

COMPOSITIONS AND METHODS FOR MAINTAINING BONE HEALTH OR REDUCING BONE LOSS.

FIELD OF THE INVENTION

[0001] The invention relates to uses of a water soluble extract of rosehip for the maintenance
5 or improvement of bone health and to treat or prevent bone disorders characterised by weakened or
fragile bones. The invention also relates to compositions comprising a water soluble extract of
rosehip useful for the maintenance or improvement of bone health and to treat or prevent bone
disorders characterised by weakened or fragile bones.

BACKGROUND TO THE INVENTION

10 [0002] As the world population ages, bone disorders are becoming more prevalent. These
disorders, such as osteopenia and osteoporosis, are costly both in financial terms and the toll the
disease takes on the quality of life of the sufferer.

[0003] Bone comprises an extracellular protein matrix (osteoid) interspersed with bone cells
(osteocytes) with a mineral component laid within the extracellular matrix consisting of calcium salts
15 and other minerals. Bone undergoes remodelling which is the process of resorption where bone is
broken down by osteoclasts and then replaced by osteoblasts (reformation). Remodelling occurs to
regulate calcium homeostasis, repair bone that has been damaged during everyday stress and to
shape bone during growth or changes in mechanical stress patterns.

[0004] Osteoclasts degrade bone at a particular area and then undergo apoptosis. Osteoblasts
20 rebuild new bone and mediate its remineralisation. During remineralisation, some osteoblasts are
encased within the calcified material and become osteocytes.

[0005] Once peak bone mass has been reached in early adulthood, the process of resorption is
nearly perfectly coupled with reformation. However, as a person ages the remodeling system is less
effectively regulated. With the onset of menopause in women, the two processes may become
25 desynchronised with resorption being dominant. Additionally, with ageing there can be losses in
bone mineral density, disruptions in bone microarchitecture and other changes which result in
increased fracture risk. Often the process of bone loss is gradual without clear symptoms presenting
until the disease is well advanced.

[0006] It is therefore important to maintain bone quality and density over time so that bone
30 does not become weakened and fragile with ageing.

[0007] Osteopenia is a condition where bone mineral density is lower than normal and has been considered by some to be a precursor to osteoporosis. However, not every person diagnosed with osteopenia will develop osteoporosis. Osteopenia is defined as a bone mineral density T score 1-2.5 standard deviations below peak bone mass (20-year-old healthy female average) as measured by DXA.

[0008] Osteoporosis is characterised by a gradual thinning and weakening of the bones which without treatment can lead to weakness of the skeleton and an increased risk of fracture.

Osteoporosis is defined as a bone mineral density 2.5 standard deviations below peak bone mass (20-year-old healthy female average) as measured by DXA.

[0009] The pathology of osteopenia and osteoporosis is an imbalance between the process of breaking down the bone (resorption) and building up the bone (reformation) by osteoclasts and osteoblasts. In a healthy individual, this process of bone resorption and bone reformation is almost perfectly balanced. If this balance is disrupted due to various reasons i.e. menopause, drugs etc, the break down of bone eventually overtakes the build up of bone and osteopenia or the more severe osteoporosis may develop.

[0010] Women are generally at a greater risk of developing osteopenia and osteoporosis because of the reduction of oestrogen production following menopause. However, elderly men and people with particular hormone disorders, or long term users of particular medications, are also susceptible to osteopenia and osteoporosis.

[0011] The treatment of bone disorders, like osteoporosis, is generally unsatisfactory, resulting in the patient suffering from side effects from the therapies prescribed to treat the bone disorder.

[0012] Osteopenia is not often diagnosed, and if diagnosed, is generally not medically treated due to the cost and length of treatment. Because sufferers of osteopenia are generally younger than people diagnosed with osteoporosis they require therapy for many years. The cost/benefit ratio of such a long term treatment is unknown and therefore osteopenia is generally not treated medically.

[0013] Various drugs are currently prescribed for the treatment of osteoporosis. Bisphosphonates are often prescribed in cases where the patient is confirmed to be suffering from osteoporosis. However, oral bisphosphonates are poorly absorbed and must be taken on an empty stomach. Additionally, they can be poorly tolerated in some people and are associated with esophagitis.

[0014] Other drugs prescribed for osteoporosis such as teriparatide and strontium ranelate are also not well tolerated by some people. Teriparatide must be given by injection and is not suitable for patients who are young, have had previous radiation therapy, or suffer from Paget's disease. Strontium ranelate has fewer side effects than bisphosphonates but has been associated with increasing the risk of a venous thromboembolism. Additionally, strontium ranelate is taken up into the bone matrix in place of calcium which results in a disproportionate increase in bone mineral density measured on DXA scanning.

[0015] There is therefore a need to provide a therapy that maintains or improves bone health, that overcomes or ameliorates at least one of the problems associated with known treatments, or that at least provides the public with a useful choice.

SUMMARY OF THE INVENTION

[0016] Accordingly, in a first aspect, the present invention relates to use of a water soluble extract of rosehip in the manufacture of a composition for treating or preventing a bone condition characterised by weakened or fragile bones.

[0017] In another aspect, the present invention relates to a composition comprising a water soluble extract of rosehip for treating or preventing a bone condition characterised by weakened or fragile bones.

[0018] In one embodiment the composition further comprises one or more agents selected from calcium, magnesium, zinc, vitamin D, vitamin K, folic acid or folate, vitamin B6 and vitamin B12.

[0019] In another aspect the present invention relates to a method for treating or preventing a bone condition characterised by weakened or fragile bones comprising administering a composition comprising an effective amount of a water soluble extract of rosehip to a subject in need thereof.

[0020] In another aspect the present invention relates to a method for treating or preventing osteoporosis comprising administering a composition comprising an effective amount of a water soluble extract of rosehip to a subject in need thereof.

[0021] In another aspect the present invention relates to a method for treating or preventing osteopenia comprising administering a composition comprising an effective amount of a water soluble extract of rosehip to a subject in need thereof.

[0022] In one embodiment the subject is need of maintained or increased bone formation, maintained or increased bone mineral density, maintained or increased bone mass (including peak bone mass), bone regeneration during fracture healing, reduced bone resorption, decreased bone loss, or maintained or increased bone strength.

5 [0023] The following embodiments may relate to any of the above aspects.

[0024] In one embodiment the composition does not include any of blueberry, blackberry, elderberry, cranberry, rosemary, clove, feverfew, nettle root, artichoke, reishi mushroom, olive extract, green tea extract, grape seed extract, resveratrol, viniferin, Aframomum melegueta, boswellia serrata extract, boswellia forte, ipriflavone, tocotrienols, evening primrose oil, INM-176, borage oil,
10 krill oil, at least one type of xanthophyll (e.g., astaxanthin), green coffee extract or ferulic acid.

[0025] In one embodiment the condition to be treated is osteoporosis or osteopenia.

[0026] In one embodiment the composition maintains or increases bone formation, maintains or increases bone density, maintains or increases bone mass, stimulates bone regeneration during fracture healing, reduces bone resorption, decreases bone loss, or maintains or increases bone
15 strength.

[0027] In one embodiment the composition is a food, confectionary, milk, milk product, milk powder, reconstituted milk, cultured milk, drinking yoghurt, set yoghurt, drink, food additive, drink additive, dietary supplement, nutritional product, medical food, nutraceutical or a pharmaceutical.

[0028] In one embodiment a composition useful in the invention further comprises one or
20 more dairy ingredients. The dairy ingredient may be selected from the group comprising recombined, powdered or fresh skim milk, reconstituted whole or skim milk powder, skim milk concentrate, skim milk retentate, concentrated milk, ultrafiltered milk retentate, milk protein concentrate, milk protein isolate, calcium depleted milk protein concentrate, low fat milk, low fat milk protein concentrate, casein, caseinate, milk fat, high CLA milk fat, cream, butter, anhydrous
25 milk fat, butter milk, butter serum, hard milk fat fractions, soft milk fat fractions, sphingolipid fractions, milk fat globular membrane fractions, phospholipid fractions, complex lipid fractions, colostrum, a colostrum fraction, colostrum protein concentrate, colostrum whey, an immunoglobulin fraction from colostrum, whey, lactoferrin, one or more lactoferrin fragments, whey protein isolate, whey protein concentrate, sweet whey, lactic acid whey, mineral acid whey,
30 reconstituted whey powder, milk minerals, a composition derived from any milk or colostrum processing stream, a composition derived from the retentate or permeate obtained by ultrafiltration or microfiltration of any milk or colostrum processing stream, a composition derived from the

breakthrough or adsorbed fraction obtained by chromatographic (including but not limited to ion and gel permeation chromatography) separation of any milk or colostrum processing stream, extracts of any of these ingredients including extracts prepared by multistage fractionation, differential crystallisation, solvent fractionation, supercritical fractionation, near supercritical
5 fractionation, distillation, centrifugal fractionation, or fractionation with a modifier (e.g. soaps or emulsifiers), hydrolysates of any of these ingredients, fractions of the hydrolysates, and any combination of any two or more of these ingredients, including combinations of hydrolysed fractions, combinations of non-hydrolysed fractions, and combinations of hydrolysed and non-hydrolysed fractions.

