

SPECIFICATION

COMPOSITION FOR INHIBITING ENDOTOXIN ENTRY INTO BLOOD

CROSS-REFERENCE TO RELATED APPLICATION

[0001]

This application enjoys the benefit of priority from the prior Japanese Patent Application No. 2016-10840 (filed January 22, 2016), the entire disclosure of which is incorporated herein by reference.

TECHNICAL FIELD

[0002]

The present invention relates to a composition for use in inhibiting endotoxin from entering the blood comprising a whey protein.

BACKGROUND ART

[0003]

Endotoxin is a type of bacterial toxin also called as bacterial endotoxin or lipopolysaccharide and is a cell wall component from Gram-negative bacteria such as *Salmonella enterica*, *Escherichia coli*, *Pseudomonas aeruginosa*, and the like. Once these Gram-negative bacteria are destroyed and a cell wall component of those bacteria, lipopolysaccharide (LPS), is released, the cell wall component exhibits its toxicity. The toxic effect of endotoxin is less powerful than exotoxin. However, it is known that, once entered in the body, endotoxin causes general symptoms such as fever, decrease in blood pressure, shock, leukopenia, thrombocytopenia, chill, headache, vomiting, muscle ache and cardiopalmus. Such symptoms are also collectively called endotoxemia or sepsis.

[0004]

When elderly individuals, or patients who have had surgery and therefore have reduced the tolerance, suffer from those endotoxin-induced symptoms, they may often experience rapid disease progression. Moreover, it has been indicated that endotoxin is also involved in causes of various diseases such as cirrhosis, alcoholic liver disorder, non-alcoholic liver disorder (NASH), acute pancreatitis,

kidney disease, Alzheimer's disease, diabetes, and arteriosclerosis (for example, Non-Patent Document 1).

REFERENCE LIST

[Non-Patent Document]

[0005]

Non-Patent Document 1: Sakaguchi S. et al., Drug Metab. Pharmacokinet., 2011; 26(1): 30-46

SUMMARY OF THE INVENTION

[0006]

In this invention, the present inventors found that when ConA-induced liver disorder mouse models were fed with a nutritional composition comprising a whey protein hydrolysate, the ingestion of the nutritional composition resulted in inhibiting endotoxin from entering the blood as well as in suppressing the progression of liver disorder. The present invention is based on these findings.

[0007]

An object of the present invention is to provide a composition for inhibiting endotoxin from entering the blood.

[0008]

According to the present invention, the invention described below will be provided.

[1] A composition for inhibiting endotoxin from entering the blood, comprising a whey protein.

[2] The composition according to [1], wherein the whey protein is in the form of hydrolysate.

[3] The composition according to [1] or [2], wherein the whey protein is one or more selected from the group consisting of α -lactalbumin, β -lactoglobulin and lactoferrin.

[4] The composition according to any of [1] to [3], wherein the whey protein is originated from one or more selected from the group consisting of milk protein concentrate (MPC), whey protein concentrate (WPC), whey protein isolate (WPI), defatted milk powder, defatted concentrated milk and raw milk.

[5] The composition according to any of [1] to [4], wherein 0.5 to 5.0 g of the whey protein is contained per 100 kcal of the composition.

- [6] The composition according to any of [1] to [5], which further comprises fermented milk protein as the protein.
- [7] The composition according to any of [1] to [6], which further comprises an oil and fat containing an n-3 fatty acid as a lipid.
- [8] The composition according to any of [1] to [7], which further comprises a saccharide containing isomaltulose as a carbohydrate.
- [9] The composition according to any of [1] to [8], which is for use in the treatment, prevention, or amelioration of a disease or symptom which is effectively treated, prevented, or ameliorated by inhibiting endotoxin from entering the blood.
- [10] The composition according to [9], wherein the disease or symptom is one or more selected from the group consisting of cirrhosis, alcoholic liver disorder, non-alcoholic liver disorder (NASH), acute pancreatitis, kidney disease, Alzheimer's disease, diabetes, arteriosclerosis, fever, decrease in blood pressure, leukopenia, thrombocytopenia, chill, headache, vomiting, muscle ache and cardiopalmus.
- [11] Use of a whey protein for the manufacture of a composition for inhibiting endotoxin from entering the blood.
- [12] A whey protein for use in inhibiting endotoxin from entering the blood.
- [13] A method of inhibiting endotoxin from entering the blood, which comprises feeding or administering an effective amount of a whey protein to a subject in need thereof.
- [0009]

According to the present invention, a composition for inhibiting endotoxin from entering the blood is provided. The whey protein contained in the composition according to the present invention utilizes a milk protein which has been long used as a food raw material, and the composition according to the present invention therefore produces less side effects and is highly safe even if it is ingested for a long time period, which is an advantage of the composition according to the present invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0010]

Fig. 1 depicts the blood aspartate transaminase (AST) activity and the blood alanine transaminase (ALT) activity in the nutritional composition group consisting of mice with liver disorder to which the nutritional composition (a test diet) has been

fed, as compared with those in the control group (the control diet-fed group). *t*-test, *: $p < 0.05$ vs the control group.

Fig. 2 depicts the blood TNF- α concentration and the blood IL-6 concentration in the nutritional composition group consisting of mice with liver disorder to which the nutritional composition (a test diet) has been fed, as compared with those in the control group (the control diet-fed group). *t*-test, *: $p < 0.05$ vs the control group, #: $p < 0.1$ vs the control group.

Fig. 3 depicts a boxplot illustrating the relationship between the presence or absence of endotoxin and the concentrations of inflammatory cytokines (TNF- α and IL-6) in the early stage.

DETAILED DESCRIPTION OF THE INVENTION

[0011]

The composition according to the present invention comprises a whey protein as an active ingredient. The term “whey” as used herein means the aqueous liquid remaining after milk fat, casein, and the like are removed from milk, and is synonymous with milk serum and milk plasma. It is known that whey is rich in protein components such as β -lactoglobulin (β -Lg), α -lactalbumin (α -La), lactoferrin, serum albumin, immunoglobulin, and the like. Moreover, the origin of the whey used in the present invention is not particularly limited, but a whey from cow milk, such as milk protein concentrate (MPC, also referred to as total milk proteins (TMP)), whey protein concentrate (WPC), whey protein isolate (WPI), defatted milk powder, defatted concentrated milk and raw milk, is preferable.

[0012]

In a preferred embodiment of the present invention, whey proteins can be used in the form of hydrolysate. A whey protein hydrolysate can be prepared by, for example, a method comprising the steps described in (i) to (v) below.

(i) A whey protein isolate (WPI, Davisco Foods International, Inc.) containing proteins at a content of about 90% (w/w) on dry matter basis is dissolved in distilled water to a protein concentration of 8% (w/v) and thereby an aqueous protein solution is obtained.

