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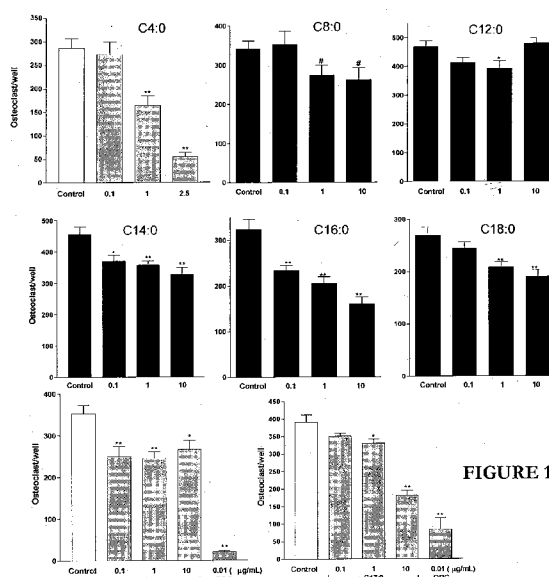


FIGURE 1

(57) Abstract: Use of carboxylic acids, or salts thereof, or ester or ether, amide, amine, ketone or aldehyde derivatives thereof, or free fatty acids, lipids, lipid compositions or dairy lipid fractions to treat or prevent conditions likely to benefit from a reduction in bone resorption or an increase in osteoblast proliferation.

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TREATMENT OR PREVENTION OF BONE CONDITIONS

FIELD OF THE INVENTION

[0001] The present invention relates to use of carboxylic acids, or salts thereof, or ester or ether or amide or amine derivatives thereof, or free fatty acids, lipids, lipid compositions or dairy lipid fractions to treat or prevent conditions likely to benefit from a reduction in bone resorption or an increase in osteoblast proliferation.

BACKGROUND

[0002] It has been reported that in the US alone, 10 million people have osteoporosis and that 50% of women and 25% of men will suffer from an osteoporotic fracture. It has also been reported that some osteoporosis sufferers prefer orally active therapies, rather than therapies delivered intravenously or subcutaneously, and also that regular administration of the therapy is undesirable. (Fraenkel et al, 2006)

[0003] There are reports exploring the links between lipids and bone (Reid, 2002). Total body fat mass is reported to be related to bone density and to fracture risk, and changes in fat mass are reported to be positively related to changes in bone density. There are reports from feeding experiments that fat ingestion influences bone turnover (Henriksen, et al, 2003; Reid, et al, 2006).

[0004] ω 3 PUFAs have been reported to reduce production of inflammatory cytokines in human osteoblastic cells whereas ω 6 PUFAs have the opposite effect (Priante, et al, 2002). It has been reported that manipulation of PUFA (poly-unsaturated fatty acid) intake results in changes in osteoblast activity *in vitro* and bone strength *in vivo* in animal studies (Watkins, et al, 2003).

[0005] The need exists for alternative therapies for osteoporosis and other conditions that are likely to benefit from a reduction in bone resorption or an increase in osteoblast proliferation so that sufferers have a wider range of options. It would be desirable to provide an alternative means for treating or preventing conditions likely to benefit from a reduction in bone resorption or an increase in osteoblast proliferation.

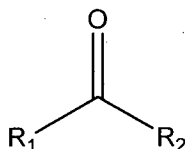
SUMMARY OF THE INVENTION

[0006] Accordingly, in one aspect the present invention relates to use of at least one agent selected from:

- (a) one or more straight chain saturated carboxylic acids or esters or salts thereof comprising 3 to 9 or 11 to 24 carbon atoms, optionally substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups,
- (b) one or more derivatives of a straight chain carboxylic acid, wherein the acid moiety is replaced with an ester, ether, amide, amine, ketone or aldehyde moiety, or a salt thereof, wherein the carboxylic acid comprises 3 to 24 carbon atoms, optionally substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups, and wherein the carboxylic acid is saturated or monounsaturated, provided that when the derivative is an amide it is not an N-acylated lysophospholipid comprising a methoxycarbonyl group or a hydroxymethyl group at the sn-2 position,
- (c) milk fat, one or more milk fat fractions, or a combination thereof,
- (d) high CLA milk fat, one or more fractions thereof, or a combination thereof, and
- (e) a combination of any two or more agents selected from any of (a) to (d),

to treat or prevent a condition likely to benefit from a reduction in bone resorption or an increase in osteoblast proliferation.

[0007] In one embodiment the agent is a compound of Formula (Ia)



or a salt thereof, wherein

R₁ is selected from straight or branched C11 to C23 alkyl optionally substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups;

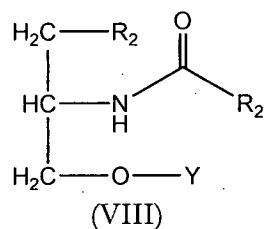
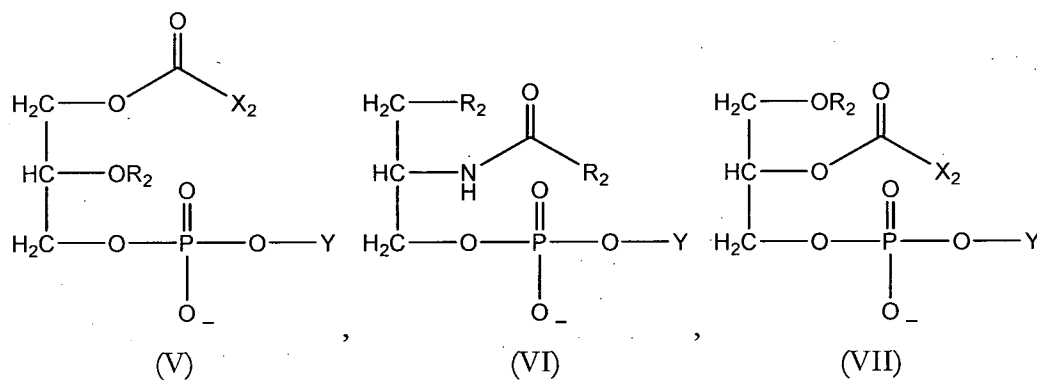
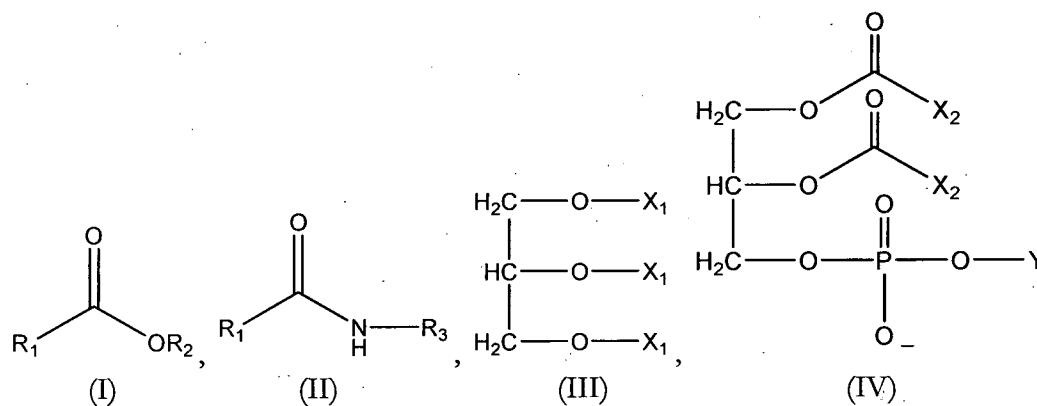
R₂ is selected from H, -CH₃, -R₁, -OH, -OR₁, -OR₃, or -NR₄R₅;

R₃ is selected from straight or branched C1 to C6 alkyl (preferably selected from methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, isobutyl, tert-butyl, pentyl, and hexyl groups), straight or branched C2 to C6 alkenyl, and straight or branched C2 to C6 alkynyl; and

R_4 and R_5 are independently selected from H, straight or branched C1 to C6 alkyl (preferably selected from methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, isobutyl, tert-butyl, pentyl, and hexyl groups), straight or branched C2 to C6 alkenyl, straight or branched C2 to C6 alkynyl.

