

DESCRIPTION

TITLE OF INVENTION: COMPOSITION FOR PROMOTING CHONDROITIN
SULFATE SYNTHESIS

5

TECHNICAL FIELD

[0001]

The present invention relates to a composition for
promoting chondroitin sulfate synthesis. The present
10 invention also relates to, for example, a method of
promoting chondroitin sulfate synthesis.

BACKGROUND ART

[0002]

15 Chondroitin sulfate is a type of acidic
mucopolysaccharides. In animals, chondroitin sulfate
exists as a proteoglycan covalently bound to a protein that
serves as a core, usually called a core protein.
Chondroitin sulfate is present in many animal tissues
20 including connective tissues such as cartilage and skin,
and brain tissues. It is abundantly present particularly
in the extracellular matrix of cartilage.

[0003]

Cartilage is a connective tissue consisting of
25 cartilage cells and a matrix surrounding the cells. In
animals such as human, cartilage makes up joints, skeleton,
and the like. For example, articular cartilage thinly
covers joint bone surfaces and has a function to keep the
joint motion fluid. A decrease in articular cartilage due
30 to aging or the like causes joint pain and the like.
Patent Literature 1 discloses a composition containing
oleuropein and/or hydroxytyrosol and also contains
quercetin for use in prevention or treatment of cartilage
destruction.

35

CITATION LIST

- Patent Literature

[0004]

Patent Literature 1: JP 2016-520603 A

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SUMMARY OF INVENTION

- Technical Problem

[0005]

10 The composition disclosed in Patent Literature 1 is
for prevention or treatment (suppression or reduction) of
cartilage destruction. However, once cartilage is reduced
due to aging or the like, the cartilage that has been
reduced cannot be increased by preventing cartilage
destruction. Patent Literature 1 nowhere examines a
15 substance effective in promoting cartilage synthesis. For
example, a component that can promote cartilage matrix
components such as chondroitin sulfate contributes to
promoting cartilage synthesis, and is useful for increasing
cartilage.

20 [0006]

The present invention aims to provide a composition
for promoting chondroitin sulfate synthesis, which promotes
chondroitin sulfate synthesis. The present invention also
aims to provide a method of promoting chondroitin sulfate
25 synthesis.

- Solution to Problem

[0007]

30 As a result of extensive studies to solve the above
issue, the present inventors found that quercetin or a
glycoside thereof has an action that promotes expression of
a glycosyltransferase involved in chondroitin sulfate
synthesis and is useful for increasing the amount of
chondroitin sulfate, maintaining cartilage function, and
35 the like.

[0008]

Specifically, the present invention relates to a composition for promoting chondroitin sulfate synthesis, a method of promoting chondroitin sulfate synthesis, and the like described below, but the present invention is not limited thereto.

(1) A composition for promoting chondroitin sulfate synthesis, the composition containing quercetin or a glycoside thereof as an active ingredient.

(2) The composition for promoting chondroitin sulfate synthesis according to (1) above, wherein the composition promotes expression of a glycosyltransferase involved in chondroitin sulfate synthesis.

(3) The composition for promoting chondroitin sulfate synthesis according to (2) above, wherein the glycosyltransferase involved in chondroitin sulfate synthesis is at least one selected from the group consisting of chondroitin sulfate N-acetylgalactosaminyltransferase 1, chondroitin sulfate N-acetylgalactosaminyltransferase 2, xylosyltransferase 2, β -1,3-galactosyltransferase 1, chondroitin sulfate synthase 1, and chondroitin polymerizing factor 2.

(4) The composition for promoting chondroitin sulfate synthesis according to (2) or (3) above, wherein the glycosyltransferase involved in chondroitin sulfate synthesis includes at least one of chondroitin sulfate N-acetylgalactosaminyltransferase 1 or chondroitin sulfate N-acetylgalactosaminyltransferase 2.

(5) The composition for promoting chondroitin sulfate synthesis according to any one of (1) to (4) above, wherein the composition is a food or beverage, a cosmetic product, or a quasi-pharmaceutical product.

(6) A method of promoting chondroitin sulfate synthesis, the method including administering quercetin or a glycoside thereof.

(7) The method according to (6) above, wherein the method promotes chondroitin sulfate synthesis by promoting expression of a glycosyltransferase involved in chondroitin sulfate synthesis.

5 (8) Use of quercetin or a glycoside thereof for promoting chondroitin sulfate synthesis.

(9) The use according to (8) above for promoting chondroitin sulfate synthesis by promoting expression of a glycosyltransferase involved in chondroitin sulfate
10 synthesis.

- Advantageous Effects of Invention

[0009]

The present invention provides a composition for
15 promoting chondroitin sulfate synthesis, which promotes chondroitin sulfate synthesis. The present invention provides a method of promoting chondroitin sulfate synthesis.

20 BRIEF DESCRIPTION OF DRAWINGS

[0010]

Fig. 1 is a graph showing the amount of chondroitin sulfate in HCH to which quercetin (5 μ M) was added.

Fig. 2 is a graph showing the expression levels of
25 glycosyltransferases involved in chondroitin sulfate synthesis and the expression level of Ext1 gene in a knee articular cartilage region of mice administered with quercetin glycoside (QG) (p: Csgalnact1 gene; q: Csgalnact2 gene; r: Chpf gene; s: Xylt2 gene; t: Ext1 gene).

30 Fig. 3 is a graph showing the amount of chondroitin sulfate in the femoral joint of mice administered with QG.

Fig. 4 is a graph showing the area of knee articular cartilage of mice administered with QG. The relative area in Fig. 4 indicates relative values of the area of the knee
35 articular cartilage in a QG administration group, when the

area of the knee articular cartilage in a control group is taken as 1.

Fig. 5A is a graph showing the expression levels of Csgalnact1 gene in cells to which quercetin was added. Fig. 5B is a graph showing the expression levels of Csgalnact2 gene in cells to which quercetin was added. Fig. 5C is a graph showing the expression levels of Xylt2 gene in cells to which quercetin was added. Fig. 5D is a graph showing the expression levels of B3galt1 gene in cells to which quercetin was added.

Fig. 6A is a graph showing the expression levels of Chsy1 gene in cells to which quercetin was added. Fig. 6B is a graph showing the expression levels of Chpf2 gene in cells to which quercetin was added. Fig. 6C is a graph showing the expression levels of Ext1 gene in cells to which quercetin was added. Fig. 6D is a graph showing the expression levels of Ext2 gene in cells to which quercetin was added.

Fig. 7 is a graph showing the amount of chondroitin sulfate in OSCV2 cells to which quercetin (5 μ M) was added.

