[DESCRIPTION]

[Invention Title]

PHARMACEUTICAL COMPOSITION FOR PREVENTING OR TREATING OBESITY COMPRISING EXTRACELLULAR VESICLE DERIVED FROM BIFIDOBACTERIUM SPP. STRAIN AS EFFECTIVE INGREDIENT, AND FOOD FOR PREVENTING OR IMPROVING OBESITY

[Technical Field]

The present invention relates to a pharmaceutical composition for preventing or treating obesity, comprising extracellular vesicles derived from a *Bifidobacterium* spp. strain as an active ingredient, and a food composition for preventing or improving obesity.

This application claims priority to and the benefit of Korean Patent Application No. 10-2024-0039320, filed on March 21, 2024, and Korean Patent Application No. 10-2025-0035393, filed on March 19, 2025, the disclosure of which is incorporated herein by reference in its entirety.

[Background Art]

Lactic acid bacteria possess a special physiological activity and are generally regarded as safe (GRAS) bacteria. They are used not only in the production of various fermented foods but are also widely utilized in dairy and fermented fruit and vegetable products that possess functional and probiotic properties. In recent years, as the consumer demand for natural supplements to replace chemical supplements has increased, lactic acid bacteria have emerged as an alternative.

Probiotics are live microorganisms that provide highly beneficial effects to the health

of host animals by improving the balance of intestinal microorganisms and enhancing the absorption of nutrients. Also, probiotics possess the characteristic of exhibiting antimicrobial activity against pathogenic microorganisms in the intestinal environment. Although probiotics include a variety of microorganisms, the genera *Lactobacillus* and *Bifidobacterium* account for the largest distribution.

Obesity is known to be a major cause of heart disease, cancer, arthritis, and diabetes. Despite the rising level of public awareness regarding the risks of obesity, there is a continuously increasing trend in the number of obese patients. Obesity develops as a result of adipogenesis, which leads to an increase in the number of adipocytes and their lipid content. Adipocytes play a key role in synthesizing and storing excess calories as triglycerides; as a result of adipogenesis, the size and number of adipocytes increase, thereby accelerating intracellular lipid accumulation. Also, obesity causes lipotoxicity and oxidative stress and induces a state of insulin resistance, which can lead to hyperglycemia and thus is the cause of various obesity-related diseases.

However, conventional treatments have side effects such as heart disease, respiratory disease, neurological disease, and the like, as well as low sustained efficacy. Also, because there have been almost no reported treatments that provide satisfactory therapeutic effects without causing side effects, there is a need for the development of new treatments for obesity and non-alcoholic fatty liver disease.

[Disclosure]

[Technical Problem]

One object of the present invention is to provide a pharmaceutical composition for preventing or treating obesity, comprising extracellular vesicles derived from a

Bifidobacterium spp. strain as an active ingredient.

Another object of the present invention is to provide a kit for preventing or treating obesity, comprising extracellular vesicles derived from a *Bifidobacterium* spp. strain and instructions.

Yet another object of the present invention is to provide a food composition for preventing or improving obesity, comprising extracellular vesicles derived from a *Bifidobacterium* spp. strain as an active ingredient.

Yet another object of the present invention is to provide a feed composition for preventing or improving obesity, comprising extracellular vesicles derived from a *Bifidobacterium* spp. strain as an active ingredient.

However, technical problems to be solved in the present invention are not limited to the above-described problems, and other problems which are not described herein will be fully understood by those of ordinary skill in the art from the following descriptions.

[Technical Solution]

The present invention provides a pharmaceutical composition for preventing or treating obesity, comprising extracellular vesicles derived from a *Bifidobacterium* spp. strain as an active ingredient.

In one example of the present invention, the *Bifidobacterium* spp. strain may be at least one selected from the group consisting of *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, and *Bifidobacterium lactis*, but is not limited thereto.

In one example of the present invention, the extracellular vesicles may be naturally secreted or artificially produced from a *Bifidobacterium* spp. strain, but are not limited thereto.

In one example of the present invention, the extracellular vesicles may have a size of 10 nm to 400 nm, but are not limited thereto.

In one example of the present invention, the composition may be characterized by at least one selected from the group consisting of the following, but is not limited thereto:

In one example of the present invention, the composition may be characterized by at least one selected from the group consisting of the following, but is not limited thereto:

a) weight loss;

b) maintenance of food intake;

c) reduction of triglycerides, low-density lipoproteins, high-density lipoproteins, and total cholesterol;

d) reduction of fat accumulation and cell size in the liver, white fat, and brown fat; and

e) reduction of fat synthesis gene expression.

In one example of the present invention, the composition may be orally administered, but is not limited thereto.

The present invention provides a kit for preventing or treating obesity, comprising extracellular vesicles derived from a *Bifidobacterium* spp. strain and instructions.

The present invention provides a food composition for preventing or improving obesity, comprising extracellular vesicles derived from a *Bifidobacterium* spp. strain as an active ingredient.

In one example of the present invention, the food may be a health functional food, but is not limited thereto.

Additionally, the present invention provides a method for preventing, improving, or treating obesity, comprising administering a pharmaceutically effective amount of a

composition comprising extracellular vesicles derived from a *Bifidobacterium* spp. strain as an active ingredient to a subject in need thereof.

Additionally, the present invention provides a use of a composition comprising extracellular vesicles derived from a *Bifidobacterium* spp. strain as an active ingredient for preventing, improving, or treating obesity.

Additionally, the present invention provides a use of a composition comprising extracellular vesicles derived from a *Bifidobacterium* spp. strain as an active ingredient in the manufacture of an agent for preventing, improving, or treating obesity.

[Advantageous Effects]

According to the pharmaceutical composition for preventing or treating obesity and the food composition for preventing or improving obesity, each comprising extracellular vesicles derived from a *Bifidobacterium* spp. strain as an active ingredient, the composition of the present invention exhibits an excellent anti-obesity effect by reducing body weight in an obese animal model and reducing elevated hepatic triglyceride and blood total cholesterol levels, the weight of white adipose tissue, and the size of adipocytes without causing a decrease in appetite, and thus may be usefully utilized as a food composition for preventing or improving obesity and/or a pharmaceutical composition for treating obesity.

[Description of Drawings]

FIG. 1 shows the results of measuring the particle size of extracellular vesicles derived from a *Bifidobacterium* spp. strain.

FIG. 2 is an experimental schematic diagram for confirming the anti-obesity effect of extracellular vesicles derived from *Bifidobacterium longum* (BL-EV) in an obese animal model established by feeding a high-calorie diet.

FIG. 3A shows changes in body shape after administration of extracellular vesicles derived from *Bifidobacterium longum* for four weeks in an obese animal model.

FIG. 3B is a graph showing the results of actual body weight (g) and body weight change rate (%) measured during administration of extracellular vesicles derived from *Bifidobacterium longum* for four weeks in an obese animal model (in the actual body weight graph, in order from top to bottom at the leftmost point of the x-axis (day 1), BL-EV, Vehicle, and Normal; in order from top to bottom at the rightmost point of the x-axis (day 29), Vehicle, Normal, and BL-EV) (in the body weight change rate graph, in order from top to bottom at the rightmost point of the x-axis (day 29), Normal, Vehicle, and BL-EV).