10 [0029] In one embodiment the amount of the water soluble extract of rosehip administered is about 1 mg to about 2000 mg per kg body weight, about 100 to about 1000 mg per kg body weight, about 50 to about 500 mg per kg body weight, or about 0.05 mg to about 300 mg per kg body weight per day.

[0030] In one embodiment calcium is calcium or a calcium salt. In one embodiment
15 magnesium is magnesium or a magnesium salt. In one embodiment zinc is zinc or a zinc salt. In one embodiment vitamin D is vitamin D or a vitamin D derivative (including but not limited to vitamin D1 [lamisterol], vitamin D2 [ergocalciferol], vitamin D3 [cholecalciferol, 1,25-dihydroxycholecalciferol], vitamin D4 [dihydrotachysterol], vitamin D5 [7-dehydrositosterol]), or a vitamin D analog. In one embodiment folic acid is folic acid or a folic acid salt i.e. folate or a
20 derivative thereof. In one embodiment vitamin B12 is vitamin B12 or a derivative thereof.

[0031] Salts useful herein include but are not limited to ammonium (NH_4^+), boron, calcium, copper, iron (ferrous, Fe^{2+} and ferric, Fe^{3+}), magnesium, manganese, phosphorus, potassium, pyridinium ($\text{C}_5\text{H}_5\text{NH}^+$), quaternary ammonium (NR_4^+), silicon, sodium, strontium, and zinc salts, or a combination thereof.

25 [0032] In one embodiment a composition useful herein further comprises a pharmaceutically acceptable carrier. In another embodiment the composition is, or is formulated as, a food, drink, food additive, drink additive, dietary supplement, nutritional product, medical food, nutraceutical, medicament, pharmaceutical, enteral or parenteral feeding product or meal replacement. In one embodiment the composition is in the form of a tablet, a caplet, a pill, a hard or soft capsule or a
30 lozenge. In one embodiment the composition is in the form of a cachet, a dispensable powder, granules, a suspension, an elixir, a liquid, or any other form that can be added to food or drink, including for example water, milk or fruit juice. In one embodiment the composition further comprises one or more constituents (such as antioxidants) which prevent or reduce degradation of

the composition during storage or after administration. These compositions may include any edible consumer product which is able to carry a water soluble extract of rosehip. Examples of suitable edible consumer products include aqueous products, baked goods, confectionary products including chocolate, gels, ice creams, reconstituted fruit products, snack bars, food bars, muesli bars, spreads, sauces, dips, dairy products including yoghurts and cheeses, drinks including dairy and non-dairy based drinks, milk, milk powders, sports supplements including dairy and non-dairy based sports supplements, fruit juice, food additives such as protein sprinkles and dietary supplement products including daily supplement tablets. Suitable nutraceutical compositions useful herein may be provided in similar forms.

10 [0033] In one embodiment the composition comprises or the method comprises administration of agents such as rosehip, calcium, magnesium, zinc, vitamin D, vitamin K, folic acid or folate, vitamin B6, vitamin B12, a dairy ingredient or a pharmaceutical agent. In one embodiment a composition useful herein comprises, consists essentially of, or consists of at least about 0.01, 0.02, 0.05, 0.07, 0.1, 0.2, 0.5, 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99, 15 99.5, 99.8 or 99.9% by weight of one or more of these agents and useful ranges may be selected between any of these foregoing values (for example, from about 0.1 to about 50%, from about 0.2 to about 50%, from about 0.5 to about 50%, from about 1 to about 50%, from about 5 to about 50%, from about 10 to about 50%, from about 15 to about 50%, from about 20 to about 50%, from about 25 to about 50%, from about 30 to about 50%, from about 35 to about 50%, from about 40 to about 50%, from about 45 to about 50%, from about 0.1 to about 60%, from about 0.2 to about 60%, from about 0.5 to about 60%, from about 1 to about 60%, from about 5 to about 60%, from about 10 to about 60%, from about 15 to about 60%, from about 20 to about 60%, from about 25 to about 60%, from about 30 to about 60%, from about 35 to about 60%, from about 40 to about 60%, from about 45 to about 60%, from about 0.1 to about 70%, from about 0.2 to about 70%, from about 0.5 to about 70%, from about 1 to about 70%, from about 5 to about 70%, from about 10 to about 70%, from about 15 to about 70%, from about 20 to about 70%, from about 25 to about 70%, from about 30 to about 70%, from about 35 to about 70%, from about 40 to about 70%, from about 45 to about 70%, from about 0.1 to about 80%, from about 0.2 to about 80%, from about 0.5 to about 80%, from about 1 to about 80%, from about 5 to about 80%, from about 10 to about 80%, from about 15 to about 80%, from about 20 to about 80%, from about 25 to about 80%, from about 30 to about 80%, from about 35 to about 80%, from about 40 to about 80%, from about 45 to about 80%, from about 0.1 to about 90%, from about 0.2 to about 90%, from about 0.5 to about 90%, from about 1 to about 90%, from about 5 to about 90%, from about 10 to about 90%, from about 15 to about 90%, from about 20 to about 90%, from about 25 to about 90%, from about 30 to about 90%, from about 35 to about 90%, from

about 40 to about 90%, from about 45 to about 90%, from about 0.1 to about 99%, from about 0.2 to about 99%, from about 0.5 to about 99%, from about 1 to about 99%, from about 5 to about 99%, from about 10 to about 99%, from about 15 to about 99%, from about 20 to about 99%, from about 25 to about 99%, from about 30 to about 99%, from about 35 to about 99%, from about 40 to about 99%, and from about 45 to about 99%).

[0034] A composition useful herein is useful for treating or preventing a bone condition characterised by weakened or fragile bones. In one embodiment, the condition to be treated is a condition that requires the maintenance of or an increase in bone mass or bone strength or both bone mass and bone strength. In one embodiment, the condition is a condition that requires a decrease in bone loss. In one embodiment, the condition is a condition that requires bone cell proliferation, bone cell differentiation, bone cell mediated mineralization or a combination of any two or more of bone cell proliferation, bone cell differentiation and bone cell mediated mineralization. In one embodiment, the condition is a condition that requires the inhibition of bone resorption, an increase in bone formation, a decrease in bone loss or a combination of any two or more of the inhibition of bone resorption, an increase in bone formation and a decrease in bone loss. In one embodiment, the condition is a condition requiring an improvement in or maintenance of bone density, bone mass, bone strength or bone health or a combination of any two or more of an improvement in or maintenance of bone density, bone mass, bone strength and bone health. In one embodiment, the condition is osteoporosis. In one embodiment the condition is osteopenia.

[0035] In one embodiment, treating or preventing a bone condition characterised by weakened or fragile bones includes one or more of maintaining or increasing bone formation, maintaining or increasing bone mineral density, maintaining or increasing bone mass (including peak bone mass), treating or preventing osteoporosis, treating or preventing osteopenia, stimulating bone regeneration during fracture healing, reducing bone resorption or increasing bone quality (for example, as measured by break stress or bone strength).

[0036] A composition useful herein is useful for administering to a subject in need thereof for the treatment of a bone condition. In one embodiment, the subject is in need of an increase in bone mass. In one embodiment, the subject is in need of treatment for a condition that requires a decrease in bone loss. In one embodiment, the subject is in need of treatment for a condition that requires bone cell proliferation, bone cell differentiation or bone cell mediated mineralization or a combination of any two or more of bone cell proliferation, bone cell differentiation and bone cell mediated mineralization. In one embodiment, the subject is in need of a treatment of a condition that requires the inhibition of bone resorption, an increase in bone formation or a decrease in bone

loss or a combination of the inhibition of bone resorption, an increase in bone formation and a decrease in bone loss. In one embodiment, the subject is in need of treatment for a condition requiring improvement or maintenance of bone density, improvement or maintenance of bone mass, improvement or maintenance of bone strength or improvement or maintenance of bone health or a combination of any two or more of these effects.

