

## MUSCLE SYNTHESIS-STIMULATING AGENT

### CROSS-REFERENCE TO RELATED APPLICATION

[0001]

5           The present application claims priority to Japanese Patent Application No. 2015-158295 (application date: August 10, 2015) which is a prior application applied to Japan. The disclosure of the prior application is herein incorporated by reference in its entirety.

10

### TECHNICAL FIELD

[0002]

          The present invention relates to a muscle synthesis-stimulating agent.

15

### BACKGROUND ART

[0003]

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

25

[0004]

          It is well known that whey protein stimulates muscle synthesis rapidly and can efficiently build muscle strength while preventing muscle fatigue, especially by ingesting the same during exercise, because whey protein contains abundant BCAA  
30       (branched-chain amino acid: valine, leucine, and isoleucine) and the rate from oral ingestion to digestion and absorption (absorption

rate) is fast (the period is short). On the other hand, in the case of casein, the rate from oral ingestion to digestion and absorption (absorption rate) is slower (the period is longer) compared with whey protein. Thus, it is thought that casein is unsuitable for those  
5 who need to stimulate muscle protein synthesis rapidly (see Patent Document 1). Therefore, whey protein is mainly used in such protein powders.

[0005]

That is, oral ingestion of whey protein is the best strategy  
10 for rapidly stimulating muscle synthesis in comparison with casein. Furthermore, whey protein can be made from whey discharged after raw milk is curdled by treatment with acid, rennet and heating and the like in the process of manufacturing natural cheeses. The utilization of whey protein offers considerable  
15 potential because whey can be reused without discarding it as industrial waste, which is advantageous to the environment.

#### PRIOR ART DOCUMENTS

Patent Documents

20 [0006]

[Patent Document 1] Japanese Translation of PCT International Application Publication No. 2009-507044

#### SUMMARY OF THE INVENTION

25 [0007]

As described above, it has been already known that whey protein stimulates muscle synthesis rapidly and, as might be expected, a specific amount of whey protein is required to be ingested for stimulating muscle synthesis. However, ingestion of a  
30 specific amount of whey protein itself has sometimes resulted in difficulties for infants and elderly people who cannot take in

abundant energy. Even for normal adults, there are concerns about the development of lifestyle-related diseases including obesity and inducement of dysfunctions in liver, kidney and the like, which could happen by excessive intake of energy and protein.

5 In addition, ingestion itself of whey protein may cause psychological burdens for people at any age. Particularly, immediately after exercise, ingestion of whey protein often becomes a burden because appetite of subjects declines due to hormone secretion by exercise, even though muscle synthesis  
10 should be rapidly stimulated. In addition, subjects who practice exercises for the purpose of beauty and or slimming are prone to demand to reduce energy intake as less as possible.

[0008]

Thus, it has been known that whey protein stimulates  
15 muscle synthesis rapidly. It is speculated that the high absorption rate of whey protein contributes to this. It has been thought that there is no other compound which stimulates muscle synthesis rapidly as well as whey protein at a lower amount of ingestion (administration) than whey protein, because the absorption rate of  
20 whey protein to the body is much rapider than that of other proteins. It is a fact that the ingestion of whey protein sometimes becomes a burden. However, to date, it has been considered that there is no such muscle synthesis-stimulating agent which exerts an equal or greater effect on stimulating muscle synthesis than  
25 whey protein at an amount less than whey protein and which is not necessarily required to be ingested at a large amount. Therefore, the problem is to provide a new muscle synthesis-stimulating agent which attains this effect.

[0009]

30 In order to solve the above-described problems, the inventors have intensively studied to find out a muscle synthesis-stimulating agent

comprising a whey protein hydrolysate as an active ingredient. Furthermore, the inventors have found out that the muscle synthesis-stimulating agent can be used as a protein source, instead of whey protein, which exhibits an equal or greater effect  
5 on stimulating muscle synthesis with smaller amount of ingestion (administration) than that of whey protein and which does not necessarily require ingestion of a large amount of protein.

[0010]

More specifically, the inventors currently have found  
10 unexpectedly a phenomenon in which a whey protein hydrolysate stimulates muscle synthesis more rapidly than the whey protein itself, despite the fact that no other protein than the whey protein believed to exist that stimulates the same level of muscle synthesis as the whey protein but with a smaller amount of ingestion  
15 (administration) than that of whey protein because of the rapid absorption rate of whey protein in the body. In consideration of the rapid absorption rate in the body, the whey protein hydrolysate of the present invention was in the same level as whey protein and was expected to stimulate muscle synthesis as well as whey  
20 protein. However, the inventors unexpectedly discovered a phenomenon that the whey protein hydrolysate of the present invention rapidly stimulates muscle synthesis greater than that of conventional whey protein. The above-mentioned phenomenon was surprising. The present invention is based on the above-  
25 mentioned findings.

[0011]

In other words, the present invention is as follows.

- [1] A muscle synthesis-stimulating agent comprising a whey protein hydrolysate an active ingredient;
- 30 [2] The muscle synthesis-stimulating agent according to [1], wherein the whey protein hydrolysate contains a dipeptide, and the

dipeptide is at least one dipeptide selected from the group consisting of

1) Ile-Leu, Leu-Ala, and

2) Ala-Leu, Val-Leu, Lys-Ile, Leu-Leu, Ile-Asn, Leu-Glu, Leu-Val  
5 and Ile-Val;

[3] The muscle synthesis-stimulating agent according to [1] or [2], wherein the whey protein hydrolysate is obtained by hydrolysis of whey protein with a proteolytic enzyme;

[4] The muscle synthesis-stimulating agent according to [3],  
10 wherein the proteolytic enzyme is one or two or more enzymes selected from the group consisting of a proteolytic enzyme derived from Bacillus, a proteolytic enzyme derived from Aspergillus, trypsin, pepsin, and flavourzyme;

[5] The muscle synthesis-stimulating agent according to [3] or [4],  
15 wherein hydrolysis with the proteolytic enzyme is performed at 20 to 80°C for 2 to 48 hours;

[6] The muscle synthesis-stimulating agent according to any of [1] to [5], comprising one unit packaging form for one meal, wherein the unit packaging form contains whey protein hydrolysate at an  
20 amount of 1 to 40 g expressed in terms of total amount of protein as the amount of ingestion for one meal;

[7] The muscle synthesis-stimulating agent according to any of [1] to [6] for a subject who performs exercise;

[8] The muscle synthesis-stimulating agent according to [7],  
25 wherein the exercise is muscle training;

[9] The agent according to any of [1] to [8], wherein the agent is a composition for stimulating muscle synthesis;

[10] The agent according to any of [1] to [9], wherein the agent is either a food and drink product or pharmaceutical product;

30 [11] A method for manufacturing a muscle synthesis-stimulating agent comprising blending with an effective amount of whey

protein hydrolysate;

[12] A method for stimulating muscle synthesis, comprising allowing a subject in need thereof to ingest an effective amount of a whey protein hydrolysate;

5 [13] The method for stimulating muscle synthesis according to [12], wherein the whey protein hydrolysate contains a dipeptide, and the dipeptide is at least one dipeptide selected from the group consisting of

1) Ile-Leu, Leu-Ala, and

10 2) Ala-Leu, Val-Leu, Lys-Ile, Leu-Leu, Ile-Asn, Leu-Glu, Leu-Val and Ile-Val;

[14] The method for stimulating muscle synthesis according to [12] or [13], wherein the whey protein hydrolysate is obtained by hydrolysis of whey protein with a proteolytic enzyme;

15 [15] The method for stimulating muscle synthesis according to [14], wherein the proteolytic enzyme is one or two or more enzymes selected from the group consisting of a proteolytic enzyme derived from Bacillus, a proteolytic enzyme derived from Aspergillus, trypsin, pepsin, and flavourzyme;

20 [16] The method for stimulating muscle synthesis according to [14] or [15], wherein hydrolysis with the proteolytic enzyme is performed at 20 to 80°C for 2 to 48 hours;

[17] The method for stimulating muscle synthesis according to any of [12] to [15], which allows a subject to ingest the whey protein hydrolysate at an amount of 1 to 40 g expressed in terms of a total  
25 amount of protein as the amount of ingestion for one meal;