[0008] In another embodiment, the agent is a compound of Formula (I), (II), (III), (IV) (V),

5 (VI), (VII) or (VIII)



15 or a salt thereof, wherein

R_1 is independently selected from straight or branched chain C2 to C23 alkyl, straight or branched chain C2 to C17 alkenyl, straight or branched chain C2 to C17 alkynyl, C3 to C6 cycloalkyl and C4 to C6 cycloalkenyl; wherein R_1 may be optionally substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups,

20 R_2 is selected from H, $-CH_3$ and R_1 ,

R_3 is selected from H, $-CH_3$, R_1 and the residue of an amino acid or protein such that $-NHR_3$ is an N-acyl amino acid or protein,

X_1 is independently selected from H, $-CH_3$, R_1 , $-CH_2-R_1$ and $-C(O)-R_1$, provided that at least one X_1 is $-CH_2-R_1$ or $-C(O)-R_1$,

5 X_2 is independently selected from H, $-CH_3$ and R_1 , provided that at least one X_2 is R_1 , and

Y is selected from H, $-CH_2-CH(OH)-CH_2OH$, $-CH_2-CH_2-NH_3^+$, $-CH-CH(NH_3^+)COO^-$, $-CH_2-CH_2N^+(CH_3)_3$, or a saccharide, wherein the saccharide is selected from a monosaccharide, a disaccharide, a trisaccharide, and a polysaccharide, including galactosyl-3-sulfate ester saccharides and saccharides comprising sialic acids (including N- or O-substituted derivatives of neuraminic acid),

provided that when the agent is a compound of Formula (I), R_1 is selected from straight or branched chain C11 to C17 alkyl optionally substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups.

[0009] In another aspect the present invention relates to a method of treating or preventing a condition likely to benefit from a reduction in bone resorption or an increase in osteoblast proliferation, the method comprising administering an effective amount of one or more agents as defined above to a subject in need thereof.

[0010] The following embodiments may relate to any of the above aspects.

[0011] In one embodiment the agent is a carboxylic acid or salt thereof selected from C12:0 to C18:0 fatty acids. In one embodiment, the agent is a compound of Formula (Ia) and R_2 is OH. In another embodiment the agent is a compound of formula (I) to (VIII) or salt thereof and R_1 is straight or branched chain C2 to C23 alkyl or straight or branched chain C2 to C17 alkenyl, preferably straight chain C2 to C23 alkyl or straight chain C2 to C17 alkenyl, more preferably straight chain C2 to C23 alkyl. In other embodiments R_1 is straight or branched chain C10 to C17 alkyl or straight or branched chain C10 to C17 alkenyl, preferably straight chain C10 to C17 alkyl or straight chain C10 to C17 alkenyl, more preferably straight chain C10 to C17 alkyl. In various embodiments R_2 is as defined as above.

[0012] In various embodiments the agent is optionally substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups wherein the position of the one or more substitutions is at one or more positions selected from carbon 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23 or 24 of the carboxylic acid or of R_1 , depending on the chain

length of the carboxylic acid or of R₁. In other embodiments where the carboxylic acid or R₁ group has a chain length of n carbon atoms, the optional one or more substitutions is at one or more positions selected from carbon n, n-1, n-2, n-3, n-4, n-5, n-6, n-7, n-8, n-9 or n-10, depending on the chain length of the carboxylic acid or of R₁. For agents having two or more R₁ groups, those R₁ may be independently substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups as described above.

[0013] In one embodiment the carboxylic acid is a fatty acid or salt thereof selected from the group comprising butyric acid (C4:0), caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1 cis-9; cis-9-octadecenoic acid), elaidic acid (C18:1 trans-9; trans-9-octadecenoic acid), vaccenic acid (C18:1 trans-11; trans-11-octadecenoic acid), cis-vaccenic acid (C18:1 cis-11; cis-11-octadecenoic acid), arachidic acid (C20:0), and behenic acid (C22:0), optionally substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups, and salts, esters and amides thereof, and combinations thereof. The optional substituent(s) may be present at any position along the carbon chain. In various embodiments, the preferred fatty acid or salt thereof is selected from C12:0 to C18:0, or combinations of any two or more thereof.

[0014] In one embodiment the carboxylic acid or salt thereof is selected from the group comprising propanoic acid (C3:0), butanoic acid (C4:0), pentanoic acid (C5:0), hexanoic acid (C6:0), heptanoic acid (C7:0), octanoic acid (C8:0), nonanoic acid (C9:0), decanoic acid (C10:0), undecanoic acid (C11:0), dodecanoic acid (C12:0), tridecanoic acid (C13:0), tetradecanoic acid (C14:0), pentadecanoic acid (C15:0), hexadecanoic acid (C16:0), heptadecanoic acid (C17:0), octadecanoic acid (C18:0), nonadecanoic acid (C19:0), eicosanoic acid (C20:0), heneicosanoic acid (C21:0), docosanoic acid (C22:0), tricosanoic acid (C23:0), and tetracosanoic acid (C24:0), optionally substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups, and salts, esters and amides thereof, and monounsaturated forms thereof, and combinations thereof. The optional substituent(s) and unsaturated bond may be present at any position along the carbon chain. In various embodiments, the preferred carboxylic acid or salt thereof is selected from C11:0 to C20:0, C11:0 to C18:0, C12:0 to C18:0 or C12:0 to C16:0, or combinations of any two or more thereof.

[0015] In one embodiment the carboxylic acid or salt thereof is a monounsaturated carboxylic acid selected from the group comprising propenoic acid (C3:1), butenoic acid (C4:1), pentenoic acid (C5:1), hexenoic acid (C6:1), heptenoic acid (C7:1), octenoic acid (C8:1), nonenoic acid (C9:1), decenoic acid (C10:1), undecenoic acid (C11:1), dodecenoic acid (C12:1), tridecenoic acid (C13:1), tetradecenoic acid (C14:1), pentadecenoic acid (C15:1), hexadecenoic acid (C16:1), heptadecenoic

acid (C17:1), and octadecenoic acid (C18:1; including C18:1c6, C18:1t6, C18:1c7, C18:1t7, C18:1c9, C18:1t9, C18:1c11, C18:1t11, C18:1c12, C18:1t12, C18:1c13, C18:1t13, C18:1c15, and C18:1t15), optionally substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups, and salts, esters and amides thereof, and combinations thereof. The optional substituent(s) and unsaturated bond may be present at any position along the carbon chain. In various embodiments, the preferred carboxylic acid or salt thereof is selected from C11:1 to C20:1, C11:1 to C18:1, C12:1 to C18:1 or C12:1 to C16:1, or combinations of any two or more thereof.

[0016] In one embodiment the carboxylic acid or salt thereof is a hydroxy fatty acid selected from alpha-hydroxy acids, beta-hydroxy acids, and polyhydroxy acids (such as ricinoleic acid (12-hydroxy-(cis)-9-octadecenoic acid) or dihydroxystearic acid).

[0017] In one embodiment, the agent is a compound of Formula (Ia), R_2 is NR_4R_5 ; and R_4 and R_5 are independently selected from H, straight or branched C1 to C6 alkyl, straight or branched C1 to C6 alkenyl, and straight or branched C2 to C6 alkynyl.

[0018] Amides useful herein include but are not limited to compounds of Formula (Ia) wherein R_4 and R_5 are independently selected H, from methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, isobutyl, tert-butyl, pentyl and hexyl groups.

[0019] In one embodiment the amide or salt thereof is selected from the group comprising propanamide (C3:0), butanamide (C4:0), pentanamide (C5:0), hexanamide (C6:0), heptanamide (C7:0), octanamide (C8:0), nonanamide (C9:0), decanamide (C10:0), undecanamide (C11:0), dodecanamide (C12:0), tridecanamide (C13:0), tetradecanamide (C14:0), pentadecanamide (C15:0), hexadecanamide (C16:0), heptadecanamide (C17:0), octadecanamide (C18:0), nonadecanamide acid (C19:0), eicosanamide acid (C20:0), heneicosanamide acid (C21:0), docosanamide acid (C22:0), tricosanamide acid (C23:0), and tetracosanamide acid (C24:0), optionally substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups, and N-substituted forms thereof, and salts, esters and amides thereof, and monounsaturated forms thereof, and combinations thereof. The optional substituent(s) and unsaturated bond may be present at any position along the carbon chain. Preferred N-substituted amides include mono-substituted and di-substituted amides wherein one or both hydrogen atoms are replaced by straight or branched C1 to C6 alkyl, straight or branched C1 to C6 alkenyl, or straight or branched C2 to C6 alkynyl.

[0020] In one embodiment the amide or salt thereof is a monounsaturated amide selected from the group comprising butenamide (C4:1), pentenamide (C5:1), hexenamide (C6:1), heptenamide (C7:1), octenamide (C8:1), nonenamide (C9:1), decenamide (C10:1), undecenamide (C11:1),

dodecenamide (C12:1), tridecenamide (C13:1), tetradecenamide (C14:1), pentadecenamide (C15:1), hexadecenamide (C16:1), heptadecenamide (C17:1), and octadecenamide (C18:1), optionally substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups, and N-substituted forms thereof, and salts, esters and amides thereof, and combinations thereof. The optional substituent(s) and unsaturated bond may be present at any position along the carbon chain. Preferred N-substituted amides include mono-substituted and di-substituted amides wherein one or both hydrogen atoms are replaced by straight or branched C1 to C6 alkyl, straight or branched C1 to C6 alkenyl, or straight or branched C2 to C6 alkynyl.

[0021] Amines useful herein include but are not limited to compounds of Formula (Ia) wherein R_2 is NR_4R_5 ; and R_4 and R_5 are independently selected H, from methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, isobutyl, tert-butyl, pentyl and hexyl groups.