FIG. 3C is a graph showing the results of measuring food intake during administration of extracellular vesicles derived from *Bifidobacterium longum* for four weeks in an obese animal model (left bar graph-Vehicle; right bar graph-BL-EV).

FIG. 3D is a graph showing the results of measuring the triglyceride level per tissue weight (mg/g) in liver tissue and the triglyceride level in the entire liver tissue (mg/liver) after administration of extracellular vesicles derived from *Bifidobacterium longum* for four weeks in an obese animal model.

FIG. 3E shows the results of measuring triglycerides, low-density lipoprotein, high-density lipoprotein, and total cholesterol in the blood after administration of extracellular vesicles derived from *Bifidobacterium longum* for four weeks in an obese animal model.

FIG. 3F shows the results of measuring the weight of white adipose tissue responsible for energy storage in the body after administration of extracellular vesicles derived from *Bifidobacterium longum* for four weeks in the obese animal model of FIG. 3E.

FIG. 3G shows the results of analyzing fat accumulation and adipocyte size in the liver, white adipose tissue, and brown adipose tissue after administration of extracellular vesicles

derived from *Bifidobacterium longum* for four weeks in the obese animal model of FIG. 3E.

FIGS. 4A to 4D show the results of analyzing the expression of lipid synthesis-related genes after treating 3T3-L1 mouse adipocytes with extracellular vesicles derived from *Bifidobacterium adolescentis* (BA-EV), extracellular vesicles derived from *Bifidobacterium bifidum* (BB-EV), extracellular vesicles derived from *Bifidobacterium breve* (BBR-EV), and extracellular vesicles derived from *Bifidobacterium lactis* (BLA-EV), respectively, and inducing fat accumulation with oleic acid.

[Best Mode]

The present invention provides a pharmaceutical composition for preventing or treating obesity, comprising extracellular vesicles derived from a *Bifidobacterium* spp. strain as an active ingredient.

The present invention relates to a pharmaceutical composition for preventing or treating obesity, comprising extracellular vesicles derived from a *Bifidobacterium* spp. strain as an active ingredient, and the like, and was completed by confirming that extracellular vesicles derived from various *Bifidobacterium* spp. strains may exhibit potent anti-obesity effects. Therefore, the extracellular vesicles of the present invention may be used in a pharmaceutical composition for preventing or treating obesity, and the like.

In the present invention, the term "*Bifidobacterium* spp. strain or strain of the genus *Bifidobacterium*" refers to a strain belonging to the genus *Bifidobacterium* and may include strains already known in the art or newly identified in the future. *Bifidobacterium* is a Gram-positive anaerobic bacterium and is one of the major types of bacteria constituting the gastrointestinal microbiota of mammals, while some *Bifidobacterium* spp. strains are known to also be used as lactic acid bacteria. The *Bifidobacterium* spp. strain according to the present invention may include, without limitation, any strain belonging to the genus *Bifidobacterium*

and is not limited to a specific type.

In one example of the present invention, it was confirmed that BL-EV (extracellular vesicles derived from *Bifidobacterium longum*), which are extracellular vesicles derived from the genus *Bifidobacterium*, exhibited an effect of reducing body weight, an effect of reducing lipids and proteins, an effect of reducing the weight of white adipose tissue, and effects of reducing fat accumulation and adipocyte size in the liver, white adipose tissue, and brown adipose tissue in an obese animal model. Additionally, in addition to *Bifidobacterium longum*, BA-EV (extracellular vesicles derived from *Bifidobacterium adolescentis*), BB-EV (extracellular vesicles derived from *Bifidobacterium bifidum*), BBR-EV (extracellular vesicles derived from *Bifidobacterium breve*), and BLA-EV (extracellular vesicles derived from *Bifidobacterium lactis*), which are extracellular vesicles derived from various species of the genus *Bifidobacterium*, were confirmed to significantly reduce the expression of lipid synthesis-related genes.

Therefore, in one example of the present invention, the *Bifidobacterium* spp. strain may be at least one selected from the group consisting of *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, and *Bifidobacterium lactis*, but is not limited thereto.

In one example of the present invention, the particle sizes of BL-EV, BA-EV, BB-EV, BBR-EV, and BLA-EV were analyzed. In this case, the particle size may refer to the diameter of the extracellular vesicles of the present invention.

In one example of the present invention, the size of the extracellular vesicles may be 10 nm to 400 nm, but is not limited thereto. For example, the size may be 10 nm to 300 nm, 10 nm to 200 nm, 10 nm to 190 nm, 10 nm to 180 nm, 10 nm to 170 nm, 10 nm to 168 nm, 10 nm to 165 nm, 10 nm to 162 nm, 30 nm to 400 nm, 30 nm to 300 nm, 30 nm to 200 nm, 30 nm to

190 nm, 30 nm to 180 nm, 30 nm to 170 nm, 30 nm to 168 nm, 30 nm to 165 nm, 30 nm to 162 nm, 60 nm to 400 nm, 60 nm to 300 nm, 60 nm to 200 nm, 60 nm to 190 nm, 60 nm to 180 nm, 60 nm to 170 nm, 60 nm to 168 nm, 60 nm to 165 nm, 60 nm to 162 nm, 90 nm to 400 nm, 90 nm to 300 nm, 90 nm to 200 nm, 90 nm to 190 nm, 90 nm to 180 nm, 90 nm to 170 nm, 90 nm to 168 nm, 90 nm to 165 nm, or 90 nm to 162 nm, but is not limited thereto.

The composition of the present invention may exhibit an anti-obesity effect by comprising extracellular vesicles derived from a strain of the genus *Bifidobacterium* as an active ingredient. In this case, the active ingredient may further include, in addition to the extracellular vesicles derived from a strain of the genus *Bifidobacterium*, a strain of the genus *Bifidobacterium* capable of producing or containing extracellular vesicles derived from a strain of the genus *Bifidobacterium*, and a culture thereof, but is not limited thereto.

In the present invention, the term “strain” includes not only live bacteria themselves obtained from a culture, such as a culture medium, but also any processed form of the strain known to those skilled in the art, and may include, for example, a bacterial cell lysate, a dried product, a frozen product, and the like, but is not limited thereto. In the present invention, the term “strain” may be used interchangeably with “bacterium,” “bacterial cell,” and the like.

In the present invention, the term “lysate” may refer to a product obtained by disrupting the cell wall of a strain through chemical treatment or the application of physical force to the strain, but is not limited thereto.

In the present invention, the term “culture” refers to an entire medium comprising a strain, metabolites thereof, remaining nutrients, and the like, obtained by culturing the strain for a predetermined period in a medium capable of supplying nutrients so that the strain may grow or survive *in vitro*. That is, the culture may refer to the culture itself obtained by culturing the strain. Additionally, the culture may include a culture obtained by removing bacterial cells

from a bacterial cell culture obtained by culturing the strain. In this case, the liquid obtained by removing bacterial cells from the culture may be interchangeably referred to as a “supernatant,” “culture supernatant,” “conditioned culture medium,” or “conditioned medium.” The supernatant may be obtained by allowing the culture to stand for a predetermined period and collecting only the upper liquid excluding the portion settled in the lower layer, removing bacterial cells through filtration, or centrifuging the culture to remove the precipitate at the bottom and collecting only the upper liquid. In addition, the culture may further include an extract of the culture, a concentrate thereof, or a lyophilizate thereof; or a culture supernatant obtained by removing the strain from the culture, as well as an extract thereof, a concentrate thereof, or a lyophilizate thereof; but is not limited thereto. The culture may be obtained by culturing the strain of the present invention in an appropriate medium (for example, an RCM (reinforced clostridial medium) medium) at any temperature selected from 10°C to 50°C, 10°C to 40°C, 20°C to 50°C, 20°C to 40°C, or 30°C to 40°C for a predetermined period, for example, 4 to 50 hours, 4 to 40 hours, 4 to 30 hours, 4 to 20 hours, or 10 to 20 hours, but is not limited thereto. A culture medium and culture conditions for culturing the strain of the present invention may be appropriately selected or modified and used by a person of ordinary skill in the art.