(ii) The aqueous solution is heat-treated at 85°C for two minutes to denature proteins. The heat-treated aqueous solution can be adjusted, for example, to a pH of about 7.5.

(iii) Subsequently, the Alcalase 2.4L enzyme (Novozymes A/S) is added at a concentration of 2.0% (w/w) relative to the proteins (substrate) and the resulting aqueous solution is maintained at 55°C for three hours to hydrolyze the substrate.

(iv) Then, the PTN 6.0S enzyme (Novozymes Japan Ltd.), a trypsin derived from pigs, is added at a concentration of 3.0% (w/w) relative to the proteins (substrate) and the resulting aqueous solution is maintained at 55°C for three hours to hydrolyze the substrate. It means that the duration of hydrolysis can be, for example, a total of six hours. The pH of the aqueous solution after completion of these hydrolysis reactions can be, for example, a pH of about 7.0.

(v) The whey protein hydrolysate is centrifuged (at 20,000 x g, for 10 minutes) and then treated with an ultrafiltration (UF) membrane with a molecular weight cut-off of 10,000 (Ultrafree-MC, Millipore Co.).

[0013]

In the method of preparing a milk protein hydrolysate, there are five optimization parameters, such as pre-heating, ratio between enzyme and substrate (E/S), pH, temperature during hydrolysis, and hydrolysis period, and the following conditions can be illustrated as an example: pre-heating at 65 to 90°C, an E/S ratio of 0.01 to 0.2, a pH of 2 to 10, a temperature of 30 to 65°C during hydrolysis, a hydrolysis period of 3 to 20 hours.

[0014]

The whey protein hydrolysate according to the present invention includes, in addition to the very protein hydrolysate, a retained liquid (retentate) remaining after treatment with the ultrafiltration membrane and a liquid that has passed through the ultrafiltration membrane (permeate), and further includes commercially available whey protein hydrolysates which have the same activity as that required in the present invention. For example, a retentate remaining after the treatment with an ultrafiltration membrane with a molecular weight cut-off between two molecular weights, where the lower limit of the molecular weight is (not less than, or more than) any of 5000, 6000, 7000, 8000, 9000, and 10000, and the upper limit of the molecular weight is (not more than, or less than) any of 15000, 20000, 25000, and 30000, preferably with an ultrafiltration membrane with a molecular weight cut-off of 10000, can be used as a milk protein hydrolysate according to the present invention.

[0015]

In the composition according to the present invention, the incorporated amount of a whey protein can be appropriately adjusted depending on the incorporated amounts of other ingredients (fermented milk protein, lipid, saccharide, and the like), as well as on the condition, symptoms, age, and body weight of a subject to whom the composition according to the present invention is fed, and on the intended use of the composition. Specifically, 0.5 to 5.0 g, preferably 1.0 to 3.0 g, more preferably 1.5 to 2.5 g, of whey protein per 100 kcal of the composition can be illustrated as an example of the incorporated amount, but the incorporated amount of a whey protein is not limited to those ranges.

[0016]

The composition according to the present invention may comprise fermented milk protein as the protein. As the fermented milk protein that can be used in the present invention, fermented products prepared from one or more selected from milk which is from farm animals such as cow, water buffalo, goat, sheep, and horse and a liquid milk product which is prepared using milk raw materials originated therefrom, such as partially defatted milk, defatted milk, reconstituted whole milk, reconstituted defatted milk, reconstituted partially defatted milk, butter, and cream by using a starter such as lactic bacteria can be used. Examples of the fermented milk protein according to the present invention include fresh cheese, natural cheese, yogurt, and whey cheese. The term “cheese” as used herein refers to solids remaining after removing the whey (milk serum) from the curd which has been prepared by fermentation of milk, butter milk, or cream with lactic bacteria, or by addition of an enzyme to milk, butter milk, or cream, regardless of the presence or absence of consolidation or aging in the process. As the starter to produce fermented milk, Lactobacillus bulgaricus and Streptococcus thermophilus can mainly be used, but the starter is not limited thereto. For example, lactic bacteria and Bifidobacterium species, such as Streptococcus lactis, Streptococcus cremoris, Streptococcus diacetylactis, Enterococcus faecium, Enterococcus faecalis, Lactobacillus casei, Lactobacillus helveticus, Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus plantarum, Lactobacillus murinus, Lactobacillus reuteri, Lactobacillus brevis, Lactobacillus gasseri, Bifidobacterium longum, Bifidobacterium bifidum, and Bifidobacterium breve, can also be used. In addition, microorganisms used in the production of fermented milk, such as bacteria belonging to the genus Propionibacterium, can also be used in combination. The composition according to

the present invention may be prepared with any fermented milk, but it can be prepared preferably with fresh cheese or yogurt, more preferably with quark or yogurt.

[0017]

The term “fresh cheese” includes many types of cheeses including cottage, quark, string, Neufchatel, cream cheese, mozzarella, ricotta, mascarpone, and the like. Among them, quark is a type of unmaturing (fresh) cheese, which has a low fat content and is characterized by fresh flavors and a sour taste.

[0018]

It is widely known that lactic bacteria have probiotic effects. Moreover, it is known that the whey from fresh cheese obtained by fermentation with lactic bacteria has antibacterial effects against *Escherichia coli*, *Vibrio parahaemolyticus*, *Bifidobacterium* species, *Bacteroides* species, and the like (see Japanese Laid-open Patent Publication No. H7-155103). Thus, probiotic effects and antibacterial effects can be expected from the addition of fermented milk protein which has been fermented by lactic bacteria to the composition according to the present invention.

[0019]

The incorporated amount of fermented milk protein can be appropriately adjusted depending on the incorporated amounts of other ingredients (milk protein hydrolysate, lipid, saccharide, and the like), as well as on the condition, symptoms, age, and body weight of a subject to whom the composition according to the present invention is fed, and on the intended use of the composition. Specifically, 0.5 to 6.0 g, preferably 1.5 to 5.0 g, more preferably 2.5 to 3.5 g, of protein converted from the fermented milk protein per 100 kcal of the composition can be illustrated as an example of the incorporated amount, but the incorporated amount of fermented milk protein is not limited to those ranges. The incorporated amount of fermented milk protein is preferably adjusted to a value within the above-described ranges in order to allow the effects owned by the fermented milk protein to be exerted and to allow the composition to be easily and continuously ingested while the good flavor of the composition is maintained.

[0020]

In the present invention, milk excluding colostrum (normal milk), that is, matured milk (mature milk) is preferably used to prepare the milk protein

hydrolysate or the fermented milk protein. The milk may be originated from any animal such as cow, water buffalo, goat, sheep, horse, or human.

