

AGENT FOR SUPPRESSION AND AMELIORATION OF REDUCTION
IN LORICRIN, TRANSGLUTAMINASE 3 ACTIVATOR, AND AGENT
FOR FORMATION PROMOTION AND STRUCTURE
REINFORCEMENT OF CORNIFIED ENVELOPE IN SKIN STRATUM
5 CORNEUM

[CROSS-REFERENCE TO RELATED APPLICATION]

[0001] This application is based upon and claims the benefit
of priority from the prior Japanese Patent Applications No.
10 264578/2014, filed on December 26, 2014; the entire contents
of which are incorporated herein by reference.

[BACKGROUND OF THE INVENTION]

[0002] Field of Invention

15 The present invention relates to an agent for
suppression and amelioration of a reduction in loricrin, a
transglutaminase 3 activator, and an agent for formation
promotion and structure reinforcement of a cornified envelope
in a skin stratum corneum. The present invention also relates
20 to an agent for formation promotion and structure
reinforcement of a cornified envelope in a skin stratum corneum,
These are useful for the prevention, suppression, or amelioration
of worsening of skin condition.

[0003] Background Art

25 An epidermal horny layer is composed of horny layer
cells formed by terminal differentiation of epidermal
keratinocytes and intercellular lipids surrounding the horny
layer cells. Intercellular lipids are composed of ceramides,
cholesterols, fatty acids and the like and have a lamellar
30 structure formed of a lipid layer and a water molecule layer.

[0004] On the other hand, horny layer cells contain keratin
fibers that are a component related to moisture retention, and
natural moisturizing factors such as amino acids. Horny layer
cells are enveloped by a very tough insoluble undercoat
35 structure through crosslinking of various protein called a
cornified envelope (known also as CE) and are very stable

against physical and chemical stimulation. In particular, loricrin formed of a granular layer among proteins constituting the cornified envelope plays a role in reinforcing the undercoat structure. This structure is completed by adhering loricrin, through transglutaminase 3, i.e., a protein crosslinked adherent enzyme, to an immature cornified envelope formed by a crosslinking reaction of proteins other than the loricrin to form a base as a foothold the cornified envelope.

[0005] Damage to skin by ultraviolet light induces abnormal turnover of skin which is likely to affect particularly a horny layer that is located outermost. It is known that, when an incomplete cornified envelope is formed by damage to and incomplete cornification of horny layer cells, the barrier function of the epidermal horny layer is deteriorated, resulting in loss of water held in the horny layer.

[0006] From the viewpoints of formation and mature promotion of the cornified envelope, for example, Japanese Patent Application Laid-Open Publication No. 303185/2008 discloses the use of specific pyrrolidone derivatives. Here studies have been made on maturation of the immature cornified envelope. As long as the present inventors know, studies have not been satisfactorily forwarded on the activation of transglutaminase 3 through suppression of a reduction in loricrin caused, for example, by damage to skin, thereby reinforcing the structure. Further, as long as the present inventors know, for this reason, oral ingestion of sphingolipids, especially sphingomyelins, have not been known up to now.

[0007] Japanese Patent Application Laid-Open Publication No. 084244/2009 discloses the effect of improving a skin barrier function, the effect of increasing the amount of ceramides, and the effect of increasing the expression of loricrin gene can be attained by continuous oral ingestion of a solution of a saturated free fatty acid having 10 to 36 carbon atoms. Here, however, oral ingestion of sphingomyelin is neither described nor suggested.

[0008] Japanese Patent Application Laid-Open Publication No.

117793/2007 discloses pharmaceutical preparations and foods and drinks containing sphingomyelins as an active ingredient and discloses sphingomyelin-derived various functions such as sialomucin secretagogue action, antiallergic action, and
5 antioxidative effect. Here, however, the function of suppressing a reduction in loricrin by sphingomyelins and activating action of transglutaminase 3 are neither described nor suggested.

[0009] Literature of Hasegawa et al. (*Hasegawa T et al.,*
10 *"Dietary glucosylceramide enhances cornified envelope formation via transglutaminase expression and involucrin production", Lipids (2011) 46, pp. 529-535*) reports that functions such as an improvement in skin barrier function of mice, an increase in expression of transglutaminase1, an
15 increase in production of involucrin, and promotion of expression of transglutaminases 1 and 3 have been observed in the administration of glucosylceramide that is a sphingolipid derived from konjac, to mice. However, it has been confirmed that glycosylceramides are different from sphingomyelins in
20 structure. Further, it has been confirmed that the action is utterly different from the action in the administration of sphingomyelins. That is, in the administration of sphingomyelins, the conservation or deterioration of transglutaminase 1 and involucrin has been actually confirmed
25 (Examples of the present invention). This is the result that is utterly opposite to that obtained in the administration of glycosylceramide.

[0010] Thus, as long as the present inventors know, the fact that the oral ingestion of sphingomyelin suppresses a reduction
30 in loricrin and activates transglutaminase 3, thereby promoting the formation of the cornified envelope to reinforce the structure, has not been known up to now.

[SUMMARY OF THE INVENTION]

35 [0011] The present inventors have unexpectedly found that the oral ingestion of sphingolipids, particularly sphingomyelins,

suppresses a reduction in loricrin and activates transglutaminase 3. The present inventors have aimed at the fact that the suppression of a reduction in loricrin and the activation of transglutaminase 3 promote the formation of a cornified envelope and reinforces the structure of an immature cornified envelope. Further, it has been also found that these ameliorate deteriorated worsened skin conditions and especially can suppress a deterioration in skin barrier function under ultraviolet irradiation. The present invention has been completed based on such finding.

[0012] Accordingly, an object of the present invention is to provide an agent for suppression and amelioration of a reduction in loricrin in a cornified envelope in a skin stratum corneum and a transglutaminase 3 activator in a cornified envelope in a skin stratum corneum. Another object of the present invention is provide an agent for formation promotion and structure reinforcement of a cornified envelope in a skin stratum corneum. A further object of the present invention is to provide a composition and food and drink that can prevent, suppress, or ameliorate a reduction in a skin barrier function caused by ultraviolet irradiation in a safe, simple, and highly cost-effective manner.

