

DESCRIPTION

USE OF CELL WALL DISRUPTED PRODUCT OF CHLORELLA FOR REDUCING SERUM RESISTIN LEVEL

5 TECHNICAL FIELD

[0001]

The present invention relates to a serum resistin level reducing agent for
reducing a serum resistin level and a food or beverage composition for
reducing a serum resistin level, as well as a method for reducing a serum
10 resistin level and a use for reducing a serum resistin level.

BACKGROUND ART

[0002]

Sugar metabolism abnormalities and blood resistin levels correlate with
15 each other [Scand J Clin Lab Invest. 2007;67(2):215-25. Associations of
resistin with inflammation and insulin resistance in patients with type 2
diabetes mellitus. Mojiminiyi OA, Abdella NA; Metabolism. 2007
May;56(5):693-8. Serum resistin level is associated with insulin sensitivity
in Japanese patients with type 2 diabetes mellitus. Tokuyama Y, Osawa H,
20 Ishizuka T, Onuma H, Matsui K, Egashira T, Makino H, Kanatsuka A], and
resistin has been reported to act on adipocytes to induce inflammation of
the adipocytes and increase cytokines that induce insulin resistance (PLoS
One. 2006 Dec 20;1:e31. Human resistin is a systemic immune-derived
proinflammatory cytokine that targets both leukocytes and adipocytes.
25 Nagaev I, Bokarewa M, Tarkowski A, Smith U).

[0003]

Therefore, there is a demand for a serum resistin level reducing agent and a food or beverage composition for reducing a serum resistin level, that are safe, suitable for oral administration, and capable of effectively reducing a blood resistin level, as well as a method for reducing a serum resistin level and a use for reducing a serum resistin level.

PRIOR ART DOCUMENTS

Non-patent Documents

[0004]

Non-patent Document 1: Scand J Clin Lab Invest. 2007;67(2):215-25. Associations of resistin with inflammation and insulin resistance in patients with type 2 diabetes mellitus. Mojiminiyi OA, Abdella NA.

Non-patent Document 2: Metabolism. 2007 May;56(5):693-8. Serum resistin level is associated with insulin sensitivity in Japanese patients with type 2 diabetes mellitus. Tokuyama Y, Osawa H, Ishizuka T, Onuma H, Matsui K, Egashira T, Makino H, Kanatsuka A.

Non-patent Document 3: PLoS One. 2006 Dec 20;1:e31. Human resistin is a systemic immune-derived proinflammatory cytokine targeting both leukocytes and adipocytes. Nagaev I, Bokarewa M, Tarkowski A, Smith U.

SUMMARY OF THE INVENTION

PROBLEMS TO BE SOLVED BY THE INVENTION

[0005]

The present invention has been developed in view of the above, and is

intended to provide a serum resistin level reducing agent and a food or beverage composition for reducing a serum resistin level, that can be safely and conveniently administered over a long period, as well as a method for reducing a serum resistin level and a use for reducing a serum resistin level
5 that can be safely and conveniently performed over a long period.

MEANS OF SOLVING THE PROBLEMS

[0006]

To accomplish the above-described object, the serum resistin level
10 reducing agent of the present invention comprises a cell wall disrupted product of a chlorella.

[0007]

The food or beverage composition for reducing a serum resistin level of the present invention comprises a cell wall disrupted product of a chlorella.

15 [0008]

The method of the present invention is a method for lowering a serum resistin level by applying a cell wall disrupted product of a chlorella.

[0009]

The use of the present invention is a use of a cell wall disrupted product of
20 a chlorella for lowering a serum resistin level.

[0010]

By applying the serum resistin level reducing agent or food or beverage composition for reducing a serum resistin level of the present invention, or by using the method for reducing a serum resistin level or cell wall
25 disrupted product of a chlorella of the present invention, a serum resistin

level can be effectively reduced.

[0011]

The serum resistin level reducing agent of the present invention may be such that it lowers the expression of the resistin gene.

5 [0012]

The food or beverage composition for reducing a serum resistin level of the present invention may be such that it lowers the expression of the resistin gene.

[0013]

10 The method for reducing a serum resistin level of the present invention may be such that it reduces the expression of the resistin gene.

[0014]

The use of a cell wall disrupted product of a chlorella of the present invention may be for reducing the expression of the resistin gene.

15 [0015]

The above-described serum resistin level reducing agent of the present invention may be such that it increases the expression of the CTBP1 gene.

