

## DESCRIPTION

TITLE OF INVENTION: INTERNAL COMPOSITION FOR LIPOLYSIS AND/OR  
WEIGHT LOSS, METHOD FOR DETERMINING EFFECTIVENESS, AND INDEX FOR  
5 QUALITY OF PLANT EXTRACT

## TECHNICAL FIELD

[0001] The present invention relates to an oral composition for lipolysis and/or weight loss,  
a method for determining efficacy, and an indicator of the quality of a plant extract.

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## BACKGROUND ART

[0002] Body fat is formed when the excess energy, resulting from energy intake exceeding  
energy expenditure, is accumulated as triglycerides within white adipocytes. The excessive  
accumulation of fat leads to obesity and weight gain, and consequently, such obesity not only  
15 causes various lifestyle diseases but also presents a significant problem in terms of cosmetic  
concerns. Obesity with predominant accumulation of visceral fat has been implicated in  
pathological conditions such as insulin resistance and arteriosclerosis, and obesity with  
predominant accumulation of subcutaneous fat has become a major concern for both men and  
women from the viewpoint of cosmetic concerns.

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[0003] Conventionally, various countermeasures aimed at restricting energy intake have  
been implemented for prevention and improvement of obesity, including dietary restrictions  
and the search for substances that inhibit carbohydrate absorption in the digestive tract.  
However, restricting energy intake can also be a factor in lowering the basal metabolic rate,  
and obesity may not be improved by this method in some cases. Furthermore, restricting  
25 energy intake can lead to a lack of essential nutrients, which may damage health. Therefore,  
it has been increasingly considered ideal, for overcoming obesity, to actively decompose the  
accumulated fat and dissipate the fat as thermal energy such that an optimal weight can be  
maintained. Under such circumstances, in recent years, extensive efforts have been made to  
search for functional components with lipolysis promoting activity among food materials, and  
30 a large number of food and drink products have been proposed.

[0004] It is known, for example, that a mixture prepared by combining salmon milt extract,  
beer yeast extract, young barley leaf extract, and tricollagen (chicken collagen) exhibits  
excellent lipolysis promoting activity (Patent Literature 1), that at least one of an extract from  
birch of the family Betulaceae and an extract from *Sasa veitchii* of the family Poaceae reduces  
35 the propensity of obesity and prevents increase of adipose tissue through the promotion of  
lipolysis in systemic or localized adipose tissue (Patent Literature 2), and that specific plants

such as common juniper or extracts thereof are useful as pharmaceuticals, food, or cosmetics that exert slimming effects such as suppression, prevention, or improvement of obesity, by promoting decomposition of triglycerides accumulated in adipose tissue (Patent Literature 3). However, the lipolysis effects and weight loss effects of these plant extracts and the like cannot be regarded sufficient at present. Moreover, Patent Literature 4 discloses that a composition containing a combination of a hydrophobic extract of licorice with an antioxidant component or the like exerts suppressing effects on body fat accumulation, promoting decomposition of body fat, and enhancing energy production, but a sufficient lipolysis promoting effect cannot be achieved by the hydrophobic extract of licorice alone.

## CITATION LIST

### Patent Literature

- [0005] Patent Literature 1: JP 2011-074051 A  
Patent Literature 2: JP 2006-045120 A  
Patent Literature 3: JP 2012-229266 A  
Patent Literature 4: WO2008/143182

## SUMMARY OF INVENTION

### Technical Problem

[0006] A problem to be solved by the present invention is to provide a novel formulation excellent in a lipolysis promoting effect, a visceral fat/body fat reducing effect, a weight reducing effect, and a BMI improving effect. Another object of the present invention is to provide a novel formulation excellent in an improving effect on insulin resistance, a reducing effect on cholesterol levels, and the like.

### Solution to Problem

[0007] The present inventors have found, as a result of intensive studies, that "Glyasperin B", that is, a specific component contained in licorice extract, exhibits excellent lipolysis promoting activity. The inventors have also found that a formulation containing licorice extract containing Glyasperin B exerts, in addition to an excellent lipolysis promoting effect, a visceral fat/body fat reducing effect, a weight reducing effect, a BMI improving effect, an insulin resistance improving effect, and a cholesterol level lowering effect. Moreover, the inventors have found that the content of Glyasperin B in a plant extract can be used as an indicator of the efficacy of the plant extract for lipolysis, weight loss, and the like. The gist of the present invention is as follows:

[0008] [1] An oral composition comprising Glyasperin B, for use in one or more of:

lipolysis, fat burning, support of fat consumption, BMI improvement, support of BMI reduction, abdominal fat reduction, visceral fat reduction, body fat reduction, waist circumference reduction, improvement of insulin resistance, and reduction of LDL cholesterol level.

5           [2] An oral composition comprising an effective amount of Glyasperin B, for use in one or more of: lipolysis, fat burning, support of fat consumption, BMI improvement, support of BMI reduction, abdominal fat reduction, visceral fat reduction, body fat reduction, waist circumference reduction, improvement of insulin resistance, and reduction of LDL cholesterol level.

10           [3] The oral composition according to [2], wherein the effective amount is 10  $\mu\text{g}$  or more per day for an adult.

            [4] The oral composition according to [1] or [2], for use in an adult having a BMI of 23  $\text{kg}/\text{m}^2$  or more and less than 30  $\text{kg}/\text{m}^2$ .

15           [5] The oral composition according to [1] or [2], for use for an adult having a visceral fat area of 100  $\text{cm}^2$  or more.

            [6] The oral composition according to [1] or [2], wherein the oral composition is ingested at a dosage of 5 to 100  $\mu\text{g}/\text{day}$ , calculated as a total amount of Glyasperin B.

            [7] A method for determining efficacy of a plant extract for lipolysis and/or weight loss in a living body, wherein the content of Glyasperin B is used as an indicator.

20           [8] The method for determining efficacy of a plant extract for lipolysis and/or weight loss according to [7], wherein the plant is licorice.

            [9] An indicator of a quality of a plant extract, wherein the quality relates to efficacy for lipolysis and/or weight loss in a living body, and the indicator comprises Glyasperin B.

            [10] The indicator according to [9], wherein the plant is licorice.

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#### Advantageous Effects of Invention

[0009] An oral composition of the present invention possesses, by containing Glyasperin B, preferably as an active ingredient, a functionally relevant component, an indicator component, or a contributing component, excellent lipolysis promoting activity and also exerts a remarkable weight reducing effect. By using the oral composition of the present invention, fat within adipocytes can be efficiently decomposed, and body fat such as subcutaneous fat and visceral fat can be reduced, resulting in remarkable weight reducing effect and BMI improving effect. Furthermore, improving effects on insulin resistance and lowering effects on cholesterol levels can also be obtained. Therefore, the oral composition of the present invention makes it possible to prevent or ameliorate obesity and lifestyle diseases over a long term, and thus, maintenance and promotion of health can be realized. Furthermore, since the

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content of Glyasperin B in a plant extract is used as an indicator of the efficacy of the plant extract for lipolysis, weight loss, and the like, it becomes possible to efficiently select a plant extract with high efficacy.

5 BRIEF DESCRIPTION OF DRAWINGS

[0010] [Fig. 1] Fig. 1 is a diagram illustrating the lipolysis promoting effect of licorice extract and Glyasperin B.

[Fig. 2] Fig. 2 is a diagram illustrating the effects of licorice extract on weight, BMI, and visceral fat level.

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DESCRIPTION OF EMBODIMENTS

[0011] Now, an oral composition, a method for determining efficacy of lipolysis and/or weight loss, and an indicator of the quality of a plant extract of the present invention will be described in detail.

15 [0012] <Oral Composition>

An oral composition of the present invention contains Glyasperin B. Glyasperin B is contained preferably, but without limitation, as an active ingredient, a functionally relevant component, an indicator component, or a contributing component. The oral composition of the present invention may contain, in addition to Glyasperin B used as an essential  
20 component, another component as long as the effects of the present invention are not impaired. Hereinafter, Glyasperin B and other components contained in the oral composition of the present invention, and the form and the like of the oral composition of the present invention will be described.

[0013] Herein, the term "active ingredient" refers to a pharmacologically active component or a physiologically active component that exhibits the intended effect, among components  
25 contained in pharmaceuticals, quasi-drugs, and the like.

[0014] Herein, the term "functionally relevant component" refers to a component contained in an oral composition such as a food product that contributes to a specific health purpose. Specifically, it is a component whose mechanism of action related to the functionality thereof  
30 has been investigated through *in vitro* tests, *in vivo* tests, or clinical trials (human trials), and that allows for direct or indirect qualitative and quantitative confirmation. It is noted that "foods with function claims" are provided to consumers with claims regarding the effects of components contained therein, and when a product is to be provided to consumers as a food with function claims, the content and quantification method of the functionally relevant  
35 component must be notified.

[0015] Assuming that extracts or essences where, although specific components capable of

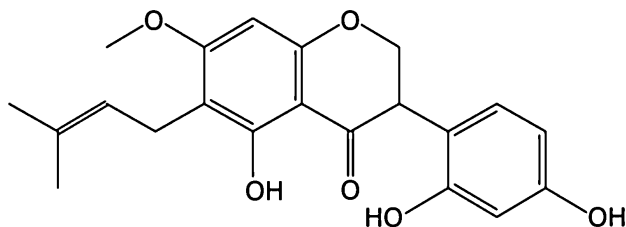
explaining a part of the scientific basis for the functionality have been identified, the entire functionality cannot be attributed only to those specific components are designated as the functionally relevant components, if mechanism of action for the claimed functionality has been investigated through *in vitro* tests, *in vivo* tests, or clinical trials (human trials), such components are herein referred to as the "indicator component".

[0016] Herein, the term "contributing component" refers to a substance corresponding to a component that directly or indirectly affects the physiological functions of the body when blended. Components corresponding to the contributing component in foods for specified health uses and foods with function claims are considered to include, but are not limited to, a "functional component", a "functionally relevant component", and an "indicator component". Moreover, a component corresponding to the contributing component in pharmaceuticals and quasi-drugs is an "active ingredient." Furthermore, it is a concept that also includes components that are implicitly linked to an effect and emphasized to consumers, with, for example, phrasing such as, "X component blended. For burn support.", in the sale and the like of food compositions except for foods with function claims.

[0017] (Glyasperin B)

Glyasperin B (hereinafter also referred to as "GB") is an isoflavanone derivative represented by the following formula. It is also referred to as 3-(2,4-dihydroxyphenyl)-5-hydroxy-2,3-dihydro-6-(3-methyl-2-butenyl)-7-methoxy-4H-1-benzopyran-4-one, 3-(2,4-dihydroxyphenyl)-5-hydroxy-6-(3-methyl-2-butenyl)-7-methoxy-3,4-dihydro-2H-1-benzopyran-4-one, or 3-(2,4-dihydroxyphenyl)-5-hydroxy-7-methoxy-6-(3-methylbut-2-en-1-yl)-3,4-dihydro-2H-1-benzopyran-4-one.

[Formula 1]



[0018] Glyasperin B is a component that has been confirmed to be contained in licorice extract, and as the Glyasperin B used in the present invention, one derived from licorice extract is preferred. It has been conventionally known that licorice extract exhibits lipolysis promoting effect, and there have been existing literatures suggesting the possibility that glabridin, which is contained in a large amount in licorice extract, exhibits the lipolysis promoting effect (see Patent Literature 4, or the like). It was, however, not clear which component within licorice extract was responsible for the lipolysis promoting activity. The

present inventors have, for the first time, discovered that Glyasperin B, among the numerous components present in licorice extract, exhibits the lipolysis promoting effect. Thus, the inventors have completed the invention of an oral composition containing Glyasperin B that exhibits the following effects: lipolysis, fat burning, support of fat consumption, BMI improvement, support of BMI reduction, abdominal fat reduction, visceral fat reduction, body fat reduction, waist circumference reduction, improvement of insulin resistance, and LDL cholesterol level reduction.

[0019] The Glyasperin B used in the present invention may be derived from licorice or may be a chemically synthesized product. It is noted that the phrase "containing Glyasperin B" herein also includes a case where a plant, such as licorice, that contains Glyasperin B is contained.