[0013] Thus, according to the present invention, there are provided that following inventions.

[0014]

<1> An agent for suppression and amelioration of a reduction in loricrin in a cornified envelope in a skin stratum corneum, the agent comprising a sphingomyelin as an active ingredient.

[0015]

<2> A transglutaminase 3 activator in a cornified envelope in a skin stratum corneum, the transglutaminase 3 activator comprising a sphingomyelin as an active ingredient.

[0016]

<3> An agent for formation promotion and structure reinforcement of a cornified envelope in a skin stratum corneum,

the agent comprising a sphingomyelin as an active ingredient.

[0017]

5 <4> Preferably, in the agent for formation promotion and structure reinforcement of a cornified envelope according to the above <1>, the formation promotion and structure reinforcement of the cornified envelope is derived from the suppression and amelioration of a reduction in loricrin and the activation of the transglutaminase 3 in a cornified envelope in a skin stratum corneum by the sphingomyelin.

10 [0018]

<5> Preferably, in the agent for suppression and amelioration of a reduction, activator, or formation promotion and structure reinforcement according to any one of the above <1> to <4>, the sphingomyelin is derived from a milk.

15 [0019]

<6> A composition for oral ingestion or enteral administration, the composition comprising an agent for suppression and amelioration of a reduction in loricrin according to the above <1> or <5>, a transglutaminase 3 activator according to the above <2> or <5>, or an agent for formation promotion and structure reinforcement of a cornified envelope according to any one of the above <3> to <5>.

[0020]

25 <7> Preferably, the composition according to the above <6> is used for prevention, suppression, or amelioration of worsening of condition of skin.

[0021]

30 <8> Preferably, in the composition according to the above <7>, the worsening of the condition of skin is a reduction in a barrier function of skin.

[0022]

<9> Preferably, in the composition according to the above <7> or <8>, the reduction in barrier function of skin is due to exposure to ultraviolet light.

35 [0023]

<10> Preferably, the composition according to any one of

the above<6> to <9> contains a sphingomyelin in an amount that allows 0.5 to 1500 mg of the sphingomyelin to be ingested per day.

[0024]

- 5 <11> Food and drink comprising an agent for suppression and amelioration of a reduction in loricrin according to the above <1> or <5>, a transglutaminase 3 activator according to the above <2> or <5>, or an agent for formation promotion and structure reinforcement of a cornified envelope according to
10 any one of the above <3> to <5>.

[0025]

<12> Food and drink, comprising a composition according to any one of the above <6> to <9>.

[0026]

- 15 <13> Preferably, the food and drink according to the above <11> or <12> contains a sphingomyelin in an amount that allows 0.2 to 45000 mg of the sphingomyelin to be ingested per package.

[0027]

- 20 <14> Preferably, the food and drink according to any one of the above <11> to <13> is a functional food, a nutritive health food, a supplement, a food for specified health use, a functional display food, or a disease risk reduction display food.

[0028]

- 25 <15> A method for suppression and amelioration of a reduction in loricrin, the method comprising allowing a sphingomyelin to be orally ingested by or to be enterally administered to a subject suffering from a reduction in the amount of loricrin in a cornified envelope in a skin stratum
30 corneum.

[0029]

- <16> A method for activating transglutaminase 3 in a cornified envelope in a skin stratum corneum, the method comprising allowing a sphingomyelin to be orally ingested by or
35 to be enterally administered to a subject suffering from a reduction in activity of transglutaminase 3 in a cornified

envelope in a skin stratum corneum.

[0030]

<17> A method for formation promotion and structure reinforcement of a cornified envelope in a skin stratum corneum, the method comprising allowing a sphingomyelin to be orally ingested by or to be enterally administered to a subject desired to realize formation promotion and structure reinforcement of a cornified envelope in a skin stratum corneum to suppress or ameliorate a reduction in loricrin in a cornified envelope in a skin stratum corneum, thereby activating transglutaminase 3.

[0031] EFFECT OF THE INVENTION

According to the present invention, the reduction in loricrin in a cornified envelope in a skin stratum corneum can be suppressed and ameliorated, and, further, transglutaminase 3 in a cornified envelope in a skin stratum corneum can be activated. The suppression of a reduction in loricrin and the activation of transglutaminase 3 contribute to the promotion of formation of the cornified envelope and, further, the reinforcement of the structure of an immature cornified envelope. In particular, the agent for reduction suppression and amelioration, the activator, or the agent for formation promotion and structure reinforcement and the composition according to the present invention can effectively realize the prevention, suppression or amelioration of deterioration in skin barrier function under ultraviolet irradiation. Further, the production promotor and composition according to the present invention are ingested through an oral or eteral route, and the raw material is derived from materials that have hitherto been used as foods. Thus, the production promotor and composition are safe and simple and highly cost-effective.

[BRIEF DESCRIPTION OF THE DRAWINGS]

[0032]

[Fig. 1] Fig. 1 is a conceptual diagram illustrating a

flow of a working example.

[Fig. 2] Fig. 2 is a graph illustrating a change in an expression level of a loricrin gene in a working example and a change in an expression level of a transglutaminase 3 gene, wherein Fig. 2(A) shows a change in an expression level of a loricrin gene and Fig. 2(B) shows a change in an expression level of a transglutaminase 3 gene. Further, in the drawing, * shows a significant difference to a control group ($p < 0.05$), and # shows a significant difference to day 0 ($p < 0.05$).

[Fig. 3] Fig. 3 is a graph illustrating a change in an expression level of a transglutaminase 1 gene and a change in an expression level of an involucrin gene in working example, wherein Fig. 3(C) shows a change in an expression level of a transglutaminase 1 gene and Fig. 3(D) shows a change in an expression level of an involucrin gene. Further, in the drawing, * shows a significant difference to a control group ($p < 0.05$), and # shows a significant difference to day 0 ($p < 0.05$).