[0037] In one embodiment the subject is in need of decreased bone resorption or decreased osteoclastogenesis or both. Accordingly, in one embodiment the invention relates to amelioration, treatment or prevention of a condition associated with net bone resorption or increased osteoclastogenesis.

[0038] In another embodiment the subject is in need of increased bone formation or increased osteoblast proliferation, increased osteoblast differentiation increased mineralization or a combination thereof. Accordingly, in this embodiment the invention relates to the amelioration, treatment or prevention of a condition associated with poor bone formation or decreased osteoblast proliferation, decreased differentiation and decreased mineralization or a combination thereof.

[0039] In one embodiment, treating or preventing a bone condition characterised by weakened or fragile bones includes decreasing bone loss, in particular bone loss associated with age in a subject.

[0040] In one embodiment the composition comprises, consists essentially of, or consists of about 0.1, 0.5, 1, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99, 99.5, 99.8 or 99.9 % by weight of one or more dairy ingredients selected from fresh, recombined or powdered whole milk or a milk derivative and useful ranges may be selected between any of these foregoing values (for example, from about 0.1 to about 50%, from about 0.2 to about 50%, from about 0.5 to about 50%, from about 1 to about 50%, from about 5 to about 50%, from about 10 to about 50%, from about 15 to about 50%, from about 20 to about 50%, from about 25 to about 50%, from about 30 to about 50%, from about 35 to about 50%, from about 40 to about 50%, and from about 45 to about 50%). The milk derivative is preferably selected from recombined, powdered or fresh skim milk, reconstituted whole or skim milk powder, skim milk concentrate, skim milk retentate, concentrated milk, ultrafiltered milk retentate, milk protein concentrate (MPC), milk protein isolate (MPI), calcium depleted milk protein concentrate (MPC), low fat milk, low fat milk protein concentrate (MPC), casein, caseinate, milk fat, high CLA milk fat, cream, butter, anhydrous milk fat (AMF), butter milk, butter serum, hard milk fat fractions, soft milk fat fractions, sphingolipid fractions, milk fat globular membrane fractions, phospholipid fractions, complex lipid fractions, colostrum, a colostrum fraction, colostrum protein concentrate (CPC), colostrum whey, an

immunoglobulin fraction from colostrum, whey, lactoferrin, one or more lactoferrin fragments, whey protein isolate (WPI), whey protein concentrate (WPC), sweet whey, lactic acid whey, mineral acid whey, reconstituted whey powder, milk minerals, a composition derived from any milk or colostrum processing stream, a composition derived from the retentate or permeate obtained by ultrafiltration or microfiltration of any milk or colostrum processing stream, a composition derived from the breakthrough or adsorbed fraction obtained by chromatographic (including but not limited to ion and gel permeation chromatography) separation of any milk or colostrum processing stream, extracts of any of these milk derivatives including extracts prepared by multistage fractionation, differential crystallisation, solvent fractionation, supercritical fractionation, near supercritical fractionation, distillation, centrifugal fractionation, or fractionation with a modifier (e.g. soaps or emulsifiers), hydrolysates of any of these derivatives, fractions of the hydrolysates, and any combination of any two or more of these derivatives, including combinations of hydrolysed fractions, combinations of non-hydrolysed fractions, and combinations of hydrolysed and non-hydrolysed fractions.

[0041] It is intended that reference to a range of numbers disclosed herein (for example, 1 to 10) also incorporates reference to all rational numbers within that range (for example, 1, 1.1, 2, 3, 3.9, 4, 5, 6, 6.5, 7, 8, 9 and 10) and also any range of rational numbers within that range (for example, 2 to 8, 1.5 to 5.5 and 3.1 to 4.7) and, therefore, all sub-ranges of all ranges expressly disclosed herein are hereby expressly disclosed. These are only examples of what is specifically intended and all possible combinations of numerical values between the lowest value and the highest value enumerated are to be considered to be expressly stated in this application in a similar manner.

[0042] In this specification where reference has been made to patent specifications, other external documents, or other sources of information, this is generally for the purpose of providing a context for discussing the features of the invention. Unless specifically stated otherwise, reference to such external documents is not to be construed as an admission that such documents, or such sources of information, in any jurisdiction, are prior art, or form part of the common general knowledge in the art.

[0043] The invention may also be said broadly to consist in the parts, elements and features referred to or indicated in the specification of the application, individually or collectively, in any or all combinations of two or more of said parts, elements or features, and where specific integers are mentioned herein which have known equivalents in the art to which the invention relates, such known equivalents are deemed to be incorporated herein as if individually set forth.

BRIEF DESCRIPTION OF THE DRAWINGS

[0044] Figure 1 is a graph showing the effect of rosehip on osteoblast differentiation as expressed by production of alkaline phosphatase compared to control ($p = 0.0093$).

[0045] Figure 2 is a graph showing the effect of rosehip extract on osteoclastogenesis. Rosehip
5 dose-dependently suppressed formation of osteoclasts. Results are compared with control at $p < 0.05$.

[0046] Figure 3 is a graph showing break stress results of a three-point bending test for the right femur. One group of rats was sham operated (Sham; $n=15$) and fed a control diet for 28 weeks. Another group was ovariectomised and fed a control diet (OVX; $n=15$) or a treatment diet
10 containing a rosehip extract (Rosehip; $n=15$) for 28 weeks. Results are shown as mean $\pm$ SEM. The results show a significant difference between Sham and OVX ($p = 0.0105$) and no significant difference between Sham and Rosehip ($p = 0.8450$) or between OVX and Rosehip ($p = 0.5754$).

[0047] Figure 4 is a graph showing maximum load for the right femur. Maximum load is defined as the maximum force that the femur can withstand during a three point bending test. 45
15 rats were fed a control diet for 3 weeks. After 3 weeks one group of rats was sham operated (Sham; $n=15$) and continued to be fed the control diet. The other 30 rats were ovariectomised. A group of the ovariectomised rats were fed the control diet (OVX; $n=15$) and another group were fed the diet supplemented with rosehip (Rosehip; $n=15$). The results are shown as mean $\pm$ SEM. The results show a significant difference between OVX and Rosehip, $p=0.035$.

[0048] Figure 5 is a graph showing break load. Break load is the force at which the femur fractured, typically less than the max force. 45 rats were fed a control diet for 3 weeks. After 3 weeks
20 one group of rats was sham operated (Sham; $n=15$) and continued to be fed the control diet. The other 30 rats were ovariectomised. A group of the ovariectomised rats were fed the control diet (OVX; $n=15$) and another group were fed the diet supplemented with rosehip (Rosehip; $n=15$).
25 The results are shown as mean $\pm$ SEM. The results show a significant difference between OVX and Rosehip, $p=0.030$.

[0049] Figure 6 is a graph showing the energy expended to break the right femur in rats. 45 rats were fed a control diet for 3 weeks. After 3 weeks one group of rats was sham operated (Sham; $n=15$) and continued to be fed the control diet. The other 30 rats were ovariectomised. A group of
30 the ovariectomised rats were fed the control diet (OVX; $n=15$) and another group were fed the diet supplemented with rosehip (Rosehip; $n=15$). The results are shown as mean $\pm$ SEM. The results show a significant difference between OVX and Rosehip, $p=0.030$.

[0050] DETAILED DESCRIPTION OF THE INVENTION

[0051] The present invention is based on the discovery that a water extract of rosehip has a positive effect on the maintenance of bone health and the treatment of bone disease such as osteoporosis or osteopenia.

5 1. Definitions

[0052] The term “comprising” as used in this specification and claims means “consisting at least in part of”. When interpreting statements in this specification and claims that include that term, the features, prefaced by that term in each statement, all need to be present but other features can also be present. Related terms such as “comprise” and “comprised” are to be interpreted in the
10 same manner.

[0053] The term “water soluble extract of rosehip” means an extract produced by a method where any food grade polar solvent such as water, acetic acid, and alcohols (such as methanol, ethanol, propanol or butanol, for example) are used to extract water soluble components from rosehips. Preferably the solvent is water.

15 **[0054]** The term “maintenance of bone health” means maintaining an individual’s bone at a healthy density for example, as defined by WHO standards for DEXA, within ± 1 SD of bone density for a 20 year old person.