[0022] In one embodiment, the agent is a compound of Formula (Ia), R_2 is OR_3 ; and R_3 is selected from straight or branched C1 to C6 alkyl, straight or branched C2 to C6 alkenyl, straight or branched C2 to C6 alkynyl, glycerol, mono-glyceride, di-glyceride, phospholipid, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylcholine, sphingomyelin, lysophospholipid, ceramide, glucoceramides, lactoceramide, ether glycerophospholipid, glycolipid, cerebroside, sulfatide, and ganglioside, and proteolipid.

[0023] Esters useful herein include but are not limited to alkyl esters (including but not limited to methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, isobutyl, tert-butyl, pentyl, and hexyl esters), glycerides (including mono-, di- and tri-glycerides), phospholipids (including phosphatidylethanolamines, phosphatidylinositols, phosphatidylserines, phosphatidylcholines and sphingomyelins), lysophospholipids (phospholipids with one fatty acid lost), ceramides (including glucoceramides and lactoceramides), ether glycerophospholipids, glycolipids (including cerebroside, sulfatides, and gangliosides) and proteolipids. Combinations of such esters may be used.

[0024] In one embodiment the agent is an ester of the carboxylic acid and glycerol, a mono-glyceride, a di-glyceride, phosphatidic acid, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, sphingomyelin, lysophospholipid, ceramide, glucoceramide, lactoceramide, a glycolipid, a cerebroside, a sulfatide, a ganglioside, or a proteolipid.

[0025] Ethers useful herein include but are not limited to compounds of Formula (Ia) wherein R_2 is OR_3 ; R_3 is selected from straight or branched C1 to C6 alkyl, straight or branched C2 to C6 alkenyl, straight or branched C2 to C6 alkynyl, glycerol, mono-glyceride, di-glyceride, phosphatidic

acid, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, sphingomyelin, lysophospholipid, ceramide, glucoceramide, lactoceramide, glycolipid, cerebroside, sulfatide, ganglioside, proteolipid, and amino acids. In one embodiment, the ether is an ether lipid, ether phospholipid, or ether glycerophospholipid.

[0026] In one embodiment the agent is an ether derivative of an ester of the carboxylic acid and glycerol, a mono-glyceride, a di-glyceride, phosphatidic acid, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, sphingomyelin, lysophospholipid, ceramide, glucoceramide, lactoceramide, a glycolipid, a cerebroside, a sulfatide, a ganglioside, or a proteolipid. In one embodiment, the ether is an ether lipid, ether phospholipid, or ether glycerophospholipid.

[0027] In one embodiment the amino acid is selected from the group comprising glycine, alanine, beta-alanine, α -methylalanine, valine, leucine, isoleucine, alloisoleucine, phenylalanine, proline, serine, homoserine, threonine, allothreonine, glutamic acid, methionine, asparagine, aspartic acid, glutamine, histidine, lysine, arginine, tyrosine, and tryptophan, and including the D or L isomers thereof, and including α , β and γ -amino acids.

[0028] In one embodiment the saccharide is a monosaccharide, disaccharide, trisaccharide or polysaccharide, including galactosyl-3-sulfate ester saccharides and saccharides comprising sialic acids (including N- or O-substituted derivatives of neuraminic acid (NANA)). In various embodiments preferred saccharides comprise monosaccharides, disaccharides, trisaccharides or polysaccharides comprising one or more pentoses or one or more hexoses (such as glucose and galactose) or a combination of one or more pentoses and one or more hexoses. Preferred saccharides include the saccharides GAL-GLU-NANA-NANA (ganglioside GD3) and GAL-GLU-NANA (ganglioside GM3).

[0029] 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, phosphorous, potassium, pyridinium ($\text{C}_5\text{H}_5\text{NH}^+$), quaternary ammonium (NR_4^+), silicon, sodium, strontium, and zinc salts, or a combination thereof.

[0030] In one embodiment the agent is naturally present in or is administered as a component of a lipid composition. Preferred lipid compositions include animal, plant and marine oils and fats and lipids produced by fermentation with microorganisms. Preferred animal fats include but are not

limited to dairy fats, particularly bovine milk fat. In one embodiment the agent or composition comprises palm oil.

[0031] In one embodiment the agent comprises or is administered as a milk fat fraction. In another embodiment the agent is milk fat or a milk fat fraction. Preferred milk fat fractions include cream, butter, anhydrous milk fat (AMF) (typically produced by phase inversion of cream or dehydration of butter), butter milk, butter serum, hard milk fat fractions from one or more stages of fractionation (including H, SH, and SSH fractions), soft milk fat fractions from one or more stages of fractionation (including S, SS, and SSS fractions); combinations of hard milk fat fractions, combinations of soft milk fat fractions, combinations of hard milk fat fractions and soft milk fat fractions, sphingolipid fractions, milk fat globule membrane fractions, phospholipid fractions, complex lipid fractions, ceramide fractions, ganglioside fractions, any combinations of any two or more thereof, hydrolysates of any one or more thereof, fractions of the hydrolysates, combinations of any two or more hydrolysates, and combinations of one or more hydrolysed and one or more non-hydrolysed fractions. In one embodiment the agent is a milk fat fraction supplemented with another agent, such as a carboxylic acid or a salt thereof or a derivative thereof, as described above.

[0032] In one embodiment the composition comprises at least about 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, 99.5, 99.8 or 99.9% by weight of one or more agents as described above and useful ranges may be selected between any of these foregoing values (for example, about 0.1 to 50, 0.2 to 50, 0.5 to 50, 1 to 50, 5 to 50, 10 to 50, 15 to 50, 20 to 50, 25 to 50, 30 to 50, 35 to 50, 40 to 50, 45 to 50, 0.1 to 60, 0.2 to 60, 0.5 to 60, 1 to 60, 5 to 60, 10 to 60, 15 to 60, 20 to 60, 25 to 60, 30 to 60, 35 to 60, 40 to 60, 45 to 60, 0.1 to 70, 0.2 to 70, 0.5 to 70, 1 to 70, 5 to 70, 10 to 70, 15 to 70, 20 to 70, 25 to 70, 30 to 70, 35 to 70, 40 to 70, 45 to 70, 0.1 to 80, 0.2 to 80, 0.5 to 80, 1 to 80, 5 to 80, 10 to 80, 15 to 80, 20 to 80, 25 to 80, 30 to 80, 35 to 80, 40 to 80, 45 to 80, 0.1 to 90, 0.2 to 90, 0.5 to 90, 1 to 90, 5 to 90, 10 to 90, 15 to 90, 20 to 90, 25 to 90, 30 to 90, 35 to 90, 40 to 90, 45 to 90, 0.1 to 99, 0.2 to 99, 0.5 to 99, 1 to 99, 5 to 99, 10 to 99, 15 to 99, 20 to 99, 25 to 99, 30 to 99, 35 to 99, 40 to 99, and 45 to 99%).

[0033] In one embodiment the composition comprises at least about 0.001, 0.01, 0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 99 or 100 grams of one or more agents as described above and useful ranges may be selected between any of these foregoing values (for example, about 0.01 to 1, 0.01 to 10, 0.01 to 19, 0.1 to 1, 0.1 to 10, 0.1 to 19, 1 to 5, 1 to 10, 1 to 19, 5 to 10, 5 to 19, 1 to 100, 10 to 100, 20 to 100, and 30 to 100 grams).

[0034] In one embodiment the composition comprises, consists essentially of, or consists of about 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, 99.5, 99.8 or 99.9% by weight of one or more agents as described above and useful ranges may be selected between any of these foregoing values (for example, about 0.1 to 50, 0.2 to 50, 0.5 to 50, 1 to 50, 5 to 50, 10 to 50, 15 to 50, 20 to 50, 25 to 50, 30 to 50, 35 to 50, 40 to 50, 45 to 50, 0.1 to 60, 0.2 to 60, 0.5 to 60, 1 to 60, 5 to 60, 10 to 60, 15 to 60, 20 to 60, 25 to 60, 30 to 60, 35 to 60, 40 to 60, 45 to 60, 0.1 to 70, 0.2 to 70, 0.5 to 70, 1 to 70, 5 to 70, 10 to 70, 15 to 70, 20 to 70, 25 to 70, 30 to 70, 35 to 70, 40 to 70, 45 to 70, 0.1 to 80, 0.2 to 80, 0.5 to 80, 1 to 80, 5 to 80, 10 to 80, 15 to 80, 20 to 80, 25 to 80, 30 to 80, 35 to 80, 40 to 80, 45 to 80, 0.1 to 90, 0.2 to 90, 0.5 to 90, 1 to 90, 5 to 90, 10 to 90, 15 to 90, 20 to 90, 25 to 90, 30 to 90, 35 to 90, 40 to 90, 45 to 90, 0.1 to 99, 0.2 to 99, 0.5 to 99, 1 to 99, 5 to 99, 10 to 99, 15 to 99, 20 to 99, 25 to 99, 30 to 99, 35 to 99, 40 to 99, and 45 to 99%).