[MODE FOR CARRYING OUT THE INVENTION]

[0033] Embodiments of the present invention will be described.

[0034]

Agent for suppression and amelioration of reduction in loricrin in cornified envelope in skin stratum corneum

As described above, the agent for suppression and amelioration of a reduction in loricrin in a cornified envelope in a skin stratum corneum according to the present invention comprises a sphingomyelin as an active ingredient. The expression "as an active ingredient" as used herein means that the agent for reduction suppression and amelioration according to the present invention contains a sphingomyelin in an amount large (an effective amount) enough to exert a function that suppresses or ameliorates a reduction in loricrin in a cornified envelope in a skin stratum corneum in vivo.

[0035] The expression "suppression and amelioration of a reduction in loricrin" as used herein means suppression or

amelioration of a reduction in loricrin in a cornified envelope in a skin stratum corneum and may embrace any means of suppression or delay of progress of the reduction, halting and maintaining of the reduction, and suppression of the reduction and an increase in loricrin after the suppression. The expression "suppression and amelioration of a reduction in loricrin" as used herein may embrace the prevention of the reduction.

[0036] The "loricrin" is a major constituent protein of an undercoat structure of a cornified envelope of skin and, for example, as described in working examples that will be described later, can assess the reduced state of loricrin in a cornified envelope in a skin stratum corneum using an expression state of a loricrin gene as a measure.

15 [0037]

Transglutaminase 3 activator

As described above, the transglutaminase 3 activator in a cornified envelope in a skin stratum corneum according to the present invention comprises a sphingomyelin as an active ingredient. The expression "as an active ingredient" as used herein means that the activator according to the present invention contains a sphingomyelin in an amount large (an effective amount) enough to exert a function that activates transglutaminase 3 in a cornified envelope in a skin stratum corneum in vivo.

[0038] The expression "activation of transglutaminase 3" as used herein is used to embrace meaning that the enzymatic activity of transglutaminase 3 in a cornified envelope in a skin stratum corneum is increased and a high level of activity is maintained.

[0039] The "transglutaminase 3" is an enzyme that allows loricrin to be adhered to a cornified envelope and enhances an undercoat structure of the cornified envelope. As described in working examples that will be described later, the activity of transglutaminase 3 in a cornified envelope in a skin stratum corneum can be assayed using an expression level of a

transglutaminase 3 gene as a measure.

[0040]

Agent for formation promotion and structure reinforcement of cornified envelope in skin stratum corneum

As described above, the agent for formation promotion and structure reinforcement of a cornified envelope in a skin stratum corneum according to the present invention comprises a sphingomyelin as an active ingredient. The expression "as an active ingredient" means that the agent for formation promotion and structure reinforcement according to the present invention contains a sphingomyelin in an amount large (an effective amount) enough to exert an effect that promotes the formation of a cornified envelope in a skin stratum corneum and/or enhances the structure.

[0041] The expression "formation promotion and structure reinforcement of a cornified envelope in a skin stratum corneum" as used herein is used to embrace meaning that the formation of a cornified envelope in a skin stratum corneum is promoted, an immature cornified envelope is matured, and the structure of the cornified envelope is reinforced.

[0042] In a preferred embodiment of the present invention, in the agent for formation promotion and structure reinforcement of a cornified envelope, the formation promotion and structure enforcement of the cornified envelope is achieved by suppression and amelioration of a reduction in a cornified envelope in a skin stratum corneum through loricrin and the activation of transglutaminase 3.

[0043]

Sphingomyelin

In the present specification, the sphingomyelin is one of sphingolipids. Sphingolipids are naturally derived, for example, derived from milk such as bovine milk, goat milk, sheep milk, and horse milk, derived from egg yolk, derived from rice, corn, and cereal, derived from konjac, and derived from beet, preferably derived from milk, more preferably derived

from bovine milk. Accordingly, as with the sphingolipid, the sphingomyelin usable in the present invention are naturally derived, preferably derived from milk, more preferably derived from bovine milk. The sphingomyelin may also be prepared by
 5 commonly used methods but may be commercially available products.

[0044] In a preferred embodiment of the present invention, the sphingomyelin is derived from milk.

[0045] The sphingomyelin derived from milk usable in the
 10 present invention preferably includes one or more molecular species selected from the group consisting of myristylsphingosylphosphorylcholine (d18:1-C14:0), tricosanylsphingosylphosphorylcholine (d18:1-C23:0), palmitylsphingosylphosphorylcholine (d18:1-C16:0),
 15 tricosenoylsphingosylphosphorylcholine (d18:1-C23:1), stearyl sphingosylphosphorylcholine (d18:1-C18:0), lignoceryl sphingosylphosphorylcholine (d18:1-C24:0), Arachidyl sphingosylphosphorylcholine (d18:1-C20:0), Norbornyl sphingosylphosphorylcholine (d18:1-C24:1),
 20 behenyl sphingosylphosphorylcholine (d18:1-C22:0), and celotyl sphingosylphosphorylcholine (d18:1-C26:0). More preferably, the milk-derived sphingomyelin includes three or more molecule species selected from the above group, more preferably five or more molecule species from the above group.

[0046] The agent for reduction suppression and amelioration, the activator, or the agent for formation promotion and structure reinforcement according to the present invention is preferably an agent for oral ingestion (that is, an agent for oral ingestion), or an agent for enteral administration (that is, an
 30 agent for enteral administration).

[0047] The agent for reduction suppression and amelioration, the activator, or the agent for formation promotion and structure reinforcement according to the present invention is preferably used for skin in worsened conditions and skin having
 35 a lowered barrier function.

