

DESCRIPTION

TITLE OF INVENTION: ERGOTHIONEINE-CONTAINING MUSCULAR
ATROPHY SUPPRESSING COMPOSITION AND SRC TYROSINE KINASE
5 INHIBITING COMPOSITION

TECHNICAL FIELD

[0001]

10 The present invention relates to an ergothioneine-containing composition for inhibiting muscle atrophy and an ergothioneine-containing composition for inhibiting Src tyrosine kinase.

BACKGROUND ART

[0002]

15 Globally, the aging population is increasing, and a vicious cycle has emerged in which elderly people who become bedridden or reduce their physical activity experience muscle atrophy, which in turn further decreases their physical activity and worsens the muscle atrophy. Continuous muscle training is the only reliable way to increase muscle mass. However, as people age, their metabolic functions decline,
20 making it difficult to maintain sufficient muscle mass through training alone. Therefore, there is a strong demand for pharmaceutical products and food or beverage products that can help suppress muscle atrophy.

[0003]

25 Muscle mass is maintained by a balance between muscle protein synthesis pathways and degradation pathways, and the synthesis pathways and the degradation pathways are influenced by different factors. Muscle protein synthesis requires materials such as amino acids and energy, and thus a diet suitable for muscle protein is necessary. However, elderly people tend to have a reduced appetite compared to younger people. Therefore, in suppressing muscle atrophy in elderly people,
30 suppressing the muscle protein degradation pathways is considered important and effective.

[0004]

35 The Src tyrosine kinase family is important as a group of intracellular signaling proteins involved in acute inflammatory responses. Phosphorylation of Src tyrosine kinase is one of the major factors that determine the expression and production levels of

inflammatory mediators. Src tyrosine kinase is expressed in muscle, and inhibition of Src tyrosine kinase can suppress muscle cell degradation.

[0005]

Ergothioneine is an amino acid found in mushrooms and the like. L-ergothioneine is present in nature. Patent Literature 1 discloses an ergothioneine-containing composition that promotes muscle differentiation. In the examples therein, culturing C2C12 cells in a medium containing ergothioneine promoted differentiation of the C2C12 cells into muscle cells, and the expression level of MyoD mRNA, which induces muscle differentiation, was increased.

CITATION LIST

- Patent Literature

[0006]

Patent Literature 1: WO 2020/004014

SUMMARY OF INVENTION

- Technical Problem

[0007]

However, Patent Literature 1 does not disclose the effect on muscle atrophy and inhibition of Src tyrosine kinase.

[0008]

The present invention aims to provide a composition for inhibiting muscle atrophy. The present invention also aims to provide a composition for inhibiting Src tyrosine kinase.

- Solution to Problem

[0009]

As a result of extensive studies to solve the above problem, the present inventors found that L-ergothioneine or a salt thereof has an effect of inhibiting muscle atrophy and inhibiting Src tyrosine kinase.

[0010]

In other words, the present invention relates to, but is not limited to, the following compositions for inhibiting muscle atrophy and the like.

(1) A composition for inhibiting muscle atrophy, containing ergothioneine or a salt thereof as an active ingredient.

(2) The composition according to (1) above, wherein the muscle atrophy is age-related muscle atrophy.

(3) The composition according to (1) or (2) above, wherein the composition inhibits muscle atrophy via an ATP-sensitive potassium channel.

5 (4) A composition for inhibiting Src tyrosine kinase, containing ergothioneine or a salt thereof as an active ingredient.

(5) The composition according to any one of (1) to (4) above, wherein the composition is an oral composition.

10 (6) The composition according to any one of (1) to (5) above, wherein the composition is a food or beverage.

(7) The composition according to any one of (1) to (6) above, wherein the composition is labeled with at least one function selected from the group consisting of “inhibiting muscle decline”, “maintaining muscle”, “enhancing muscle”, “inhibiting muscle strength decline”, “maintaining muscle strength”, “enhancing muscle strength”,
15 “enhancing walking function”, “maintaining walking function”, “enhancing motor function”, “maintaining motor function”, “maintaining muscle that weakens with aging”, “maintaining muscle strength that weakens with aging”, and “maintaining physical fitness”.