[0020] The type of the licorice is not especially limited, and examples include plants belonging to the genus *Glycyrrhiza* of the family Fabaceae, such as *Glycyrrhiza uralensis* Fisch. et DC. (Ural licorice), *Glycyrrhiza inflata* BAT. (inflated licorice), *Glycyrrhiza glabra* L. (Chinese licorice), *Glycyrrhiza glabra* L. var. *glandulifera* Regel et Herder (licorice fern), *Glycyrrhiza aspera*, *Glycyrrhiza echinata* L. (Shina-kanzo), and *Glycyrrhiza pallidiflora* Maxim (dog licorice).

[0021] As a method for obtaining Glyasperin B derived from licorice, a method of purification from licorice extract can be employed. Specifically, the licorice extract refers to a licorice extract obtained by extracting a pulverized material of the whole or a part of a licorice plant (e.g., the root, stem, stolon, leaf, flower, or fruit) with a solvent, and the licorice extract thus obtained may be subjected to spray drying or freeze drying. Examples of the extraction solvent include water, alcohols such as methanol and ethanol, and a mixed solvent of water and an alcohol or a ketone such as acetone. Among these, water, alcohol, or hydrous alcohol is preferred, and hot water, ethanol, or hydrous ethanol is more preferred as the extraction solvent.

[0022] The alcohol concentration in the hydrous alcohol is 0.1% by mass to 99.9% by mass, preferably 10% by mass to 99.9% by mass, more preferably 30% by mass to 70% by mass, further preferably 40% by mass to 60% by mass, and particularly preferably 50% by mass. Alternatively, the alcohol concentration is 0.1% (v/v) to 99.9% (v/v), preferably 10% (v/v) to 99.9% (v/v), more preferably 30% (v/v) to 70% (v/v), further preferably 40% (v/v) to 60% (v/v), and particularly preferably 50% (v/v).

[0023] A licorice extract aqueous solution is prepared by adding distilled water to a dried product of the licorice extract to dissolve it. An organic solvent such as ethyl acetate is added in a half volume relative to the volume of this aqueous solution, and the resultant is mixed, and allowed to separate into two layers, and an operation of recovering an organic

solvent layer such as ethyl acetate from this state is repeated a plurality of times, preferably about three times. After adding anhydrous sodium sulfate to the obtained organic solvent layer, an organic fraction can be obtained by filtering the resultant. The organic fraction is concentrated with an evaporator, and then a fraction eluted with hexane:ethyl acetate = 1:1 (v/v) or the like by silica gel column chromatography is subjected to repeated purification by high performance liquid chromatography using an ODS column (acetonitrile:water = 60:40 or 45:55 (both v/v)), and thus, a compound (Glyasperin B) can be obtained.

[0024] The content of Glyasperin B in the oral composition of the present invention is 0.00001% by mass to 50% by mass, preferably 0.0001% by mass to 20% by mass, more preferably 0.0005% by mass to 10% by mass, and further preferably 0.001% by mass to 5% by mass.

[0025] (Other Components)

The oral composition of the present invention may contain, in addition to Glyasperin B, a carrier, an excipient, a solvent, and other optional components as long as the effects of the present invention are not impaired.

[0026] When the oral composition of the present invention contains Glyasperin B, Glyasperin B purified from licorice extract as described above, or chemically synthesized Glyasperin B may be blended, or alternatively, licorice extract itself may be blended in such an amount that the final content of Glyasperin B in the oral composition falls within the numerical range specified above. Moreover, from the viewpoint of lipolysis promoting activity, it is preferable that the oral composition of the present invention has the content of Glyasperin B within the numerical range specified above, and that either it contains no licorice-derived components other than Glyasperin B, or if it contains a licorice-derived component in addition to Glyasperin B, a ratio of the content of Glyasperin B to the content of glycyrrhizic acid (a value obtained by dividing the content of Glyasperin B by the content of glycyrrhizic acid) is preferably 0.001 or more based on glycyrrhizic acid, which is a quantitative indicator for licorice extract in the Japanese Pharmacopoeia, used as the comparative standard.

[0027] The oral composition of the present invention is used in the form of a food or drink product, a food with function claims, a food for specified health uses, a food with nutrient function claims, a cosmetic product, a quasi-drug, a pharmaceutical, a cosmetic preparation, or the like, for efficiently decomposing fat and reducing body fat such as subcutaneous fat and visceral fat.

[0028] When the oral composition of the present invention is prepared as a food or drink product, it may be formulated into a desired form by mixing, in addition to Glyasperin B, a sweetener, a colorant, a preservative, a thickener, a stabilizer, a gelling agent, a paste, an

antioxidant, a color developer, a bleaching agent, a mold inhibitor (a fungicide), yeast food, a gum base, a flavoring, an acidulant, a seasoning, an emulsifier, a pH adjuster, kansui, a leavening agent, a nutritional fortifier, and other food and drink materials. When the oral composition of the present invention is prepared in a food or drink form, the form is not especially limited. Examples include supplement-type foods such as a gel, a granule, a fine granule, a capsule, a tablet, a powder, a liquid, and a semi-solid preparations; drink products such as a carbonated drink, a soft drink, a milk drink, an alcoholic beverage, a fruit juice drink, teas, and a nutritional drink; powdered beverages such as powdered juice and powdered soup; confectioneries such as gum, a tablet, a candy, a cookie, a gummy, a rice cracker, a biscuit, and a jelly; bread, noodles, cereals, a jam, and a seasoning. These food products are used as food and drink products for efficiently decomposing fat and reducing body fat such as subcutaneous fat and visceral fat, and these can also be used as, for example, in addition to general food and drink products, nutraceuticals such as a nutritional supplement, a food with function claims, a food for specified health uses, and food for patients.

[0029] When the oral composition of the present invention is prepared as a pharmaceutical product (including a quasi-drug), in addition to Glyasperin B, other medically active ingredients, pharmaceutically acceptable carriers, additives, and the like may be optionally blended as needed. Specific examples of the pharmaceutically acceptable carriers and additives include a binder, a disintegrant, a lubricant, a wetting agent, a buffer, a preservative, and a flavoring agent. When the oral composition of the present invention is prepared as a pharmaceutical product, the form is not especially limited. Examples include an injection, a topical agent, an inhalant, a suppository, a film preparation, a troch, a liquid, a powder, a tablet, a granule, a capsule, a syrup, an eye drop, an eye wash, and a nasal drop. Among these, forms suitable for oral administration (namely, an oral pharmaceutical product) are preferred. Specific examples of such forms include a troch, a liquid, a powder, a tablet, a granule, a capsule, and a syrup. These pharmaceutical products (including quasi-drugs) are used as a pharmaceutical product for efficiently decomposing fat and reducing body fat such as subcutaneous fat and visceral fat.

[0030] The Glyasperin B contained in the oral composition of the present invention may be used also as a cosmetic preparation (including a functional cosmetic preparation) or a topical quasi-drug. For preparing it as a cosmetic preparation or a topical quasi-drug, a pharmaceutically or cosmetically acceptable carrier (e.g., water, an oil component or the like) may be blended in addition to Glyasperin B to formulate the resultant into a desired form. The form of the cosmetic preparation is not especially limited as long as it is applicable to the skin. Examples include forms of a liquid, an emulsion, a powder, a solid, a suspension, a cream, an ointment, a mousse, a granule, a tablet, a gel, a jelly, a paste, a gel, an aerosol, a

spray, a liniment, and a pack. These cosmetic preparations are used as cosmetic preparations for efficiently decomposing fat and reducing subcutaneous fat, and the like.

[0031] The dosage and application amount of the oral composition of the present invention can be appropriately determined depending on the age, weight, health condition, degree of obesity, disease state, and the like of a user, and the dosage in terms of a total amount of Glyasperin B per day for an adult can be, although not limited to, 0.1  $\mu\text{g}$  or more, 0.5  $\mu\text{g}$  or more, 1  $\mu\text{g}$  or more, 5  $\mu\text{g}$  or more, 10  $\mu\text{g}$  or more, or 12  $\mu\text{g}$  or more, and 5000  $\mu\text{g}$  or less, 1000  $\mu\text{g}$  or less, 500  $\mu\text{g}$  or less, 100  $\mu\text{g}$  or less, 60  $\mu\text{g}$  or less, 20  $\mu\text{g}$  or less, 15  $\mu\text{g}$  or less, or the like. For example, the dosage in terms of the total amount of Glyasperin B per day for an adult is 0.1  $\mu\text{g}$  to 5000  $\mu\text{g}$ , preferably 0.5  $\mu\text{g}$  to 1000  $\mu\text{g}$ , more preferably 1  $\mu\text{g}$  to 500  $\mu\text{g}$ , further preferably 1  $\mu\text{g}$  to 200  $\mu\text{g}$ , still further preferably 1  $\mu\text{g}$  to 100  $\mu\text{g}$ , particularly preferably 5  $\mu\text{g}$  to 100  $\mu\text{g}$ , more particularly preferably 5  $\mu\text{g}$  to 60  $\mu\text{g}$ , further particularly preferably 10  $\mu\text{g}$  to 50  $\mu\text{g}$ , and most preferably 10 to 20  $\mu\text{g}$ .

[0032] When the oral composition provided by the present invention is to be orally administered to a human, Glyasperin B is administered preferably in an effective amount. Herein, the term "effective amount" refers to a dosage at which the intended effect is observed, no matter whether through a direct effect or an indirect effect. The effective amount varies depending on the age and the weight, and can be, for example, 1  $\mu\text{g}$  or more, 3  $\mu\text{g}$  or more, 5  $\mu\text{g}$  or more, 7  $\mu\text{g}$  or more, 10  $\mu\text{g}$  or more, or 12  $\mu\text{g}$  or more, and can be 1 mg or less, 500  $\mu\text{g}$  or less, 200  $\mu\text{g}$  or less, 100  $\mu\text{g}$  or less, 60  $\mu\text{g}$  or less, 50  $\mu\text{g}$  or less, 20  $\mu\text{g}$  or less, or the like per day for an adult in terms of the total amount of Glyasperin B described above. For example, the amount in terms of the total amount of Glyasperin B per day for an adult is 1  $\mu\text{g}$  or more and 1 mg or less, preferably 1  $\mu\text{g}$  or more and 500  $\mu\text{g}$  or less, more preferably 1  $\mu\text{g}$  or more and 200  $\mu\text{g}$  or less, further preferably 1  $\mu\text{g}$  or more and 100  $\mu\text{g}$  or less, still further preferably 5  $\mu\text{g}$  or more and 100  $\mu\text{g}$  or less, particularly preferably 5  $\mu\text{g}$  or more and 60  $\mu\text{g}$  or less, more particularly preferably 10  $\mu\text{g}$  or more and 50  $\mu\text{g}$  or less, further particularly preferably 10  $\mu\text{g}$  or more and 20  $\mu\text{g}$  or less, and most preferably 14  $\mu\text{g}$ .

[0033] The oral composition of the present invention is preferably used for an adult with a BMI of 23  $\text{kg}/\text{m}^2$  or more and less than 30  $\text{kg}/\text{m}^2$ , and more preferably used for an adult with a BMI of 23  $\text{kg}/\text{m}^2$  or more and less than 25  $\text{kg}/\text{m}^2$  from the viewpoint of easily obtaining a weight reducing effect. According to the criteria of the Japan Society for the Study of Obesity, a BMI of 18.5  $\text{kg}/\text{m}^2$  or more and less than 25  $\text{kg}/\text{m}^2$  is classified as normal weight, and a BMI of 25  $\text{kg}/\text{m}^2$  or more and less than 30  $\text{kg}/\text{m}^2$  is classified as obesity degree 1. The oral composition of the present invention is preferably used for an adult categorized as having so-called normal weight to slightly obese (obesity degree 1), and is particularly preferably used for an adult who is not classified as obese but is positioned slightly toward the obese side

in the normal weight region. A BMI (Body Mass Index) is also designated as a body mass index, and is a physique index that represents the obesity degree, calculated from weight and height using the following formula. A BMI is used as an international index for adults.

