in vascular endothelial function", "maintaining vascular health", "maintaining vascular flexibility", "dilating blood vessels to maintain blood flow", "promoting blood circulation", "improving blood flow to maintain body temperature", "improving blood flow to prevent or reduce sensitivity to the cold", "improving blood flow to prevent or improve shoulder stiffness", "preventing or reducing swelling", and "improving blood flow to improve skin conditions".

25

Claim 8. Use of a composition for improving vascular endothelial function, the composition comprising a sesamin-class compound and a docosahexaenoic acid-class compound, wherein the docosahexaenoic acid-class compound is at least one compound selected from the group consisting of docosahexaenoic acid, a compound containing docosahexaenoic acid as a constituent fatty acid, and salts thereof, and in the composition, a sesamin equivalent weight of the sesamin-class compound is 2 to 150 relative to a docosahexaenoic acid equivalent weight of the

35

docosahexaenoic acid-class compound taken as 100.