In the present invention, the term “concentrate” may be obtained by concentrating the above-described culture itself or a supernatant obtained from the culture by a method such as centrifugation or filtration.

In the present invention, the term “extract” refers to a product extracted from the above-described culture or a concentrate thereof, and may include an extract solution, a dilution or concentrate of the extract solution, a dried product or lyophilizate obtained by drying the extract solution, a preparation or purified product thereof, or a fraction obtained by

fractionating the same.

In the present invention, the term “bacterial cell” refers to the strain itself and may include the strain itself isolated and selected from a biological sample or the like, or the strain isolated from a culture in which the strain has been cultured. The bacterial cells may be obtained by centrifuging the culture and collecting the portion settled in the lower layer, or may be obtained by allowing the culture to stand for a predetermined period and then removing the upper liquid because the bacterial cells settle in the lower layer of the culture by gravity, but are not limited thereto and may be obtained by a method commonly used in the art.

In the present invention, the term “extracellular vesicle (EV)” refers to a membrane-structured vesicle surrounded by a membrane and released from a cell, and is a structure responsible for intercellular signal transmission and material transport. Extracellular vesicles may be classified as exosomes, microvesicles, or apoptotic bodies according to their size and origin. Exosomes are formed in multivesicular endosomes and then released by fusion with the cell membrane, and mainly comprise proteins, lipids, RNA, and the like. Microvesicles are released by directly budding from the cell membrane and play an important role in interactions with the surrounding environment. Apoptotic bodies are formed by fragmentation of the cytoplasm during the process of cell death and are associated with immune responses. These extracellular vesicles are found in body fluids such as blood, saliva, and urine, and are involved in various physiological processes and disease progression.

Extracellular vesicles may reflect the state of the cells of origin (donor cells) that secrete them, exhibit various biological activities depending on the cells from which they are secreted, and play an important role in intercellular interactions by transferring genetic materials and proteins between cells. Additionally, cell-derived materials comprising extracellular vesicles have effects of stimulating immune cells to combat diseases and

facilitating the breakdown and absorption, through the metabolic processes of microorganisms, of substances that humans are unable to digest. Extracellular vesicles are membrane-structured vesicles having distinct internal and external regions, contain cellular plasma membrane lipids, plasma membrane proteins, nucleic acids, cytoplasmic components, and the like, and may be smaller than the cells from which they are derived.

In the present invention, the term “extracellular vesicle” may be used interchangeably with “extracellular vesicle” and may be referred to as a “vesicle,” or the like.

The extracellular vesicles according to the present invention may be produced during *in vitro* culture of the strain. Specifically, the vesicles according to the present invention may be naturally secreted from a strain of the genus *Bifidobacterium* or artificially produced (isolated) by subjecting the bacteria to heat treatment, pressure treatment, or the like, but are not limited thereto. Therefore, in one example of the present invention, the extracellular vesicles may be naturally secreted from or artificially produced from a strain of the genus *Bifidobacterium*, but are not limited thereto.

In one example of the present invention, the composition may be characterized by at least one selected from the group consisting of the following, but is not limited thereto:

a) weight loss;

b) maintenance of food intake;

c) reduction of triglycerides, low-density lipoproteins, high-density lipoproteins, and total cholesterol;

d) reduction of fat accumulation and cell size in the liver, white fat, and brown fat; and

e) reduction of fat synthesis gene expression.

In one example of the present invention, it was confirmed that treatment with BA-EV, BB-EV, BBR-EV, and BLA-EV, which are extracellular vesicles derived from

Bifidobacterium adolescentis, *Bifidobacterium bifidum*, *Bifidobacterium breve*, and *Bifidobacterium lactis*, respectively, exhibited a markedly superior anti-obesity effect through a reduction in the expression of lipid synthesis-related genes. In this case, Fasn, Acc1, Scd1, and Pparg were analyzed as lipid synthesis-related genes.

In the present invention, "Fasn (Fatty Acid Synthase)" is an enzyme responsible for fatty acid synthesis and primarily promotes the synthesis of palmitic acid (C16:0). Fasn is a multifunctional enzyme that synthesizes fatty acids in the cytoplasm using acetyl-CoA and malonyl-CoA. Fasn is known to exhibit increased expression in various diseases associated with lipid metabolism, particularly obesity and cancer.

In the present invention, "Acc1 (Acetyl-CoA Carboxylase 1)" is an enzyme that converts acetyl-CoA into malonyl-CoA and regulates the rate-determining step of fatty acid biosynthesis. Acc1 primarily regulates fatty acid synthesis in the liver and adipose tissue and is associated with insulin signaling. When the activity of Acc1 is increased, lipid synthesis is promoted, and when the activity of Acc1 is inhibited, fatty acid oxidation is increased.

In the present invention, "Scd1 (Stearoyl-CoA Desaturase 1)" is a Δ9-fatty acid desaturase that converts saturated fatty acids into unsaturated fatty acids. Scd1 contributes to the regulation of membrane lipid composition and signal transduction by converting palmitic acid and stearic acid into oleic acid and the like. Increased expression of Scd1 may be associated with obesity, insulin resistance, and hepatic steatosis.

In the present invention, "Pparg (Peroxisome Proliferator-Activated Receptor Gamma)" is a nuclear receptor transcription factor and plays a key role in the regulation of adipocyte differentiation and lipid metabolism. Pparg is primarily expressed in adipose tissue, enhances insulin sensitivity, and exerts anti-inflammatory effects. Thiazolidinedione drugs, which are agonists of Pparg, are used for the treatment of type 2 diabetes.

The pharmaceutical composition according to the present invention may be used by being formulated, according to commonly used methods, into a form such as powders, granules, sustained-release-type granules, enteric granules, liquids, eye drops, elixirs, emulsions, suspensions, spirits, troches, aromatic water, lemonades, tablets, sustained-release-type tablets, enteric tablets, sublingual tablets, hard capsules, soft capsules, sustained-release-type capsules, enteric capsules, pills, tinctures, soft extracts, dry extracts, fluid extracts, injections, capsules, perfusates, or a preparation for external use, such as plasters, lotions, pastes, sprays, inhalants, patches, sterile injectable solutions, or aerosols. The preparation for external use may have a formulation such as creams, gels, patches, sprays, ointments, plasters, lotions, liniments, pastes, or cataplasmas.