20 (8) A method for inhibiting muscle atrophy, including administering ergothioneine or a salt thereof to a subject.

(9) A method for inhibiting Src tyrosine kinase, including administering ergothioneine or a salt thereof to a subject.

(10) Use of ergothioneine or a salt thereof for inhibiting muscle atrophy.

25 (11) Use of ergothioneine or a salt thereof for inhibiting Src tyrosine kinase.

- Advantageous Effects of Invention

[0011]

The present invention can provide a composition for inhibiting muscle atrophy. In addition, the present invention can provide a composition for inhibiting Src tyrosine
30 kinase.

BRIEF DESCRIPTION OF DRAWINGS

[0012]

FIG. 1 is a graph showing changes in lean body mass percentage (Δ lean body mass percentage (%)) in a test food group and a control food group.
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FIG. 2 is a graph showing the evaluation results of the inhibitory activity of L-ergothioneine against Src tyrosine kinase.

FIG. 3 is a graph showing the evaluation results of the inhibitory activity of L-ergothioneine against substrate binding to ATP-sensitive potassium channels.

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DESCRIPTION OF EMBODIMENTS

[0013]

The composition for inhibiting muscle atrophy of the present invention contains ergothioneine or a salt thereof as an active ingredient.

10 Ergothioneine is a type of sulfur-containing amino acid. In the present invention, ergothioneine is preferably L-ergothioneine.

The salt of ergothioneine is not limited as long as it is a pharmacologically acceptable salt or a dietarily acceptable salt, and it may be either an acid salt or a basic salt. Examples of the acid salt include inorganic acid salts such as hydrochloride, sulfate, nitrate, and phosphate; and organic acid salts such as acetate, citrate, maleate, malate, oxalate, lactate, succinate, fumarate, and propionate. Examples of the basic salt include alkali metal salts such as sodium salt and potassium salt, and alkaline earth metal salts such as calcium salt and magnesium salt.

15 [0014]

20 Ergothioneine or a salt thereof is not limited in any way by its form, production method, or the like. Ergothioneine or a salt thereof that can be used may be a chemically synthesized product or a purified extract of a natural product. L-ergothioneine is abundantly present in golden/yellow oyster mushrooms (scientific name: *Pleurotus cornucopiae* var. *citrinopileatus*) belonging to the genus *Pleurotus* of the family *Pleurotaceae*. L-ergothioneine is also present in mushrooms such as common mushrooms (scientific name: *Agaricus bisporus*) including white button mushrooms, cremini mushrooms, and portabella mushrooms; grey oyster mushrooms (scientific name: *Pleurotus ostreatus*), shiitake (scientific name: *Lentinula edodes*), hen-of-the-woods (scientific name: *Grifola Frondosa*), reishi mushrooms (scientific name: *Ganoderma lucidum*), lion's mane mushrooms (scientific name: *Hericium erinaceus*), Yanagi-matsutake (scientific name: *Agrocybe aegerita*), girolles (scientific name: *Cantharellus cibarius*), porcini mushrooms (scientific name: *Boletus edulis*), and common morel mushrooms (scientific name: *Morchella esculenta*). When L-ergothioneine is obtained from a natural product, preferably, it is extracted from a golden/yellow oyster mushroom, for example. Ergothioneine or a salt thereof can also

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be produced by microbial fermentation. Ergothioneine or a salt thereof may be in an isolated form.

[0015]

5 L-ergothioneine or a salt thereof is present in natural products as well as food and beverages. These are compounds that have been consumed. Thus, for example, long-term ingestion of ergothioneine or a salt thereof is less likely to cause problems in terms of safety. The present invention can provide a highly safe composition for inhibiting muscle atrophy.