Fig. 8 is a view showing names of enzymes involved in synthetic pathways of chondroitin sulfate (CS) and heparan sulfate (HS).

DESCRIPTION OF EMBODIMENTS

[0011]

The composition for promoting chondroitin sulfate synthesis of the present invention contains quercetin or a glycoside thereof as an active ingredient. Hereinafter, the composition for promoting chondroitin sulfate synthesis of the present invention is also simply referred to as "the composition of the present invention".

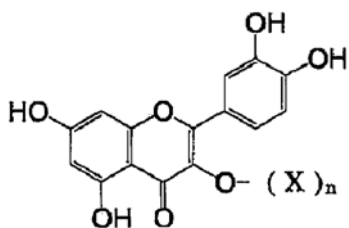
[0012]

As used herein, the term "quercetin" refers to quercetin that is a compound belonging to the flavonol

class which is one class of polyphenols. In the present invention, the term "quercetin glycoside" refers to a glycoside of the quercetin. Specifically, it is a collective term for a series of compounds in which one or more monosaccharides are glycosidically linked to a hydroxy group at position 3 of quercetin. A quercetin glycoside is a compound represented by the following formula. In the following formula, (X)_n represents a glycan. X represents a monosaccharide, and n is an integer of 1 or more. The quercetin glycoside may include one compound or two or more compounds.

[0013]

[Chem. 1]



[0014]

Examples of the monosaccharide of the glycan, which is glycosidically linked to quercetin and represented by X, include glucose, rhamnose, galactose, and glucuronic acid. Glucose and rhamnose are preferred. In addition, n is not limited as long as it is 1 or more, but it is preferably 1 to 16, more preferably 1 to 8. When n is 2 or more, the X moiety may contain one type of monosaccharide or multiple types of monosaccharides. In other words, when n is 2 or more, (X)_n may be a glycan containing one type of monosaccharide or a glycan containing multiple types of monosaccharides. The quercetin glycoside in the present invention includes a quercetin glycoside obtained by treating an existing quercetin glycoside with an enzyme or the like for transglycosylation. Specific examples of the quercetin glycoside as used herein include rutin, enzyme-

treated rutin, quercitrin, and isoquercitrin. In the present invention, the quercetin glycoside is particularly preferably an enzyme-treated product of rutin. Preferred examples of the enzyme-treated product of rutin include
5 isoquercitrin obtained by treating rutin with an enzyme to remove a rhamnose glycan, a product in which a glycan containing 1 to 7 glucose units are bound to isoquercitrin obtained by treating with a glycosyltransferase, and a product mainly containing a mixture thereof. In one
10 embodiment, for example, a preferred quercetin glycoside is a compound in which 1 to 8 glucose units are glycosidically linked to the hydroxy group at position 3 of quercetin (e.g., isoquercitrin or a glycoside in which 1 to 7 glucose units are bound to isoquercitrin).

15 Ingested quercetin glycoside is converted into aglycon (quercetin) by monosaccharide breakdown in the intestinal tract, and is then absorbed into the body.
[0015]

In the present invention, quercetin or a glycoside
20 thereof may be obtained from any source and by any process. Examples of plants known to contain a large amount of quercetin or a glycoside thereof include buckwheat, Japanese pagoda trees, caper, apples, tea, onions, grapes, broccoli, nalta jute, raspberries, cowberries, cranberries,
25 prickly pears, leaf vegetables, and citrus fruits. Quercetin or a glycoside thereof can be obtained from these plants.

Synthetic quercetin or a glycoside thereof can also be used. The composition of the present invention may
30 contain a raw material derived from a plant, such as a plant extract containing quercetin or a glycoside thereof, as long as the effect of the present invention is achieved. Purified or isolated quercetin or a glycoside thereof may also be used.

35 [0016]

Quercetin or a glycoside thereof is present in natural products and food and beverages. It is a compound that has a history of consumption. Thus, daily ingestion of quercetin or a glycoside thereof, for example, is less likely to cause problems in terms of safety. The present invention can provide a composition for promoting chondroitin sulfate synthesis, which contains a highly safe substance as an active ingredient.

[0017]

Chondroitin sulfate has a structure in which sulfuric acid is bound to a glycan having a repeating structure of a disaccharide unit in which D-glucuronic acid is bound to N-acetyl-D-galactosamine. The glycan of chondroitin sulfate is synthesized by glycosyltransferase.

Fig. 8 is a view showing names of enzymes involved in synthetic pathways of chondroitin sulfate (CS) and heparan sulfate (HS) (reference: The Journal of Biochemistry, 87(6): 744-748 (2015)).

In cartilage or the like, a chondroitin sulfate (CS) chain is added to a core protein of proteoglycan as follows. First, xylose (Xyl), two galactose (Gal) molecules, and D-glucuronic acid (GlcA) are sequentially transferred to the serine residue (Ser) in the chondroitin sulfate binding sequence in the core protein, whereby a Xyl-Gal-Gal-GlcA glycan is synthesized. Xyl is transferred to the serine residue by xylosyltransferase (XylT). Gal is transferred to Xyl by β -1,4-galactosyltransferase (B4GalT). The second Gal is transferred to the first Gal by β -1,3-galactosyltransferase (B3GalT). GlcA is transferred to Gal by β -1,3-glucuronyl transferase (B3GaT). Next, N-acetyl-D-galactosamine (GalNAc) is transferred to GlcA at the non-reducing end (the side that extends). GalNAc is transferred by chondroitin sulfate N-acetylgalactosaminyltransferase 1 (CSGalNacT1) or chondroitin sulfate N-acetylgalactosaminyltransferase 2

(CSGalNacT2). Subsequently, GlcA and GalNac are sequentially and alternately transferred to GalNac at the non-reducing end, thus extending the glycan. The glycan extension process proceeds by CSGalNacT1, CSGalNacT2, chondroitin polymerizing factor 2 (Chpf2), and chondroitin sulfate synthases 1, 2, and 3 (ChSy1, ChSy2, and ChSy3). CSGalNacT1 and CSGalNacT2 each have a transference activity to transfer GalNac to GlcA. Chpf2 has a transglycosylation activity to transfer GlcA to GalNac. ChSy1, ChSy2, and ChSy3 each exhibit a transglycosylation activity to transfer GalNac to GlcA and a transglycosylation activity to transfer GlcA to GalNac. Promoting expression of a glycosyltransferase involved in chondroitin sulfate synthesis can promote chondroitin sulfate synthesis.