[0048] As demonstrated in working examples that will be

described later, according to the present invention, in such a state that the skin barrier function has been lowered, for example, through a reduction in loricrin of a cornified envelope of skin, the barrier function of skin can be maintained or
5 ameliorated by the suppression and amelioration of a reduction in loricrin. Likewise, as demonstrated in working examples that will be described later, according to the present invention, in such a state that the barrier function of skin has been lowered, for example, through a reduction in activity of transglutaminase
10 3 in a cornified envelope of skin, the barrier function of skin can be maintained or ameliorated by activating transglutaminase 3. Further, the suppression and amelioration of a reduction in loricrin and the activation of transglutaminase 3 in a cornified envelope in a skin stratum corneum can contribute to formation
15 promotion and structure reinforcement of the cornified envelope, whereby skin in worsened conditions and skin having a lowered barrier function can be ameliorated.

[0049] For this reason, more preferably, the agent for reduction suppression and amelioration, the activator, or the
20 agent for formation promotion and structure reinforcement according to the present invention is used for skin in worsened conditions and skin having a lowered barrier function.

[0050]

Use

25 The sphingomyelin as an active ingredient in the present invention can suppress and ameliorate a reduction in loricrin in a cornified envelope in a skin stratum corneum and can further activate transglutaminase 3 in a cornified envelope in a skin stratum corneum (an working example that will be
30 described later). Thus, the formation of the cornified envelope can be promoted, and, further, the structure of an immature cornified envelope can be reinforced. Further, the sphingomyelin as an active ingredient in the present inventing has the effect of preventing, suppressing, or ameliorating
35 worsening of skin.

[0051] Here worsening in skin conditions includes, for

example, a lowering in skin barrier function, drying of skin, roughening of skin, lowered water content of horny layer, and atopic dermatitis. Maintaining the barrier function is also embraced in the lowering in the barrier function of skin.

5 Further, the lowering in function of skin is preferably a lowering in barrier function of skin under ultraviolet irradiations.

[0052] The expression "prevention, suppression, or amelioration" in worsened conditions is used in meaning embracing regulation, delay of progress, alleviation, prevention
10 of onset, and prevention of recurrence.

[0053] In another embodiment of the present invention, treatment, prevention, or amelioration of conditions that can be treated, prevented, or ameliorated can be realized by suppression and amelioration of a reduction in loricrin, or
15 activation of transglutaminase 3.

[0054] According to another preferred one aspect of the present invention, there is provided a method for prevention, suppression, or amelioration of worsening of skin conditions, the method comprising allowing a sphingomyelin to be orally
20 ingested by or to be enterally administered to a subject suffering from a reduction in the amount of loricrin in a cornified envelope in a skin stratum corneum.

[0055] According to still another preferred one aspect of the present invention, there is provided a method for prevention, suppression, or amelioration of worsening of skin conditions, the
25 method comprising allowing a sphingomyelin to be orally ingested by or to be enterally administered to a subject suffering from a lowering in activity of transglutaminase 3 in a cornified envelope in a skin stratum corneum.

30 [0056] According to a further preferred one aspect of the present invention, there is provided a method for prevention, suppression, or amelioration of worsening of skin conditions, the method comprising allowing a sphingomyelin to be orally ingested by or to be enterally administered to a subject in which
35 the formation promotion and structure reinforcement of a cornified envelope in a horny layer of skin is desired, to

suppress or ameliorate a reduction in loricrin in a cornified envelope in a skin stratum corneum and to active transglutaminase 3, whereby formation promotion and structure reinforcement of the cornified envelope in a horny layer of skin is achieved.

[0057] According to another aspect of the present invention, there is provided a sphingomyelin for suppression or amelioration of a reduction in loricrin in a cornified envelope in a skin stratum corneum.

10 [0058] According to still another aspect of the present invention, there is provided a sphingomyelin for activation of transglutaminase 3 in a cornified envelope in a skin stratum corneum.

[0059] According to a further aspect of the present invention, there is provided a sphingomyelin for formation promotion and/or structure reinforcement of a cornified envelope in a skin stratum corneum.

[0060] According to an another preferred one aspect of the present invention, there is provided use of a sphingomyelin for suppression and amelioration of a reduction in loricrin in a cornified envelope in a skin stratum corneum. According to a still another preferred one aspect of the present invention, there is provided use of a sphingomyelin for the manufacture of a composition for oral ingestion or enteral administration for suppression and amelioration of loricrin in a cornified envelope in a skin stratum corneum. Here the above use may be non-therapeutic use.

[0061] According to a still another preferred one aspect of the present invention, there is provided use of a sphingomyelin for activation of transglutaminase 3 in a cornified envelope in a skin stratum corneum. According to a further preferred one aspect of the present invention, there is provided use of a sphingomyelin for the manufacture of a composition for oral ingestion or enteral administration for activation of transglutaminase 3 in a cornified envelope in a skin stratum corneum. Here the above use may be non-therapeutic use.

[0062] According to a further preferred one aspect of the present invention, there is provided use of a sphingomyelin for formation promotion and/or structure reinforcement of a cornified envelope in a skin stratum corneum. According to another preferred one aspect of the present invention, there is provided use of a sphingomyelin for the manufacture of a pharmaceutical product of a composition for oral ingestion or enteral administration for formation promotion and/or structure reinforcement of a cornified envelope in a skin stratum corneum. Here the above use may be non-therapeutic use.

[0063]

Composition and food and drink

As described above, according to the present invention, there is provided a composition for oral ingestion or enteral administration, the composition comprising an agent for reduction suppression and amelioration, an activator, or an agent for formation promotion and structure reinforcement according to the present invention. Further, according to the present invention, there is provided food and drink comprising an agent for reduction suppression and amelioration, an activator, or an agent for formation promotion and structure reinforcement according to the present invention.

[0064] The composition and food and drink may be manufactured by a manufacturing method comprising adding an agent for reduction suppression and amelioration, an activator, or an agent for formation promotion and structure reinforcement according to the present invention to material ingredients of the composition and the food and drink.

[0065] Preferably, the composition for oral ingestion or enteral administration according to the present invention is used for prevention, suppression, or amelioration of worsened conditions of skin. Thus, according to the present invention, there is provided a composition for prevention, suppression, or amelioration of worsened conditions of skin.