[0016]

10 Muscle atrophy refers to the shrinkage of muscle mass. Muscle atrophy is broadly classified into two types: muscle atrophy caused by diseases affecting the muscles themselves (myogenic muscle atrophy), and muscle atrophy resulting from damage to the motor neurons that directly transmit movement commands to the muscles (neurogenic muscle atrophy). Even in the absence of such diseases affecting the
15 muscles or neurons, muscles naturally shrink when not used; this condition is known as disuse muscle atrophy. In addition, the total muscle mass in the body decreases with age (age-related muscle atrophy). In one embodiment, the composition of the present invention is used preferably for inhibition of age-related muscle atrophy, and more preferably for prevention of age-related muscle atrophy.

20 [0017]

As described in EXAMPLES below, consumption of L-ergothioneine suppressed a decrease in lean body mass percentage compared to when L-ergothioneine was not consumed.

Lean body mass (LBM) refers to the body weight excluding fat weight, and is
25 an indicator for assessing the proportion of muscle in the body. Measuring changes in lean body mass allows one to assess changes in muscle mass. Lean body mass percentage is the proportion of lean body mass relative to total body weight. A decrease in lean body mass percentage indicates a reduction in muscle mass. Therefore, suppressing the decrease in lean body mass percentage indicates that the
30 reduction in muscle mass has been inhibited. Accordingly, ergothioneine can be used as an active ingredient for the inhibition of muscle atrophy.

[0018]

In addition, as described in EXAMPLES below, L-ergothioneine inhibited Src tyrosine kinase. Also, L-ergothioneine inhibited substrate binding to ATP-sensitive
35 potassium (K_{ATP}) channels.

Src tyrosine kinase is expressed in muscle, and inhibition of Src tyrosine kinase is known to suppress muscle cell degradation. Thus, ergothioneine exerts the effect of inhibiting muscle atrophy by inhibiting Src tyrosine kinase and thereby suppressing muscle cell degradation.

5 K_{ATP} channels are also expressed in muscle and are known to be involved in the regulation of the muscle atrophy process. Accordingly, ergothioneine is considered to have the effect of inhibiting muscle atrophy via ATP-sensitive potassium channels. [0019]

 The composition of the present invention is used to inhibit muscle atrophy.
10 The inhibition of muscle atrophy encompasses preventing muscle atrophy, delaying the progression of muscle atrophy, suppressing the progression of muscle atrophy, and the like. The phrase "preventing muscle atrophy" as used herein encompasses suppressing the onset, delaying the onset, reducing the incidence rate, reducing the risk of onset, and the like. The inhibition of muscle atrophy leads to the effects such as inhibiting
15 muscle mass decline, maintaining muscle mass, and the like. [0020]

 The composition of the present invention may be an oral composition or a parenteral composition, preferably an oral composition.

 The oral composition may specifically be a food or beverage, an oral
20 pharmaceutical product, an oral quasi-pharmaceutical product, feed, or the like, preferably a food or beverage or an oral pharmaceutical product, more preferably a food or beverage. The composition of the present invention may be a material, preparation, or the like to be added to a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like.
25 [0021]

 The composition of the present invention is applicable for therapeutic use (medical use) and non-therapeutic use (non-medical use). The "non-therapeutic" is a concept that does not include medical activities, i.e., a concept that does not include methods of surgery, therapy, or diagnosis of humans.
30 [0022]

 The composition of the present invention can contain optional additives and optional components in addition to ergothioneine or a salt thereof, as long as the effects of the present invention are not impaired. Such additives and components can be selected according to the composition form or the like. When the composition of the
35 present invention is provided as an oral composition, the composition may contain any

additives and the like that can be commonly used for oral compositions such as food and beverages, pharmaceutical products, quasi-pharmaceutical products, feed, and the like.

[0023]

5 When the composition of the present invention is provided as a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like, any common method can be used for production.

 The oral composition of the present invention may be in any form such as a solid (e.g., powder, granule, tablet), a liquid, or a paste.