[0021]

The composition according to the present invention may also comprise any protein other than whey proteins and fermented milk proteins, and examples of such proteins include plant and animal proteins, such as whole milk powder, defatted milk powder, partially defatted milk powder, casein, α -casein, β -casein, κ -casein, soybean protein, chicken egg protein, and meat protein, and hydrolysates thereof; and various milk-derived components, such as butter, whey (milk serum) minerals, cream, whey, non-protein nitrogen, and sialic acid. Additionally, the composition according to the present invention may comprise peptides and amino acids such as casein phosphopeptides and lysine.

[0022]

The composition according to the present invention may comprise a lipid as an ingredient. Examples of the lipid that can be incorporated into the composition according to the present invention include phospholipids and edible oils and fats. Examples of the phospholipid that can be used in the composition according to the present invention include milk phospholipids, soybean lecithin, and egg yolk lecithin, and these phospholipids may be used singly or in combination.

[0023]

Examples of the edible oil and fat that can be used in the composition according to the present invention include fatty acid-containing oils and fats. Examples of the fatty acids include saturated fatty acids, monounsaturated fatty acids, and polyunsaturated fatty acids. Among those fatty acid-containing oils and fats, oils and fats containing monounsaturated fatty acids such as oleic acid are preferable.

[0024]

The incorporated amount of monounsaturated fatty acids such as oleic acid can be not less than 25%, preferably not less than 30%, more preferably 30 to 50%, in the fatty acid composition of the composition according to the present invention, but the incorporated amount of monounsaturated fatty acids is not limited to those ranges. Furthermore, polyunsaturated fatty acids such as n-3 fatty acids (for example, docosahexaenoic acid, eicosapentaenoic acid, linolenic acid) or n-6 fatty acids (for example, linoleic acid, arachidonic acid), or medium-chain fatty acids such as caprylic acid, capric acid, or lauric acid can be added.

[0025]

The composition according to the present invention may preferably comprise an n-3 fatty acid as a lipid. Examples of the n-3 fatty acid that is contained in the composition according to the present invention include EPA, DHA, α -linolenic acid, DPA, and the like, and preferably include EPA, DHA, and/or α -linolenic acid, and more preferably include EPA or/and DHA. Examples of n-3 fatty acid-containing oils and fats include shiso oil, linseed oil, perilla oil, fish oil, rapeseed oil, soybean oil, salad oil, flaxseed oil, and the like. In the present invention, the n-3 fatty acids may be contained directly or in the form of oils and fats, such as fish oil.

[0026]

Moreover, the composition according to the present invention may preferably comprise a medium-chain fatty acid triglyceride (MCT: medium-chain triglyceride) as a lipid. MCTs are characterized in that they are quickly absorbed in the body and easily converted into energy, and induce reduction of fat deposition in the body. Examples of MCT-containing oils and fats include palm oil, palm kernel oil, medium-chain fatty acid-containing oils and fats, and the like. In the present invention, MCTs may be contained directly or in the form of oils and fats, such as palm kernel oil.

[0027]

Moreover, the composition according to the present invention can comprise a fatty acid, such as oleic acid, palmitic acid, palmitoleic acid, linoleic acid, stearic acid, linolenic acid, and arachidonic acid, and preferably oleic acid, as a lipid. Examples of oils and fats containing any of those fatty acids include high oleic sunflower oil, rapeseed oil, and olive oil, high oleic safflower oil, soybean oil, corn oil, palm oil, and the like, each of which contains a high level of oleic acid. Sunflower oil, rapeseed oil, olive oil, and oils mixed with olive oil can also be used. Linoleic acid, arachidonic acid, γ -linolenic acid, and the like are n-6 fatty acids. Examples of n-6 fatty acid-containing oils and fats include safflower oil, sunflower oil, soybean oil, linseed oil, corn oil, peanut oil, and the like.

[0028]

The composition according to the present invention may comprise any edible oil and fat other than those mentioned above, and examples of such edible oils and fats include animal oils and fats, such as lard, fish oil, and fractionated oils thereof, hydrogenated oils thereof, and transesterified oils thereof; and plant oils and fats,

such as safflower oil, palm oil, fractionated oils thereof, hydrogenated oils thereof, and transesterified oils thereof.

[0029]

The incorporated amount of edible oils and fats, including fatty acid-containing oils and fats, can be appropriately adjusted depending on the incorporated amounts of other ingredients (milk protein hydrolysate, fermented milk protein, saccharide, and the like), as well as on the condition, symptoms, age, and body weight of a subject to whom the composition according to the present invention is fed, and on the intended use of the composition. Specifically, a total of 0.5 to 5.0 g, preferably 1.0 to 4.0 g, more preferably 2.0 to 3.0 g, of edible oils and fats per 100 kcal of the composition can be illustrated as an example of the incorporated amount, but the incorporated amount of edible oils and fats is not limited to those ranges. Moreover, the incorporated amount of MCTs can be adjusted to 0.3 to 1.0 g, preferably 0.5 to 0.7g, per 100 kcal of the composition, and the incorporated amount of n-3 fatty acids can be adjusted to 0.03 to 0.10 g, preferably 0.05 to 0.07g, per 100 kcal of the composition. The incorporated amount of lipid components is preferably adjusted to a value within the above-described ranges in order to allow the effects owned by the lipid components to be exerted and to allow the composition to be easily and continuously ingested while the good flavor of the composition is maintained. In the present invention, if 1 kcal of the composition corresponds to 1 mL of the composition, the phrase “per 100 kcal of the composition” may be replaced with the phrase “per 100 mL of the composition”.

[0030]

The composition according to the present invention may comprise a saccharide as a carbohydrate. Examples of the saccharide that can be incorporated into the composition according to the present invention include sugar alcohols (sorbitol, xylitol, maltitol, and the like), honey, granulated sugar, glucose, fructose, invert sugar, isomaltulose, dextrin, and the like. Preferably, at least isomaltulose can be incorporated as a saccharide.

[0031]

Isomaltulose (molecular weight: 342.297; CAS No.: 13718-94-0) is a disaccharide composed of a glucose molecule and a fructose molecule, which are linked by an α -1,6 bond. Isomaltulose is a structural isomer of sucrose and is also called 6-O-(α -D-glucopyranosyl)-D-fructose, isomaltulose, or palatinose, and is

used in sweeteners and the like. A very small amount of isomaltulose is present in honey, sugarcane, and the like. Additionally, isomaltulose can also be produced by allowing a Protaminobacter rubrum-derived α -glucosyltransferase and the like to act on sucrose to convert the α -1,2 bond of sucrose into an α -1,6 bond. The sweet taste of isomaltulose is similar to that of sucrose, but the sweetness of isomaltulose is about half that of sucrose. Orally ingested isomaltulose is degraded by isomaltase in the digestive tract and, similarly to sucrose, digested to glucose and fructose, and then absorbed (Aida N. et al., Journal of Japan Society of Nutrition and Food Sciences, Vol. 36(3): 169-173, 1983). Since isomaltose, panose, isomaltotriose, and the like which are digested by isomaltase compete with isomaltulose for the digestion, it is said that the digestion and absorption of these sugars is inhibited by ingestion of isomaltulose (Journal of Japan Society of Nutrition and Food Sciences, 36(3), pp. 169-173(1983)). Isomaltulose has a calorie content of 4 kcal/g. In the present invention, isomaltulose includes palatinose syrup, reduced palatinose, starch syrup-like palatinose, or the like. Starch syrup-like palatinose is a liquid substance in the form of starch syrup, containing oligosaccharides including tetrasaccharides, hexasaccharides, octasaccharides and the like as main components, which are produced by dehydration condensation of isomaltulose.