5            Calculation formula:  $BMI = \text{body weight (kg)} / \text{height}^2 \text{ (m)}$

[0034] The oral composition of the present invention is preferably used for an adult whose visceral fat area is 100 cm<sup>2</sup> or more from the viewpoint of easily obtaining a weight reducing effect. The visceral fat area can be calculated according to a conventional method by  
10 performing an abdominal CT scan and taking a plurality of slice images at an interval of, for example, 1 cm centered on the umbilical region.

[0035] Another embodiment of the present invention provides a weight reducing agent, a visceral fat/body fat reducing agent, a BMI improving agent, an insulin resistance improving agent, or a cholesterol level lowering agent, all containing Glyasperin B as an active  
15 ingredient.

[0036] The present invention also encompasses the aforementioned weight reducing agent, visceral fat/body fat reducing agent, BMI improving agent, insulin resistance improving agent, or cholesterol level lowering agent, and since Glyasperin B is contained, these agents possess both excellent lipolysis promoting activity and exhibit a remarkable weight reducing  
20 effect. When the weight reducing agent, visceral fat/body fat reducing agent, BMI improving agent, insulin resistance improving agent, or cholesterol level lowering agent of the present invention are used, fat in adipocytes can be efficiently decomposed, and body fat such as subcutaneous fat and visceral fat can be reduced, resulting in remarkable weight reducing effect and BMI improving effect. Moreover, the improving effects on insulin resistance and  
25 lowering effects on cholesterol levels can also be obtained. Therefore, the weight reducing agent, visceral fat/body fat reducing agent, BMI improving agent, insulin resistance improving agent, or cholesterol level lowering agent of the present invention can prevent and improve obesity and lifestyle-related diseases for a long period, and thus, maintenance and promotion of health can be realized. It is noted that the weight reducing agent, visceral  
30 fat/body fat reducing agent, BMI improving agent, insulin resistance improving agent, or cholesterol level lowering agent of the present invention have the same configuration as the above-mentioned oral composition of the present invention that contains Glyasperin B as an active ingredient, and therefore, the description provided in the section of the oral composition of the present invention can also be used directly as a specific description of it.

35 [0037] <Method for Determining Efficacy of Plant Extract for Lipolysis and/or Weight Loss>

The present invention also encompasses an invention relating to a method for determining the efficacy of a plant extract for lipolysis and/or weight loss in a living body, using the content of Glyasperin B as an indicator. As described above, a formulation containing Glyasperin B as an active ingredient possesses excellent lipolysis promoting activity and exhibits a remarkable weight reducing effect. Furthermore, it can efficiently decompose fat in adipocytes, and thus can reduce body fat such as subcutaneous fat and visceral fat, resulting in remarkable weight reducing effect and BMI improving effect. Additionally, the improving effects on insulin resistance and lowering effects on cholesterol levels can be also obtained. Since the aforementioned effects also increase in proportion to the content of Glyasperin B, it becomes possible to determine the efficacy of the plant extract for lipolysis and/or weight loss in a living body by using the content of Glyasperin B in the plant extract as an indicator. The plant extract is not especially limited as long as it contains Glyasperin B, but licorice extract is particularly preferred.

[0038] Now, the determination method of the present invention will be described in detail.

The determination method of the present invention includes a step of measuring the content of Glyasperin B in a plant extract (hereinafter, also referred to as the "measuring step").

Moreover, the method preferably includes a step of comparing the content of Glyasperin B thus obtained with a Glyasperin B content used as a reference (hereinafter, also referred to as the "comparing step").

[0039] A method for measuring the content of Glyasperin B in the plant extract in the measuring step is not especially limited, and the measurement can be performed by any method known to those skilled in the art. Examples include a method using Western blotting, and a method using ELISA. In either the method using Western blotting or the method using ELISA, the content of Glyasperin B can be measured with high accuracy by using an anti-Glyasperin B antibody that specifically binds to Glyasperin B. In the method using Western blotting, the content of Glyasperin B in a sample can be calculated by comparing the intensity of the obtained Glyasperin B-specific blot with the intensity of a standard sample (positive control) of a known concentration.

[0040] The Glyasperin B used as the reference in the determination method of the present invention can be obtained, for example, through extraction from a natural source, expression of a recombinant nucleic acid encoding Glyasperin B, or chemical synthesis.

[0041] In the determination method of the present invention, the content of Glyasperin B in the extract to be evaluated is compared with the content of the Glyasperin B used as the reference through this comparing step. The amount of the Glyasperin B used as the reference can be appropriately selected according to the purpose and subject of the evaluation.

[0042] <Indicator of Quality of Plant Extract>

The present invention also encompasses an invention relating to an indicator of the quality of a plant extract, where the quality is related to the efficacy of lipolysis and/or weight loss in a living body, and the indicator comprises Glyasperin B. As described above, a formulation containing Glyasperin B as an active ingredient possesses excellent lipolysis promoting activity and exhibits a remarkable weight reducing effect. Furthermore, it can efficiently decompose fat in adipocytes, and thus can reduce body fat such as subcutaneous fat and visceral fat, resulting in remarkable weight reducing effect and BMI improving effect. Additionally, the improving effects on insulin resistance and lowering effects on cholesterol levels can be also obtained. Since the aforementioned effects also increase in proportion to the content of Glyasperin B, it is possible to determine the efficacy of the plant extract for lipolysis and/or weight loss in a living body by using Glyasperin B in the plant extract as an indicator. A plant of the plant extract is preferably licorice.

[0043] The indicator of the quality of a plant extract of the present invention corresponds to Glyasperin B in the above-described method for determining efficacy of a plant extract for lipolysis and/or weight loss. The description provided above regarding the determination method can also be used as a specific description of the indicator of the present invention.

#### EXAMPLES

[0044] The present invention will now be described in more detail with reference to examples, but the scope of the present invention is not limited to these.

[0045] As described below, in order to evaluate the influence on visceral fat obtained when a subject has ingested a test food (a hard capsule containing licorice extract) for 12 weeks, a randomized, double-blind, parallel-group comparison trial was conducted using a control food (a hard capsule not containing licorice extract) as a control.

[0046] <Study 1>

##### 1. Study Food (Composition, Packaging, etc.)

A test food contained 100 mg of licorice extract (manufactured by MG Pharma Co., Ltd.; derived from Ural licorice) in one capsule, and was further composed of excipients. A control food did not contain licorice extract and was composed of excipients colored with caramel color. The shape of both the study foods was a white hard capsule, and each was packaged in an aluminum pouch containing 30 capsules, and an identification code was displayed on each study food. The inability to distinguish the study food and the packaging was reviewed and approved by IRB. The nutritional components and relevant components of the study foods are shown in Table 1 below. The amount of Glyasperin B contained in the test food was 14 µg per recommended daily intake.

[0047] [Table 1]

Table 1. Licorice Extract Content and Nutritional Components in Study Formulations [per capsule (per day)]

|                       | Test Formulation | Control Formulation |
|-----------------------|------------------|---------------------|
| Licorice Extract (mg) | 100              | 0                   |
| Calories (kcal)       | 1.150            | 0.765               |
| Protein (g)           | 0.016            | 0.000               |
| Lipid (g)             | 0.006            | 0.004               |
| Carbohydrates (g)     | 0.264            | 0.288               |
| Salt Equivalent (mg)  | 0.651            | 1.984               |

## [0048] 2. Subjects

The target sample size for this study was calculated to be 34 subjects per group, totaling 68 subjects. This calculation was based on the need for the enrollment of 26 to 35 subjects per group to achieve 80 to 90% power with a significance level of 0.05, assuming a large effect size (0.8) for the study food in the parallel-group comparison trial between 2 groups. Considering the possibility of dropouts and discontinuation during the study, the entry number was set to 80 (40 subjects per group). Subjects were publicly recruited from among volunteers registered with the contract research organization, and a total of 81 individuals (50 males, 31 females) who met the following selection criteria, did not meet exclusion criteria, and were deemed appropriate for the participation in this study by an investigator were enrolled as subjects.

### [0049] (Selection Criteria)

Those who met the following conditions in the screening examination were selected.

(1) Males and females aged 20 years old or older and under 65 years old; (2) individuals with a BMI of 23.0 kg/m<sup>2</sup> or more and less than 30.0 kg/m<sup>2</sup>; (3) individuals with a visceral fat area of 100 cm<sup>2</sup> or more; and (4) individuals who had received a sufficient explanation of the objective and content of this study, had the capacity to consent, fully understood the study, and willingly volunteered to participate, and were able to provide written consent for participation in this study.

### (Exclusion Criteria)

(1) Individuals currently suffering from, or with a history of, severe cardiovascular disorders, hepatic dysfunction, renal dysfunction, respiratory disorders, endocrine disorders, or metabolic disorders; (2) individuals receiving treatment for chronic diseases such as dyslipidemia, hypertension, or diabetes mellitus; (3) individuals with psychiatric disorders such as depression, schizophrenia, or bulimia nervosa; (4) individuals regularly using pharmaceuticals or quasi-drugs that may affect the evaluation of this study for body fat, triglycerides, weight, cholesterol, or energy metabolism and the like; (5) individuals regularly using supplements/health foods (such as foods for specified health uses, and foods with function claims) that may affect the evaluation of this study for body fat, triglycerides, body

weight, cholesterol, or energy metabolism and the like; (6) individuals who have the potential to cause allergic reactions to the study foods; (7) individuals with metal implants in the abdominal area to be targeted for CT scan; (8) individuals with implantable medical devices such as a cardiac pacemaker or an implantable cardioverter-defibrillator; (9) individuals with claustrophobia; (10) individuals with a smoking habit; (11) individuals with a habit of heavy alcohol consumption (pure alcohol 60 g/day or more); (12) individuals with an extremely irregular dietary habits; (13) individuals working night shifts or on a day-and-night rotating shift system; (14) individuals who have not had a bowel movement for five days or more; (15) individuals who have symptoms of menopause; (16) individuals with hypertension, pseudoaldosteronism, hypokalemia, or myopathy; (17) individuals regularly using pharmaceuticals containing licorice or food primarily containing licorice; (18) individuals who are prone to developing edema or weakness; (19) individuals with a urination disorder; (20) individuals who have undergone blood sampling (such as blood donation) exceeding 200 mL within one month, or 400 mL within three months prior to the start of the study; (21) individuals who have participated in another clinical trial within the past four months, are currently participating in another clinical trial, or plan to participate in another clinical trial during the study period; (22) individuals who are pregnant or breastfeeding, or who plan to become pregnant; and (23) any other individual deemed inappropriate by the investigator.

### [0050] 3. Study Protocol

This study was performed as a randomized, placebo-controlled, double-blind, parallel-group comparison trial. Examinations were conducted at the start of the intake of the study food, and after 4 weeks, 8 weeks, and 12 weeks of the intake. Subjects enrolled by the investigator were assigned to groups by an allocation manager who was not involved in the study execution, using random numbers generated for randomization. At this point, it was confirmed that there were no significant differences between the groups with respect to the allocation factors, such as the sex and age at the time of consent, the visceral fat area, BMI, hip circumference, and waist circumference at the time of the screening examination.

[0051] During the study period, subjects were instructed to ingest one capsule of the study food (test food for the test food group, control food for the control food group) once daily with water or lukewarm water before dinner. However, if unable to take it before dinner, the subjects were instructed to ingest one capsule once daily at a feasible time.

[0052] During the study period, the subjects were instructed to maintain their usual habits for food and drink as much as possible, and were prohibited from ingesting health foods (e.g., foods for specified health uses, and foods with function claims) that could potentially affect the evaluation of this study for body fat, triglycerides, weight, cholesterol, energy metabolism and the like, as well as drinking grapefruit juice. Moreover, the subjects were instructed to

maintain their usual alcohol intake habits and were prohibited from drinking amounts that deviated from their normal intake. Additionally, alcohol intake was prohibited on the day before the examination, and the intake of anything other than water was prohibited after 21:00. Intake of anything other than water was also prohibited for 4 hours prior to the abdominal CT scan. During the study period, the subjects were instructed to maintain their usual exercise habits (frequency, type of exercise, and the like) as much as possible, and were prohibited from changing their exercise habits. During the study period, if a subject needed to use a new pharmaceutical, he/she was required to obtain permission from the investigator, except in emergency cases. Vaccination was prohibited for 2 weeks prior to the examination day. Blood donation was prohibited during the study period.