[0016]

The above-described food or beverage composition for reducing a serum
20 resistin level of the present invention may be such that it increases the expression of the CTBP1 gene.

[0017]

The method for reducing a serum resistin level of the present invention may be such that it increases the expression of the CTBP1 gene.

25 [0018]

The use of a cell wall disrupted product of a chlorella of the present invention may be such that it increases the expression of the CTBP1 gene.

[0019]

The CTBP1 gene negatively controls the expression of the resistin gene.

5 [0020]

The above-described serum resistin level reducing agent of the present invention may be such that it increases the expression of the CTBP2 gene.

[0021]

10 The above-described food or beverage composition for reducing a serum resistin level of the present invention may be such that it increases the expression of the CTBP2 gene.

[0022]

The method for reducing a serum resistin level of the present invention may be such that it increases the expression of the CTBP2 gene.

15 [0023]

The use of a cell wall disrupted product of a chlorella of the present invention may be such that it increases the expression of the CTBP2 gene.

[0024]

The CTBP2gene negatively controls the expression of the resistin gene.

20 [0025]

The above-described serum resistin level reducing agent of the present invention may be such that it increases the expression of the RXR-alpha gene.

[0026]

25 The above-described food or beverage composition for reducing a serum

resistin level of the present invention may be such that it increases the expression of the RXR-alpha gene.

[0027]

The method for reducing a serum resistin level of the present invention
5 may be such that it increases the expression of the RXR-alpha gene.

[0028]

The use of a cell wall disrupted product of a chlorella of the present invention may be such that it increases the expression of the RXR-alpha gene.

10 [0029]

The RXR-alpha gene negatively controls the expression of the resistin gene.

[0030]

The chlorella in the serum resistin level reducing agent of the present invention may be *Chlorella pyrenoidosa*.

15 [0031]

The chlorella in the food or beverage composition for reducing a serum resistin level of the present invention may be *Chlorella pyrenoidosa*.

[0032]

The chlorella in the method for reducing a serum resistin level of the
20 present invention may be *Chlorella pyrenoidosa*.

[0033]

The chlorella in the use of a cell wall disrupted product of a chlorella of the present invention may be *Chlorella pyrenoidosa*.

[0034]

25 The serum resistin level reducing agent of the present invention may be an

agent for oral administration.

[0035]

In the method for reducing a serum resistin level of the present invention,
a cell wall disrupted product of a chlorella may be applied by oral
5 administration.

[0036]

In the use of a cell wall disrupted product of a chlorella of the present
invention, a cell wall disrupted product of a chlorella may be used for oral
administration.

10 [0037]

"A serum resistin level reducing agent" in the present invention may be
reworded to "a substance for reducing a serum resistin level."

[0038]

A method for reducing a serum resistin level by applying the serum resistin
15 level reducing agent or food or beverage composition for reducing a serum
resistin level of the present invention may be reworded to a method for
reducing a serum resistin level by applying a cell wall disrupted product of
a chlorella.

20 EFFECT OF THE INVENTION

[0039]

The serum resistin level reducing agent and food or beverage composition
for reducing a serum resistin level of the present invention can be safely
and conveniently administered over a long period to effectively reduce a
25 serum resistin level. The method for reducing a serum resistin level and

use for reducing a serum resistin level of the present invention can be safely and conveniently performed over a long period to effectively reduce a serum resistin level.

5 BRIEF DESCRIPTION OF DRAWINGS

[0040]

[Figure 1] Results of measurement of serum resistin levels obtained before the test period (0w) and after the end of the test period (12w).

[Figure 2] Results of measurement of two probes for resistin mRNA, i.e.,
10 A_33_P3350863 and A_23_P119222, obtained before the test period (0w) and after the end of the test period (12w).

[Figure 3] Results of measurement of the expression of the CTBP1 gene, the CTBP2gene, and the RXR-alpha gene, which are genes that negatively control the expression of the resistin gene, obtained before the test period
15 (0w) and after the end of the test period (12w).

MODES FOR CARRYING OUT THE INVENTION

[0041]

The chlorella in the present invention refers to a single-cell green alga
20 belonging to the genus Chlorella, and is exemplified by Chlorella pyrenoidosa, Chlorella ellipsoidea, Chlorella vulgaris, and Chlorella regularis. The most suitable chlorella for the present invention is Chlorella pyrenoidosa.

[0042]

25 The cell wall disrupted product of a chlorella in the serum resistin level

reducing agent and food or beverage composition for reducing a serum resistin level of the present invention, as well as the method for reducing a serum resistin level and use thereof for reducing a serum resistin level of the present invention can, for example, be obtained as described below.