[0032]

It is also known that harmful bacteria are removed and a proliferative environment for useful bacteria is provided in the intestine by an aqueous solution prepared by dissolving a combination of a crystalloid osmotic pressure regulator such as isomaltulose and a colloid osmotic pressure regulator such as dextrin or indigestible dextrin in water to adjust the osmotic pressure to a value of 200 to 440 mOsm/L (WO 2004/067037). Thus, from the incorporation of isomaltulose into the composition according to the present invention, a positive effect on improvement of the intestinal environment is expected in those who have ingested the composition.

[0033]

The incorporated amount of isomaltulose can be appropriately adjusted depending on the incorporated amounts of other ingredients (milk protein hydrolysate, fermented milk protein, lipid, and the like), as well as on the condition, symptoms, age, and body weight of a subject to whom the composition according to the present invention is fed, and on the intended use of the composition. Specifically, 4 to 15 g, preferably 6 to 8 g, of isomaltulose per 100 kcal of the

composition can be illustrated as an example of the incorporated amount, but the incorporated amount of isomaltulose is not limited to those ranges.

[0034]

The incorporated amount of isomaltulose can be appropriately adjusted depending on the incorporated amounts of other ingredients (whey protein, fermented milk protein, lipid, and the like), as well as on the condition, symptoms, age, and body weight of a subject to whom the composition according to the present invention is fed, and on the intended use of the composition. Specifically, 4 to 15 g, preferably 6 to 8 g, of isomaltulose per 100 kcal of the composition can be illustrated as an example of the incorporated amount, but the incorporated amount of isomaltulose is not limited to those ranges. The incorporated amount of isomaltulose is preferably adjusted to a value within the above-described ranges in order to allow the effects owned by isomaltulose to be exerted and to allow the composition to be easily and continuously ingested while the good flavor of the composition is maintained.

[0035]

The composition according to the present invention may comprise any saccharide raw material other than those mentioned above, and examples of such a saccharide raw material include processed starches such as soluble starch, British starch, oxidized starch, starch ester, and starch ether.

[0036]

The calorie content of the composition according to the present invention can be controlled by adjusting the incorporated amounts of protein, lipid, and saccharide. The calorie content of the composition according to the present invention can be adjusted to 50 to 150 kcal, preferably 80 to 120 kcal, per 100 mL of the composition in the form of liquid, but the calorie content of the composition is not limited to those ranges.

[0037]

Moreover, in the composition according to the present invention, the energy ratios of protein, lipid and saccharide to the overall composition can be determined nearly in accordance with the description in Recommended Dietary Allowances for the Japanese, 6th revision. Specifically, 15 to 25% of energy from protein, 20 to 30% of energy from lipid, and 45 to 65% of energy from saccharide can be illustrated as an example, but the energy ratios are not limited to those ranges.

[0038]

The composition according to the present invention may further comprise dietary fiber. Dietary fiber refers to substances in foods that are not hydrolyzed by human digestive enzymes, and dietary fibers are classified into water-soluble dietary fiber and insoluble dietary fiber depending on the difference in compatibility with water. Examples of water-soluble dietary fiber include indigestible oligosaccharides such as lactulose, lactitol, or raffinose. Among those dietary fibers, the indigestible oligosaccharides are known to have physiological functions, such as reaching the colon as undigested fiber and contributing to the activation and growth of intestinal Bifidobacterium species, and improving the intestinal environment, which means that the indigestible oligosaccharides have beneficial effects on intestinal function. Other water-soluble dietary fibers include pectin (protopectin, pectinic acid, pectic acid), guar gum and its enzyme degradation products, tamarind seed gum, and the like.

[0039]

In the composition according to the present invention, water, vitamins, minerals, organic acids, organic bases, fruit juice, flavors, emulsifiers, thickeners, stabilizing agents, and the like can be used in addition to the above proteins, lipid, saccharide, and dietary fibers. Examples of the vitamins include vitamin A, carotene, B vitamins, vitamin C, D vitamins, vitamin E, K vitamins, vitamin P, vitamin Q, niacin, nicotinic acid, pantothenic acid, biotin, inositol, choline, folic acid, and the like. Examples of the minerals include calcium, potassium, magnesium, sodium, copper, iron, manganese, zinc, selenium, and the like. Examples of the organic acids include malic acid, citric acid, lactic acid, tartaric acid, erythorbic acid, and the like. Moreover, the composition according to the present invention can comprise a material having an effect to reduce the odor of feces (for example, 0.005% to 0.5% of champignon extract), a carotenoid formulation (for example, 0.00001% to 0.0002% of a formulation containing α -carotene, β -carotene, lycopene, lutein, or the like), and an antioxidant (such as catechin, polyphenol). Two or more of these ingredients may be used in combination, and synthetic products and/or food materials containing large amounts of these ingredients may also be used.

[0040]

According to a preferred embodiment of the present invention, provided is a nutritional composition for inhibiting endotoxin from entering the blood, which

comprises a whey protein and fermented milk protein and further comprises an edible oil and fat containing an n-3 fatty acid and a saccharide containing isomaltulose as a lipid and a carbohydrate, respectively. The nutritional composition can preferably be provided in the form of a liquid composition. In that case, the content of whey protein per 100 kcal of the composition can be adjusted to 0.5 to 5.0 g, the content of fermented milk protein per 100 kcal of the composition can be adjusted to 0.5 to 6.0 g, the content of n-3 fatty acid per 100 kcal of the composition can be adjusted to 0.03 to 0.10 g, and the content of isomaltulose per 100 kcal of the composition can be adjusted to 4 to 15 g.

[0041]

The composition according to the present invention can be produced by methods known in the art. For example, the composition according to the present invention can be produced by blending a part or all of the above-described ingredients and then homogenizing the ingredients as necessary. The term “homogenizing” as used herein refers to thoroughly mixing all the incorporated ingredients to a homogeneous mixture and, moreover, mechanically pulverizing fat globules and other coarse particle components to prevent floating and aggregation of fat and the like, as well as to keep the composition in a uniformly emulsified state.