[18] The method for stimulating muscle synthesis according to any of [12] to [17], wherein the subject is a subject who performs exercise;

30 [19] The method for stimulating muscle synthesis according to [18], wherein the exercise is muscle training;

[20] Use of a whey protein hydrolysate for manufacturing a muscle synthesis-stimulating agent;

[21] Use of a whey protein hydrolysate as a muscle synthesis-stimulating agent;

5 [22] A whey protein hydrolysate for stimulating muscle synthesis.  
[0012]

In the present invention, a muscle synthesis-stimulating agent comprising a whey protein hydrolysate as an active ingredient can be provided. The muscle synthesis-stimulating  
10 agent of the present invention can be used as a source of protein, instead of whey protein, which attains an equal or greater effect on stimulating a muscle synthesis with smaller amount of ingestion (administration) than that of whey protein and which does not necessarily require ingestion of a large amount of the protein.  
15 Because the muscle synthesis-stimulating agent of the present invention uses a whey protein hydrolysate as an active ingredient, the burden of a subject in digestion and degradation of the whey protein hydrolysate is less than that of whey protein. Thus, the muscle synthesis-stimulating agent of the present invention is  
20 particularly advantageous in that it can provide an excellent muscle synthesis-stimulating action while relieving the burden of the subject in digestion and exhibiting the effect at an amount of ingestion less than that of whey protein. Also, whey protein is usually discharged after raw milk is curdled by treatment with acid,  
25 rennet and heating and the like in the process of manufacturing natural cheeses and discarded as industrial waste. The whey protein hydrolysate contained in the muscle synthesis-stimulating agent of the present invention as an active ingredient reuses this whey as a raw material. The present invention therefore offers  
30 considerable advantages for economy and environment.

### BRIEF DESCRIPTION OF THE DRAWINGS

[0013]

[Figure 1] Figure 1 shows the difference in the skeletal muscle fractional synthesis rate (FSR (%/day) between the muscle synthesis-stimulating agent of the present invention and whey protein when they are orally administered.

[0014]

The muscle synthesis-stimulating agent of the present invention is a muscle synthesis-stimulating agent in which a whey protein hydrolysate is an active ingredient.

[0015]

An "active ingredient" means an ingredient that is required in order to exhibit an effect on stimulating muscle synthesis. As one aspect, an "active ingredient" means an ingredient that is required to attain an effect on stimulating muscle synthesis for a subject in need of rapid-acting stimulation of muscle synthesis.

[0016]

"Stimulation of muscle synthesis" means to stimulate the synthesis of muscles by ingesting a whey protein hydrolysate and absorbing it as a nutrient in the body. Therefore, the muscle synthesis-stimulating agent of the present invention is used for stimulation of muscle synthesis and a state attributed to this. For example, the purposes of stimulation of muscle synthesis include increase in muscle mass, enhancement of muscle growth, the repair of damaged muscle tissues, the recovery of muscle strength, muscle augmentation, muscle strength improvement, promotional effects for health and athletic performance improvement along with stimulating muscle synthesis, and preferably damaged muscle tissue repair, muscle strength improvement, and promotional effects for health and athletic performance improvement along with stimulation of muscle synthesis. In addition, the stimulating

muscle synthesis also includes an anabolic effect of the muscle protein from amino acids. "Rapid stimulation of muscle synthesis" means to stimulate muscle synthesis by immediate absorption of a whey protein hydrolysate. The stimulation of muscle synthesis of the present invention includes stimulation of muscle synthesis in healthy subjects.

[0017]

A "subject in need of rapid stimulation of muscle synthesis" means a subject who needs the stimulation of muscle synthesis in order to repair muscle tissues and enhance muscle growth. For example, it means a subject who performs exercise to stimulate muscle synthesis, to prevent muscle loss and to promote the recovery 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 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.

[0018]

Subjects ingesting the muscle synthesis-stimulating agent of 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, infants and/or children etc. who need make muscle in the stages of growth and development, and patients suffering from muscle weakness disease (for example, sarcopenia) who need to stimulate muscle synthesis by exercise. Furthermore, such subjects may include animals other than humans (livestock, such as horses and cows, pets, such as dogs

and cats, and ornamental animals reared in zoo).

[0019]

The timing when a subject in need of rapid stimulation of muscle synthesis is allowed to ingest (administered with) the muscle synthesis-stimulating agent in the present invention may be before exercise, during or after exercise. Here, the timing of allowing ingestion is preferably after exercise, more preferably right after exercise. Because the muscle synthesis-stimulating agent 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, and 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).

[0020]

Rapidly stimulating muscle synthesis is equal to or greater than that of whey protein means, stimulating muscle synthesis in a level equal to or greater than those seen when whey protein with the same protein content is orally ingested (administration). In addition, stimulating muscle synthesis also includes the anabolic action of the muscle protein from amino acids. Here, stimulating muscle synthesis 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, when the FSR in the study group of the muscle synthesis-stimulating agent of the present invention is equivalent to or more than the FSR in the control group in which whey protein is orally ingested in the same amount of protein concentration which is said to have the rapid stimulation of muscle

synthesis, stimulation of the muscle synthesis can be determined as significant.

[0021]

"Protein content" means the total amount of amino acids  
5 contained in various proteins. Here, the protein content may be determined and calculated by conventional methods such as the Kjeldahl method or 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-  
10 protein conversion factor (generally 6.25) to determine the amount of protein. For example, the protein content in the whey protein hydrolysate calculated as discussed above is theoretically the same as the protein content in whey protein before hydrolysis, because the whey protein hydrolysate of the present invention is obtained  
15 by hydrolysis of whey protein.

[0022]

The whey protein hydrolysate of the present invention is also referred to as whey peptide. Means and/or methods for the preparation thereof are not particularly limited to as far as all or a  
20 part of whey protein as a raw material is hydrolyzed. Whey protein used for the present invention may be isolated or purified whey protein, and raw materials or food and drink products containing whey protein and casein at a specific protein mass ratio (for example, milk protein mixtures and the like) can be used.

25 [0023]

As isolated or purified whey protein, for example, a stock solution of whey protein (sweet whey, acid whey and the like), a concentrate thereof, dried form thereof (whey powder and the like), frozen matter thereof and the like, and a reduction solution  
30 thereof and the like may be used. Furthermore, desalted whey, a whey protein concentrate (WPC), a whey protein isolate (WPI),  $\alpha$ -

lactalbumin ( $\alpha$ -La),  $\beta$ -lactoglobulin ( $\beta$ -Lg), immunoglobulin, lactoferrin and the like, and a reduction solution thereof may be used. Also, as a raw material or a food and drink product containing whey protein, for example, fresh milk, pasteurized milk  
5 (cow milk and the like), 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) and a concentrate thereof, dried matter  
10 thereof, and frozen matter thereof and the like may be used. At this time, as a raw material or a food and drink product containing whey protein, commercial products or in-house preparations may be used.

[0024]

15 The whey protein hydrolysate of the present invention can be prepared by hydrolysis of whey protein as described above once or more. For example, a whey protein hydrolysate may be prepared by at least partially hydrolyzing whey protein during the step of isolation and purification, and then further another  
20 hydrolysis may be carried out. The whey protein hydrolysate of the present invention can be prepared by hydrolysis of whey protein by a well-known method, preferably by using a proteolytic enzyme. The proteolytic enzyme is not particularly limited as far as the effect on stimulating muscle synthesis in the present  
25 invention is attained, and the types or reaction conditions can be optionally determined by reference to descriptions such as in the Japan patent 5645360. For example, proteolytic enzymes include a proteolytic enzyme derived from microorganisms in Bacillus or Aspergillus; proteolytic enzymes derived from plants such as  
30 papain derived from a papaya, bromelain derived from a pineapple, Actinidin derived from a kiwi and the like; and proteolytic enzymes

derived from animals such as pancreatin, trypsin, chymotrypsin and the like. These proteolytic enzymes can be used alone or in combination of two or more. In addition, commercially available products can be used as a proteolytic enzyme. Commercially  
5 available proteolytic enzymes include, for example, protease M Amano (Amano enzyme), protease N Amano (Amano enzyme), protease P Amano (Amano enzyme), protease A Amano (Amano enzyme), and trypsin (Novo Nordisk), pepsin (Wako Pure Chemical Industries. Ltd.), umamizyme (Amano enzyme), flavourzyme  
10 (Novo Nordisk), but are not limited to them. According to a favorable embodiment of the present invention, a proteolytic enzyme used for hydrolysis of whey protein is one or more kinds selected from the group consisting of a proteolytic enzyme derived from Bacillus, a proteolytic enzyme derived from Aspergillus,  
15 trypsin, pepsin and flavourzyme.  
[0025]

The reaction temperature of hydrolysis of whey protein by a proteolytic enzyme to obtain the whey protein hydrolysate of the present invention is preferably 20 to 80°C, more preferably 25 to  
20 75°C, further preferably 30 to 70°C, further preferably 35 to 65°C, further preferably 40 to 60°C, and particularly preferably 45 to 55°C. When the reaction temperature is 20°C or more, whey protein can be hydrolyzed efficiently by a proteolytic enzyme, which is preferable. In addition, when the reaction temperature is  
25 80°C or less, the deactivation of the proteolytic enzyme by its denaturation is inhibited, which is preferable.

