

MUSCLE SYNTHESIS PROMOTER

BACKGROUND OF THE INVENTION

[0001]

5 The present patent application is based on an earlier Japanese Patent Application No. 2013-189703 (application date: September 12, 2013). The present patent application claims priority to Japanese Patent Application No. 2013-189703. The entire contents of Japanese Patent Application No. 2013-189703 are incorporated
10 herein by reference.

[0002]

Technical Field

The present invention relates to a muscle synthesis promoter intended for use in a subject in need of rapid absorption of protein.

15 [0003]

Technical Background

Recently, many people (subjects) orally ingest nutrition supplements (supplements) containing proteins as a major component when they exercise in order to facilitate muscle synthesis,
20 to prevent muscle loss, and to promote the recovery of muscle mass for the purpose of improving their health and athletic performance. Such nutrition supplements include a protein powder whose major components are known to be whey protein (lactoserum protein) and casein protein from cow milk as a raw material.

25 [0004]

It is thought that whey protein can build muscle strength efficiently while preventing muscle fatigue, because the period from oral ingestion to digestion and absorption (absorption rate) is short (short period) and whey protein contains abundant BCAA
30 (branched-chain amino acids: valine, leucine, and isoleucine). On the other hand, casein protein is metabolized more slowly and continuously compared with whey protein. Therefore, the period from oral ingestion to digestion and absorption (absorption rate) is long (long period). Thus, it is thought that casein protein is
35 unsuitable for those who need rapid absorption of protein (refer to Patent literature 1). Therefore, whey protein is mainly used in such

protein powders.

[0005]

Through exploitation of the difference in their absorption rates, a mixed protein in which whey protein exhibiting rapid
5 absorption and casein protein exhibiting sustained absorption are mixed at the ratio of 1 to 1 has been developed (for example, DNS Protein Slow Loading).

[0006]

However, whey protein likely aggregates when heated
10 because it has a low heat resistance (thermal stability) leading to heat denaturation. Actually, methods except those for ingesting protein powders dissolved in water are not recommended. Thus, the protein powder undissolved in water causes a powdery taste in the mouth and does not convey high palatability. In addition, there
15 was a problem of the decrease in the absorption efficiency when whey protein was excessively heated, because it was solidified due to its heat denaturation.

PRIOR ART DOCUMENTS

20 [0007]

Patent Literatures

Patent literature 1: Japanese Translation of PCT International Application Publication No. 2009-507044

25

SUMMARY OF THE INVENTION

[0008]

As described above, to the knowledge of the inventors, there has been no such material or composition having high heat resistance and excellent processability while having rapid muscle
30 synthesis promoting effect equivalent to that of whey protein.

[0009]

The inventors have currently discovered unexpectedly a phenomenon in which a milk protein mixture formed by substituting a majority of whey protein with casein protein that
35 has so far been said to have no rapid-acting property according to conventional technologies because of slow rate of protein

absorption has a rapid muscle synthesis promoting activity equivalent to that of whey protein. The milk protein is composed of about 80% casein protein and about 20% whey protein, and is generally known to have absorbability similar to that of casein protein alone (refer to Judy A. Driskell, "Sports Nutrition Fats and Proteins" CRC Press, p149, 2007). Because of this, the above-mentioned phenomenon was surprising. Furthermore, the inventors have also found that this milk protein mixture has higher heat resistance and better processability compared with whey protein alone. The present invention is based on the above-mentioned findings.

[0010]

Namely, an object of the present invention is to provide a muscle synthesis (buildup) promoter that has a rapid muscle synthesis promoting activity equivalent to that of whey protein.

[0011]

According to the present invention, the following inventions are provided.

(1) A muscle synthesis promoter for use in a subject in need of rapid absorption of protein, comprising a milk protein mixture containing casein protein and whey protein as an effective ingredient, wherein the muscle synthesis promoter has a protein content ratio of casein protein to whey protein (casein protein/whey protein) of 2 to 20.

(2) The muscle synthesis promoter according to (1), wherein the milk protein mixture is ingested so that the total amount of protein for one meal is 0.13 to 0.7 g/kg body weight.

(3) The muscle synthesis promoter according to (1) or (2), comprising one unit packaging form for one meal, wherein the unit packaging form contains the milk protein mixture in which the total amount of protein is adjusted to 8 to 40 g as the intake amount for one meal.

(4) The muscle synthesis promoter according to any one of (1) to (3), wherein the subject in need of rapid absorption of protein is a subject who performs exercise.

(5) The muscle synthesis promoter according to (4), wherein the exercise is muscle training.

(6) The muscle synthesis promoter according to any one of (1) to (5), wherein the muscle synthesis promoter is in a liquid form.

(7) A food and drink product packed in a container provided with muscle synthesis promoting activity due to rapid protein absorption, produced by blending the muscle synthesis promoters according to any one of (1) to (6) and heat sterilizing, the product being in a unit packaging form for one meal, wherein the milk protein mixture is adjusted to contain the total amount of protein at 8 to 40 g as the intake amount for one meal.

(8) A milk beverage packed in a container provided with muscle synthesis promoting activity due to rapid protein absorption, produced by blending and heat sterilizing wherein a milk protein mixture containing casein protein and whey protein at the protein content ratio of the casein protein to the whey protein (casein protein/whey protein) of 2 to 20 is blended with one kind of material or more selected from the group consisting of raw milk, pasteurized milk, concentrated milk, powdered milk and reconstituted milk, wherein the milk protein mixture is adjusted to make the total amount of protein at 8 g/200 ml or more and the milkfat at 8 g/200 ml or less.

(9) A method for producing a milk beverage packed in a packaging container and provided with muscle synthesis promoting activity due to rapid protein absorption, comprising a step of blending a milk protein mixture containing casein protein and whey protein at the protein content ratio of casein protein to whey protein (casein protein/whey protein) of 2 to 20 with one kind of material or more selected from the group consisting of raw milk, pasteurized milk, concentrated milk, powdered milk and reconstituted milk, and a step of heat sterilization, wherein the milk protein mixture is adjusted to make the total amount of protein at 8 g/200 ml or more and the milkfat at 8 g/200 ml or less.