10 [0024]

 For example, when the composition of the present invention is provided as a food or beverage, components usable in food or beverages (e.g., food materials and optional food additives) can be added to ergothioneine or a salt thereof to provide various food or beverages. The food or beverage is not limited. Examples thereof
15 include general food or beverages, health foods, health supplements, health drinks, foods with function claims, foods for specified health uses, and foods for the sick. The health foods, health supplements, foods with function claims, foods for specified health uses, and the like can be used in various forms of preparations such as fine granules, tablets, granules, powders, capsules, chewable tablets, dry syrups, syrups, liquid agents,
20 beverages, energy drinks, and liquid foods.

[0025]

 When the composition of the present invention is provided as a pharmaceutical product or a quasi-pharmaceutical product, for example, a pharmacologically acceptable carrier, an optional additive, or the like can be added to ergothioneine or a salt thereof to
25 provide various dosage forms of pharmaceutical products or quasi-pharmaceutical products. Such a carrier, additive, or the like may be any pharmacologically acceptable one that can be used in pharmaceutical products or quasi-pharmaceutical products. Examples thereof include excipients, binders, disintegrants, lubricants, antioxidants, and colorants. One or more of these can be used. The form of
30 administration (ingestion) of the pharmaceutical product or the quasi-pharmaceutical product may be oral or parenteral (e.g., transdermal, transmucosal, enteral, or injection) administration. When the composition of the present invention is provided as a pharmaceutical product or a quasi-pharmaceutical product, it is preferably an oral pharmaceutical product or an oral quasi-pharmaceutical product. Examples of dosage
35 forms for oral administration of a pharmaceutical product or a quasi-pharmaceutical

product include liquids, tablets, powders, fine granules, granules, sugar-coated tablets, capsules, suspensions, emulsions, and chewable tablets. Examples of the dosage forms for parenteral administration include injections, intravenous agents, ointments, lotions, adhesive skin patches, suppositories, nasal agents, and transpulmonary agents (inhalants). The pharmaceutical product may be for non-human animals.

[0026]

When the composition of the present invention is provided as feed, ergothioneine or a salt thereof is simply added to feed. The feed includes feed additives. Examples of the feed include feed for farm animals such as cows, pigs, chickens, sheep, and horses; feed for small animals such as rabbits, rats, and mice; and pet food for animals such as dogs, cats, and birds.

[0027]

The subject (administration subject) who ingests the composition of the present invention or to whom the composition is administered is not limited. The administration subject is preferably a human or non-human mammal, more preferably a human.

In one embodiment, the administration subject may be, for example, a subject needing or wanting inhibition of muscle atrophy. Examples of such a subject include middle-aged and older people. In one embodiment, the composition of the present invention is suitable as a composition for inhibiting muscle atrophy for middle-aged and older people. The term "middle-aged and older people" includes the elderly. The middle-aged and older people may be those who are 40 years old and older, for example. The elderly may be people who are 60 years old and older or 65 years old and older, for example. The administration subject may be a healthy person. For example, the composition of the present invention can be used in healthy people for purposes such as inhibition of muscle atrophy. In the present invention, particularly preferred subjects are healthy people who are 50 years old and older but under 65 years old, and most preferred subjects are healthy people who are 50 years old and older but under 55 years old.

[0028]

The amount of ergothioneine or a salt thereof in the composition of the present invention is not limited and can be set according to the form of the composition or the like.

The amount of ergothioneine or a salt thereof in the composition of the present invention in terms of ergothioneine is, for example, preferably 0.0001% by weight or

more, more preferably 0.001% by weight or more, while preferably 90% by weight or less, more preferably 50% by weight or less.

In one embodiment, the amount of ergothioneine or a salt thereof in the composition of the present invention in terms of ergothioneine is preferably 0.0001 to 90% by weight, more preferably 0.001 to 50% by weight. The amount of ergothioneine or a salt thereof can be measured by high-performance liquid chromatography (HPLC).