As the carrier, the excipient, and the diluent that may be included in the pharmaceutical composition according to the present invention, lactose, dextrose, sucrose, oligosaccharides, sorbitol, mannitol, xylitol, erythritol, maltitol, starch, acacia rubber, alginate, gelatin, calcium phosphate, calcium silicate, cellulose, methyl cellulose, microcrystalline cellulose, polyvinylpyrrolidone, water, methyl hydroxybenzoate, propyl hydroxybenzoate, talc, magnesium stearate, and mineral oil may be used.

For formulation, commonly used diluents or excipients such as fillers, thickeners, binders, wetting agents, disintegrants, and surfactants are used.

As additives of tablets, powders, granules, capsules, pills, and troches according to the present invention, excipients such as corn starch, potato starch, wheat starch, lactose, white sugar, glucose, fructose, D-mannitol, precipitated calcium carbonate, synthetic aluminum silicate, dibasic calcium phosphate, calcium sulfate, sodium chloride, sodium hydrogen carbonate, purified lanolin, microcrystalline cellulose, dextrin, sodium alginate, methyl cellulose, sodium carboxymethylcellulose, kaolin, urea, colloidal silica gel, hydroxypropyl

starch, hydroxypropyl methylcellulose (HPMC), HPMC 1928, HPMC 2208, HPMC 2906, HPMC 2910, propylene glycol, casein, calcium lactate, and Primojel®; and binders such as gelatin, Arabic gum, ethanol, agar powder, cellulose acetate phthalate, carboxymethylcellulose, calcium carboxymethylcellulose, glucose, purified water, sodium caseinate, glycerin, stearic acid, sodium carboxymethylcellulose, sodium methylcellulose, methylcellulose, microcrystalline cellulose, dextrin, hydroxycellulose, hydroxypropyl starch, hydroxymethylcellulose, purified shellac, starch, hydroxypropyl cellulose, hydroxypropyl methylcellulose, polyvinyl alcohol, and polyvinylpyrrolidone may be used, and disintegrants such as hydroxypropyl methylcellulose, corn starch, agar powder, methylcellulose, bentonite, hydroxypropyl starch, sodium carboxymethylcellulose, sodium alginate, calcium carboxymethylcellulose, calcium citrate, sodium lauryl sulfate, silicic anhydride, 1-hydroxypropylcellulose, dextran, ion-exchange resin, polyvinyl acetate, formaldehyde-treated casein and gelatin, alginic acid, amylose, guar gum, sodium bicarbonate, polyvinylpyrrolidone, calcium phosphate, gelled starch, Arabic gum, amylopectin, pectin, sodium polyphosphate, ethyl cellulose, white sugar, magnesium aluminum silicate, a di-sorbitol solution, and light anhydrous silicic acid; and lubricants such as calcium stearate, magnesium stearate, stearic acid, hydrogenated vegetable oil, talc, lycopodium powder, kaolin, Vaseline, sodium stearate, cacao butter, sodium salicylate, magnesium salicylate, polyethylene glycol (PEG) 4000, PEG 6000, liquid paraffin, hydrogenated soybean oil (Lubri wax), aluminum stearate, zinc stearate, sodium lauryl sulfate, magnesium oxide, Macrogol, synthetic aluminum silicate, silicic anhydride, higher fatty acids, higher alcohols, silicone oil, paraffin oil, polyethylene glycol fatty acid ether, starch, sodium chloride, sodium acetate, sodium oleate, dl-leucine, and light anhydrous silicic acid may be used.

As additives of liquids according to the present invention, water, dilute hydrochloric

acid, dilute sulfuric acid, sodium citrate, monostearic acid sucrose, polyoxyethylene sorbitol fatty acid esters (twin esters), polyoxyethylene monoalkyl ethers, lanolin ethers, lanolin esters, acetic acid, hydrochloric acid, ammonia water, ammonium carbonate, potassium hydroxide, sodium hydroxide, prolamine, polyvinylpyrrolidone, ethylcellulose, and sodium carboxymethylcellulose may be used.

In syrups according to the present invention, a white sugar solution, other sugars or sweeteners, and the like may be used, and as necessary, a fragrance, a colorant, a preservative, a stabilizer, a suspending agent, an emulsifier, a viscous agent, or the like may be used.

In emulsions according to the present invention, purified water may be used, and as necessary, an emulsifier, a preservative, a stabilizer, a fragrance, or the like may be used.

In suspensions according to the present invention, suspending agents such as acacia, tragacanth, methylcellulose, carboxymethylcellulose, sodium carboxymethylcellulose, microcrystalline cellulose, sodium alginate, hydroxypropyl methylcellulose (HPMC), HPMC 1828, HPMC 2906, HPMC 2910, and the like may be used, and as necessary, a surfactant, a preservative, a stabilizer, a colorant, and a fragrance may be used.

Injections according to the present invention may include: solvents such as distilled water for injection, a 0.9% sodium chloride solution, Ringer's solution, a dextrose solution, a dextrose+sodium chloride solution, PEG, lactated Ringer's solution, ethanol, propylene glycol, non-volatile oil-sesame oil, cottonseed oil, peanut oil, soybean oil, corn oil, ethyl oleate, isopropyl myristate, and benzene benzoate; cosolvents such as sodium benzoate, sodium salicylate, sodium acetate, urea, urethane, monoethylacetamide, butazolidine, propylene glycol, the Tween series, amide nicotinate, hexamine, and dimethylacetamide; buffers such as weak acids and salts thereof (acetic acid and sodium acetate), weak bases and salts thereof (ammonia and ammonium acetate), organic compounds, proteins, albumin, peptone, and gums; isotonic

agents such as sodium chloride; stabilizers such as sodium bisulfite (NaHSO3) carbon dioxide gas, sodium metabisulfite (Na2S2O5), sodium sulfite (Na2SO3), nitrogen gas (N2), and ethylenediamine tetraacetic acid; sulfating agents such as 0.1% sodium bisulfide, sodium formaldehyde sulfoxylate, thiourea, disodium ethylenediaminetetraacetate, and acetone sodium bisulfite; a pain relief agent such as benzyl alcohol, chlorobutanol, procaine hydrochloride, glucose, and calcium gluconate; and suspending agents such as sodium CMC, sodium alginate, Tween 80, and aluminum monostearate.

In suppositories according to the present invention, bases such as cacao butter, lanolin, Witepsol, polyethylene glycol, glycerogelatin, methylcellulose, carboxymethylcellulose, a mixture of stearic acid and oleic acid, Subanal, cottonseed oil, peanut oil, palm oil, cacao butter + cholesterol, lecithin, lanette wax, glycerol monostearate, Tween or span, imhausen, monolan(propylene glycol monostearate), glycerin, Adeps solidus, buytyrum Tego-G, cebes Pharma 16, hexalide base 95, cotomar, Hydrokote SP, S-70-XXA, S-70-XX75(S-70-XX95), Hydrokote 25, Hydrokote 711, idropostal, massa estrarium (A, AS, B, C, D, E, I, T), masa-MF, masupol, masupol-15, neosuppostal-N, paramount-B, supposiro OSI, OSIX, A, B, C, D, H, L, suppository base IV types AB, B, A, BC, BBG, E, BGF, C, D, 299, suppostal N, Es, Wecoby W, R, S, M, Fs, and tegester triglyceride matter (TG-95, MA, 57) may be used.

Solid preparations for oral administration include tablets, pills, powders, granules, capsules, and the like, and such solid preparations are formulated by mixing the composition with at least one excipient, e.g., starch, calcium carbonate, sucrose, lactose, gelatin, and the like. In addition to simple excipients, lubricants such as magnesium stearate and talc are also used.