[0018]

As shown in Fig. 8, in heparan sulfate (HS) chain addition, GalNac is transferred by Exostosin1 (Ext1), Exostosin2 (Ext2), Ext-like 1 (Extl1), Ext-like 2 (Extl2), and Ext-like 3(Extl3) to the non-reducing end of the Xyl-Gal-Gal-GlcA glycan added to the serine residue of the core protein. Subsequently, GlcA and GalNac are sequentially and alternately transferred to GalNac at the non-reducing end, thus extending the glycan.

[0019]

As described in Examples described later, when cells were cultured in the presence of quercetin, the expression levels of genes (glycosyltransferase genes) encoding glycosyltransferases involved in chondroitin sulfate synthesis such as a gene (Csgalnact1) encoding CSGalNacT1 and a gene (Csgalnact2) encoding CSGalNacT2 were increased, compared to when cultured in the absence of quercetin. When cells such as chondrocytes were cultured in the presence of quercetin, the amount of chondroitin sulfate was significantly increased, compared to a control group.

An animal fed with quercetin glycoside showed a significant

increase in the expression levels of genes such as Csgalnact1 and Csgalnact2 encoding glycosyltransferases involved in chondroitin sulfate synthesis in the knee joint, and thus showed a significant increase in the amount of chondroitin sulfate in the joint. Further, an animal fed with a quercetin glycoside showed an increase in the area of articular cartilage.

[0020]

Thus, quercetin or a glycoside thereof has an action that promotes chondroitin sulfate synthesis and an action that promotes expression of a glycosyltransferase involved in chondroitin sulfate synthesis. Quercetin or a glycoside thereof can be used as an active ingredient for promoting chondroitin sulfate synthesis and for promoting expression of a glycosyltransferase involved in chondroitin sulfate synthesis. In the present invention, promoting chondroitin sulfate synthesis and promoting expression of a glycosyltransferase involved in chondroitin sulfate synthesis are preferably promoting chondroitin sulfate synthesis in the body of a subject and promoting expression of the glycosyltransferase in the body of a subject, respectively.

[0021]

Examples of glycosyltransferases involved in chondroitin sulfate synthesis include chondroitin sulfate N-acetylgalactosaminyltransferase 1 (CSGalNacT1), chondroitin sulfate N-acetylgalactosaminyltransferase 2 (CSGalNacT2), xylosyltransferase (XylT), β -1,3-galactosyltransferase (B3GalT), β -1,4-galactosyltransferase (B4GalT), chondroitin sulfate synthases 1 to 3 (ChSy1, ChSy2, ChSy3), and chondroitin polymerizing factor 2 (Chpf2). Five types of glycosyltransferases (Ext1, Ext2, EXTL1 (Extl1), EXTL2 (Extl2), and EXTL3 (Extl3)) involved in synthesis of heparan sulfate chains are not included in the glycosyltransferases involved in chondroitin sulfate

synthesis in the present invention.

[0022]

The composition for promoting chondroitin sulfate synthesis of the present invention can promote expression
5 of a glycosyltransferase involved in chondroitin sulfate synthesis. In one embodiment, the composition of the present invention can be used for promoting chondroitin sulfate synthesis by promoting expression of a glycosyltransferase involved in chondroitin sulfate
10 synthesis. The glycosyltransferase involved in chondroitin sulfate synthesis is preferably at least one selected from the group consisting of chondroitin sulfate N-acetylgalactosaminyltransferase 1, chondroitin sulfate N-acetylgalactosaminyltransferase 2, xylosyltransferase 2, β -
15 1,3-galactosyltransferase 1 (BGalT1), chondroitin sulfate synthase 1, and chondroitin polymerizing factor 2. Preferably, the composition of the present invention can promote expression of one or more of these glycosyltransferases. In a preferred embodiment, the
20 composition of the present invention can promote expression of chondroitin sulfate N-acetylgalactosaminyltransferase 1 and/or chondroitin sulfate N-acetylgalactosaminyltransferase 2, and can be used for such a purpose. CSGalNacT1 and CSGalNacT2 are considered to be
25 important enzymes that serve as starting points for chondroitin sulfate synthesis. More preferably, the composition of the present invention can promote expression of chondroitin sulfate N-acetylgalactosaminyltransferase 1, chondroitin sulfate N-acetylgalactosaminyltransferase 2,
30 xylosyltransferase 2, and chondroitin polymerizing factor 2.

The "promoting expression of a glycosyltransferase" described above includes either one or both of promoting expression of a gene encoding a glycosyltransferase and promoting expression of the glycosyltransferase at the
35 protein level. The "promoting expression of a gene" may be

promoting mRNA expression, preferably promoting transcription to mRNA. The "promoting expression of the glycosyltransferase at the protein level" includes promoting expression during translation.

5 [0023]

In one embodiment, the composition of the present invention can be preferably used, for example, for promoting expression of a glycosyltransferase involved in chondroitin sulfate synthesis in chondrocytes and for
10 promoting chondroitin sulfate synthesis in chondrocytes. Promoting chondroitin sulfate synthesis in cartilage can promote cartilage synthesis. In one embodiment, the composition of the present invention can be used for promoting cartilage synthesis. Promoting cartilage
15 synthesis makes it possible to increase cartilage. Promoting cartilage synthesis is useful for preventing a decrease in cartilage, for example.

[0024]

It has been reported that promoting chondroitin
20 sulfate synthesis in the brain promotes regeneration of nerve cells in the hippocampus. Promoting chondroitin sulfate synthesis in osteoblasts can promote bone formation.

Quercetin or a glycoside thereof has an action that promotes expression of a glycosyltransferase involved in
25 chondroitin sulfate synthesis in nerve cells and osteoblasts, and can promote chondroitin sulfate synthesis. Quercetin or a glycoside thereof promotes chondroitin sulfate synthesis in nerve cells (preferably, hippocampal nerve cells) and is useful for protecting nerves.
30 Protecting nerves is likely to result in a cognitive function improving effect. Quercetin or a glycoside thereof promotes chondroitin sulfate synthesis in osteoblasts and is useful for promoting bone formation. In one embodiment, the composition of the present invention
35 can be used for protecting nerves or promoting bone

formation.

[0025]

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

The composition of the present invention can be
10 provided in the form of a food or beverage, a cosmetic product, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like. The composition of the present invention may be a food or beverage, a cosmetic product, a pharmaceutical product, a quasi-pharmaceutical product,
15 feed, or the like by itself for use in promoting chondroitin sulfate synthesis, or may be a material, a preparation, or the like that is added to these products.