[0066] In one embodiment of the present invention, the composition for prevention, suppression, or amelioration of

worsened conditions of skin according to the present invention is a pharmaceutical composition.

[0067] In the present invention, the pharmaceutical composition is one that has been prepared as an oral preparation or a parenteral preparation according to a conventional method using additives acceptable in formulation. Oral preparations are preferred from the viewpoint of simplicity. Oral preparations may be in the form of solid preparations such as tablets, powders, fine subtilaes, granules, capsules, pills, and sustained preparation, and liquid preparations such as solutions, suspensions, and emulsions. Additives acceptable in the formulation include, for example, excipients, stabilizers, preservatives, wetting agents, emulsifier, lubricants, sweetening agents, colorants, perfume, buffering agents, antioxidants, and pH adjustors.

[0068] Optional ingredients may be if necessary added to food and drink according to the present invention. Ingredients that may be optionally added are not particularly limited. Examples thereof include ingredients, sweetening agents, and acidulant commonly incorporated in food and drink, juice or soup of vegetables, fruits, and nuts and seeds, nutrients thereof such as extracts, vitamins, minerals, and amino acids, useful microorganisms such as lactobacillus, bifidobacteria, and Propionibacterium and culture products thereof, functional saccharides such as oligosaccharides, existing functional materials such as royal jellys, collagens, ceramides, glucosamines, astaxanthins, and polyphenols, perfumes, pH adjustors, excipients, acidulants, colorants, emulsifiers, and preservatives.

[0069] In the present invention, food and drink are not particularly limited as long as the food and drink are those other than pharmaceutical compositions and can be orally ingested, such as solutions, suspensions, emulsions, powders, and solid molded products. Specifically, examples of food and drink include milk products such as milk beverages, yoghurts, lactobacillus beverages, fermented beverages, ice creams,

creams, and cheeses; beverages, for example, powder beverages and sport beverages such as refreshing beverages, fruit beverages, vegetable beverages, soymilk beverages, coffee beverages, tea beverages, jelly beverages, cocoas and smoothies, powder beverages for nutrient enhancement, powder foods for cosmetic use, powder soups, sources of steamed breads, concentrated beverages, and alcoholic beverages; flour products such as breads, pastas, noodles, bread mixes, powders for deep-fried foods, and bread crumbs; confectioneries, for example, confectioneries for deserts such as chocolates, gums, lollipops, cookies, gummies, snack bars, Japanese confectioneries, jellies, and puddings; seasoners such as processed seasonings, flavor seasoning agents, and cooking mixes; retort foods of curries, pasta sauces, pot-au-feu, stews, and Japanese foods; fats and oils such as processed fats and oils, butters, margarines, spreads, and mayonnaises; ready-to-eat foods such as freeze-dried foods; agricultural processed products such as agricultural canned foods, jams and marmalades, pickles, cooked beans, cereals, and porridges of rice and vegetables; fishery processed products; livestock processed products; frozen products such as pizzas, rice casseroles, gratins, ordinary dishes, and fried foods; liquid foods; and feed stuffs for animals, tablets, and cosmetics for oral use.

[0070] In one preferred embodiment of the present invention, the food and drink is a milk beverage (that may include processed milk), a fermented milk, a refreshing beverage, jelly beverages, tablets, powder foods for beauty purposes, powder beverages, liquid diets, or liquid formula milk. In a more preferred embodiment, the food and drink is a milk beverage (that may include a processed milk) and a fermented milk.

[0071] Further, functional foods, nutritive health foods, supplements, health foods, food for specified health use, functional display foods, nutritive functional foods, foods for sick persons, modified milk powders for infants, milk powders for

parturients or nursing women, or those classified into food and drink products with disease risk reduction claim are embraced in the food and drink. In one preferred embodiment of the present invention, functional foods, nutritive health foods, 5 supplements, foods for specified health use, functional display foods, or disease risk reduction display foods may be mentioned as the food and drink.

[0072] The disease risk reduction display is a display of food and drink having a possibility of reducing a disease risk and 10 may be a display established or approved based on standards established by Joint FAO/WHO Food Standards Program Codex Alimentarius Commission (Codex Commission) or by reference to the standards.

[0073] The food and drink according to the present invention can be provided, for example, as foods suitable for 15 consumers who expect to ameliorate or alleviate worsening of skin conditions, foods suitable for amelioration of worsening of skin conditions, that is, as the so-called foods for specified health use or functional display foods.

[0074] According to another aspect of the present invention, there is provided food and drink with a display of function that prevents, suppresses, or ameliorates worsening of skin 20 conditions.

[0075] The content of the active ingredient in the food and drink and the composition may vary depending, for example, 25 upon type, form, and purposes of prevention and amelioration of the composition and thus cannot be unconditionally determined. In the composition of the present invention, however, the composition of the present invention may be one 30 in which the effective amount has been regulated so that 0.5 to 1500 mg, preferably 1 to 1000 mg, more preferably 5 to 500 mg, of sphingomyelin can be ingested per day per adult (weight: 60 kg).

[0076] The content of the sphingomyelin can be measured 35 by conventional methods, for example, by liquid chromatography using AQUASIL SP100 (4.6 X 250 mm, Senshu

Scientific Co., Ltd.).

[0077] In this case, preferably, a solution composed of a mixture of a 0.5 mM phosphate-citrate buffer solution (pH 3.0) and methanol at a ratio of 5 : 95 is used as a mobile phase.

5 Detection may be carried out under conditions of a measurement time of 20 min, a mobile phase flow rate of 0.6 mL/min, and a column temperature of 40°C at an absorbance of 205 nm. For example, a sphingomyelin (derived from milk, manufactured by Nagara Science Co., Ltd.) may be used as a
10 reference substance, and the sphingomyelin may be quantitatively determined by an area ratio.