In addition, the reaction temperature of hydrolysis of whey protein by a hydrolytic enzyme to obtain the whey protein hydrolysate of the present invention can be set in one, or two or  
30 more steps in view of the optimal temperature of the hydrolytic enzyme used. When the reaction temperature is determined in two

steps or more, its sequence is optional.

[0026]

The reaction time for hydrolysis of whey protein by a hydrolytic enzyme to obtain the whey protein hydrolysate of the present invention is preferably 2 to 48 hours, more preferably 2 to 36 hours, further preferably 2 to 24 hours, further preferably 2 to 18 hours, further preferably 2 to 14 hours, further preferably 4 to 12 hours, further preferably 6 to 10 hours, and particularly preferable 7 to 9 hours. When the reaction time is 2 hours or more, much whey protein can be hydrolyzed, which is preferable. Also, when the reaction time is 48 hours or less, whey protein can be hydrolyzed efficiently, which is preferable.

In addition, when the reaction temperature is set in two steps or more for hydrolysis of whey protein by a hydrolytic enzyme to obtain the whey protein hydrolysate of the present invention, the temperature in two steps or more can be optionally set among the reaction times described above.

[0027]

An amount of addition of a hydrolytic enzyme when whey protein is subjected to hydrolysis by a hydrolytic enzyme to obtain the whey protein hydrolysate of the present invention is, without particular limitation, preferably 0.5 to 10 g per 100 g of protein, more preferably 0.7 to 5 g, further preferably 0.8 to 4 g, and particularly preferably 1 to 2 g, as far as the effect on stimulating muscle synthesis of the present invention is attained.

[0028]

The reaction pH of hydrolysis of whey protein by a hydrolytic enzyme to obtain the whey protein hydrolysate of the present invention is preferably around an optimal pH of the hydrolytic enzyme used. For example, when a proteolytic enzyme derived from Bacillus and a proteolytic enzyme derived from Aspergillus are

concomitantly used, pH is preferably 5 to 9, more preferably 5.5 to 8.5, further preferably 6 to 8, and particularly preferably 6.5 to 7.5.

In addition, the reaction pH when whey protein is subjected to hydrolysis by a protein hydrolytic enzyme to obtain the whey protein hydrolysate of the present invention can be set in one step or two or more steps in view of the optimum pH of the hydrolytic enzyme to be used. When the reaction pH in two or more steps is determined, its sequence is optional.

[0029]

As an index for the degradation of the whey protein hydrolysate of the present invention, inclusion of a specific dipeptide can be used. The specific dipeptide here is preferably Ile-Leu, Leu-Ala, Ala-Leu, Val-Leu, Lys-Ile, Leu-Leu, Ile-Asn, Leu-Glu, Leu-Val and Ile-Val, and a case including at least one dipeptide selected from the group consisting of those dipeptides. Here, Ile means isoleucine, Leu means leucine, Ala means alanine, Val means valine, Lys means lysine, Asn means asparagine, and Glu means glutamic acid, respectively. The above dipeptides can be measured by LC/MS, specifically by a method described in Test Example 1, mentioned below.

[0030]

For the above specific dipeptide, more preferable is a case containing a specific dipeptide that fills the following conditions (1) and (2).

At least one dipeptide selected from the group consisting of

(1) Ile-Leu, Leu-Ala, and

(2) Ala-Leu, Val-Leu, Lys-Ile, Leu-Leu, Ile-Asn, Leu-Glu, Leu-Val and Ile-Val.

In other words, it is particularly preferable that Ile-Leu and Leu-Ala in (1) are contained in the whey protein hydrolysate of the

present invention, and it is particularly preferable that at least one dipeptide among Ala-Leu, Val-Leu, Lys-Ile, Leu-Leu, Ile-Asn, Leu-Glu, Leu-Val and Ile-Val in (2) is contained in the whey protein hydrolysate of the present invention.

5 [0031]

When inclusion of a specific dipeptide Ile-Leu is used as an index for degradation in the whey protein hydrolysate of the present invention, its content is preferably 0.5 mg or more per 1 g of the protein content of the whey protein hydrolysate (the protein  
10 content of whey protein before hydrolysis), more preferably 0.5 to 10 mg per 1 g of protein content, further preferably 0.5 to 8 mg per 1 g of protein content, further preferably 0.5 to 6 mg per 1 g of protein content, and particularly preferably 0.5 to 4 mg per 1 g of protein content. When the content of dipeptide Ile-Leu is 0.5 mg  
15 or more per 1 g of the protein content of the whey protein hydrolysate, the effect on stimulating muscle synthesis of the present invention is further exhibited, which is preferable. In addition, when the content of dipeptide Ile-Leu is 10 mg or less per 1 g of the protein content of the whey protein hydrolysate, the  
20 whey protein hydrolysate of the present invention can be prepared efficiently, which is preferable.

[0032]

When inclusion of a specific dipeptide Leu-Ala is used as an index for degradation in the whey protein hydrolysate of the present invention, its content is preferably 0.5 mg or more per 1 g  
25 of the protein content of the whey protein hydrolysate (the protein content of whey protein before hydrolysis), more preferably 0.5 to 10 mg per 1 g of protein content, further preferably 0.5 to 8 mg per 1 g of protein content, further preferably 0.5 to 6 mg per 1 g of  
30 protein content, and particularly preferably 0.5 to 4 mg per 1 g of protein content. When the content of dipeptide Leu-Ala is 0.5 mg

or more per 1 g of the protein content of the whey protein hydrolysate, the effect on stimulating muscle synthesis of the present invention is further exhibited, which is preferable. Also, when the content of dipeptide Leu-Ala is 10 mg or less per 1 g of the protein content of the whey protein hydrolysate, the whey protein hydrolysate of the present invention can be prepared efficiently, which is preferable.

[0033]

When inclusion of a specific dipeptide Ala-Leu is used as an index for degradation in the whey protein hydrolysate of the present invention, its content is preferably 0.2 mg or more per 1 g of the protein content of the whey protein hydrolysate (the protein content of whey protein before hydrolysis), more preferably 0.2 to 10 mg per 1 g of protein content, further preferably 0.2 to 8 mg per 1 g of protein content, further preferably 0.2 to 6 mg per 1 g of protein content, and particularly preferably 0.2 to 5 mg per 1 g of protein content. When the content of dipeptide Ala-Leu is 0.2 mg or more per 1 g of the protein content of the whey protein hydrolysate, the effect on stimulating muscle synthesis of the present invention is further exhibited, which is preferable. In addition, when the content of dipeptide Ala-Leu is 10 mg or less per 1 g of the protein content of the whey protein hydrolysate, the whey protein hydrolysate of the present invention can be prepared efficiently, which is preferable.

[0034]

When inclusion of a specific dipeptide Val-Leu is used as an index for degradation in the whey protein hydrolysate of the present invention, its content is preferably 1 mg or more per 1 g of the protein content of the whey protein hydrolysate (the protein content of whey protein before hydrolysis), more preferably 1 to 15 mg per 1 g of protein content, further preferably 1 to 12 mg per 1

g of protein content, further preferably 1 to 10 mg per 1 g of protein content, and particularly preferably 1 to 8 mg per 1 g of protein content. When the content of dipeptide Val-Leu is 1 mg or more per 1 g of the protein content of the whey protein hydrolysate, the effect on stimulating muscle synthesis of the present invention is further exhibited, which is preferable. In addition, when the content of dipeptide Val-Leu is 15 mg or less per 1 g of the protein content of the whey protein hydrolysate, the whey protein hydrolysate of the present invention can be prepared efficiently, which is preferable.