[0035] In one embodiment the composition comprises, consists essentially of, or consists of at least about 0.1, 0.5, 1, 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50% by weight of 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, cream, butter, anhydrous milk fat (AMF), cheese, butter milk, butter serum, hard milk fat fractions, soft milk fat fractions, sphingolipid fractions, milk fat globule membrane fractions, phospholipid fractions, complex lipid fractions, colostrum, a colostrum fraction, colostrum protein concentrate (CPC), colostrum whey, an immunoglobulin fraction from colostrum, whey, whey protein isolate (WPI), whey protein concentrate (WPC), sweet whey, lactic acid whey, mineral acid whey, reconstituted whey powder, 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 and/or non-hydrolysed fractions.

5 **[0036]** In one embodiment the composition 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 or pharmaceutical. In one embodiment the invention relates to use of one or more agents described above in the manufacture of a composition such as a medicament for the treatment or prevention of
10 a condition as described herein. In one embodiment the composition is in the form of a tablet, a caplet, a pill, a hard or soft capsule or a 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
15 reduce degradation of the composition during storage or after administration. In one embodiment the composition is or is formulated as a food, drink, food additive, drink additive, dietary supplement, nutritional product, medical food, enteral or parenteral feeding product, meal replacement, or cosmeceutical. These compositions may include any edible consumer product which is able to carry fatty acids or lipid. Examples of suitable edible consumer products include
20 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 (including natural cheese and processed cheese) and milk fat products (including butters and ghees), drinks including dairy and non-dairy based drinks, milk, milk powders, sports supplements including dairy and non-dairy based sports supplements, fruit juice,
25 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.

[0037] In one embodiment the composition may further comprise another bone-health agent, such as calcium, fluoride, magnesium, zinc, calcium salts, fluoride salts, magnesium salts, zinc salts, vitamin A, vitamin B6, vitamin C, vitamin D, vitamin D derivatives (including but not limited to
30 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, and combinations thereof. In another embodiment the composition may further comprise one or more omega-3 or omega-6 fatty acids, or a source thereof. Suitable sources include marine oils.

[0038] In one embodiment the condition is likely to benefit from or the subject is in need of decreased bone resorption or decreased osteoclastogenesis or both. Accordingly, in one embodiment the invention relates to treatment or prevention of a condition associated with net bone resorption or increased osteoclastogenesis.

[0039] In another embodiment the condition is likely to benefit from or the subject is in need of increased bone formation or increased osteoblast proliferation or both. Accordingly, in this embodiment the invention relates to treatment or prevention of a condition associated with poor bone formation or decreased osteoblast proliferation.

[0040] In another embodiment the condition is a skeletal disorder. Examples of such disorders include but are not limited to bone fracture, bone damage following surgery, osteoporosis, rheumatoid arthritis, osteoarthritis, 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, osteogenesis imperfecta and oral bone erosion (such as peritonitis or osteonecrosis of the jaw, particularly of alveolar bone). In one embodiment the condition is osteoporosis, osteoarthritis or oral bone erosion. In another embodiment the condition is osteoporosis.

[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.

BRIEF DESCRIPTION OF THE DRAWINGS

[0043] Figure 1 is a graph showing the effects of saturated fatty acids on osteoclastogenesis in bone marrow cultures. Each fatty acid has been assessed at the same 3 concentrations, shown in $\mu\text{g/mL}$. The number of carbon atoms in the fatty acid is denoted by the number after the "C". Data are mean $\pm$ SE. Significant differences from control are shown as follows: * $P < 0.05$, ** $P < 0.01$, by Dunnett's test; # $P < 0.05$, by t-test only.

[0044] Figure 2 is a graph showing the effect of 18-carbon fatty acids on osteoclastogenesis in bone marrow cultures. The fatty acids differ in the number of their double bonds (indicated by the number after the colon) and according to whether the double bonds are in a *cis* (c) or *trans* (t) configuration. C18:1 is C18:1c9, oleic acid. Each fatty acid has been assessed at the same 3 concentrations. Data are mean $\pm$ SE, and are ratios of the number of osteoclasts formed in the experimental wells to those in the control wells for that experiment (t/c). Significant differences from control are shown as follows: * $P < 0.05$, ** $P < 0.01$, by Dunnett's test; # $P < 0.05$, by t-test only.

[0045] Figure 3 is a graph showing the effect of $\omega 3$ and $\omega 6$ fatty acids on osteoclastogenesis in bone marrow cultures. C18:0 is shown for comparison. Each fatty acid has been assessed at 3 concentrations as indicated on the figure. The low solubility of C20:4 and C 20:5 reduced the maximum concentrations that could be studied. Data are mean $\pm$ SE, and are ratios of the number of osteoclasts formed in the experimental wells to those in the control wells for that experiment (t/c). Significant differences from control are shown as follows: * $P < 0.05$, ** $P < 0.01$, by Dunnett's test; # $P < 0.05$, by t-test only.

[0046] Figure 4 is a graph showing the effect of palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2) acids on thymidine incorporation into primary cultures of fetal rat osteoblasts at 24 hours. Significant differences from control are shown as follows: * $P < 0.05$, ** $P < 0.01$, by Dunnett's test.

[0047] Figure 5 is a graph showing the effect of palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2) acids on bone resorption (assessed as Ca^{45} release) in cultures of neonatal mouse calvariae. No significant effects were detected at concentrations of 1 and 10 $\mu\text{g/mL}$.

[0048] Figure 6 is six graphs showing the effect of phospholipid or ganglioside fractions and hydrolysed phospholipid or ganglioside fractions on osteoclastogenesis in bone marrow cultures.

Phospholipid fractions were prepared and tested as described in the examples. (A) PL2-2 (hydrolysed PL1-2 that contained phosphatidylcholine and phosphatidylinositol in similar amounts, a small amount of phosphatidylserine, and the first of the three sphingomyelin peaks), (B) PL2-3 (hydrolysed PL1-3 that contained a large amount of phosphatidylethanolamine and ceramides and lysophospholipid), (C) PL3-1 (Phospholipid Concentrate PC600™ phospholipid fraction), (D) PL3-2 (hydrolysed PL3-1), (E) PL3-3 (Ganglioside G600™ ganglioside fraction), and (F) PL3-4 (hydrolysed PL3-3). Significant differences from control are shown as follows: (A) to (C), (E) and (F) - *: $P < 0.05$; **: $P < 0.01$; ANOVA test. (D) - **: $P < 0.01$, ANOVA test; #: $P < 0.05$, t-test.

[0049] Figure 7 is seven graphs showing the effect of milk fat fractions and hydrolysed milk fat fractions on osteoclastogenesis in bone marrow cultures. Milk fat fractions were prepared and tested as described in the examples and significant differences from control are shown as follows. (A) MF1-1 (CLA-rich milk fat), *: $P < 0.05$; **: $P < 0.01$, ANOVA test; #: $P < 0.05$, t-test. (B) MF1-2 (a milk fat fraction rich in saturated fats), *: $P < 0.05$; **: $P < 0.01$, ANOVA test. (C) MF2-1 (hydrolysed MF1-1), **: $P < 0.01$, ANOVA test; #: $P < 0.05$, t-test. (D) MF2-2 (hydrolysed MF1-2), **: $P < 0.01$, ANOVA test; #: $P < 0.05$, t-test. (E) MF3-1 (CLA-rich milk fat with a higher CLA concentration than MF1-1), **: $P < 0.01$, Dunnett's test. (F) MF3-2 (partially hydrolysed MF3-1, neutralised with acetic acid), *: $P < 0.05$, **: $P < 0.01$, Dunnett's test. (G) MF3-3 (partially hydrolysed MF3-1, neutralised with hydrochloric acid), **: $P < 0.01$, Dunnett's test.

[0050] Figure 8 is a graph showing the effect of hydrolysed milk fat fractions on osteoblast proliferation. (*: $P < 0.05$), Dunnett's test.

[0051] Figure 9 is four graphs showing the effect on osteoclastogenesis in bone marrow cultures of the following. Significant differences from control are shown. (A) glycerol (**: $P < 0.01$, ANOVA test), (B) monopalmitin ($P < 0.0001$ ANOVA, Dunnett's, **: $P < 0.01$), (C) dipalmitin (**: $P < 0.01$), and (D) hydrolysed tripalmitin ($P < 0.0001$ ANOVA, Dunnett's, **: $P < 0.01$).

[0052] Figure 10 is four graphs showing the effect on osteoclastogenesis in bone marrow cultures of the following. Significant differences from control are shown. (A) hydrolysed soybean oil (**: $P < 0.01$, Dunnett's test), (B) hydrolysed palm oil (**: $P < 0.01$; #: $P < 0.05$, t-test), (C) hydrolysed coconut oil (*: $P < 0.05$, Dunnett's test; #: $P < 0.05$, in t-test only) and (D) hydrolysed safflower oil (**: $P < 0.01$ over control in ANOVA test).

