ABSORPTION PROMOTER OF SPHINGOLIPID

[CROSS-REFERENCE TO RELATED APPLICATIONS]

[0001]

The present application is based upon and claims the benefit of the priority from prior Japanese Patent Application No. 2015-000745, filed on January 6, 2015; the entire contents of which are incorporated herein by reference.

[BACKGROUND OF THE INVENTION]

[0002] Technical Field

The present invention relates to a sphingolipid absorption promoting agent (Absorption Promoter of Sphingolipid). The present invention also relates to a composition for oral intake or enteric administration containing a fermented product and/or a cultured product of a lactic acid bacterium and/or a bifidobacterium and additional sphingolipid.

[0003] Related Art

Sphingolipid is a component for constituting a cell membrane of a eukaryote and it is a general name of lipids having sphingoid base. It has been found to exhibit an effect of improving skin barrier function, which is based on oral intake.

[0004]

Sphingomyelin is one of the sphingolipids that are derived from milk. Sphingomyelin is known to exhibit an effect of preventing or improving deteriorated skin conditions (for example, impaired skin barrier function, dryness and roughness of skin, reduced moisture amount in keratinous layer, and atopic dermatits), in which the effect is based on oral intake.

[0005]

Furthermore, sphingomyelin is known to have an activity of suppressing cancer (colon cancer) (Sugawara Tatsuya, "Digestion and Absorption of Sphingolipid as Functional Food Components", Nippon Eiyo Shokuryo Gakkaishi, Vol. 66, No. 4, pp.177-183 (2013)), a skin-beautifying activity (Japanese Patent Application

Laid-Open Publication No. 2008-184428 A), an activity of promoting infant brain development (Japanese Patent No. 3203485 B1), an activity of enhancing mitochondria function (Japanese Patent Application Laid-Open Publication No. 2011-157329 A), an activity of enhancing motor skills (Japanese Patent Application Laid-Open Publication No. 2014-141496 A), an activity of suppressing accumulation of visceral fat and promoting increased adiponectin concentration in blood (anti-hyperglycemic activity and anti-hyperlipidemia activity) (Japanese Patent Application Laid-Open Publication No. 2007-320900 A), and an activity of preventing infection (Japanese Patent Application Laid-Open Publication No. 2008-037788 A).

[0006]

Sphingomyelin is a material consisting of ceramide and phosphocholine. It is hydrolyzed by sphingomyelinase to yield ceramide and phosphocholine. Furthermore, ceramide is hydrolyzed by ceramidase to yield sphingoid base and fatty acid, and it is mostly absorbed, as fatty acid, by epithelial cells of small intestine and part of sphingoid base is re-synthesized to sphingomyelin or ceramide. Meanwhile, it has been suggested that sphingomyelin is decomposed by enteric bacteria.

[0007]

Glucosylceramide is a material in which glucose is bonded to ceramide consisting of sphingoid base and phosphocholine. It is hydrolyzed by glucoceramidase to yield ceramide and glucose. Galactosylceramide is a material in which galactose is bonded to ceramide consisting of sphingoid base and phosphocholine.

[0008]

When orally taken, lipids are generally emulsified by bile acid, decomposed by lipase to yield fatty acid and monoglyceride, and absorbed in small intestine. Furthermore, the monoglyceride is resynthesized, in epithelial cells of small intestine, with triacylglycerol to produce chylomicron, which is transported to a lymphatic vessel, and, after passing through thoracic duct, it enters systemic circulation.

[0009]

Meanwhile, it is known that sphingomyelin and ceramide have a poor digestion · absorption property. As a reason for that, the low activity of sphingomyelinase in alimentary canal or discharge of absorbed sphingoid base by an action of a transporter expressed therein are considered.

[0010]

As such, for a case in which sphingolipids including sphingomyelin, which is expected to exhibit various activities described above, are orally taken, an intake means capable of enhancing absorption property of sphingolipid and has safety, convenience, and efficiency has been desired.

[0011]

With regard to enhancement of digestion and absorption of lipids, a nutrition composition in which bile acid or bile acid salt is blended for enhancing digestion and absorption of lipids is described in JP 3920969 B1, for example. However, in the document, no description is given for sphingolipid and, as bile acid itself has bitter taste, such effect has to be considered when it is used as a food product.

[0012]

Fermented milk (yoghurt) is a fermented food product that is prepared by fermentation according to addition of a lactic acid bacterium or yeast to milk, and due to good taste and cosmetic or health effect, it is widely consumed.

[0013]

In Japanese Patent Application Laid-Open Publication No. H7 (1995)-327633 A, for example, a processed chitosan food product in which chitosan, adlay, and yoghurt powder are admixed with one another is disclosed. In the document, the yoghurt powder is described to have an activity of supplementing digestion and absorption of chitosan. However, the activity related to digestion and absorption described therein is based on the activity of active lecithin in yoghurt powder, and there is no description or suggestion included in the document regarding the promoted digestion and absorption of sphingolipid.

[SUMMARY OF THE INVENTION]

[0014]

The inventors of the present invention have unexpectedly found that, when fermented milk (yoghurt) is taken simultaneously with sphingomyelin, the amount of sphingomyelin absorbed into a living body can be increased, that is, sphingomyelin absorption is enhanced. The inventors of the present invention further found that ceramide absorption is also enhanced at that time. In addition, based on those, the inventors of the present invention found that, by taking a fermented product and/or a cultured product of a lactic acid bacterium and/or a bifidobacterium, sphingolipid absorption can be promoted. Furthermore, as the amount of sphingolipid including sphingomyelin absorbed into a living body is increased, an improvement of various beneficial activities or effects other than improved skin barrier function, which is expected in conjunction with absorption of sphingolipid, can be obtained. Furthermore, by preparing an oral or enteric composition which contains yoghurt and sphingomyelin in an amount higher than the yoghurt amount, the inventors of the present invention actually confirmed the increased amount of sphingomyelin absorbed into a living body. The present invention is based on those findings.

[0015]

Accordingly, an object of the present invention is to provide, as a means capable of enhancing or promoting sphingolipid absorption at the time of having sphingolipid, a means for safe, convenient, and economic (or efficient) absorption.

[0016]

Namely, the following inventions are provided by the present invention.

[0017]

<1> A sphingolipid absorption promoting agent including, as an active ingredient, a fermented product and/or a cultured product of a lactic acid bacterium and/or a bifidobacterium.

[0018]

<2> With regard to the sphingolipid absorption promoting

agent of <1> above, it is advantageous that the sphingolipid is sphingomyelin and absorption of sphingomyelin into a living body is promoted by the agent.

[0019]

<3> With regard to the sphingolipid absorption promoting agent of <2> above, it is advantageous that absorption of sphingomyelin derived from milk into a living body is promoted by the agent.

[0020]

<4> With regard to the sphingolipid absorption promoting agent of any one of <1> to <3> above, it is advantageous that the sphingolipid is at least one of ceramide, glucosylceramide, and galactosylceramide.

[0021]

<5> With regard to the sphingolipid absorption promoting agent of any one of <1> to <4> above, it is advantageous that the fermented product and/or cultured product of a lactic acid bacterium and/or a bifidobacterium is a milk fermented product and/or a milk cultured product of a lactic acid bacterium and/or a bifidobacterium.

[0022]

<6> A sphingolipid absorption promoting agent having polysaccharides as an active ingredient.

[0023]

<7> A composition for oral intake or enteric administration containing the sphingolipid absorption promoting agent of any one of <1> to <6> above and sphingolipid.

[0024]

<8> A composition for oral intake or enteric administration containing a fermented product and/or a cultured product of a lactic acid bacterium and/or a bifidobacterium and additional sphingolipid.

[0025]

<9> With regard to the composition for oral intake or enteric administration of <7> or <8> above, it is advantageous that, as for the blending ratio between the fermented product

and/or cultured product of a lactic acid bacterium and/or a bifidobacterium and sphingolipid, the fermented product and/or cultured product of a lactic acid bacterium and/or a bifidobacterium (dry weight (powder)) is contained at 1 to 10000 mg per 1 mg of the sphingolipid.

[0026]

<10> With regard to the composition for oral intake or enteric administration of <7> or <8> above, it is advantageous that, as for the blending ratio between the fermented product and/or cultured product of a lactic acid bacterium and/or a bifidobacterium and sphingolipid, the fermented product and/or cultured product of a lactic acid bacterium and/or a bifidobacterium (wet weight) is contained at 0.01 to 100 g per 1 mg of the sphingolipid.

[0027]

<11> With regard to the composition for oral intake or enteric administration of any one of <7> to <10> above, it is advantageous that the sphingolipid is sphingomyelin.

[0028]

<12> With regard to the composition for oral intake or enteric administration of any one of <7> to <11> above, it is advantageous that the composition is used for an in vivo activity that is induced by absorption of sphingomyelin into a living body.

[0029]

<13> With regard to the composition for oral intake or enteric administration of <12> above, it is advantageous that the in vivo activity of <7> above is selected from a group consisting of prevention or improvement of deteriorated skin conditions, an activity of suppressing cancer, a skin-beautifying activity, an activity of promoting infant brain development, an activity of enhancing mitochondria function, an activity of enhancing motor skills, an activity of suppressing accumulation of visceral fat and promoting increased adiponectin concentration in blood, and an activity of preventing infection.

[0030]

<14> With regard to the composition for oral intake

or enteric administration of any one of <7> to <11> above, it is advantageous that the composition is used for prevention, suppression, or improvement of deteriorated skin conditions.

[0031]

<15> With regard to the composition of <14> above, it is advantageous that the deteriorated skin conditions is a deteriorated barrier function of skin.

[0032]

<16> A food and drink product containing the sphingolipid absorption promoting agent of any one of <1> to <6> above or the composition of any one of <7> to <15> above.

[0033]

<17> With regard to the food and drink product of <16> above, it is advantageous that the food and drink product is a functional food, a health and nutrition food, a supplement, a food for specific health use, a food labeled with functionality, or a food tagged with low disease risk label.

[0034]

<18> A method for promoting sphingolipid absorption including oral intake or enteric administration of, together with sphingolipid, a fermented product and/or a cultured product of a lactic acid bacterium and/or a bifidobacterium to a subject who is in need of intake of sphingolipid.

[0035]

<19> With regard to the method for promoting sphingolipid absorption of <18> above, it is advantageous that the sphingolipid is sphingomyelin.

[0036]

According to the sphingolipid absorption promoting agent of the present invention, sphingomyelin absorption can be enhanced or promoted when sphingomyelin is taken, and sphingomyelin can be absorbed safely, conveniently, and economically (or efficiently). The yoghurt used for the absorption promoting agent or composition of the present invention is, from the viewpoint that it has been conventionally used as a food product, highly safe, convenient for actual consumption, and economically advantageous.