[0035]

When inclusion of a specific dipeptide Lys-Ile is used as an index for degradation in the whey protein hydrolysate of the present invention, its content is preferably 0.2 mg or more per 1 g of the protein content of the whey protein hydrolysate (the protein content of whey protein before hydrolysis), more preferably 0.2 to 15 mg per 1 g of protein content, further preferably 0.2 to 12 mg per 1 g of protein content, further preferably 0.2 to 10 mg per 1 g of protein content, and particularly preferably 0.2 to 8 mg per 1 g of protein content. When the content of dipeptide Lys-Ile is 0.2 mg or more per 1 g of the protein content of the whey protein hydrolysate, the effect on stimulating muscle synthesis of the present invention is further exhibited, which is preferable. Also, when the content of dipeptide Lys-Ile is 15 mg or less per 1 g of the protein content of the whey protein hydrolysate, the whey protein hydrolysate of the present invention can be prepared efficiently, which is preferable.

[0036]

When inclusion of a specific dipeptide Leu-Leu is used as an index for degradation in the whey protein hydrolysate of the present invention, its content is preferably 0.2 or more per 1 g of

the protein content of the whey protein hydrolysate (the protein content of whey protein before hydrolysis), more preferably 0.2 to 25 mg per 1 g of protein content, further preferably 0.2 to 20 mg per 1 g of protein content, further preferably 0.2 to 18 mg per 1 g of protein content, and particularly preferably 0.2 to 16 mg per 1 g of protein content. When the content of dipeptide Leu-Leu is 0.2 mg or more per 1 g of the protein content of the whey protein hydrolysate, the effect on stimulating muscle synthesis of the present invention is further exhibited, which is preferable. In addition, when the content of dipeptide Leu-Leu is 25 mg or less per 1 g of the protein content of the whey protein hydrolysate, the whey protein hydrolysate of the present invention can be prepared efficiently, which is preferable.

[0037]

When inclusion of a specific dipeptide Ile-Asn is used as an index for degradation in the whey protein hydrolysate of the present invention, its content is preferably 1 mg or more per 1 g of the protein content of the whey protein hydrolysate (the protein content of whey protein before hydrolysis), more preferably 1 to 10 mg per 1 g of protein content, further preferably 1 to 8 mg per 1 g of protein content, further preferably 1 to 6 mg per 1 g of protein content, and particularly preferably 1 to 5 mg per 1 g of protein content. When the content of dipeptide Ile-Asn is 1 mg or more per 1 g of the protein content of the whey protein hydrolysate, the effect on stimulating muscle synthesis of the present invention is further exhibited, which is preferable. In addition, when the content of dipeptide Ile-Asn is 10 mg or less per 1 g of the protein content of the whey protein hydrolysate, the whey protein hydrolysate of the present invention can be prepared efficiently, which is preferable.

[0038]

When inclusion of a specific dipeptide Leu-Glu is used as an index for degradation in the whey protein hydrolysate of the present invention, its content is preferably than 0.5 mg or more per 1 g of the protein content of the whey protein hydrolysate (the protein content of whey protein before hydrolysis), more preferably 0.5 to 12 mg per 1 g of protein content, further preferably 0.5 to 10 mg per 1 g of protein content, further preferably 0.5 to 8 mg per 1 g of protein content, and particularly preferably 0.5 to 6 mg per 1 g of protein content. When the content of dipeptide Leu-Glu is 0.5 mg or more per 1 g of the protein content of the whey protein hydrolysate, the effect on stimulating muscle synthesis of the present invention is further exhibited, which is preferable. In addition, when the content of dipeptide Leu-Glu is 12 mg or less per 1 g of the protein content of the whey protein hydrolysate, the whey protein hydrolysate of the present invention can be prepared efficiently, which is preferable.

[0039]

The whey protein hydrolysate of the present invention is in a liquid form right after the preparation, but it can be subjected to cryopreservation, drying (spray drying, lyophilization), concentration, thermal sterilization (indirect thermal sterilization such as sterilization with plate heat exchanger, thermal sterilization with tubular-type heat exchanger, batch-type thermal sterilization, thermal sterilization by electrical heating, thermal sterilization by microwave, retort heating and the like; direct thermal sterilization such as steam injection, steam infusion and the like), non-thermal sterilization (photolytic sterilization, radiation sterilization, high-voltage pulsed sterilization) to increase its preserving property. Above all, the form treated with drying (spray drying, lyophilization) is preferable due to the simplicity of handling it.

[0040]

As far as the effect of the present invention, enhancing muscle synthesis-stimulating effect compared with whey protein itself, is exerted, the whey protein hydrolysate of the present invention can be added with other food raw materials and/or food additive raw materials. For example, for the purpose of imparting palatability to the muscle synthesis-stimulating agent of the present invention, sweeteners (sugars, liquid sugars, fructose, maltose, soft brown sugars and the like), sugar alcohols (erythritol and the like), high-potency sweeteners (stevia, aspartame, sucralose, acesulfame potassium), acidulants, polysaccharide thickeners, spices, fruit juice, emulsifiers, or vegetable juice can be added. In addition, to impart additional functions to the muscle synthesis-stimulating agent of the present invention, minerals (calcium, iron, manganese, magnesium, zinc and the like), vitamins, functional materials, probiotic lactic acid bacteria and the like can be added to provide the muscle synthesis-stimulating agent of the present invention.

[0041]

The muscle synthesis-stimulating agent of the present invention can be produced in a powdery form packaged in a container for the market. Well-known materials such as paper, bags, boxes, cans, bottles, and the like can be used for the container. The capacity for the container is, not particularly limited, for example, 1 to 5000 g, 2 to 4000 g, 3 to 3000 g, 4 to 2000 g, 4 to 1000 g, 4 to 500 g, 4 to 250 g, 4 to 200 g, 4 to 100 g, 4 to 50 g, and 4 to 30 g. Above all, for example, the capacity is 300 to 5000 g, 300 to 4000 g, 300 to 3000 g, 300 to 2000 g, 300 to 1500 g, 300 to 1400 g, 300 to 1300 g, 300 to 1200 g, 300 to 1100 g for a family size (for multiple administrations), and 1 to 500 g, 2 to 250 g, 3 to 200 g, 4 to 100 g, 4 to 50 g, 4 to 40 g, 4 to 30 g, 5 to 30 g, 10 to 30 g, 15 to 30 g for a personal size (individual

administration).

[0042]

The muscle synthesis-stimulating agent of the present invention can be produced in the form of a beverage in a container (a beverage in which a powder is dispersed and dissolved) for the market. Well-known containers such as paper, soft bag, PET bottles, cans, bottles and the like can be used. The capacity for the container is, not particularly limited, for example, 1 to 5000 g, 2 to 4000 g, 3 to 3000 g, 4 to 2000 g, 5 to 1000 g, 6 to 800 g, 7 to 1300 g, 80 to 1200 g, and 90 to 1100 g. Above all, for example, the capacity is 300 to 5000 g, 300 to 4000 g, 300 to 3000 g, 300 to 2000 g, 300 to 1500 g, 300 to 1400 g, 300 to 1300 g, 300 to 1200 g, 300 to 1100 g for a family size (for multiple drinks), and 10 to 500 g, 20 to 500 g, 30 to 500 g, 40 to 500 g, 50 to 500 g, 60 to 500 g, 70 to 500 g, 80 to 500 g, 90 to 500 g for a personal size (individual drink).

[0043]

According to one embodiment of the present invention, for example, the muscle synthesis-stimulating agent of the present invention is ingested (administered) so that the protein content in a whey protein hydrolysate is preferably more than 0.05 g/kg body weight for one meal, more preferably 0.05 to 0.67 g/kg body weight, further preferably 0.05 to 0.33 g/kg body weight, more preferably 0.07 to 0.33 g/kg body weight, further preferably 0.07 to 0.25 g/kg body weight, and particularly preferably 0.08 to 0.25 g/kg body weight. Here, the typical body weight of the subject is set at about 60 kg.