DETAILED DESCRIPTION OF THE INVENTION

[0053] The examples below demonstrate inhibition of osteoclastogenesis in bone marrow cultures by saturated fatty acids. This effect is related to fatty acid chain length, and is maximal for palmitic acid (C16:0).

- 5 [0054] Accordingly, in one aspect the present invention relates to use of one or more agents as described above to treat or prevent a condition likely to benefit from a reduction in bone resorption or an increase in osteoblast proliferation. Methods of conversion of a carboxylic acid to an ester or ether or amide or amine are known in the art. Many of the compounds of Formula (Ia) or Formula (I) to (VIII) will be readily commercially available.

10 1. Definitions

[0055] The terms “alkenyl” and “alkynyl” refer to straight or branched chain groups having one double or triple bond, respectively.

- [0056] The terms “anhydrous milk fat” and “AMF” are used interchangeably herein and refer to the milk fat fraction produced by phase inversion of cream, or dehydration of butter. Milk fat
 15 may be any mammalian milk fat including but not limited to bovine, sheep, goat, pig, mouse, water buffalo, camel, yak, horse, donkey, llama or human milk fat, with bovine milk fat being a preferred source. Methods commonly used for the preparation of AMF are disclosed in Bylund (Ed., 1995), incorporated herein by reference in its entirety. Preferred AMF is typically about 60%, about 70%, about 80%, about 90%, about 95%, greater than about 95%, about 96%, about 97%, about 98%,
 20 about 99%, about 99.5%, or 100% fat, with AMF of about 99% fat, 99.5% fat or greater being more preferred. AMF is frequently further fractionated into “hard”(H) and “soft”(S) fractions, the latter can be further fractionated into “soft hard”(SH) and “soft soft”(SS) fractions, the latter can again be further fractionated into “soft soft hard”(SSH) and “soft soft soft”(SSS) fractions. As will be appreciated, each fraction differs in fatty acid composition. Non-limiting exemplary fatty acid
 25 compositions for AMF and derivative fractions are shown in Tables 1 to 5 below.

Table 1. Exemplary AMF composition

Fatty acid component	Mean (% w/w)	Min (% w/w)	Max (% w/w)
c4:0 (butyric acid)	3.6	3.3	4.1
c6:0 (caproic acid)	2.2	1.9	2.4
c8:0 (caprylic acid)	1.2	1.1	1.4
c10:0 (capric acid)	2.6	2.2	2.8
c10:1 (2-decenoate)	0.3	0.3	0.3

Fatty acid component	Mean (% w/w)	Min (% w/w)	Max (% w/w)
c12:0 (lauric acid)	2.9	2.5	3.2
c12:1 (11-dodecenoic acid)	0.1	0.1	0.1
c13:0 br (tridecanoic acid br)	0.1	0.1	0.1
c13:0 (tridecanoic acid)	0.1	0.1	0.1
c14:0 br (myristic acid br)	0.2	0.1	0.2
c14:0 (myristic acid)	10.4	9.5	10.8
c14:1 (myristoleic acid)	0.9	0.6	1.0
c15:0 iso	0.4	0.3	0.5
c15:0 ante-iso	0.6	0.5	0.7
c15:0 (pentadecanoic acid)	1.4	1.1	1.5
c16:0 br	0.3	0.2	0.3
c16:0 (palmitic acid)	28.7	25.4	30.4
c16:1 (palmitoleic acid)	1.9	1.6	2.0
c17:0 iso	0.7	0.6	0.7
c17:0 ante-iso	0.5	0.5	0.5
c17:0 (margaric acid)	0.7	0.6	0.8
c17:1	0.3	0.3	0.4
c18:0 (stearic acid)	11.5	10.8	13.6
c18:1 (oleic acid)	23.4	21.8	26.4
c18:2 (linoleic acid)	1.4	1.3	1.7
c18:2 conj	1.3	1.0	1.8
c18:3	0.8	0.7	0.9
c20:0 (arachidic acid)	0.2	0.1	0.2
c20:1	0.3	0.2	0.3

Table 2. Exemplary Hard Fraction (Fraction H) composition

Fatty acid component	Mean (% w/w)	Min (% w/w)	Max (% w/w)
c4:0 (butyric acid)	2.0	1.8	2.1
c6:0 (caproic acid)	1.3	1.2	1.4
c8:0 (caprylic acid)	0.8	0.8	0.9
c10:0 (capric acid)	2.2	1.9	2.4
c10:1 (2-decenoate)	0.2	0.1	0.2
c12:0 (lauric acid)	3.0	2.6	3.4
c12:1 (11-dodecenoic acid)	0.0	0.0	0.1
c13:0 br (tridecanoic acid br)	0.1	0.1	0.1
c13:0 (tridecanoic acid)	0.1	0.1	0.1
c14:0 br (myristic acid br)	0.1	0.1	0.2
c14:0 (myristic acid)	11.8	10.7	12.6
c14:1 (myristoleic acid)	0.6	0.3	0.7
c15:0 iso	0.4	0.3	0.5
c15:0 ante-iso	0.5	0.4	0.6
c15:0 (pentadecanoic acid)	1.6	1.2	1.7
c16:0 br	0.3	0.3	0.3
c16:0 (palmitic acid)	34.8	31.5	36.6
c16:1 (palmitoleic acid)	1.3	1.1	1.6

Fatty acid component	Mean (% w/w)	Min (% w/w)	Max (% w/w)
c17:0 iso	0.8	0.7	0.8
c17:0 ante-iso	0.5	0.5	0.6
c17:0 (margaric acid)	0.9	0.8	0.9
c17:1	0.2	0.2	0.3
c18:0 (stearic acid)	15.2	13.9	19.7
c18:1 (oleic acid)	17.0	15.5	19.8
c18:2 (linoleic acid)	1.3	1.1	1.5
c18:2 conj	0.8	0.6	1.1
c18:3	0.5	0.4	0.6
c20:0 (arachidic acid)	0.2	0.2	0.3
c20:1	0.2	0.1	0.2

Table 3. Exemplary Soft Hard Fraction (Fraction SH) composition

Fatty acid component	Mean (% w/w)	Min (% w/w)	Max (% w/w)
c4:0 (butyric acid)	4.0	3.7	4.3
c6:0 (caproic acid)	2.4	2.1	2.6
c8:0 (caprylic acid)	1.2	1.1	1.4
c10:0 (capric acid)	2.4	2.2	2.7
c10:1 (2-decenoate)	0.3	0.2	0.3
c12:0 (lauric acid)	2.5	2.3	2.7
c12:1 (11-dodecenoic acid)	0.1	0.0	0.1
c13:0 br (tridecanoic acid br)	0.1	0.1	0.1
c13:0 (tridecanoic acid)	0.1	0.1	0.1
c14:0 br (myristic acid br)	0.1	0.1	0.2
c14:0 (myristic acid)	9.8	9.0	10.3
c14:1 (myristoleic acid)	0.8	0.5	0.9
c15:0 iso	0.4	0.3	0.4
c15:0 ante-iso	0.5	0.4	0.6
c15:0 (pentadecanoic acid)	1.4	1.1	1.5
c16:0 br	0.2	0.2	0.3
c16:0 (palmitic acid)	32.8	29.8	34.0
c16:1 (palmitoleic acid)	1.5	1.3	1.8
c17:0 iso	0.6	0.6	0.7
c17:0 ante-iso	0.4	0.4	0.5
c17:0 (margaric acid)	0.8	0.8	0.9
c17:1	0.3	0.2	0.3
c18:0 (stearic acid)	13.2	12.5	16.1
c18:1 (oleic acid)	19.5	17.4	22.2
c18:2 (linoleic acid)	1.3	1.2	1.5
c18:2 conj	1.2	1.0	1.6
c18:3	0.7	0.6	0.7
c20:0 (arachidic acid)	0.2	0.2	0.3
c20:1	0.2	0.2	0.3

Table 4. Exemplary Soft Soft Hard Fraction (Fraction SSH) composition

Fatty acid component	Mean (% w/w)	Min (% w/w)	Max (% w/w)
c4:0 (butyric acid)	4.0	3.9	4.3
c6:0 (caproic acid)	2.4	2.2	2.6
c8:0 (caprylic acid)	1.4	1.2	1.6
c10:0 (capric acid)	2.8	2.4	3.4
c10:1 (2-decenoate)	0.3	0.3	0.3
c12:0 (lauric acid)	3.2	2.7	3.8
c12:1 (11-dodecenoic acid)	0.1	0.1	0.1
c13:0 br (tridecanoic acid br)	0.1	0.1	0.1
c13:0 (tridecanoic acid)	0.1	0.1	0.1
c14:0 br (myristic acid br)	0.2	0.1	0.2
c14:0 (myristic acid)	11.5	10.6	12.2
c14:1 (myristoleic acid)	0.9	0.7	1.0
c15:0 iso	0.4	0.4	0.5
c15:0 ante-iso	0.6	0.6	0.7
c15:0 (pentadecanoic acid)	1.4	1.2	1.5
c16:0 br	0.3	0.2	0.3
c16:0 (palmitic acid)	28.6	25.7	30.0
c16:1 (palmitoleic acid)	1.8	1.6	2.0
c17:0 iso	0.7	0.6	0.7
c17:0 ante-iso	0.5	0.5	0.5
c17:0 (margaric acid)	0.7	0.6	0.8
c17:1	0.3	0.3	0.4
c18:0 (stearic acid)	10.6	10.2	11.3
c18:1 (oleic acid)	22.2	20.3	24.8
c18:2 (linoleic acid)	1.4	1.3	1.5
c18:2 conj	1.3	1.1	1.7
c18:3	0.8	0.8	1.0
c20:0 (arachidic acid)	0.2	0.1	0.2
c20:1	0.2	0.0	0.3