The composition for promoting chondroitin sulfate synthesis of the present invention can be provided as an
20 agent or the like, for example, but it is not limited thereto. The agent can be directly provided as a composition, or can be provided as a composition containing the agent. In one embodiment, the composition for promoting chondroitin sulfate synthesis of the present
25 invention can also be referred to as a "chondroitin sulfate synthesis promoting agent".

The composition of the present invention may be an oral composition or a parenteral composition, preferably an oral composition. It is possible to promote chondroitin
30 sulfate synthesis in a subject by oral ingestion, oral administration, or parenteral administration of the composition of the present invention. The oral composition is, for example, a food or beverage, an oral pharmaceutical product, a quasi-pharmaceutical product, or feed,
35 preferably a food or beverage or an oral pharmaceutical

product, more preferably a food or beverage. The parenteral composition is, for example, a cosmetic product, a parenteral pharmaceutical product, or a parenteral quasi-pharmaceutical product.

5 [0026]

The composition of the present invention may contain optional additives and optional components in addition to quercetin or a glycoside thereof, as long as the effect of the present invention is not impaired. Such additives and components can be selected according to the composition form or the like. Those that can be used generally in food and beverages, cosmetic products, pharmaceutical products, quasi-pharmaceutical products, feed, and the like can be used. When the composition of the present invention is provided as a food or beverage, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like, any production method may be used, and such a product can be produced by a common method.

[0027]

20 For example, when the composition of the present invention is provided as a food or beverage, a component usable in food or beverages (e.g., a food material or an optional food additive) can be added to quercetin or a glycoside thereof to provide various types of foods or beverages. Non-limiting examples of the food or beverages include general foods and beverages, health foods, health beverages, foods with function claims, foods for specified health uses, dietary supplements, and foods and beverages for the sick. The health foods, foods with function claims, foods for specified health uses, dietary supplements, and the like can be used, for example, in various forms of preparations such as fine granules, tablets, granules, powders, capsules, chewable tablets, dry syrups, syrups, liquids, and liquid foods.

35 [0028]

When the composition of the present invention is provided as a cosmetic product, a cosmetically acceptable carrier, additive, and the like can be added to quercetin or a glycoside thereof. The cosmetic product may be in any product form.

[0029]

When the composition of the present invention is provided as a pharmaceutical product or a quasi-pharmaceutical product, for example, a pharmacologically acceptable carrier, an optional additive, or the like can be added to quercetin or a glycoside thereof to provide pharmaceutical products or quasi-pharmaceutical products in various dosage forms. Such a carrier, an additive, or the like may be any pharmacologically acceptable one that can be used in pharmaceutical products or quasi-pharmaceutical products. Examples thereof include excipients, binders, disintegrants, lubricants, antioxidants, and colorants. One or more of these can be used. The form of administration (ingestion) of the pharmaceutical product or the quasi-pharmaceutical product may be, for example, oral or parenteral administration (e.g., transdermal, transmucosal, or enteral administration, or injection). When the composition of the present invention is provided as a pharmaceutical product or a quasi-pharmaceutical product, the composition is preferably an oral pharmaceutical product or a quasi-pharmaceutical product. Examples of dosage forms for oral administration include liquids, tablets, powders, fine granules, granules, sugar-coated tablets, capsules, suspensions, emulsions, and chewable tablets. Examples of dosage forms for parenteral administration include injections, intravenous drips, and topical agents (e.g., patches, creams, and ointments). The pharmaceutical product may be a pharmaceutical product for non-human animals.

[0030]

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

[0031]

The amount of quercetin or a glycoside thereof in the
10 composition of the present invention is not limited, and can be set according to the composition form and the like. The amount of quercetin or a glycoside thereof in the composition of the present invention, for example, in terms of quercetin, is preferably 0.01 wt% or more, more
15 preferably 0.1 wt% or more and is preferably 80 wt% or less, more preferably 50 wt% or less. In one embodiment, the amount of quercetin or a glycoside thereof in terms of quercetin in the composition of the present invention is preferably 0.01 to 80 wt%, more preferably 0.1 to 50 wt%.

20 The quercetin or a glycoside thereof can be measured by a known method, such as HPLC.

[0032]

The composition of the present invention is usually ingested by or administered to a subject. The
25 administration route of the composition of the present invention is not limited. The composition can be ingested or administered by a method suitable for the form. In one embodiment, the composition of the present invention is preferably orally ingested (orally administered). The
30 dosage (intake) of the composition of the present invention is not limited, as long as it is the amount that produces an effect of promoting chondroitin sulfate synthesis and an effect of promoting expression of a glycosyltransferase involved in chondroitin sulfate synthesis. The dosage may
35 be suitably set according to the dosage form,

administration method, and the like.

[0033]

In one embodiment, when a human (adult) is subjected to ingestion or administration of the composition of the present invention, the dosage of quercetin or a glycoside thereof in terms of quercetin is preferably 0.3 mg or more, more preferably 1.0 mg or more, still more preferably 10 mg or more, and is preferably 4000 mg or less, more preferably 2000 mg or less, still more preferably 1000 mg or less per 60 kg body weight per day. In one embodiment, the dosage of quercetin or a glycoside thereof in terms of quercetin to a human (adult) is preferably 0.3 to 4000 mg, more preferably 1.0 to 2000 mg, still more preferably 1.0 to 1000 mg, particularly preferably 10 to 1000 mg per 60 kg body weight per day. Preferably, the above amount of the composition is ingested or administered in one or more portions per day, for example, in one to several portions (for example, two or three portions) per day. In one embodiment, preferably, the above amount of quercetin or a glycoside thereof is orally ingested by or orally administered to a human. In one embodiment of the present invention, the composition of the present invention can be used for subjecting a human to ingestion or administration of the above amount of quercetin or a glycoside thereof per 60 kg body weight per day.

[0034]

Preferably, the composition of the present invention is continuously ingested or administered. Continuous ingestion or administration of quercetin or a glycoside thereof is likely to increase the effect of promoting chondroitin sulfate synthesis. In one embodiment, the composition of the present invention is continuously ingested or administered for preferably one week or more, more preferably four weeks or more, more preferably eight weeks or more.

[0035]

The composition of the present invention can be used for preventing or alleviating a condition or disease likely to be prevented or alleviated by promoting chondroitin sulfate synthesis. Examples of such a condition or disease include conditions or diseases caused by a decrease in the amount of chondroitin sulfate, such as knee osteoarthritis, hip osteoarthritis, spinal stenosis, and osteoporosis. In one embodiment, the composition of the present invention can be used for preventing or alleviating cognitive function decline or for preventing or alleviating dementia. As used herein, the expression "preventing a condition or disease" encompasses preventing the onset of a condition or disease, delaying the onset of a condition or disease, reducing the incidence of a condition or disease, reducing the onset risk of a condition or disease, and the like. The expression "alleviating a condition or disease" encompasses helping a subject recover from a condition or disease, alleviating a symptom of a condition or disease, favorably changing a symptom of a condition or disease, delaying or preventing the progress of a condition or disease, and the like.