[0044]

"One meal" means each state in which rapid-acting stimulation of muscle synthesis is required. For example, the muscle synthesis-stimulating agent of the present invention may

be ingested in one meal not at one time but at several times by dividing one meal. In terms of rapidly raising the blood levels of amino acids, when injection is divided into several instances, the timing of the allowing ingestion (administration) of the muscle synthesis-stimulating agent of the present invention to a subject in need of rapid-acting stimulation of muscle synthesis is preferably within 2 hours after exercise, more preferably within 1 hour after exercise, further preferably within 30 minutes after exercise, and particularly preferably within 5 minutes after exercise.

10 [0045]

A method by which a subject in need of rapid-acting stimulation of muscle synthesis is allowed to ingest (administered with) the muscle synthesis-stimulating agent of the present invention can be appropriately chosen from the following: 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.

[0046]

According to another embodiment of the present invention, for example, the muscle synthesis-stimulating agent of the present invention is composed of one unit packaging form for one meal, wherein the whey protein hydrolysate in the unit is preferably adjusted as the amount of ingestion for one meal to 1 g or more expressed in terms of total content of protein, specifically 1 to 40 g, preferably 2 to 20 g, more preferably 3 to 20 g, further preferably 4 to 15 g, and particularly preferably 5 to 15 g. Here, "one unit packaging form for one meal" means that the amount of ingestion 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 ingestion for one meal (usage, dosage) on the packaging container or on a homepage and the like. There is less gelatinization of the muscle synthesis-stimulating agent of the present invention by heating than with whey protein because it uses a whey protein hydrolysate as an active ingredient. Therefore, suitable properties can be maintained for the process of manufacturing drink, jelly and the like. Also, the muscle synthesis-stimulating agent of the present invention exhibits an effect on stimulating muscle synthesis even at a small quantities. Therefore, convenience for children and elderly people can be improved in any package forms such as a bite-sized block, a mini-canned beverage that can be swallowed by the mouthful, and the like, which is easy to ingestion or swallowing.

[0047]

According to a favorable embodiment of the present invention, for the muscle synthesis-stimulating agent of the present invention, a whey protein hydrolysate contains dipeptides composed of Ile-Leu, Leu-Ala, Ala-Leu, Val-Leu, Lys-Ile, Leu-Leu, Ile-Asn, Leu-Glu, Leu-Val and Ile-Val. According to a more favorable embodiment of the present invention, for the muscle synthesis-stimulating agent of the present invention, a whey

protein hydrolysate contains dipeptides composed of Ile-Leu, Leu-Ala, Val-Leu, Lys-Ile, Leu-Leu, Ile-Asn, Leu-Glu, Leu-Val and Ile-Val.

[0048]

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

[0049]

20           Also, as described above, the muscle synthesis-stimulating agent of the present invention can be provided by itself alone, and in the form of a composition in which a plurality of ingredients is blended thereto. Therefore, according to one embodiment of the present invention, a composition for stimulating muscle synthesis is  
25 provided which contains the whey protein hydrolysate as an active ingredient. The form of these compositions, not limited as long as the effect on stimulating muscle synthesis is exerted, can include, for example, a solid form (a powder form, a granular form, a capsule form and a block form), a liquid form, a gel form, a glue-like form, a paste form and the like, and preferably a powder form.  
30 The composition includes a food and drink composition and

pharmaceutical composition.

[0050]

According to a favorable embodiment of the present invention, a food and drink product for stimulating muscle  
5 synthesis is provided which contains the whey protein hydrolysate as an active ingredient. In the present invention, the form of a food and drink product is not particularly limited as long as in the orally ingestible form such as solution, suspension, liquid emulsion, powder, and solid molding product, and the like. Specifically  
10 included are instant foods such as instant noodle, retort pouch, canned foods, microwave oven foods, instant soup, miso soup, freeze-dried foods and the like; beverages such as refreshing drink, fruit juice, vegetable drink, soy milk, coffee drink, tea drink, powdered drink, concentration drink, alcoholic drink and the like;  
15 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 source, processed tomato seasoning, flavor seasoning, seasoning  
20 cooking mixture, 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, yogurts, lactic acid bacteria beverage, ice cream, cream and the like; processed agricultural products such as agricultural  
25 canned food, jam and marmalade, cereal and the like; and frozen foods. A food and drink product is preferably a dairy product, and more preferably milk drink, lactic acid bacteria beverage.

[0051]

Also, the food and drink product includes health foods,  
30 functional foods, nutritional supplementary foods, foods with function claims, foods for specified health use, foods for patients,

modified dry milk for infants, powdered milk for pregnant women or lactating women, and a food and drink product with description of usage for stimulating muscle synthesis.

[0052]

5           According to another embodiment of the present invention, a pharmaceutical product for stimulating muscle synthesis is provided which contains the whey protein hydrolysate as an active ingredient. A pharmaceutical product may mean an agent prepared as an oral preparation or a parenteral preparation by  
10   adding pharmaceutically acceptable additives according to a conventional method. In the case of oral preparation, solid preparations such as tablets, powders, fine granules, granules, capsules, pills, controlled release agents and the like; liquid preparations such as solution, suspension, emulsion and the like  
15   may be used. In the case of parenteral preparation, injection and suppository may be used. In terms of simplicity, an oral formulation is preferable. Pharmaceutically acceptable additives include, for example, excipients, stabilizers, preservatives, wetting agents, emulsifiers, lubricants, sweeteners, coloring agents,  
20   flavors, buffers, antioxidants, pH adjusters and the like. A pharmaceutical product of the present invention can be used to treat or prevent diseases that require stimulating muscle synthesis, specifically, muscle damage disorders (for example, sarcopenia).

[0053]

25           Also, as described above, the muscle synthesis-stimulating agent of the present invention can be provided by blending an effective dose of the whey protein hydrolysate and optionally acceptable additives for oral ingestion. Therefore, according to another embodiment of the present invention, a method for  
30   manufacturing a muscle synthesis-stimulating agent comprising blending with an effective dose of the whey protein hydrolysate is

provided. The method for manufacturing the muscle synthesis-stimulating agent of the present invention may further comprise a step of heating and homogenization of a mixture obtained by blending a whey protein hydrolysate. The present invention  
5 includes such an embodiment.

[0054]

In addition, according to another embodiment of the present invention, a method for stimulating muscle synthesis is provided, the method comprising allowing a subject in need thereof to ingest  
10 an effective amount of the whey protein hydrolysate. According to another embodiment of the present invention, the method described above excludes medical interference for humans. Here, "medical interference toward humans" means an action of allowing humans to ingest (administering to humans) a pharmaceutical  
15 product, requiring doctor's prescription. Also, in the embodiment described above, the subject is preferably a healthy subject.

[0055]

In addition, the method for stimulating muscle synthesis of the present invention can be used to improve diseases or  
20 conditions associated with muscular damage in a subject. Therefore, the method for stimulating muscle synthesis is preferably a treatment method for diseases or conditions associated with muscular damage, and more preferably the method for improving sarcopenia. Herein, "improvement" is not only to  
25 treat established diseases or conditions but also to prevent diseases or conditions that may develop in the future. In addition, the method for stimulating muscle synthesis can be performed according to the explanation of the muscle synthesis-stimulating agent in the present DESCRIPTION.

30 [0056]

According to one preferable embodiment of the present

invention, in the method for stimulating muscle synthesis of the present invention, the protein content in a whey protein hydrolysate can be preferably ingested (administered) at 0.05 g or more /kg body weight for one meal, more preferably 0.05 to 0.67 g/kg body weight, further preferably 0.05 to 0.33 g/kg body weight, more preferably 0.07 to 0.33 g/kg body weight, further preferably 0.07 to 0.25 g/kg body weight, and particularly preferably 0.08 to 0.25 g/kg body weight. Here, the typical body weight of the subject is set at about 60 kg. Because the muscle synthesis-stimulating agent in the present invention exhibits sufficient effect even at small amounts as compared to whey protein, the burden for children and elderly people can be lessened. [0057]

According to a preferable embodiment of the present invention, in the method for stimulating muscle synthesis of the present invention, the protein content in a whey protein hydrolysate can be allowed to ingest at 1 g or more for one meal, specifically, 1 to 40 g, preferably 2 to 20 g, more preferably 3 to 20 g, further preferably 4 to 15 g, and particularly preferably 5 to 15 g. [0058]

According to one embodiment of the present invention, use of the whey protein hydrolysate for stimulation of muscle synthesis is provided. According to one preferable embodiment of the present invention, the use of the present invention is non-therapeutic. [0059]

According to one embodiment of the present invention, use of the whey protein hydrolysate for manufacturing food and drink products and pharmaceutical products for stimulation of muscle synthesis is provided.