(10) A method for promoting muscle synthesis(excluding medical practice toward humans), comprising administering a milk protein mixture containing casein protein and whey protein at a protein content ratio of casein protein to whey protein (casein protein/whey protein) of 2 to 20 to a subject in need of rapid absorption of protein.

(11) A method for promoting muscle synthesis, comprising administering a milk protein mixture containing casein protein and whey protein at a protein content ratio of casein protein to whey protein (casein protein/whey protein) of 2 to 20 to a subject in need of rapid absorption of protein.

(12) The method for promoting muscle synthesis according to (10) or (11), wherein the milk protein mixture is administered so that the total amount of protein for one meal is 0.13 to 0.7 g/kg body weight.

(13) The method for promoting muscle synthesis according to (12), wherein the milk protein mixture is administered so that the total amount of protein for one meal is 8 to 40 g.

(14) The method for promoting muscle synthesis according to any one of (10) to (13), wherein the milk protein mixture is administered to a subject in need of rapid absorption of protein in a warm liquid form.

(15) The method for promoting muscle synthesis according to any one of (10) to (14), wherein the subject in need of rapid absorption of protein is a subject who performs exercise.

(16) The method for promoting muscle synthesis according to (15), wherein the subject performing exercise is a subject right after performing exercise.

(17) The method for promoting muscle synthesis according to any one of (15) or (16), wherein the exercise is muscle training.

[0012]

The muscle synthesis promoter of the present invention exhibits a rapid muscle synthesis promoting effect equivalent to that of whey protein, and has higher heat resistance and better processability compared with the solo use of whey protein. Therefore, the muscle synthesis promoter of the present invention can be expected to achieve a rapid muscle synthesis promoting activity and is advantageous in that it can be provided as an inexpensive product form that can be commercially produced in a large-scale.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013]

[Figure 1] Figure 1 illustrates the skeletal muscle fractional synthesis rates (FSR) in the triceps brachii muscle and gastrocnemius muscle
5 when various concentrations of milk protein solution are orally ingested (administered).

[Figure 2] Figure 2 illustrates a change in FSR associated with a swimming exercise.

[Figure 3] Figure 3 illustrates the serum concentrations of 3MeHis
10 when various concentrations of milk protein solution are orally ingested (administered).

DETAILED DESCRIPTION OF THE INVENTION

[0014]

15 The muscle synthesis promoter of the present invention is intended for use in a subject in need of rapid absorption of protein, comprising a milk protein mixture containing casein protein and whey protein as an effective ingredient, wherein the muscle synthesis promoter has a protein ratio of casein protein to whey
20 protein (casein protein/whey protein) of 2 to 20.

[0015]

"Effective ingredient" refers to a component that is required to attain the of muscle synthesis promoting effect for a subject in need of rapid absorption of protein.

25 [0016]

"A subject in need of rapid absorption of protein" means a subject who needs the promotion in the synthesis of muscle protein in order to synthesis muscle tissues and enhance muscle growth. For example, it means a subject who performs exercise to promote
30 muscle synthesis, to prevent muscle loss and to promote the recovery (repair) of muscle mass in order to improve health and athletic performance. Here, the exercise is preferably muscle training (resistance exercise) because it facilitates muscle synthesis by adding a load to the muscle, and more preferably a long-term
35 muscle training, for example, continuous muscle training for 30 minutes to 5 hours at a frequency of 1 to 7 days per week. Muscle

training includes, for example, workouts with a dumbbell, squat exercise and training machine exercise.

[0017]

Subjects ingesting the muscle synthesis promoter in the present invention include, for example, sport players, sport enthusiasts (athletes), persons who require exercise in order to improve their lifestyle related diseases, people such as the elderly who need exercise for better health, and patients suffering from muscle disorders (for example, sarcopenia) who need to promote muscle synthesis by exercise. Furthermore, such subjects may include animals other than humans (domestic animals, such as horses and cows, pets, such as dogs and cats, and ornamental animals reared in zoo).

[0018]

The timing when the muscle synthesis promoter in the present invention is taken by (administered to) a subject in need of rapid absorption of protein may be before exercise, during or after exercise. Here, the timing of ingestion is preferably after exercise, more preferably right after exercise. Because the muscle synthesis promoter in the present invention inhibits the degradation of skeletal muscle (catabolism) due to exercise and promotes anabolism from amino acids to protein, the timing of administration is preferably right after exercise, especially within 3 hours after exercise, more preferably within 2 hours after exercise, further preferably within 1 hour after exercise, and particularly preferably within 30 minutes after exercise (refer to Levenhagen et al. *Am J Physiol Endocrinol Metab* (2001), 280, E982-E993).

[0019]

A rapid muscle synthesis promoting activity equivalent to that of whey protein means an equivalent degree of absorption rate and an equivalent degree of protein synthesis promoting activity to those seen when a protein containing whey protein as a major component is orally ingested (or administered). In addition, the muscle synthesis promoting activity also includes the anabolic effect of the muscle protein from amino acids. Here, the muscle synthesis promoting activity is confirmed by, for example, measuring a triceps

brachii muscle fractional synthesis rate (FSR) in a study group within one hour after the administration of a sample material after a swimming test in rats. Namely, compared with the FSR in a control group in which a protein comprising whey protein which is said to have the rapid muscle synthesis promoting activity as a major component is orally ingested, when the FSR in the study group shows equivalent to or more than that in the control group, the muscle synthesis promoting activity can be affirmed as significant (refer to Example 1 (2)). Also, the muscle synthesis promoting activity includes the inhibition of muscle protein degradation, namely, a preventive activity of catabolism from muscle protein to amino acids. Here, the inhibition of muscle protein degradation can be confirmed, for example, by measuring the concentration of 3-methylhistidine within 1 hour after the administration of a sample material after a swimming test in rats (refer to Example 1 (3)).

[0020]

"The protein content" refers to the quantity of protein as a macromolecule of amino acids, which are contained in a variety of proteins such as milk protein, whey protein or casein protein. "The protein content ratio" refers to the ratio of the amount of protein as a macromolecule of amino acids, which are contained in a variety of proteins such as milk protein, whey protein or casein protein. Here, the protein amount may be determined, for example, based on known information on the Standard Tables of Food Composition in Japan, or based on conventional methods such as the Kjeldahl method and the Lowry method. Actually, in the Kjeldahl method, the amount of nitrogen contained in various proteins is measured and the measured value is multiplied by a nitrogen-protein conversion factor (generally 6.25) to determine the amount of protein.