[0029]

Herein, in the case of ergothioneine, the expression regarding the amount of ergothioneine or a salt thereof in terms of ergothioneine or an expression similar thereto refers to the amount of ergothioneine; whereas in the case of a salt of ergothioneine, the expression or an expression similar thereto refers to a value obtained by multiplying the number of moles of the salt by the molecular weight of ergothioneine.

[0030]

The composition of the present invention can be ingested or administered using an appropriate method depending on the form thereof. In one embodiment, preferably, the composition of the present invention is orally ingested (orally administered). The administration amount (intake) of the composition of the present invention is not limited. The administration amount may be any amount sufficient to achieve the effect of inhibiting muscle atrophy and may appropriately be set according to the form of administration, administration method, the weight of the subject, and the like.

[0031]

In one embodiment, when the composition of the present invention is orally ingested by or administered to a human (adult) as a subject, the administration amount of ergothioneine or a salt thereof (in terms of ergothioneine) per day is preferably 2 mg or more, more preferably 5 mg or more, still more preferably 7 mg or more, while preferably 50 mg or less, more preferably 25 mg or less, still more preferably 20 mg or less. In one embodiment, the administration amount of the composition of the present invention as the amount of ergothioneine or a salt thereof (in terms of ergothioneine) to a human (adult) per day is preferably 2 to 50 mg, more preferably 5 to 25 mg, still more preferably 5 to 20 mg, further more preferably 7 to 20 mg. Preferably, the compound in the above amount is ingested or administered in one or more portions per day, for example, in one to several portions (e.g., two or three portions) per day. In one embodiment, preferably, ergothioneine or a salt thereof in the above amount is orally ingested by or administered to a human. The above administration amount may be an

administration amount per 60 kg body weight. In one embodiment, the composition of the present invention can be used to enable daily ingestion or administration of ergothioneine or a salt thereof in the above amount to a human per 60 kg body weight, for example.

5 [0032]

The amount of ergothioneine or a salt thereof in the composition of the present invention per adult daily intake is preferably 2 to 50 mg, more preferably 5 to 25 mg, still more preferably 5 to 20 mg, particularly preferably 7 to 20 mg, in terms of ergothioneine.

10 [0033]

The composition of the present invention may, for example, be labeled with a function of inhibiting muscle atrophy; or a function resulting from inhibition of muscle atrophy.

The composition of the present invention may, for example, be labeled with at least one function selected from the group consisting of “inhibiting muscle decline”, “maintaining muscle”, “enhancing muscle”, “inhibiting muscle strength decline”, “maintaining muscle strength”, “enhancing muscle strength”, “enhancing walking function”, “maintaining walking function”, “enhancing motor function”, “maintaining motor function”, “maintaining muscle that weakens with aging”, “maintaining muscle strength that weakens with aging”, and “maintaining physical fitness”.

In one embodiment, preferably, the composition of the present invention is a food or beverage labeled with one or more functions described above. The label may be one indicating use of the composition for obtaining the function. The label may be attached to the composition itself, or may be attached to a container or package of the composition.

25 [0034]

As described above, ergothioneine or a salt thereof has the effect of inhibiting Src tyrosine kinase. Ergothioneine or a salt thereof can be used as an active ingredient for inhibiting Src tyrosine kinase. The composition of the present invention can also be used as a composition for inhibiting Src tyrosine kinase. A composition for inhibiting Src tyrosine kinase that contains ergothioneine or a salt thereof as an active ingredient is also one embodiment of the present invention.

The active ingredient, active ingredient content, dosage, administration subjects, and other features of the composition for inhibiting Src tyrosine kinase are the same as those of the above-mentioned composition for inhibiting muscle atrophy.

[0035]

As described above, ergothioneine or a salt thereof inhibits substrate binding to K_{ATP} channels. Thus, ergothioneine or a salt thereof can also be used as an active ingredient for inhibiting substrate binding to K_{ATP} channels. A composition for
5 inhibiting substrate binding to K_{ATP} channels that contains ergothioneine or a salt thereof as an active ingredient is also an embodiment of the present invention.