[0055] The term “weakened or fragile bones” means an individual has a bone density below the healthy density for example, as defined by WHO standards for DEXA, within ± 1 SD of bone
20 density for a 20 year old person.

[0056] An “effective amount” is the amount required to confer a therapeutic effect. The interrelationship of dosages for animals and humans (based on milligrams per meter squared of body surface) is described by Freireich, et al. (1966). Body surface area can be approximately determined from height and weight of the subject. See, e.g., Scientific Tables, Geigy
25 Pharmaceuticals, Ardley, New York, 1970, 537. Effective doses also vary, as recognized by those skilled in the art, dependent on route of administration, carrier usage, species and individual genetic variation, and the like.

[0057] The term “oral administration” includes oral, buccal, enteral and intra-gastric administration.

[0058] The term “pharmaceutically acceptable carrier” is intended to refer to a carrier including but not limited to an excipient, diluent, auxiliary or combination thereof that can be administered to a subject as a component of a composition of the invention that does not reduce the activity of the composition and is not toxic when administered in doses sufficient to deliver an effective amount of a rosehip extract. The formulations can be administered orally, nasally and topically.

[0059] A “subject” in accordance with the invention is an animal, preferably a mammal, more preferably a mammalian companion animal or human. Preferred companion animals include cats, dogs and horses.

[0060] The term “treat” and its derivatives should be interpreted in their broadest possible context. The term should not be taken to imply that a subject is treated until total recovery. Accordingly, “treat” broadly includes amelioration or prevention or amelioration and prevention of the onset of the symptoms or severity of a particular condition; for example preventing or ameliorating a reduction in bone density, preventing or otherwise reducing the risk of developing osteoporosis, preventing or otherwise reducing the risk of developing osteopenia, or preventing or ameliorating other disease symptoms. The term “treat” also broadly includes the maintenance of good bone health and building bone quality for disease or disorder prevention.

2. Rosehip

[0061] Rosehip, sometimes called rosehaw, is a fruit found on the rose plant (*Rosa spp.*) which contains several vitamins including high levels of vitamin C. Other vitamins found in rosehip are vitamin A, D and E. Rose hip also contains high levels of iron and some essential fatty acids and antioxidants.

[0062] The fruit of several *Rosa* species can be used to make the water soluble extract of rosehip useful in the present invention. These species include but are not limited to *Rosa canina*, *Rosa dumalis* subsp. *boissieri*, *Rosa dumalis* subsp. *antalyensis*, *Rosa villosa*, *Rosa pulverulenta*, *Rosa moyesii* *Rosa gallica*, *Rosa condita*, *Rosa rugosa* and *Rosa pisiformis*.

[0063] A possible extraction process of rosehip is as follows. Fresh rose hips are macerated or dried rose hips are powdered. The macerated or powdered rosehips are mixed with distilled water or other aqueous solutions (e.g. buffers) in a ratio of 1:5 (w/w), to allow extraction of the water soluble components from the rosehips. The aqueous solvent may be replaced one or more times, preferably 3 times, and each amount of solvent is allowed to remain in contact with the plant material for several hours, preferably 12, 24 or 48 hours, preferably with continuous stirring. After

filtration or centrifugation of the total extracts, the water solutions can then be freeze or spray dried to form a powder that can be used as the water extract for use herein.

[0064] This extract can be further fractionated by standard methods of ion exchange chromatography or membrane separation or other such separation techniques that are known in the art. See for example Ion Exchange Chromatography & Chromatofocusing, Principles and Methods, Amersham Biosciences Limited 2004, Code 11-0004-21, Edition AA, <http://www.amersham.com>.

3. Methods of treatment or prevention

[0065] The compositions and uses of the compositions described herein are useful for treating or preventing a bone condition characterised by weakened or fragile bones, such as osteoporosis or osteopenia.

[0066] In one embodiment, the condition to be treated is a condition that requires an increase in bone mass. In one embodiment, the condition is a condition that requires a decrease in bone loss. In one embodiment, the condition is a condition that requires bone cell proliferation, bone cell differentiation, or bone cell mediated mineralisation, or a combination of two or more thereof. In one embodiment, the condition is a condition that requires the inhibition of bone resorption, an increase in bone formation, or a decrease in bone loss, or a combination of two or more thereof. In one embodiment, the condition is a condition requiring an improvement or maintenance of bone strength, bone density, or bone mass, or a combination of two or more thereof. In one embodiment, the condition is osteoporosis. In one embodiment the condition is osteopenia.

4. Compositions useful according to the invention

[0067] Described herein is a composition comprising, consisting essentially of or consisting of a water soluble extract of rosehip and one or more ingredients selected from calcium, magnesium, zinc, vitamin D, vitamin K, folic acid or folate, vitamin B6 and vitamin B12.

[0068] Also described is a composition consisting essentially of a water soluble extract of rosehip and one or more ingredients selected from calcium, magnesium, zinc, vitamin D, folic acid, folate, and vitamin B12, wherein the composition is formulated for simultaneous, separate or sequential administration of the water soluble extract of rosehip and the one or more ingredients.

[0069] Also described herein is a composition comprising a water soluble extract of rosehip and one or more dairy ingredients.

[0070] A composition useful herein includes any composition that can carry a water soluble extract of rosehip, including any consumer product and any pharmaceutical product that is able to carry a water soluble extract of rosehip. The composition may be formulated as a food, drink, food additive, drink additive, dietary supplement, nutritional product, medical food, nutraceutical, medicament or pharmaceutical. Preferably, a composition of the invention is formulated as a powder, liquid, food bar, spread, sauce, ointment, tablet or capsule. Suitable foods and drinks include dairy and non-dairy foods and drinks. In one embodiment, the composition is a milk powder, milk drink, yoghurt, yoghurt powder, yoghurt drink, soy, acidified beverages, UHT, pasteurised, butter or cheese. Appropriate formulations may be prepared by an art skilled worker with regard to that skill and the teaching of this specification.

[0071] A nutraceutical composition for use according to the invention can be a dietary supplement (e.g., a capsule, a mini-bag, or a tablet) or a food product (e.g., milk, juice, a soft drink, a herbal tea-bag, or confectionary). The composition can also include other nutrients, such as protein, carbohydrate, lipids, vitamins, minerals, or amino acids. The composition can be in a form suitable for oral use, such as a tablet, a hard or soft capsule, an aqueous or oil suspension, or a syrup; or in a form suitable for parenteral use, such as an aqueous propylene glycol solution, or a buffered aqueous solution. The amount of the active ingredient in the nutraceutical composition depends to a large extent on a subject's specific need. The amount also varies, as recognized by those skilled in the art, dependent on administration route, species, genetic/ physiological predisposition and possible co-usage of other bone-enhancing agents

[0072] Foods, food additives or food supplements comprising a rosehip extract for use according to the invention include any edible consumer product which is able to carry a water soluble plant extract. Examples of suitable edible consumer products include confectionary products, reconstituted fruit products, snack bars, muesli bars, bakery products, spreads, dips, dairy products including yoghurts and cheeses, drinks including dairy and non-dairy based drinks, milk powders, sports supplements including dairy and non-dairy based sports supplements, food additives such as protein sprinkles and dietary supplement products including daily supplement tablets. Suitable nutraceutical compositions useful herein may be provided in similar forms.

[0073] The compositions useful herein may be formulated to allow for administration to a subject by any chosen route, including but not limited to oral or nasal administration.

[0074] Preferably the composition useful herein is administered orally.

[0075] As will be appreciated, the dose of the composition administered, the period of administration, and the general administration regime may differ between subjects depending on such variables as the severity of symptoms of a subject, the type of disorder to be treated, the mode of administration chosen, and the age, sex and general health of a subject. However, by way of general example, the inventors contemplate administration of from about 1 mg to about 2000 mg per kg body weight of rosehip per day, preferably about 100 to 1000 mg per kg body weight of rosehip per day or about 50 to about 500 mg per kg body weight of rosehip per day. In one embodiment, the inventors contemplate administration of from about 0.05 mg to about 300 mg per kg body weight of rosehip.

[0076] Preferably the composition useful herein is administered at least once a day. Preferably, the composition useful herein is administered 2-3 times a day. Alternatively, the composition useful herein may be administered once a week.