[0021]

The milk protein mixture used in the present invention may contain whey protein and casein protein at a particular ratio. The milk protein mixture may be prepared, though not limited to, by mixing whey protein and casein protein, each isolated and purified separately, to make up a particular ratio. Or it may be prepared by mixing a raw material or a food and drink product containing whey

protein and casein protein at a particular ratio, wherein whey protein and casein protein are not separately isolated and purified. In the milk protein mixture used in the present invention, because the step for isolation or purification is not required and thus its manufacturing cost is low, it is preferable to use a raw material or a food and drink product containing whey protein and casein protein at a specific protein content ratio as major ingredients and to blend whey protein and casein protein that are separately purified and prepared with the raw material to make up a specific protein content ratio, when needed.

[0022]

As whey protein, which is isolated or purified separately from casein protein, for example, a stock solution of whey protein (sweet whey, acid whey or the like), a concentrate thereof, dried form thereof (whey powder or the like), frozen matter thereof, a hydrolysate thereof or the like, a reduction solution thereof or the like may be used. Furthermore, desalted whey, a whey protein concentrate (WPC), a whey protein isolate (WPI), α -lactalbumin (α -La), β -lactoglobulin (β -Lg), immunoglobulin, lactoferrin or the like, or a reduction solution thereof may be used. And, as casein protein, which is isolated and purified separately from whey protein, for example, a casein isolate (natural cheese such as fresh cheese, sodium caseinate, calcium caseinate or the like), a concentrate thereof, a dried matter thereof, a frozen matter thereof, a hydrolysate thereof or the like, or a reduction solution thereof or the like may be used. Furthermore, a purified matter such as α -casein, β -casein, κ -casein and a reduction solution thereof may be used. Also, as a raw material or a food and drink product already containing whey protein and casein protein at a specific protein content ratio, for example, fresh milk, pasteurized milk (cow milk), nonfat milk, ingredient adjustment milk, processed milk, dairy products (concentrated milk, powdered milk, condensed milk, fermented milk (yogurt and the like), lactic acid bacteria beverage, processed cheeses, ice creams, creams), a milk protein concentrate (MPC), a concentrate thereof dried matter thereof, frozen matter thereof, a hydrolysate thereof or the like and a reduction solution thereof or the

like may be used. As these ingredients, commercial products or preparations prepared in-house may be used.

[0023]

5 The milk protein mixture used in the present invention is composed of whey protein and casein protein at a particular protein content ratio, specifically, the milk protein mixture has a protein content ratio of casein protein to whey protein (casein protein/whey protein) of 2 to 20, preferably 2 to 10, more preferably 2 to 6, further preferably 2 to 4, and particularly preferably 2 to 3.5. Furthermore,
10 the milk protein mixture used in the present invention is strikingly different from that made by the conventional techniques in terms of intentional adjustment (design) of the protein content ratio of casein protein to whey protein (casein protein/whey protein).

[0024]

15 The muscle synthesis promoter of the present invention has high heat resistance (thermal stability) exhibiting the effect of heat resistance. Here, exhibiting the effect of heat resistance means that protein does not undergo solidification (aggregation) by heat denaturation even when it is treated at a particular temperature,
20 specifically, protein does not undergo heat denaturation even under heat sterilization under conditions and methods generally used in the processing of cow milk. Namely, in the muscle synthesis promoter of the present invention, exhibiting the effect of heat resistance means that the protein is hardly heat denaturated, even when heat
25 sterilization is performed, more specifically, at 62 to 65°C for 30 minutes (LTLT treatment: Low-Temperature-Long-Time Treatment), or by methods that have equivalent or larger microbicidal effect, for example, heat sterilization at 120 to 150°C for 1 to 30 seconds (UHT treatment: Ultra-high temperature processing), heat sterilization at
30 more than 72°C for more than 15 seconds (HTST treatment: high-temperature short-time treatment), or heat sterilization at 75°C or more for 15 minutes or more. In these cases, heat sterilization methods include known methods such as a batch type in which the treatment solution is stirred while being heated in a tank, an indirect
35 heating method in which a plate-type heat exchanger or tube-type heat exchanger is used, a direct heating method in which a steam

injection-type heating device or a steam-infusion type heating device is used, or an electric heating system in which a joule heating apparatus is used.

[0025]

5 According to one embodiment of the present invention, for example, in the muscle synthesis promoter of the present invention, the milk protein mixture is ingested (administered) so that the total amount of protein for one meal is 0.13 g /kg body weight or more, specifically 0.13 to 0.7 g/kg body weight, preferably 0.13 to 0.6 g/kg
10 body weight, more preferably 0.13 to 0.5 g/kg body weight, further preferably 0.15 to 0.4 g/kg body weight, and particularly preferably 0.2 to 0.4 g/kg body weight. Here, the typical body weight of the subject is set at about 60 kg.

[0026]

15 “One meal” means each state in which rapid absorption of protein is required. For example, the muscle synthesis promoter of the present invention may be ingested in one meal not only at one time but also at several times by dividing one meal. In terms of rapidly raising the blood levels of amino acids, when administration is
20 divided into several instances, the timing of the ingestion (administration) of the muscle synthesis promoter of the present invention to a subject in need of rapid absorption of protein is preferably within 2 hours after exercise, more preferably within 1 hour after exercise, further preferably within 30 minutes after
25 exercise, and particularly preferably within 5 minutes after exercise.

[0027]

 A method for ingesting (administering) the muscle synthesis promoter of the present invention to a subject in need of rapid absorption of protein can be appropriately chosen from the following:
30 oral ingestion (oral administration), enteral administration and administration from gastric fistula and the like depending on the subject and the purpose of use, and oral ingestion is preferable.