[0036]

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

In one embodiment, the administration subject may be a subject needing or wanting to promote chondroitin sulfate synthesis or a subject needing or wanting to prevent or alleviate a condition or disease caused by a decrease in the amount of chondroitin sulfate. A decrease in the amount of chondroitin sulfate may be a decrease in the amount of chondroitin sulfate due to aging, and may be a decrease in the amount of chondroitin sulfate due to aging

among middle-aged people.

In one embodiment, examples of the administration subject in the present invention include middle-aged people. The term "middle-aged people" encompasses the elderly. The elderly are preferred subjects among the middle-aged people. In the present invention, the middle-aged people may be people who are 40 years old and older, for example. The elderly may be people who are 60 years old and older or 65 years old and older, for example. In one embodiment, the composition of the present invention is suitably used as a composition for promoting chondroitin sulfate synthesis for middle-aged people.

The composition of the present invention can also be used by a healthy person for a purpose such as preventing a condition or disease likely to be prevented or alleviated by promoting chondroitin sulfate synthesis, for example.

[0037]

The composition of the present invention may be labeled with a function claim based on the effect of promoting chondroitin sulfate synthesis. The composition for promoting chondroitin sulfate synthesis of the present invention may be labeled with one or more function claims such as "maintaining the amount of cartilage", "reducing or preventing cartilage wear", "reducing or preventing cartilage degeneration", "alleviating knee joint trouble", "maintaining knee joint function", "reducing concerns about knee joint during movement", "alleviating knee joint discomfort", "improving knee bending and stretching movements", "maintaining knee joint function that declines due to aging", "protecting knee joint cartilage so as to help people who are feeling a decline in locomotive function such as walking, standing up, and sitting down to perform smooth bending and stretching of the knees and improve walking ability". These function claim labels may indicate that these functions are obtainable by promoting

chondroitin sulfate synthesis.

In one embodiment, preferably, the composition of the present invention is a food or beverage labeled as described above. These labels may be labels indicating use
5 for obtaining these functions. These labels may be attached to the composition itself or a container or a package of the composition.

[0038]

The present invention also encompasses the following
10 method and use:

a method of promoting chondroitin sulfate synthesis, the method including administering quercetin or a glycoside thereof; and

use of quercetin or a glycoside thereof for promoting
15 chondroitin sulfate synthesis.

The method and the use may be therapeutic or non-therapeutic. Administration (ingestion) of quercetin or a glycoside thereof can increase the expression level of glycosyltransferase involved in chondroitin sulfate
20 synthesis. Administration of quercetin or a glycoside thereof can promote chondroitin sulfate synthesis. The method may be a method of promoting chondroitin sulfate synthesis by promoting expression of a glycosyltransferase involved in chondroitin sulfate synthesis. The use may be
25 use of quercetin or a glycoside thereof for promoting chondroitin sulfate synthesis by promoting expression of a glycosyltransferase involved in chondroitin sulfate synthesis. Promoting chondroitin sulfate synthesis may be, for example, promoting chondroitin sulfate synthesis in
30 cartilage. Quercetin or a glycoside thereof can be used for preventing or alleviating a condition or disease expected to be prevented or alleviated by promoting chondroitin sulfate synthesis described above.

[0039]

35 In the method and the use, preferably, quercetin or a

glycoside thereof is administered to (ingested by) a subject at least once a day, for example, one to several times (e.g., two to three times) a day. The use is preferably for humans or non-human mammals, more preferably for humans. Quercetin or a glycoside thereof is as described above.

[0040]

The method and the use only require use of quercetin or a glycoside thereof in an amount (effective amount) that produces an action that promotes chondroitin sulfate synthesis. Preferred dosage, administration subject, administration method, and the like of quercetin or a glycoside thereof are as described above for the composition for promoting chondroitin sulfate synthesis of the present invention. Quercetin or a glycoside thereof may be administered as is, or may be administered in the form of a composition containing quercetin or a glycoside thereof. For example, the composition of the present invention described above may be used. Preferably, quercetin or a glycoside thereof is orally administered (ingested).

[0041]

Quercetin or a glycoside thereof can be used for producing a food or beverage, a cosmetic product, a pharmaceutical product, a quasi-pharmaceutical product, feed, or the like for use in promoting chondroitin sulfate synthesis. In one embodiment, the present invention encompasses use of quercetin or a glycoside thereof for producing a composition for promoting chondroitin sulfate synthesis. The composition for promoting chondroitin sulfate synthesis, its preferred embodiment, and the like are as described above for the composition of the present invention.

[0042]

The present invention is specifically described below with reference to examples. A series of animal experiments was performed based on a plan approved by the relevant
5 chief through evaluation of the in-house animal experiment committee, in compliance with the animal welfare management laws and other related laws and regulations. The present invention is not limited to these examples.

[0043]

10 <Example 1: Effect of quercetin on promoting chondroitin sulfate synthesis 1 (*in vitro*)>

(1) Culturing of cells

Human chondrocytes (HCH) were cultured in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum (Sigma-
15 Aldrich) until the cells were completely confluent.

[0044]

(2) Quantification of the amount of chondroitin sulfate using cells

Quercetin (5 μ M) was added to the medium containing
20 HCH, and the cells were cultured. Quercetin was added in the form of a solution in dimethyl sulfoxide (DMSO). The amount of chondroitin sulfate in the cultured cells four days after the addition was quantified by cell ELISA based on an anti-chondroitin sulfate antibody (CS-56). Control
25 cells were cultured in the same manner except that DMSO was added, and chondroitin sulfate was quantified. The difference in mean between the groups was tested by Student's t test, with the significance level being 5% or lower (*: $p < 0.05$ vs. control).

30 [0045]

Fig. 1 shows the results (*: $p < 0.05$ vs. control). Fig. 1 is a graph showing the amount of chondroitin sulfate in HCH to which quercetin (5 μ M) was added. In Fig. 1, the relative CS level on the vertical axis indicates relative
35 values of the chondroitin sulfate (CS) level in the cells

to which quercetin was added, when the CS level in the control cells to which DMSO was added is taken as 1 (both values are expressed as means $\pm$ standard deviation, with N = 4). In Fig. 1, "quercetin" indicates the cells cultured in the medium to which quercetin was added. Addition of quercetin (5 μ M) resulted in a significant increase in the amount of chondroitin sulfate produced in HCH.