[0077] Preferably, the composition useful herein is administered as a prophylactic before the onset of menopause. Alternatively, the composition useful herein is administered after the onset of menopause for maintaining or increasing bone health or for treating or preventing a bone condition.

[0078] It should be understood that a person of ordinary skill in the art will be able without undue experimentation, having regard to that skill and this disclosure, to determine an effective dosage regime (including daily dose and timing of administration) for a given condition.

[0079] The efficacy of a composition useful according to this invention can be evaluated both *in vitro* and *in vivo*. See, e.g., the examples below. Briefly, the composition can be tested for its ability to promote osteoblast development and activity or inhibit osteoclastogenesis, limit osteoclast activity, or reduce osteoclast numbers *in vitro*. For *in vivo* studies, the composition can be administered to an animal (e.g., a rat) and its effects on bone tissues are then accessed. Based on the results, an appropriate dosage range and administration route can be determined.

[0080] The compositions useful herein may be used alone or in combination with one or more other therapeutic agents. The therapeutic agent may be a food, drink, food additive, drink additive, food component, drink component, dietary supplement, nutritional product, medical food, nutraceutical, medicament or pharmaceutical. The therapeutic agent is preferably effective to promote osteoblast development and activity, inhibit osteoclastogenesis reduce osteoclast numbers or limit osteoclast activity.

[0081] When used in combination with another therapeutic agent, the administration of a composition useful herein and the other therapeutic agent may be simultaneous or sequential.

Simultaneous administration includes the administration of a single dosage form that comprises all components or the administration of separate dosage forms at substantially the same time.

Sequential administration includes administration according to different schedules, preferably so that there is an overlap in the periods during which the composition useful herein and other therapeutic agent are provided.

[0082] In one embodiment, a composition useful herein includes or is administered simultaneously or sequentially with milk components such as whey protein, whey protein fractions (including acidic or basic whey protein fractions or a combination thereof), glycomacropeptide, lactoferrin or a functional lactoferrin variant, or a lactoferrin fragment, a vitamin D, vitamin D derivative, vitamin D analog, or calcium and its salts or combinations thereof. Useful milk component-containing compositions include compositions such as a food, drink, food additive, drink additive, dietary supplement, nutritional product, medical food or nutraceutical. Milk fractions enriched for these components may also be employed.

[0083] It should be understood that the additional therapeutic agents listed above (both food based and pharmaceutical agents) may also be employed in a method according to the invention where they are administered separately, simultaneously or sequentially with a composition useful herein.

[0084] Compositions useful herein may further include another bone-health agent, such as calcium, fluoride, magnesium, zinc, calcium salts, fluoride salts, magnesium salts, zinc salts, vitamin A, folate, or folic acid, or vitamin B12, vitamin B6, vitamin C, vitamin D, vitamin D derivatives (including but not limited to vitamin D (including vitamin D1 [lamisterol], vitamin D2 [ergocalciferol], vitamin D3 [cholecalciferol, 1,25-dihydroxycholecalciferol], vitamin D4 [dihydrotachysterol] and vitamin D5 [7-dehydrositosterol], and vitamin D analogs), vitamin E, vitamin E derivatives, vitamin E analogs, vitamin K, vitamin K derivatives, vitamin K analogs, vitamin K2, whey protein, whey protein fractions (including acidic or basic whey protein fractions or a combination thereof), glycomacropeptide, lactoferrin, a functional lactoferrin variant, a functional lactoferrin fragment, isoflavones, phytoestrogens, prebiotics or probiotics, or a combination of any two or more of these, that may impact on mineral absorption, polyunsaturated fatty acids, and combinations thereof.

[0085] Also within the scope of this invention is the use of a pharmaceutical composition that contains rosehip or components thereof. The pharmaceutical composition can be used to prevent and treat bone-related disorders described above. The pharmaceutical composition can further include an effective amount of another bone-enhancing agent. The pharmaceutically acceptable

carrier includes a solvent, a dispersion medium, a coating, an antibacterial and antifungal agent, and an isotonic and absorption delaying agent

[0086] A pharmaceutical composition useful in the invention may be formulated with an appropriate pharmaceutically acceptable carrier (including excipients and diluents) selected with regard to the intended route of administration and standard pharmaceutical practice. For example, a composition of the invention can be administered orally as a powder, liquid, tablet, effervescent tablet or capsule. Suitable formulations may contain additional agents as required, including emulsifying agents, antioxidants, flavouring or colouring agents, and may be adapted for immediate-, delayed-, modified-, sustained-, pulsed- or controlled-release.

[0087] In one preferred embodiment, a composition for use according to the invention is formulated for ingestion.

[0088] Capsules can contain any standard pharmaceutically acceptable materials such as gelatin or cellulose. Tablets can be formulated in accordance with conventional procedures by compressing mixtures of the active ingredients with a solid carrier and a lubricant. Examples of solid carriers include but are not limited to starch and sugar bentonite. Active ingredients can also be administered in a form of a hard shell tablet or a capsule containing a binder, e.g., lactose or mannitol, a conventional filler, and a tableting agent.

[0089] Effervescent tablets can be formulated using any conventional procedures and may include any suitable pharmaceutically acceptable effervescent material. Examples of effervescent materials include but are not limited to, citric acid, malic acid, sodium bicarbonate, potassium bicarbonate, sodium carbonate and potassium carbonate.

[0090] The compositions of the invention can be formulated into dosage forms for different administration routes utilizing conventional methods. For example, it can be formulated in a capsule, a gel seal, or a tablet for oral administration.

[0091] A suitable pharmaceutical composition may be formulated with appropriate pharmaceutically acceptable excipients, diluents or carriers selected with regard to the intended dosage form and standard pharmaceutical formulation practice. A dosage form useful herein can be administered orally as a powder, liquid, tablet or capsule. Suitable dosage forms may contain additional agents as required, including emulsifying, antioxidant, flavouring or colouring agents. Dosage forms useful herein may be adapted for immediate, delayed, modified, sustained, pulsed or controlled release of the active components.

[0092] A composition comprising rosehip may be used to treat or prevent skeletal disorders. Examples of such disorders include, but are not limited to osteoporosis, hepatic osteodystrophy, osteomalacia, rickets, osteitis fibrosa cystica, renal osteodystrophy, osteosclerosis, osteopenia, fibrogenesis-imperfecta ossium, secondary hyperparathyroidism, hypoparathyroidism, hyperparathyroidism, chronic renal disease, sarcoidosis, glucocorticoid-induced osteoporosis, idiopathic hypercalcemia, Paget's disease, and osteogenesis imperfecta.

[0093] The rosehip extract may be used alone or in combination with one or more other therapeutic agents (nutraceuticals, pharmaceuticals or medical foods, for example). When used in combination with another therapeutic agent the administration of the two agents may be separate, simultaneous or sequential. Simultaneous administration includes the administration of a single dosage form that comprises both agents and the administration of the two agents in separate dosage forms at substantially the same time. Sequential administration includes the administration of the two agents according to different schedules, preferably so that there is an overlap in the periods during which the two agents are provided. Suitable agents with which the compositions of the invention can be co-administered include other bone growth agents or bone disease treatments, and other suitable agents known in the art. Such agents are preferably administered parenterally, preferably by intravenous, subcutaneous, intramuscular, intraperitoneal, intramedullary, epidural, intradermal, transdermal (topical), transmucosal, intra-articular, and intrapleural, as well as oral, inhalation, and rectal administration.