[0028]

 The muscle synthesis promoter of the present invention can
35 be in any form as long as its effect is exhibited. For example, the form includes a solid form, a liquid form, a gel form, a glue-like form,

a paste-like form and the like. The muscle synthesis promoter of the present invention may be used in solid form such as a powder form, a granular form, a capsule form or a block-like form. A liquid form is preferable because of its excellent processability. For example, the muscle synthesis promoter of the present invention can be adequately subjected to heat sterilization because it has a sufficient heat resistance property even though it contains water. Therefore, for example, the muscle synthesis promoter of the present invention can have a long-term shelf life in a refrigerated state and at room temperature. In the muscle synthesis promoter of the present invention, water content can be appropriately adjusted to, specifically 10 to 95 wt%, preferably 20 to 95 wt%, more preferably 30 to 90 wt%, and further preferably 40 to 90 wt%. The muscle synthesis promoter of the present invention can efficiently promote muscle synthesis by adjusting the water content (or solid content) at a predetermined value, unlike conventional health drinks and sports drinks containing high water content.

[0029]

According to another embodiment of the present invention, for example, the muscle synthesis promoter of the present invention is preferably composed of one unit packaging form for one meal, wherein the milk protein mixture in the unit packaging form is adjusted so that the total amount of protein is 8 g or more as the amount of intake for one meal, specifically 8 to 40 g, preferably 8 to 20 g, more preferably 9 to 20 g, further preferably 9 to 15 g, and particularly preferably 10 to 15 g. Here, "one unit packaging form for one meal" means that the amount of intake for one meal is in predetermined form. For example, it means not only common foods but also forms, such as beverages (health drinks and the like), health supplemental foods, health functional foods and supplements as food and drink products, that can be orally ingested at a specific amount. In "a unit packaging form for one meal", for example, when the form is a liquid beverage, jelly (in a gel form, a glue-like form and a paste form) and a solid form food (a powder form, a granular form, a capsule form and a block form), a packaging container such as a metallic can, a glass bottle (such as a bottle), a plastic container (a

PET bottle and the like), a pack, a pouch, a film container, a paper carton can be used, wherein the specific amount (dosage) can be defined by those containers. Or it includes forms in which a specific amount can be defined by indicating the amount of intake for one meal (usage, dosage) on the packaging container or on a homepage and the like.

[0030]

According to another embodiment of the present invention, for example, when the muscle synthesis promoter of the present invention is in the form of a health drink, a packaging container such as a metallic can, a glass bottle (such as a bottle), a plastic container (such as a PET bottle) packed (filled) with the health drink can be used, wherein the packaging container contains 8 to 40 g of the milk protein suspended or dissolved in the drink so that the drink-up volume (dosage) for one meal is adjusted to 100 to 1000 ml by varying water content. Also, the drink in the form of a large-capacity packaging container can be mentioned, in which the amount of several meals (for several occasions) is put together, wherein 8 to 40 g of the milk protein is suspended or dissolved and information about the amount of intake for one meal (usage, dosage) (for example, 100 to 1000 ml) is displayed on the packaging container or on a homepage.

[0031]

The muscle synthesis promoter of the present invention is not limited with respect to its manufacturing steps (manufacturing procedures) as long as the muscle synthesis promoter is manufactured to contain predetermined milk protein mixture and the muscle synthesis promoter can be produced according to, for example, manufacturing steps of producing cow milk, component adjustment milk, processed milk, a milk beverage and the like. The muscle synthesis promoter of the present invention can be produced, for example, by blending (adjusting) various components such as milk protein, milkfat and the like with raw milk (unprocessed milk), pasteurized milk, concentrated milk, powdered milk and reconstituted milk according to a known production method (manufacturing method). Or it can be produced by blending

(adjusting) various components such as milk protein, milkfat and the like with component adjustment milk and processed milk (such as low-fat milk or nonfat milk).

[0032]

5 According to one embodiment of the present invention, for example, the muscle synthesis promoter of the present invention can provide a composition containing a muscle synthesis promoter. In other words, the muscle synthesis promoter of the present invention may be used as it is, but it may be added as a raw
10 material (material) or an additive to various compositions for oral ingestion (for oral administration) such as a food and drink product or a pharmaceutical preparation to obtain a composition having rapid muscle synthesis promoting effect (for example, a pharmaceutical composition or a food and drink composition), as
15 long as the muscle synthesis promoting activity is exerted. Thus, according to one embodiment of the present invention, a composition containing the muscle synthesis promoter of the present invention is provided.

[0033]

20 "A food and drink product" (a food and drink composition) is other than a pharmaceutical preparation (a pharmaceutical composition) and may have an orally ingestible form such as an solution, suspension, liquid emulsion, powder and a solid molded product, and not particularly limited. A food and drink product
25 specifically includes instant foods such as instant noodle, retort pouch, canned foods, microwave oven foods, instant soup, miso soups, freeze-dried foods and the like; beverages such as refreshing drink, fruit juice, vegetables drink, soy milk, coffee drink, tea drink, powdery drink, concentration drink, alcoholic drink and the like;
30 wheat flour product such as bread, pasta, noodle, a cake mix, bread crumb and the like; confectionery such as candy, caramel, chewing gum, chocolate, cookie, biscuit, cake, pie, snack, cracker, Japanese sweet, dessert cake and the like; seasoning such as sauce, processed tomato seasoning, flavor seasoning, seasoning cooking mixture, tare
35 sauce, dressings, soups, curry or stew mix and the like; oils and fats such as possessed oil and fat, butter, margarine, mayonnaise and the

like; dairy products such as milk beverage, fermented milk (yogurt and the like), lactic acid bacteria beverage, natural cheese, processed cheese, ice cream, cream and the like; processed agricultural products such as agricultural canned food, jam and marmalade, cereal and the like; and frozen foods.

[0034]

Also, the food and drink product includes health foods, functional foods, nutritional supplementary foods, foods for specified health use, foods for patients, modified dry milk for infants, powdered milk for pregnant women or lactating women, and categories such as foods with an indication of usage for rapid muscle synthesis promoting.

[0035]

A "pharmaceutical preparation" (a pharmaceutical composition) means a preparation prepared as an oral preparation or a parenteral preparation by adding pharmaceutically acceptable additives according to a conventional method. When a pharmaceutical preparation is an oral preparation, solid preparations such as tablets, powders, fine granules, granules, capsules, pills, controlled release tablets and the like; liquid preparations such as solution, suspension, emulsion and the like may be used. When a pharmaceutical preparation is parenteral preparation, injection and suppository may be used. Here, an oral preparation is preferable as a pharmaceutical preparation because of its simplicity in ingestion by (or administration to) patients, and the like. Pharmaceutically acceptable additives include, for example, excipients, stabilizers, preservatives, wetting agents, emulsifiers, lubricants, sweeteners, coloring agents, flavors, buffers, antioxidants, pH regulators and the like.