[0053] This study was conducted in accordance with the spirit of the Declaration of Helsinki (2013) - Tokyo, Venice, Hong Kong, Somerset West, Edinburgh revisions, Washington, Tokyo annotations added, Seoul revision, Fortaleza revision - and followed the "Ethical Guidelines for Life Sciences and Medical Research involving Human Subjects (established on March 23, 2021, enforced on June 30, 2021, partially revised on March 10, 2022). Prior to the implementation, approval was obtained from Koseikai Fukuda Naika Clinic Institutional Review Board (IRB) (Approval Number: IRB-20220716-2). Moreover, this study was registered with the University Hospital Medical Information Network (UMIN) Clinical Trial Registry System (UMIN trial ID: UMIN000048430).

#### [0054] 4. Examination Items

##### 1) Physical Examination and Abdominal CT Scan

As physical examination items, a height (only at the screening examination), body composition assessment using InBody 770 (InBody Japan Inc.) (including a weight, a BMI, a body fat percentage, a muscle mass, a segmental fat mass, a basal metabolic rate, and other body composition component analyses), a waist circumference, a hip circumference, a blood pressure, and pulse were measured, and CT scan was performed on a subject who met the selection criteria. The weight was measured using DST-210N (Muratec-KDS Corporation) only at the time of the CT scan. The BMI was calculated from the height and the weight, and the BMI at the time of the CT scan was used as a value before intake.

[0055] The abdominal CT scan was performed using Discovery 710 or Discovery MIDR (GE Healthcare Japan Corp.), three slices of images were taken at 1 cm intervals centering on the umbilical region, and average values of the visceral fat area, the subcutaneous fat area, and the total fat area were calculated. If the image taken in the umbilical region included the kidneys or ilium, rescanning was performed centering on the fourth lumbar vertebra.

##### [0056] 2) Diet Record and Activity Record

During the study period, subjects were instructed to record their alcohol intake,

pharmaceutical use, dietary details and the like in a life record. During the study food intake period, the subjects were instructed to also record their intake of the study foods.

Furthermore, for three days prior to each examination day, the subjects were instructed to record not only the contents but also the quantity of their meals. The subjects were instructed to wear an actigraphy device (SAT-1, Medithink Inc.) during the study period, except while sleeping, so as to check steps, Active steps, and moderate-intensity activity time every two weeks. The health status of the subjects was assessed after 4, 8, and 12 weeks of the intake through interviews and medical examinations to grasp occurrence of subjective and objective symptoms, and the like.

### 10 [0057] 3) Evaluation of Fatigue and Sleep

For the evaluation of fatigue, subjects conducted evaluation by the VAS method at their home upon waking, at the start of intake, and after 4, 8, and 12 weeks of the intake. For the evaluation of sleep, subjects conducted evaluation using the OSA sleep inventory MA version at their home upon waking, at the start of intake, and after 4, 8, and 12 weeks of the intake.

### 15 [0058] 4) Blood Test and Urine Test

For a blood test, blood samples were collected before the intake, and after 4, 8, and 12 weeks of the intake, and the following parameters were analyzed: WBC, RBC, Hb, Ht, MCV, MCH, MCHC, Plt, white blood cell differential, total protein, Alb, A/G ratio, AST, ALT,  $\gamma$ -GTP, creatinine, blood glucose, total cholesterol (T-Cho), HDL cholesterol (HDL-Cho), LDL cholesterol (LDL-Cho), triglyceride (TG), CPK, uric acid (UA), urea nitrogen (UN), ALP, LDH, Na, K, Cl, Ca, Mg, P, total bilirubin (T-Bil), HbA1c, glycoalbumin, insulin, and ketone body fraction (total ketone bodies, acetoacetic acid, 3-hydroxybutyric acid). A urine test was performed before the intake, and after 12 weeks of the intake, and glucose, protein, occult blood, and urobilinogen tests were performed.

### 25 [0059] 5. Statistical Analysis

The primary evaluation item was the visceral fat area measured by abdominal CT scan. Secondary evaluation items were body weight, BMI, subcutaneous fat area and total fat area measured by abdominal CT scan, body fat percentage, body fat mass, muscle mass, segmental fat mass, and basal metabolic rate measured by InBody 770, waist circumference, hip circumference, waist-to-hip ratio, VAS (fatigue), the OSA sleep inventory MA version, and ketone body fraction, T-Cho, LDL-Cho, HDL-Cho, TG, blood glucose, HbA1c, insulin, and glycoalbumin obtained by the blood test.

[0060] The statistical analysis was performed using statistical software SPSS Ver. 26 (IBM Japan, Ltd.), and the level of significance was set at 5% for the two-sided test. Furthermore, to confirm the trend based on subject attributes, subgroup analyses were performed based on

sex, and on a subgroup with a BMI before the intake of 23 kg/m<sup>2</sup> or more and less than 25 kg/m<sup>2</sup>, and a subgroup with a BMI of 25 kg/m<sup>2</sup> or more and less than 30 kg/m<sup>2</sup>. For values at the time of each examination and changes from values before the start of intake to the time of each examination, an average and a standard deviation were calculated for each group. A difference in the average between the test food group and the control food group at the time of each examination was subjected to unpaired t-test for comparison of the difference between the groups. Moreover, a difference in the average between the value before the intake and the value at the time of each examination in each of the respective study food groups was analyzed using Dunnett's multiple comparison test for comparison of the difference depending on the intake period of the study food. For the abdominal CT scan, paired t-test was conducted for comparison of the difference depending on the intake period of the study food. No multiple comparison correction was applied to the paired t-test.

[0061] (Results)

#### 1. Analysis Subjects

A total of 81 subjects were enrolled in this study (41 in the test food group and 40 in the control food group). However, after randomization, one subject in the test food group was discontinued from the study because he/she started a treatment that met the exclusion criterion (2), and thus the study commenced with 80 subjects. A total of 78 subjects (39 in the test food group and 39 in the control food group) were defined as the full analysis set (FAS), excluding one subject in the test food group who was identified to meet the exclusion criterion (15) after the start of the intake of the study food, and one subject in the control food group who never ingested the study food and withdrew from the study due to personal reasons. Furthermore, the Per-Protocol Set (PPS) was defined as 76 subjects (38 in the test food group and 38 in the control food group) because one subject in the test food group discontinued the study due to the onset of autoimmune pancreatitis and one subject in the control food group discontinued the study due to personal reasons during the study food intake period. In addition, two cases of partial exclusion from the analysis occurred in the control food group, where data for certain evaluation items were excluded from the analysis only for those specific items due to concerns regarding data reliability. In one case, LDH, K, and insulin from the blood test after 4 weeks of the intake were excluded from the analysis because hemolysis was observed in the blood sample. In another case, blood glucose and insulin from the blood test after 8 weeks of the intake were excluded from the analysis because diet intake on the day of the examination (violation of management instructions) was suspected. The subject demographics of the subjects included in subjects for the efficacy analysis are shown in Table 2.

[0062] [Table 2]

Table 2. Subject Demographics

|              | n  | Male | Female | Age<br>(years old) | Visceral Fat<br>Area<br>(cm <sup>2</sup> )<br>before intake | BMI<br>(kg/m <sup>2</sup> )<br>before intake | Waist<br>Circumference<br>(cm)<br>before intake | Hip<br>Circumference<br>(cm)<br>before intake |
|--------------|----|------|--------|--------------------|-------------------------------------------------------------|----------------------------------------------|-------------------------------------------------|-----------------------------------------------|
| Test food    | 38 | 24   | 14     | 54.2±6.0           | 129.91±19.06                                                | 26.1±1.5                                     | 93.9±5.7                                        | 100.9±4.4                                     |
| Control Food | 38 | 23   | 15     | 53.7±7.4           | 131.16±19.66                                                | 26.2±1.9                                     | 93.9±7.0                                        | 101.1±5.4                                     |

Average ± Standard Deviation

[0063] No significant differences were observed between the test food group and the control food group in any population, including the subgroups by FAS, PPS, and BMI, and the subgroups by sex and age at the time of consent, that is, the allocation factors, and visceral fat area, BMI, waist circumference, and hip circumference at the screening.

## [0064] 2. Analysis Results

### (Abdominal Fat Area)

Changes in the visceral fat area, the subcutaneous fat area, and the total fat area in the Per-Protocol Set (PPS) are shown in Table 3. There were no significant differences observed between the test food group and the control food group regarding the visceral fat area that was the primary evaluation item. In the changes over time in the test food group, the visceral fat area and the total fat area showed a significant decrease after 12 weeks of the intake compared to the values before the intake.

[0065] [Table 3]

Table 3. Abdominal Fat Area

|                                             |                |              | Before Intake<br>Test Food: n=38<br>Control Food: n=38 | After 12 Weeks of Intake<br>Test Food: n=37<br>Control Food: n=38 |
|---------------------------------------------|----------------|--------------|--------------------------------------------------------|-------------------------------------------------------------------|
| Visceral Fat Area<br>(cm <sup>2</sup> )     | Measured Value | Test Food    | 129.9±19.1                                             | 124.0±22.9 §                                                      |
|                                             |                | Control Food | 131.2±19.7                                             | 126.4±24.9                                                        |
|                                             | Δ              | Test Food    |                                                        | -6.5±18.9                                                         |
|                                             |                | Control Food |                                                        | -4.7±20.1                                                         |
| Subcutaneous Fat Area<br>(cm <sup>2</sup> ) | Measured Value | Test Food    | 214.0±52.8                                             | 211.2±52.1                                                        |
|                                             |                | Control Food | 219.2±73.1                                             | 221.8±78.0                                                        |
|                                             | Δ              | Test Food    |                                                        | -3.5±17.9                                                         |
|                                             |                | Control Food |                                                        | 2.5±14.7                                                          |
| Total Fat Area<br>(cm <sup>2</sup> )        | Measured Value | Test Food    | 344.0±55.9                                             | 335.2±57.2 §                                                      |
|                                             |                | Control Food | 350.4±77.6                                             | 348.2±83.8                                                        |
|                                             | Δ              | Test Food    |                                                        | -10.0±27.9                                                        |
|                                             |                | Control Food |                                                        | -2.2±27.8                                                         |

average ± standard deviation, Δ: average ± standard deviation of change from before intake  
comparison between groups (unpaired t-test): no significant difference and trend  
comparison with before intake (paired t-test): § p<0.05

## [0066] (Body Measurement Items)

Changes in the weight, BMI, body fat mass, muscle mass, segmental fat mass, waist

circumference, and hip circumference in the PPS, and changes in the blood pressure and pulse rate in the FAS are shown in Table 4. Regarding the blood pressure and pulse rate, after 4 weeks of the intake, the test food group showed significantly higher values in the systolic blood pressure and the diastolic blood pressure, and a significantly higher change in the pulse rate, compared to the control food group, but no significant changes were observed thereafter. For all the other evaluation items, there were no significant differences observed between the test food group and the control food group.