Examples of liquid preparations for oral administration include suspensions, liquids for internal use, emulsions, syrups, and the like, and these liquid preparations may include, in

addition to simple commonly used diluents, such as water and liquid paraffin, various types of excipients, for example, a wetting agent, a sweetener, a fragrance, a preservative, and the like. Preparations for parenteral administration include an aqueous sterile solution, a non-aqueous solvent, a suspension, an emulsion, a freeze-dried preparation, and a suppository. Non-limiting examples of the non-aqueous solvent and the suspension include propylene glycol, polyethylene glycol, a vegetable oil such as olive oil, and an injectable ester such as ethyl oleate.

The pharmaceutical composition according to the present invention is administered in a pharmaceutically effective amount. In the present invention, "the pharmaceutically effective amount" refers to an amount sufficient to treat diseases at a reasonable benefit/risk ratio applicable to medical treatment, and an effective dosage level may be determined according to factors including types of diseases of patients, the severity of disease, the activity of drugs, sensitivity to drugs, administration time, administration route, excretion rate, treatment period, and simultaneously used drugs, and factors well known in other medical fields.

The composition according to the present invention may be administered as an individual therapeutic agent or in combination with other therapeutic agents, may be administered sequentially or simultaneously with therapeutic agents in the related art, and may be administered in a single dose or multiple doses. It is important to administer the composition in a minimum amount that can obtain the maximum effect without any side effects, in consideration of all the aforementioned factors, and this may be easily determined by those of ordinary skill in the art.

The pharmaceutical composition of the present invention may be administered to a subject via various routes. All administration methods can be predicted, and the pharmaceutical composition may be administered via, for example, oral administration,

subcutaneous injection, intraperitoneal injection, intravenous injection, intramuscular injection, intrathecal (space around the spinal cord) injection, sublingual administration, administration via the buccal mucosa, intrarectal insertion, intravaginal insertion, ocular administration, intra-aural administration, intranasal administration, inhalation, spraying via the mouth or nose, transdermal administration, percutaneous administration, or the like.

The pharmaceutical composition of the present invention is determined depending on the type of a drug, which is an active ingredient, along with various related factors such as a disease to be treated, administration route, the age, gender, and body weight of a patient, and the severity of diseases.

As used herein, the “subject” refers to a subject in need of treatment of a disease, and more specifically, refers to a mammal such as a human or a non-human primate, a mouse, a rat, a dog, a cat, a horse, and a cow.

As used herein, the "administration" refers to providing a subject with a predetermined composition of the present invention by using an arbitrary appropriate method.

The term "prevention" as used herein means all actions that inhibit or delay the onset of a target disease. The term "treatment" as used herein means all actions that alleviate or beneficially change a target disease and abnormal metabolic symptoms caused thereby via administration of the pharmaceutical composition according to the present invention. The term "alleviation" as used herein means all actions that reduce the degree of parameters related to a target disease, e.g., symptoms via administration of the composition according to the present invention.

The present invention provides a kit for preventing or treating obesity, comprising extracellular vesicles derived from a strain of the genus *Bifidobacterium* (*Bifidobacterium* spp.), or a composition comprising the extracellular vesicles as an active ingredient; and instructions.

In the present invention, the term “kit” refers to a tool that enables the prevention or treatment of obesity using the extracellular vesicles derived from a strain of the genus *Bifidobacterium* according to the present invention. The kit of the present invention may include, in addition to the materials, other components, compositions, solutions, devices, and the like conventionally required for the storage and treatment thereof. As a specific example, in this case, each component may be applied one or more times without limitation on the number of applications, the order in which each material is applied is not limited, and the respective materials may be applied simultaneously or at different times.

In the present invention, the kit may include a container; instructions; and the like. The container may serve to package the materials and may also serve to store and secure the materials. The container may take the form of, for example, a bottle, tub, sachet, envelope, tube, ampoule, or the like, and may be formed partially or entirely from plastic, glass, paper, foil, wax, or the like. The container may be provided with a fully or partially removable closure that is initially part of the container or may be attached to the container by mechanical, adhesive, or other means, and may also be provided with a stopper that permits access to the contents by a needle. The kit may include an outer package, and the outer package may include instructions regarding the use of the components.

The present invention provides a food composition for preventing or ameliorating obesity, comprising extracellular vesicles derived from a strain of the genus *Bifidobacterium* (*Bifidobacterium* spp.) as an active ingredient.

In one example of the present invention, the food may be a health functional food, but is not limited thereto.

In the present invention, the term “food” refers to a natural product or processed product containing one or more nutrients, preferably refers to a product that has undergone a

certain degree of processing and is in a state suitable for direct consumption, and, in its ordinary meaning, encompasses health functional foods, beverages, food additives, beverage additives, and the like.

In the present invention, the term “health functional food (functional food),” which is synonymous with a food for special health use (FoSHU), refers to a food having high medicinal and medical effects that has been processed so that biological regulatory functions are efficiently exhibited in addition to providing nutrition, and may be prepared in the form of a tablet, capsule, pill, granule, powder, liquid, flake, paste, syrup, gel, jelly, bar, or film formulation. As used herein, the term “functionality” refers to obtaining effects useful for health purposes, such as regulating nutrients or exerting physiological actions with respect to the structure and function of the human body.

In the present invention, the type of health functional food is not particularly limited. Specifically, examples of foods to which the composition of the present invention may be added include dairy products including ice cream products, various soups, beverages, teas, health drinks, alcoholic beverages, vitamin complexes, and the like, and particularly include all food groups to which added value has been imparted so that the functions of the corresponding foods in the ordinary sense act and are exhibited for a specific purpose, or foods designed so that physiological regulatory functions of a food composition relating to biological defense, rhythm regulation, disease prevention and recovery, and the like are sufficiently exhibited in the body.

In the present invention, the term “food additive” refers to a substance used by addition to, mixing with, infiltration into, or any other method with respect to food in the manufacture, processing, or preservation of the food, and should be harmless to the human body when consumed over a long period, as with a health functional food.

When the composition of the present invention is used as a food additive, the food additive may be added in the form of the composition of the present invention as it is or used together with another food or food ingredient, and may be appropriately used according to a conventional method.

The amount of the active ingredient mixed may be appropriately determined according to the intended use (prevention, health, or therapeutic treatment). Generally, in the manufacture of a food or beverage, the composition of the present invention may be added in an amount of 15 wt% or less, or 10 wt% or less, based on the raw materials. However, in the case of long-term consumption for health and hygiene purposes or health regulation purposes, the amount may be at or below the range, and because there is no problem in terms of safety, the active ingredient may also be used in an amount exceeding the range.

In the present invention, the composition may include various food-acceptable auxiliary additives and may further include suitable carriers, excipients, and diluents conventionally used in the manufacture of foods.

