

COMPOSITIONS COMPRISING A COMBINATION OF Fisetin AND OLEUROPEIN FOR USE IN TREATING BONE DISORDERS

Technical field of the invention

The present invention relates to bone health and in particular to use of a composition comprising
5 a combination of oleuropein and/or metabolite thereof and fisetin and/or metabolite thereof for
prevention or treatment of bone disorders or maintenance of bone health. The present invention
also relates to compositions and methods for enhancing bone quality, bone growth and/or bone
strength.

Background of the invention

10 Bone is a tissue that undergoes constant remodelling as a result of the destruction and de novo
synthesis of bone tissue in a complex process involving two main types of cells, the osteoblasts
which produce new bone tissue and the osteoclasts which destroy bone, respectively. The
osteoblasts, the cells responsible for bone formation, differentiate from precursor cells and
express and secrete many enzymes and many structural proteins of the bone matrix, including
15 collagen type I, osteocalcin, osteopontin and alkaline phosphatase. The osteoclasts are
multinucleated cells which are responsible for bone loss in a process generally designated bone
resorption.

In a healthy adult, man or animal, the joint action of the osteoblasts and osteoclasts makes
possible the maintenance of the bone mass over time and simultaneously ensures remodelling of
20 bone tissue by resorption and de novo synthesis of bone. However, in osteoporotic individuals, an
imbalance in the process of bone remodelling is produced which culminates in a loss of bone
which proceeds at a more rapid rate than the rate of formation. Although this imbalance exists to
a certain extent in most individuals as they age, it is much more severe and occurs at a younger
age in osteoporotic individuals.

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Thus, in man and other mammals a great variety of disorders are related to abnormal metabolism
of bone resorption and bone formation, leading to an imbalance in metabolism or bone
remodelling.

Of the pathological disorders related to an imbalance in bone metabolism, particular mention may be made of the disorders or diseases such as osteoporosis, Paget's disease, bone loss or osteolysis observed close to a prosthesis, metastatic bone diseases, hypercalcemia due to a cancer, multiple myelomas and periodontal diseases. Some of the disorders or diseases of bone metabolism may be caused by long-term immobilisation, for example long-term hospitalisation or even after a period of weightlessness. Of the disorders linked to abnormal bone resorption, the most common is osteoporosis, the most frequent manifestation of which is observed in women after the start of menopause. Osteoporosis is a systemic skeletal disease characterised by a reduction of the bone mass and a deterioration of the microarchitecture of bone tissue, associated with an increase of the fragility of the bone and its susceptibility to fracture.

Since osteoporosis, like other disorders associated with bone loss, constitutes a chronic disorder, its prevention and its treatment must be planned in the long term.

It is currently accepted that early treatment must be preferred because the two critical phases for bone capital are: the period of growth during which the maximal bone mass (peak bone mass) is acquired; ageing which conditions the rate of loss of bone mass. The prevention of osteoporosis must hence no longer be restricted to the elderly individual.

Moreover, in man and animals, there are many conditions characterised by the need to increase bone formation. For example, in the case of bone fractures, it is necessary to stimulate bone growth in order to accelerate complete repair of the bone. This need is also present in the periodontal diseases, the metastatic diseases of bone, the osteolytic diseases and the conditions under which repair of the connective tissue is required, for example for the cicatrisation or regeneration of defects or traumatisms of cartilage. The stimulation of bone growth is also required in the case of primary and secondary hyperparathyroidism, as well as in osteoporosis associated with diabetes and in osteoporosis associated with glucocorticoids.

Although there exists to-day a large variety of active compounds for stimulating bone formation and/or inhibiting bone resorption, there is a constant need for new active compounds, in particular owing to the limited success of the current treatments.

Furthermore, in view of the chronic character of some conditions caused by an imbalance in bone metabolism, there is a need for new active compounds which it will be possible to use in the long term in man and animals, and which are likely to be available in the form of a food additive, for example in the form of a nutritional composition.

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Moreover, at an earlier stage, the growth and development of the human skeleton requires an adequate supply of many different nutritional factors. Classical nutrient deficiencies are associated with stunting (e.g. energy, protein, Zn), rickets (e.g. vitamin D) and other bone abnormalities (e.g. Cu, Zn, vitamin C). There is evidence to suggest that peak bone mass and later

10 fracture risk are influenced by the pattern of growth and nutritional exposures in childhood.

However, it is challenging to define dietary reference values using bone health as a criterion, and the question of what type of diet constitutes the best support for optimal bone growth and development remains open (see e.g. Prentice, A., et al., 2006. Proceedings of the Nutrition Society, 65(4), pp.348-360).

15 Several approaches may be taken to improve the intake of growth-limiting nutrients, including administration of micronutrient supplements, fortification of food with micronutrients or improved dietary intake. However, particularly in populations where dietary quality is poor, several micronutrient deficiencies can co-occur, in which case growth may be affected by more than one growth-limiting nutrient (see e.g. Rivera, J.A., et al., 2003. The Journal of nutrition, 20 133(11), pp.4010S-4020S).

Thus, there is also a demand for new nutritional interventions to enhance bone growth and/or bone strength, particularly in children suffering from stunted growth and/or faltering growth.