[0060]

According to one embodiment of the present invention, the whey protein hydrolysate for stimulation of muscle synthesis is provided.

5 [0061]

According to the muscle synthesis-stimulating agent of the present invention, its muscle synthesis-stimulating effect can be enhanced as compared with whey protein itself, which is not hydrolyzed. For example, in an animal experiment in Example 10 (Test Example 1) described later, it was shown that the muscle synthesis-stimulating agent of the present invention stimulated muscle synthesis higher than that of whey protein which was not hydrolyzed when a specific amount of protein content in the muscle synthesis-stimulating agent of the present invention was ingested 15 (administered). Also, in an animal experiment in Example (Test Example 1) described later, when the muscle synthesis-stimulating agent of the present invention was ingested (administered) at 0.5 g of protein/kg body weight, the muscle synthesis-stimulating agent stimulated muscle synthesis with a tendency to rise from the equal level when whey protein was ingested (administered) at 2.0 20 g of protein/kg body weight.

[0062]

In other words, the muscle synthesis-stimulating agent of the present invention can be expected to exhibit a strong muscle 25 synthesis-stimulating effect by ingestion (administration) of a specific amount of protein content when compared with whey protein that has been well known to have a rapid-acting muscle synthesis-stimulating effect. In addition, to obtain a muscle synthesis-stimulating effect equal to that when whey protein is 30 ingested, the amount of the protein content in the muscle synthesis-stimulating agent of the present invention can be

reduced for ingestion. Thus, it can be easily ingested (administered) because its effect can be expected to be attained at a small amount. Furthermore, the digestion burden can be reduced because the whey protein hydrolysate, an active ingredient, has been already partially broken down. Until now, ingestion of whey protein itself has sometimes resulted in difficulties for infants and elderly people who cannot take in abundant energy. Even for normal adults, there are concerns about the development of lifestyle-related diseases including obesity and inducement of dysfunctions in liver and kidney, which could happen by excessive intake of energy and protein. Further, ingestion itself of whey protein may cause psychological burdens for people at any age. Particularly, ingestion of whey protein often becomes a burden because appetite of subjects declines due to hormone secretion by exercise, even though muscle synthesis should be immediately promoted right after exercise. In addition, subjects who practice exercises for the purpose of beauty or slimming are prone to demand to reduce energy intake. On the other hand, the problems can be solved by the whey protein hydrolysate of the present invention because it can attain a muscle synthesis stimulating effect at an amount of ingestion less than that of whey protein.

### EXAMPLES

[0063]

In the following, the present invention will be further explained by referring to the following Examples, but not limited to these Examples. In addition, "protein" in "mg/g protein" and "g protein/kg body weight" described later indicate protein content.

[0064]

[Example 1]

Fifty grams of whey protein was dissolved in 1 L of water. The solution of dissolved whey protein was adjusted to pH 7 with a pH adjuster and 500 mg of protease derived from Bacillus (protease M Amano, Amano enzyme) and 500 mg of protease derived from Aspergillus (protease N Amano, Amano enzyme) were combined and added to the solution and whey protein was hydrolyzed at 50°C for 8 hours. After heating deactivation of proteases, the solution obtained was pulverized to powder by lyophilization to obtain the muscle synthesis-stimulating agent in

Example 1.

[0065]

[Test example 1]

The powder form of the muscle synthesis-stimulating agent in Example 1 was dissolved in a 0.1% trifluoroacetic acid aqueous solution at a dilution of 1,000 times (volume ratio), and Ile-Leu, Ile-Trp, Ala-Leu, Val-Leu, Asp-Leu, Lys-Ile, Leu-Leu, Ile-Asn, Leu-Ala and Leu-Glu were quantitated using LC/MS under the following conditions (unit is mg/g protein).

The conditions of LC/MS are specifically as follows.

Analysis conditions

Column: Develosil ODS-HG-3 (15 mm × 2 mm)

Mobile phase: A solution: 0.05% TFA aqueous solution

B solution: 0.05% TFA acetonitrile solution

A gradient was applied starting from 3 vol% of solution B at 0 min to 20 vol% of solution B at 40 min (ratio to the total volume of solution A and solution B, respectively) after the initiation of elution of mobile phase.

Column temperature: 35°C

Flow rate: 0.2 mL/min

MS conditions

Ionization: API-ES positive

SIM ion: m/z 245

Drying gas: 10 L/min at 350°C

Nebulizer: 25 psig

Fragmentor: 30 V

5 EM gain: 1

[0066]

The muscle synthesis-stimulating agent in Example 1 contained 3.82 mg/g protein of Ile-Leu, 1.53 mg/g protein of Ala-Leu, 4.70 mg/g protein of Val-Leu, 4.82 mg/g protein of Lys-Ile, 10 1.56 mg/g protein of Leu-Leu, 3.98 mg/g protein of Ile-Asn, 0.75 mg/g protein of Leu-Ala, and 4.35 mg/g protein of Leu-Glu.

[0067]

[Test example 2]

The muscle synthesis-stimulating effect of the muscle synthesis-stimulating agent in Example 1 was verified as follows.

(1) Swimming test in rats

Male SD rats with initial body weight of 130 to 170 g (obtained from CLEA Japan Inc.) were used as experimental animals.

20 Rats were acclimated to swimming exercise for 30 minutes (water temperature at 34°C) after preliminarily bred for 3 to 5 days with solid feed (product made in Oriental Yeast Co., Ltd.). After fasting the rats for 16 hours from the second day to the third day of the acclimatization of swimming exercise, the rats were divided 25 into five groups to avoid developing variability in body weight in each study groups (8 rats in each test group). Subsequently, the rats were subjected to swimming exercise for 2-hours. Immediately after the swimming exercise, the rats were administrated with water (i.e., 0 g of protein/kg body weight), or 30 2.5wt% 10wt% solution of a muscle synthesis-stimulating agent, or 2.5wt% 10wt% solution of whey protein at 20 mL/kg body

weight (i.e., 0.5 g protein/kg body weight or 2.0 g protein/kg body weight). Forty five minutes after the oral administration, a solution of deuterium-labeled phenylalanine (prepared by dissolving 225 mg of deuterium-labeled phenylalanine in 10 mL of physiological saline) was injected in tail vein at 2.0 mL/kg body weight. Fifteen minutes after the tail vein injection (namely, 60 minutes after the oral administration), the rats were dissected under isoflurane anesthesia. After exsanguination from the inferior vena cava, triceps brachii muscle was excised, washed with saline, and immediately frozen with liquid nitrogen. The time of the tail vein injection and the cryopreservation of the triceps brachii muscle were exactly recorded. The samples were stored at -80°C until analysis.

[0068]

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

The skeletal muscle fractional synthesis rate (FSR) of the excised and preserved triceps brachii muscle was measured according to the description in the prior art document (A.Kanda et al., Br J Nutr, Sep. 28, 981-987, 2013). Specifically, the amounts of phenylalanine (Phe) and deuterium-labeled phenylalanine (Phe (Ring-D5)) which were existing in assimilated protein of the skeletal muscle cells or in a free state in the skeletal muscle cells were quantitated using LC/MS/MS (Waters). 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: 215 nm.