[0036]

The present invention also encompasses the following methods:
a method for inhibiting muscle atrophy, including administering ergothioneine
10 or a salt thereof to a subject; and
a method for inhibiting Src tyrosine kinase, including administering ergothioneine or a salt thereof to a subject.

The method may be therapeutic or non-therapeutic.

[0037]

15 The present invention also encompasses the following uses:
use of ergothioneine or a salt thereof for inhibiting muscle atrophy; and
use of ergothioneine or a salt thereof for inhibiting Src tyrosine kinase.

The use may be therapeutic or non-therapeutic.

Administering ergothioneine or a salt thereof to a subject enables inhibition of
20 muscle atrophy.

[0038]

In the above methods and uses, the preferred embodiments of ergothioneine or a salt thereof are the same as described above for the composition of the present invention. Ergothioneine or a salt thereof may include one type of ergothioneine or a
25 salt thereof or two types of ergothioneine or a salt thereof. In the above methods and uses, preferably, ergothioneine or a salt thereof is administered to (ingested by) a subject at least once a day, for example, one to several times (e.g., two or three times) a day. In the above methods and uses, ergothioneine or a salt thereof is preferably orally administered (ingested). The above uses are preferably for humans or non-human
30 mammals, more preferably for humans.

[0039]

In the above methods and uses, ergothioneine or a salt thereof is simply used in an amount (effective amount) sufficient to achieve a desired effect. The preferred administration amount of ergothioneine or a salt thereof, preferred administration
35 subjects, and the like are as described above for the composition of the present

invention. Ergothioneine or a salt thereof may be administered directly or may be administered in the form of a composition containing the ergothioneine or a salt thereof. For example, the composition of the present invention described above may be used.

[0040]

5 The present invention encompasses use of ergothioneine or a salt thereof for producing a composition for inhibiting muscle atrophy.

 The present invention encompasses use of ergothioneine or a salt thereof for producing a composition for inhibiting Src tyrosine kinase.

10 The present invention also relates to ergothioneine or a salt thereof for use in inhibiting muscle atrophy.

[0041]

 The numerical range defined by the lower limit and the upper limit herein, i.e., "the lower limit to the upper limit", includes the lower limit and the upper limit. For example, the range defined by "1 to 2" means 1 or more and 2 or less, with 1 and 2
15 being included. Herein, the range may be any combination of any upper limit and any lower limit.

EXAMPLES

[0042]

20 The present invention is described in further detail below with reference to examples. The present invention is not limited to these examples.

[0043]

<Example 1>

(Evaluation of muscle atrophy in human subjects)

25 To evaluate the effect of a supplement containing ergothioneine on human lean body mass percentage, a placebo-controlled, randomized, double-blind, parallel-group comparative study was conducted. In the study, the subjects were healthy adults who were 50 years old and older but 55 years old and younger (test food group: 12 people, control food group: 11 people, total: 23 people). They ingested a capsule with 8 mg of
30 ergothioneine (test food) or a capsule without ergothioneine (control food) once a day for 12 weeks.

[0044]

 The lean body mass percentage was assessed using a body composition analyzer DC-430A, separate type (Tanita Corporation). First, before the start of the
35 study (before the ingestion of the test food or control food), the participants' lean body

mass percentage was assessed using the body composition analyzer. After 12 weeks of ingesting either the test food or the control food, the lean body mass percentage was assessed again (Week 12 assessment).

[0045]

5 (Evaluation foods)

Two types of evaluation foods were prepared, designed to be indistinguishable in appearance, flavor, and the like.

- Test food: capsule with the test substance (L-ergothioneine, 8 mg)

- Control food: capsule without the test substance

10 In addition to the test substance, the ingredients used in both evaluation foods included dextrin, calcium stearate, and hydroxypropyl methylcellulose. The control food was produced using the same ingredients as the test food, except that it did not contain the test substance (L-ergothioneine).