[BRIEF DESCRIPTION OF THE DRAWINGS]

[0037]

FIG. 1 is a graph illustrating the results of Example 1 (transition of the amount of ceramide molecular species in lymphatic fluid (accumulated value));

FIG. 2 is a graph illustrating the results of Example 1 (transition of the amount of sphingomyelin molecular species in lymphatic fluid (accumulated value));

FIGS. 3A to 3D are graphs illustrating the results of Example 2 (change in the amount of ceramide molecular species in blood serum) (in the figure, * means a significant difference ($P < 0.05$) compared to an MPL group);

FIG. 4 is a graph illustrating the results of Example 2 (area under the time curve of the amount of ceramide molecular species in blood serum) (in the figure, * means a significant difference ($P < 0.05$) compared to the MPL group);

FIG. 5 is a graph illustrating the transition of moisture amount in keratinous layer of Test 1 of Example 3 (there is a significant difference ($P < 0.05$) among different Roman numerals);

FIG. 6 is a graph illustrating the transition of transdermal evaporation amount of moisture in Test 1 of Example 3 (there is a significant difference ($P < 0.05$) among different Roman numerals);

FIG. 7 is a graph illustrating the results of Example 4 (area under the time curve of the amount of ceramide molecular species in blood serum) (in the figure, * means a significant difference ($P < 0.05$) compared to the MPL group, and # means a significant difference ($P < 0.05$) compared to the non-fermented milk group);

FIG. 8 is a graph illustrating the results of Example 5 (area under the time curve of the amount of ceramide molecular species in blood serum) (in the figure, * means a significant difference ($P < 0.05$) compared to the MPL group);

FIG. 9 is a graph illustrating the results of Example 6 (area under the time curve of the amount of ceramide molecular species in blood serum) (in the figure, * means a significant difference ($P < 0.05$) compared to the MPL group);

FIG. 10 is a graph illustrating the results of Example 7 (area under the time curve of the amount of ceramide molecular species in blood serum) (in the figure, * means a significant difference ($P < 0.05$) compared to the MPL group);

FIG. 11 is a graph illustrating the results of Example 8 (area under the time curve of the amount of ceramide molecular species in blood serum) (in the figure, * means a significant difference ($P < 0.05$) compared to the MPL group);

FIG. 12 is a graph illustrating the results of Example 9 (area under the time curve of the amount of ceramide molecular species in blood serum) (in the figure, * means a significant difference ($P < 0.05$) compared to the MPL group); and

FIG. 13 is a graph illustrating the results of Example 10 (area under the time curve of the amount of ceramide molecular species in blood serum) (in the figure, * means a significant difference ($P < 0.05$) compared to the MPL group).

[MODE FOR CARRYING OUT THE INVENTION]

[0038]

Hereinbelow, embodiments of the present invention will be described.

[0039]

Sphingolipid absorption promoting agent

The sphingolipid absorption promoting agent of the present invention has, as an active ingredient, a fermented product and/or a cultured product of a lactic acid bacterium and/or a bifidobacterium as described above. As described herein, the expression "... has, as an active ingredient, ..." means that the absorption promoting agent of the present invention contains the fermented product and/or cultured product of a lactic acid bacterium and/or a bifidobacterium in a use amount (active amount) that is sufficient for having exhibition of the activity of promoting sphingolipid absorption in a living body.

[0040]

Furthermore, as described herein, the expression "promoting absorption" of sphingolipid means promoted absorption of

sphingolipid into a living body. Furthermore, as described herein, the expression "absorption" typically means that the sphingolipid, which is orally taken or enterically administered either simultaneously or sequentially with the absorption promoting agent of the present invention, or sphingolipid, which is already fully taken into a living body, is delivered to a circulation system (blood vessel system or lymph system) of a living body via a biomembrane of an alimentary canal or an absorption duct. Accordingly, the actually absorbed sphingolipid can exhibit various activities in a living body, either as sphingolipid or as a derivative substance thereof.

[0041]

Furthermore, as described herein, the expression "promoting" means, compared to a case in which an absorption promoting agent is not used, absorption of sphingolipid is promoted, and increase of absorption amount, increase of absorption speed, and improvement of lowered absorption property are all encompassed by it. Meanwhile, according to a preferred aspect of the present invention, the term "promoting" is used with a meaning of having increased absorption amount of sphingolipid into a living body.

[0042]

The sphingolipid described herein is derived from a natural source, and examples thereof include those derived from milk such as cow milk, goat milk, sheep milk, or horse milk, those derived from grains like soybean, rice, and corn, those derived from konjak, and those derived from beet. It is preferably those derived from milk. It is more preferably those derived from cow milk. Sphingomyelin, glucosylceramide, and galactosylceramide are included in the sphingolipid. The sphingolipid is preferably sphingophospholipid, and more preferably sphingomyelin. They can be prepared by a known method from natural raw materials, but a commercially available product can be also used.

[0043]

In the present invention, the sphingomyelin which may be used is one of sphingolipids, and like the sphingolipid described

above, it is derived from a natural source. It is preferably the one derived from milk. It is more preferably the one derived from cow milk. The sphingomyelin can be prepared by a known method from natural raw materials, but a commercially available product can be also used.

[0044]

According to a preferred aspect of the present invention, absorption of sphingomyelin derived from milk into a living body is promoted by a sphingolipid absorption promoting agent.

[0045]

In the present invention, sphingolipid (for example, sphingomyelin) absorption can be evaluated by measuring the amount of sphingolipid in a living body. In that case, based on constant molecular species of sphingomyelin, absorption of sphingomyelin can be also evaluated by measuring the amount of the molecular species taken into a living body. Alternatively, by measuring the amount of a derivative substance of sphingomyelin, for example, the amount of ceramide which is produced according to hydrolysis of sphingomyelin, taken into a living body, the sphingomyelin absorption can be evaluated.

[0046]

In general, examples of sphingomyelin, in particular, the molecular species contained in sphingomyelin derived from milk, include those described below.

Namely, there are sphingomyelin molecular species having phosphocholine or phosphoethanolamine bonded to a ceramide structure in which sphingosine or dihydrosphingosine with 16 to 18 carbon chain number and a fatty acid with 14 to 26 carbon chain number are bonded to each other via an amide bond.

[0047]

With regard to the sphingomyelin absorption, based on any one of those molecular species or a combination of two or more of them, the evaluation can be made by measuring their amount or amount of their derivative substances.

[0048]

As described above, sphingomyelin is a substance consisting

of ceramide and phosphocholine, and by an action of sphingomyelinase, it is hydrolyzed into ceramide and phosphocholine. Ceramide is also hydrolyzed into sphingoid base and fatty acid by ceramidase. As such, in addition to promoted absorption of sphingomyelin by a sphingolipid absorption promoting agent, absorption of ceramide into a living body is also promoted.

For such reasons, by the sphingolipid absorption promoting agent of the present invention, ceramide absorption into a living body is also promoted.

[0049]

According to another aspect of the present invention, an agent for promoting absorption of ceramide, glucosylceramide, and/or galactosylceramide, containing, as an active ingredient, a fermented product and/or a cultured product of a lactic acid bacterium and/or a bifidobacterium is also provided. According to the agent for promoting absorption of lipids like ceramide, glucosylceramide, and/or galactosylceramide of the present invention, absorption of ceramide, glucosylceramide, and/or galactosylceramide, or a combination of two or more of them into a living body is also promoted.

[0050]

In the present invention, examples of the molecular species included in ceramide, glucosylceramide, or galactosylceramide include those described below.

Namely, there are ceramide molecular species in which sphingosine, dihydrosphingosine, sphingadienine, phytosphingosine, or hydroxysphingosine with 16 to 18 carbon chain number and a fatty acid or a hydroxy fatty acid with 14 to 26 carbon chain number are bonded to each other via an amide bond, glucosylceramide molecular species having glucose bonded to the aforementioned ceramide molecular species, and galactosylceramide molecular species having galactose bonded to the aforementioned ceramide molecular species.

[0051]

As described herein, the expression "fermented product and/or cultured product of a lactic acid bacterium and/or a

bifidobacterium" is used to have a meaning which includes a fermented product of a lactic acid bacterium and a bifidobacterium, a fermented product of a lactic acid bacterium, a fermented product of a bifidobacterium, a cultured product of a lactic acid bacterium and a bifidobacterium, a cultured product of a lactic acid bacterium, a cultured product of a bifidobacterium, and a combination product thereof.

[0052]

As described herein, the expression "lactic acid bacterium" is a general name of microbes which assimilate glucose and produce lactic acid at yield of 50% or more per sugar. In terms of physiological activity, it is a coccus or a bacillus of gram positive bacteria, and it has a property including no mobility, no spore-forming ability, and catalase-negative property. Because the lactic acid bacterium is traditionally consumed all over the world as fermented milk or the like, it can be said that it is a microbe with very high safety. The a lactic acid bacterium are currently classified into 11 genera including genus Lactococcus, genus Lactobacillus, genus Leuconostoc, genus Pediococcus, genus Streptococcus, genus Wissella, genus Tetragenococcus, genus Oenococcus, genus Enterococcus, genus Vagococcus, and genus Carnobacterium. In the present invention, those lactic acid bacterium can be used. Among them, examples of a preferred lactic acid bacterium include genus Lactobacillus, that is, Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus, Lactobacillus lactis, and Lactobacillus gasseri. In particular, a mixed starter of Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus is preferably used.

[0053]

As described herein, the expression "bifidobacterium" is a general name of microbes which assimilate glucose and produce acetic acid or lactic acid, and it is a microbe belonging to genus Bifidobacterium. Examples thereof include Bifidobacterium longum, Bifidobacterium infantis, Bifidobacterium adolescentis, and Bifidobacterium breve.

[0054]

According to one preferred aspect of the present invention, the lactic acid bacterium include a combination of a bulgaricus bacterium and a thermophilus bacterium, and it is more preferably a combination of a bulgaricus bacterium and a thermophilus bacterium.

[0055]

Furthermore, as described herein, the expression "fermented product" means a cultured product itself which is obtained according to fermentation by a lactic acid bacterium and/or a bifidobacterium. As described herein, the expression "fermented product" includes a fermented product and a treated product of a lactic acid bacterium and/or a bifidobacterium, for example, a culture filtrate liquid or a culture supernatant that is obtained by bacteria removal according to filtration · centrifuge or membrane separation of the above cultured product (cultured product of a lactic acid bacterium and/or a bifidobacterium), a concentrate obtained by concentration of the culture filtrate liquid · culture supernatant or the fermented product of a lactic acid bacterium and/or a bifidobacterium by using an evaporator or the like, a paste product, a diluted product, or a dried product (obtained by freeze dry, heating, or de-pressurizing) of them.

Furthermore, for producing a treated product in the present invention, the aforementioned treating process including a bactericidal treatment like filtration, centrifuge, and membrane separation, precipitation, concentration, preparation into a paste, dilution, and drying can be used either singly or in combination of two or more of them.

[0056]

Furthermore, as described herein, examples of a medium for the "cultured product" include a non-fat milk powder medium added with yeast extract and a MRS medium.