Additionally, in addition to the foregoing, the composition of the present invention may contain various nutrients, vitamins, electrolytes, flavoring agents, coloring agents, pectic acid and salts thereof, alginic acid and salts thereof, organic acids, protective colloidal thickeners, pH adjusting agents, stabilizers, preservatives, glycerin, alcohol, carbonating agents used in carbonated beverages, and the like. In addition, the composition of the present invention may contain fruit pulp for the manufacture of natural fruit juices, fruit juice beverages, and vegetable beverages. These ingredients may be used independently or in combination. Although the proportion of such additives is not particularly important, it is generally selected within a range of 0.01-0.20 parts by weight per 100 parts by weight of the composition of the present invention, but is not limited thereto, and may be an optimal or arbitrary amount

depending on the type, function, and the like of the product in which the composition is used.

In the present invention, the type of food is not particularly limited. Examples of foods to which the materials may be added include meat, sausages, bread, chocolate, candies, snacks, confectionery products, pizza, instant noodles, other noodle products, chewing gum, dairy products including ice cream products, various soups, beverages, teas, health drinks, alcoholic beverages, vitamin complexes, and the like, and may include all health functional foods in the ordinary sense, but are not limited thereto.

Additionally, the composition according to the present invention may also be added to health beverages and may contain various flavoring agents, natural carbohydrates, and the like as additional ingredients, as in conventional beverages. The natural carbohydrates described above include monosaccharides such as glucose and fructose, disaccharides such as maltose and sucrose, polysaccharides such as dextrin and cyclodextrin, and sugar alcohols such as xylitol, sorbitol, and erythritol. As a sweetener, natural sweeteners such as thaumatin and stevia extract, synthetic sweeteners such as saccharin and aspartame, and the like may be used. The proportion of the natural carbohydrates may generally be about 0.01-0.20 g, or about 0.04-0.10 g, per 100 mL of the composition of the present invention, but is not limited thereto, and may be a conventional amount added in the art, may include a maximum range to enhance the efficacy of the composition of the present invention, and may include an optimal or arbitrary amount determined in consideration of synergistic effects with other materials added together.

The present invention provides a feed composition for preventing or ameliorating obesity, comprising extracellular vesicles derived from a strain of the genus *Bifidobacterium* (*Bifidobacterium* spp.) as an active ingredient.

In the present invention, the term “feed” refers to food intended to be eaten, consumed, and digested by an animal. The feed may provide organic or inorganic nutrients necessary for

maintaining the life of an animal. In this case, the term “feed” may encompass a “feed additive,” and, in the present invention, the feed additive may be a substance added to feed and used for auxiliary purposes, such as promoting the healthy growth of an animal, improving productivity, or improving the quality of life of an animal. Therefore, the present invention provides a feed additive composition for preventing or ameliorating obesity, comprising extracellular vesicles derived from a strain of the genus *Bifidobacterium* (*Bifidobacterium* spp.) as an active ingredient. In the present invention, when the feed composition is used as a feed additive, the feed composition may exhibit an anti-obesity effect by being added to and fed with conventional feed.

In the present invention, the feed may be fed to an animal alone, may be fed while further comprising a carrier, diluent, excipient, additive, and oxidation inhibitor, or may be fed while further comprising an antioxidant, preservative, surfactant, seasoning agent, texturizing agent, stabilizer, coloring agent, or flavoring agent. Additionally, in the present invention, when the term “feed” refers to a feed additive, the feed may be administered alone or together with feed according to a method conventionally employed in the art. In addition, in the present invention, when the term “feed” refers to a feed additive, the matters described in the present invention or conventionally employed in the art may be applied.

In the present invention, the term “carrier” refers to a material suitable for use in feed that may support an active ingredient to improve dispersibility and has excellent chemical stability and adsorptivity, and includes organic carriers and inorganic carriers. The organic carrier is a material containing a large amount of crude fiber and may include corn flour, corn cob powder, wheat bran, rice bran powder, defatted rice bran, bran, corn stalk powder, peanut shell powder, and the like. The inorganic carrier is a mineral mainly classified into calcium salts and silicon oxides, is used in the production of trace element premixes, and may include

calcium carbonate, silicate, vermiculite, zeolite, sepiolite, and the like.

In the present invention, the term “diluent” refers to a material that uniformly distributes an additive raw material throughout a material and dilutes the high-concentration additive raw material into a low-concentration premix or preblend, may separate trace ingredients from one another and reduce interactions between active ingredients, thereby increasing the stability of the active ingredients without affecting the physicochemical properties of the relevant materials, and includes organic diluents and inorganic diluents. The organic diluent may include corn flour, degerminated corn flour, dextrose (glucose), sucrose, semolina including wheat bran, roasted soybean flour, wheat middlings, corn gluten meal, and the like, and the inorganic diluent may include limestone, calcium dihydrogen phosphate, shell powder, kaolin, salt, and sodium sulfate.

In the present invention, the term “excipient” refers to a wetting agent that induces the inherent viscosity of a material, a binder that binds materials, a disintegrant that disintegrates a flake-shaped mass of the entire material into a large number of fine particles, a filtration aid that reduces friction between particles, or an anti-adherent that prevents adhesion of materials, and may include magnesium stearate, talc, vegetable oil, magnesium lauryl sulfate, starch, starch slurry, water, inorganic salts, dextrin, powdered sugar, and the like.

In the present invention, the term “additive” may refer to a nutritional feed additive, general feed additive, or medicinal feed additive. The nutritional feed additive refers to a substance used in a small or trace amount that is added to feed to balance the nutrients in the feed, improve feed utilization, and directly exert a nutritional effect on an animal, and may be an amino acid, an amino acid salt or an analog thereof, a vitamin or a vitamin analog, a co-mineral component or a conjugate (chelate) thereof, a microbial enzyme preparation, or non-protein nitrogen.

The general feed additive refers to a non-nutritive substance that is added to feed to improve feed utilization, ensure the quantity and quality of the feed, and benefit the health or metabolism of an animal, and may include a growth promoter, an antiparasitic health agent, a flavoring and feeding stimulant, a feed modifier, a feed formulation agent, a feed preservative, and a herbal medicine additive, and the growth promoter may be butyric acid, calcium butyrate, sodium butyrate, tannic acid, p-thymol, a p-thymol ester, a p-thymol salt, 2-hydroxybenzoic acid, a β-acid, a β-acid ester, a β-acid salt, a hexahydro-β-acid, a hexahydro-β-acid ester, a hexahydro-β-acid salt, benzoic acid or calcium benzoate, zinc oxide, zinc sulfate, or zinc chloride.

In the present invention, the feed composition may include a feed additive or medicinal feed additive. The medicinal feed additive has functions of preventing animal diseases and promoting animal growth, and may include a veterinary drug premix substance that is mixed with a carrier or diluent and may be added to feed for long-term use. For example, the medicinal feed additive may be a feed antibiotic, and the feed antibiotic may be polymyxin, salinomycin, avilamycin, bacitracin, virginiamycin, nosiheptide, flavomycin, enramycin, leucomycin, olaquindox, oxytetracycline, or chlortetracycline.

In the present invention, the term “oxidation inhibitor” refers to a substance capable of reacting with and neutralizing free radicals, and may be a carotenoid (including beta-carotene, lycopene, and lutein), selenium, coenzyme Q10 (ubiquinone), tocotrienol, soy isoflavone, S-adenosylmethionine, glutathione, taurine, N-acetylcysteine, vitamin E, vitamin C, lipoic acid, L-carnitine, or arginine, but is not limited thereto.