[0036]

The muscle synthesis promoter of the present invention exhibits a heat-resistant effect. Therefore, a composition prepared by blending (adding and mixing) the muscle synthesis promoter of the present invention may preferably be provided in a liquid product form and also as a product for warm dispensing by using a hot vending machine and the like, because the muscle synthesis

promoter of the present invention exerts a heat-resistant effect.

[0037]

Furthermore, a composition prepared by blending (adding, mixing) the muscle synthesis promoter of the present invention may be in any form as long as its effect is exhibited. For example, it may be in a solid form, a liquid form, a gel form, a glue-like form, a paste form and the like. The composition prepared by blending the muscle synthesis promoter of the present invention may be in a powder form, a granular form, a capsule form, a block form among solid forms. However, a liquid form is preferable because of its excellent processability.

[0038]

According to one embodiment of the present invention, provided are a food and drink product having muscle synthesis promoting activity of protein due to rapid absorption and a method for production thereof, wherein the product is prepared by blending (adding, mixing) the muscle synthesis promoter of the present invention and heat sterilization, is composed of a unit packaging form for one meal, wherein the milk protein mixture in a unit packaging form is adjusted so that the total amount of protein for one meal is 8 to 40 g as the amount of intake for one meal, preferably 8 to 20 g, more preferably 9 to 20 g, further preferably 9 to 15 g and particularly preferably 10 to 15 g. Here, an operation (step) of "blending (adding, mixing) and heat sterilizing" to a predetermined composition means an operation of preparing eventually a predetermined composition and sterilization. Although it is not particularly limited, such operations include mixing all the necessary components to prepare a predetermined composition and then the composition is subjected to heat sterilization. Or all the necessary components are individually heat sterilized followed by mixing them to prepare a predetermined composition. However, in the present invention, the order and frequency of this blending (adding, mixing) and heat sterilization are arbitrary.

[0039]

In these cases, the food and drink product (the food and drink product of the present invention) is preferably a food and drink

product packed in a packaging container, more preferably a food and drink product packed in a package as a warm product. Namely, because the food and drink product has heat resistance, the proteins contained in it are not extremely denatured even if it is sold under warm conditions. While a conventional food and drink product constituted with whey protein alone or whey protein as a major component cannot be served as a warm food and drink product outdoor in winter, the food and drink product can be served as a warm food and drink product outdoor in winter (especially, under cold conditions) and allow consumers to ingest (drink). Therefore, the realization of a food and drink product having muscle synthesis promoting activity is expected to meet consumers' new needs. Here, a food and drink product for sales under warm conditions includes, for example, a beverage for warm dispensing housed in a heated vending machine or double-boiled warming, a beverage and a food for warm dispensing which is contained in a cookware at amount needed and is heated by a stove burner, an electric cooking stove, a microwave oven and the like. Furthermore, for warm dispensing, a food and drink product is generally stored at about 30 to 80°C or about 50 to 80°C.

[0040]

According to a preferable embodiment of the present invention, the food and drink product is a milk beverage. The reason is, because the food and drink product has heat resistance, the protein does not extremely undergo denaturation even if it is subjected to heat sterilization. Here, "a milk beverage" means a beverage that contains a solid milk fraction (a mixture of nonfat milk solid and milkfat) at 3.0 wt% or more. This milk beverage can be blended with, for example, vitamins, minerals, oligosaccharides, lactose, fruit juices and the like.

[0041]

In a method for producing the food and drink product of the present invention, its manufacturing steps (manufacturing procedures) are not limited as long as the food and drink product is manufactured to contain a predetermined milk protein mixture (at a predetermined concentration), and it can be produced, for example,

according to a manufacturing process for cow milk, component adjustment milk, processed milk, a milk beverage and the like. The food and drink product of the present invention can be produced, for example, by blending (adjusting) various components such as milk protein and milkfat with unprocessed milk (raw milk), pasteurized milk, concentrated milk, powdered milk and reconstituted milk according to known production methods (manufacturing procedures). Or it can be produced by blending (adjusting) various components such as milk protein and milkfat with component adjustment milk and processed milk (such as low-fat milk or nonfat milk).
[0042]

According to another embodiment of the present invention, provided is a milk beverage having the muscle synthesis promoting activity due to rapid protein absorption packed in a packaging container, wherein a milk protein mixture, containing casein protein and whey protein at a protein content ratio of casein protein to whey protein (casein protein/whey protein) of 2 to 20, is mixed with one or more materials selected from the group consisting of raw milk, pasteurized milk, concentrated milk, powdered milk and reconstituted milk, and is subjected to heat sterilization, wherein the milk protein mixture is prepared so that the total amount of protein is 8 g or more/200 ml, preferably 8 to 20 g/200 ml, more preferably 9 to 20 g/200 ml, further preferably 9 to 15 g/200 ml and particularly preferably 10 to 15 g/200 ml, and the amount of milkfat is 8 g or less/200 ml, preferably 0.1 to 8 g/200 ml, more preferably 0.1 to 6 g/200 ml, further preferably 0.5 to 4 g/200 ml and particularly preferably 0.5 to 3 g/200 ml.
[0043]

According to another embodiment of the present invention, provided is a method for producing a milk beverage having the muscle synthesis promoting activity due to rapid protein absorption packed in a packaging container, comprising a step of blending a milk protein mixture, containing casein protein and whey protein at a protein content ratio of casein protein to whey protein (casein protein/whey protein) of 2 to 20, with one or more materials selected from the group consisting of raw milk, pasteurized milk,

concentrated milk, powdered milk and reconstituted milk, and a step of performing heat sterilization, wherein the milk protein mixture is prepared so that the total amount of protein is 8 g or more/200 ml, preferably 8 to 20 g/200 ml, more preferably 9 to 20 g/200 ml, further preferably 9 to 15 g/200 ml and particularly preferably 10 to 15 g/200 ml, and the amount of milkfat is 8 g less/200 ml, preferably 0.1 to 8 g/200 ml, more preferably 0.1 to 6 g/200 ml, further preferably 0.5 to 4 g/200 ml and particularly preferably 0.5 to 3 g/200 ml.