5 Specifically, first, an aqueous suspension of chlorella powder having a chlorella concentration of 10 to 25% by weight is adjusted to a temperature of not more than 10 degrees Celsius. Subsequently, this suspension is fed to a continuous wet pulverizer as described below, and pulverized so that the temperature of the slurry just after disruption will not be more than 40
10 degrees Celsius. Subsequently, the chlorella slurry thus obtained is immediately cooled to a temperature of not more than 10 degrees Celsius, whereby a chlorella with a disrupted cell wall can be obtained without resulting in quality deterioration.

[0043]

15 The above-described continuous wet pulverizer has a large number of glass beads 0.5 to 1.5 mm in diameter enclosed in a closed cylinder with a cooling jacket. The volume of the glass beads is 80 to 85% of the capacity of the closed cylinder, and the material in the influent liquid is ground by mixing and rotating the glass beads with the influent liquid.

20 [0044]

The chlorella having a cell wall thus disrupted may be used as it is, and may also be used after being subjected to an appropriate treatment, for example, vacuum drying followed by milling.

[0045]

The cell wall disrupted product of a chlorella in the serum resistin level reducing agent and food or beverage composition for reducing a serum resistin level of the present invention, as well as the method for reducing a serum resistin level and the use for reducing a serum resistin level of the present invention is suitable for oral administration. The dose of a cell wall disrupted product of *Chlorella pyrenoidosa* (or a cell wall disrupted product of any other chlorella) for an adult human is appropriately about 1 to 35 g per day. However, because cell wall disrupted product of *Chlorella pyrenoidosa* and the like are not toxic to the human body, this dose range is not construed as limiting the present invention.

[0046]

Although the dosage form for oral administration in the serum resistin level reducing agent, method for reducing a serum resistin level, and use thereof for reducing a serum resistin level of the present invention is not subject to limitations, the dosage form can be prepared as, for example, powders, tablets, hard capsules, and soft capsules.

[0047]

To form a wide variety of dosage forms, various excipients, binders, disintegrating agents, lubricants, coating agents, coloring agents, taste-masking agents, odor-masking agents, plasticizers, and the like may be used as appropriate.

[0048]

Examples of excipients include saccharides (lactose, sucrose, glucose, mannitol), starches (potato, wheat, corn), inorganic substances (calcium

carbonate, calcium sulfate, sodium hydrogen carbonate, sodium chloride), crystalline cellulose, powdered plants (powdered glycyrrhiza, powdered gentian), and the like.

[0049]

5 Examples of binders include starch glue liquid, gum arabic, gelatin, sodium alginate, methyl cellulose (MC), ethyl cellulose (EC), polyvinylpyrrolidone (PVP), polyvinyl alcohol (PVA), hydroxypropyl cellulose (HPC), carboxymethyl cellulose (CMC), and the like.

[0050]

10 Examples of disintegrating agents include starches, agar, powdered gelatin, crystalline cellulose, CMC-Na, CMC-Ca, calcium carbonate, sodium hydrogen carbonate, sodium alginate, and the like.

[0051]

Examples of lubricants include magnesium stearate, talc, hydrogenated
15 vegetable oils, macrogol, silicone oil, and the like.

[0052]

Examples of coating agents include sugar coatings (sucrose, HPC, shellac), gelatin coatings (gelatin, glycerin, sorbitol), film coatings [hydroxypropylmethyl cellulose (HPMC), EC, HPC, PVP], enteric coatings
20 [hydroxypropylmethyl cellulose phthalate (HPMCP), cellulose acetate phthalate (CAP)], and the like.

[0053]

Examples of coloring agents include water-soluble food dyes, lake pigments, and the like. Examples of taste-masking agents include lactose,
25 sucrose, glucose, mannitol, and the like. Examples of odor-masking agents

include aromatic essential oils, light screens (titanium oxide), and the like. Examples of plasticizers include phthalic acid esters, vegetable oils, polyethylene glycol, and the like.

[0054]

5 The food or beverage composition for reducing a serum resistin level of the present invention can, for example, be prepared in the form of a food or beverage composition for nutritional food, nutritional supplement, liquid beverage, or the like, and that contains a cell wall disrupted product of a chlorella, and may contain various components that do not interfere with
10 the effect of the present invention.