[0057]

According to one preferred aspect of the present invention, the "fermented product and/or cultured product of a lactic acid bacterium and/or a bifidobacterium" is a milk fermented product and/or a milk cultured product of a lactic acid bacterium and/or a

bifidobacterium. According to a more preferred aspect of the present invention, the "fermented product and/or cultured product of a lactic acid bacterium and/or a bifidobacterium" is fermented milk (yoghurt). Herein, the yoghurt can be preferably a supernatant of fermented milk. According to an even more preferred aspect of the present invention, the "fermented product and/or cultured product of a lactic acid bacterium and/or a bifidobacterium" is polysaccharides. Herein, the polysaccharides are preferably those derived from a fermented product and/or a cultured product.

[0058]

Thus, according to another aspect of the present invention, a sphingolipid absorption promoting agent containing polysaccharides as an active ingredient is provided.

[0059]

As described herein, the expression "milk fermented product and/or milk cultured product", that is, "fermented product of milk or cultured product of milk", means a product which is obtained by fermenting or culturing raw materials containing milk. Examples of the milk include animal milk like cow milk and a processed product thereof (for example, non-fat milk, whole-fat milk powder, non-fat milk powder, condensed milk, casein, whey, raw cream, compound cream, butter, butter milk powder, or the like), and plant oils like soy bean oil derived from soy bean.

[0060]

Examples of the raw materials of a fermented milk include a raw material mix for fermented milk.

The raw material mix for fermented milk is a mixture containing a raw material milk and other components, and it can be obtained by, for example, dissolving by heating the raw materials that are commonly used for producing fermented milk like raw material milk, water, and other optional components (for example, sucrose, sugars, sweetener, acid-taste agent, mineral, vitamin, flavor or the like) followed by mixing. Furthermore, for the fermented milk, a thickening agent or a gelling agent like pectin, guar gum, xanthan gum, carageenan, and processed starch can be

used in a culture of a non-fat milk powder or a decomposed product of whey. The milk includes milk before sterilization and milk after sterilization. In specific content (materials) of the raw material milk, water, raw milk, sterilized milk, non-fat milk, whole fat milk powder, non-fat milk powder, whole fat milk concentrate, non-fat milk concentrate, buttermilk, butter, and cream may be included. It is also possible that a whey protein concentrate (WPC), a whey protein isolate (WPI), α-lactoalbumin (α-La), β-lactoglobulin (β-Lg), or the like are contained.

[0061]

The method for producing fermented milk includes a step of combining a raw material mix in which raw materials are admixed (combined) with one another. For the step of combining a raw material mix, a condition commonly used for producing fermented milk can be suitably adopted. In addition, the method for producing fermented milk of the present invention includes, like a method of a related art, a step of sterilizing (heating) a raw material mix, a step of cooling a raw material mix, a step of adding a starter, a step of fermentation, and a step of cooling fermented milk. It is preferable that those steps are included in the aforementioned order. Meanwhile, for those steps, a condition commonly used for producing fermented milk can be suitably adopted.

[0062]

As for the medium for culturing a lactic acid bacterium or the like, a commonly used medium can be used. Namely, as long as it is a medium which suitably contains a nitrogen source, inorganic substances, and other nutrients other than main carbon source, any medium can be used. As for the carbon source, lactose, glucose, sucrose, fructose, starch hydrolyzate, waste molasses, or the like can be used depending on the assimilation property of a bacterium to be used. As for the nitrogen source, a material containing organic nitrogen including a hydrolyzate of casein, a hydrolyzate of whey protein, α-lactoalbumin, β-lactoglobulin, glycomacropeptide, and a hydrolyzate of soy bean protein can be used. In addition, as an agent for promoting proliferation, meat

extract, fish meat extract, yeast extract or the like can be used.

[0063]

As for the conditions for culturing a lactic acid bacterium or the like, an anaerobic environment is preferable. However, it could be a slightly aerobic environment formed by static liquid culture which is commonly used. For culture in an anaerobic environment, a known method like culture under carbon gas layer can be applied. However, other methods can be also used. In general, culture temperature is preferably 30 to 47°C, more preferably 35 to 46°C, and even more preferably 37°C to 45°C. The pH of a medium for culturing a lactic acid bacterium or the like is preferably maintained at 6 to 7. However, as long as it is temperature allowing growth of bacteria, other pH conditions are also acceptable. In general, time for culturing a lactic acid bacterium or the like is preferably 1 to 48 hours, more preferably 8 to 36 hours, and even more preferably 10 to 24 hours.

[0064]

According to a representative example of the fermented milk (yoghurt) of the present invention, solid matter of fat free milk is 8% by weight or more, and the number of a lactic acid bacterium or the number of yeast is 10,000,000/ml or more. For example, it is 10^{11}/ml or less.

[0065]

Use

The fermented product and/or cultured product of a lactic acid bacterium and/or a bifidobacterium as an active ingredient of the present invention has an activity of promoting sphingolipid absorption (Examples 1 and 2 that will be described below), and in general, it also has an activity of promoting ceramide absorption. As such, by using the sphingolipid absorption promoting agent of the present invention, the sphingolipid absorption in a living body can be enhanced. Thus, various activities that are exhibited in a living body by the presence of sphingolipid or a derivative substance thereof can be further enhanced. Of course, the same holds true for a case in which the sphingolipid is sphingomyelin, and, because the sphingomyelin absorption in a living body can be

enhanced by using the sphingomyelin absorption promoting agent of the present invention, various activities that are exhibited in a living body by the presence of sphingomyelin or a derivative substance thereof can be further enhanced.

[0066]

Similarly, when the sphingolipid is ceramide, glycosylceramide, or galactosylceramide, by using the absorption promoting agent for ceramide, glycosylceramide, or galactosylceramide of the present invention, absorption of ceramide, glycosylceramide, or galactosylceramide in a living body can be enhanced. Accordingly, various activities that are exhibited in a living body by the presence of for ceramide, glycosylceramide, or galactosylceramide, and a derivative substance thereof can be further enhanced.

[0067]

According to the increased amount of sphingomyelin or a derivative substance thereof present in a living body, the effect of enhancing, improving, increasing or preventing deteriorated skin conditions (for example, impaired skin barrier function, dryness and roughness of skin, reduced moisture amount in keratinous layer, and atopic dermatitis) can be exhibited.

[0068]

Furthermore, according to the increased amount of sphingomyelin or a derivative substance thereof present in a living body, an activity of suppressing cancer (colon cancer) (Sugawara Tatsuya, "Digestion and Absorption of Sphingolipid as Functional Food Components", Nippon Eiyo Shokuryo Gakkaishi, Vol. 66 (2013), No. 4, pp.177-183), a skin-beautifying activity (JP 2008-184428 A), an activity of promoting infant brain development (JP 3203485 B1), an activity of enhancing mitochondria function (JP 2011-157329 A), an activity of enhancing motor skills (JP 2014-141496 A), an activity of suppressing accumulation of visceral fat and promoting increased adiponectin concentration in blood (anti-hyperglycemic activity and anti-hyperlipidemia activity) (JP 2007-320900 A), and an activity of preventing infection (JP 2008-037788 A) can be exhibited.

[0069]

Accordingly, those effects are obtained as the sphingolipid absorption is promoted by the sphingolipid absorption promoting agent of the present invention.

[0070]

Furthermore, as illustrated in Example 3 which is described below, by promoting the sphingolipid absorption, the skin barrier function can be further improved for a state in which the skin barrier function is impaired by ultraviolet ray.

[0071]

As described herein, the deteriorated skin conditions include impaired skin barrier function, dryness and roughness of skin, reduced moisture amount in keratinous layer, and atopic dermatitis, for example. The deteriorated skin conditions also include maintaining the barrier function. Furthermore, the deteriorated skin conditions preferably indicate deteriorated skin conditions under irradiation of ultraviolet ray.

[0072]

Furthermore, as described herein, the expression "preventing, suppressing, or improving" the deteriorated conditions is used to have a meaning which includes control of such conditions, delay of progress, alleviation of the conditions, prevention of an occurrence of symptoms, and prevention of recurrence or the like.

[0073]

Thus, according to the present invention, the amount of sphingolipid absorbed into a living body can be increased by promoting sphingolipid absorption. As a result, the in vivo activity (function, efficacy) which is induced by absorption of sphingolipid into a living body can be enhanced, improved, or increased. Meanwhile, as described herein, the expression "in vivo activity" indicates an activity which is induced by absorption of sphingolipid into a living body. Specific examples thereof include the various effects as described above. Thus, according to the present invention, an agent for enhancing the in vivo activity (function) induced by absorption of sphingolipid into a living body (an enhancer for function accompanied with sphingolipid absorption

(intake)) can be provided.

[0074]

Composition for oral intake or enteric administration

As described above, according to the present invention, a composition for oral intake or enteric administration containing the active ingredient of the present invention, that is, a fermented product and/or a cultured product of a lactic acid bacterium and/or a bifidobacterium, and sphingolipid is provided. It can be also said that the composition is obtained by including, as an active ingredient, the sphingolipid absorption promoting agent of the present invention.

[0075]

In many cases, sphingolipid is included in a fermented product and/or a cultured product of a lactic acid bacterium and/or a bifidobacterium itself. However, according to a preferred aspect of the present invention, the composition of the present invention is obtained by containing a fermented product and/or a cultured product of a lactic acid bacterium and/or a bifidobacterium and additional sphingolipid. As such, the composition of the present invention is distinguished from a fermented product and/or a cultured product of a lactic acid bacterium and/or a bifidobacterium like conventional yoghurt or the like.

As described herein, the expression "additional sphingolipid" means, for a case in which sphingolipid is already contained in a fermented product and/or a cultured product of a lactic acid bacterium and/or a bifidobacterium, the sphingolipid which is additionally present in the composition while it is higher than the amount of the sphingolipid itself contained in a fermented product and/or a cultured product of a lactic acid bacterium and/or a bifidobacterium.

[0076]

As for the additional sphingolipid, sphingolipid itself can be used as it is. Examples thereof include milk-derived phospholipid concentrate (MPL) containing a high amount of sphingomyelin. It is also possible to be obtained from cream, butter, or butter serum, which is a byproduct generated during production of butter oil, as a

fraction insoluble in acetone (solvent) and/or ethanol. Butter serum is also used in the field of a commercially available milk, and therefore obtainable (for example, manufactured by Tatua, New Zealand). Butter serum has a high content of MFGM, in which sphingomyelin is localized, and thus is suitable as a raw material.

[0077]

For separation of phospholipids, in which sphingomyelin is localized, from butter serum, the property of phospholipids being insoluble in acetone and/or ethanol is utilized. Through a multiple acetone extractions, an acetone-soluble fraction containing neutral lipids is removed and then an acetone-insoluble fraction containing concentrated phospholipids is obtained. The acetone-insoluble fraction is subjected to vacuum concentration to remove acetone and then the concentrate is subjected to freeze drying after sterilization. The dried product is pulverized, so that a milk-derived phospholipid fraction is obtained and it can be used for the composition.