10 [0044]

"Reconstituted milk" means milk in a liquid form, wherein the reconstituted milk is prepared by dispersing/dissolving a raw milk material such as powdered milk or concentrated milk into water. The raw milk material includes, for example, whole powdered milk, nonfat powdered milk, whole concentrated milk, nonfat concentrated milk, butter, cream, condensed milk and the like, and is not particularly limited as long as it is prepared by removing water from raw milk, pasteurized milk (such as cow milk), skimmed milk, component adjustment cow milk, processed milk and the like, wherein they may be used by mixing one or more of them. In the milk beverage, no aggregation of whey protein by heat denaturation is observed (refer to Example 2), therefore, the milk beverage retains the rapid absorption effect of protein similar to that of the muscle synthesis promoter of the present invention.

25 [0045]

According to another embodiment of the present invention, provided is a method for promoting muscle synthesis, comprising administering a milk protein mixture containing casein protein and whey protein at a protein content ratio of casein protein to whey protein (casein protein/whey protein) of 2 to 20 to a subject in need of rapid absorption of protein.

[0046]

According to a preferable embodiment of the present invention, provided is a method for promoting muscle synthesis (excluding medical practice toward humans), comprising administering a milk protein mixture containing casein protein and

whey protein at a protein content ratio of casein protein to whey protein (casein protein/whey protein) of 2 to 20 to a subject in need of rapid absorption of protein. Here, "medical practice toward humans" means an action of making humans to take (administering) a pharmaceutical preparation, requiring doctor's prescription. The method for promoting muscle synthesis of the present invention can be implemented by using the muscle synthesis promoter of the present invention according to the contents described in the present description.

10 [0047]

According to a preferable embodiment of the present invention, in the method for promoting muscle synthesis of the present invention, the milk protein mixture can be ingested (administered) so that the total amount of protein for one meal is 15 0.13 g or more/kg body weight, specifically, 0.13 to 0.7 g/kg body weight, preferably 0.13 to 0.6 g/kg body weight, more preferably 0.13 to 0.5 g/kg body weight, further preferably 0.15 to 0.4 g/kg body weight, and particularly preferably 0.2 to 0.4 g/kg body weight. [0048]

20 According to a preferable embodiment of the present invention, in the method for promoting muscle synthesis of the present invention, the milk protein mixture can be ingested so that the total amount of protein for one meal is 8 g or more, specifically, 8 to 40 g, preferably 8 to 20 g, more preferably 9 to 20 g, further 25 preferably 9 to 15 g, and particularly preferably 10 to 15 g. [0049]

According to an embodiment of the present invention, in the method for promoting muscle synthesis of the present invention, the milk protein mixture can be ingested in a warm liquid form to a 30 subject in need of rapid absorption of protein. [0050]

According to an embodiment of the present invention, provided is a use of the muscle synthesis promoter to a subject in need of rapid absorption of protein, wherein the muscle synthesis 35 promoter comprises a milk protein mixture containing casein protein and whey protein at a protein content ratio of casein protein to whey

protein (casein protein/whey protein) of 2 to 20. The use of the muscle synthesis promoter in the present invention can be implemented according to the contents described in the present description.

5 [0051]

According to an embodiment of the present invention, provided is a use of the muscle synthesis promoter for the production of a food and drink product and a pharmaceutical preparation for a subject in need of rapid absorption of protein to promote muscle
10 synthesis, wherein the muscle synthesis promoter comprises a milk protein mixture containing casein protein and whey protein at a protein ratio of casein protein to whey protein (casein protein/whey protein) of 2 to 20. The use of the muscle synthesis promoter in the present invention can be implemented according to the contents
15 described in the present description.

[0052]

According to one embodiment of the present invention, though specific experimental results are omitted here, the muscle synthesis promoter of the present invention can exhibit not only an
20 excellent muscle synthesis promoting activity (muscle synthesis promoting effect) but also an excellent water retaining activity in the body (water retaining effect in the body). Therefore, a particular effect can be expected in the present invention, which cannot be obtained by the use of conventional pharmaceutical preparations or
25 food and drink products containing whey protein as a major component.

[0053]

The present invention will be explained in detail by referring to the following Examples, but not limited to these Examples.

30

EXAMPLES

[0054]

Example 1: Muscle synthesis promoting activity by a milk protein mixture

35 (1) A swimming test in rats

Male Sprague-Dawley rats with initial body weight of 140 to

160 g (obtained from CLEA Japan Inc.) were used as experimental animals. The rats were divided into 6 groups consisting of 9 to 10 rats in each test block (group) to avoid developing variability in body weight among groups. All the rats were raised in a stainless-steel cage individually with a light period between 7:00 and 19:00 and a dark period between 19:00 and 7:00 at room temperature of $22\pm 2^{\circ}\text{C}$.

[0055]

The rats underwent a preliminary breeding for 3 to 4 days using solid feed (Oriental Yeast Co., Ltd.) and a swimming test in rats was conducted (let rats exercise) according to the following protocol.

[0056]

[Protocol of a swimming test (exercise) in rats]

Day 1: Rats were subjected to a swimming exercise for a total of 4 hours without weight loading, consisting of a swimming exercise for 2 hours, a break for 45 minutes, and a swimming exercise for 2 hours. After the exercise, the rats were fed (solid feed MF) *ad libitum*.

Day 2: Rats were subjected to a swimming exercise for a total of 6 hours without weight loading, consisting of a swimming exercise for 3 hours, a break for 45 minutes, and a swimming exercise for 3 hours. After the exercise, the rats were fed (solid feed MF) *ad libitum*.

Day 3: Rats were subjected to a swimming exercise for a total of 6 hours without weight loading, consisting of a swimming exercise for 3 hours, a break for 45 minutes, and a swimming exercise for 3 hours. After the exercise, the rats were fed 5 g of controlled diet (powder diet MF).