Table 5. Exemplary Soft Soft Soft Fraction (Fraction SSS) composition

Fatty acid component	Mean (% w/w)	Min (% w/w)	Max (% w/w)
c4:0 (butyric acid)	4.4	4.0	4.7
c6:0 (caproic acid)	2.7	2.4	2.8
c8:0 (caprylic acid)	1.6	1.4	1.8
c10:0 (capric acid)	3.4	2.8	3.7
c10:1 (2-decenoate)	0.4	0.3	0.4
c12:0 (lauric acid)	3.7	3.2	4.1
c12:1 (11-dodecenoic acid)	0.1	0.1	0.1
c13:0 br (tridecanoic acid br)	0.2	0.1	0.2
c13:0 (tridecanoic acid)	0.1	0.1	0.1
c14:0 br (myristic acid br)	0.2	0.2	0.2
c14:0 (myristic acid)	10.2	9.5	11.0
c14:1 (myristoleic acid)	1.2	0.8	1.3

Fatty acid component	Mean (% w/w)	Min (% w/w)	Max (% w/w)
c15:0 iso	0.5	0.4	0.5
c15:0 ante-iso	0.8	0.7	0.9
c15:0 (pentadecanoic acid)	1.1	0.9	1.2
c16:0 br	0.3	0.2	0.3
c16:0 (palmitic acid)	20.0	18.4	21.1
c16:1 (palmitoleic acid)	2.6	2.2	3.0
c17:0 iso	0.6	0.5	0.6
c17:0 ante-iso	0.5	0.5	0.5
c17:0 (margaric acid)	0.4	0.4	0.5
c17:1	0.5	0.5	0.6
c18:0 (stearic acid)	6.7	5.8	7.8
c18:1 (oleic acid)	30.8	28.2	33.4
c18:2 (linoleic acid)	1.9	1.7	2.1
C18:2 conj	1.7	1.3	2.3
C18:3	1.3	1.1	1.4
C20:0 (arachidic acid)	0.1	0.1	0.1
C20:1	0.3	0.1	0.4

[0057] The term “comprising” as used in this specification means “consisting at least in part of”. When interpreting statements in this specification which include that term, the features, prefaced by that term in each statement or claim, 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 same manner.

[0058] An “effective amount” is the amount required to confer 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 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, and the like.

[0059] The term “high CLA milk fat” means milk fat that comprises a higher level of c-9, t-11 conjugated linoleic acid (CLA) or a salt, ester or precursor thereof than normal milk fat, and, optionally, a higher level of one or more other CLA isomers. High CLA milk fat may prepared by known techniques including but not limited to supplementary free fatty acid feeding of pasture fed cows by, for example, feeding cows with fish oil and/or plant oils such as sunflower oil according to known methods. High CLA milk fat is typically prepared from whole milk but may also be prepared from colostrum. A typical composition of high CLA milk fat is described in published international PCT application WO 2005/107736 that is hereby incorporated by reference. High CLA milk fat may also be prepared by supplementing milk fat with CLA. In one embodiment the high CLA milk

fat comprises at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45 or 50% by weight CLA, preferably c-9, t-11 CLA or a salt, ester or precursor thereof and useful ranges may be selected between any of these forgoing values (e.g. from about 4% to about 8%). Preferably the high CLA milk fat comprises at least about 2% c-9, t-11 CLA by weight, preferably about 2 to 10% c-9, t-11 CLA by weight, more preferably about 4 to 7% c-9, t-11 CLA by weight and most preferably about 5% c-9, t-11 CLA by weight. In one embodiment the high CLA milk fat comprises CLA isomers which comprise at least about 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 99% by weight: c-9, t-11 CLA or a salt, ester or precursor thereof and useful ranges may be selected between any of these forgoing values (e.g. from about 80% to about 95%). Preferably the high CLA milk fat includes CLA isomers comprising at least about 50% c-9, t-11 CLA by weight, preferably about 70 to 80% c-9, t-11 CLA by weight. The term "conjugated linoleic acid" (CLA) refers to one or more CLA isomers selected from isomers of 9,11-octadecadienoic acid and 10,12-octadecadienoic acid, in free or esterified form, or salts thereof, or mixtures thereof, including the cis-9,cis-11, cis-9,trans-11, trans-9,cis-11, trans-9,trans-11, cis-10,cis-12, cis-10,trans-12, trans-10,cis-12, and trans-10,trans-12 isomers, preferably the cis-9,cis-11, cis-9,trans-11, trans-10,cis-12, and cis-10,cis-12 isomers, preferably the cis-9,trans-11 isomer, as described in published United States patent US 5,585,400 incorporated herein by reference.

[0060] The term "milk fat" includes mammalian milk lipids and lipid fractions, lipid hydrolysates, and lipid fraction hydrolysates. Preferred milk fats are dairy fats, particularly bovine milk fats. Preferred milk fat has one or more of palmitic acid, oleic acid, stearic acid, or myristic acid as the most abundant fatty acid(s) present, preferably palmitic, oleic, stearic and myristic acids are the most abundant fatty acids present. In particularly preferred embodiments, the milk fat has a) substantially the same percentage by weight of palmitic acid as does normal bovine milk fat (between about 23%(w/w) and about 32%(w/w), typically about 28%(w/w) – see Table 1.2, PF Fox and PLH McSweeney eds, Advanced Dairy Chemistry Volume 2 - Lipids, 3rd Ed, Springer NY, NY (2006) ISBN-10:0-387-26364-0); b) substantially the same percentage by weight of oleic acid as does normal bovine milk fat (between about 15%(w/w) and about 22%(w/w), typically about 17%(w/w) – see Fox and McSweeney *ibid*); c) substantially the same percentage by weight of stearic acid as does normal bovine milk fat (between about 10%(w/w) and about 15%(w/w), typically about 12%(w/w) – see Fox and McSweeney *ibid*); d) substantially the same percentage by weight of myristic acid as does normal bovine milk fat (between about 9%(w/w) and about 12%(w/w), typically about 11%(w/w) – see Fox and McSweeney *ibid*); e) any two of a), b), c), or d) above; f) any three of a), b), c), or d) above; g) each of a), b), c), and d) above. Anhydrous milk fat (AMF) is preferred, particularly AMF having substantially the same percentage by weight palmitic, oleic and stearic acid composition as normal bovine milk fat, more preferably substantially the same fatty acid

composition as normal bovine milk fat (see Fox and McSweeney *ibid*). Preferred milk fat fractions include cream, butter, anhydrous milk fat (AMF) (typically produced by phase inversion of cream or dehydration of butter), butter milk, butter serum, hard milk fat fractions from one or more stages of fractionation (including H, SH, and SSH fractions), soft milk fat fractions from one or more stages of fractionation (including S, SS, and SSS fractions), combinations of hard milk fat fractions, combinations of soft milk fat fractions, combinations of hard milk fat fractions and soft milk fat fractions, sphingolipid fractions, milk fat globule membrane fractions, phospholipid fractions, and complex lipid fractions, ceramide fractions, ganglioside fractions, and any combinations thereof of any two or more thereof, and hydrolysates thereof of any one or more thereof, and fractions of the hydrolysates, combinations of any two or more hydrolysates, and combinations of one or more hydrolysed and/or one or more non-hydrolysed fractions. In one embodiment the agent is a milk fat fraction supplemented with another agent, such as a carboxylic acid or a salt thereof or a derivative thereof, as described above.

[0061] The term “oral administration” includes oral, buccal, enteral and intra-gastric administration.

[0062] The term “parenteral administration” includes but is not limited to topical (including administration to any dermal, epidermal, mucosal or bone surface), subcutaneous, intravenous, intraperitoneal, and intramuscular administration.

[0063] The term “pharmaceutically acceptable carrier” is intended to refer to a carrier including but not limited to an excipient, diluent or auxiliary, or combination thereof, that can be administered to a subject as a component of a composition described herein 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 compound or composition useful herein. The formulations can be administered orally, nasally or parenterally (including topically, intramuscularly, intraperitoneally, subcutaneously and intravenously).