[0078] In another embodiment of the present invention, the content of the active ingredient in the food and drink can be specified per package. For example, for the food and drink, the
15 content of the sphingomyelin may be 5 to 1500 mg, preferably 6 to 1000 mg, more preferably 7 to 500 mg, and, for the composition, the content of the sphingomyelin may be 0.5 to 1500 mg, preferably 1 to 1000 mg, more preferably 5 to 500 mg. The amount (content) per package is not limited to a
20 single ingestion dose and may be an ingestion dose for a plurality of times of administration or for a plurality of days (for example, 30 days). For example, for the food and drink, the content of the sphingomyelin may be 5 to 45000 mg, preferably 6 to 30000 mg, more preferably 7 to 15000 mg, and, for the
25 composition, the content of the sphingomyelin may be 0.5 to 45000 mg, preferably 1 to 30000 mg, more preferably 5 to 15000 mg.

[0079] In conventional general milk beverages and fermented milks, the content of phospholipids per g of lipid is
30 20 mg. On the other hand, in milk beverages and fermented milks according to the present invention, the content of phospholipids per g of lipid may be 25 to 3000 mg, preferably 30 to 2500 mg, more preferably 40 to 2000 mg, still more preferably 50 to 1500 mg. Further, in conventional general
35 milk beverages and fermented milks, the content of sphingomyelin per g of lipid is 6 mg. On the other hand, in

milk beverages and fermented milks according to the present invention, the content of sphingomyelin per g of lipid is preferably 8 to 1250 mg, more preferably 9 to 1000 mg, still more preferably 10 to 750 mg.

5 [0080] That is, in one preferred embodiment of the present invention, milk beverages and fermented milks having an intentionally enhanced sphingomyelin content may be used.

[0081] Milk beverages and fermented milks according to the present invention may be such that the lipid is contained in an
10 ordinary amount (for example, in an amount of 3% by weight) and the sphingomyelin is 0.2 to 60 mg/g, preferably 0.25 to 50 mg/g, more preferably 0.3 to 40 mg/g, still more preferably 0.35 to 30 mg/g.

[0082] On the other hand, in milk beverages and fermented
15 milks according to the present invention, when the lipid is contained in a small amount (for example, in the case of a low-fat milk in an amount of 1% by weight), the content of the sphingomyelin may be 0.05 to 60 mg/g, preferably 0.1 to 50 mg/g, more preferably 0.15 to 40 mg/g, still more preferably
20 0.2 to 30 mg/g.

[0083] In milk beverages, fermented milks, refreshing beverages, jelly beverages, tablets, and powder foods for beauty use, when the lipid is hardly contained (in the case of no-fat products), the content of the sphingomyelin may be 0.02
25 to 60 mg/g, preferably 0.04 to 50 mg/g, more preferably 0.06 to 40 mg/g, still more preferably, 0.08 to 30 mg/g.

[0084] Accordingly, according to a further preferred one aspect of the present invention, there are provided a milk beverage or a fermented milk comprising an additional
30 sphingomyelin, and a composition or food and drink comprising the milk beverage or the fermented milk. For example, yoghurts may be mentioned as the composition or the food and drink.

[0085] For example, for yoghurts, yoghurts comprising an
35 additional sphingomyelin can be provided. Not only yoghurts comprising a commonly used amount of sphingomyelin but also

yoghurts having a sphingomyelin concentration intentionally enhanced by separately adding the sphingomyelin thereto mentioned as the yoghurts. Here the expression "additional sphingomyelin" means a sphingomyelin separately added in the preparation of yoghurts having an intentionally enhanced sphingomyelin concentration rather than the sphingomyelin contained in a commonly used amount in yoghurts.

5 [0086] Thus, according to another preferred aspect of the present invention, there is provided a yoghurt comprising an agent for suppression and amelioration of a reduction in loricrin according to the present invention, a transglutaminase 3 activator according to the present invention, or an agent for formation promotion and structure reinforcement of a cornified envelope according to the present invention.

10 [0087] According to a still another preferred one aspect of the present invention, there is provided a yoghurt having a function of suppressing and ameliorating a reduction of loricrin, the yoghurt comprising an additional sphingomyelin. According to a further preferred one aspect of the present invention, there is provided a yoghurt having a function of activating transglutaminase 3, the yoghurt comprising an additional sphingomyelin. According to another preferred one aspect of the present invention, there is provided a yoghurt having a function of formation promotion and structure reinforcement of a cornified envelope, the yoghurt comprising an additional sphingomyelin.

15 [0088] In another aspect of the present invention, there is provided a method for suppression and amelioration of a reduction in loricrin, the method comprising allowing a sphingomyelin to be orally ingested by or to be enterally administered to a subject suffering from a reduction in the amount of loricrin in a cornified envelope in a skin stratum corneum. The amount of loricrin means the amount of loricrin present in a cornified envelope in skin stratum corneum, and may be assayed, for example, in terms of an expression level of a loricrin gene.

[0089] In still another aspect of the present invention, there is provided a method for activation of transglutaminase 3 in a cornified envelope in a skin stratum corneum, the method comprising allowing a sphingomyelin to be orally ingested by or
5 to be enterally administered to a subject suffering from a lowering in activity of transglutaminase 3 in a cornified envelope in a skin stratum corneum. Here the activity of transglutaminase 3 may be assayed, for example, in terms of an expression level of a transglutaminase 3 gene.

10 [0090] According to a further aspect of the present invention, there is provided a method for formation promotion and structure reinforcement of a cornified envelope in a skin stratum corneum, the method comprising allowing a sphingomyelin to be orally ingested by or to be enterally
15 administered to a subject desired to realize formation promotion and structure reinforcement of a cornified envelope in a skin horny layer to suppress or ameliorate a reduction in loricrin in a cornified envelope in a skin stratum corneum, thereby activating transglutaminase 3.

20 [0091] Here, in the method, preferably, medical use is excluded. The subject is preferably human being or mammals other than human being.