[0057]

After 3 days of the above-mentioned exercise, the rats were subjected to a swimming exercise for 2 hours without weight loading. A milk beverage (milk protein solution) was prepared with the concentrations of protein shown in Table 1 using milk protein and MilliQ water. After this 3 days exercise, a milk beverage and MilliQ water were orally administered to the rats at 24.24 ml/kg BW (test blocks 1 to 5 and comparison block 1). Here, a milk protein concentrate (MPC480, Fonterra Co-operative Group Limited, milk protein 77.2 wt%, milkfat 1.2 wt%, lactose 9.2 wt%, ash 7.1 wt%,

water 5.3 wt%) was used for the milk protein. This MPC480 is a mixture of casein protein and whey protein prepared from skimmed milk by removing lactose and ash using membrane separation (UF treatment, MF treatment and the like) to leave casein protein and whey protein as main components, and is in a dry powder form. The ratio of the protein in MPC480 is casein protein: whey protein= about 8:2 in the case of raw milk (cow milk) (refer to A general dictionary of milk, edited by Kunio Yamauchi and Kenkichi Yokoyama).

[0058]

Forty five minutes after the oral ingestion, a solution of 0.4 ml/200 g body weight of deuterium-labeled phenylalanine (prepared by dissolving 22.5 mg of deuterium-labeled phenylalanine in 1 ml of physiological saline) was injected in tail vein. Fifteen minutes after the injection (namely, 60 minutes after the oral ingestion), the rats were dissected under isoflurane anesthesia. After exsanguination was conducted from an abdominal portion of the vena cava, triceps brachii muscle (sample) was excised and washed with physiological saline, and immediately after that, it was frozen with liquid nitrogen. The time of injections and cryopreservation of the sample were accurately recorded. The samples were stored at -80°C until analysis.

[0059]

[Table 1]

Table 1: Blending of milk protein solutions at various concentrations

	Protein amount (Amount of intake of the protein) (g/kg BW)	Concentration of MPC480 (g/100 ml)
Test block 1	0.39	2.06
Test block 2	0.77	4.13
Test block 3	1.54	8.25
Test block 4	3.09	16.5
Test block 5	4.63	24.8
Comparison block 1	0	0

[0060]

(2) Measurement of skeletal muscle fractional synthesis rate (FSR)

The skeletal muscle fractional synthesis rate (FSR) of samples was measured according to the description in the prior art document (A.Kanda et al., Br J Nutr, Feb. 7, 1-7, 2013). Specifically, phenylalanine (Phe) and stable isotope-labeled phenylalanine (Phe(Ring-D5)), that are present in skeletal muscle cells or in free form in the blood in skeletal muscle, were quantified by LC/MS/MS (Waters), respectively. The conditions of measurement are as follows. Device: LC/MS/MS (Waters), column: ACQUITY UPLC BEH C18 (Waters), mobile phase: 0.05% TFA water/0.05% TFA containing acetonitrile, injection volume 3 µl, flow rate 0.3 ml/min, and UV detection at 215 nm.

[0061]

Here, skeletal muscle fractional synthesis rate (FSR) was calculated according to the following formula.

$$\text{FSR (\%/day)} = (\text{Eb} \times 100) / (\text{Ea} \times t)$$

Ea: enrichment of supernatant sample

Eb: enrichment of skeletal muscle sample

t: period between the injection of inject solution and start of cryopreservation of a sample (Unit: day)

$$\text{Enrichment} = \text{Phe(Ring - D5)} / (\text{Phe} + \text{Phe(Ring - D5)})$$

[0062]

The values obtained were statistically processed to obtain mean ± SD. Specifically, one-way analysis of variance was carried out, and then the Tukey multiple comparison test was carried out. Significance level was set at 5% (p<0.05). The results are shown in Figure 1. In case where there is a significant difference in the Figure, different letters were added. For example, a group with letter a (comparison block 1 and the like) and a group with letter b (test block 3) or a group with letter c (test block 4 and the like) are shown to have a significant difference between them. Moreover, for example, there is no significant difference between a group with letter a (comparison block 1 and the like) or letter b (test block 3) and a group with letter ab (test block 2).

[0063]

As a result, it was confirmed that the synthesis of skeletal muscle was promoted in a dose-dependent manner when the milk protein was orally ingested.

5 [0064]

It is noted that FSR has been demonstrated to be significantly decreased by the swimming exercise for 2 hours in the prior art document (A.Kanda et al., Br J Nutr, Feb. 7, 1-7, 2013) (refer to Figure 2).

10 [0065]

(3) Measurement of concentration of 3-methylhistidine (3MeHis) in the blood

By using LC/MS/MS (Waters), the plasma concentration of 3MeHis in the samples was measured as one of indicators of the degradation of skeletal muscles. The measurement conditions are as follows. Device: LC/MS/MS (Waters), column: ACQUITY UPLC BEH C18 (Waters), mobile phase: 0.05% TFA waster/0.05% TFA containing acetonitrile, injection volume 3 μ l, and flow rate 0.3 ml/min.

20 [0066]

The values obtained were statistically processed to determine mean $\pm$ SD. Specifically, one-way analysis of variance was carried out, and then the Tukey multiple comparison test was carried out. Significance level was set at less than 5% ($p < 0.05$).

25 The results are shown in Figure 3. In case where there is a significant difference in the Figure, different letters were added.

[0067]

As a result, it was demonstrated that the degradation of skeletal muscle after the exercise was suppressed in a dose-dependent manner when the milk protein was orally ingested.

30 [0068]

Example 2: Muscle synthesis promoting activity by a milk beverage containing milk protein

(1) Preparation (production) of a milk beverage containing milk protein

35 Milk protein was added to and mixed with raw milk (milk

protein 3.3 wt%, milkfat 3.8 wt%, lactose 4.8 wt%, ash 0.7 wt%,
water 87.4 wt%, obtained from Meiji Co., Ltd.) to prepare a raw milk
material in such a way that the concentration of milk protein was
adjusted to the concentrations described in Table 2. And according
5 to a routine procedure for producing cow milk, a milk beverage (a
milk protein solution) containing high concentration of the milk
protein (test blocks 6 and 7) was obtained by subjecting the raw milk
material to heat sterilization at 130°C for 2 seconds by an indirect
heating method using a plate-type heat exchanger followed by
10 cooling to 10°C or below. Furthermore, raw milk (obtained from
Meiji Co., Ltd.) without adding the milk protein was also subjected to
heat sterilization followed by cooling to 10°C or below to obtain cow
milk (test block 8). Here, a milk protein concentrate (MPC480,
Fonterra Co-operative Group Limited, milk protein 77.2 wt%, milkfat
15 1.2 wt%, lactose 9.2 wt%, ash 7.1 wt% and water 5.3 wt%) was
used as the milk protein that was added to a milk beverage. This
MPC480 is a mixture of casein protein and whey protein prepared
from skimmed milk by removing lactose and ash using membrane
separation (UF treatment, MF treatment and the like) to leave casein
20 protein and whey protein as main components, is in a dry powder
form. The ratio of protein in MPC480 is casein protein: whey
protein=about 8:2 as in the case of raw milk (cow milk) (A general
dictionary of milk, edited by Kunio Yamauchi and Kenkichi
Yokoyama).