[0055]

Such a serum resistin level reducing agent and food or beverage composition for reducing a serum resistin level can be safely and conveniently administered over a long period to effectively reduce a serum
15 resistin level. Such a method for reducing a serum resistin level and use for reducing a serum resistin level can be safely and conveniently performed over a long period to effectively reduce a serum resistin level.

EXAMPLES

20 [0056]

A test was performed to determine the effects of oral administration of a cell wall disrupted product of a chlorella on a serum resistin level, the expression of the resistin gene, and the expression of genes that negatively control the expression of the resistin gene.

25 [0057]

1. Production of test substance

[0058]

A dry powder of *Chlorella pyrenoidosa* with a disrupted cell wall as a test substance (hereinafter also simply referred to as "chlorella") was produced as described below.

[0059]

An aqueous suspension of *Chlorella pyrenoidosa* powder having a *Chlorella pyrenoidosa* concentration of 10 to 25% by weight, previously adjusted to a temperature of not more than 10 degrees Celsius, was fed to a continuous wet pulverizer (product name: Dyno-Mill Model KD, manufactured by WAB, Inc.) that had a large number of glass beads 0.5 to 1.5 mm in diameter enclosed in a closed cylinder with a cooling jacket, in a volume 80 to 85% of the capacity of the closed cylinder, and that ground the material in the influent liquid by mixing and rotating the glass beads with the influent liquid, and pulverized so that the temperature of the slurry just after disruption would not be more than 40 degrees Celsius. Subsequently, the *Chlorella pyrenoidosa* slurry thus obtained was immediately cooled to a temperature of not more than 10 degrees Celsius, vacuum dried, and then milled, whereby dry powdered *Chlorella pyrenoidosa* with a disrupted cell wall (a chlorella powder with a disrupted cell wall, produced by SUN CHLORELLA Corporation), which served as the test substance, was obtained.

[0060]

2. Test method

[0061]

The test substance, 3.83 g (7.66 g per day), was orally administered to each of 28 subjects (42- to 49-year-old males with an HbA1c level of not less than 5.3% and less than 6.1%) before breakfast and before dinner every day for 12 weeks (test period).

5 [0062] Peripheral blood was drawn before oral administration of the test substance on the first day of the test period (0w) and before breakfast on the day after the end of the test period (12w), and used as samples for measurement of serum resistin levels, the expression of the resistin gene, and the expression of genes that negatively control the expression of the
10 resistin gene. Taking any food or beverage other than water was prohibited between 21:00 o'clock of the day before the first day of the test period and the time of blood drawing on the first day.

[0063]

3. Measuring methods

15 (1) Resistin levels

[0064]

Each sample was analyzed for resistin by ELISA using a human resistin ELISA kit (product name: Human Resistin ELISA, manufactured by BioVendor Laboratorni medicina a.s.), and the absorbance from each well
20 was measured using a microplate reader with a main wavelength of 450 nm and a secondary wavelength of 630 nm to determine the resistin level.

(2) Gene expression

[0065]

Total RNA was extracted from each sample in a vacuum blood drawing tube
25 for RNA isolation (product name: PAXgene Blood RNA Tube, manufactured

by BD) using an RNA extraction kit (product name: PAXgene Blood miRNA Kit, manufactured by QIAGEN).

[0066]

The yield of the extracted total RNA was determined using a spectrophotometer (product name: NanoDrop 1000, manufactured by Thermo Scientific), and the degree of degradation was determined using an analyzer (product name: Agilent 2100 Bioanalyzer, manufactured by Agilent Technologies).

[0067]

Microarray analysis was performed using a gene expression microarray (product name: SurePrint G3 Human GE 8x60K Microarray, manufactured by Agilent Technologies). Labeled cRNA was prepared from 100 ng of the total RNA using a gene expression microarray labeling kit (product name: Low Input Quick Amp Labeling Kit, manufactured by Agilent Technologies) in accordance with the standard protocol thereof. Labeling was achieved by the one-color method using Cyanine 3.

[0068]

Microarray scans were performed using a microarray scanner (product name: G2505C DNA microarray scanner, manufactured by Agilent Technologies), and digitization was performed using microarray image digitizing software (product name: Feature Extraction Software ver.10.7.3.1, manufactured by Agilent Technologies).

[0069]

4. Results

[0070]

Results of measurement of serum resistin levels, the expression of the resistin gene, and the expression of genes that negatively control the expression of the resistin gene obtained before the test period (0w) and after the end of the test period (12w) are as follows:

(1) Serum resistin levels

[0071]

The results of measurement of serum resistin levels before the test period (0w) and after the end of the test period (12w) are shown in Figure 1; the serum resistin level decreased significantly with oral administration of the test substance. The mean serum resistin level in the subjects was 5.47 ng/mL before the test period (0w) and 5.02 ng/mL after the end of the test period (12w).