[0078]

Furthermore, there is a method of extracting sphingomyelin in which whole lipids which have been extracted from powder of chicken skin and subjected to a drying treatment are extracted with a mixed solvent containing aliphatic hydrocarbon-based solvent and a water soluble ketone-based solvent, an in soluble part containing sphingomyelin as a main component is extracted with a mixed solvent containing water and a water soluble ketone-based solvent, and non-lipid components included in a soluble part are removed. A known method like a method of extracting from fishes and shell fishes, animals and birds, or the like can be used. From the viewpoint of flavor, sphingomyelin derived from milk is preferable.

[0079]

With regard to the composition of the present invention, the fermented product and/or cultured product of a lactic acid bacterium and/or a bifidobacterium can be yoghurt in various forms like set type yoghurt (solid-like fermented milk), soft type yoghurt (paste-like fermented milk), and drink yoghurt (liquid-like

fermented milk), and it can be also yoghurt containing grains, fruits, vegetables, nutritional components, or the like.

[0080]

According to the composition for oral intake or enteric administration of the present invention, the absorption property of sphingolipid into a living body is preferably increased. More preferably, the absorption property of sphingomyelin into a living body is increased. Namely, it can be said that the composition for oral intake or enteric administration of the present invention is a "highly sphingolipid absorbing", and preferably "highly sphingomyelin absorbing" composition for oral intake or enteric administration. Meanwhile, the "highly sphingolipid absorbing" or "highly sphingomyelin absorbing" can be confirmed by, like Example 1 or Example 2 described below, comparing the absorption amount of sphingomyelin in the case of yoghurt of a related art or the like.

[0081]

From the viewpoint of the highly sphingolipid absorbing property or highly sphingomyelin absorbing property, in the composition for oral intake or enteric administration of the present invention, a blending ratio between the fermented product and/or cultured product of a lactic acid bacterium and/or a bifidobacterium and sphingolipid is such that the fermented product and/or cultured product of a lactic acid bacterium and/or a bifidobacterium (dry weight (powder)) is contained at 1 to 10000 mg, more preferably at 5 to 5000 mg, and even more preferably at 10 to 750 mg per 1 mg of the sphingolipid.

[0082]

Similarly, from the viewpoint of the highly sphingolipid absorbing property or highly sphingomyelin absorbing property, in the composition for oral intake or enteric administration of the present invention, a blending ratio between the fermented product and/or cultured product of a lactic acid bacterium and/or a bifidobacterium and sphingolipid is such that the fermented product and/or cultured product of a lactic acid bacterium and/or a bifidobacterium (wet weight) is contained at 0.01 to 100 g,

preferably at 0.05 to 50 g, and more preferably at 0.1 to 7.5 g per 1 mg of the sphingolipid.

[0083]

The composition for oral intake or enteric administration of the present invention is preferably used for an in vivo activity which is induced by absorption of sphingomyelin into a living body. In addition, the composition for oral intake or enteric administration of the present invention is more preferably used for a case in which the in vivo activity is selected from a group consisting of prevention or improvement of deteriorated skin conditions, an activity of suppressing cancer, a skin-beautifying activity, an activity of promoting infant brain development, an activity of enhancing mitochondria function, an activity of enhancing motor skills, an activity of suppressing accumulation of visceral fat and promoting increased adiponectin concentration in blood, and an activity of preventing infection.

[0084]

The composition for oral intake or enteric administration of the present invention is preferably used for preventing, suppressing, or improving deteriorated skin conditions. Herein, the deteriorated skin conditions are preferably a deteriorated skin barrier function. Furthermore, the deteriorated skin barrier function preferably indicates the one caused by irradiation of ultraviolet ray.

[0085]

As a preferred aspect, the composition of the present invention can be a pharmaceutical composition.

In the present invention, the pharmaceutical composition indicates a composition which is prepared, according to a common method, as an oral preparation or a parenteral preparation by using in combination an additive that is acceptable in terms of formulation. From the viewpoint of simplicity, an oral preparation is preferable. Examples of the form of an oral preparation include a solid formulation like a tablet, powder, a microgranule, a granule, a capsule, a pill, and a sustained-release preparation, and a liquid formulation like a solution, a suspension, and an emulsion. Examples of the additive which is acceptable in terms of

formulation include a vehicle, a stabilizer, a preservative, a wetting agent, an emulsifying agent, a lubricant, a sweetener, a coloring agent, a flavor, a buffer, an anti-oxidant, and a pH adjusting agent.

[0086]

Furthermore, according to the present invention, a food and drink product obtained by containing the sphingolipid absorption promoting agent of the present invention or the aforementioned composition for oral intake or enteric administration is provided.

[0087]

Those composition and food and drink product of the present invention can be produced by a production method which includes adding the absorption promoting agent of the present invention or an active ingredient thereof to raw material ingredients of the composition and food and drink product.

[0088]

The food and drink product of the present invention can be added with, if necessary, any ingredient. The ingredient which can be added is not particularly limited, but ingredients that are generally blended in a food and drink product, that is, a sweetener, an acidulant, a juice or an extract of a vegetable, a fruit, or a nut, a nutrient like vitamin, mineral and amino acid, a useful microorganism including a lactic acid bacterium, a bifidobacterium, a useful microorganism like propionic acid or a cultured product thereof, sugars having functional property like oligosaccharides, a known substance with functional property like royal jelly, collagen, ceramide, glucosamine, astaxanthin, and polyphenol, a flavor, a pH adjusting agent, a vehicle, an acidulant, a coloring agent, an emulsifying agent, or a preservative can be blended.

[0089]

As described herein, the expression "food and drink product" means a product other than a pharmaceutical composition, and it is not particularly limited if it is in the orally takable form of a solution, an emulsion, powder, or a solid-molded product. Specific examples thereof include a dairy product including a milk drink, yoghurt, a drink of a lactic acid bacterium, a fermented milk drink, ice creams, creams, and cheeses; a drink including a soft drink, a

fruit juice drink, a vegetable drink, a soy bean milk drink, a coffee drink, a tea drink, a jelly drink, a cocoa, a powder drink like smoothie or a sports powder drink, a powder drink with fortified nutrition, a powder food product for cosmetic use, a powder soup, a base for steamed bread, a concentrated drink, and an alcohol drink; a wheat product including bread, pasta, noodle, cake mix, powder for deep-frying, and bread powder; snacks including chocolate, gum, candy, cookie, gummi, snack, Japanese-style confectionery, jelly, and a dessert snack like pudding; seasonings including processed seasonings, flavored seasonings, and a cooked mix; a retort food product including curry, pasta sauce, pot au feu, and Japanese-style food product; oils and fats including processed oils and fats, butter, margarine, spread, and mayonnaise; instant food products including a freeze-dry food product; a processed agricultural product including a canned agricultural product, jam · marmalades, pickles, soy bean cooked with soy sauce, cereal, and rice porridge; a processed sea product; a processed livestock product; a frozen food product including pizza, doria, gratin, garnish, and fry; a formula, and also feeds for an animal, a tablet, and a cosmetic product used for oral care.

[0090]

According to a preferred aspect of the present invention, the food and drink product is a milk drink (it may contain processed milk), fermented milk, a soft drink, a jelly drink, a tablet, a powder food product for cosmetic use, a powder food product, a formula, or an infant dry milk powder formula in liquid state. According to a more preferred aspect of the present invention, the food and drink product is a milk drink, fermented milk, a soft drink, a jelly drink, a tablet of an infant dry milk powder formula, or a powder food product for cosmetic use. According to an even more preferred aspect of the present invention, the food and drink product is a milk drink (it may contain processed milk) or fermented milk.

[0091]

Furthermore, the food and drink product also encompasses those that are classified into a functional food, a health and

nutrition food, a supplement, a health food, a food for specific health use, a food labeled with functionality, a food with nutritional function, a food for a patient, dry milk powder formula for an infant, milk powder for a pregnant or breastfeeding woman, or a food tagged with low disease risk label. According to a preferred aspect of the present invention, the food and drink product is a functional food, a health and nutrition food, a supplement, a food for specific health use, a food labeled with functionality, or a food tagged with low disease risk label.

[0092]

Meanwhile, as described herein, "low disease risk label" means a label for a food and drink product which has a possibility of lowering a disease risk, and it can be a label that is designated or recognized on the basis of the standard set by FAO/WHO Joint Commission for Food Standard (Codex Alimentarius Commission) or with reference to the standard.

[0093]

Thus, the food and drink product of the present invention can be provided as, for example, a food product that is suitable for a consumer who expects to have improvement or alleviation of deteriorated skin conditions or a food product that is suitable for improvement of deteriorated skin conditions, that is, a so-called food for specific health use or a food labeled with functionality.

[0094]

With regard to the food and drink product and composition of the present invention, the content of sphingomyelin in the food and drink product and composition is preferably set within a pre-determined range to obtain a desired effect that is accompanied with absorption of sphingomyelin. Specific content may vary depending on type of the food and drink product or composition, the form, or purpose like prevention · improvement, and thus it is difficult to set uniformly the content. However, in the composition of the present invention, it can be adjusted to an active amount which enables intake of milk-derived sphingomyelin in an amount of 0.5 to 1500 mg, preferably 1 to 1000 mg, and more preferably 5 to 500 mg by an adult (body weight: 60 kg) per day.

[0095]

Content of sphingolipid, for example, sphingomyelin, can be measured by a commonly used method. However, it can be also measured according to the descriptions of Example 1 and Example 2 that will be described below. Furthermore, it can be also measured by using liquid chromatography and using AQUASIL SP100 (4.6×250 mm, Senshu Scientific Co., Ltd.) as a column, for example.

[0096]

At that time, as a mobile phase, a solution in which 0.5 mM phosphoric acid-citric acid buffer solution (pH: 3.0) and methanol are admixed with each other at ratio of 5 to 95 can be used. By setting the measurement time at 20 minutes, flow rate of the mobile phase at 0.6 mL/minute, column temperature at 40°C, and absorbance at 205 nm, the detection can be made. As a reference material, sphingomyelin (derived from milk, manufactured by Nagara Science, Co., Ltd.) can be used, for example. Quantification can be made based on area ratio.

[0097]

According to other aspect of the present invention, content of the active ingredient in the food and drink product and the composition can be defined by the content per single package form. For example, in case of the food and drink product, the sphingolipid content is 5 to 1500 mg, preferably 6 to 1000 mg, and more preferably 7 to 500 mg. Furthermore, in case of the composition, the sphingolipid content is 0.5 to 1500 mg, preferably 1 to 1000 mg, and more preferably 5 to 500 mg. Furthermore, the amount (content) per single package form is not limited to a single intake amount, and it may include the whole intake amount of several intakes or several days (for example, 30 days). For example, in case of the food and drink product, the sphingolipid content is 5 to 45000 mg, preferably 6 to 30000 mg, and more preferably 7 to 15000 mg. Furthermore, in case of the composition, the sphingolipid content is 0.5 to 45000 mg, preferably 1 to 30000 mg, and more preferably 5 to 15000 mg.