[0067] [Table 4-1]

Table 4. Body Measurement Items

|                                      |                |              | Before Intake<br>Test Food:<br>n=38<br>Control Food:<br>n=38 | After 4 Weeks<br>of Intake<br>Test Food:<br>n=38<br>Control Food:<br>n=38 | After 8 Weeks<br>of Intake<br>Test Food:<br>n=36<br>Control Food:<br>n=37 | After 12 Weeks<br>of Intake<br>Test Food:<br>n=37<br>Control Food:<br>n=38 |
|--------------------------------------|----------------|--------------|--------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Weight<br>(kg)                       | Measured Value | Test Food    | 72.3±8.7                                                     | 71.7±8.6 §§                                                               | 71.6±8.4 §§                                                               | 71.4±8.8 §§                                                                |
|                                      |                | Control Food | 73.1±8.9                                                     | 72.7±9.2                                                                  | 72.8±9.3                                                                  | 72.7±9.3                                                                   |
|                                      | Δ              | Test Food    |                                                              | -0.6±1.1                                                                  | -0.7±1.1                                                                  | -0.7±1.4                                                                   |
|                                      |                | Control Food |                                                              | -0.4±1.1                                                                  | -0.4±1.2                                                                  | -0.4±1.5                                                                   |
| BMI<br>(kg/m <sup>2</sup> )          | Measured Value | Test Food    | 26.1±1.5                                                     | 25.8±1.5 §§                                                               | 25.9±1.5 §§                                                               | 25.7±1.6 §§                                                                |
|                                      |                | Control Food | 26.2±1.9                                                     | 26.0±2.0                                                                  | 26.0±2.0                                                                  | 26.0±2.0                                                                   |
|                                      | Δ              | Test Food    |                                                              | -0.2±0.4                                                                  | -0.2±0.4                                                                  | -0.3±0.5                                                                   |
|                                      |                | Control Food |                                                              | -0.2±0.4                                                                  | -0.2±0.4                                                                  | -0.1±0.5                                                                   |
| Body Fat Mass<br>(kg)                | Measured Value | Test Food    | 22.0±3.9                                                     | 21.9±4.0                                                                  | 22.2±4.1                                                                  | 22.1±4.2                                                                   |
|                                      |                | Control Food | 22.9±5.2                                                     | 22.7±5.6                                                                  | 23.0±5.6                                                                  | 23.0±5.7                                                                   |
|                                      | Δ              | Test Food    |                                                              | -0.1±1.1                                                                  | 0.0±1.2                                                                   | 0.1±1.4                                                                    |
|                                      |                | Control Food |                                                              | -0.2±1.0                                                                  | 0.1±1.0                                                                   | 0.1±1.3                                                                    |
| Muscle Mass<br>(kg)                  | Measured Value | Test Food    | 47.5±8.8                                                     | 47.0±8.4 §                                                                | 46.7±8.1 §§                                                               | 46.5±8.3 §§                                                                |
|                                      |                | Control Food | 47.4±8.4                                                     | 47.2±8.5                                                                  | 47.1±8.5                                                                  | 46.9±8.6 §                                                                 |
|                                      | Δ              | Test Food    |                                                              | -0.5±1.5                                                                  | -0.7±1.3                                                                  | -0.8±1.2                                                                   |
|                                      |                | Control Food |                                                              | -0.2±0.8                                                                  | -0.5±0.6                                                                  | -0.4±1.1                                                                   |
| Body Fat Mass<br>(Right Arm)<br>(kg) | Measured Value | Test Food    | 1.5±0.4                                                      | 1.5±0.4                                                                   | 1.5±0.4                                                                   | 1.5±0.4                                                                    |
|                                      |                | Control Food | 1.6±0.6                                                      | 1.6±0.6                                                                   | 1.6±0.6                                                                   | 1.6±0.6                                                                    |
|                                      | Δ              | Test Food    |                                                              | 0.0±0.1                                                                   | 0.0±0.1                                                                   | 0.0±0.1                                                                    |
|                                      |                | Control Food |                                                              | 0.0±0.1                                                                   | 0.0±0.1                                                                   | 0.0±0.1                                                                    |
| Body Fat Mass                        | Measured Value | Test Food    | 1.5±0.4                                                      | 1.5±0.4                                                                   | 1.5±0.4                                                                   | 1.5±0.4                                                                    |
|                                      |                | Control Food | 1.6±0.6                                                      | 1.6±0.6                                                                   | 1.6±0.6                                                                   | 1.6±0.6                                                                    |

|                                |                |              |          |          |          |          |
|--------------------------------|----------------|--------------|----------|----------|----------|----------|
| (Left Arm) (kg)                | $\Delta$       | Test Food    |          | 0.0±0.1  | 0.0±0.1  | 0.0±0.1  |
|                                |                | Control Food |          | 0.0±0.1  | 0.0±0.1  | 0.0±0.1  |
| Body Fat Mass (Core) (kg)      | Measured Value | Test Food    | 11.2±1.9 | 11.1±2.0 | 11.3±2.1 | 11.3±2.1 |
|                                |                | Control Food | 11.5±2.4 | 11.4±2.6 | 11.6±2.6 | 11.7±2.7 |
|                                | $\Delta$       | Test Food    |          | -0.1±0.6 | 0.1±0.6  | 0.1±0.8  |
|                                |                | Control Food |          | -0.1±0.5 | 0.1±0.6  | 0.2±0.7  |
| Body Fat Mass (Right Leg) (kg) | Measured Value | Test Food    | 3.3±0.6  | 3.3±0.6  | 3.3±0.6  | 3.3±0.6  |
|                                |                | Control Food | 3.5±0.8  | 3.4±0.9  | 3.5±0.9  | 3.5±0.9  |
|                                | $\Delta$       | Test Food    |          | 0.0±0.2  | 0.0±0.2  | 0.0±0.2  |
|                                |                | Control Food |          | -0.1±0.2 | 0.0±0.2  | 0.0±0.2  |
| Body Fat Mass (Left Leg) (kg)  | Measured Value | Test Food    | 3.3±0.6  | 3.3±0.6  | 3.3±0.6  | 3.3±0.6  |
|                                |                | Control Food | 3.5±0.8  | 3.4±0.9  | 3.5±0.9  | 3.4±0.9  |
|                                | $\Delta$       | Test Food    |          | 0.0±0.2  | 0.0±0.2  | 0.0±0.2  |
|                                |                | Control Food |          | -0.1±0.2 | 0.0±0.2  | -0.1±0.2 |

[0068] [Table 4-2]

Table 4 (continued)

|                                |                |              | Before Intake<br>Test Food:<br>n=38<br>Control Food:<br>n=38 | After 4 Weeks<br>of Intake<br>Test Food:<br>n=38<br>Control Food:<br>n=38 | After 8 Weeks<br>of Intake<br>Test Food:<br>n=36<br>Control Food:<br>n=37 | After 12 Weeks<br>of Intake<br>Test Food:<br>n=37<br>Control Food:<br>n=38 |
|--------------------------------|----------------|--------------|--------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Waist Circumference (cm)       | Measured Value | Test Food    | 93.9±5.7                                                     | 93.9±5.2                                                                  | 94.0±5.6                                                                  | 93.2±5.6                                                                   |
|                                |                | Control Food | 93.9±7.0                                                     | 94.2±7.4                                                                  | 94.6±7.4 §                                                                | 93.8±7.2                                                                   |
|                                | $\Delta$       | Test Food    |                                                              | 0.0±1.8                                                                   | 0.2±1.8                                                                   | -0.5±1.9                                                                   |
|                                |                | Control Food |                                                              | 0.3±1.5                                                                   | 0.7±1.5                                                                   | -0.1±1.6                                                                   |
| Hip Circumference (cm)         | Measured Value | Test Food    | 100.9±4.4                                                    | 101.1±4.2                                                                 | 101.1±4.0                                                                 | 99.9±4.2 §§                                                                |
|                                |                | Control Food | 101.1±5.4                                                    | 101.6±5.6                                                                 | 102.2±5.5 §§                                                              | 100.9±5.4                                                                  |
|                                | $\Delta$       | Test Food    |                                                              | 0.3±1.7                                                                   | 0.4±1.6                                                                   | -0.8±1.8                                                                   |
|                                |                | Control Food |                                                              | 0.5±2.2                                                                   | 0.9±2.1                                                                   | -0.2±2.5                                                                   |
| Waist-to-Hip Ratio             | Measured Value | Test Food    | 0.93±0.04                                                    | 0.93±0.03                                                                 | 0.93±0.03                                                                 | 0.93±0.04                                                                  |
|                                |                | Control Food | 0.93±0.05                                                    | 0.93±0.04                                                                 | 0.93±0.04                                                                 | 0.93±0.04                                                                  |
|                                | $\Delta$       | Test Food    |                                                              | 0.00±0.02                                                                 | 0.00±0.02                                                                 | 0.00±0.02                                                                  |
|                                |                | Control Food |                                                              | 0.00±0.02                                                                 | 0.00±0.02                                                                 | 0.00±0.03                                                                  |
| Systolic Blood Pressure (mmHg) | Measured Value | Test Food    | 120±11                                                       | 122±15                                                                    | 119±12                                                                    | 120±12                                                                     |
|                                |                | Control Food | 121±12                                                       | 115±12 ]* §§                                                              | 115±13 §§                                                                 | 118±12                                                                     |
|                                | $\Delta$       | Test Food    |                                                              | 2±13 ]**                                                                  | -1±11                                                                     | 1±13                                                                       |

|                                 |                |              |       |         |       |          |
|---------------------------------|----------------|--------------|-------|---------|-------|----------|
|                                 |                | Control Food |       | -6±9    | -6±11 | -3±11    |
| Diastolic Blood Pressure (mmHg) | Measured Value | Test Food    | 72±8  | 76±9 §§ | 73±8  | 74±8     |
|                                 |                | Control Food | 74±8  | 73±9    | 72±9  | 73±9     |
|                                 | Δ              | Test Food    |       | 4±8     | 1±7   | 2±8      |
|                                 |                | Control Food |       | 0±6 ]** | -2±6  | -1±7     |
| Pulse Rate (beats /min)         | Measured Value | Test Food    | 69±9  | 74±14 § | 72±10 | 74±15 §§ |
|                                 |                | Control Food | 70±10 | 70±8    | 73±12 | 75±11 §§ |
|                                 | Δ              | Test Food    |       | 5±10    | 4±9   | 5±11     |
|                                 |                | Control Food |       | 0±6 ]*  | 3±7   | 5±7      |

average ± standard deviation, Δ: average ± standard deviation of change from before intake

\* Regarding systolic blood pressure, diastolic blood pressure, and pulse rate, before intake, test food: n = 39, control food: n = 39, after 4 weeks of intake, test food: n = 38, control food: n = 38, after 8 weeks of intake, test food: n = 37, control food: n = 37, after 12 weeks of intake, test food: n = 37, control food: n = 38

\* Regarding waist circumference, hip circumference, and waist-to-hip ratio, after 8 weeks of intake, test food: n = 37, control food: n = 37

comparison between groups (unpaired t-test): \*\* p<0.01, \* p<0.05

comparison with before intake (Dunnett's multiple comparison test): §§ p<0.01, § p<0.05

#### 10 [0069] (Blood Test and Urine Test)

Changes in blood test items (secondary evaluation items) in the PPS are shown in Table 5. Regarding T-Cho, after 4 weeks of the intake, the test food group showed a significant suppression of the decrease in the change from the value before the intake compared to the control food group. Regarding blood glucose, the test food group showed predominantly higher values compared to the control food group from before the start of the intake, and showed significantly higher values after 4 weeks of the intake, but no difference was observed thereafter. Regarding insulin levels, the test food group showed a significant decrease compared to the control food group. Moreover, regarding the other blood test items and urine test items in the FAS, no clinically problematic changes were observed in any of the subjects.