Summary of the invention

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The inventors have surprisingly demonstrated that a combination of oleuropein (or oleuropein aglycone) and fisetin synergistically activates mitochondrial function at the cellular level, via mitochondrial calcium elevation. Mitochondria are the primary source of aerobic energy production in mammalian cells and maintain a large Ca²⁺ gradient across their inner membrane, 30 providing a signaling potential for this molecule. Furthermore, mitochondrial Ca²⁺ plays a role in

the mitochondria in the regulation of ATP generation and potentially contributes to the orchestration of cellular metabolic homeostasis. Specifically, activation of mitochondrial Ca^{2+} import increases cellular energy metabolism (Glancy & Balaban, Biochemistry, Role of mitochondrial Ca^{2+} in the regulation of cellular energetics (2012), 246:237-247). In bone tissue
5 and cells, mitochondria play a central role in cell energy metabolism and bone formation (Sautchuk and Eliseev, Bone Report, Cell energy metabolism and bone formation (2022), 27:16:101594). Therefore, in osteoprogenitors mitochondria energy metabolism is activated during osteogenic differentiation. In addition, osteoblasts are heavily dependent on mitochondrial oxidative phosphorylation/mitochondrial energy production. Furthermore, bone
10 aging is associated with mitochondrial dysfunction.

Thus, one aspect of the invention relates to a composition comprising an effective amount of a combination of oleuropein and/or metabolite thereof and fisetin and/or derivative thereof for use to prevent or treat bone disorders or maintenance of bone health in an individual.

In another aspect, the present invention provides a combination of oleuropein and/or metabolite
15 thereof and fisetin and/or derivative thereof for use in enhancing bone quality, bone growth and/or bone strength in an individual.

In a last aspect, the present invention relates to a method of manufacturing a composition for use according to the invention.

Additional features and advantages are described herein and will be apparent from the following
20 Figures and Detailed Description.

Brief description of the figures

FIG. 1 represents chemical structure of Fisetin (A) and Oleuropein (B)

FIG. 2 is a showing that the effect of the combination of Fisetin with Oleuropein is greater than the effect of fisetin or oleuropein alone on mitochondrial activation, via mitochondrial Ca^{2+} rise,
25 in HeLa cells. The bar chart shows the effect of oleuropein (3 μM , black), fisetin (3 μM , gray) and the combination of 3 μM fisetin + 3 μM oleuropein on the integrated mitochondrial calcium rise, evoked by 100 μM histamine. Results are expressed as mean $\pm$ SEM from n = 6 experiments. *

indicates statistically significant difference of the measured vs. theoretical difference in mitochondrial calcium at $P < 0.05$ (one-way ANOVA test).

FIG. 3 is a graph showing that Fisetin synergizes with Oleuropein to activate mitochondria, via mitochondrial Ca^{2+} rise, in HeLa cells. To quantify the synergistic effect of the combination of oleuropein and fisetin on mitochondrial activation, the expected theoretical effect (sum between 5 oleuropein effect and oleuropein effect, extracted from the data in fig.2) and the real measured effect of the combination (fisetin + oleuropein, extracted from data in fig. 2) were compared. Results are expressed as mean $\pm$ SEM from $n = 6$ experiments. * indicates statistically significant difference of the measured vs. theoretical difference in mitochondrial calcium at $P < 0.05$ 10 (Student's test).

Detailed description of the invention

Definitions

Prior to discussing the present invention in further details, the following terms and conventions will first be defined:

15 In the context of the present invention, mentioned percentages are weight/weight percentages unless otherwise stated.

The term "and/or" used in the context of the "X and/or Y" should be interpreted as "X", or "Y", or "X and Y".

Numerical ranges as used herein are intended to include every number and subset of numbers 20 contained within that range, whether specifically disclosed or not. Further, these numerical ranges should be construed as providing support for a claim directed to any number or subset of numbers in that range. For example, a disclosure of from 1 to 10 should be construed as supporting a range of from 1 to 8, from 3 to 7, from 4 to 9, from 3.6 to 4.6, from 3.5 to 9.9, and so forth.

25 The terms "prevent" and "prevention" mean to administer a composition as disclosed herein to an individual is not showing any symptoms of the condition to reduce or prevent development of

at least one symptom associated with the condition. Furthermore, "prevention" includes reduction of risk, incidence and/or severity of a condition or disorder.

As used herein, an "effective amount" is an amount that treats or prevents a deficiency, treats or prevents a disease or medical condition in an individual, or, more generally, reduces symptoms,
5 manages progression of the disease, or provides a nutritional, physiological, or medical benefit to the individual.

"Animal" includes, but is not limited to, mammals, which includes but is not limited to rodents; aquatic mammals; domestic animals such as dogs, cats and other pets; farm animals such as sheep, pigs, cows and horses; and humans. Where "animal," "mammal" or a plural thereof
10 used, these terms also apply to any animal that is capable of the effect exhibited or intended to be exhibited by the context of the passage, e.g., an animal benefitting from improved mitochondrial calcium import. While the term "individual" or "subject" is often used herein to refer to a human, the present disclosure is not so limited. Accordingly, the term "individual" or "subject" refers to any animal, mammal or human that can benefit from the methods and
15 compositions disclosed herein.

The term "pet" means any animal which could benefit from or enjoy the compositions provided by the present disclosure. For example, the pet can be an avian, bovine, canine, equine, feline, hircine, lupine, murine, ovine, or porcine animal, but the pet can be any suitable animal. The term "companion animal" means a dog or a cat.

20 A "subject" or "individual" is a mammal, preferably a human. The term "elderly" in the context of a human means an age from birth of at least 60 years, preferably above 63 years, more preferably above 65 years, and most preferably above 70 years. The term "older adult" in the context of a human means an age from birth of at least 45 years, preferably above 50 years, more preferably above 55 years, and includes elderly individuals. The term "older adult" in the context of a human
25 means an age from birth of at least 45 years, preferably above 50 years, more preferably above 55 years, and includes elderly individuals. In an embodiment, the individual is menopausal or perimenopausal individual, in particular menouposal or perimenopausal woman.