[0098]

In a common milk drink or fermented milk of a related art, phospholipid is contained at 20 mg per 1 g of lipids. However, in the milk drink or fermented milk of the present invention, phospholipid is contained at 25 to 3000 mg, preferably 30 to 2500 mg, more preferably 40 to 2000 mg, and even more preferably 50 to 1500 mg per 1 g of lipids. Furthermore, in a common milk drink or fermented milk of a related art, sphingomyelin is contained at 6 mg per 1 g of lipids. However, in the food and drink product or the composition of the present invention, sphingomyelin is contained at 7 to 1500 mg, preferably 8 to 1250 mg, more preferably 9 to 1000 mg, and even more preferably 10 to 750 mg per 1 g of lipids. Namely, in a preferred aspect of the present invention, a product with intentionally increased sphingomyelin content is used.

[0099]

In the milk drink or fermented milk of the present invention, if lipids are contained in a typical amount (for example, lipids are contained at 3% by weight), phospholipid may be contained at 1 to 300 mg/g, preferably 1.5 to 250 mg/g, more preferably 2 to 200 mg/g, and even more preferably 2.5 to 150 mg/g. Furthermore, in the food and drink product or the composition of the present invention, if lipids are contained in a typical amount, sphingomyelin may be contained at 0.2 to 60 mg/g, preferably 0.25 to 50 mg/g, more preferably 0.3 to 40 mg/g, and even more preferably 0.35 to 30 mg/g.

[0100]

In a common milk drink or fermented milk of a related art, phospholipid is contained at 20 mg per 1 g of lipids. However, in the milk drink or fermented milk of the present invention, phospholipid is contained at 25 to 3000 mg, preferably 30 to 2500 mg, more preferably 40 to 2000 mg, and even more preferably 50 to 1500 mg per 1 g of lipids. Furthermore, in the food and drink product or the composition of the present invention, if lipids are contained in a small amount, sphingomyelin may be contained at 0.05 to 60 mg/g, preferably 0.1 to 50 mg/g, more preferably 0.15 to 40 mg/g, and even more preferably 0.2 to 30 mg/g.

[0101]

Furthermore, in a common milk drink or fermented milk of a related art, phospholipid is contained at 20 mg per 1 g of lipids. However, in the milk drink or fermented milk of the present invention, phospholipid is contained at 25 to 3000 mg, preferably 30 to 2500 mg, more preferably 40 to 2000 mg, and even more preferably 50 to 1500 mg per 1 g of lipids. Furthermore, in the milk drink, fermented milk, soft drink, jelly drink, tablet, or powder product for cosmetic use, if lipids are almost not contained (fat free), sphingomyelin may be contained at 0.02 to 60 mg/g, preferably 0.04 to 50 mg/g, more preferably 0.06 to 40 mg/g, and even more preferably 0.08 to 30 mg/g.

[0102]

According to another aspect of the present invention, a method for promoting sphingolipid absorption including oral intake or enteric administration of, together with sphingolipid, a fermented product and/or a cultured product of a lactic acid bacterium and/or a bifidobacterium to a subject who is in need of intake of sphingolipid is provided. Herein, the sphingolipid is preferably sphingomyelin.

[0103]

According to another aspect of the present invention, a method for promoting ceramide absorption including oral intake or enteric administration of, together with sphingolipid, a fermented product and/or a cultured product of a lactic acid bacterium and/or a bifidobacterium to a subject who is in need of intake of sphingolipid is provided.

[0104]

Meanwhile, medial use is preferably excluded from the aforementioned method. In addition, the subject described above is preferably a human, or a mammal other than human.

[0105]

Furthermore, the aforementioned method may be a method for promoting sphingolipid absorption including continuous oral intake or enteric administration for 2 days or longer of, together with sphingolipid, a fermented product and/or a cultured product of

a lactic acid bacterium and/or a bifidobacterium to a subject who is in need of intake of sphingolipid, or a method for promoting ceramide absorption including the same for a subject who is in need of intake of sphingolipid.

[EXAMPLES]

[0106]

The present invention is further illustrated by Examples that are not intended as a limitation of the invention.

[0107]

Example 1: Test for evaluating sphingomyelin absorption property in simultaneous administration of yoghurt and sphingomyelin

To a rat, sphingomyelin derived from milk is administered only or yoghurt powder and sphingomyelin derived from milk are administered simultaneously, and the absorption amount of sphingomyelin, which is incorporated to lymphatic fluid, was evaluated.

[0108]

[Experimental methods]

The experiments were carried out according to the method of Sugawara, et. al. (J. Lipid Res. 2010, 51, 1761-1769). Specifically, it is as described below.

[0109]

(1-1) Administration of sphingomyelin and lymphatic fluid recovery

A rat (SD, male, weight: about 350 g) was acclimated for 1 week. After that, a cannula was placed in the thoracic lymph node and stomach. By using a syringe pump, a buffer solution (glucose: 139 mM, sodium chloride: 85 mM) was transported overnight (about 12 hours) to the inside of the stomach (flow rate: 3 mL/min).

[0110]

Then, corresponding to each test solution (A), (B), and (C) that will be described below, the rats were divided into three groups (a, b, and c). Starting 1 hour before administration of each test solution to the inside of the stomach, lymphatic fluid was collected from the three groups (a, b, and c) in a spitz tube which

has been treated with EDTA.

[0111]

Furthermore, by using a syringe pump, each test solution (A), (B), and (C) (3 mL for each) was transported for 1 minute to the inside of the stomach (flow rate: 3 mL/min). Then, by using a syringe pump, the buffer solution was transported for 6 hours to the inside of the stomach (flow rate: 3 mL/min).

[0112]

Then, lymphatic fluid was collected every hour from the three groups (a, b, and c) in a spitz tube which has been treated with EDTA.

[0113]

(1-2) Test solution

Each test solution (A), (B), and (C) was prepared as described below.

(A) Triolein: 200 mg, bovine serum albumin: 50 mg, and sodium taurocholate: 200 mg were admixed with one another which has been treated with EDTA.

[0114]

(B) In a solution in which triolein: 195 mg, bovine serum albumin: 50 mg, and sodium taurocholate: 200 mg are admixed with one another, milk-derived sphingomyelin (SM, purity of 98%, manufactured by Nagara Science Co., Ltd.): 5 mg was emulsified under ultrasonication.

[0115]

(C) In a solution in which triolein: 192 mg, bovine serum albumin: 50 mg, and sodium taurocholate: 200 mg are admixed with one another, milk-derived sphingomyelin (SM, purity of 98%, manufactured by Nagara Science Co., Ltd.): 4.75 mg was emulsified under ultrasonication. In addition, yoghurt powder (YG, fat: about 0%, Meiji Bulgaria Yoghurt Fat Zero, obtained from Meiji Co., Ltd.): 250 mg was prepared.

[0116]

(1-3) Constitution of group for evaluation

The three groups (a, b, and c) corresponding to the test solution (A), (B), and (C) will be described below, and the details

are given in Table 1. Meanwhile, there were 5 rats for each group.

(a) Control group: the test solution (A): 3 mL/rat (SM: 0 mg/rat), administered to the inside of stomach.

(b) SM group: the test solution (B): 3 mL/rat (SM: 5 mg/rat), administered to the inside of stomach.

(c) SM+YG group: the test solution (C): 3 mL/rat (SM: 4.75 mg/rat) together with YG: 250 mg/rat, administered to the inside of stomach.

[0117]

[Table 1]

Table 1: Group constitution and composition of test solution (3 mL/rat) for animal test

	a. Control group	b. SM group	c. SM+YG group
Triolein	200	195	192 **
Bovine serum albumin	50	50	50
Sodium taurocholate	200	200	200
Sphingomyelin	–	5	4.75 *
Yoghurt powder	–	–	250

* Blending amount of sphingomyelin was set such that, after being added with yoghurt powder, total amount of the sphingomyelin is 5 mg/rat.

** Blending amount of triolein was set such that total lipid amount remains the same in the 3 groups.

[0118]

(1-4) Index for evaluation

The absorption amount of sphingomyelin in rat lymphatic fluid was evaluated after administering the test solution. Specifically, by following a change the ceramide molecular species (d16:1-C16:0) and sphingomyelin molecular species (d16:1-C16:0 SM), which are absent in rat lymphatic fluid but present specifically in milk-derived sphingomyelin, as an index, comparison of the

difference in the absorption property was made.

[0119]

(1-5) Method for analysis

Extraction of lipids from lymphatic fluid, which has been collected from the rat, was performed according to the method of Folch, et. al. (J. Biol. Chem., 1957, 226, 497-509).

Specifically, physiological saline: 800 μL was added to the lymphatic fluid : 200 μL (when the lymphatic fluid has been recovered at 2.5 mL/h). Then, after adding a mixture of chloroform-methanol (2 : 1 (v/v)): 4 mL, it was shaken (200 rpm, for 15 minutes). Subsequently, after centrifuge (2000 rpm, for 10 minutes), the bottom layer was transferred to another tube, concentrated by centrifuge, and added with methanol: 500 μL to prepare a sample for analysis of ceramide. Furthermore, according to 10 times dilution with methanol, a sample for analysis of sphingomyelin was prepared.

[0120]

As a standard material, the ceramide (d18:1-C16:0, obtained from Avanti Polar Lipids) and sphingomyelin (d18:1-C16:0 SM, obtained from Avanti Polar Lipids) were used. By using those standard materials in an equivalent amount and using LC/MS/MS (ACQUITY premier XE (manufactured by Waters), quantification of ceramide (d16:1-C16:0) and sphingomyelin (d16:1-C16:0 SM) was performed. At that time, as a column, ACQUITY UPLC BEH C18 (2 mm × 100 mm, manufactured by Waters) was used. As a mobile phase A, ammonium acetate (5 mM)/methanol (95%) was used. As a mobile phase B, ammonium acetate (5 mM)/methanol was used.

[0121]

In addition, after having a gradient such that it starts from 100% of the mobile phase A but the mobile phase B is 100% after 30 minutes, the mobile phase B is maintained at 100% for 2 minutes. Then, 3 minutes later, it was switched to 100% of the mobile phase A. Herein, the measurement time for single sample by LC/MS/MS was 35 minutes, the flow rate of the mobile phase was 0.4 mL/minute, and the column temperature was set at 40°C.

Then, by using electro · spray · ionization, detection was made at positive mode. As a parameter for analysis by LC/MS/MS, the setting are as follows: the capillary voltage was 3000 V, the source temperature was 120°C, the desolvation temperature was 400°C, the flow rate of gas for desolvation was 850 L/hours, the flow rate of cone gas was 50 L/hour, and the cone voltage was 40 V/hour.