(2) Expression of the resistin gene

[0072]

The results of measurement with two probes for resistin mRNA, i.e., A_33_P3350863 and A_23_P119222, before the test period (0w) and after the end of the test period (12w) are shown in Figure 2; the expression of the resistin gene was significantly suppressed with oral administration of the test substance.

(3) Expression of genes that negatively control the expression of the resistin gene

[0073]

The results of measurement of the expression of the CTBP1 gene, CTBP2 gene, and RXR-alpha gene, which negatively control the expression of the resistin gene, before the test period (0w) and after the end of the test

period (12w) are shown in Figure 3; the expression of each of the aforementioned genes that negatively control the expression of the resistin gene was significantly increased with oral administration of the test substance.

CLAIMS

[Claim 1] A serum resistin level reducing agent comprising a cell wall disrupted product of a chlorella.

[Claim 2] The serum resistin level reducing agent according to Claim 1
5 wherein the agent reduces the expression of the resistin gene.

[Claim 3] The serum resistin level reducing agent according to Claim 1 or 2 wherein the agent increases the expression of the CTBP1 gene.

[Claim 4] The serum resistin level reducing agent according to Claim 1 or 2 wherein the agent increases the expression of the CTBP2 gene.

10 [Claim 5] The serum resistin level reducing agent according to Claim 1 or 2 wherein the agent increases the expression of the RXR-alpha gene.

[Claim 6] The serum resistin level reducing agent according to any one of Claims 1 to 5 wherein the chlorella is *Chlorella pyrenoidosa*.

[Claim 7] The serum resistin level reducing agent according to any one of
15 Claims 1 to 6 wherein the agent is an agent for oral administration.

[Claim 8] A food or beverage composition for reducing a serum resistin level wherein the composition comprises a cell wall disrupted product of a chlorella.

[Claim 9] The food or beverage composition for reducing a serum resistin
20 level according to Claim 8 wherein the composition reduces the expression of the resistin gene.

[Claim 10] The food or beverage composition for reducing a serum resistin level according to Claim 8 or 9 wherein the composition increases the expression of the CTBP1 gene.

25 [Claim 11] The food or beverage composition for reducing a serum resistin

level according to Claim 8 or 9 wherein the composition increases the expression of the CTBP2 gene.

[Claim 12] The food or beverage composition for reducing a serum resistin level according to Claim 8 or 9 wherein the composition increases the
5 expression of the RXR-alpha gene.

[Claim 13] The food or beverage composition for reducing a serum resistin level according to one of Claims 8 to 12 wherein the chlorella is *Chlorella pyrenoidosa*.

[Claim 14] A method of reducing a serum resistin level by applying a cell
10 wall disrupted product of a chlorella.

[Claim 15] The method according to Claim 14 wherein the method reduces the expression of the resistin gene.

[Claim 16] The method according to Claim 14 or 15 wherein the method increases the expression of the CTBP1 gene.

15 [Claim 17] The method according to Claim 14 or 15 wherein the method increases the expression of the CTBP2 gene.

[Claim 18] The method according to Claim 14 or 15 wherein the method increases the expression of the RXR-alpha gene.

[Claim 19] The method according to one of Claims 14 to 18 wherein the
20 chlorella is *Chlorella pyrenoidosa*.

[Claim 20] The method according to one of Claims 14 to 20 wherein the method comprises applying a cell wall disrupted product of a chlorella by oral administration.

[Claim 21] A use of a cell wall disrupted product of a chlorella for
25 reducing a serum resistin level.

[Claim 22] The use according to Claim 21 wherein the use reduces the expression of the resistin gene.

[Claim 23] The use according to Claim 21 or 22 wherein the use increases the expression of the CTBP1 gene.

5 [Claim 24] The use according to Claim 21 or 22 wherein the use increases the expression of the CTBP2 gene.

[Claim 25] The use according to Claim 21 or 22 wherein the use increases the expression of the RXR-alpha gene.

10 [Claim 26] The use according to one of Claims 21 to 25 wherein the chlorella is *Chlorella pyrenoidosa*.

[Claim 27] The use according to one of Claims 21 to 26 wherein the use comprises oral administration of a cell wall disrupted product of a chlorella.