An "oral nutrition supplement" or "ONS" is a composition comprising at least one macronutrient and/or at least one micronutrient, for example in a form of sterile liquids, semi-solids or powders,

and intended to supplement other nutritional intake such as that from food. Non-limiting examples of commercially available ONS products include MERITENE®, BOOST®, NUTREN® and SUSTAGEN®. In some embodiments, an ONS can be a beverage in liquid form that can be consumed without further addition of liquid, for example an amount of the liquid that is one
5 serving of the composition.

A “kit” means that the components of the kit are physically associated in or with one or more containers and considered a unit for manufacture, distribution, sale, or use. Containers include, but are not limited to, bags, boxes, cartons, bottles, packages of any type or design or material, over-wrap, shrink-wrap, affixed components (e.g., stapled, adhered, or the like), or combinations
10 thereof.

All references to singular characteristics or limitations of the present invention shall include the corresponding plural characteristic or limitation, and vice versa, unless otherwise specified or clearly implied to the contrary by the context in which the reference is made.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as
15 commonly understood by one of ordinary skill in the art.

Composition for use

The present inventors have shown that the provision of a combination of oleuropein and/or metabolite thereof and fisetin and/or derivative synergistically improves mitochondrial functions which are altered in bone disorders for example.
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In an embodiment, the said composition is for use in stimulating bone formation and/or inhibiting bone resorption; ii) improving bone quality; iii) preventing or treating disorders linked to an imbalance in the relationship between bone formation and bone resorption.

25 By "stimulation of bone formation" is meant according to the invention, the capacity of the combination of oleuropein plus fisetin to the present invention induces an increase of the bone density at a given dose.

The specialist skilled in the art may also have recourse to any of the other tests also described in

the examples, such as the measurement of the resistance of bone to rupture or also to the measurement of alkaline phosphatase activity and the measurement of the accumulation of calcium in the osteoblast cells.

- 5 By "inhibition of bone resorption" is meant according to the invention inhibition of the destructive activity of bone tissue by the osteoclast cells. In order to verify that the supply of the composition in man or animals inhibits bone resorption, the specialist skilled in the art can measure the urinary excretion of desoxypyridinoline as described in the examples, a diminution of the expression of desoxypyridinoline being the reflection of inhibition of bone resorption.

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The supply of the composition according to the invention to an animal organism induces simultaneously a stimulation of bone formation and inhibition of bone resorption, the overall increase of bone mineralisation, and hence of the bone density, being the result of the induction of these two mechanisms.

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In order to determine whether a subject presents a state of reduced bone mass and as a consequence requires a supply of the composition according to the present invention, the specialist skilled in the art will be able to refer in particular to the report of the World Health Organisation (WHO) of 1994 entitled "Assessment of fracture risk and its application to screening
20 for post-menopausal osteoporosis" (WHO Technical Series-843).

The composition according to the present invention is also designed for Individuals presenting symptoms of bone deficit or likely to suffer from bone deficit, i.e. from an imbalance in the relationship between bone formation and bone resorption which, if it continues, induces a
25 diminution of the bone mass. A composition according to the invention is also designed for individuals presenting symptoms of bone deficit resulting from a fracture, an operation or also a dental disease.

The composition for use according to any preceding claim, characterised in that said composition
30 is designed to treat a bone deficit resulting from a fracture.

In particular the composition is designed to prevent or treat diseases selected from osteoporosis,

Paget's disease, bone loss or osteolysis observed close to a prosthesis, metastatic bone diseases, hypercalcemia due to a cancer, multiple myelomas, periodontal diseases or osteoarthritis.

As has already been mentioned above, many disorders linked to an imbalance of bone

5 metabolism, such as osteoporosis, develop gradually over a long period of time and require chronic treatments. Their prevention or their treatment can hence be carried out by means of a regular supply of the composition according to the present invention, preferably in the form of a nutritional composition.

10 Similarly, a regular nutritional supply of the composition according to the present invention to young growing individuals, humans or animals, is such as to make possible the production of a high bone density and an elevated peak bone mass by stimulation of bone formation when these individuals attain adult age.

15 A regular nutritional supply of the composition according to the present invention is also useful for preventing the bone loss that occurs with ageing and/or to protect bone cells during bone aging.

In another embodiment, the said composition is designed to stimulate bone formation in young individuals in the growth phase.

A composition such as defined above is also designed to promote bone quality, bone growth in
20 young individuals in order to obtain individuals possessing high bone density and, if possible, concomitantly a high peak bone mass. In particular, a composition according to the invention is useful during the growth phase of man as well as other mammals, in particular of pedigree dogs, and also racehorses.

25 As used herein "bone quality" may refer to aspects of bone composition and structure that contribute to bone strength independently of bone mineral density. These include bone turnover, microarchitecture, mineralisation, microdamage and the composition of bone matrix and mineral. Methods to measure bone quality are known in the art.

30 As used herein, "promoting bone growth and/or strength" may refer to the support of normal

bone growth and/or strength, for example during childhood and adolescence. Supporting normal bone growth and/or strength may result in normal bone anatomy and physiology. Suitable methods and parameters to determine bone growth and bone strength will be known to the skilled person (see e.g. Donnelly, E., 2011. Clinical Orthopaedics and Related Research, 469(8), pp.2128-2138). Suitably, normal bone growth and/or strength may be determined using one or more bone parameter selected from: trabecular bone volume fraction (BV/TV), bone mineral density (BMD), bone mineral content (BMC), cortical bone volume (Ct.BV), medio-lateral diameter, antero-posterior diameter, bone ultimate force (FMax), and bone stiffness. In some embodiments, normal bone growth and/or strength is determined using one or more bone parameter selected from: bone mineral density (BMD), trabecular bone volume fraction (BV/TV), cortical bone volume (Ct.BV), and bone ultimate force (FMax). Suitable methods to determine these parameters will be available to the skilled person.