[0046]

<Method of quantifying the gene expression level>

10 In the following examples, RNAs were isolated from cells or tissues to measure the gene expression level in the cells or tissues by the following method.

cDNAs were synthesized from the isolated RNAs using High-Capacity cDNA Reverse Transcriptional Kits (Thermo Fisher Scientific). In Quantstudio Real Time PCR System (ThermoFisher), quantitative PCR was performed using TaqMan Fast Universal PCR Mastermix (Thermo Fisher Scientific) to quantify the expression levels of genes encoding glycosyltransferases (glycosyltransferase genes) involved in synthesis of chondroitin sulfate or heparan sulfate. The difference in mean between the groups was tested by Dunnett's t test, with the significance level being 5% or lower (*: $p < 0.05$ vs. quercetin 0 μ M). Table 1 shows the types of genes quantified in Examples 2 and 3, proteins encoded by the genes, and PCR primers and probes used for the measurement. Fig. 8 shows positions of the enzymes subjected to gene analysis in the glycosaminoglycan synthetic pathway.

[0047]

30 [Table 1]

Gene symbol	General preferred protein names	Forward primers (5'-3')	PrimeTime qPCR probes	Reverse primers(5' -3')
Csgalnact1	CS-N-acyetyl-galactosaminyltransferase 1	GAAGGGACTGGATGTTGGAG (SEQ ID NO: 1)	56- FAM/TCGGAGTTC/ZEN/CTCAACACTTGT AGGC(SEQ ID NO: 2)/3IABkFQ	AAATACCTTCTTCCCTGGCTG (SEQ ID NO: 3)
Csgalnact2	CS-N-acyetyl-galactosaminyltransferase 2	CCATTGTCTATGCCAACCCAGG (SEQ ID NO: 4)	56- FAM/CAGGTCATC/ZEN/CCAAAGCCAAAG TCTCT(SEQ ID NO: 5)/3IABkFQ	TTCCATGTGGAATCCACCG (SEQ ID NO: 6)
Xylt2	Xylosyltransferase 2	GCCACTGAATATCCAACGAG (SEQ ID NO: 7)	56- FAM/TGAACCTGG/ZEN/AATTGTCTCGCC CAT(SEQ ID NO: 8)/3IABkFQ	TCACACTCATGGAAGGCCG (SEQ ID NO: 9)
B3galt1	Beta-1,3-galactosyltransferase 1	GCCTACTTTAAGCTACGTGGT (SEQ ID NO: 10)	56- FAM/CTTGGGCTC/ZEN/CAGGTCATTGAG GG(SEQ ID NO: 11)/3IABkFQ	TGGCTTCTCTGTTCTCGTGATG (SEQ ID NO: 12)
Chsy1	Chondroitin sulfate synthase 1	GGAACCTTCTCTTCGTGGGAG (SEQ ID NO: 13)	56- FAM/TTGTCTTTGG/ZEN/ACCATGTTCTGT AGCG(SEQ ID NO: 14)/3IABkFQ	GATGTATCTGAACCCCTCGCTAG (SEQ ID NO: 15)
Chpf2	Chondroitin polymerizing factor 2	CTGAAGACCAGACATAGGGTTG (SEQ ID NO: 16)	56- FAM/TTTCTGTTG/ZEN/CTGACCTCGTTG ACTCC(SEQ ID NO: 17)/3IABkFQ	GCACAGTCTCAAGGATGAGTAG (SEQ ID NO: 18)
Ext1	Exostosin-1	TTGAAGTCTTTACAGGCGGG (SEQ ID NO: 19)	56- FAM/ACTGGAGCT/ZEN/GAAAGTGTGAT TGGGA(SEQ ID NO: 20)/3IABkFQ	TGAGAGCAGGATGAAATAGCG (SEQ ID NO: 21)
Ext2	Exostosin-2	TGGGATCGAGGAACAAATCAC (SEQ ID NO: 22)	56- FAM/AGCTCCAGG/ZEN/CAACATATTGAA CAGCA(SEQ ID NO: 23)/3IABkFQ	GTAAGTCCAGGTAGAAAAAGCCG (SEQ ID NO: 24)
Gapdh	glyceraldehyde-3-phosphate dehydrogenase	AATGGTGAAGGTCGGTGTG (SEQ ID NO: 25)	5TexRd- XN/TGCAAAATGGCAGCCCTGGTG(SEQ ID NO: 26)/3IABkFQ	GTGGAGTCATACTGGAACATGTAG (SEQ ID NO: 27)

[0048]

<Example 2: Effect of quercetin glycoside on promoting chondroitin sulfate synthesis (*in vivo*)>

(1) Administration of quercetin glycoside to mice

5 Each of 36-week-old C57BL/6J wild type (WT) mice and Csgalnact1 gene knockout (T1-KO) mice was assigned into a group to drink distilled water (control group) or a group to drink distilled water containing 4.5 g/L quercetin glycoside (QG) (QG administration group). Each group
10 contained mice of the same body weight. The WT mice (WT-QG) and T1-KO mice (KO-QG) in the respective QG administration groups were fed with the distilled water containing the quercetin glycoside for 18 weeks. The
15 intake of quercetin glycoside per body weight per day was 117 mg/kg in terms of quercetin. The WT mice (WT-control) and the T1-KO mice (KO-control) in the respective control groups were fed with distilled water for 18 weeks. All the
20 mice were kept alone in separate cages. Each cage was provided with a device that can measure voluntary running on a saucer-shaped wheel (Muromachi Kikai Co., Ltd.) for
25 exercise loads to encourage the mice to perform a certain amount of voluntary exercise. Knee joint tissues were collected 18 weeks after the start of ingestion of drinking water. Using the collected knee joint tissues, the gene
30 expression levels, the amount of chondroitin sulfate, and the amount of articular cartilage were measured. The quercetin glycoside used in Example 2 was enzyme-treated isoquercitrin obtainable by treating isoquercitrin with glycosyltransferase, in which a glycan containing 1 to 7 glucose units is bonded to isoquercitrin.

[0049]

(2) Quantification of gene expression level using murine tissues

35 RNAs were isolated from the collected knee joint using RNeasy Mini Kit (QIAGEN). cDNAs were synthesized

from the isolated RNAs by the method described in the method of quantifying the gene expression level described above, and the expression level of each glycosyltransferase gene involved in chondroitin sulfate or heparan sulfate synthesis was quantified by quantitative RT-PCR (N = 12). The quantified genes are Csgalnact1, Csgalnact2, Chpf2, Xylt2, and Ext1 shown in Table 1. The difference in mean between the groups was tested by Student's t test, with the significance level being 5% or lower (*: $p < 0.05$ vs. control).