[0069]

[Table 2]

Table 2: Blending of milk protein solutions at various concentrations and FSR

		Milk protein amount (g/200 ml)	Protein amount (Amount of protein intake) (g/kg BW)	FSR (%/day)
Test block 6	Milk beverage	8.2	2.06	6.7
Test block 7	Milk beverage	10.4	2.60	7.7
Test block 8	Cow milk	6.8	1.71	6.0

5

[0070]

Precipitates due to protein aggregation (solidification) by heating were not found in any of these milk beverage obtained and cow milk.

10 [0071]

(2) Swimming test in rats and measurement of a skeletal muscle fractional synthesis rate (FSR)

Except that raw milk (test block 8), milk beverages (test blocks 6 and 7) and milliQ wate (comparison block 1) shown in Table 2 were orally administered to rats at 24.24 ml/kg BW, a swimming test in rats was carried out in the same method described in the above-mentioned Example 1. Subsequently, skeletal muscle fractional synthesis rate (FSR) was measured (calculated).

15 [0072]

20 As a result, FSR in the test blocks 6 and 7 were 6.7%/day and 7.7%/day, respectively, resulting in high values.

[0073]

In this case, in the test blocks 6 and 7, it was confirmed that the rapid muscle synthesis promoting activity was obtained that is equal to the case when whey protein alone is orally ingested.

25

[0074]

In addition, when whey protein was orally ingested at a protein amount of 3.11 g/kg BW 2 hours after the exercise on a treadmill, it has been confirmed that FSR is 8.8%/day in the prior art
5 document (Anthony et al., J.Nutr.137, 357-362 (2007)).

CLAIMS

1. A muscle synthesis promoter for use in a subject in need of rapid absorption of protein, comprising a milk protein mixture containing casein protein and whey protein as an effective ingredient, wherein the muscle synthesis promoter has a protein content ratio of casein protein to whey protein (casein protein/whey protein) of 2 to 20.
2. The muscle synthesis promoter according to Claim 1, wherein the milk protein mixture is ingested so that the total amount of protein for one meal is 0.13 to 0.7 g/kg body weight.
3. The muscle synthesis promoter according to Claim 1 or 2, comprising one unit packaging form for one meal, wherein the unit packaging form contains the milk protein mixture in which the total amount of protein is adjusted to 8 to 40 g as the intake amount for one meal.
4. The muscle synthesis promoter according to any one of Claims 1 to 3, wherein the subject in need of rapid absorption of protein is a subject who performs exercise.
5. The muscle synthesis promoter according to Claim 4, wherein the exercise is muscle training.
6. The muscle synthesis promoter according to any one of Claims 1 to 5, wherein the muscle synthesis promoter is in a liquid form.
7. A food and drink product packed in a container provided with muscle synthesis promoting activity due to rapid protein absorption, produced by blending the muscle synthesis promoters according to any one of Claims 1 to 6 and heat sterilizing, the product being in a unit packaging form for one meal, wherein the milk protein mixture is adjusted to contain the total amount of protein at 8 to 40 g as the intake amount for one meal.

8. A milk beverage packed in a container provided with muscle synthesis promoting activity due to rapid protein absorption, produced by blending and heat sterilizing wherein a milk protein mixture containing casein protein and whey protein at the protein content ratio of the casein protein to the whey protein (casein protein/whey protein) of 2 to 20 is blended with one kind of material or more selected from the group consisting of raw milk, pasteurized milk, concentrated milk, powdered milk and reconstituted milk, wherein the milk protein mixture is adjusted to make the total amount of protein at 8 g/200 ml or more and the milkfat at 8 g/200 ml or less.

9. A method for producing a milk beverage packed in a packaging container and provided with muscle synthesis promoting activity due to rapid protein absorption, comprising a step of blending a milk protein mixture containing casein protein and whey protein at the protein content ratio of casein protein to whey protein (casein protein/whey protein) of 2 to 20 with one kind of material or more selected from the group consisting of raw milk, pasteurized milk, concentrated milk, powdered milk and reconstituted milk, and a step of heat sterilization, wherein the milk protein mixture is adjusted to make the total amount of protein at 8 g/200 ml or more and the milkfat at 8 g/200 ml or less.

10. A method for promoting muscle synthesis(excluding medical practice toward humans), comprising administering a milk protein mixture containing casein protein and whey protein at a protein content ratio of casein protein to whey protein (casein protein/whey protein) of 2 to 20 to a subject in need of rapid absorption of protein.

11. A method for promoting muscle synthesis, comprising administering a milk protein mixture containing casein protein and whey protein at a protein content ratio of casein protein to whey protein (casein protein/whey protein) of 2 to 20 to a subject in need of rapid absorption of protein.

12. The method for promoting muscle synthesis according to Claim 10 or 11, wherein the milk protein mixture is administered so that the total amount of protein for one meal is 0.13 to 0.7 g/kg body weight.
13. The method for promoting muscle synthesis according to Claim 12, wherein the milk protein mixture is administered so that the total amount of protein for one meal is 8 to 40 g.
14. The method for promoting muscle synthesis according to any one of Claims 10 to 13, wherein the milk protein mixture is administered to a subject in need of rapid absorption of protein in a warm liquid form.
15. The method for promoting muscle synthesis according to any one of Claims 10 to 14, wherein the subject in need of rapid absorption of protein is a subject who performs exercise.
16. The method for promoting muscle synthesis according to Claim 15, wherein the subject performing exercise is a subject right after performing exercise.
17. The method for promoting muscle synthesis according to any one of Claims 15 or 16, wherein the exercise is muscle training.