[0046]

15 The average lean body mass percentage (%) of the participants in the test food group was used as the lean body mass percentage of the test food group. The average lean body mass percentage (%) of the participants in the control food group was used as the lean body mass percentage of the control food group. For each group, the change in lean body mass percentage (Δ lean body mass percentage (%)) was calculated by
20 subtracting the pre-study assessment lean body mass percentage from the Week 12 assessment lean body mass percentage. The change in lean body mass percentage (Δ lean body mass percentage (%)) before the study was set to 0.

[0047]

FIG. 1 is a graph showing changes in lean body mass percentage (Δ lean body mass percentage (%)) in a test food group and a control food group. As a result, the decrease in lean body mass percentage was more suppressed in the test food group than in the control food group. This result revealed that L-ergothioneine inhibits muscle atrophy.

25 In FIG. 1, black squares (■) represent the test food group, and white squares (□) represent the control food group. On the horizontal axis, “0W” indicates the pre-study assessment, and “12W” indicates the Week 12 assessment.

[0048]

<Example 2>

(Evaluation of Src tyrosine kinase inhibitory activity)

35 To evaluate the molecular targets of L-ergothioneine in retaining the lean body

weight, an in vitro assay was conducted on Src tyrosine kinase. The in vitro assay was outsourced to Eurofins Panlabs and performed according to the following procedure.

[0049]

Human recombinant Src tyrosine kinase expressed in insect cells was used.

5 The Src tyrosine kinase at a concentration of 0.023 $\mu\text{g/mL}$ was pre-incubated at 37°C for 15 minutes in HEPES buffer (pH 7.4) with L-ergothioneine (L-ergothioneine concentration: 10 μM or 1000 μM). The reaction was then initiated by adding 0.2 mg/mL poly(Glu:Tyr), 10 μM ATP, and 0.25 μM $\text{Ci}[\gamma\text{-}^{32}\text{P}]\text{ATP}$. After a 30-minute incubation, the reaction was terminated by adding 3% H_3PO_4 . The inhibitory activity of L-ergothioneine was evaluated based on the amount of $[\text{}^{32}\text{P}]\text{poly}(\text{Glu:Tyr})$ formed in
10 the reaction solution. A smaller amount of $[\text{}^{32}\text{P}]\text{poly}(\text{Glu:Tyr})$ indicates greater inhibition of Src tyrosine kinase activity.

[0050]

FIG. 2 is a graph showing the evaluation results of the inhibitory activity of L-
15 ergothioneine against Src tyrosine kinase. The concentration (μM) along the horizontal axis indicates the concentration of L-ergothioneine.

The Src tyrosine kinase inhibitory activity in percentage (%) was determined by the following procedure. The amount of $[\text{}^{32}\text{P}]\text{Poly}(\text{Glu:Tyr})$ in the reaction solution (control) without L-ergothioneine was defined as representing 100% Src tyrosine kinase activity. The relative Src tyrosine kinase activity (%) relative to the control was
20 determined based on the amount of $[\text{}^{32}\text{P}]\text{Poly}(\text{Glu:Tyr})$ in the reaction solution with L-ergothioneine. The Src tyrosine kinase inhibitory activity (%) was then determined by subtracting the relative Src tyrosine kinase activity (%) in the presence of L-ergothioneine from the 100% Src tyrosine kinase activity of the control.

25 L-ergothioneine exhibited Src tyrosine kinase inhibitory activity. Src tyrosine kinase is expressed in muscle and inhibition thereof is known to suppress muscle cell degradation. Thus, L-ergothioneine presumably inhibited muscle cell degradation by inhibiting Src tyrosine kinase, thereby exerting the above effect of inhibiting muscle atrophy.

30 [0051]

<Example 3>

(Evaluation of inhibitory activity against substrate binding to ATP-sensitive potassium channels)

To evaluate the molecular targets of L-ergothioneine in retaining the lean body
35 weight, an in vitro assay was conducted on ATP-sensitive potassium channels. The in

vitro assay was outsourced to Eurofins Panlabs and performed according to the following procedure.