The combination of the present invention may promote catch-up growth e.g. in individuals with stunted growth and/or faltering growth. As used herein, "catch-up growth" may refer to height velocity above the limits of normal for age for at least 1 year after a transient period of growth inhibition and may be complete or incomplete (see e.g. Wit, J.M. and Boersma, B., 2002. Journal of Pediatric Endocrinology and Metabolism, 15, pp.1229-1242).

Suitable method and parameters to determine catch-up growth will be known to the skilled person. Suitably, catch-up growth may be determined using height velocity or height standard deviation score (see e.g. Frongillo, E.A., Leroy, J.L. and Lapping, K., 2019. Advances in Nutrition, 10(3), pp.372-379 and Desmond, C. and Casale, D., 2017. PloS one, 12(12), p.e0189135).

In some embodiments, the catch-up growth is determined in absolute terms of linear growth (i.e. the height deficit from the healthy reference population mean is reduced). In some embodiments, the catch-up growth is determined in relative terms of linear growth (i.e. the height-for-age z-score is improved and/or passes the -2SD or -1SD cut-off points).

Individual

The individual may be any suitable individual. Suitably, the individual may be a mammal. In preferred embodiments, the individual is a human. In other embodiments, the individual is an

animal, preferably wherein the animal is a pet. A pet may be an animal selected from dogs, cats, birds, fish, rodents such as mice, rats, and guinea pigs, rabbits, etc. In some embodiments, the pet is a small dog breed.

- 5 In some embodiments, the individual is an elderly or older adult. In another embodiment, the individual is menopausal or perimenopausal individual, in particular menopause or perimenopausal woman.

The term “elderly” in the context of a human means an age from birth of at least 60 years, preferably above 63 years, more preferably above 65 years, and most preferably above 70 years.

- 10 The term “older adult” in the context of a human means an age from birth of at least 45 years, preferably above 50 years, more preferably above 55 years, and includes elderly individuals. The term “older adult” in the context of a human means an age from birth of at least 45 years, preferably above 50 years, more preferably above 55 years, and includes elderly individuals.

- 15 In some embodiments, the individual is a juvenile, an adolescent, a child. The term “juvenile” may refer to an individual that has not yet reached adulthood. The term “adolescent” may refer to an individual during the period from the onset of puberty to adulthood. The term “child” may refer to an individual between the stages of birth and puberty.

- 20 In preferred embodiments, the individual is about 3 years of age or older. In some embodiments, the individual is about 4 years of age or older or about 5 years of age or older. In preferred embodiments, the individual is about 10 years of age or younger. In some embodiments, the individual is about 9 years of age or younger, about 8 years of age or younger, about 7 years of age or younger, about 6 years of age or younger, or about 5 years of age or younger.

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In some embodiments, the individual is about 3 years to about 10 years of age, about 3 years to about 9 years of age, about 3 years to about 8 years of age, about 3 years to about 7 years of age, about 3 years to about 6 years of age, or about 3 years to about 5 years of age.

- 30 The present invention is particularly suitable for children who were born preterm or with low-birth weight or experienced intra-uterine growth retardation or who suffered from growth stunting because of malnutrition or experienced disease such as Crohn’s disease and/or celiac

disease and/or cancer or who were treated with drugs leading to malabsorption, anorexia and/or metabolic bone disease, such as chemotherapy drugs and/or corticosteroids. The present invention is particularly preferred for use in children who were born preterm or with low-birth weight or experienced intra-uterine growth retardation, or with intra-uterine malnutrition or who
5 suffered growth delay. The present invention is also suitable for children at risk of bone disease, having a family history of bone disease, or having already experienced at least one, preferably several, episode(s) of fracture.

In some embodiments, the individual suffered from and/or is suffering from stunted growth. The
10 definition of stunting may refer to the "height for age" value to be less than two standard deviations of the WHO Child Growth Standards median (see e.g. De Onis, M. and Branca, F., 2016. Maternal & child nutrition, 12, pp.12-26).

In some embodiments, the individual suffered from and/or is suffering from faltering growth. The
15 term "faltering growth" may describe a pattern of slower weight gain than expected for age and sex in children and other adolescents (see e.g. King, C. and Davis, T., 2010. European journal of clinical nutrition, 64(1), pp.S11-S13). In some embodiments, the individual suffered from and/or is suffering from growth stunting and/or faltering growth because of malnutrition or experienced disease such as anorexia, Crohn's disease and/or celiac disease. In some embodiments, the
20 individual suffered from and/or is suffering from growth stunting and/or faltering growth because of treatment with drugs leading to malabsorption, anorexia and/or metabolic bone disease, such as chemotherapy drugs and/or corticosteroids.

In some embodiments, the individual was born preterm or with low-birth weight or experienced
25 intra-uterine growth retardation. The term "preterm infant" may refer to an infant born at least than 37 weeks gestational age. The term "low birth weight infant" may refer to an infant having a live-born weight less than 2,500 g.

Ingredients- main bioactive compounds

30 The oleuropein and fisetin are the main bioactive molecules according to present invention.

Oleuropein is a polyphenol found in the fruit, the roots, the trunk and more particularly in the leaves of plants belonging to the Oleaceae family, and especially *Olea europaea*.