[0094] Suitable agents with which the compositions useful herein can be co-administered include alpha v beta 3 integrin receptor antagonists, antiestrogens or SERMs (Selective Estrogen Receptor Modulators) (including but not limited to tamoxifen, raloxifene, lasofoxifene, toremifene, azorxifene, clomiphene, droloxifene, idoxifene, levormeloxifene, zuclomiphene, enclomiphene, nafoxidene, and salts thereof), antiresorptive agents, bisphosphonates (including but not limited to alendronate, clodronate, etidronate, ibandronate, incadronate, minodronate, neridronate, olpadronate, pamidronate, piridronate, risedronate, tiludronate, zoledronate, and pharmaceutically acceptable salts thereof), calcium receptor antagonists, calcium supplements, cathepsin K inhibitors, Dual Action Bond Agents (DABAs) (including but not limited to strontium ranelate), estrogen and estrogen derivatives (including but not limited to 17 beta-estradiol, estrone, conjugated estrogen, equine estrogen, and 17 beta-ethynyl estradiol), flavonoids, folic acid, folate, vitamin B12, osteoanabolic agents, osteoprotegerin, progestin and progestin derivatives (including but not limited to norethindrone and medroxy-progesterone acetate), vacuolar ATPase inhibitors, antagonists of VEGF, thiazolidinediones, calcitonin, protein kinase inhibitors, parathyroid hormone (PTH), PTH analogs, recombinant parathyroid hormone, growth hormone secretagogues, growth hormone

releasing hormone, inhibitors of hyperhomocysteinemia insulin-like growth factor, bone morphogenetic protein (BMP), inhibitors of BMP antagonism, prostaglandin derivatives, fibroblast growth factors, statins (including but not limited to lovastatin, simvastatin, pravastatin, fluvastatin, atorvastatin, cerivastatin, rosuvastatin, and pitavastatin), calcium, fluoride, magnesium, zinc, calcium salts, fluoride salts, magnesium salts, zinc salts, vitamin A, vitamin A derivatives, vitamin A analogs, vitamin B6, vitamin C, vitamin C derivatives, vitamin C analogs, vitamin D, vitamin D derivatives (including but not limited to vitamin D (including vitamin D1 [lamisterol], vitamin D2 [ergocalciferol], vitamin D3 [cholecalciferol, 1,25-dihydroxycholecalciferol], vitamin D4 [dihydrotachysterol] and vitamin D5 [7-dehydrositosterol], and vitamin D analogs), vitamin E, vitamin E derivatives, vitamin E analogs, vitamin K, vitamin K derivatives, vitamin K analogs, vitamin K2, whey protein, whey protein fractions (including acidic or basic whey protein fractions or a combination thereof), glycomacropeptide, lactoferrin, a functional lactoferrin variant, a functional lactoferrin fragment, milk fat, high CLA milk fat, milk fat fractions, isoflavones, phytoestrogens, prebiotics or probiotics or a combination of any two or more of these agents, that may impact on mineral absorption, polyunsaturated fatty acids and combinations thereof, and other suitable agents known in the art.

[0095] Published international patent applications WO 03/082921 and WO 2007/043900 describe useful lactoferrin and lactoferrin fragments and hydrolysates and are incorporated herein by reference.

20 [0096] Published international patent application WO 2008/147228 describes fatty acids, fatty acid derivatives and milk fat compositions including high CLA milk fat and milk fat fractions that are able to treat or prevent bone conditions that would benefit from a reduction in bone resorption and is incorporated herein by reference.

25 [0097] Additionally, it is contemplated that a composition in accordance with the invention may be formulated with additional active ingredients which may be of benefit to a subject in particular instances. For example, therapeutic agents that target the same or different facets of the disease process may be used.

[0098] Various aspects of the invention will now be illustrated in non-limiting ways by reference to the following examples.

EXAMPLES

Cell culture, assay materials

[0099] All cell culture materials were purchased from Invitrogen. MC3T3-E1 preosteoblast cells were obtained from ATCC, Manassas, VA, USA. Rosehip extract was purchased from Paninkret, Germany.

EXAMPLE 1 – OSTEOLAST DIFFERENTIATION

[00100] MC3T3-E1/4 cells were seeded at 0.55×10^5 cells/ml in 48-well plates with 0.3ml per well. Quadruplicate wells per treatment were used within each experiment and experiments were replicated three times to ensure consistency of results. Culture media was α -MEM with 10% FCS.

Treatments were added 24 hours following initial seeding of plates to allow cells to adhere to wells. Following 3 days of culture, media was changed to α -MEM with 10% FCS, 10mM β -glycerophosphate and 50 μ g/ml ascorbic acid with and without treatments. Media was changed every 2-3 days. Following 10 days of culture, media was removed from wells and cells were washed with PBS. Cells were incubated with 200 μ l of alkaline phosphatase test reagent (0.05M p-nitrophenyl phosphate in TBS pH 9.5) for 1 hr at 37°C. Two samples (100 μ l) of test reagent from each well were transferred to a 96-well plate and absorbance read at 405nm using an ELx808 Ultra microplate reader (Bio-Tek Instruments Inc., Vermont, USA). Cells were washed again with PBS, fixed with 1% formaldehyde for 15 minutes and cell number was determined by crystal violet assay. Cells were incubated for two hours with 300 μ L 1% crystal violet (dissolved in PBS) and then were washed under running water for 15 minutes to remove excess dye and allowed to air dry. Triton-X 100 (0.2% in dd H₂O), 500 μ L/well, was added to dried plates and plates were incubated at room temperature for 2 hours to solubilise absorbed dye. Duplicate aliquots of 100 μ L were taken from each well and transferred to a 96-well plate. Absorbance was read using a ELx808 Ultra microplate reader (Bio-Tek Instruments, Inc. Vermont, USA) at 550nm using 0.2% Triton-X 100 as a blank. Alkaline phosphatase activity on a per cell basis was determined by dividing the absorbance measurement at 405nm (a measure of p-nitrophenyl production or alkaline phosphatase activity) by the absorbance measurement at 550nm (from the crystal violet assay, a measure of cell number). The resultant index figure was used to represent alkaline phosphatase activity on a per cell basis. The results are shown in Figure 1. Administration of rosehip increased differentiation by 17% compared to control.

EXAMPLE 2 – OSTEOCLAST FORMATION

[00101] Osteoclasts can be generated from the murine macrophage RAW 264.7 cell line. RAW 264.7 cells were seeded into 24 well plates containing coverslips and treated with murine RANK-L. The effect of a compound on osteoclast formation was tested by the addition of the compound to the RANK-L containing cell culture media at different concentrations. These cells were incubated for 5 days with a media change on day 3. The coverslips were then fixed and stained for TRAP and then counterstained with hematoxylin. Large multinucleated osteoclasts were quantified. Osteoclasts appeared as large multinucleated cells staining purple red and may form even larger giant cells. The effect of an agent on osteoclast formation is quantified by taking photomicrographs of three random regions on each coverslip and then counting the number of TRAP(+) cells with greater than three nuclei and expressing this as "number of TRAP(+) cells with greater than three nuclei per unit area". TRAP stain was also solubilised and measured colorimetrically. These results are presented as graphs. In the first step of the validation of the *in vitro* assay, a cell density titration was performed.

[00102] In order to ascertain the optimum cell density to seed cells, cells were seeded at 2×10^3 - 6×10^4 cells/ml with 35 ng/ml RANK-L. From this a density of 1.5×10^4 cells/ml was chosen for further plates. A RANK-L titration (1 ng/ml – 35 ng/ml) was then performed using the chosen cell density. From these two results a cell seeding density of 1.5×10^4 cells/ml and RANK-L concentration of 15 ng/ml were chosen as the optimal conditions under which to observe osteoclastogenesis in all future *in vitro* assays. The results are shown in Figure 2. Administration of rosehip resulted in a decrease in osteoclast numbers by 29% as compared to control. At 10ug/ml no osteoclast like cells could be observed compared to control. Cell viability was not affected.

EXAMPLE 3 – BONE RESORPTION

[00103] This study is designed to assess whether diets supplemented with rosehip are able to impact bone quality in the OVX rat model.

Animals

[00104] 44 female rats aged 5.5 months were fed a casein based diet for two weeks (week -2 to week 0). At week -2 the rats underwent *in vivo* DEXA scanning under anaesthesia. At week 0 the rats were either sham operated (Sham; n=15) or ovariectomised (n=30). Sham rats underwent anaesthesia by isoflurane inhalation and an incision was made but the ovaries left intact. Ovaries were removed from the ovariectomised animals. The ovariectomised rats were randomly assigned to either a control group (OVX) and fed a casein control diet or a treatment group (Rosehip) and fed a Rosehip extract at 0.5% w/w of the control diet for 28 weeks.

[00105] Confirmation of successful ovariectomy was determined by measuring uterus weights. Both the OVX (0.14 g; $p < 0.0001$) and Rosehip (0.13 g; $p < 0.0001$) groups had significantly lower uterus weight than Sham (0.86 g).

[00106] At week 28, the rats were fasted overnight and then euthanised by exsanguination (heart puncture under anaesthesia). After heart puncture, the animals were exposed to 100% CO₂. The bodies were dissected and various bones collected and fixed for further analysis.

Diets

[00107] Animals were fed a balanced semi-synthetic diet consisting of 15% (w/w) casein (as caseinate), 5% (w/w) cellulose, 5% (w/w) corn oil, 0.5% (w/w) calcium, 62% (w/w) starch and added vitamins, minerals and amino acids to meet the requirements according to AIN93M (National Research Council, 1995).