[0052]

A membrane fraction (containing K_{ATP} channels, available from Eurofins Cerep) in an amount of 400 μg from rat cerebral cortex was incubated at 22°C for 60 minutes in a 50 mM Tris-HCl buffer (pH 7.4) with 0.1 nM [^3H]glibenclamide and L-ergothioneine (L-ergothioneine concentration: 100 μM or 1000 μM). Glibenclamide is a K_{ATP} channel inhibitor. Nonspecific binding was determined in the presence of 1 μM glibenclamide (without L-ergothioneine). Since excess glibenclamide prevents [^3H]glibenclamide from binding to K_{ATP} channels, the radioactivity detected therein represents nonspecific binding. The membrane fraction was filtered and washed, and the substrate-binding inhibitory activity of L-ergothioneine was evaluated based on the measured radioactivity. A larger amount of [^3H]glibenclamide binding to K_{ATP} channels results in higher radioactivity in the membrane fraction.

15 [0053]

FIG. 3 is a graph showing the evaluation results of the inhibitory activity of L-ergothioneine against substrate binding to ATP-sensitive potassium channels. The concentration (μM) along the horizontal axis indicates the concentration of L-ergothioneine. The inhibitory activity (%) against substrate binding to K_{ATP} channels was determined by the following procedure. From the radioactivity of the membrane fraction in the reaction solution without L-ergothioneine (control) taken as 100% and the radioactivity of the membrane fraction in the reaction solution with L-ergothioneine, the relative activity (%) of the membrane fraction in the reaction solution with L-ergothioneine (100 μM or 1000 μM) relative to the control was determined. When the binding of glibenclamide to K_{ATP} channels is inhibited by L-ergothioneine, the radioactivity of the membrane fraction decreases. The inhibitory activity (%) against substrate binding to K_{ATP} channels was determined by subtracting the relative activity (%) of the membrane fraction with L-ergothioneine from the radioactivity (100%) of the membrane fraction in the control.

30 [0054]

L-ergothioneine inhibited the binding of glibenclamide as a substrate to ATP-sensitive potassium channels. ATP-sensitive potassium channels are also expressed in muscle and are known to be involved in the regulation of the muscle atrophy process. Thus, L-ergothioneine presumably exerted the above effect of inhibiting muscle atrophy via ATP-sensitive potassium channels.

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CLAIMS

Claim 1. A composition for inhibiting muscle atrophy, comprising ergothioneine or a salt thereof as an active ingredient.

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Claim 2. The composition according to claim 1, wherein the muscle atrophy is age-related muscle atrophy.

Claim 3. The composition according to claim 1 or 2, wherein the composition inhibits muscle atrophy via an ATP-sensitive potassium channel.

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Claim 4. A composition for inhibiting Src tyrosine kinase, comprising ergothioneine or a salt thereof as an active ingredient.

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Claim 5. The composition according to claim 1 or 4, wherein the composition is an oral composition.

Claim 6. The composition according to claim 5, wherein the composition is a food or beverage.

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Claim 7. The composition according to claim 1 or 4, wherein the composition is labeled with at least one function selected from the group consisting of “inhibiting muscle decline”, “maintaining muscle”, “enhancing muscle”, “inhibiting muscle strength decline”, “maintaining muscle strength”, “enhancing muscle strength”, “enhancing walking function”, “maintaining walking function”, “enhancing motor function”, “maintaining motor function”, “maintaining muscle that weakens with aging”, “maintaining muscle strength that weakens with aging”, and “maintaining physical fitness”.

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Claim 8. A method for inhibiting muscle atrophy, comprising administering ergothioneine or a salt thereof to a subject.

Claim 9. A method for inhibiting Src tyrosine kinase, comprising administering ergothioneine or a salt thereof to a subject.

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Claim 10. Use of ergothioneine or a salt thereof for inhibiting muscle atrophy.

5 Claim 11. Use of ergothioneine or a salt thereof for inhibiting Src tyrosine
kinase.