[0042]

Heat treatment or heat sterilization can be performed in the production of the composition according to the present invention. Conditions commonly used in food sterilization can be used as conditions for heat sterilization, and heat sterilization can be performed using common equipment. For example, sterilization, retort (pressurizing and heating) pasteurization, autoclaving, and the like at a temperature of 62 to 65°C for 30 minutes, at a temperature of 72°C or higher for not less than 15 seconds, at a temperature of 72°C or higher for not less than 15 minutes, or at a temperature of 120 to 150°C for 1 to 5 seconds, or at a temperature of 121 to 124°C for 5 to 20 minutes, or at a temperature of 105 to 140°C can be employed, but the conditions are not limited to those examples. Heat sterilization can be performed preferably under pressure.

[0043]

Moreover, a method comprising heating and sterilizing a liquid composition in advance and then aseptically packaging the composition in a container (for example, a combinational method of the UHT sterilization method and an aseptic

packaging method), a method comprising packaging a liquid nutritional composition in a container and then heating and sterilizing the composition together with the container (for example, the autoclaving method), a method comprising packaging a composition in a can container or a various type of container used for liquid foods or oral or tube feeding (so-called soft bag, nutrition bag, or the like) and performing retort pasteurization (for example, at a temperature of 115 to 145°C for 5 to 10 seconds), and a method comprising packaging a composition in a can container or a various type of container used for liquid foods or oral or tube feeding (so-called soft bag, nutrition bag, or the like) and performing retort pasteurization and subsequent heat sterilization at a temperature of about 140 to 145°C for about 5 to 8 seconds and then cooling the composition for aseptic packaging can be illustrated, but the heat treatment or heat sterilization is not limited to the above. Starter bacteria from fermented milk as a raw material (such as lactic bacteria, Bifidobacterium species, or Propionibacterium species) are killed by heat treatment or heat sterilization.

[0044]

In cases where the composition according to the present invention is provided in liquid form, the osmotic pressure of the composition can be 500 to 1000 mOsm/L, and preferably 550 to 750 mOsm/L.

[0045]

In cases where the composition according to the present invention is provided in liquid form, the viscosity of the composition can be 20 to 100 cp (1cp = 0.001 Pa·s), preferably 25 to 60 cp, and more preferably 30 to 50 cp, when measured at room temperature; however, the viscosity of the composition is not limited to those ranges.

[0046]

In cases where the composition according to the present invention is provided in liquid form, the composition can be adjusted to a pH of 4.6 or less, preferably a pH of 3.0 to 4.3, and more preferably a pH of 3.8 to 4.2, but the pH of the composition is not limited to those ranges.

[0047]

As indicated in Example below, the composition comprising a whey protein according to the present invention inhibits endotoxin from entering the blood. Thus, the composition according to the present invention can be used as a composition for inhibiting endotoxin from entering the blood and as an agent for inhibiting endotoxin

from entering the blood, as well as can be used in a method of inhibiting endotoxin from entering the blood.

[0048]

Namely, according to the present invention, provided is a method of inhibiting endotoxin from entering the blood, which comprises feeding or administering an effective amount of a whey protein or the composition according to the present invention to a subject in need thereof. The fed or administered subject is a human or a non-human animal (for example, a non-human mammal).

[0049]

According to the present invention, use of a whey protein or a composition comprising the same for the manufacture of a composition for inhibiting endotoxin from entering the blood is also provided. According to the present invention, use of a whey protein or a composition comprising the same for the manufacture of an agent for inhibiting endotoxin from entering the blood is further provided. According to the present invention, a whey protein or a composition comprising the same for use in inhibiting endotoxin from entering the blood is also further provided.

[0050]

The term “endotoxin” as used herein refers to a cell wall component from Gram-negative bacteria such as *Salmonella enterica*, *Escherichia coli*, *Pseudomonas aeruginosa*, and the like, and is also referred to as bacterial endotoxin or lipopolysaccharide. The presence or absence of “inhibiting endotoxin from entering the blood” can be evaluated based on the concentration of endotoxin in serum (see Example 1). The phrase “inhibiting endotoxin from entering the blood” is intended to be used with a meaning that includes reduced endotoxin entry into blood.

[0051]

The use of the composition according to the present invention may be use in human and non-human animals, and in samples derived therefrom, and is intended for both therapeutic use and non-therapeutic use. The term “non-therapeutic” means excluding the act of performing surgery on, treating, or diagnosing a human (*i.e.*, medical practices on humans), specifically, excluding a procedure in which a physician or an individual who is directed by a physician performs surgery on, treats, or diagnoses a human.

[0052]

As indicated in Example below, the composition according to the present invention is considered to be able to inhibit endotoxin from entering the blood. It has been known so far that an increased concentration of endotoxin in blood is associated with the development and progression of diseases such as liver disorder (Michelena J et al., *Hepatology* 2015; 62(3): 762-772; Verdam FJ et al., *J Clin Gastroenterol.* 2011; 45(2): 149-152; Wig JD et al., *J Clin Gastroenterol.* 1998; 26(2): 121-124; Wong J et al., *Semin Dial.* 2015; 28(1): 59-67; Bester J et al., 2015; 6(34): 35284-35303). Moreover, it has been known that an increased concentration of endotoxin in blood is associated with the manifestation of symptoms, such as fever, decrease in blood pressure, leukopenia, thrombocytopenia, chill, headache, vomiting, muscle ache, cardiopalmus, and the like (Doorduyn J et al., *Shock* 2015; 44(4): 316-322). As further indicated in Example below, it was confirmed that the concentrations of AST and ALT in blood, indexes of liver disorder, were significantly decreased in liver disorder mouse models by feeding them with the composition according to the present invention (Example 1). Thus, in the present invention, the composition according to the present invention can be used for the treatment, prevention, or amelioration of diseases and symptoms which are effectively treated, prevented, or ameliorated by inhibiting endotoxin from entering the blood. Moreover, the composition according to the present invention can be used as a therapeutic agent, a prophylactic agent, and an ameliorative agent for diseases and symptoms which are effectively treated, prevented, or ameliorated by inhibiting endotoxin from entering the blood, as well as can be used in methods of treating, preventing, and ameliorating diseases and symptoms which are effectively treated, prevented, or ameliorated by inhibiting endotoxin from entering the blood. The therapeutic method, prophylactic method and ameliorative method according to the present invention can be performed by feeding or administering the composition according to the present invention or an effective amount of a whey protein to a subject in need thereof. The fed or administered subject is a human or a non-human animal (for example, a non-human mammal).