The feed may be a raw material containing water or a dry material from which water has been removed. When the feed is a dry material, the feed may be in the form of pellets, which are particulate masses or pieces, or may be in powder form. The pellets may have a

spherical, cylindrical, or elliptical shape. In the present invention, the raw material or dry material may be provided in a formulation conventionally used in the art. For example, the powder form may be dissolved in a solvent to be fed (e.g., water) and provided as a solution formulation, but is not limited thereto.

Additionally, in the present invention, the feed composition may be mixed with and fed together with a conventionally known feed composition for preventing or ameliorating obesity or a newly developed feed composition for preventing or ameliorating obesity. When the feed composition of the present invention further includes a feed composition for preventing or ameliorating obesity, it is important that an amount capable of achieving the maximum effect with the minimum amount without side effects be mixed, and this may be readily determined by a person skilled in the art. When the feed composition is consumed in combination with a known composition having an effect of preventing or ameliorating obesity or another feed composition for preventing or ameliorating obesity, the compositions may be consumed simultaneously, separately, or sequentially, and may be consumed in a single or multiple intake. Considering all of the factors, it is important to consume an amount capable of achieving the maximum effect with the minimum amount without side effects, and this may be readily determined by a person skilled in the art.

[Mode for Invention]

Hereinafter, preferred examples are presented to help understand the present invention. However, the following examples are provided only to help understand the present invention more easily, and the contents of the present invention are not limited by the following examples.

[EXAMPLES]

Example 1: Extraction and size measurement analysis of extracellular vesicles derived from *Bifidobacterium* spp. strains

Example 1-1: Extraction of extracellular vesicle derived from *Bifidobacterium* spp. strain

To extract extracellular vesicles (EVs) derived from *Bifidobacterium* spp. strains, the strains were cultured in a reinforced clostridial medium (RCM) (MB cell, MB-R1602) in an anaerobic chamber at 37 °C for 18 to 72 hours. The culture medium was centrifuged at 2000 × g for 20 minutes, and the supernatant was then collected to remove cell debris and waste from the culture medium using a 0.22 μm filter. An EV was extracted from each culture medium using a 300 kDa molecular weight tangential flow filtration (TFF) membrane filter-based TFF system, and diluted and purified in phosphate-buffered saline (PBS). Finally, the EVs derived from the *Bifidobacterium* spp. strains dispersed in PBS were extracted. EVs derived from *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, and *Bifidobacterium lactis* were extracted using the *Bifidobacterium* spp. strains, and were named BL-EV, BA-EV, BB-EV, BBR-EV, and BLA-EV, respectively.

Example 1-2: Size measurement analysis of EVs derived from *Bifidobacterium* spp. strains

The sizes of BL-EV, BA-EV, BB-EV, BBR-EV, and BLA-EV extracted in Example 1-1 were measured using a nanoparticle tracer (Zetaview, Particle Metrix GmbH) and confirmed as shown in FIG. 1.

Example 2: Construction of obese animal model and establishment of

experimental conditions for anti-obesity activity of BL-EV

To confirm the anti-obesity activity of the BL-EV of the present invention, an obese animal model was constructed. At this time, a diet-induced obesity (DIO) animal model was used as the obese animal model. Specifically, an obese animal model was induced by feeding mice a high-fat diet (60% fat) for 4 months, and the BL-EV of the present invention was fed to the obese animal model along with the high-fat diet (FIG. 2). At this time, obese mice having an average body weight of 40 g were selected, and BL-EV was orally administered daily along with the high-fat diet at a dose of 1 x 10^{12} particles for 4 weeks, while the control was orally administered PBS.

Example 3: Confirmation of anti-obesity effect by EV derived from *Bifidobacterium longum* in obese animal model

Example 3-1: Weight loss effect

To confirm the effect of BL-EV on weight loss, when BL-EV was orally administered to the obese animal model prepared in Example 2, the final volume changes of the mouse body shape and body weight were measured daily for 4 weeks and compared.

As a result, the body volume of the obesity group (Vehicle) increased compared to the normal group (Normal), whereas the BL-EV-administered group (hereinafter referred to as a BL-EV group) was visually observed to have a body volume similar to that of the normal group (FIG. 3A).

Also, as a result of daily weight measurements, the actual weight (g) and the percentage of weight change increased in the normal group, while the actual weight (g) and the percentage of weight change remained similar in the obesity group. On the other hand, the BL-EV group showed a tendency for the actual weight (g) and the percentage of weight change

to decrease significantly. Specifically, it was confirmed that the weight increased by 25% in the obese group compared to the normal group, but the weight decreased by 30% in the BL-EV group (FIG. 3B).

In addition, when the food intake of the obesity group and the BL-EV group was checked at weekly intervals, it was confirmed that the food intake of the obesity group and the BL-EV group did not show a statistically significant difference, confirming that they were at the same level (FIG. 3C).

Example 3-2: Lipid and protein reduction effects

To confirm the effects of BL-EV on lipids and proteins, BL-EV was orally administered to an obese animal model for 4 weeks, and triglycerides in liver tissue and triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and total cholesterol in the blood were measured. At this time, the triglycerides in liver tissue were evaluated by quantifying the triglycerides according to the manufacturer's procedure using a triglyceride assay kit (Fluorometric, Reducing Samples; ab65336).

As a result (FIG. 3D), the concentration of triglycerides per unit of liver tissue weight (mg/g) increased in the obesity group (Vehicle) that was fed a high-fat diet compared to the normal group (Normal) that was not fed a high-fat diet.

On the other hand, it was confirmed that the increased concentration of triglycerides decreased in mice administered BL-EV. Also, it was confirmed that the concentration of triglycerides included in the whole liver per individual also decreased in the same way.

Blood biochemical analysis was commissioned to TNP Bio Co., Ltd. To analyze changes in total cholesterol concentration in the blood, the concentrations of triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were measured, and total

cholesterol was calculated as LDL + HDL + 20% TG.

As a result (FIG. 3E), it was confirmed that the total cholesterol increased in the obesity group (Vehicle) that was fed a high-fat diet compared to the normal group (Normal) that was not fed a high-fat diet, whereas it was confirmed that the increased concentration of total cholesterol decreased in the BL-EV group.

Example 3-3: White fat weight reduction effect

To confirm the effect of BL-EV on white fat weight, BL-EV was orally administered to the obese animal model prepared in Example 2 for 4 weeks, and the total amount of white fat (Subcutaneous + Visceral) in the mouse trunk was then measured.

As a result (FIG. 3F), it was confirmed that the weight of white fat increased in the obesity group (Vehicle) that was fed a high-fat diet compared to the normal group (Normal) that was not fed a high-fat diet, whereas the increased weight of white fat decreased in the BL-EV group.

Examples 3-4: Effects on reduction of fat accumulation and adipocyte size in liver, white fat, and brown fat

To determine the effect of BL-EV on fat accumulation in the liver, white fat, and brown fat, BL-EV was orally administered to the obese animal model prepared in Example 2 for 4 weeks. The collected tissues were fixed in a 10% formalin solution, followed by paraffin block preparation and slide preparation. Thereafter, hematoxylin-eosin staining was performed. In this case, this process was commissioned to TNP Bio Co., Ltd.

As a result (FIG. 3G), the obesity group (Vehicle) that was fed a high-fat diet showed increased fat accumulation in the liver and brown fat compared to the normal group (Normal),

and showed an increase in the size of adipocytes in white fat. However, it was confirmed that the fat accumulation and the increase in the size of white fat cells were reduced in the BL-EV group.