[0064] A “subject” is an animal, preferably a mammal, more preferably a mammalian companion animal or human. Preferred companion animals include cats, dogs and horses.

[0065] 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 and/or prevention of the onset of the symptoms or severity of a particular condition; for example reduction in the degree of bone loss experienced by a subject.

[0066] The term "variant" refers to a naturally occurring (an allelic variant, for example) or non-naturally occurring (an artificially generated mutant, for example) polypeptide or polypeptide fragment that varies from the predominant wild-type amino acid sequence of a polypeptide of a given species or a fragment thereof by the addition, deletion or substitution of one or more amino acids.

[0067] The term "vitamin D analog" refers to any compound that will bind and activate a vitamin D receptor (VDR). The VDR is a ligand-activated intracellular receptor that acts as a transcription factor and binds vitamin D response elements (VDREs) in the promoter/enhancer regions of genes. Assays for assessing VDR binding are known; for example, immunoassays that measure the expression of genes regulated by vitamin D. Therefore, candidate vitamin D analogs may be readily assessed without undue experimentation for use according to the present invention.

2. Sources of fatty acids

[0068] In one embodiment the carboxylic acid or compound is a fatty acid selected from butyric acid (C4:0), caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1 cis-9; cis-9-octadecenoic acid), elaidic acid (C18:1 trans-9; trans-9-octadecenoic acid), vaccenic acid (C18:1 trans-11; trans-11-octadecenoic acid), cis-vaccenic acid (C18:1 cis-11; cis-11-octadecenoic acid), arachidic acid (C20:0), and behenic acid (C22:0), optionally substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups, and salts, esters and amides thereof, and combinations thereof. The optional substituent(s) may be present at any position along the carbon chain. Preferred fatty acids include butyric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, and elaidic acid, salts, esters and amides thereof, and combinations thereof. These fatty acids are the major components of bovine milk fat.

[0069] Fatty acids may be derived from many plant and animal sources, or produced by bacterial fermentation.

[0070] Plant sources of palmitic, oleic and linoleic acids include canola (rapeseed), coconut, corn, olive, palm, peanut, safflower, and soybean oils. Of these oils, canola and soybean oils also contain useful levels of linolenic acid. Coconut oil contains useful levels of lauric and myristic acids.

[0071] Marine oils, such as fish oils (including anchovy, cod herring, mackerel, orange roughy, salmon, sardine, trout, tuna, shark, and swordfish oils) and shellfish oils (including mussel oils,

including Lyprinol™ green-lipped mussel oil), also provide a useful source of fatty acids, particularly omega-3 fatty acids.

[0072] Cereals, vegetable oils and eggs are useful sources of omega-6 fatty acids

3. Dairy lipids and lipid fractions

5 [0073] Dairy lipids are discussed comprehensively by Fox and McSweeney (2006), hereby incorporated by reference. Fractionation of dairy lipids is discussed in the Dairy Processing Handbook, 1995 and by Illingworth, 2002, and Rombaut et al, 2006(b), all hereby incorporated by reference in their entirety. Seasonal variation of milk fat is discussed by Fox and McSweeney (2006).

10 [0074] Examples of dairy lipid fractions useful according to the invention include cream (typically about 20 to about 40% fat by weight, preferably about 40% fat by weight), butter, anhydrous milk fat (AMF) (typically produced by phase inversion of cream or dehydration of butter), butter milk, butter serum, hard milk fat fractions, soft milk fat fractions, sphingomyelin fractions, milk fat globule membrane fractions, phospholipid fractions, and complex lipid fractions, and combinations thereof, and hydrolysates thereof. Lipids may be hydrolysed by treatment with
15 lipases (Fox and McSweeney (2006), Chapter 15 by HC Deeth and CH Fitz-Gerald).

[0075] Buttermilk, butter serum, and beta serum are discussed by Bylund, 1995, Rombaut et al, 2005, Rombaut et al, 2006(a), and Rombaut et al, 2006(b), for example, all incorporated herein by reference. Buttermilk is a term used to describe the aqueous liquid phase obtained from traditional butter production using a butter making process which may be a batch (churn) process or a
20 continuous (Fritz) process. Buttermilk is also a term used to describe the aqueous by-product produced by the cream concentration step of the traditional method of producing AMF from cream. This traditional method involves concentration then phase inversion of cream to produce oil that is further concentrated and polished to produce AMF. Finally, buttermilk is also a term used to describe a combination of the secondary skim and beta serum by-products of a two-serum process
25 for AMF production. In that two-serum process, the by-product from the cream concentration step is further separated to produce secondary skim and the by-product from the oil concentration step is further separated to produce beta-serum. In the first two instances, the buttermilk is produced before any phase inversion has occurred. In the third instance, the buttermilk is a combination of secondary skim produced before phase inversion and beta serum produce after phase inversion.
30 Concentration and polishing in these processes is typically achieved by centrifugation. Phase inversion is typically achieved by homogenisation. It should be understood that the source of these dairy lipid fractions may be milk or colostrum or a combination thereof.

[0076] Useful starting materials for fractionation include cream, AMF, butter milk, butter serum, or beta serum, from milk or colostrum or a combination thereof.

[0077] Multistage fractionation of milk fat may be carried out by differential crystallisation.

Milk fat fractions are heated to a set temperature and the crystallised or solid ("stearin" – hard fraction) and liquid ("olein" – soft fraction) fractions are separated. Multi-step fractionation refers to re-fractionation in a subsequent step of a product of a previous fractionation step. Successive soft fractions may be produced by fractionating parent soft fractions into soft and hard sub-fractions.

[0078] Other fractionation methods include phase inversion, interesterification, glycerolysis,

solvent fractionation (such as with ethanol, water, or acetone, used alone or sequentially), supercritical fractionation (see Astaire, et al, 2003, for example), near supercritical fractionation (see WO 2004/066744, for example), distillation, centrifugal fractionation, suspension crystallisation, dry crystallisation, fractionation with a modifier (e.g. soaps or emulsifiers), and combinations of these methods, all as known in the art.

[0079] Lipids present in the compositions of the invention may be fully or partially modified,

whether naturally, chemically, enzymatically, or by any other methods known in the art, including, for example, glycosylated, sialylated, esterified, phosphorylated or hydrolysed. Lipid hydrolysates may be prepared using known techniques, including but not limited to acid hydrolysis, base hydrolysis, enzymatic hydrolysis using a lipase, for example as described in Fox and McSweeney

((2006), Chapter 15 by HC Deeth and CH Fitz-Gerald), and microbial fermentation. One method of base hydrolysis includes adding 1% KOH (in ethanol) and heating for 10 minutes. Hydrolysed material may be neutralised with acetic acid or hydrochloric acid.

[0080] Milk fat globule membrane material may be isolated according to the acidification

method of Kanno & Dong-Hyun, 1990, and further fractionated into complex lipid and lipoprotein fractions by the addition of methanol, as described by Kanno et al, 1975. A phospholipid fraction may be isolated by extracting the lipid mixture with acetone according to the procedure of Purthi et al, 1970. Lipid residue may be further enriched in complex lipids by the selective extraction of simple lipids with pentane.

[0081] Phospholipid fractions useful herein include the Phospholipid Concentrate PC500™

phospholipid fraction (available from Fonterra Co-operative Group Limited, New Zealand). The PC500™ phospholipid fraction is a spray dried milk phospholipid concentrate with a typical composition of 77-95% total lipids, from about 50% neutral lipids and from about 30% polar lipids;

and a typical lipid composition of 1.5-5 % phosphatidyl serine, 12-18% phosphatidyl choline, 6-9% phosphatidyl ethanolamine, and 6.7-9% sphingomyelin; and a typical fatty acid composition of butyric acid (4:0) 1.8%, capric acid (10:0) 0.3%, lauric acid (12:0) 0.5%, myristic acid (14:0) 7.4%, myristoleic acid (14:1) 14.1%, pentadecanoic acid (15:0) 1.0%, palmitic acid (16:0) 26.0%, palmitoleic acid (16:1) 1.7%, margaric acid (17:0) 0.6%, heptadecenoic acid (17:1) 0.3%, stearic acid (18:0) 11.9%, oleic acid (18:1) 39.0%, linoleic acid (18:2) 5.0%, linolenic (18:3) 2.0%, arachidic acid (20:0) 0.3%, and cholesterol 0.8%.

[0082] Another phospholipid fraction useful herein includes the Phospholipid Concentrate PC600™ phospholipid fraction (available from Fonterra Co-operative Group Limited, New Zealand). The PC600™ phospholipid fraction is a freeze dried milk phospholipid concentrate with a typical composition of 75% polar lipids, 8.0% neutral lipids, <12% ash, and <4% moisture; a typical lipid composition of phosphatidyl serine 3-4%, phosphatidyl choline >36%, phosphatidyl ethanolamine >9%, and sphingomyelin >18%; and a typical fatty acid composition of myristic acid (14:0) 6.6%, palmitic acid (16:0) 27.1%, palmitoleic acid (16:1) 1.3%, margaric acid (17:0) 2.3%, stearic acid (18:0) 14%, oleic acid (18:1) 38.2%, linoleic acid (18:2) 6.5%, linolenic acid (18:3) 2%, cholesterol 0.1%, and others 2%.