[0053]

Examples of the diseases which are effectively treated, prevented, or ameliorated by inhibiting endotoxin from entering the blood include diseases such as liver disorder including cirrhosis, alcoholic liver disorder, non-alcoholic liver disorder (NASH) and the like, and acute pancreatitis, kidney disease, Alzheimer's

disease, diabetes, and arteriosclerosis. Moreover, examples of the symptoms which are effectively treated, prevented, or ameliorated by inhibiting endotoxin from entering the blood include fever, decrease in blood pressure, leukopenia, thrombocytopenia, chill, headache, vomiting, muscle ache, cardiopalmus, and the like.

[0054]

The composition and blocking agent according to the present invention, as well as the therapeutic agent, prophylactic agent, and ameliorative agent according to the present invention can be provided in the form of a medicament, a quasi-drug, a food product, a feed product, and the like, and can be achieved as described below. Moreover, the method of inhibiting endotoxin from entering the blood according to the present invention, as well as the therapeutic method, prophylactic method and ameliorative method according to the present invention can be performed as described below.

[0055]

The composition according to the present invention can be fed or administered orally as a medicament or a dietary supplement to human and non-human animals. Oral drugs include tablets (including sugar-coated tablets), pills, capsules, granules, powders, syrups, and the like. These formulations can be formulated with pharmaceutically acceptable carriers, according to procedures commonly used in the art. Examples of the pharmaceutically acceptable carriers include excipients, binders, disintegrators, lubricants, odor masking agents, solubilizing agents, suspending agents, coating agents, flavoring agents, buffers, thickeners, coloring agents, stabilizing agents, emulsifiers, and the like. Additionally, a suitable amount of calcium may be added, and, furthermore, suitable amounts of vitamins, minerals, organic acids, saccharides, amino acids, peptides, and the like may be added.

[0056]

In the present invention, parenteral administration, such as tube feeding, nasal tube feeding, infusion, and suppository, to human and non-human animals is feasible depending on the form of the composition according to the present invention. For example, the composition according to the present invention taking the form of a viscous liquid composition or a semi-solid composition can be administered even to humans and non-human animals in which chewing or swallowing function is reduced

and thus oral ingestion or oral administration is impossible. Even though the chewing or swallowing function is reduced due to aging or the like, by feeding or administering the composition according to the present invention through a parenteral route, the endotoxin entry into blood is blocked, as well as treatment, prevention, and amelioration of diseases such as liver disorder and of symptoms associated with the diseases can be expected in the humans and the non-human animals. Furthermore, the composition according to the present invention can have functions as a nutritional supplement and as an energy supplement depending on its ingredients, and can therefore supply various nutritional components and energy to humans and non-human animals in which chewing or swallowing function is reduced. [0057]

The composition according to the present invention not only uses daily food materials such as whey protein and its hydrolysate as a raw material, but also has an effect to inhibit endotoxin from entering the blood. Therefore, the composition can be provided as a daily ingestible food, as a food ingestible as a dietary supplement, and, furthermore, as a functional nutritional food intended for nutritional supplementation. The composition according to the present invention can also be provided mixed with various foods. [0058]

In cases where the composition according to the present invention is provided as a food, the food contains an effective amount of the composition according to the present invention. The phrase “containing an effective amount” of the composition according to the present invention, as used herein, refers to the content of the composition according to the present invention in each food product to be taken in a range as will be described below when the food product is taken in such an amount that the food product is usually eaten by individuals. Moreover, the term “food” is used to refer inclusively to health foods, functional foods, health-promoting foods (for example, foods for specified health uses, functional nutritional foods, dietary supplements, foods with function claims), and foods for special dietary use (for example, foods for infants, foods for pregnant women, foods for diseased people). The composition according to the present invention contains ingredients that can be used as foods, and is therefore available as a liquid food, an oral or tube-feeding nutrient, a beverage, a gel-like food (particularly, a so-called functional food), and the like for the nutritional management of patients receiving oral or enteral nutrition,

elderly individuals, infants, and the like, in expectation of inhibiting endotoxin from entering the blood and of effects associated therewith.

[0059]

The form of the “food product” is not particularly limited, and it may be in the form of liquid, as in the case of, for example, a beverage and a liquid food, or in the form of paste, semi-liquid, gel, solid, or powder. Moreover, various foods (milk, soft drink, sour milk beverage, yogurt, cheese, bread, biscuit, cracker, pizza crust, modified milk powder, liquid foods, foods for special dietary use, foods for diseased people, nutritional foods, frozen foods, processed foods, other commercial foods, and the like) may be supplemented with the composition according to the present invention and the resulting foods may be ingested, regardless of whether the foods have a liquid form, a solid form, or powder form. Moreover, if the nutritional composition is in a powder form when it is used, it can be produced by means of, for example, spray drying, lyophilization, or the like.

[0060]

The daily ingested or administered amount of the composition according to the present invention as a medicament or a food product is not particularly limited because it varies depending on the condition, age, symptoms, and body weight of a subject who ingests the composition of the present invention, the subject’s intention to use the composition, and whether the composition according to the present invention is the sole nutritional ingredient or not. In the case of ingestion and administration intended for inhibiting endotoxin from entering the blood and for effects associated therewith, for example, the composition according to the present invention can be fed or administered to an adult individual such that the adult individual can ingest 0.5 to 80 g, preferably 2 to 42 g, more preferably 6 to 25 g, of whey protein per day. Moreover, the composition according to the present invention has an aspect of a nutritional composition, and an amount of the composition corresponding to 100 to 1600 kcal, preferably 200 to 1400 kcal, more preferably 400 to 1000 kcal, can be illustrated as examples of the daily ingested amount and of the daily administered amount for adult individuals. In the case of healthy adult individuals, a daily ingested amount and a daily administered amount corresponding to, for example, up to 3000 kcal are acceptable. Additionally, the ingested amount can be determined by an attending physician of a subject who ingests the composition of the present invention.

[0061]

The composition according to the present invention can be provided in the daily ingested amount, which is effective in inhibiting endotoxin from entering the blood, and the like. In this case, the composition according to the present invention may be packaged such that the daily effective ingested amount of the composition can be ingested, whereas the packaging form may be a single packaging form or a multi-packaging form as long as the packaging form allows ingestion of the daily effective ingested amount of the composition. Preferably, if the composition of the present invention is provided in a certain packaging form, a description of the ingested amount is printed on the package, or a document describing the ingested amount is further provided as package insert, so that the daily effective ingested amount is ingested. Moreover, if the daily effective ingested amount is provided in a multi-packaging form, a set of multiple packages each containing the daily effective ingested amount of the composition of the present invention can also be provided for ease of ingestion.

[0062]

The packaging form to provide the composition according to the present invention is not particularly limited as long as it specifies a particular amount, and examples of the packaging form include containers capable of accommodating the composition of the present invention, such as packing paper, envelope, soft bag, paper container, can, bottle, and capsule.