[0122]

[Results]

The results were as illustrated in FIG. 1 and FIG. 2.

[0123]

In FIG. 1, transition of the amount of ceramide molecular species (d16:1-C16:0) is illustrated. Herein, 4, 5, or 6 hours after the oral administration of each test solution, the amount of ceramide molecular species was found to be higher in the SM+YG group compared to the SM group.

In FIG. 2, transition of the amount of sphingomyelin molecular species (d16:1-C16:0 SM) is illustrated. Herein, 3, 4, 5, or 6 hours after the oral administration of each test solution, the amount of sphingomyelin molecular species was found to be higher in the SM+YG group compared to the SM group.

[0124]

From the above results, it was found that, according to simultaneous oral intake of sphingomyelin and yoghurt, not only the ceramide absorption is promoted but also the sphingomyelin absorption is promoted.

[0125]

<u>Example 2: Test for evaluating sphingomyelin absorption property in simultaneous intake of yoghurt and phospholipid concentrate with high content of milk-derived sphingomyelin (MPL)</u>

To a rat, MPL is orally administered only or yoghurt and MPL are administered simultaneously by the oral route, and the amount of ceramide in blood was evaluated.

[0126]

[Experimental methods]

(2-1) Oral administration of MPL and blood collecting

A rat (SD, male, weight: about 300 g) was acclimated for 1

week. After that, the rat was fasted for 16 hours and divided into 2 groups. Then, each test solution (D) and (E) that will be described below was orally administered. Blood was taken from the tail vein, before the administration and 90 minutes, 180 minutes, 270 minutes, or 360 minutes after the administration. Further, the blood serum was obtained according to a common method.

[0127]

(2-2) Constitution of group for evaluation

(d) MPL group: the test solution (D): dose of MPL: 540 mg (100 mg in terms of the amount of sphingomyelin) was administered per 1 kg of body weight.

(e) MPL+YG group: the test solution (E): dose of yoghurt (Meiji Bulgaria Yoghurt Fat Zero, obtained from Meiji Co., Ltd.): 11.3 g and MPL: 535 mg (100 mg in terms of the total amount of sphingomyelin) was administered per 1 kg of body weight.

[0128]

(2-3) Index for evaluation

The amount of ceramide in rat blood serum was evaluated after administering orally the test solution.

Specifically, by following a change in the ceramide molecular species (d16:1-C16:0, d16:1-C22:0, d16:1-C23:0, d16:1-C24:0), which are absent in rat blood serum but present specifically in milk-derived sphingomyelin, as an index, comparison of the difference in blood serum concentration was made.

[0129]

(2-4) Method for analysis

Extraction of blood serum lipids, which has been collected from the rat, was performed according to the method of Folch, et. al. (J. Biol. Chem., 1957, 226, 497-509)

Specifically, physiological saline: 200 μL was added to the blood serum : 50 μL. Then, after adding a mixture of chloroform-methanol (2 : 1 (v/v)): 1 mL, the extraction was performed. Subsequently, after centrifuge (2000 rpm, for 10 minutes), the bottom layer was transferred to another tube, concentrated by centrifuge, and added with methanol: 200 μL to prepare a sample

for analysis of ceramide.

[0130]

As a standard material, the ceramide (d18:1-C16:0, d18:1-C22:0, d18:1-C23:0, d18:1-C24:0, obtained from Avanti Polar Lipids) were used. By using those standard materials in an equivalent amount and using LC/MS/MS (ACQUITY premier XE (manufactured by Waters), quantification of the ceramide (d16:1-C16:0, d16:1-C22:0, d16:1-C23:0, d16:1-C24:0) was performed. At that time, as a column, ACQUITY UPLC BEH C18 (2 mm × 100 mm, manufactured by Waters) was used. As a mobile phase A, ammonium acetate (5 mM)/methanol (95%) was used. As a mobile phase B, ammonium acetate (5 mM)/methanol was used.

[0131]

In addition, after having a gradient such that it starts from 100% of the mobile phase A but the mobile phase B is 100% after 30 minutes, the mobile phase B is maintained at 100% for 2 minutes. Then, 3 minutes later, it was switched to 100% of the mobile phase A. Herein, the measurement time for single sample by LC/MS/MS was 35 minutes, the flow rate of the mobile phase was 0.4 mL/minute, and the column temperature was set at 40°C. Then, by using electro · spray · ionization, detection was made at positive mode. As a parameter for analysis by LC/MS/MS, the setting are as follows: the capillary voltage was 3000 V, the source temperature was 120°C, the desolvation temperature was 400°C, the flow rate of gas for desolvation was 850 L/hours, the flow rate of cone gas was 50 L/hour, and the cone voltage was 40 V/hour.

[0132]

[Results]

The results were as illustrated in FIGS. 3A to 3D and FIG. 4.

[0133]

In FIGS. 3A to 3D, a change in the amount of ceramide molecular species (d16:1-C16:0, d16:1-C22:0, d16:1-C23:0, d16:1-C24:0) in blood serum is illustrated. Compared to the MLP group, a significant higher value was obtained from the MPL+YG group, that is, 90 and 180 minutes after the administration of the test solution in the ceramide molecular species (d16:1-C16:0), 90,

180, and 270 minutes after the administration of the test solution in the ceramide molecular species (d16:1-C22:0, d16:1-C23:0), and 90, 180, 270, and 360 minutes after the administration of the test solution in the ceramide molecular species (d16:1-C24:0).

[0134]

In FIG. 4, the area under the time curve of the amount of ceramide molecular species in blood serum was illustrated. In any of the ceramide molecular species, a significantly higher value of the area under the time curve was obtained from the MPL+YG group compared to the MPL group.

[0135]

It was found that, according to simultaneous intake of yoghurt and phospholipid concentrate with high content of milk-derived sphingomyelin by the oral route, sphingomyelin absorption is promoted.

[0136]

Example 3: Effect of yoghurt and sphingolipid on deteriorated skin barrier function under ultraviolet ray irradiation

A hairless mouse having deteriorated skin barrier function due to ultraviolet ray irradiation was allowed to have oral intake of sphingomyelin, or sphingomyelin and yoghurt for 3 days, and the effect on the skin barrier function (moisture amount in keratinous layer and transepidermal evaporation amount of water (Transepidermal Water Loss: TEWL)) was evaluated.

As a sphingolipid, milk-derived sphingomyelin (SM, purity of 98%, Nagara Science Co., Ltd.) was used.

[0137]

[Experimental methods]

(3-1) Induction of state with deteriorated skin barrier function by ultraviolet ray irradiation

A hairless mouse (Hos: HR-1, female, 4-week old, Hoshino Animal Laboratory, Inc.) was acclimated for 1 week. After that, it was irradiated with ultraviolet ray (UV-B (GL20SE, SANKYO DENKI Co. Ltd)) at conditions of 20 mJ/cm^2.

[0138]

(3-2) Constitution of group for evaluation

By using a tester for measuring the deteriorated skin barrier function, the effect of oral administration of sphingomyelin or sphingomyelin and yoghurt on skin state was evaluated. There were 8 animals for each test group. Relationship between the group constitution (constitution of group for evaluation) for the animal test and content of sphingomyelin and yoghurt is described below.

[0139]

Control group (group for control): Group administered with typical feeds.

SM group: Group administered orally with sphingomyelin at 10 mg/kg/day

SM+YG group: Group administered orally with sphingomyelin and yoghurt at 10 mg/kg/day and 11.3 g/kg/day, respectively (with the proviso that, for both of the SM group and the SM+YG group, collagen was administered additionally via the oral route at ratio of 1000 mg/kg/day, in addition to sphingomyelin and yoghurt).

[0140]

(3-3) Method for evaluation test

For each test group, the moisture amount in keratinous layer and TEWL were measured. The skin barrier function was evaluated based on them.

[0141]

(3-3-1) Moisture amount in keratinous layer

The moisture amount in keratinous layer was measured 5 times/animal by using Corneometer (Courage and Khazaka Electronic GmbH). Among the obtained values, average of the 3 measurement values except the highest and the lowest values was taken and used as a moisture amount in keratinous layer.

[0142]

(3-3-2) Transepidermal evaporation amount of water (TEWL)

The transepidermal evaporation amount of water (TEWL) was measured with Tewemeter MPA580 (Courage and Khazaka Electronic GmbH), by using for 20 seconds a probe heated to 29°C. From the obtained measurement results, the average of 3

measurements was taken and used as a transepidermal evaporation amount of water.

[0143]

[Results of evaluation]

(Moisture amount in keratinous layer)

The results were as illustrated in FIG. 5.

In FIG. 5, the moisture amount in keratinous layer was higher in the SM group and the SM+YG group compared to the moisture amount in keratinous layer in the Control group. Furthermore, the SM+YG group exhibited a higher value than the SM group. From the results, it was considered that, compared to the SM group, the SM+YG group has more suppressed (improved) deteriorated skin barrier function.

[0144]

(Transepidermal evaporation amount of water (TEWL))

The results were as illustrated in FIG. 6.

In FIG. 6, the TEWL value was lower in the SM group compared to the TEWL value of the Control group. Furthermore, the SM+YG group exhibited a lower value than the SM group. From the results, it was considered that, compared to the SM group, the SM+YG group has more suppressed (improved) deteriorated skin barrier function.

[0145]

It was demonstrated that, according to oral intake of sphingolipid and yoghurt (fermented milk), the deteriorated skin barrier function caused by ultraviolet ray is further suppressed (improved). It was also demonstrated that, as sphingomyelin and yoghurt (fermented milk) are continuously taken for 3 days or longer, the deteriorated skin barrier function can be more easily suppressed (improved).

[0146]

<u>Example 4: Test for evaluating sphingomyelin absorption property in simultaneous intake of non-fermented milk and MPL</u>

Ceramide amount in blood was evaluated for each of a case in which only MPL is administered via the oral route to a rat, a case in which yoghurt and MPL are administered simultaneously via the

oral route to a rat, and a case in which non-fermented milk and MPL are administered simultaneously via the oral route to a rat.

[0147]

[Experimental methods]

(4-1) A rat (SD, male, weight: about 270 g) was acclimated for 1 week. After that, the rat was fasted for 16 hours and divided into 3 groups. Then, each test solution (F), (G), and (H) that will be described below was orally administered. Blood was taken from the tail vein, at each time point including before the administration and 90 minutes, 180 minutes, 270 minutes, or 360 minutes after the administration. Further, the blood serum was obtained according to a common method.

[0148]

(4-2) Constitution of group for evaluation

(f) MPL group: the test solution (F): dose of MPL: 540 mg (100 mg in terms of the amount of sphingomyelin) was administered per 1 kg of body weight.

(g) MPL+YG group: the test solution (G): dose of yoghurt (Meiji Bulgaria Yoghurt Fat Zero, obtained from Meiji Co., Ltd.): 11.3 g and MPL: 535 mg (100 mg in terms of the total amount of sphingomyelin) was administered per 1 kg of body weight.