[0069]

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 (existing in skeletal muscle cells in a free state)

Eb: Enrichment (existing as being assimilated into protein of the skeletal muscle cells)

5 t: The time interval between tail vein administration and cryopreserving of the triceps brachii muscle (Unit: day)

Enrichment =  $\text{Phe(Ring - D5)} / (\text{Phe} + \text{Phe(Ring - D5)})$   
[0070]

10 The mean  $\pm$  SE of each study group was determined from the values obtained. Statistical analysis was carried out by using a multiple comparison test with a Dunnett method in all the groups against water administration group (0 g of protein/kg body weight) as a control group. Also, Student's t-test was performed between  
15 the group administered with the muscle synthesis-stimulating agent of Example 1 and the group administered with whey protein when the same amounts of protein were administered in both groups. In either case, the significance level was set at less than 5% ( $p < 0.05$ ). The results are shown in Figure 1.

20 [0071]

The skeletal muscle fractional synthesis rate in rats administered with whey protein significantly increased only at a dose of 2.0 g of protein/kg body weight as compared to the water administration group (0 g of protein /kg weight). By contrast, the  
25 skeletal muscle fractional synthesis rate in rats significantly increased when rats were administered the muscle synthesis-stimulating agent in Example 1 at the doses of both 0.5 g of protein/kg body weight and 2.0 g of protein /kg body weight as compared to the water administration group (Figure 1).  
30 Furthermore, the skeletal muscle fractional synthesis rate in rats administered with 0.5 g of protein/kg body weight of the muscle

synthesis-stimulating agent in Example 1 was significantly higher than the skeletal muscle fractional synthesis rate in rats administrated with 0.5 g of protein/kg body weight of the whey protein (Figure 1).

5 [0072]

The skeletal muscle fractional synthesis rate in rats administrated with 2.0 g of protein/kg body weight of the muscle synthesis-stimulating agent in Example 1 tended to rise from the equal level when compared to the skeletal muscle fractional  
10 synthesis rate in rats administrated with 2.0 g of protein/kg body weight of the whey protein, which has the same protein content (Figure 1). Also, the skeletal muscle fractional synthesis rate in rats administrated 0.5 g of protein/kg body weight of the muscle synthesis-stimulating agent in Example 1 also tended to rise from  
15 the equal level when compared to the skeletal muscle fractional synthesis rate in rats administrated 2.0 g of protein/kg body weight of the whey protein (Figure 1). As shown in Figure 1, the muscle synthesis-stimulating agent of the present invention stimulated muscle synthesis greater than that of whey protein. And it was  
20 confirmed that the difference in this effect became particularly remarkable at administration of a small quantity.

[0073]

Based on the results above, it was found that the muscle synthesis-stimulating agent of the present invention showed an  
25 equal effect on stimulating muscle synthesis with smaller amount of administration than that of whey protein that has been known to have an effect on stimulating muscle synthesis rapidly. It was also found that the muscle synthesis-stimulating agent of the present invention retained an excellent muscle synthesis-stimulating action  
30 and that the amount of administration could be reduced even though the active ingredient of the muscle synthesis-stimulating

agent of the present invention is a hydrolysate. Therefore, it was confirmed that the muscle synthesis-stimulating agent of the present invention is advantageous for performing the stimulation of muscle synthesis effectively while relieving a digestion burden for subjects whose visceral functions have decreased in kidney, stomach and the like.

[0074]

[Example 2]

Fifty grams of whey protein was dissolved in 1 L of water. The solution dissolving whey protein was adjusted to pH 7 with a pH adjuster, and 500 mg of protease derived from Bacillus (protease M Amano, Amano Enzyme) was added to the solution and whey protein was hydrolyzed at 50°C for 8 hours. After heating deactivation of proteases, the solution obtained was pulverized to powder by lyophilization to obtain the muscle synthesis-stimulating agent in Example 2.

[0075]

Based on Test Example 1, the content of dipeptide in the muscle synthesis-stimulating agent in Example 2 was measured. The muscle synthesis-stimulating agent in Example 2 contained 2.39 mg/g protein of Ile-Leu, 0.64 mg/g protein of Ala-Leu, 2.35 mg/g protein of Val-Leu, 1.97 mg/g protein of Lys-Ile, 0.67 mg/g protein of Leu-Leu, 2.73 mg/g protein of Ile-Asn, 1.22 mg/g protein of Leu-Ala, and 1.90 mg/g protein of Leu-Glu.

[0076]

[Example 3]

Fifty grams of whey protein was dissolved in 1 L of water. The solution dissolving whey protein was adjusted to pH 7 with a pH adjuster, and 500 mg of protease derived from Aspergillus (protease N Amano, Amano Enzyme) was added to the solution and whey protein was hydrolyzed at 50°C for 8 hours. After

heating deactivation of proteases, the solution obtained was pulverized to powder by lyophilization to obtain the muscle synthesis-stimulating agent in Example 3.

[0077]

5           Based on Test example 1, the content of dipeptide in the muscle synthesis-stimulating agent in Example 3 was measured. The muscle synthesis-stimulating agent in Example 3 contained 3.74 mg/g protein of Ile-Leu, 1.98 mg/g protein of Ala-Leu, 2.13 mg/g protein of Val-Leu, 1.07 mg/g protein of Lys-Ile, 8.20 mg/g  
10 protein of Leu-Leu, 2.40 mg/g protein of Ile-Asn, 1.77 mg/g protein of Leu-Ala, and 1.08 mg/g protein of Leu-Glu.

[0078]

[Example 4]

Fifty grams of whey protein was dissolved in 1 L of water.  
15 The solution dissolving whey protein was adjusted to pH 7 with a pH adjuster, 500 mg of trypsin (Novo Nordisk) was added, the solution was subjected to reaction at 37°C for 4 hours and then 500 mg of protease derived from Bacillus (protease M Amano, Amano Enzyme) was added. The solution was hydrolyzed at 50°C  
20 for 4 hours. After heating deactivation of proteases, the solution obtained was pulverized to powder by lyophilization to obtain the muscle synthesis-stimulating agent in Example 4.

[0079]

Based on Test example 1, the content of dipeptide in the  
25 muscle synthesis-stimulating agent in Example 4 was measured. The muscle synthesis-stimulating agent in Example 2 contained 3.16 mg/g protein of Ile-Leu, 4.77 mg/g protein of Ala-Leu, 5.33 mg/g protein of Val-Leu, 4.84 mg/g protein of Lys-Ile, 1.07 mg/g protein of Leu-Leu, 1.65 mg/g protein of Ile-Asn, 0.83 mg/g  
30 protein of Leu-Ala, and 4.82 mg/g protein of Leu-Glu.

[0080]

[Example 5]

Fifty grams of whey protein was dissolved in 1 L of water. The solution dissolving whey protein was adjusted to pH 7 with a pH adjuster, 500 mg of trypsin (Novo Nordisk) was added, the  
5 solution was subjected to reaction at 37°C for 4 hours and then whey protein was hydrolyzed by protease derived from *Aspergillus* (protease N Amano, Amano Enzyme) at 50°C for 4 hours. After heating deactivation of proteases, the solution obtained was pulverized to powder by lyophilization to obtain the muscle  
10 synthesis-stimulating agent in Example 5.

[0081]

Based on Test example 1, the content of dipeptide in the muscle synthesis-stimulating agent in Example 5 was measured. The muscle synthesis-stimulating agent in Example 5 contained  
15 3.57 mg/g protein of Ile-Leu, 2.04 mg/g protein of Ala-Leu, 2.60 mg/g protein of Val-Leu, 0.74 mg/g protein of Lys-Ile, 4.89 mg/g protein of Leu-Leu, 1.61 mg/g protein of Ile-Asn, 1.96 mg/g protein of Leu-Ala, and 0.71 mg/g protein of Leu-Glu.

[0082]

20 [Example 6]

Fifty grams of whey protein was dissolved in 1 L of water. The solution dissolving whey protein was adjusted to pH 7 with a pH adjuster, 500 mg of pepsin (Wako Pure Chemical Industries. Ltd.) was added, the solution was subjected to reaction at 37°C for  
25 4 hours and then 500 mg of protease derived from *Bacillus* (protease M Amano, Amano Enzyme) was added to the solution and whey protein was hydrolyzed at 50°C for 4 hours. After heating deactivation of proteases, the solution obtained was pulverized to powder by lyophilization to obtain the muscle  
30 synthesis-stimulating agent in Example 6.