[0092] The above method may be a method for suppression and amelioration of a reduction in loricrin for a subject suffering
25 from a lowering in amount of loricrin in a cornified envelope in a skin stratum corneum, the method comprising, preferably, allowing a sphingomyelin to be orally ingested or enterally administered continuously for two days or more, a method for activating transglutaminase 3 in a cornified envelope in a skin
30 stratum corneum, for a subject suffering from a lowering in activity of transglutaminase 3 in a cornified envelope in a skin stratum corneum, a method for formation promotion and structure reinforcement of a cornified envelope in a skin stratum corneum.

[EXAMPLES]

[0093] The present invention is further illustrated by Examples that are not intended as a limitation of the invention.

[0094]

5 Test: Effect of sphingomyelin in worsening in skin barrier function under ultraviolet irradiation

Hairless mice were irradiated with ultraviolet light (GL20SE, manufactured by Sankyo Electric Co., Ltd.) to worsen a barrier function of skin, and the effect of continuous oral
10 ingestion of sphingomyelin were assayed.

[0095]

<Experimental method>

(1) Worsening of barrier function of skin by ultraviolet irradiation

15 Hairless mice (Hos: HR-1, female, 8 weeks old) were grouped and, after one week, were irradiated with ultraviolet light (UV-B) at 20mJ/cm². In the test, individuals were necessary for each measurement and collection of skin, and, thus, 48 mice (in total) were used and were grouped so that
20 each group consisted of 8 mice.

[0096]

(2) Construction of group and procedure of test

A test system of ultraviolet irradiation mode animals prepared in the above (1) was used to assay the effect of oral
25 ingestion (oral administration) of a sphingomyelin (purity 98%, manufactured by Nagara Science Co., Ltd.) on gene expression of skin.

[0097] Each group was tested under the following conditions. The sphingomyelin was orally administered to the mice in 9
30 days from grouping.

(i) Control group: sphingomyelin was administered on 0 day.

(ii) Control group: sphingomyelin was administered on first day.

35 (iii) Control group: sphingomyelin was administered on second day.

(iv) Sphingomyelin ingestion group: sphingomyelin was administered on 0 day.

(v) Sphingomyelin ingestion group: sphingomyelin was administered on first day.

5 (vi) Sphingomyelin ingestion group: sphingomyelin was administered on second day.

[0098] In the control group on 0 day, the control group on first day, and the control group on second day, a usual feed was ingested, and a solvent (a 5% aqueous solution of ethanol) was orally administered (10mL/kg-BW/day). In the sphingomyelin ingestion group on 0 day, the sphingomyelin ingestion group on first day, and the sphingomyelin ingestion group on second day, the sphingomyelin (purity: 98%, Nagara Science Co., Ltd.):146 mg/10mL-solvent (a 5% aqueous solution of ethanol) was orally administered (10mL/kg-BW/day).

[0099] Specifically, in the test, the expression level of a loricrin gene and a transglutaminase 3 gene were quantitatively determined before irradiation (on 0 day), on first day after the irradiation, and on second day after the irradiation. In this case, the expression level of the transglutaminase 1 gene and the involucrin gene was also quantitatively determined.

[0100] (Method for quantitative determined of expression level of each of loricrin, transglutaminase 3, transglutaminase1, and involucrin genes)

25 Total RNA was extracted using RNeasy Fibrous Tissue Mini kit (QIAGEN) from a skin sample collected from each individual, and, from 1.5 µg of total RNA extracted from skin, complementary DNA (cDNA) synthesis and a reverse transcription reaction were carried out with RivertAid First Strand cDNA Synthesis Kit (Thermo SCIENTIFIC).

[0101] mRNA expression level of loricrin (Loricrin) (Assay ID: Mm01962650_s1), transglutaminase 3 (Transglutaminase 3) (Assay ID:Mm00436999_m1), transglutaminase1 (Transglutaminase 1) (Assay ID: Mm00498375_m1), and involucrin (Involcrin) (Mm00515219_s1) that are target genes for each sample were measured with a 7500 real-time PCR

System (manufactured by Applied Biosystems) (synthesized cDNA: RT-PCR method, primer: TaqmanGene Expression Assay (Applied Biosystems)).

[0102] At the same time, an mRNA expression level of
5 glyceraldehyde-3-phosphate dehydrogenase(GAPDH)) (Assay ID: Mm99999915_g1) as an internal reference was measured.

[0103] Quantification was carried out by a $\Delta\Delta\text{Ct}$ method, and, for the expression level of a target gene of skin samples for each group, the ratio of a target gene expression level for
10 each group to a target gene expression level for the control group on 0 day was calculated as a relative expression level.

[0104] Specifically, the relative expression level was calculated by the following calculation equation.

15 $\Delta\text{Ct} = (\text{Ct value of target gene for each individual sample}) - (\text{Ct value of GAPDH gene for each individual sample})$

$\Delta\Delta\text{Ct} = (\Delta\text{Ct value of target gene for each individual sample}) - (\Delta\text{Ct average value for control group on 0 day})$

20

Gene expression level = $2^{-\Delta\Delta\text{Ct}}$

Relative expression level = $(2^{-\Delta\Delta\text{Ct}} \text{ for target gene for each individual sample}) / (2^{-\Delta\Delta\text{Ct}} \text{ average value for target gene for control group on 0 day})$
25

[0105]

(3) Test results

30 A change in expression of gene related to formation promotion and structure reinforcement of a cornified envelope was evaluated by an expression level of a loricrin gene and a transglutaminase 3 gene.

[0106] Here, an expression level of the loricrin gene for the sphingomyelin group on 0 day, the sphingomyelin group on first
35 day, and the sphingomyelin group on second day, higher than that for the control group on 0 day, the control group on first

day, and the control group on second day means that a reduction in the expression of loricrin was suppressed. An expression level of a transglutaminase 3 gene for the sphingomyelin group on 0 day, the sphingomyelin group on first day, and the sphingomyelin group on second day, higher than that for the control group on 0 day, the control group on first day, and the control group on second day means that an increase in the expression of the transglutaminase 3 gene was promoted.