(h) MPL+ non-fermented milk group: the test solution (H): dose of non-fat milk (Meiji Non-fat Milk, obtained from Meiji Co., Ltd.): 1.3 g and MPL: 537 mg (100 mg in terms of the total amount of sphingomyelin) was administered per 1 kg of body weight.

[0149]

(4-3) Index for evaluation

The amount of ceramide in rat blood serum was evaluated after administering orally the test solution.

Specifically, the ceramide molecular species (d16:1-C16:0, d16:1-C22:0, d16:1-C23:0, d16:1-C24:0) were used as an index.

[0150]

[Results]

The results were as illustrated in FIG. 7.

[0151]

In FIG. 7, the area under the time curve of the amount of ceramide molecular species in blood serum was illustrated. Compared to the MPL group, the area under the time curve of any ceramide molecular species was significantly higher in the MPL group, MPL+non-fermented milk group, and MPL+YG group in the order.

[0152]

When phospholipid concentrate derived from milk in which sphingomyelin is contained in high content and non-fermented milk are simultaneously taken via the oral route, sphingomyelin absorption is promoted. In this regard, it was found that, when phospholipid concentrate derived from milk in which sphingomyelin is contained in high content and yoghurt are simultaneously taken via the oral route, sphingomyelin absorption is further promoted.

[0153]

Example 5: Test for evaluating sphingomyelin absorption property in simultaneous intake of yoghurt fraction and MPL

Ceramide amount in blood was evaluated for each of a case in which only MPL is administered via the oral route to a rat, a case in which yoghurt and MPL are administered simultaneously via the oral route to a rat, a case in which yoghurt fraction supernatant and MPL are administered simultaneously via the oral route to a rat, and a case in which yoghurt fraction precipitate and MPL are administered simultaneously via the oral route to a rat.

[0154]

[Experimental methods]

(5-1) Method for preparing yoghurt fraction

Yoghurt (Meiji Bulgaria Yoghurt Fat Zero, obtained from Meiji Co., Ltd.) was centrifuged for 10 minutes at 6000 rpm to obtain a yoghurt fraction supernatant and a yoghurt fraction precipitate.

[0155]

(5-2)

A rat (SD, male, weight: about 270 g) was acclimated for 1 week. After that, the rat was fasted for 16 hours and divided into 3 groups. Then, each test solution (I), (J), (K), and (L) that will be described below was orally administered. Blood was taken from

the tail vein, at each time point including before the administration and 90 minutes, 180 minutes, 270 minutes, or 360 minutes after the administration. Further, the blood serum was obtained according to a common method.

[0156]

(5-3) Constitution of group for evaluation

(i) MPL group: the test solution (I): dose of MPL: 540 mg (100 mg in terms of the amount of sphingomyelin) was administered per 1 kg of body weight.

(j) MPL+YG group: the test solution (J): dose of yoghurt (Meiji Bulgaria Yoghurt Fat Zero, obtained from Meiji Co., Ltd.): 11.3 g and MPL: 535 mg (100 mg in terms of the total amount of sphingomyelin) was administered per 1 kg of body weight.

(k) MPL+ YG supernatant group: the test solution (J): dose of yoghurt supernatant: 743 mg and MPL: 539 mg (100 mg in terms of the total amount of sphingomyelin) was administered per 1 kg of body weight.

(l) MPL+ YG supernatant group: the test solution (K): dose of yoghurt precipitate: 455 mg and MPL: 536 mg (100 mg in terms of the total amount of sphingomyelin) was administered per 1 kg of body weight.

[0157]

(5-4) Index for evaluation

The amount of ceramide in rat blood serum was evaluated after administering orally the test solution.

Specifically, the ceramide molecular species (d16:1-C16:0, d16:1-C22:0, d16:1-C23:0, d16:1-C24:0) were used as an index.

[0158]

[Results]

The results were as illustrated in FIG. 8.

[0159]

In FIG. 8, the area under the time curve of the amount of ceramide molecular species in blood serum was illustrated. Compared to the MPL group, the area under the time curve of any ceramide molecular species was significantly higher in the MPL+YG group and MPL+YG supernatant group. However, no significant

difference was found between the MPL group and MPL+YG precipitate group.

[0160]

It was found that, when phospholipid concentrate derived from milk in which sphingomyelin is contained in high content and yoghurt supernatant fraction are simultaneously taken via the oral route, sphingomyelin absorption is promoted. Based on the results, it was believed that a component for promoting the sphingomyelin absorption is contained in the yoghurt supernatant fraction.

[0161]

Example 6: Test for evaluating sphingomyelin absorption property in simultaneous intake of lactic acid and MPL

Ceramide amount in blood was evaluated for each of a case in which only MPL is administered via the oral route to a rat, a case in which yoghurt and MPL are administered simultaneously via the oral route to a rat, and a case in which lactic acid and MPL are administered simultaneously via the oral route to a rat.

[0162]

[Experimental methods]

(6-1)

A rat (SD, male, weight: about 270 g) was acclimated for 1 week. After that, the rat was fasted for 16 hours and divided into 3 groups. Then, each test solution (L), (M), and (N) that will be described below was orally administered. Blood was taken from the tail vein, at each time point including before the administration and 90 minutes, 180 minutes, 270 minutes, or 360 minutes after the administration. Further, the blood serum was obtained according to a common method.

[0163]

(6-2) Constitution of group for evaluation

(l) MPL group: the test solution (L): dose of MPL: 540 mg (100 mg in terms of the amount of sphingomyelin) was administered per 1 kg of body weight.

(m) MPL+YG group: the test solution (M): dose of yoghurt (Meiji Bulgaria Yoghurt Fat Zero, obtained from Meiji Co., Ltd.):

11.3 g and MPL: 535 mg (100 mg in terms of the total amount of sphingomyelin) was administered per 1 kg of body weight.

(n) MPL+ lactic acid group: the test solution (N): dose of lactic acid (obtained from Wako Pure Chemical Industries, Ltd.): 109 mg (lactic acid amount and pH were set at the same level as those of the yoghurt) and MPL: 540 mg (100 mg in terms of the amount of sphingomyelin) was administered per 1 kg of body weight.

[0164]

(6-3) Index for evaluation

The amount of ceramide in rat blood serum was evaluated after administering orally the test solution.

Specifically, the ceramide molecular species (d16:1-C16:0, d16:1-C22:0, d16:1-C23:0, d16:1-C24:0) were used as an index.

[0165]

[Results]

The results were as illustrated in FIG. 9.

[0166]

In FIG. 9, the area under the time curve of the amount of ceramide molecular species in blood serum was illustrated. Compared to the MPL group, the area under the time curve of any ceramide molecular species was significantly higher in the MPL+YG group. However, no significant difference was found between the MPL group and MPL+lactic acid group.

[0167]

Based on the above results, it was considered that the lactic acid in yoghurt is not a component which is capable of promoting absorption of sphingomyelin.

[0168]

<u>Example 7: Test for evaluating sphingomyelin absorption property in simultaneous intake of whey protein and MPL</u>

Ceramide amount in blood was evaluated for each of a case in which only MPL is administered via the oral route to a rat, a case in which yoghurt and MPL are administered simultaneously via the oral route to a rat, and a case in which whey protein and MPL are administered simultaneously via the oral route to a rat.

[0169]

[Experimental methods]

(7-1)

A rat (SD, male, weight: about 270 g) was acclimated for 1 week. After that, the rat was fasted for 16 hours and divided into 3 groups. Then, each test solution (O), (P), and (Q) that will be described below was orally administered. Blood was taken from the tail vein, at each time point including before the administration and 90 minutes, 180 minutes, 270 minutes, or 360 minutes after the administration. Further, the blood serum was obtained according to a common method.

[0170]

(7-2) Constitution of group for evaluation

(o) MPL group: the test solution (O): dose of MPL: 540 mg (100 mg in terms of the amount of sphingomyelin) was administered per 1 kg of body weight.

(p) MPL+YG group: the test solution (P): dose of yoghurt (Meiji Bulgaria Yoghurt Fat Zero, obtained from Meiji Co., Ltd.): 11.3 g and MPL: 535 mg (100 mg in terms of the total amount of sphingomyelin) was administered per 1 kg of body weight.

(q) MPL+ whey protein group: the test solution (Q): dose of whey protein (ALACEN 8899, obtained from Fonterra Co-operative Group): 109 mg (it was set at the same level as the whey protein amount in the yoghurt) and MPL: 540 mg (100 mg in terms of the amount of sphingomyelin) was administered per 1 kg of body weight.

[0171]

(7-3) Index for evaluation

The amount of ceramide in rat blood serum was evaluated after administering orally the test solution.

Specifically, the ceramide molecular species (d16:1-C16:0, d16:1-C22:0, d16:1-C23:0, d16:1-C24:0) were used as an index.

[0172]

[Results]

The results were as illustrated in FIG. 10.

[0173]

In FIG. 10, the area under the time curve of the amount of ceramide molecular species in blood serum was illustrated. Compared to the MPL group, the area under the time curve of any ceramide molecular species was significantly higher in the MPL+YG group. However, no significant difference was found between the MPL group and MPL+whey protein group.

[0174]

Based on the above results, it was considered that the whey protein in yoghurt is not a component which is capable of promoting absorption of sphingomyelin.

[0175]

Example 8: Test for evaluating sphingomyelin absorption property in simultaneous intake of polysaccharides and MPL

Ceramide amount in blood was evaluated for each of a case in which only MPL is administered via the oral route to a rat, a case in which yoghurt and MPL are administered simultaneously via the oral route to a rat, and a case in which polysaccharides and MPL are administered simultaneously via the oral route to a rat.

[0176]

[Experimental methods]

(8-1) Purification of polysaccharides

Purification of polysaccharides was performed according to the method by Cerning, et. al. (J. Dairy Sci., 1992, 75, 692-699). A yoghurt supernatant fraction was treated with Pronase (manufactured by Roche) to hydrolyze proteins. After adding ethanol in the amount of 3 times, it was frozen overnight and centrifuged to obtain a precipitate containing polysaccharides. The precipitate was filtered through an ultrafiltration membrane (Merck Millipore, Centriprep Ultracel YM-3) to remove the protein hydrolyzates. As a result, the polysaccharides were purified.

[0177]

(8-2)

A rat (SD, male, weight: about 270 g) was acclimated for 1 week. After that, the rat was fasted for 16 hours and divided into 3 groups. Then, each test solution (R), (S), and (T) that will be described below was orally administered. Blood was taken from

the tail vein, at each time point including before the administration and 90 minutes, 180 minutes, 270 minutes, or 360 minutes after the administration. Further, the blood serum was obtained according to a common method.

[0178]

(8-3) Constitution of group for evaluation

(r) MPL group: the test solution (R): dose of MPL: 540 mg (100 mg in terms of the amount of sphingomyelin) was administered per 1 kg of body weight.