Mechanical properties of bone

[00108] The right femurs were scraped clean of adhering flesh and stored in phosphate buffered saline solution at -20°C . Before biomechanical testing, the bones were thawed. The length of the femurs was measured using an electronic calliper. The midpoint was marked with a waterproof pen and the width and thickness of the femurs at midpoint were also recorded. The femurs were then held at 23°C to be at room temperature before and during the test. The femurs were placed in a testing jig constructed for a three point bending test. The distance between the supporting rods had a fixed length of 12 mm. Load was applied at a constant deformation rate of 50 mm min^{-1} . Maximum load (N), stiffness (N mm^{-2}) and energy (J) were measured using a Shimadzu Ezi-test texture analyzer (Kyoto, Japan). The results are shown in Figure 3. The amount of energy required to break the right femur in a three point bending test was 118, 96 and 101 N per mm^2 respectively for sham, OVX and rosehip. Sham and OVX were significantly different ($p = 0.0105$). Rosehip was not significantly different from sham or OVX but was intermediate in its effect of making the bone more resilient to breaking.

EXAMPLE 4 – BONE RESORPTION AND MECHANICAL PROPERTIES

[00109] This study was designed to assess whether diets supplemented with Rosehip were able to inhibit bone resorption or affect bone biochemical and mechanical properties in the OVX rat model.

Animals

[00110] 45 female Sprague Dawley rats at approx. 5 months of age were housed singly in shoebox cages, with Milli-Q water ad libitum. For three weeks to acclimatize them to the diet and their environment, they were all fed a nutritionally adequate casein based diet consisting of 14 % (w/w) Caseinate, 5 % (w/w) Cellulose, 5 % (w/w) Vitamins, 5 % (w/w) Minerals, 1.3 % (w/w) Calcium carbonate, 4 % (w/w) Corn oil, 5.7 % (w/w) Sucrose and 60 % (w/w) Wheat cornstarch.

[00111] No vitamin C was added to any of the diets and the vitamin mix did not contain any vitamin C. The addition of rosehip to their diet contributed approximately 0.08% (w/w) vitamin C.

[00112] After three weeks, 30 animals were ovariectomised and the other 15 were sham operated to mimic ovariectomy without actual removal of the ovaries. From this time on, 15 randomly selected ovariectomised rats and the 15 sham operated animals were fed the standard casein based diet, while the other 15 ovariectomised animals received the base diet supplemented with 2 % rosehip extract. After 24 weeks the animals were euthanized as described in example 3, their right femur removed and prepared for bone biomechanical testing using 3 point breaking test regime as described in example 3. The results were compared by 1-way ANOVA.

Biochemical Analysis

[00113] Biomechanical Analysis was performed on the right femurs of the OVX, Sham and Rosehip animals. Maximum load, break load and energy were all calculated using a Shimadzu Ezi-test texture analyzer (Kyoto, Japan). The maximum load is defined as the maximum recorded force applied to the femur during a three point bending test. The break load is defined as force at which the bone fractures during the three point bending test. The force is measured by a force transducer. The unit breaks the bone and gathers and records force and displacement. This was plotted to produce a force-displacement curve which clearly identified maximum and break loads. The software automatically identifies these points.

[00114] Energy is a measure of the energy expended to break the bone and was also calculated using the Shimadzu Ezi-test texture analyzer (Kyoto, Japan) using the force recordings and the known points at which the break and max force measurements were made.

[00115] These results show that bones from animals fed 2% rosehip extract required more force/energy to break than OVX animals, and an equivalent force/energy to Sham animals. The maximum load is shown in Figure 4, the break load is shown in Figure 5 and the energy is shown in Figure 6.

INDUSTRIAL APPLICATION

[00116] The present invention has utility in maintaining bone health or ameliorating imbalances in bone remodelling leading to bone loss.

[00117] The described compositions may be employed as foods, drinks, food additives, drink
5 additives, dietary supplements, nutritional products, medical foods, nutraceuticals, medicaments or pharmaceuticals.

[00118] Those persons skilled in the art will understand that the above description is provided by way of illustration only and that the invention is not limited thereto.

REFERENCES

- Freireich EJ, Gehan EA, Rall DP, Schmidt LH, Skipper HE (1966) Quantitative comparison of toxicity to anticancer agents in mouse, rat, hamster, dog, monkey and man. *Cancer Chemother Rep* 50: 219–244
- 5 Gillies, R. Didier, N., Denton, M. Determination of cell number in monolayer cultures, *Analytical Biochemistry*, 1986, 159:109-113)
- Green JH, Booth C, Bunning R. Post prandial metabolic responses to milk enriched with milk calcium are different from responses to milk enriched with calcium carbonate. *Asia Pacific J Clin Nutr* 2003; 12:109-19
- 10 Murray T. Calcium nutrition and osteoporosis. *CMAJ* 1996; 155:935-9.
- National Research Council .1995. *Nutrient requirements of laboratory animals*. 4th Edition. National Academic Press. Washington, DC.
- Zikan V, Roubal P, Haast T, Stepan JJ. “Acute effects of calcium carbonate and milk on the calcium-parathyroid axis and bone resorption in healthy women.” In: Bruckhardt P, Dawson-Hughes B, Heaney RP eds. *Nutritional Aspects of Osteoporosis: Proceedings of the 4th International Symposium Lausanne, May 2000*. Academic Press, London, 2001. 131-140.
- 15

WHAT WE CLAIM IS:

1. Use of a water soluble extract of rosehip in the manufacture of a composition for treating or preventing a bone condition characterised by weakened or fragile bones.
2. A use of claim 1, wherein the composition maintains or increases bone formation, maintains or increases bone mineral density, maintains or increases bone mass, stimulates bone regeneration during fracture healing, reduces bone resorption, decreases bone loss, or maintains or increases bone strength.
3. A use of claim 1 or 2, wherein the condition is osteoporosis or osteopenia.
4. A use of any one of claims 1 to 3, wherein the composition provides about 1 mg to about 2000 mg per kg body weight, about 100 to about 1000 mg per kg body weight, about 50 to about 500 mg per kg body weight, or about 0.05 mg to about 300 mg per kg body weight of rosehip per day.
5. A composition comprising a water soluble extract of rosehip for treating or preventing a bone condition characterised by weakened or fragile bones.
6. A composition of claim 5, wherein the composition maintains or increases bone formation, maintains or increases bone mineral density, maintains or increases bone mass, stimulates bone regeneration during fracture healing, reduces bone resorption, decreases bone loss, or maintains or increases bone strength.
7. A composition of claim 5 or 6, wherein the condition is osteoporosis or osteopenia.
8. A composition of any one of claims 5 to 7, wherein the composition provides about 1 mg to about 2000 mg per kg body weight, about 100 to about 1000 mg per kg body weight, about 50 to about 500 mg per kg body weight, or about 0.05 mg to about 300 mg per kg body weight of rosehip per day.
9. A use of any one of claims 1 to 4 or a composition of any one of claims 5 to 8, wherein the composition further comprises one or more dairy ingredients.
10. A use or composition of claim 9, wherein the dairy ingredient is selected from the group comprising recombined, powdered or fresh skim milk, reconstituted whole or skim milk powder, skim milk concentrate, skim milk retentate, concentrated milk, ultrafiltered milk retentate, milk protein concentrate, milk protein isolate, calcium depleted milk protein concentrate, low fat milk,

low fat milk protein concentrate, casein, caseinate, milk fat, high CLA milk fat, cream, butter, anhydrous milk fat, butter milk, butter serum, hard milk fat fractions, soft milk fat fractions, sphingolipid fractions, milk fat globular membrane fractions, phospholipid fractions, complex lipid fractions, colostrum, a colostrum fraction, colostrum protein concentrate, colostrum whey, an immunoglobulin fraction from colostrum, whey, lactoferrin, one or more lactoferrin fragments, whey protein isolate, whey protein concentrate, sweet whey, lactic acid whey, mineral acid whey, reconstituted whey powder, milk minerals, a composition derived from any milk or colostrum processing stream, a composition derived from the retentate or permeate obtained by ultrafiltration or microfiltration of any milk or colostrum processing stream, a composition derived from the breakthrough or adsorbed fraction obtained by chromatographic (including but not limited to ion and gel permeation chromatography) separation of any milk or colostrum processing stream, extracts of any of these ingredients including extracts prepared by multistage fractionation, differential crystallisation, solvent fractionation, supercritical fractionation, near supercritical fractionation, distillation, centrifugal fractionation, or fractionation with a modifier (e.g. soaps or emulsifiers), hydrolysates of any of these ingredients, fractions of the hydrolysates, and any combination of any two or more of these ingredients, including combinations of hydrolysed fractions, combinations of non-hydrolysed fractions, and combinations of hydrolysed and non-hydrolysed fractions.