In an embodiment, at least a portion of the oleuropein is obtained by extraction, e.g., by
5 extraction from a plant such as a plant belonging to the Oleaceae family, preferably one or more of the stems, the leaves, the fruits or the stones of a plant belonging to the Oleaceae family such as *Olea europaea* (olive tree), a plant of genus *Ligustrum*, a plant of genus *Syringa*, a plant of genus *Fraxinus*, a plant of genus *Jasminum* and a plant of genus *Osmanthus*. Additionally or alternatively, at least a portion of the oleuropein and / or metabolites can be obtained by
10 chemical synthesis.

Non-limiting examples of suitable metabolites of oleuropein include oleuropein aglycone, hydroxytyrosol, elenolic acid, homovanillyl alcohol, isohomovanillyl alcohol, glucuronidated forms thereof, sulfated forms thereof, derivatives thereof, and mixtures thereof.

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Fisetin (7,3',4'-flavon-3-ol) (see Fig 1) is a polyphenol found in many plants, where it serves as a yellow/ochre colouring agent. It is also found in many fruits and vegetables, such as strawberries, apples, persimmons, grape, onions and cucumbers.

20 In an embodiment, at least a portion of the fisetin is obtained by known means, e.g., by extraction from a plant/vegetable/fruit source of fisetin. Additionally or alternatively, at least a portion of the fisetin and/or metabolites can be obtained by chemical synthesis.

Non-limiting examples of suitable metabolites of fisetin include glucuronidated forms thereof,
25 sulfated forms thereof, derivatives, and mixtures. In a preferred embodiment, the derivative is gerardol.

The fisetin may be from any suitable source and may be isolated and/or chemically synthesized. In a preferred embodiment oleuropein and fisetin and derivatives are obtained from plant
30 sources. For example, oleuropein may be obtained from olive plants, For example, fisetin may be obtained from strawberries, apples, persimmons, grape, onions, cucumbers and others.

The effective amount of each of the oleuropein and/or metabolite thereof and fisetin and/or derivative thereof varies with the particular composition, the age and condition of the recipient, and the particular disorder or disease being treated. Nevertheless, in a general embodiment, 0.001 mg to 1.0 g can be administered to the individual per day, preferably from 0.01 mg to 0.9 g
5 per day, more preferably from 0.1 mg to 750 mg per day, more preferably from 0.5 mg to 500 mg per day, and most preferably from 1.0 mg to 200 mg per day. Moreover, the inventors found that the active dose of oleuropein or derivative in the combination, may be lowered for an equal efficacy.

10 In some embodiments, the combination of oleuropein or metabolite and the fisetin or derivative is administered in a composition further comprising calcium. At least a portion of the calcium can be one or more calcium salts, such as calcium acetate, calcium carbonate, calcium chloride, calcium citrate, calcium glutubionate, calcium gluconate, calcium lactate or mixtures thereof. In a general embodiment, 0.1 g to 1.0 g of the calcium is administered to the individual per day,
15 preferably from 125 mg to 950 g of the calcium per day, more preferably from 150 mg to 900 mg of the calcium per day, more preferably from 175 mg to 850 mg of the calcium per day, and most preferably from 200 mg - 800 mg of the calcium per day.

In an alternative embodiment, the combination of oleuropein and fisetin can be administered
20 sequentially with calcium in separate compositions. The term "sequentially" means that the calcium and the at least one of oleuropein or metabolite thereof are administered in a successive manner such that the at least one of oleuropein or metabolite thereof is administered at a first time without the calcium, and the calcium is administered at a second time (before or subsequent to the first time) without the combination of oleuropein and fisetin. The time between sequential
25 administrations may be, for example, one or several seconds, minutes or hours in the same day; one or several days or weeks in the same month; or one or several months in the same year.

The at least one oleuropein or metabolite thereof and fisetin or derivative may be formulated in a particular ratio. In some embodiments, the formulation may comprise these components in the
30 following exemplary ratios: 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:20 and each of these ratios can be Fisetin: OLE in some embodiments and OLE: Fisetin in other embodiments. Preferably, the ratio OLE: fisetin is between 1:1 to 1:10.

In some embodiments, the oleuropein or metabolite thereof and the fisetin or derivative thereof are the only polyphenols in the composition and/or the only polyphenols administered to the individual.

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The composition can comprise an effective amount of at least one of oleuropein or metabolite thereof. For example, a single serving or dose of the composition can comprise the effective amount, and a package can contain one or more of the servings or doses. Optionally the composition can further comprise calcium.

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In another embodiment, the oleuropein and/or derivative can be provided by any of the compositions and methods disclosed by WO 2019/092068 and WO 2019/092066, each entitled “Bioconversion of oleuropein” and “Method of selecting a probiotic”, and WO 2019/092069 entitled “Homovanillyl alcohol (HVA), HVA isomer, methods of making compositions comprising
15 such compounds, and methods of using such compounds”, each incorporated herein by reference in its entirety.

Ingredients- further bioactive compound

20 The compositions for use according to the invention may also comprise at least one further bioactive compound selected from the group consisting of antioxidants, anti-inflammatory compounds, glycosaminoglycans, prebiotics, fibres, probiotics, fatty acids, enzymes, minerals, trace elements and/or vitamins.

25 The term “bioactive” in the context of the present application means that the compound contributes to the health of an individual, or has an effect on the human body, beyond that of meeting basic nutritional need.

The at least one further bioactive compound may be from a natural source. Thus the compounds
30 may be from extracts of plants, animals, fish, fungi, algae, microbial fermentation. Minerals are considered as from natural source also within this definition.

In a preferred embodiment, enzymes may be proteases such as trypsin, or enzyme extracts such as bromelain, for example.