[0050]

Fig. 2 shows the results (*: $p < 0.05$ vs. control). Fig. 2 is a graph showing the expression levels of the glycosyltransferases (Csgalnact1, Csgalnact2, Chpf2, and Xylt2) involved in chondroitin sulfate synthesis and the expression level of Ext1 gene in the knee articular tissue of the mice administered with quercetin glycoside (QG) (means $\pm$ standard deviation, with N = 12). In Fig. 2, p indicates Csgalnact1; q indicates Csgalnact2; r indicates Chpf2; s indicates Xylt2; and t indicates Ext1. In Fig. 2, the relative mRNA level on the vertical axis indicates relative values of the mRNA level in genes in each group, when the mRNA level in genes in the WT mouse control group (WT-control) is taken as 1.

[0051]

In the case of the WT mice, the QG administration group (WT-QG) showed a significant increase in the expression levels of Csgalnact1, Csgalnact2, Chpf2, and Xylt2, compared to the control group (WT-control). In contrast, no changes in the expression level of Ext1 were found. In the case of T1-KO mice, the QG administration group (KO-QG) showed a significant increase in the expression level of Csgalnact2, Chpf2, and Xylt2, compared to the control group (KO-control). In contrast, no changes in the expression level of Ext1 were found.

[0052]

(3) Quantification of the amount of chondroitin sulfate using murine tissue

Protein components were removed from the collected
 5 knee joint tissues by trichloroacetic acid precipitation, and glycosaminoglycan fractions were concentrated by ethanol precipitation. After desalting, glycosaminoglycan was digested by chondroitinase, and disaccharide
 10 chondroitin sulfate fluorescently labeled with 2-aminobenzamide was quantified by high performance liquid chromatography (Shimadzu Corporation) under the following conditions (N = 6).

[0053]

<HPLC conditions>

15 Column: DOCOSIL SP100 5.0 μ m, 4.6 $\times$ 100 mm (Senshu Scientific Co., Ltd.)
 Guard column: Myghtysil RP-18GP 5.0 μ m, 4.6 $\times$ 10 mm (Kanto Chemical Co., Inc.)
 Eluent:

20 A. Distilled water
 B. 0.2 M NaCl
 C. 10 mM Tetrabutyl ammonium hydrogen sulfate
 D. 50% Acetonitril

Gradient conditions:

25 0-10 min: A (70%), B (1%), C + D (29%) (C (12%), D (17%))
 10-11 min: A (67%), B (4%), C + D (29%) (C (12%), D (17%))
 11-20 min: A (61%), B (10%), C + D (29%) (C (12%), D (17%))
 20-26 min: A (53%), B (18%), C + D (29%) (C (12%), D (17%))
 26-28 min: A (35%), B (36%), C + D (29%) (C (12%), D (17%))
 30 28-37 min: A (18%), B (53%), C + D (29%) (C (12%), D (17%))
 37-57 min: A (70%), B (1%), C + D (29%) (C (12%), D (17%))

Flow rate (eluent): 1.1 mL/min

Reaction solution:

A. 0.5% 2-Cyanoacetate solution
 35 B. 0.25 M NaOH

Flow rate (reaction solution): 0.7 mL/min

Amount of injection: 50 μ L

Fluorescence monitor: EX 346 nm, EM 410 nm, AT 64, Gain $\times 100$

5 Integrator: End Time 40 min, Speed 2 min/min, AT 512
[0054]

The difference in mean between the groups was tested by Student's t test, with the significance level being 5% or lower (*: $p < 0.05$ vs. control).

10 [0055]

Fig. 3 shows the results (*: $p < 0.05$ vs. control). Fig. 3 is a graph showing the amount of chondroitin sulfate in the knee joint of the mice administered with quercetin glycoside (QG) (means $\pm$ standard deviation, with $N = 6$).

15 In Fig. 3, the relative CS level on the vertical axis indicates relative values of the chondroitin sulfate (CS) level in each group, when the CS level in the WT mice control group is taken as 1. In both cases of WT and T1-KO mice, the QG administration groups (WT-QG and KO-QG) showed
20 a significant increase in the amount of chondroitin sulfate, compared to the control groups (WT-control and KO-control). Ingestion of quercetin glycoside increased the amount of chondroitin sulfate in the knee joint.

[0056]

25 (4) Quantification of the amount of articular cartilage using murine tissues

The collected knee joint tissues were fixed with formalin, and continuous paraffin sections were prepared to produce samples. A central portion with the largest area
30 in the joint was completely flattened, and 20 sections per tissue were selected every 100 μ m and stained with HE such that each section would have the same range of area variation for measurement. The area of the articular cartilage was quantified using image analysis software
35 "imageJ" ($N = 18$). The difference in mean between the

groups was tested by Student's t test, with the significance level being 5% or lower (*: $p < 0.05$ vs. control).

[0057]

5 Fig. 4 shows the results (*: $p < 0.05$ vs. control).
 Fig. 4 is a graph showing the area of knee articular
 cartilage of the mice administered with QG. The relative
 area in Fig. 4 indicates relative values of the area of
 cartilage in each group when the area of cartilage in the
 10 control group of WT mice is taken as 1.

In the case of the WT mice, the QG administration
 group (WT-QG) showed a significant increase in the area of
 the articular cartilage, compared to the control group (WT-
 control). In contrast, in the case of the T1-KO mice, the
 15 QG administration group (KO-QG) showed an increase tendency,
 compared to the control group (KO-control), but no
 significant changes were found.

[0058]

<Example 3: Effect of quercetin on promoting chondroitin
 20 sulfate synthesis 2 (*in vitro*)>

(1) Culturing of various cells

C6 cells (glial cells), SKOV3 cells (ovarian adenoma
 cells), C1300 cells (neuroblastoma cells), KINGS1 cells
 (glial cells), NM-CG1 cells (glial cells), MC3T3-E1 cells
 25 (osteoblasts), and OSCV2 cells (osteoclasts) were cultured
 in Dulbecco's Modified Eagle Medium with 10% fetal bovine
 serum (Sigma-Aldrich) until the cells were completely
 confluent.