[0070] [Table 5]

Table 5. Blood Test (Secondary Evaluation Items)

|                              |                |              | Before Intake<br>Test Food:<br>n=38<br>Control Food:<br>n=38 | After 4 Weeks<br>of Intake<br>Test Food:<br>n=38<br>Control Food:<br>n=38 | After 8 Weeks<br>of Intake<br>Test Food:<br>n=37<br>Control Food:<br>n=37 | After 12 Weeks<br>of Intake<br>Test Food:<br>n=37<br>Control Food:<br>n=38 |
|------------------------------|----------------|--------------|--------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Total Ketone Bodies (mmol/L) | Measured Value | Test Food    | 91±88                                                        | 113±130                                                                   | 95±84                                                                     | 103±105                                                                    |
|                              |                | Control Food | 92±102                                                       | 107±118                                                                   | 86±72                                                                     | 103±107                                                                    |
|                              | Δ              | Test Food    |                                                              | 22±78                                                                     | 3±78                                                                      | 12±62                                                                      |

|                                      |                |              |          |          |            |            |
|--------------------------------------|----------------|--------------|----------|----------|------------|------------|
|                                      |                | Control Food |          | 15±93    | -5±126     | 11±128     |
| Aceto-acetic Acid (mmol/L)           | Measured Value | Test Food    | 25±21    | 33±33    | 31±24      | 30±24      |
|                                      |                | Control Food | 26±23    | 33±31    | 30±20      | 33±31      |
|                                      | Δ              | Test Food    |          | 9±23     | 6±21       | 5±16       |
|                                      |                | Control Food |          | 7±25     | 5±29       | 8±35       |
| 3-Hydroxybutyric Acid (mmol/L)       | Measured Value | Test Food    | 66±68    | 79±99    | 64±61      | 73±82      |
|                                      |                | Control Food | 67±81    | 74±87    | 56±53      | 70±77      |
|                                      | Δ              | Test Food    |          | 13±58    | -3±57      | 7±47       |
|                                      |                | Control Food |          | 8±70     | -9±98      | 4±94       |
| T-Cho (mg/dL)                        | Measured Value | Test Food    | 211±29   | 218±27 § | 218±31 §   | 222±29 §§  |
|                                      |                | Control Food | 211±32   | 209±30   | 209±32     | 215±33     |
|                                      | Δ              | Test Food    |          | 7±18     | 7±20       | 10±16      |
|                                      |                | Control Food |          | -3±23 ]* | -2±25      | 4±27       |
| LDL-Cho (direct method) (mg/dL)      | Measured Value | Test Food    | 130±22   | 132±21   | 131±25     | 136±23     |
|                                      |                | Control Food | 133±27   | 130±25   | 127±24     | 133±26     |
|                                      | Δ              | Test Food    |          | 2±15     | 0±15       | 4±13       |
|                                      |                | Control Food |          | -3±22    | -6±22      | -1±24      |
| LDL-Cho (Friedewald formula) (mg/dL) | Measured Value | Test Food    | 129±24   | 133±24   | 134±26     | 137±23 §§  |
|                                      |                | Control Food | 129±27   | 129±28   | 127±26     | 133±29     |
|                                      | Δ              | Test Food    |          | 4±17     | 4±15       | 7±13       |
|                                      |                | Control Food |          | -1±21    | -2±22      | 3±25       |
| HDL-Cho (mg/dL)                      | Measured Value | Test Food    | 58±13    | 60±15    | 63±16 §§   | 64±17 §§   |
|                                      |                | Control Food | 56±14    | 56±14    | 57±14      | 59±16 §§   |
|                                      | Δ              | Test Food    |          | 2±7      | 4±8        | 5±9        |
|                                      |                | Control Food |          | 0±5      | 1±5        | 3±6        |
| TG (mg/dL)                           | Measured Value | Test Food    | 118±59   | 125±71   | 109±45     | 109±56     |
|                                      |                | Control Food | 129±64   | 120±62   | 122±66     | 121±72     |
|                                      | Δ              | Test Food    |          | 6±68     | -7±39      | -11±43     |
|                                      |                | Control Food |          | -9±47    | -7±47      | -9±58      |
| Blood Glucose (mg/dL)                | Measured Value | Test Food    | 98±11    | 97±11    | 97±12      | 98±10      |
|                                      |                | Control Food | 92±7 ]** | 91±7 ]*  | 93±8       | 94±7 §     |
|                                      | Δ              | Test Food    |          | 0±9      | -1±9       | 0±8        |
|                                      |                | Control Food |          | 0±5      | 2±6        | 3±6        |
| HbA1c (%)                            | Measured Value | Test Food    | 5.5±0.3  | 5.5±0.3  | 5.4±0.3 §§ | 5.4±0.3 §§ |
|                                      |                | Control Food | 5.5±0.3  | 5.5±0.2  | 5.4±0.2 §§ | 5.4±0.2 §§ |

|                   |                |              |                |                   |                |                   |
|-------------------|----------------|--------------|----------------|-------------------|----------------|-------------------|
|                   | $\Delta$       | Test Food    |                | -0.1 $\pm$ 0.2    | -0.1 $\pm$ 0.2 | -0.2 $\pm$ 0.2    |
|                   |                | Control Food |                | 0.0 $\pm$ 0.2     | -0.1 $\pm$ 0.2 | -0.1 $\pm$ 0.2    |
| Insulin (mU/mL)   | Measured Value | Test Food    | 8.7 $\pm$ 6.2  | 9.3 $\pm$ 6.6     | 9.0 $\pm$ 4.1  | 8.0 $\pm$ 3.0     |
|                   |                | Control Food | 9.2 $\pm$ 4.9  | 9.0 $\pm$ 4.2     | 9.7 $\pm$ 4.1  | 10.0 $\pm$ 4.3 ]* |
|                   | $\Delta$       | Test Food    |                | 0.5 $\pm$ 8.3     | 0.2 $\pm$ 6.2  | -0.9 $\pm$ 6.0    |
|                   |                | Control Food |                | -0.2 $\pm$ 3.2    | 0.8 $\pm$ 2.7  | 0.8 $\pm$ 3.1     |
| Glyco-albumin (%) | Measured Value | Test Food    | 13.5 $\pm$ 1.2 | 13.4 $\pm$ 1.1 §  | 13.6 $\pm$ 1.2 | 13.5 $\pm$ 1.2    |
|                   |                | Control Food | 13.4 $\pm$ 0.9 | 13.1 $\pm$ 0.9 §§ | 13.4 $\pm$ 0.9 | 13.2 $\pm$ 0.8 §  |
|                   | $\Delta$       | Test Food    |                | -0.2 $\pm$ 0.4    | 0.1 $\pm$ 0.4  | 0.0 $\pm$ 0.4     |
|                   |                | Control Food |                | -0.2 $\pm$ 0.4    | 0.0 $\pm$ 0.4  | -0.2 $\pm$ 0.4    |

average  $\pm$  standard deviation,  $\Delta$ : average  $\pm$  standard deviation of change from before intake

\* Regarding insulin, LDH, K after 4 weeks of intake, control food: n = 37, regarding blood glucose and insulin after 8 weeks of intake, control food: n = 36

comparison between groups (unpaired t-test): \*\* p<0.01, \* p<0.05

5 comparison with before intake (Dunnett's multiple comparison test): §§ p<0.01, § p<0.05,

[0071] (Activity Meter)

Regarding the physical activity during the study period, the number of steps, the number of Active steps, and moderate-intensity physical activity time were checked every two weeks, but there were no significant differences observed between the test food group and the control food group.

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[0072] (Subgroup Analysis)

The results of the analysis of the subgroup with a BMI of 23 kg/m<sup>2</sup> or more and less than 25 kg/m<sup>2</sup> before the start of the intake are shown in Table 6. Regarding the visceral fat area of the evaluation items for abdominal fat area, in the analysis of the subgroup with a BMI of 23 kg/m<sup>2</sup> or more and less than 25 kg/m<sup>2</sup> before the start of the intake, the test food group showed a significant lower value compared to the control food group after 12 weeks of the intake. Furthermore, a significant decrease was observed in the change from before the study in the subcutaneous fat area and the total fat area after 12 weeks of the intake.

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Regarding the body measurement items, the test food group showed a significant decrease in the change from before the intake in the weight and BMI compared to the control food group after 12 weeks of the intake. Moreover, regarding the blood test evaluation items, the test food group showed a significant increase in the change from before the intake in the total ketone bodies, acetoacetic acid, and 3-hydroxybutyric acid compared to the control food group after 12 weeks of the intake. Significant increases were observed in HDL-Cho after 4, 8, and 12 weeks of the intake. Regarding TG, a significant lower value and a significant decrease in the change from before the start of the intake were observed after 12 weeks of the intake. Regarding blood glucose levels, a significant higher value was observed before the

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intake and after 4 and 8 weeks of the intake. Regarding insulin, a significant suppression of the increase in the change from before the intake was observed after 12 weeks of the intake.

[0073] [Table 6-1]

Table 6. Subgroup Analysis for Subjects with BMI before intake of 23 kg/m<sup>2</sup> or more and less than 25 kg/m<sup>2</sup>

|                                          |                |              | Before Intake<br>Test Food:<br>n=10<br>Control Food:<br>n=12 | After 4 Weeks<br>of Intake<br>Test Food:<br>n=10<br>Control Food:<br>n=12 | After 8 Weeks<br>of Intake<br>Test Food:<br>n=10<br>Control Food:<br>n=12 | After 12 Weeks<br>of Intake<br>Test Food:<br>n=10<br>Control Food:<br>n=12 |
|------------------------------------------|----------------|--------------|--------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Visceral Fat Area (cm <sup>2</sup> )     | Measured Value | Test Food    | 127.5±23.4                                                   |                                                                           |                                                                           | 115.2±23.3 §                                                               |
|                                          |                | Control Food | 137.8±19.8                                                   |                                                                           |                                                                           | 138.9±25.1 ]*                                                              |
|                                          | Δ              | Test Food    |                                                              |                                                                           |                                                                           | -12.3±16.7                                                                 |
|                                          |                | Control Food |                                                              |                                                                           |                                                                           | 1.0±17.9                                                                   |
| Subcutaneous Fat Area (cm <sup>2</sup> ) | Measured Value | Test Food    | 189.4±44.0                                                   |                                                                           |                                                                           | 179.5±40.6 §                                                               |
|                                          |                | Control Food | 164.4±30.6                                                   |                                                                           |                                                                           | 168.1±32.1                                                                 |
|                                          | Δ              | Test Food    |                                                              |                                                                           |                                                                           | -9.9±12.6                                                                  |
|                                          |                | Control Food |                                                              |                                                                           |                                                                           | 3.7±10.2 ]*                                                                |
| Total Fat Area (cm <sup>2</sup> )        | Measured Value | Test Food    | 317.0±57.5                                                   |                                                                           |                                                                           | 294.7±54.0 §                                                               |
|                                          |                | Control Food | 302.2±38.9                                                   |                                                                           |                                                                           | 306.9±44.2                                                                 |
|                                          | Δ              | Test Food    |                                                              |                                                                           |                                                                           | -22.3±26.5                                                                 |
|                                          |                | Control Food |                                                              |                                                                           |                                                                           | 4.7±22.7 ]*                                                                |
| Weight (kg)                              | Measured Value | Test Food    | 65.7±7.0                                                     | 65.3±7.0                                                                  | 65.8±7.0                                                                  | 64.7±7.1 §§                                                                |
|                                          |                | Control Food | 68.3±7.4                                                     | 68.0±7.3                                                                  | 68.1±7.3                                                                  | 68.3±7.2                                                                   |
|                                          | Δ              | Test Food    |                                                              | -0.3±0.9                                                                  | -0.6±1.0                                                                  | -1.0±1.0                                                                   |
|                                          |                | Control Food |                                                              | -0.3±1.0                                                                  | -0.2±0.7                                                                  | 0.0±1.0 ]*                                                                 |
| BMI (kg/m <sup>2</sup> )                 | Measured Value | Test Food    | 24.2±0.3                                                     | 24.1±0.5                                                                  | 24.0±0.5                                                                  | 23.9±0.5 §§                                                                |
|                                          |                | Control Food | 24.2±0.5                                                     | 24.1±0.6                                                                  | 24.1±0.5                                                                  | 24.2±0.6                                                                   |
|                                          | Δ              | Test Food    |                                                              | -0.1±0.3                                                                  | -0.2±0.3                                                                  | -0.4±0.3                                                                   |
|                                          |                | Control Food |                                                              | -0.1±0.4                                                                  | -0.1±0.3                                                                  | 0.0±0.4 ]*                                                                 |
| Total Ketone Bodies (mmol/L)             | Measured Value | Test Food    | 117±119                                                      | 157±198                                                                   | 135±129                                                                   | 154±156                                                                    |
|                                          |                | Control Food | 125±158                                                      | 121±140                                                                   | 91±98                                                                     | 68±83                                                                      |
|                                          | Δ              | Test Food    |                                                              | 40±91                                                                     | 18±82                                                                     | 37±70                                                                      |
|                                          |                | Control Food |                                                              | -5±66                                                                     | -34±188                                                                   | -57±87 ]*                                                                  |
| Acetoacetic Acid (mmol/L)                | Measured Value | Test Food    | 31±28                                                        | 44±42                                                                     | 43±37                                                                     | 42±37                                                                      |
|                                          |                | Control Food | 32±33                                                        | 37±37                                                                     | 30±26                                                                     | 21±20                                                                      |
|                                          | Δ              | Test Food    |                                                              | 13±24                                                                     | 12±23                                                                     | 11±19 ]*                                                                   |
|                                          |                | Control Food |                                                              |                                                                           |                                                                           |                                                                            |

|                                |                |              |        |         |         |           |
|--------------------------------|----------------|--------------|--------|---------|---------|-----------|
| /L)                            |                | Control Food |        | 5±19    | -2±39   | -11±20    |
| 3-Hydroxybutyric Acid (mmol/L) | Measured Value | Test Food    | 86±92  | 113±158 | 92±93   | 112±119   |
|                                |                | Control Food | 94±126 | 84±103  | 61±73   | 47±64     |
|                                | Δ              | Test Food    |        | 27±72   | 6±61    | 26±53     |
|                                |                | Control Food |        | -10±53  | -32±150 | -47±69 ]* |
| T-Cho (mg/dL)                  | Measured Value | Test Food    | 219±37 | 225±32  | 224±41  | 228±38    |
|                                |                | Control Food | 207±20 | 210±22  | 211±23  | 216±27    |
|                                | Δ              | Test Food    |        | 6±17    | 5±15    | 9±16      |
|                                |                | Control Food |        | 3±15    | 3±17    | 9±22      |