Nutritional compositions

5 The compositions for use according to the invention may be nutritional compositions or pharmaceutical compositions, and may be for human or veterinary use. Thus, in preferred embodiments, the composition for use according to the invention is a nutritional composition. By "nutritional composition" is meant in the context of the present application a composition which is a source of nutrition to an individual.

10

The nutritional products or compositions of the invention may be a source of complete nutrition or may be a source of incomplete nutrition. As used herein, "complete nutrition" includes nutritional products and compositions that contain sufficient types and levels of macronutrients (protein, fats and carbohydrates) and micronutrients to be sufficient to be a sole source of

15 nutrition for the animal to which it is being administered to. Patients can receive 100% of their nutritional requirements from such complete nutritional compositions.

As used herein, "incomplete nutrition" includes nutritional products or compositions that do not contain sufficient levels of macronutrients (protein, fats and carbohydrates) or micronutrients to be sufficient to be a sole source of nutrition for the animal to which it is being administered to.

20 Partial or incomplete nutritional compositions can be used as a nutritional supplement.

The combination of oleuropein and fisetin can be administered in any composition that is suitable for human and/or animal consumption. In a preferred embodiment, it is administered to the individual orally or enterally (e.g. tube feeding). For example, it can be administered to the

25 individual in a beverage, a food product, a capsule, a tablet, a powder or a suspension.

Non-limiting examples of suitable compositions for the include food compositions, dietary supplements, dietary supplements (e.g., liquid ONS), complete nutritional compositions, beverages, pharmaceuticals, oral nutritional supplement, medical food, nutraceuticals, food for special medical purpose (FSMP), powdered nutritional products to be reconstituted in water or

30 milk before consumption, food additives, medicaments, drinks, petfood, and combinations thereof.

Nutritional composition ingredients*Protein source*

In an embodiment, the compositions for use according to the invention include a source of protein. The protein source may be dietary protein including, but not limited to animal protein
5 (such as milk protein, meat protein or egg protein), vegetable protein (such as soy protein, wheat protein, rice protein, and pea protein), or combinations thereof. In an embodiment, the protein is selected from the group consisting of whey, chicken, corn, caseinate, wheat, flax, soy, carob, pea or combinations thereof.

Carbohydrate source

10 In an embodiment, the compositions include a source of carbohydrates. Any suitable carbohydrate may be used in the present compositions including, but not limited to, starch, sucrose, lactose, glucose, fructose, corn syrup solids, maltodextrin, modified starch, amylose starch, tapioca starch, corn starch, xylitol, sorbitol or combinations thereof.

Fat source

15 In an embodiment, the compositions include a source of fat. The source of fat may include any suitable fat or fat mixture. For example, the fat source may include, but is not limited to, vegetable fat (such as olive oil, corn oil, sunflower oil, high-oleic sunflower, rapeseed oil, canola oil, hazelnut oil, soy oil, palm oil, coconut oil, blackcurrant seed oil, borage oil, lecithins, and the like), animal fats (such as milk fat), or combinations thereof. The source of fat may also be less
20 refined versions of the fats listed above (e.g., olive oil for polyphenol content).

Flavourings etc.

In addition, compositions for use according to the invention may also comprise natural or artificial flavours, for example fruit flavours like banana, orange, peach, pineapple or raspberry or other plant flavours like vanilla, cocoa, coffee, etc.

25 Nutritional composition formats

The nutritional compositions may include, besides the main bioactive components and any further bioactive components, and optionally one or more of a protein, carbohydrate and fat source, any number of optional additional food ingredients, including conventional food additives
30 (synthetic or natural), for example one or more acidulants, additional thickeners, buffers or

agents for pH adjustment, chelating agents, colorants, emulsifiers, excipient, flavor agent, mineral, osmotic agents, a pharmaceutically acceptable carrier, preservatives, stabilizers, sugar, sweeteners, texturizers, and/or vitamins. The optional ingredients can be added in any suitable amount.

5

The nutritional composition may be provided in any suitable format. Examples of nutritional composition formats in which the composition for use according to the invention may be provided include solutions, ready-for-consumption compositions (e.g. ready-to-drink compositions or instant drinks), liquid comestibles, soft drinks, juice, sports drinks, milk drinks, milk-shakes, yogurt drinks, soup, etc.

10

In another embodiments, the nutritional compositions may be provided in the form of a concentrate, a powder, or granules (e.g. effervescent granules), which are diluted with water or other liquid, such as milk or fruit juice, to yield the ready-for-consumption composition. Further nutritional composition formats include, baked products, dairy products, desserts, confectionery products, cereal bars, and breakfast cereals. Examples of dairy products include milk and milk drinks, yoghurts and other cultured milk products, ice creams and cheeses. Examples of baked products include bread, biscuits and cakes.

15

In one embodiment, the composition for use according to the invention may also be available in a great variety of formats designed as animal foods, in particular for the dog or the cat, whether in a wet form, semi-wet form or dry form, in particular in the form of biscuits.

20

Routes of administration

The nutritional compositions of the present disclosure may be administered by any means suitable for human administration, and in particular for administration in any part of the gastrointestinal tract. In a preferred embodiment, the composition is for oral administration, preferably wherein the combination is administered simultaneously.

25

Enteral administration, oral administration and administration through a tube or catheter are all covered by the present disclosure. The nutritional compositions may also be administered by means selected from oral, rectal, sublingual, sublabial, buccal, topical, etc.