[0063]

To better exert the effects of the composition according to the present invention, it is continuously administered or fed preferably in a period of not less than one week, and the administration and ingestion period is more preferably from 1 to 4 weeks, and particularly preferably from 1 to 2 weeks. The term “continuously” as used herein means continuing daily administration or ingestion. If the composition according to the present invention is provided in a certain packaging form, a set of packages intended for a certain time period (for example, one week) each containing the effective ingested amount of the composition of the present invention may also be provided for continuous ingestion.

EXAMPLES

[0064]

The present invention will be described in more details by way of example below, but the present invention is not limited to the example.

[0065]

Example 1: Confirmation of the blocking effect of a nutritional composition according to the present invention on the endotoxin entry into blood

To confirm the blocking effect of a nutritional composition according to the present invention on the endotoxin entry into blood, an assessment as described below was performed using liver disorder mouse models generated by administration of concanavalin A (ConA).

[0066]

(1) Animal diets in the study

The liquid food product “Meiji MEIN” (manufactured by Meiji Co., Ltd.) was used as a test diet (a nutritional composition according to the present invention). The liquid food product “Meibalance HP” (manufactured by Meiji Co., Ltd.) was used as a control diet. Powders of the respective liquid food products obtained by lyophilization were used as animal diets in the study. The component compositions of the test diet and the control diet (in 100 mL) and the raw material composition of the test diet in terms of protein, saccharide, fiber and lipid (in 100mL) were as indicated in Tables 1 and 2 below.

[0067]

Table 1: Component compositions of the control diet and the test diet

Components		Control diet	Test diet
		Component composition / 100 mL	Component composition / 100 mL
Macronutrients	Protein (g)	5.0	5.0
	Carbohydrate (g)	15.3	14.5
	Saccharide (g)	14.1	13.3
	Fiber (g)	1.2	1.2
	Lipid (g)	2.5	2.8
	MCT (g)	-	0.59
	EPA, DHA (g)	-	0.06
	Ash (g)	0.67	0.6
	Water (g)	84.3	84.4
Vitamins	Vitamin A (μg RE)	60	150
	Vitamin D (μg)	0.50	0.75
	Vitamin E (mg)	3.0	5.0
	Vitamin K (μg)	5.0	3.4
	Vitamin B1 (mg)	0.15	0.25
	Vitamin B2 (mg)	0.20	0.30
	Niacin (mg)	1.5	3.0
	Vitamin B6 (mg)	0.30	0.30

	Vitamin B12 (µg)	0.60	0.60
	Folic acid (µg)	50	50
	Biotin (µg)	15	7.5
	Pantothenic acid (mg)	0.6	1.2
	Vitamin C (mg)	16	50
	Choline (mg)	1.8	9.2
	Carnitine (mg)	-	15
Minerals	Sodium (mg)	110	70
	Potassium (mg)	100	80
	Calcium (mg)	70	80
	Magnesium (mg)	30	20
	Phosphorus (mg)	70	70
	Iron (mg)	1.0	1.0
	Zinc (mg)	1.0	1.0
	Copper (mg)	0.05	0.05
	Manganese (mg)	0.23	0.175
	Chromium (µg)	3.0	2.96
	Molybdenum (µg)	2.5	2.5
	Selenium (µg)	6.0	5.0
	Iodine (µg)	15	9.7
	Chlorine (mg)	110	80
Energy	From all components (kcal)	100	100
	From proteins (%kcal)	20	20
	From carbohydrates (%kcal)	57.5	55
	From lipids (%kcal)	22.5	25
Osmotic pressure (mOsm/L)		420	600
pH (20°C)		6.7	4.0

RE: retinol equivalents, MCT: medium-chain fatty acid triglycerides, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid

[0068]

Table 2: Raw material composition of the test diet

Components	Test diet	
	Raw materials	Raw material composition / 100 mL
Protein (g)	Whey protein hydrolysate	2.0
	Fermented milk protein	3.0
Saccharide (g)	Isomaltulose	7.0
	Dextrin	6.3
Fiber (g)	Indigestible dextrin	0.9
	Pectin	0.3
Lipid (g)	Edible oils and fats	2.6
	· Processed oils and fats	(1.8)
	· MCT	(0.6)
	· Refined fish oils	(0.2)
	Milk phospholipids	0.1
	Soy lecithin	0.1

MCT: medium-chain fatty acid triglycerides

[0069]

(2) Test conditions

Six-week-old female Balb/c mice (purchased from Japan SLC, Inc.) were reared for one week prior to dividing them into four groups such that the average body weight in each group was equal, and were assigned to a control diet-fed normal mice group (n = 5), a nutritional composition-fed normal mice group (n = 5), a control diet-fed liver disorder mice group (n = 15), and a nutritional composition-fed liver disorder mice group (n = 14).

[0070]

Mice in the control diet-fed normal mice group and the control diet-fed liver disorder mice group were reared with the control diet for 14 days. Mice in the nutritional composition-fed normal mice group and the nutritional composition-fed liver disorder mice group were reared with the test diet for 14 days. The mice in the control diet-fed liver disorder mice group and the nutritional composition-fed liver disorder mice group (hereinafter sometimes collectively referred to as “the liver disorder mice groups”) were administered on day 14 via tail vein with ConA (manufactured by Sigma-Aldrich Co, LLC) at a dose of 20 mg/kg, whereas the mice in the control diet-fed normal mice group and the nutritional composition-fed normal mice group (hereinafter sometimes collectively referred to as “the normal mice groups”) were not administered with ConA.

[0071]

(3) Measurement items

The liver disorder mice groups underwent 0.3-mL blood sampling from tail vein with a heparin-treated syringe at two hours after the administration of ConA, and exsanguination from the abdominal vena cava with a heparin-treated syringe under isoflurane anesthesia on the next day (day 15). The mice in the normal mice groups underwent exsanguination from the abdominal vena cava with a heparin-treated syringe under isoflurane anesthesia 14 days after the beginning of rearing. The blood was collected aseptically, and the supernatant after centrifugation at 3000 rpm for one minute was recovered as platelet-rich plasma, which was used to detect the number of live bacteria and to detect endotoxin. The blood collected from tail vein and the blood remaining after taking a portion of the above platelet-rich plasma were centrifuged at 12,000 rpm for five minutes, and the resulting plasma was used for measurement of cytokines and for blood biochemistry.

[0072]

(i) AST and ALT activities

For the liver disorder mice groups, the obtained plasma (the plasma obtained 24 hours after the administration of ConA) was used individually to measure the aspartate transaminase (AST) activity and the alanine transaminase (ALT) activity, which served as indexes of liver disorder. The Fuji DRY-CHEM NX500i (manufactured by Fujifilm Co.) was used for the measurement.