(s) MPL+YG group: the test solution (S): dose of yoghurt (Meiji Bulgaria Yoghurt Fat Zero, obtained from Meiji Co., Ltd.): 11.3 g and MPL: 535 mg (100 mg in terms of the total amount of sphingomyelin) was administered per 1 kg of body weight.

(t) MPL+ whey protein group: the test solution (T): dose of polysaccharides: 52.5 mg (it was set at the same level as the polysaccharides in the yoghurt) and MPL: 540 mg (100 mg in terms of the amount of sphingomyelin) was administered per 1 kg of body weight.

[0179]

(8-4) Index for evaluation

The amount of ceramide in rat blood serum was evaluated after administering orally the test solution.

Specifically, the ceramide molecular species (d16:1-C16:0, d16:1-C22:0, d16:1-C23:0, d16:1-C24:0) were used as an index.

[0180]

[Results]

The results were as illustrated in FIG. 11.

[0181]

In FIG. 11, the area under the time curve of the amount of ceramide molecular species in blood serum was illustrated. Compared to the MPL group, any of the ceramide molecular species exhibited a significantly higher value of area under the time curve in the MPL+YG group and MPL+ polysaccharides group.

[0182]

When phospholipid concentrate derived from milk in which sphingomyelin is contained in high content and polysaccharides in

yoghurt are simultaneously taken via the oral route, sphingomyelin absorption is promoted. Thus, it is considered that the polysaccharides in yoghurt is one of the components which promote sphingomyelin absorption.

[0183]

Example 9: Test for evaluating sphingomyelin absorption property in simultaneous intake of yoghurt prepared by a lactic acid bacterium and/or a bifidobacterium and MPL

Ceramide amount in blood was evaluated for each of a case in which only MPL is administered via the oral route to a rat and a case in which yoghurt prepared by a lactic acid bacterium and/or a bifidobacterium and MPL are administered simultaneously via the oral route to a rat.

[0184]

(9-1)

A rat (SD, male, weight: about 270 g) was acclimated for 1 week. After that, the rat was fasted for 16 hours and divided into 3 groups. Then, each test solution (U), (V), (W), (X), and (Y) that will be described below was orally administered. Blood was taken from the tail vein, at each time point including before the administration and 90 minutes, 180 minutes, 270 minutes, or 360 minutes after the administration. Further, the blood serum was obtained according to a common method.

[0185]

(9-2) Constitution of group for evaluation

(u) MPL group: the test solution (U): dose of MPL: 540 mg (100 mg in terms of the amount of sphingomyelin) was administered per 1 kg of body weight.

(v) MPL+YG (1) group: the test solution (V): dose of yoghurt (1) (prepared by lactic acid bacterium Lactobacillus Gasseri and Streptococcus thermophilus, Meiji Probio Yoghurt LG21, Fat zero, obtained from Meiji Co., Ltd.): 11.3 g and MPL: 535 mg (100 mg in terms of the total amount of sphingomyelin) was administered per 1 kg of body weight.

(w) MPL+YG (2) group: the test solution (W): dose of yoghurt (2) (prepared by lactic acid bacterium Lactobacillus

delbrueckii subsp. bulgaricus and Streptococcus thermophilus, Meiji Probio Yoghurt R-1, Fat zero, obtained from Meiji Co., Ltd.): 11.3 g and MPL: 535 mg (100 mg in terms of the total amount of sphingomyelin) was administered per 1 kg of body weight.

(x) MPL+YG (3) group: the test solution (W): dose of yoghurt (3) (prepared by lactic acid bacterium Lactobacillus Gasseri, a bifidobacterium Bifidobacterium longum, Nature Megumi, Fat 0 (zero), obtained from Megmilk Snow Brand Company, Limited): 11.3 g and MPL: 535 mg (100 mg in terms of the total amount of sphingomyelin) was administered per 1 kg of body weight.

(y) MPL+YG (4) group: the test solution (Y): dose of yoghurt (4) (prepared by bifidobacterium Bifidobacterium longum, Bifidus BB536 Plain Yoghurt, Fat zero, obtained from Morinaga Milk Industry Co., Ltd.): 11.3 g and MPL: 535 mg (100 mg in terms of the total amount of sphingomyelin) was administered per 1 kg of body weight.

[0186]

(9-3) Index for evaluation

The amount of ceramide in rat blood serum was evaluated after administering orally the test solution.

Specifically, the ceramide molecular species (d16:1-C16:0, d16:1-C22:0, d16:1-C23:0, d16:1-C24:0) were used as an index.

[0187]

[Results]

The results were as illustrated in FIG. 12.

[0188]

In FIG. 12, the area under the time curve of the amount of ceramide molecular species in blood serum was illustrated. Compared to the MPL group, any of the ceramide molecular species exhibited a significantly higher value of area under the time curve in the MPL+YG (1) group, MPL+YG (2) group, MPL+YG (3) group, or MPL+YG (4) group.

[0189]

In this regard, it is considered that, when phospholipid concentrate derived from milk in which sphingomyelin is contained in high content and yoghurt prepared by various kinds of a lactic

acid bacterium and/or a bifidobacterium are simultaneously taken via the oral route, sphingomyelin absorption is promoted.

[0190]

Example 10: Test for evaluating glucosylceramide absorption property in simultaneous intake of yoghurt and glucosylceramide

Ceramide amount in blood was evaluated for each of a case in which only glucosylceramide (GC) is administered via the oral route to a rat and a case in which yoghurt and glucosylceramide L are administered simultaneously via the oral route to a rat.

[0191]

[Experimental methods]

(10-1)

A rat (SD, male, weight: about 270 g) was acclimated for 1 week. After that, the rat was fasted for 16 hours and divided into 3 groups. Then, each test solution (Y) and (Z) that will be described below was orally administered. Blood was taken from the tail vein, at each time point including before the administration and 90 minutes, 180 minutes, 270 minutes, or 360 minutes after the administration. Further, the blood serum was obtained according to a common method.

[0192]

(10-2) Constitution of group for evaluation

(y) GC group: the test solution (Y): dose of GC: 1613 mg (100 mg in terms of glucosylceramide, Nippon Ceramide RPS, obtained from Nippon Flour Mills Company, Limited) was administered per 1 kg of body weight.

(z) GC+YG group: the test solution (Z): dose of yoghurt (Meiji Bulgaria Yoghurt Fat Zero, obtained from Meiji Co., Ltd.): 11.3 g and GC: 1613 mg were administered per 1 kg of body weight.

[0193]

(10-3) Index for evaluation

After having the test solution, the amount of ceramide in rat blood serum was evaluated.

Specifically, according to the report by Sugawara, et. al. (J. Lipid Res., 2010, 51, 1761-1769), the blood serum ceramide

molecular species (d18:2-C16:0, d18:2-C23:0), which are known to increase after administration of glucosylceramide, were used as an index.

[0194]

[Results]

The results were as illustrated in FIG. 13.

[0195]

In FIG. 13, the area under the time curve of the amount of ceramide molecular species in blood serum was illustrated. Compared to the GC group, any of the ceramide molecular species exhibited a significantly higher value of area under the time curve in the GC+YG group.

[0196]

It was found that, according to simultaneous intake by the oral route of glucosylceramide and yoghurt, absorption of glucosylceramide is promoted.

CLAIMS

1. A sphingolipid absorption promoting agent comprising, as an active ingredient, a fermented product and/or a cultured product of a lactic acid bacterium and/or a bifidobacterium.

2. The sphingolipid absorption promoting agent according to claim 1, wherein the sphingolipid is sphingomyelin and the agent promotes absorption of sphingomyelin into a living body.

3. The sphingolipid absorption promoting agent according to claim 2, wherein the agent promotes absorption of sphingomyelin derived from milk into a living body.

4. The sphingolipid absorption promoting agent according to any one of claims 1 to 3, wherein the sphingolipid is at least one of ceramide, glucosylceramide, and galactosylceramide.

5. The sphingolipid absorption promoting agent according to any one of claims 1 to 4, wherein the fermented product and/or the cultured product of a lactic acid bacterium and/or a bifidobacterium is a milk fermented product and/or a milk cultured product of a lactic acid bacterium and/or a bifidobacterium.

6. A sphingolipid absorption promoting agent comprising polysaccharides as an active ingredient.

7. A composition for oral intake or enteric administration comprising the sphingolipid absorption promoting agent according to any one of claims 1 to 6 and sphingolipid.

8. A composition for oral intake or enteric administration comprising a fermented product and/or a cultured product of a lactic acid bacterium and/or a bifidobacterium and additional sphingolipid.

9. The composition for oral intake or enteric administration according to claim 7 or 8, wherein, with regard to a blending ratio between the fermented product and/or the cultured product of a lactic acid bacterium and/or a bifidobacterium and sphingolipid, the fermented product and/or the cultured product of a lactic acid bacterium and/or a bifidobacterium (dry weight (powder)) is contained at 1 to 10000 mg per 1 mg of the sphingolipid.

10. The composition for oral intake or enteric administration according to claim 7 or 8, wherein, with regard to a blending ratio between the fermented product and/or the cultured product of a lactic acid bacterium and/or a bifidobacterium and sphingolipid, the fermented product and/or the cultured product of a lactic acid bacterium and/or a bifidobacterium (wet weight) is contained at 0.01 to 100 g per 1 mg of the sphingolipid.

11. The composition for oral intake or enteric administration according to any one of claims 7 to 10, wherein the sphingolipid is sphingomyelin.

12. The composition for oral intake or enteric administration according to any one of claims 7 to 11, wherein the composition is used for an in vivo activity that is induced by absorption of sphingomyelin into a living body.

13. The composition for oral intake or enteric administration according to claim 12, wherein the induced in vivo activity is selected from a group consisting of prevention or improvement of deteriorated skin conditions, an activity of suppressing cancer, a skin-beautifying activity, an activity of promoting infant brain development, an activity of enhancing mitochondria function, an activity of enhancing motor skills, an activity of suppressing accumulation of visceral fat and promoting increased adiponetin concentration in blood, and an activity of preventing infection.

14. The composition for oral intake or enteric administration

according to any one of claims 7 to 11, wherein the composition is used for prevention, suppression, or improvement of deteriorated skin conditions.

15. The composition according to claim 14, wherein the deteriorated skin condition is a deteriorated barrier function of skin.

16. A food and drink product comprising the sphingolipid absorption promoting agent according to any one of claims 1 to 6 or the composition according to any one of claims 7 to 15.

17. The food and drink product according to claim 16, wherein the food and drink product is a functional food, a health and nutrition food, a supplement, a food for specific health use, a food labeled with functionality, or a food tagged with low disease risk label.

18. A method for promoting sphingolipid absorption, comprising oral intake or enteric administration of, together with sphingolipid, a fermented product and/or a cultured product of a lactic acid bacterium and/or a bifidobacterium to a subject who is in need of intake of sphingolipid.

19. The method for promoting sphingolipid absorption according to claim 18, wherein the sphingolipid is sphingomyelin.