30

The nutritional compositions may be administered in any known form including, for example, tablets, capsules, liquids, chewables, soft gels, sachets, powders, syrups, liquid suspensions, emulsions and solutions in convenient dosage forms. In soft capsules, the active ingredients are preferably dissolved or suspended in suitable liquids, such as fatty oils, paraffin oil or liquid

5 polyethylene glycols. Optionally, stabilizers may be added.

If the nutritional compositions are administered by tube feeding, the nutritional compositions may be used for short term or long term tube feeding.

Method of manufacturing a nutritional composition of the invention

10 The invention relates in a further aspect to a method for manufacturing a nutritional composition for use according to the invention, said method comprising the step of:

- providing ingredients for a nutritional composition comprising a combination of oleuropein and/or metabolite thereof and fisetin and/or derivative thereof, and mixing, such that the nutritional composition comprises the combination of oleuropein and/or

15 metabolite thereof and fisetin and/or derivative thereof.

Pharmaceutical composition for use.

In a further embodiment, the invention relates to a composition for use to inhibit or prevent
20 cartilage degeneration according to the invention, wherein the composition is a pharmaceutical composition. By pharmaceutical means a composition, other than a nutritional composition, wherein a substance is used on or in the body to prevent, diagnose, alleviate, treat, or cure a disease in humans or animals in medicine. According to the present invention, the pharmaceutical may be used for inhibiting or decreasing cartilage degeneration.

25

The pharmaceutical may be for use by a human. It may alternatively be a veterinary composition, for example suited for a dog, cat, or horse, in particular a thoroughbred horse.

In one preferred embodiment, the pharmaceutical composition of the invention comprises a combination of oleuropein or metabolite thereof and fisetin or derivative.

In another preferred embodiment, the pharmaceutical composition of the invention comprises oleuropein or metabolite thereof and fisetin or derivative.

The invention further relates to uses of the pharmaceutical according to the invention, as described herein as use of the compositions of the invention.

5

A pharmaceutical composition for use according to the invention comprising a combination of oleuropein or metabolite thereof and fisetin or derivative in combination with at least one excipient selected from the group constituted by the pharmaceutically acceptable excipients.

Procedures for the preparation of pharmaceutical compositions according to the invention can

10 easily be found by the specialist skilled in the art, for example in the handbook Remington's Pharmaceutical Sciences, Mid. Publishing Co, Easton, Pa., USA. Physiologically acceptable excipients, vehicles and adjuvants are also described in the handbook entitled "Handbook of Pharmaceutical Excipients, Second edition, American Pharmaceutical Association, 1994. In order to formulate a pharmaceutical composition according to the invention, the specialist skilled in the

15 art will advantageously be able to refer to the latest edition of the European Pharmacopoeia or the Pharmacopoeia of the United States of America (USP). The specialist skilled in the art will in particular be able advantageously to refer to the fourth edition "2002" of the European Pharmacopoeia or also to the edition USP 25-NF 20 of the American Pharmacopoeia (U.S. Pharmacopoeia).

20

Advantageously, a pharmaceutical composition such as defined above is suitable for oral, parenteral or intravenous administration. When the pharmaceutical composition for use according to the invention comprises at least one pharmaceutically or physiologically acceptable excipient, it is in particular an excipient appropriate for administration of the composition by the

25 oral route or an excipient suitable for administration of the composition by the parenteral route.

A pharmaceutical composition for use according to the invention is available indifferently in a solid or liquid form. For oral administration, a solid pharmaceutical composition in the form of tablets, capsules or gelatine capsules will be preferred.

30 In liquid form, a pharmaceutical composition in the form of an aqueous or non-aqueous suspension, or also in the form of a water-in-oil or oil-in-water emulsion will be preferred.

Solid pharmaceutical forms may comprise, as vehicles, adjuvants or excipients, at least one diluent, one flavour, one solubilising agent, one lubricant, one suspension agent, one binder, one disintegrating agent and one encapsulating agent. Such compounds are for example magnesium carbonate, magnesium stearate, talc, lactose, pectin, dextrin, starch, gelatine, cellulosic materials, 5 cocoa butter, etc. The compositions in liquid form may also comprise water, possibly as a mixture with propylene glycol or polyethylene glycol, and possibly also colouring agents, flavours, stabilisers and thickening agents.

The present disclosure also provides a kit comprising a combination of oleuropein and/or a 10 metabolite thereof and fisetin and/or a derivative in one or more containers. In an embodiment of the kit, the one or more containers comprise at least one first container that stores the oleuropein and/or metabolite separately from the fisetin and/or derivative, which is stored in at least one second container, and the kit further comprises instructions for admixing the oleuropein with the fisetin into a unit dosage form.

15

In an embodiment of the kit, the combination can be provided together in one or more prepackaged unit dosage forms, for example in separate containers that each contain a dried powder such that each container contains one prepackaged unit dosage form.

In another embodiment, the kit can comprise a plurality of compositions for admixing together to 20 form one or more of the compositions disclosed herein. For example, the kit can contain two or more dried powders in separate containers relative to each other, the separate powders each containing a portion of the final unit dosage form. As a non-limiting example of such an embodiment, the kit can contain one or more first containers that house the oleuropein and can also contain one or more second containers that house the fisetin. The content of one of the first 25 containers can be admixed with one of the second containers to form at least a portion of the unit dosage form of the composition.

The above examples of administration do not require continuous daily administration with no interruptions. Instead, there may be some short breaks in the administration, such as a break of 30 two to four days during the period of administration. The ideal duration of the administration of the composition can be determined by those of skill in the art.

Combination of disclosures

It should be noted that embodiments and features described in the context of one of the aspects of the present invention also apply to the other aspects of the invention.