[0083]

Based on Test Example 1, the content of dipeptide in the muscle synthesis-stimulating agent in Example 6 was measured. The muscle synthesis-stimulating agent in Example 6 contained 1.47 mg/g protein of Ile-Leu, 0.75 mg/g protein of Ala-Leu, 4.92 mg/g protein of Val-Leu, 0.54 mg/g protein of Lys-Ile, 2.21 mg/g protein of Leu-Leu, 1.59 mg/g protein of Ile-Asn, 2.80 mg/g protein of Leu-Ala, and 0.62 mg/g protein of Leu-Glu.

[0084]

[Example 7]

10 Fifty grams of whey protein was dissolved in 1 L of water. The solution dissolving whey protein was adjusted to pH 7 with a pH adjuster, 500 mg of pepsin (Wako Pure Chemical Industries. Ltd.) was added, the solution was subjected to reaction at 37°C for 4 hours and then whey protein was hydrolyzed by protease derived from *Aspergillus* (protease N Amano, Amano Enzyme) at 50°C for 4 hours. After heating deactivation of proteases, the solution obtained was pulverized to powder by lyophilization to obtain the muscle synthesis-stimulating agent in Example 7.

[0085]

20 Based on Test Example 1, the content of dipeptide in the muscle synthesis-stimulating agent in Example 7 was measured. The muscle synthesis-stimulating agent in Example 7 contained 2.86 mg/g protein of Ile-Leu, 2.28 mg/g protein of Ala-Leu, 3.90 mg/g protein of Val-Leu, 0.81 mg/g protein of Lys-Ile, 13.37 mg/g protein of Leu-Leu, 2.82 mg/g protein of Ile-Asn, 3.08 mg/g protein of Leu-Ala, and 0.97 mg/g protein of Leu-Glu.

[0086]

[Example 8]

30 Fifty grams of whey protein was dissolved in 1 L of water. The solution dissolving whey protein was adjusted to pH 7 with a pH adjuster, and 500 mg of flavourzyme (Novo Nordisk) was added

to the solution and whey protein was hydrolyzed at 50°C for 6 hours. After heating deactivation of proteases, the solution obtained was pulverized to powder by lyophilization to obtain the muscle synthesis-stimulating agent in Example 8.

5 [0087]

Based on Test example 1, the content of dipeptide in the muscle synthesis-stimulating agent in Example 8 was measured. The muscle synthesis-stimulating agent in Example 8 contained 0.62 mg/g protein of Ile-Leu, 0.35 mg/g protein of Ala-Leu, 1.58  
10 mg/g protein of Val-Leu, 1.54 mg/g protein of Lys-Ile, 0.20 mg/g protein of Leu-Leu, 1.64 mg/g protein of Ile-Asn, 1.05 mg/g protein of Leu-Ala, and 1.40 mg/g protein of Leu-Glu.

[0088]

[Example 9]

15 Fifty grams of whey protein was dissolved in 1 L of water. The solution dissolving whey protein was adjusted to pH 7 with a pH adjuster, and 500 mg of protease derived from Aspergillus (umamizyme, Amano enzyme) was added to the solution and whey protein. The solution was hydrolyzed at 50°C for 6 hours. After  
20 heating deactivation of proteases, the solution obtained was pulverized to powder by lyophilization to obtain the muscle synthesis-stimulating agent in Example 9.

[0089]

Based on Test example 1, the content of dipeptide in the  
25 muscle synthesis-stimulating agent in Example 9 was measured. The muscle synthesis-stimulating agent in Example 3 contained 2.78 mg/g protein of Ile-Leu, 1.09 mg/g protein of Ala-Leu, 6.25 mg/g protein of Val-Leu, 6.45 mg/g protein of Lys-Ile, 1.85 mg/g protein of Leu-Leu, 4.93 mg/g protein of Ile-Asn, 3.93 mg/g  
30 protein of Leu-Ala, and 5.87 mg/g protein of Leu-Glu.

[0090]

[Example 10]

Fifty grams of whey protein was dissolved in 1 L of water. The solution dissolving whey protein was adjusted to pH 7 with a pH adjuster, and 500 mg of protease derived from *Aspergillus* (protease A Amano, Amano Enzyme) was added to the solution and whey protein was hydrolyzed at 50°C for 6 hours. After heating deactivation of proteases, the solution obtained was pulverized to powder by lyophilization to obtain the muscle synthesis-stimulating agent in Example 10.

10 [0091]

Based on Test example 1, the content of dipeptide in the muscle synthesis-stimulating agent in Example 10 was measured. The muscle synthesis-stimulating agent in Example 8 contained 2.17 mg/g protein of Ile-Leu, 0.64 mg/g protein of Ala-Leu, 4.23 mg/g protein of Val-Leu, 2.72 mg/g protein of Lys-Ile, 2.22 mg/g protein of Leu-Leu, 3.23 mg/g protein of Ile-Asn, 2.97 mg/g protein of Leu-Ala, and 2.48 mg/g protein of Leu-Glu.

[0092]

[Example 11]

20 Fifty grams of whey protein was dissolved in 1 L of water. The solution dissolving whey protein was adjusted to pH 7 with a pH adjuster, and 500 mg of protease derived from *Aspergillus* (protease P Amano, Amano Enzyme) was added to the solution and whey protein was hydrolyzed at 50°C for 6 hours. After heating deactivation of proteases, the solution obtained was pulverized to powder by lyophilization to obtain the muscle synthesis-stimulating agent in Example 11.

[0093]

30 Based on Test example 1, the content of dipeptide in the muscle synthesis-stimulating agent in Example 11 was measured. The muscle synthesis-stimulating agent in Example 3 contained

1.85 mg/g protein of Ile-Leu, 0.66 mg/g protein of Ala-Leu, 5.19 mg/g protein of Val-Leu, 3.83 mg/g protein of Lys-Ile, 1.74 mg/g protein of Leu-Leu, 3.70 mg/g protein of Ile-Asn, 3.54 mg/g protein of Leu-Ala, and 3.56 mg/g protein of Leu-Glu.

### CLAIMS

1. A muscle synthesis-stimulating agent comprising a whey protein hydrolysate as an active ingredient.
2. The muscle synthesis-stimulating agent according to Claim 1, wherein the whey protein hydrolysate contains a dipeptide, and the dipeptide is at least one dipeptide selected from the group consisting of
  - 1) Ile-Leu, Leu-Ala, and
  - 2) Ala-Leu, Val-Leu, Lys-Ile, Leu-Leu, Ile-Asn, Leu-Glu, Leu-Val and Ile-Val.
3. The muscle synthesis-stimulating agent according to Claim 1 or 2, wherein the whey protein hydrolysate is obtained by hydrolysis of whey protein with a proteolytic enzyme.
4. The muscle synthesis-stimulating agent according to Claim 3, wherein the proteolytic enzyme is one or two or more enzymes selected from the group consisting of a proteolytic enzyme derived from Bacillus, a proteolytic enzyme derived from Aspergillus, trypsin, pepsin, and flavourzyme.
5. The muscle synthesis-stimulating agent according to Claim 3 or 4, wherein hydrolysis with the proteolytic enzyme is performed at 20 to

80°C for 2 to 48 hours.

6. The muscle synthesis-stimulating agent according to any of Claims 1 to 5, comprising one unit packaging form for one meal, wherein the unit packaging form contains whey protein hydrolysate at an amount of 1 to 40 g expressed in terms of total amount of protein as the amount of ingestion for one meal.

7. The muscle synthesis-stimulating agent according to any of Claims 1 to 6 for a subject who performs exercise.

8. The muscle synthesis-stimulating agent according to Claim 7, wherein the exercise is muscle training.

9. The agent according to any of Claims 1 to 8, wherein the agent is a composition for stimulating muscle synthesis.

10. The agent according to any of Claims 1 to 9, wherein the agent is either a food and drink product or pharmaceutical product.

11. A method for manufacturing a muscle synthesis-stimulating agent, comprising blending with an effective amount of whey protein hydrolysate.

12. A method for stimulating muscle synthesis, comprising allowing a subject in need thereof to ingest an effective amount of a whey protein hydrolysate.
13. Use of a whey protein hydrolysate for manufacturing a muscle synthesis-stimulating agent.
14. Use of a whey protein hydrolysate as a muscle synthesis-stimulating agent.
15. A whey protein hydrolysate for stimulating muscle synthesis.