[0073]

(ii) Concentrations of TNF- α and IL-6 in plasma

For the liver disorder mice groups, the obtained plasma (the plasma obtained 2 hours after the administration of ConA) was used individually to measure the concentrations of inflammatory cytokines produced in the early stage after ConA challenge, TNF- α and IL-6. The Mouse Inflammation Kit (manufactured by Nippon Becton Dickinson Company, Ltd.) was used for the measurement, and the measurement was performed on the BD FACS Verse (manufactured by BD Biosciences).

[0074]

(iii) The presence or absence of detected endotoxin in plasma

For the normal mice groups and the liver disorder mice groups, it was confirmed whether or not endotoxin was detected in the obtained platelet-rich plasma (the plasma obtained 24 hours after the administration of ConA). To confirm the presence or absence of detected endotoxin, the concentration of endotoxin was measured on the Toxinometer ET-6000 (manufactured by Wako Pure Chemical Industries, Ltd.) using the Limulus ES-F Single Test wako (manufactured by Wako Pure Chemical Industries, Ltd.). No detection of endotoxin was determined when the concentration of endotoxin in plasma was below the detection limit (0.02 EU/mL), whereas detection of endotoxin was determined when the concentration of endotoxin in plasma was at or above the detection limit.

[0075]

The results of (i) to (iii) were expressed as mean $\pm$ standard error (SE), and were statistically analyzed by *t*-test (*: $p < 0.05$) using the IBM SPSS software for statistical analysis.

[0076]

(4) Results

During the rearing period, the body weight of mice was steadily increased in any group and no significant difference was observed among the groups.

[0077]

(i) AST and ALT activities

The results of measuring the AST and ALT activities are shown in Fig. 1. In the nutritional composition-fed liver disorder mice group, both the AST and ALT activities were significantly lower as compared with those in the control diet-fed liver disorder mice group. These results indicated that the nutritional composition according to the present invention suppressed the development and progression of liver disorder.

[0078]

(ii) Concentrations of TNF- α and IL-6 in plasma

The results of measuring the concentrations of TNF- α and IL-6 in plasma are shown in Fig. 2. The nutritional composition-fed liver disorder mice group exhibited a decreasing trend for the concentration of TNF- α , as compared with the control diet-fed liver disorder mice group. Additionally, in the nutritional composition-fed liver disorder mice group, the concentration of IL-6 was significantly lower as compared with the control diet-fed liver disorder mice group. These results indicated that the nutritional composition according to the present invention inhibited the production of the inflammatory cytokines in the early stage, which suppressed the development and progression of liver disorder.

[0079]

(iii) The presence or absence of detected endotoxin in plasma

The number of mice individuals from which endotoxin was detected in plasma is shown for every group in Table 3. The number of live bacteria in plasma from the normal mice groups and the liver disorder mice groups was examined by culture method, but no live bacteria were detected (data not shown).

Table 3: Groups with or without endotoxin detected in plasma

	Endotoxin	
	+	-
Control diet-fed normal mice group	0	5
Nutritional composition-fed normal mice group	0	5
Control diet-fed liver disorder mice group	6	9
Nutritional composition-fed liver disorder mice group	2	12

[0080]

As seen from the result in Table 3, in the normal mice groups, no endotoxin was detected in all individuals from both the control group and the nutritional composition group. On the other hand, in the liver disorder mice groups, endotoxin was detected in six out of 15 individuals (40%) from the control group and in two out of 14 individuals (14%) from the nutritional composition group. Additionally, the concentration of endotoxin in plasma from the individuals in which detection of endotoxin was determined ranged from 0.022 to 0.047 EU/mL (3.14-6.71 pg/mL), indicating that those individuals were clearly different from the individuals in which endotoxin was not detected.

[0081]

Accordingly, it was indicated that the nutritional composition according to the present invention can inhibit endotoxin from entering the blood and thereby suppress the development and progression of liver disorder.

[0082]

While liver disorder induced by administration of ConA is caused by production of inflammatory cytokines in the early stage and the endotoxin entry into blood, the present example demonstrated that the nutritional composition according to the present invention not only inhibited endotoxin from entering the blood but also inhibited the production of inflammatory cytokines in the early stage. To confirm whether the production of inflammatory cytokines in the early stage is affected by endotoxin, a boxplot was created from the data on the concentration of inflammatory cytokines in the early stage and the presence or absence of detected endotoxin, which suggested no correlation between the production of inflammatory cytokines in the early stage and the inhibited entry of endotoxin into blood (Fig. 3). Accordingly, the nutritional composition according to the present invention is considered to reduce liver disorder by inhibiting endotoxin from entering the blood and by inhibiting the production of inflammatory cytokines in the early stage through a pathway which is different from that in the above process.

CLAIMS

1. A composition for inhibiting endotoxin from entering the blood, comprising a whey protein.
2. The composition according to claim 1, wherein the whey protein is in the form of hydrolysate.
3. The composition according to claim 1 or 2, wherein the whey protein is one or more selected from the group consisting of α -lactalbumin, β -lactoglobulin and lactoferrin.
4. The composition according to any one of claims 1 to 3, wherein the whey protein is originated from one or more selected from the group consisting of milk protein concentrate (MPC), whey protein concentrate (WPC), whey protein isolate (WPI), defatted milk powder, defatted concentrated milk and raw milk.
5. The composition according to any one of claims 1 to 4, wherein 0.5 to 5.0 g of the whey protein is contained per 100 kcal of the composition.
6. The composition according to any one of claims 1 to 5, which further comprises fermented milk protein as the protein.
7. The composition according to any one of claims 1 to 6, which further comprises an oil and fat containing an n-3 fatty acid as a lipid.
8. The composition according to any one of claims 1 to 7, which further comprises a saccharide containing isomaltulose as a carbohydrate.
9. The composition according to any one of claims 1 to 8, which is for use in the treatment, prevention, or amelioration of a disease or symptom which is effectively treated, prevented, or ameliorated by inhibiting endotoxin from entering the blood.
10. The composition according to claim 9, wherein the disease or symptom is one or more selected from the group consisting of cirrhosis, alcoholic liver disorder, non-alcoholic liver disorder (NASH), acute pancreatitis, kidney disease, Alzheimer's disease, diabetes, arteriosclerosis, fever, decrease in blood pressure, leukopenia, thrombocytopenia, chill, headache, vomiting, muscle ache and cardiopalmus.
11. Use of a whey protein for the manufacture of a composition for inhibiting endotoxin from entering the blood.
12. A whey protein for use in inhibiting endotoxin from entering the blood.

13. A method of inhibiting endotoxin from entering the blood, which comprises feeding or administering an effective amount of a whey protein to a subject in need thereof.