Example 4: Confirmation of anti-obesity effects by EVs derived from *Bifidobacterium* spp. strains other than *Bifidobacterium longum*

In Example 3, it was confirmed that the EV derived from *Bifidobacterium longum*, which is a *Bifidobacterium* spp. strain, exhibited anti-obesity effects through various activities. In Example 4, the anti-obesity activities of EVs derived from the Bifidobacterium spp. strains other than *Bifidobacterium longum* were confirmed.

First, in addition to the EV (BL-EV) derived from *Bifidobacterium longum*, an EV derived from *Bifidobacterium adolescentis* (hereinafter referred to as "BA-EV"), an EV derived from *Bifidobacterium bifidum* (hereinafter referred to as "BB-EV"), an EV derived from *Bifidobacterium breve* (hereinafter referred to as "BBR-EV"), and an EV derived from *Bifidobacterium lactis* (hereinafter referred to as "BLA-EV") were prepared in the same manner as in Example 1.

To confirm the anti-obesity efficacy of each extracted EV, an analysis experiment was performed on the expression levels of lipid synthesis-related genes using 3T3-L1 adipocytes with induced excessive fat accumulation. Specifically, cells were cultured in a DMEM medium containing 10% bovine calf serum and 1% penicillin-streptomycin, and inoculated into a 6-well plate when the flask was filled to approximately 70%. Thereafter, the cells were cultured for another 2 days until the cells reached 100% confluency, and the medium was replaced with a differentiation medium (DMEM-based medium containing 10% fetal bovine serum, 1% penicillin-streptomycin, 500 μM isobutyl-1-methylxanthine, 1 μM dexamethasone,

10 μg/mL insulin, and 0.5 μM rosiglitazone) (Day 0 of differentiation). Then, the cells were cultured until day 4 of differentiation by replacing the medium with DMEM containing 10 μg/mL insulin and 0.5 μM Rosiglitazone every two days. On day 4 of differentiation, 50 μL of BA-EV, BB-EV, BBR-EV, and BLA-EV were added to each well to achieve a final concentration of 1×10^8 particles/1.5 mL, while the control (Vehicle) was treated with the same volume of PBS. Two hours after treatment, 500 μM oleic acid was added to induce fat accumulation for 72 hours. Subsequently, RNA was extracted, cDNA was synthesized, and real-time PCR was performed to analyze the expression of lipid synthesis-related genes.

[Table 1]

NO.	**NAME.**	**SEQUENCE**
1	Fasn-F	GCTGCGGAAACTTCAGGAAAT
2	Fasn-R	AGAGACGTGTCACTCCTGGACTT
3	Acc1-F	TGACAGACTGATCGCAGAGAAAG
4	Acc1-R	TGGAGAGCCCCACACACA
5	Scd1-F	CCCCTGCGGATCTTCCTTAT
6	Scd1-R	AGGGTCGGCGTGTGTTTCT
7	Pparg-F	GTACTGTCGGTTTCAGAAGTGCC
8	Pparg-R	ATCTCCGCCAACAGCTTCTCCT

As a result (FIGS. 4A to 4D), it was confirmed that the expression of Fasn, Acc1, Scd1, and Pparg, which are representative lipid synthesis-related genes, was significantly reduced in the group treated with the EVs derived from the *Bifidobacterium* spp. strains compared to the control (Vehicle). In particular, in the BA-EV treated group, the expression of all the lipid synthesis-related genes showed a tendency to decrease to a statistically significant level.

In conclusion, it was proven that, as the EVs derived from the *Bifidobacterium* spp.

strains, BL-EV exhibited various anti-obesity activities in the obese animal model, and BA-EV, BB-EV, BBR-EV, and BLA-EV had excellent anti-obesity effects by reducing the expression of the fat synthesis-related genes.

The foregoing description of the present invention is intended for illustrative purposes, and it will be understood by those skilled in the art that various modifications can be made thereto in other specific forms without departing from the technical spirit or essential features of the present invention. Therefore, the embodiments described above should be understood as illustrative in all respects and not restrictive.

[Industrial Applicability]

The present invention relates to a pharmaceutical composition for preventing or treating obesity, comprising extracellular vesicles derived from a strain of the genus *Bifidobacterium* as an active ingredient, and a food composition for preventing or ameliorating obesity, wherein the composition of the present invention has effects of reducing body weight in an obese animal model, reducing hepatic triglyceride levels, blood total cholesterol levels, and white adipose tissue weight, which are increased in the obese animal model, and reducing fat accumulation in the liver and brown adipose tissue and the size of white adipocytes, without causing a decrease in appetite. Additionally, the extracellular vesicles of the present invention have an effect of inhibiting lipid synthesis in adipocytes in which fat accumulation has been induced. As described above, the extracellular vesicles derived from a strain of the genus *Bifidobacterium* according to the present invention exhibit an excellent anti-obesity effect and thus have industrial applicability.

[CLAIMS]

[Claim 1]

A pharmaceutical composition for preventing or treating obesity, comprising an extracellular vesicle derived from a *Bifidobacterium* spp. strain as an active ingredient.

[Claim 2]

The pharmaceutical composition of claim 1, wherein the *Bifidobacterium* spp. strain is any one or more selected from the group consisting of *Bifidobacterium longum*, *Bifidobacterium adolescentis*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, and *Bifidobacterium lactis*.

[Claim 3]

The pharmaceutical composition of claim 1, wherein the extracellular vesicle has a size of 10 nm to 400 nm.

[Claim 4]

The pharmaceutical composition of claim 1, wherein the extracellular vesicle is naturally secreted or artificially produced from the *Bifidobacterium* spp. strain.

[Claim 5]

The pharmaceutical composition of claim 1, wherein the composition is characterized by any one or more selected from the group consisting of:

a) weight loss;

b) maintenance of food intake;

c) reduction of triglycerides, low-density lipoproteins, high-density lipoproteins, and total cholesterol;

d) reduction of fat accumulation and cell size in the liver, white fat, and brown fat; and

e) reduction of fat synthesis gene expression.

[Claim 6]

A kit for preventing or treating obesity, comprising an extracellular vesicle derived from a *Bifidobacterium* spp. strain, and instructions.

[Claim 7]

A food composition for preventing or improving obesity, comprising an extracellular vesicle derived from a *Bifidobacterium* spp. strain as an active ingredient.

[Claim 8]

The food composition of claim 7, wherein the food is a health functional food.

[Claim 9]

A feed composition for preventing or improving obesity, comprising an extracellular vesicle derived from a *Bifidobacterium* spp. strain as an active ingredient.

[Claim 10]

A method of improving or treating obesity, comprising:

administering a pharmaceutically effective amount of a composition comprising an extracellular vesicle derived from a *Bifidobacterium* spp. strain as an active ingredient to a

subject in need thereof.

[Claim 11]

Use of a composition comprising an extracellular vesicle derived from a *Bifidobacterium* spp. strain as an active ingredient for the prevention, improvement, or treatment of obesity.

[Claim 12]

Use of a composition comprising an extracellular vesicle derived from a *Bifidobacterium* spp. strain as an active ingredient for the manufacture of preparations for the prevention, improvement, or treatment of obesity