[0107] On the other hand, for the expression level of the transglutaminase 1 gene, an expression level of the transglutaminase 1 gene for the sphingomyelin group on 0 day, the sphingomyelin group on first day, and the sphingomyelin group on second day lower than that for the control group on 0 day, the control group on first day, and the control group on second day means that an increase in the expression of the transglutaminase 1 was suppressed. Further, an expression level of an involucrin 1 gene for the sphingomyelin group on 0 day, the sphingomyelin group on first day, and the sphingomyelin group on second day lower than that for the control group on 0 day, the control group on first day, and the control group on second day means that an increase in the expression of the involucrin gene was suppressed.

[0108] The results were as illustrated in Figs. 2 and 3.

[0109] As can be seen from Fig. 2(A), the expression level of the loricrin gene for the sphingomyelin ingestion on first day was higher than that for the control group on first day (one day after ultraviolet irradiation).

As can be seen from Fig. 2(B), the expression level of the transglutaminase 3 gene for the sphingomyelin group on first day and the sphingomyelin group on second group was higher than that for the control group on first day (one day after ultraviolet irradiation) and the control group on second day (second day after ultraviolet irradiation).

[0110] As can be seen from Fig. 3(A), the expression level of the transglutaminase 1 gene for the sphingomyelin ingestion

group on first day, the sphingomyelin ingestion group on second day, and the sphingomyelin ingestion group on third day was lower than that for the control group on first day, the control group on second day, and the control group on third day (control groups) (respectively first, second, and third days after ultraviolet irradiation).

As can be seen from Fig. 3(B), the expression level of the involucrin gene for the sphingomyelin ingestion group on first day, the sphingomyelin ingestion group on second day, and the sphingomyelin ingestion group on third day was lower than that for the control group on first day, the control group on second day, and the control group on third day (SM groups) (respectively first, second, and third days after ultraviolet irradiation).

[0111] These results suggest that the oral ingestion of the sphingomyelin suppresses a reduction in expression of the loricrin gene caused by ultraviolet irradiation and, at the same time, promotes an increase in expression of the transglutaminase 3 gene to realize formation promotion and structure reinforcement of the cornified envelope.

[0112] Further, the oral ingestion of the sphingomyelin did not promote an increase in expression of the transglutaminase 1 gene and the involucrin gene, suggesting that the oral ingestion of the sphingomyelin uniquely contributes to the promotion of expression of unique genes related to formation promotion and structure reinforcement of the cornified envelope (here the loricrin gene and the transglutaminase 3 gene).

CLAIMS

1. An agent for suppression and amelioration of a reduction in loricrin in a cornified envelope in a skin stratum corneum, the agent comprising a sphingomyelin as an active ingredient.
2. A transglutaminase 3 activator in a cornified envelope in a skin stratum corneum, the transglutaminase 3 activator comprising a sphingomyelin as an active ingredient.
3. An agent for formation promotion and structure reinforcement of a cornified envelope in a skin stratum corneum, the agent comprising a sphingomyelin as an active ingredient.
4. The agent for formation promotion and structure reinforcement of a cornified envelope according to claim 3, wherein the formation promotion and structure reinforcement of the cornified envelope is derived from the suppression and amelioration of a reduction in loricrin and the activation of the transglutaminase 3 in a cornified envelope in a skin stratum corneum by the sphingomyelin.
5. The agent for suppression and amelioration of a reduction, activator, or formation promotion and structure reinforcement according to any one of claims 1 to 4, wherein the sphingomyelin is derived from a milk.
6. A composition for oral ingestion or enteral administration, the composition comprising an agent for suppression and amelioration of a reduction in loricrin according to claim 1 or 5, a transglutaminase 3 activator according to claim 2 or 5, or an agent for formation promotion and structure reinforcement of a cornified envelope according to any one of claims 3 to 5.

7. The composition according to claim 6, for prevention, suppression, or amelioration of worsening of condition of skin.
8. The composition according to claim 7, wherein the worsening of the condition of skin is a reduction in a barrier function of skin.
9. The composition according to claim 7 or 8, wherein the reduction in barrier function of skin is due to exposure to ultraviolet light.
10. The composition according to any one of claims 6 to 9, which contains a sphingomyelin in an amount that allows 0.5 to 1500 mg of the sphingomyelin to be ingested per day.
11. Food and drink comprising an agent for suppression and amelioration of a reduction in loricrin according to claim 1 or 5, a transglutaminase 3 activator according to claim 2 or 5, or an agent for formation promotion and structure reinforcement of a cornified envelope according to any one of claims 3 to 5.
12. The food and drink, comprising a composition according to any one of claims 6 to 9.
13. The food and drink according to claim 11 or 12, which contains a sphingomyelin in an amount that allows 0.2 to 45000 mg of the sphingomyelin to be ingested per package.
14. The food and drink according to any one of claims 11 to 13, which is a functional food, a nutritive health food, a supplement, a food for specified health use, a functional display food, or a disease risk reduction display food.
15. A method for suppression and amelioration of a reduction in loricrin, the method comprising allowing a

sphingomyelin to be orally ingested by or to be enterally administered to a subject suffering from a reduction in the amount of loricrin in a cornified envelope in a skin stratum corneum.

16. A method for activating transglutaminase 3 in a cornified envelope in a skin stratum corneum,

the method comprising allowing a sphingomyelin to be orally ingested by or to be enterally administered to a subject suffering from a reduction in activity of transglutaminase 3 in a cornified envelope in a skin stratum corneum.

17. A method for formation promotion and structure reinforcement of a cornified envelope in a skin stratum corneum,

the method comprising allowing a sphingomyelin to be orally ingested by or to be enterally administered to a subject desired to realize formation promotion and structure reinforcement of a cornified envelope in a skin stratum corneum to suppress or ameliorate a reduction in loricrin in a cornified envelope in a skin stratum corneum, thereby activating transglutaminase 3.