[0074] [Table 6-2]

Table 6 (continued)

|                                 |                |              | Before Intake<br>Test Food:<br>n=10<br>Control Food:<br>n=12 | After 4 Weeks<br>of Intake<br>Test Food:<br>n=10<br>Control Food:<br>n=12 | After 8 Weeks<br>of Intake<br>Test Food:<br>n=10<br>Control Food:<br>n=12 | After 12 Weeks<br>of Intake<br>Test Food:<br>n=10<br>Control Food:<br>n=12 |
|---------------------------------|----------------|--------------|--------------------------------------------------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|----------------------------------------------------------------------------|
| LDL-Cho (direct method) (mg/dL) | Measured Value | Test Food    | 134±24                                                       | 133±26                                                                    | 131±31                                                                    | 137±29                                                                     |
|                                 |                | Control Food | 132±18                                                       | 134±17                                                                    | 132±15                                                                    | 134±24                                                                     |
|                                 | Δ              | Test Food    |                                                              | -1±15                                                                     | -3±14                                                                     | 3±14                                                                       |
|                                 |                | Control Food |                                                              | 2±14                                                                      | 0±16                                                                      | 2±21                                                                       |
| HDL-Cho (mg/dL)                 | Measured Value | Test Food    | 65±18                                                        | 67±17                                                                     | 69±17                                                                     | 72±16 §§                                                                   |
|                                 |                | Control Food | 54±8                                                         | 52±9 ]*                                                                   | 54±10 ]*                                                                  | 55±11 ]**                                                                  |
|                                 | Δ              | Test Food    |                                                              | 2±8                                                                       | 4±6                                                                       | 7±6                                                                        |
|                                 |                | Control Food |                                                              | -2±5                                                                      | 0±5                                                                       | 1±7                                                                        |
| TG (mg/dL)                      | Measured Value | Test Food    | 96±25                                                        | 113±50                                                                    | 100±22                                                                    | 77±15                                                                      |
|                                 |                | Control Food | 124±59                                                       | 141±92                                                                    | 120±62                                                                    | 137±86 ]*                                                                  |
|                                 | Δ              | Test Food    |                                                              | 18±62                                                                     | 5±30                                                                      | -19±27                                                                     |
|                                 |                | Control Food |                                                              | 17±44                                                                     | -4±27                                                                     | 13±40 ]*                                                                   |
| Blood Glucose (mg/dL)           | Measured Value | Test Food    | 98±12                                                        | 98±12                                                                     | 99±9                                                                      | 98±10                                                                      |
|                                 |                | Control Food | 88±8 ]*                                                      | 89±6 ]*                                                                   | 92±5 ]*                                                                   | 92±6 §                                                                     |
|                                 | Δ              | Test Food    |                                                              | 0±7                                                                       | 1±7                                                                       | 0±6                                                                        |
|                                 |                | Control Food |                                                              | 0±5                                                                       | 3±6                                                                       | 4±7                                                                        |
| HbA1c (%)                       | Measured Value | Test Food    | 5.5±0.3                                                      | 5.4±0.3                                                                   | 5.4±0.4 §                                                                 | 5.3±0.3 §§                                                                 |
|                                 |                | Control Food | 5.6±0.3                                                      | 5.5±0.2                                                                   | 5.4±0.2 §§                                                                | 5.4±0.2 §§                                                                 |
|                                 | Δ              | Test Food    |                                                              | -0.1±0.2                                                                  | -0.1±0.2                                                                  | -0.2±0.2                                                                   |

|                   |                |              |          |           |            |             |
|-------------------|----------------|--------------|----------|-----------|------------|-------------|
|                   |                | Control Food |          | -0.1±0.2  | -0.2±0.2   | -0.2±0.2    |
| Insulin (mU/mL)   | Measured Value | Test Food    | 5.7±2.1  | 7.8±4.5   | 7.0±3.3    | 6.5±2.1     |
|                   |                | Control Food | 5.7±2.5  | 7.9±3.2 § | 8.7±3.0 §§ | 8.6±2.9 §§  |
|                   | Δ              | Test Food    |          | 2.1±3.9   | 1.3±2.0    | 0.8±1.4     |
|                   |                | Control Food |          | 2.2±2.1   | 2.9±1.8    | 2.9±1.4 ]** |
| Glyco-albumin (%) | Measured Value | Test Food    | 13.8±1.0 | 13.7±1.1  | 14.0±1.1   | 13.8±1.2    |
|                   |                | Control Food | 13.5±0.9 | 13.3±0.8  | 13.5±0.7   | 13.3±0.6    |
|                   | Δ              | Test Food    |          | -0.2±0.4  | 0.1±0.4    | 0.0±0.4     |
|                   |                | Control Food |          | -0.1±0.5  | 0.0±0.4    | -0.2±0.5    |

average ± standard deviation, Δ: average ± standard deviation of change from before comparison between groups (unpaired t-test): \*\* p<0.01, \* p<0.05

comparison with before intake (Dunnett's multiple comparison test): §§ p<0.01, § p<0.05

[0075] In the analysis of the subgroup with a BMI of 25 kg/m<sup>2</sup> or more and less than 30 kg/m<sup>2</sup> before the start of the intake, and the subgroup by sex, there were no significant differences observed between the test food group and the control food group for any of the evaluation items.

[0076] (Discussion)

This study investigated the effect of a food containing licorice extract on body fat reduction in healthy adult men and women with a BMI of 23 kg/m<sup>2</sup> or more and less than 30 kg/m<sup>2</sup>, and an abdominal visceral fat area of 100 cm<sup>2</sup> or more, by the randomized, placebo-controlled, double-blind, parallel-group comparison trial, in which either the capsule containing 100 mg of licorice extract (test food) or the capsule not containing licorice extract (control food) was ingested once daily for 12 weeks. As a result, in the subgroup with a BMI of 23 kg/m<sup>2</sup> or more and less than 25 kg/m<sup>2</sup>, the test food group showed a significantly lower value for the visceral fat area after 12 weeks of the intake compared to the control food group, and a significant reduction was observed in the change from before the start of the study for the subcutaneous fat area and the total fat area, as well as in the change from before the start of the study for the weight and BMI. Thus, it was revealed that licorice extract exhibits a body fat reducing effect also in humans, and accordingly, a decrease in the weight and BMI was observed.

[0077] Regarding the mechanism of the body fat reducing effect of licorice extract, a study described below confirmed its ability to promote the decomposition of TG in adipocytes in a cell test. Suppression of body fat accumulation and a reduction in adipose cell size have been confirmed also in animal testing, and thus, results supporting the results of the cell test have also been reported. The body fat reducing effect was also observed in this study, in which the test food group showed a significant increase in the total ketone bodies, acetoacetic

acid, and 3-hydroxybutyrate compared to the control food group. Elevation of blood ketone bodies is an indicator of fatty acid consumption via  $\beta$  oxidation, and can also be observed in cases of insulin resistance, but no increase in blood glucose or insulin levels was observed, and therefore, the elevation was considered not due to the exacerbation of insulin resistance, but due to the enhancement of energy consumption that is reported also for other functional foods and the like.

[0078] These results suggest that when TG was decomposed in adipocytes by the intake of licorice extract, free fatty acids were released, and then rapidly consumed as an energy source through  $\beta$  oxidation, and thus, the body fat reducing effect was brought about.

[0079] It was confirmed by the study described below that Glyasperin B contained in the licorice extract accounts for substantially 100% of the contribution to the TG decomposition in adipocytes. Based on these, it was considered that the body fat reducing effect resulting from the intake of licorice extract was achieved by Glyasperin B.

[0080] The safety of the licorice extract was also confirmed in this study. As a result, no adverse events attributable to the intake of licorice extract, including abnormalities in clinical laboratory values, were observed, and it was believed that there are no issues with the safety of long-term intake of licorice extract.

[0081] (Conclusion)

It was revealed that the food containing licorice extract has a body fat reducing effect in healthy adult men and women classified as slightly obese (with a BMI of 23 kg/m<sup>2</sup> or more and less than 25 kg/m<sup>2</sup>, and an abdominal visceral fat area of 100 cm<sup>2</sup> or more). Moreover, one aspect of the mechanism of action was considered to be the decomposition of TG in adipocytes, accompanied by the enhancement of fatty acid consumption ( $\beta$  oxidation).

[0082] <Study 2>

Male C57BL/6J mice of five weeks old were divided into groups of six mice each, and fed, for 8 weeks, "MF (Oriental Yeast Co., Ltd.), high-fat high-sucrose powdered feed (hereinafter abbreviated as HFS, D12079BM, Research Diet), or HFS mixed with licorice extract. After 8 weeks of the intake, weight and visceral fat weight (a total weight of fat surrounding the epididymis, perinephric fat, and mesenteric fat) were measured. The licorice extract was prepared by obtaining, through 50% (v/v) ethanol extraction process, a liquid from a powder resulting from pulverizing the roots and stolons of a licorice plant, drying the liquid using an evaporator and freeze-drying, and finally pulverizing the resultant to obtain the licorice extract. The extract was mixed into the respective feeds at a concentration of 0.3% for feeding. The amount of Glyasperin B contained in the licorice extract, and the weight gain and the visceral fat weight of the mice are shown in Table 7.

[0083] [Table 7]

Table 7. Amount of Glyasperin B in Licorice Extract, and Weight Gain and Visceral Fat

|                | Amount of Glyasperin B<br>in Licorice Extract<br>( $\mu\text{g/g}$ ) | Weight Gain<br>(g)   | Visceral Fat Weight<br>(g) |
|----------------|----------------------------------------------------------------------|----------------------|----------------------------|
| Test Example 1 | 100.0                                                                | 10.38 $\pm$ 0.78     | 2.03 $\pm$ 0.26            |
| Test Example 2 | 122.9                                                                | 9.78 $\pm$ 0.67      | 1.88 $\pm$ 0.16            |
| Test Example 3 | 137.5                                                                | 7.88 $\pm$ 0.38      | 1.48 $\pm$ 0.11            |
| Test Example 4 | 148.1                                                                | 7.09 $\pm$ 0.50 *    | 1.13 $\pm$ 0.14 *          |
| Test Example 5 | 0                                                                    | 10.13 $\pm$ 0.99 vs. | 2.14 $\pm$ 0.34 vs.        |

average  $\pm$  standard deviation, Student's t-test (\*  $p < 0.05$ , \*\*  $p < 0.01$ , vs. Test Example 5)

#### [0084] (Conclusion)

Mice that ingested a licorice extract with a higher content of Glyasperin B showed a slower weight gain and had a lower visceral fat weight. This suggests that Glyasperin B is significantly involved in the body fat and visceral fat reducing effect of licorice extract, which is also involved in the results of Test Example 1.