[0059]

30 (2) Quantification of the amount of chondroitin sulfate
 using cells

DMSO or quercetin in an amount of 0.5 μM , 1 μM , or 5
 μM was added to various cells (C6 cells, SKOV3 cells, C1300
 cells, KINGS1 cells, NM-CG1 cells, and MC3T3-E1 cells).
 35 RNAs were isolated from the cells two hours after the

addition, using RNeasy Mini Kit (QIAGEN). cDNAs were synthesized from the isolated RNAs by the method of quantifying the gene expression level described above, and the expression level of a glycosyltransferase gene involved in chondroitin sulfate or heparan sulfate synthesis was quantified by quantitative RT-PCR. The difference in mean between the groups was tested by Dunnett's t test, with the significance level being 5% or lower (*: $p < 0.05$ vs. quercetin 0 μ M). The quantified genes are Csgalnact1, Csgalnact2, Xylt2, B3galt1, Chsy1, Chpf2, Ext1, and Ext2 shown in Table 1.

[0060]

Fig. 5A, Fig. 5B, Fig. 5C, Fig. 5D, Fig. 6A, Fig. 6B, Fig. 6C, and Fig. 6D show the results (*: $P < 0.05$, vs. quercetin 0 μ M). Fig. 5A is a graph showing the expression levels of Csgalnact1 gene in the cells to which quercetin was added. Fig. 5B is a graph showing the expression levels of Csgalnact2 gene in the cells to which quercetin was added. Fig. 5C is a graph showing the expression levels of Xylt2 gene in the cells to which quercetin was added. Fig. 5D is a graph showing the expression levels of B3galt1 gene in the cells to which quercetin was added. Fig. 6A is a graph showing the expression levels of Chsy1 gene in the cells to which quercetin was added. Fig. 6B is a graph showing the expression levels of Chpf2 gene in the cells to which quercetin was added. Fig. 6C is a graph showing the expression levels of Ext1 gene in the cells to which quercetin was added. Fig. 6D is a graph showing expression levels of Ext2 gene in the cells to which quercetin was added.

In Fig. 5A to Fig. 5D and Fig. 6A to Fig. 6D, "a" indicates C6 cells; "b" indicates SKOV3 cells; "c" indicates C1300 cells; "d" indicates KINGS1 cells; "e" indicates NM-CG1 cells; and "f" indicates MC3T3-E1SK cells. In each of Fig. 5A to Fig. 5D and Fig. 6A to Fig. 6D, each

gene expression level was expressed as a relative mRNA level. The relative mRNA level indicates relative values (means $\pm$ standard deviation, with N = 8) of the mRNA level in the cells to which quercetin was added (quercetin concentration of 0.5 μ M, 1 μ M or 5 μ M), when the mRNA level of each gene in the control cells to which DMSO was added (quercetin concentration: 0 μ M) is taken as 1.

[0061]

Addition of quercetin (5 μ M) resulted in a significant increase in the expression levels of both Csgalnact1 and Csgalnact2 in all the cells (Fig. 5A and Fig. 5B). Addition of quercetin (5 μ M) resulted in a significant increase in the expression level of Xylt2 in C6 cells, C1300 cells, KINGS1 cells, NM-CG1 cells, and MC3T3-E1 cells (Fig. 5C); in the expression level of B3galt1 in SKOV3 cells, KINGS1 cells, and MC3T3-E1 cells (Fig. 5D); in the expression level of Chsy1 in SKOV3 cells, C1300 cells, KINGS1 cells, NM-CG1 cells, and MC3T3-E1 cells (Fig. 6A); and in the expression level of Chpf2 in SKOV3 cells, C1300 cells, KINGS1 cells, and MC3T3-E1 cells (Fig. 6B). In contrast, no significant changes in the expression levels of Ext1 and Ext2 involved in heparan sulfate synthesis were found in any cells (Fig. 6C and Fig. 6D).

[0062]

(3) Quantification of the amount of chondroitin sulfate using cells

Quercetin (5 μ M) was added to the medium containing OSCV2 cells, and the cells were cultured. The amount of chondroitin sulfate in the cultured cells four days after the addition was quantified by cell ELISA based on an anti-chondroitin sulfate antibody (CS-56). Control cells were cultured in the same manner except that DMSO was added, and chondroitin sulfate was quantified. The difference in mean between the groups was tested by Student's t test, with the significance level being 5% or lower (*: $p < 0.05$ vs.

control).

[0063]

Fig. 7 shows the results (*: $p < 0.05$ vs. control).
Fig. 7 is a graph showing the amount of chondroitin sulfate
in the OSCV2 cells to which quercetin (5 μ M) was added. In
Fig. 7, the relative CS level on the vertical axis
indicates relative values of the chondroitin sulfate (CS)
level in the cells to which quercetin was added, when the
CS level in the control cells to which DMSO was added is
taken as 1 (both values are expressed as means $\pm$ standard
deviation, with $N = 4$). In Fig. 7, "quercetin" indicates
the cells cultured in the medium to which quercetin was
added. Addition of quercetin (5 μ M) resulted in a
significant increase in the amount of chondroitin sulfate.

[0064]

Based on the above results, it was found that
quercetin or a glycoside thereof has an action that
promotes expression of a glycosyltransferase involved in
chondroitin sulfate synthesis, and an action that promotes
chondroitin sulfate synthesis. Quercetin or a glycoside
thereof has an action that increases the amount of
articular cartilage.

SEQUENCE LISTING FREE TEXT

SequenceListing.txt

CLAIMS

Claim 1. Use of quercetin or a quercetin glycoside in the manufacture of a medicament for preventing or
5 alleviating spinal stenosis, or osteoporosis by promoting chondroitin sulfate synthesis.

Claim 2. The use according to claim 1,
wherein the medicament promotes expression of a
10 glycosyltransferase involved in chondroitin sulfate synthesis.

Claim 3. The use according to claim 2,
wherein the glycosyltransferase involved in
15 chondroitin sulfate synthesis is at least one selected from the group consisting of chondroitin sulfate N-acetylgalactosaminyltransferase 1, chondroitin sulfate N-acetylgalactosaminyltransferase 2, xylosyltransferase 2, β -1,3-galactosyltransferase 1, chondroitin sulfate synthase
20 1, and chondroitin polymerizing factor 2.

Claim 4. The use according to claim 2 or 3,
wherein the glycosyltransferase involved in
chondroitin sulfate synthesis comprises at least one of
25 chondroitin sulfate N-acetylgalactosaminyltransferase 1 or chondroitin sulfate N-acetylgalactosaminyltransferase 2.

Claim 5. The use according to any one of claims 1 to
4,
30 wherein the medicament is a food or beverage, a cosmetic product, or a quasi-pharmaceutical product.