5

The compositions for use according to the invention are herein described in different parameters, such as the ingredients, nutritional composition formats, uses, target groups etc. It should be noted that embodiments and features described in the context of one of the parameters of the composition for use according to the invention, may also be combined with other embodiments

10 and features described in the context of another parameter, unless expressly stated otherwise.

All patent and non-patent references cited in the present application, are hereby incorporated by reference in their entirety.

The invention will now be described in further details in the following non-limiting examples.

15

EXAMPLE

The following non-limiting example present experimental data supporting the compositions and methods disclosed herein.

To test the effect of oleuropein (or oleuropein aglycone), fisetin and their combination in living
20 cells, the inventors measured mitochondrial calcium elevation in HeLa cells. HeLa cells were purchased from ATCC. HeLa cells were seeded in 96-well plates at a density of 50000 cells per well in minimal essential medium (DMEM, Gibco), high glucose, + 10% fetal calf serum. Mitochondrial calcium measurements were carried out using HeLa cells infected with the adenovirus (from Sirion biotech) expressing the mitochondrially targeted calcium sensor mitochondrial mutated aequorin
25 (Montero et al., 2004). For aequorin reconstitution, 24 hours after infection, cells were incubated for 2 h at room temperature (22 °C) in standard medium (145 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, 10 mM glucose and 10 mM Hepes, pH 7.4) with 1 μM wild-type coelenterazine. For treatment, compounds were directly added to the cell culture or myotubes cultures 2 hours before measurements. Luminescence was measured at the FLIPR Tetra Aequorin (Molecular Devices).
30 Mitochondrial calcium rise was obtained by stimulating the cells with 100 μM histamine. Calibration of the luminescence data into calcium concentration was carried out using an algorithm,

as described previously (Alvarez & Montero, 2002). Custom module analysis based on Excel (Microsoft) and GraphPad Prism 7.02 (GraphPad) software was used for quantification.

As shown in **FIG. 2** the effect of the combination of Oleuropein with Fisetin is greater than the effect of Fisetin or Oleuropein alone on mitochondrial activation, via mitochondrial Ca^{2+} rise, in
5 HeLa cells. Also, **FIG. 3**, shows that fisetin synergizes with Oleuropein to activate mitochondria, via mitochondrial Ca^{2+} rise, in HeLa cells.

Claims

1. Composition comprising an effective amount of a combination of oleuropein and/or metabolite thereof and fisetin and/or derivative for use to prevent or treat bone disorders or maintenance of bone health in an individual.
- 5 2. The composition according to Claim 1, characterised in that said composition is suitable for oral administration.
3. The combination for use according to any preceding claim, wherein the individual is a human or an animal, preferably wherein the animal is a pet.
4. The composition for use according to any preceding claim, wherein the metabolite of
10 oleuropein is selected from the group consisting of oleuropein aglycone, hydroxytyrosol, elenolic acid, homovanillyl alcohol, isohomovanillyl alcohol, glucuronidated forms thereof, sulfated forms thereof, derivatives thereof, and mixtures thereof.
5. The composition for use according to any preceding claim, wherein the metabolite of fisetin is selected from the group consisting of glucuronidated forms thereof, sulfated forms
15 thereof, derivatives and mixtures thereof, preferably gerardol.
6. The composition for use according to any preceding claim, further comprising calcium.
7. The composition for use according to any preceding claim, wherein the composition further comprises at least one compound selected from the group consisting of antioxidants, anti-inflammatory compounds, glycosaminoglycans, prebiotics, fibres, probiotics, fatty acids,
20 enzymes, minerals, trace elements and/or vitamins.
8. The composition for use according to any preceding claim, wherein the composition is selected from the group consisting of food compositions, dietary supplements, nutritional compositions, oral nutritional supplement, medical food, nutraceuticals, beverages, powdered nutritional products to be reconstituted in water or milk before consumption, food
25 additives, food for special medical purpose (FSMP) medicaments, drinks, petfood, and combinations thereof.
9. The composition for use according to any preceding claim, wherein the composition is in a form of a solid powder, a powdered stick, a capsule or a solution.

10. The composition for use according to any preceding claim, wherein the use is for i) stimulating bone formation and/or inhibiting bone resorption; ii) improving bone quality; iii) preventing or treating disorders linked to an imbalance in the relationship between bone formation and bone resorption.
- 5 11. The composition for use according to any preceding claim, characterised in that said composition is designed to prevent the bone loss which occurs with aging and/or to protect bone cells during bone aging.
12. The composition for use according to any preceding claim, characterised in that said composition is designed to treat a bone deficit resulting from a fracture.
- 10 13. The composition for use according to any preceding claim, characterised in that said composition is designed to prevent or treat diseases selected from osteoporosis, Paget's disease, bone loss or osteolysis observed close to a prosthesis, metastatic bone diseases, hypercalcemia due to a cancer, multiple myelomas, periodontal diseases or osteoarthritis.
- 15 14. The composition for use according to any preceding claim, characterised in that said composition is designed to i) stimulate bone formation in a young individual in the growth phase; ii) enhance bone quality, bone growth and/or bone strength in a child or juvenile individual
15. The combination for use according to any preceding claim, wherein the individual suffered from and/or is suffering from stunted growth and/or faltering growth.
- 20 16. The combination for use according to any preceding claim, wherein the combination promotes catch-up growth, preferably wherein catch-up growth is determined using height velocity.
17. A method of manufacturing a nutritional composition for use according to any of the preceding claims, comprising the steps of providing one or more ingredients for a nutritional
- 25 composition, oleuropein or metabolite thereof and fisetin and/or derivative and optionally further calcium, and mixing.