#### [0085] <Study 3> Preparation of Glyasperin B

A licorice extract aqueous solution was prepared by adding 10 ml of distilled water per g of licorice extract to dissolve it. An operation in which a half volume of ethyl acetate was added to the resultant aqueous solution, mixed, and allowed to separate into two layers, and an ethyl acetate layer was recovered from this state was repeated three times. To the thus obtained ethyl acetate layer, anhydrous sodium sulfate was added, and the resultant was filtered to obtain an organic fraction. The organic fraction was first concentrated using an evaporator, and then subjected to silica gel column chromatography for eluting a fraction with hexane:ethyl acetate = 1:1 (v/v), and this fraction was subjected to repeated purification by high performance liquid chromatography using an ODS column (acetonitrile:water = 60:40, 45:55 (both v/v)), and thus, a compound (Glyasperin B) was obtained. The Glyasperin B (GB) obtained here was used as a test substance in the following study utilizing mouse preadipocytes 3T3-L1.

[0086] The licorice extract was obtained by the following method. The stems, roots, and stolons of a licorice plant were pulverized to obtain a licorice powder. 50 g of the licorice powder was extracted with 500 mL of 50% hydrous ethanol at 20°C, and the thus obtained extract was then dried using freezing and an evaporator to yield a 50% hydrous ethanol extract of licorice.

[0087] The spectral data for the compound are shown below, which exhibited a very good agreement with the spectral data reported in a literature (Zeng L. et al., Heterocycles 34:575-587 (1992)) for Glyasperin B isolated from plants of the genus *Glycyrrhiza*, and there were no inconsistencies with the structural information, and therefore, the compound was identified as Glyasperin B (GB).

[0088] <sup>1</sup>H-NMR (deuterated acetone) ppm: 1.63 (3H, s), 1.74 (3H, s), 3.24 (2H, d, J = 7.0 Hz), 3.90 (3H, s), 4.27 (1H, dd, J = 5.5 and 10.5 Hz), 4.48 (1H, dd, J = 5.0 and 11.5 Hz), 4.62 (1H, t, J = 10.5 Hz), 5.17 (1H, t, J = 7.0 Hz), 6.13 (1H, s), 6.33 (1H, dd, J = 2.0 and 8.5 Hz), 6.45 (1H, d, J = 2.0 Hz), 6.94 (1H, d, J = 8.5 Hz), 8.30 (1H, bs), 8.63 (1H, bs), 12.48 (1H, s)  
 5 <sup>13</sup>C-NMR (deuterated acetone) ppm: 17.6, 21.4, 25.7, 47.2, 56.2, 71.0, 91.3, 103.5, 103.7, 107.6, 109.7, 113.5, 123.3, 131.1, 131.5, 156.8, 158.7, 161.1, 162.7, 165.8, 198.7  
 ESI+-MS m/z: 393.13059 [M+Na]<sup>+</sup> (calculated, C<sub>21</sub>H<sub>22</sub>O<sub>6</sub>Na: 393.13086)

[0089] The Glyasperin B (GB) contained in the licorice extract was quantitatively determined by high performance liquid chromatography described in detail below. The  
 10 results showed that the content of the Glyasperin B (GB) was 132.6 µg per g of licorice extract. The glycyrrhizic acid contained in the licorice extract was 123 mg per g of the licorice extract, and a value (ratio) of Glyasperin B (GB) content/glycyrrhizic acid content was 0.00107.

[0090] (Conditions for Analysis of Glyasperin B (GB))  
 15 Column: TSKgel ODS-100V 5 µm (Tosoh Corporation), 4.6 mm (inner diameter) × 150 mm (length)  
 Column temperature: 40°C  
 Mobile phase: acetonitrile:water = 45:55 (v/v)  
 Flow rate: 1 ml/min  
 20 Detection wavelength: 290 nm  
 GB retention time: 33.2 min

[0091] <Study 4> Examination on Lipolysis Effect of Licorice Extract and Glyasperin B (GB)  
 Mouse preadipocytes 3T3-L1 were cultured for two days in a differentiation-  
 25 inducing medium (0.5 mM Isobutyl-methylxanthine, 1 µM Dexamethasone, and 1 µg/mL Insulin in 10% FBS/DMEM), and then, the medium was replaced with 10% FBS/DMEM containing 1 µg/mL Insulin, and the cells were cultured further for 7 days. The test substances (licorice extract and GB) were added on the ninth day from the start of the differentiation induction, and the resultant cells were subsequently cultured for 19 hours. As  
 30 the licorice extract, the licorice extract used in Study 1 (final concentration: 100 µg/mL, 200 µg/mL, or 300 µg/mL) was used, and as the GB, the GB prepared in Study 1 (final concentration: 13.5 ng/mL, 27 ng/mL, or 40 ng/mL) was used. The test was conducted with 3 samples per group. Glycerol released into the culture medium from the triglycerides accumulated in the cells was quantitatively determined by LaboAssay (TM) Triglyceride  
 35 (FUJIFILM Wako Pure Chemical Corporation). The results are illustrated in Fig. 1. The amount of glycerol was shown as an average ± a standard deviation. Each symbol in Fig. 1

indicates the following: \*\*  $p < 0.01$  vs. untreated (Student's t-test).

[0092] As illustrated in Fig. 1, the amount of glycerol released increased in a GB concentration-dependent manner, which indicates that lipolysis within the cells occurred in a GB concentration-dependent manner, and thus, it was found that GB possesses lipolysis promoting activity in adipocytes. On the other hand, glycerol was released from the adipocytes also by the licorice extract, but when the concentration of the licorice extract was 300  $\mu\text{g/mL}$ , the amount of glycerol released was reduced compared to that released when the concentration was 100  $\mu\text{g/mL}$  or 200  $\mu\text{g/mL}$ . The concentrations of the licorice extract, 100  $\mu\text{g/mL}$ , 200  $\mu\text{g/mL}$ , and 300  $\mu\text{g/mL}$ , respectively correspond to 13.5 ng/mL, 27 ng/mL, and 40 ng/mL in terms of the amount of GB contained. Although the licorice extract exhibited an excellent lipolysis effect, the lipolysis effect decreased when the concentration was higher, which suggests that the licorice extract contains various components, and some of these may inhibit lipolysis. From this, it can be said that in order to obtain a higher lipolysis effect, it is preferable to use a purified product of GB rather than a licorice extract.

[0093] <Study 5> Effect of Licorice Extract on Weight, BMI, Visceral Fat Level (indicator value for visceral fat accumulation)

A subject (a 59-year-old male) ingested the licorice extract used in Study 1 at a dose of 200 mg per day for 100 days. The subject was provided with a recording sheet and instructed to record his weight, BMI, and visceral fat level, measured with a Tanita body composition analyzer, before the start of the intake of the licorice extract (Day 0), on day 30, day 45, day 72, day 83, day 92 of the intake, and a day after the final intake (day 101). Changes in weight, BMI, and visceral fat level from before the start of the intake are illustrated in Fig. 2.

[0094] As illustrated in Fig. 2, the intake of the licorice extract containing GB resulted in a decrease in the visceral fat level first, followed by a subsequent decrease in weight and BMI. Considering also the results of Study 3 described above, it is believed that the GB contained in the licorice extract efficiently decomposed fat in adipocytes, leading to a reduction in body fat, such as visceral fat.

[0095] <Study 6>

Male C57BL/6J mice of five weeks old were divided into groups of six mice each, and fed, for 8 weeks, "MF (Oriental Yeast Co., Ltd.), high-fat high-sucrose powdered feed (hereinafter abbreviated as HFS, D12079BM, Research Diet), or HFS mixed with licorice extract. After 8 weeks of the intake, visceral fat weight (a total weight of fat surrounding the epididymis, perinephric fat, and mesenteric fat) was measured. Licorice extracts (Test Examples 1 to 5) were prepared by obtaining, through 50% (v/v) ethanol extraction process, a liquid from a powder resulting from pulverizing the roots and stolons of a licorice plant,

drying the liquid using an evaporator and freeze-drying, and finally pulverizing the resultant to obtain the licorice extract. Each of the extracts was mixed, for feeding, into the respective feeds in an amount (%) shown in Table 8 below. The human equivalent Glyasperin dosage ( $\mu\text{g/day}$ ) and the visceral fat weight suppression rate (%) in mice are both shown in Table 8.

5 [0096] [Table 8]

|                | Amount of Licorice<br>Extract Mixed in Feed<br>(%) | Human Equivalent<br>Glyasperin B Dosage<br>( $\mu\text{g/day}$ ) | Visceral Fat Weight<br>Suppression Rate<br>(%) |
|----------------|----------------------------------------------------|------------------------------------------------------------------|------------------------------------------------|
| Test Example 1 | 0.1                                                | 9.7                                                              | 24                                             |
| Test Example 2 | 0.2                                                | 19.3                                                             | 40                                             |
| Test Example 3 | 0.3                                                | 29.1                                                             | 43                                             |
| Test Example 4 | 0.2                                                | 31.6                                                             | 45                                             |
| Test Example 5 | 0.3                                                | 47.6                                                             | 47                                             |

[0097] (Conclusion)

As shown in the table above, a significant visceral fat weight suppression effect was observed in mice under a condition of a Glyasperin dosage (intake) corresponding to a human equivalent dosage of  $9.7 \mu\text{g/day}$  to  $47.6 \mu\text{g/day}$ .

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#### Industrial Applicability

[0098] An oral composition of the present invention possesses, by containing Glyasperin B, excellent lipolysis promoting activity and also exerts a remarkable body weight reducing effect. By using the oral composition of the present invention, fat within adipocytes can be efficiently decomposed, and body fat such as subcutaneous fat and visceral fat can be reduced, resulting in remarkable weight reducing effect and BMI improving effect. Furthermore, improving effects on insulin resistance and lowering effects on cholesterol levels can also be obtained. Therefore, the oral composition of the present invention makes it possible to prevent or ameliorate obesity and lifestyle diseases over a long term, and thus, maintenance and promotion of health can be realized. Furthermore, since the content of Glyasperin B in a plant extract is used as an indicator of the efficacy of the plant extract for lipolysis, weight loss, and the like, it becomes possible to efficiently select a plant extract with high efficacy.

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## CLAIMS

- [Claim 1] An oral composition comprising Glyasperin B, for use in one or more of: lipolysis, fat burning, support of fat consumption, BMI improvement, support of BMI  
5 reduction, abdominal fat reduction, visceral fat reduction, body fat reduction, waist circumference reduction, improvement of insulin resistance, and reduction of LDL cholesterol levels.
- [Claim 2] An oral composition comprising an effective amount of Glyasperin B, for use in one or more of: lipolysis, fat burning, support of fat consumption, BMI improvement, support  
10 of BMI reduction, abdominal fat reduction, visceral fat reduction, body fat reduction, waist circumference reduction, improvement of insulin resistance, and reduction of LDL cholesterol levels.
- [Claim 3] The oral composition according to claim 2, wherein the effective amount is 10  $\mu\text{g}$  or more per day for an adult.
- 15 [Claim 4] The oral composition according to claim 1 or 2, for use in an adult having a BMI of 23  $\text{kg}/\text{m}^2$  or more and less than 30  $\text{kg}/\text{m}^2$ .
- [Claim 5] The oral composition according to claim 1 or 2, for use in an adult having a visceral fat area of 100  $\text{cm}^2$  or more.
- [Claim 6] The oral composition according to claim 1 or 2, wherein the oral composition is  
20 ingested at a dosage of 5 to 100  $\mu\text{g}/\text{day}$ , calculated as a total amount of Glyasperin B.
- [Claim 7] A method for determining efficacy of a plant extract for lipolysis and/or weight loss in a living body, wherein the content of Glyasperin B is used as an indicator.
- [Claim 8] The method for determining efficacy of a plant extract for lipolysis and/or weight loss according to claim 7, wherein the plant is licorice.
- 25 [Claim 9] An indicator of a quality of a plant extract, wherein the quality relates to efficacy for lipolysis and/or weight loss in a living body, and the indicator comprises Glyasperin B.
- [Claim 10] The indicator according to claim 9, wherein the plant is licorice.