11. A use or composition of any one of claims 1 to 10, wherein the composition further comprises one or more agents selected from calcium, magnesium, zinc, vitamin D, vitamin K, folic acid or folate, vitamin B6 and vitamin B12.
12. A use or composition of any one of claims 1 to 11, wherein the composition is a food, confectionary, milk, milk product, milk powder, reconstituted milk, cultured milk, yoghurt, drinking yoghurt, set yoghurt, drink, food additive, drink additive, dietary supplement, nutritional product, medical food, nutraceutical or a pharmaceutical.
13. A method for treating or preventing a bone condition characterised by weakened or fragile bones comprising administering a composition comprising an effective amount of a water soluble extract of rosehip to a subject in need thereof.
14. A method of claim 13, wherein the condition is osteopenia or osteoporosis.
15. A method of claim 13, wherein the composition maintains or increases bone formation, maintains or increases bone density, maintains or increases bone mass, stimulates bone regeneration during

fracture healing, reduces bone resorption, decreases bone loss, or maintains or increases bone strength.

16. A method of claim 13, wherein the subject is need of maintained or increased bone formation, maintained or increased bone mineral density, maintained or increased bone mass, bone regeneration during fracture healing, reduced bone resorption, decreased bone loss, or maintained or increased bone strength.
17. A method of claim 13, wherein the amount of the water soluble extract of rosehip administered is about 1 mg to about 2000 mg per kg body weight, about 100 to about 1000 mg per kg body weight, about 50 to about 500 mg per kg body weight, or about 0.05 mg to about 300 mg per kg body weight per day.
18. A method of claim 13, wherein the composition comprises one or more dairy ingredients.
19. A method of claim 18, wherein the dairy ingredient is selected from the group comprising recombined, powdered or fresh skim milk, reconstituted whole or skim milk powder, skim milk concentrate, skim milk retentate, concentrated milk, ultrafiltered milk retentate, milk protein concentrate, milk protein isolate, calcium depleted milk protein concentrate, low fat milk, low fat milk protein concentrate, casein, caseinate, milk fat, high CLA milk fat, cream, butter, anhydrous milk fat, butter milk, butter serum, hard milk fat fractions, soft milk fat fractions, sphingolipid fractions, milk fat globular membrane fractions, phospholipid fractions, complex lipid fractions, colostrum, a colostrum fraction, colostrum protein concentrate, colostrum whey, an immunoglobulin fraction from colostrum, whey, lactoferrin, whey protein isolate, whey protein concentrate, sweet whey, lactic acid whey, mineral acid whey, reconstituted whey powder, milk minerals, a composition derived from any milk or colostrum processing stream, a composition derived from the retentate or permeate obtained by ultrafiltration or microfiltration of any milk or colostrum processing stream, a composition derived from the breakthrough or adsorbed fraction obtained by chromatographic (including but not limited to ion and gel permeation chromatography) separation of any milk or colostrum processing stream, extracts of any of these ingredients including extracts prepared by multistage fractionation, differential crystallisation, solvent fractionation, supercritical fractionation, near supercritical fractionation, distillation, centrifugal fractionation, or fractionation with a modifier (e.g. soaps or emulsifiers), hydrolysates of any of these ingredients, fractions of the hydrolysates, and any combination of any two or more of these ingredients, including combinations of hydrolysed fractions, combinations of non-hydrolysed fractions, and combinations of hydrolysed and non-hydrolysed fractions.

20. A method of claim 13, wherein the composition further comprises one or more agents selected from calcium, magnesium, zinc, vitamin D, folic acid or folate, and vitamin B12.
21. A method of claim 13, wherein the composition or extract is a food, confectionary, milk, milk product, milk powder, reconstituted milk, cultured milk, yoghurt, drinking yoghurt, set yoghurt, drink, food additive, drink additive, dietary supplement, nutritional product, medical food, nutraceutical or a pharmaceutical.
22. A method for treating or preventing osteoporosis comprising administering a composition comprising an effective amount of a water soluble extract of rosehip to a subject in need thereof.
23. A method of claim 22, wherein the composition maintains or increases bone formation, maintains or increases bone density, maintains or increases bone mass, stimulates bone regeneration during fracture healing, reduces bone resorption, decreases bone loss, or maintains or increases bone strength.
24. A method of claim 22, wherein the subject is need of maintained or increased bone formation, maintained or increased bone mineral density, maintained or increased bone mass, bone regeneration during fracture healing, reduced bone resorption, decreased bone loss, or maintained or increased bone strength.
25. A method of claim 22, wherein the amount of the water soluble extract of rosehip administered is about 1 mg to about 2000 mg per kg body weight, about 100 to about 1000 mg per kg body weight, about 50 to about 500 mg per kg body weight, or about 0.05 mg to about 300 mg per kg body weight per day.
26. A method of claim 22, wherein the composition comprises one or more dairy ingredients.
27. A method of claim 26, wherein the dairy ingredient is selected from the group comprising recombined, powdered or fresh skim milk, reconstituted whole or skim milk powder, skim milk concentrate, skim milk retentate, concentrated milk, ultrafiltered milk retentate, milk protein concentrate, milk protein isolate, calcium depleted milk protein concentrate, low fat milk, low fat milk protein concentrate, casein, caseinate, milk fat, high CLA milk fat, cream, butter, anhydrous milk fat, butter milk, butter serum, hard milk fat fractions, soft milk fat fractions, sphingolipid fractions, milk fat globular membrane fractions, phospholipid fractions, complex lipid fractions, colostrum, a colostrum fraction, colostrum protein concentrate, colostrum whey, an immunoglobulin fraction from colostrum, whey, lactoferrin, whey protein isolate, whey protein

concentrate, sweet whey, lactic acid whey, mineral acid whey, reconstituted whey powder, milk minerals, a composition derived from any milk or colostrum processing stream, a composition derived from the retentate or permeate obtained by ultrafiltration or microfiltration of any milk or colostrum processing stream, a composition derived from the breakthrough or adsorbed fraction obtained by chromatographic (including but not limited to ion and gel permeation chromatography) separation of any milk or colostrum processing stream, extracts of any of these ingredients including extracts prepared by multistage fractionation, differential crystallisation, solvent fractionation, supercritical fractionation, near supercritical fractionation, distillation, centrifugal fractionation, or fractionation with a modifier (e.g. soaps or emulsifiers), hydrolysates of any of these ingredients, fractions of the hydrolysates, and any combination of any two or more of these ingredients, including combinations of hydrolysed fractions, combinations of non-hydrolysed fractions, and combinations of hydrolysed and non-hydrolysed fractions.

28. A method of claim 22, wherein the composition further comprises one or more agents selected from calcium, magnesium, zinc, vitamin D, folic acid or folate, and vitamin B12.
29. A method of claim 22, wherein the composition or extract is a food, confectionary, milk, milk product, milk powder, reconstituted milk, cultured milk, yoghurt, drinking yoghurt, set yoghurt, drink, food additive, drink additive, dietary supplement, nutritional product, medical food, nutraceutical or a pharmaceutical.
30. A method for treating or preventing osteopenia comprising administering a composition comprising an effective amount of a water soluble extract of rosehip to a subject in need thereof.
31. A method of claim 30, wherein the composition maintains or increases bone formation, maintains or increases bone density, maintains or increases bone mass, stimulates bone regeneration during fracture healing, reduces bone resorption, decreases bone loss, or maintains or increases bone strength.
32. A method of claim 30, wherein the subject is need of maintained or increased bone formation, maintained or increased bone mineral density, maintained or increased bone mass, bone regeneration during fracture healing, reduced bone resorption, decreased bone loss, or maintained or increased bone strength.
33. A method of claim 30, wherein the amount of the water soluble extract of rosehip administered is about 1 mg to about 2000 mg per kg body weight, about 100 to about 1000 mg per kg body weight,

about 50 to about 500 mg per kg body weight, or about 0.05 mg to about 300 mg per kg body weight per day.