[0083] Another phospholipid fraction useful herein includes the Phospholipid Concentrate PC700™ phospholipid fraction (available from Fonterra Co-operative Group Limited, New Zealand). The PC700™ phospholipid fraction is a freeze dried milk phospholipid concentrate with a typical composition of 85% lipids, 10% ash, 2% lactose, and 2.5% moisture; a typical lipid composition of phosphatidyl serine 3%, phosphatidyl choline 31%, phosphatidyl ethanolamine 8.7%, and sphingomyelin 16.5%; and a typical fatty acid composition of myristic acid (14:0) 5.4%, palmitic acid (16:0) 20.9%, palmitoleic acid (16:1) 1.3%, margaric acid (17:0) 0.5%, stearic acid (18:0) 10.5%, oleic acid (18:1) 30.5%, linoleic acid (18:2) 4.3%, linolenic acid (18:3) 1.8%, and arachidonic acid 0.5%.

[0084] Ganglioside fractions useful herein include the Ganglioside GL500™ and G500™ and G600™ ganglioside fractions (available from Fonterra Co-operative Group Limited, New Zealand). The GL500™ ganglioside fraction is a crude extract and the G500™ ganglioside fraction is a spray dried milk ganglioside concentrate with good powder flowability. The G500™ ganglioside fraction has a typical composition of lipids 34.0%, moisture 3.2%, ash 5.0%, and lactose 56.0%; a typical lipid composition of ganglioside GD3 0.6% and ganglioside GM3 0.5%; and a typical fatty acid composition of myristic acid (14:0) 5.6%, palmitic acid (16:0) 18.4%, palmitoleic acid (16:1) 1.2%, margaric acid (17:0) 0.5%, stearic acid (18:0) 14.9%, oleic acid (18:1) 31.0%, linoleic acid (18:2) 3.8%, linolenic acid (18:3) 1.5%, and arachidonic acid (20:4) 0.5%.

[0085] The Ganglioside G600™ ganglioside fraction is a spray dried milk ganglioside concentrate with good powder flowability. The G600™ ganglioside fraction has a typical composition of lipids 30.0%, moisture 3.5%, ash 8.3%, and lactose 58.0%; a typical lipid composition of ganglioside GD3 1.4%, ganglioside GM3 0.3%, phosphatidyl serine 4.5%,
5 phosphatidyl choline 5.1%, phosphatidyl ethanolamine 2.0%, and sphingomyelin 1.7%; and a typical fatty acid composition of myristic acid (14:0) 4.7%, palmitic acid (16:0) 16.4%, palmitoleic acid (16:1) 1.2%, margaric acid (17:0) 0.5%, stearic acid (18:0) 17.0%, oleic acid (18:1) 33.4%, linoleic acid (18:2) 4.2%, linolenic acid (18:3) 1.4%, and arachidonic acid (20:4) 0.6%.

[0086] Fractionation methods useful to produce milk fat fractions useful herein are also
10 described in published international patent applications WO 2004/066744, WO 2006/041316, WO 2007/123424, and WO 2007/123425 that are each incorporated herein by reference in their entirety.

4. Compositions useful according to invention

[0087] A composition useful herein may be formulated as a food, drink, food additive, drink
15 additive, dietary supplement, nutritional product, medical food, enteral or parenteral feeding product, meal replacement, cosmeceutical, or pharmaceutical. Appropriate formulations may be prepared by an art skilled worker with regard to that skill and the teaching of this specification.

[0088] In one embodiment, compositions useful herein include any edible consumer product
20 which is able to carry carboxylic acids, fatty acids or lipid. Examples of suitable edible consumer products include powders, liquids, 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 (such as milk drinks and yogurt 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. Within this embodiment, a composition useful herein may also
25 be an infant formula, in powder or liquid form. Suitable nutraceutical compositions useful herein may be provided in similar forms.

[0089] 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
30 A, 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 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, and combinations thereof.

[0090] 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 parenteral (including topical, subcutaneous, intramuscular and intravenous) administration.

[0091] Thus, a pharmaceutical composition useful according to the invention may be formulated with an appropriate pharmaceutically acceptable carrier (including excipients, diluents, auxiliaries, and combinations thereof) selected with regard to the intended route of administration and standard pharmaceutical practice. For example, a composition useful according to the invention can be administered orally as a powder, liquid, tablet or capsule, or topically as an ointment, cream or lotion. Suitable formulations may contain additional agents as required, including emulsifying, antioxidant, flavouring or colouring agents, and may be adapted for immediate-, delayed-, modified-, sustained-, pulsed- or controlled-release.

[0092] Capsules can be formed from 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 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. Pharmaceutical compositions can also be administered via the parenteral route. Examples of parenteral dosage forms include aqueous solutions, isotonic saline or 5% glucose of the active agent, or other well-known pharmaceutically acceptable excipient. Cyclodextrins, or other solubilising agents well-known to those familiar with the art, can be utilized as pharmaceutical excipients for delivery of the therapeutic agent.

[0093] The efficacy of a composition useful according to the 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 inhibit osteoclastogenesis *in vitro*. For *in vivo* studies, the composition can be fed to or injected into an animal (e.g., a mouse) and its effects on bone tissues are then accessed. Based on the results, an appropriate dosage range and administration route can be determined.

[0094] 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 attenuate one or more of the symptoms of a condition associated with bone resorption or osteoclastogenesis.

[0095] 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.

[0096] 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, 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, insulin-like growth factor, bone morphogenetic protein (BMP), inhibitors of BMP antagonism, prostaglandin derivatives, fibroblast growth factors, soy isoflavones, 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 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 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, and combinations thereof, and other suitable agents known in the art.

[0097] 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 lactoferrin fragment, vitamin D or calcium, 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. Useful lactoferrins, fragments and compositions are described in international patent applications WO 03/082921 and WO2007/043900, both incorporated herein by reference in their entirety.

[0098] 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.

[0099] 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/or 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 a composition useful herein is administered per day, preferably about 50 to about 1500 mg per kg per day. In one embodiment, the inventors contemplate administration of from about 0.05 mg to about 250 mg per kg body weight of a pharmaceutical composition useful herein. In another embodiment where the agent is delivered as an oral composition, the inventors contemplate administration of about 1 gram to about 100 grams per day of a composition described above, such as milk fat, preferably about 10 to 100, about 20 to 100, about 30 to 100, about 40 to 100, about 50 to 100, about 60 to 100, about 70 to 100, about 80 to 100, about 90 to 100, about 20 to 80, about 30 to 80, about 40 to 80, about 50 to 80, about 60 to 80, about 30 to 60, about 40 to 60 or about 50 to 60 grams per day. It should be appreciated that administration may include a single daily dose or administration of a number of discrete divided doses as may be appropriate.

[0100] Various aspects of the invention will now be illustrated in non-limiting ways by reference to the following examples.

EXAMPLES

Osteoblast-like cell culture for assessing osteoblast proliferation

[0101] Osteoblasts were isolated from 20-day fetal rat calvariae, as previously described (Cornish, et al, 1999). Briefly, calvariae were excised and the frontal and parietal bones, free of suture and periosteal tissue, were collected. The calvariae were sequentially digested using collagenase and the cells from third and fourth digests were collected, pooled and washed. Cells were grown in T75 flasks in 10% fetal bovine serum (FBS) (GibcoBRL, Life Technologies, Auckland, New Zealand)/ Dulbecco's modified eagle medium (DMEM) (GIBCO, Invitrogen Corporation, Auckland, New Zealand) for 2 days and then changed to 10% FBS/ minimum essential medium (MEM) (GIBCO, Invitrogen Corporation, Auckland, New Zealand) and grown to 90% confluency. Cells were then seeded into 24 well plates in 5% FBS/ MEM for 24 hours. Cells were growth-arrested in 0.1% bovine serum albumin (BSA) (ICPbio, Auckland, New Zealand) for 24 h. Fresh media and experimental compounds were then added for a further 24 h. Cells were pulsed with [³H]-thymidine 4 hours before the end of the experimental incubation. The experiment was terminated and cell counts or thymidine incorporation assessed. There were 6 wells in each group and each experiment was repeated 3 or 4 times.

Bone marrow culture for assessing osteoclastogenesis

[0102] Bone marrow was obtained from the long bones of normal Swiss male mice aged 4-6 weeks, as previously described (Cornish, et al, 1999). Marrow cells were placed in 90 mm petri dishes for two hours and non-adherent cells collected and grown in 48-well plates. 1,25(OH)₂D₃ (10⁻⁸M) was added (day 0) to all wells except to negative controls. The cultures were fed 0.5 mL of fresh medium on day 2 and 0.5 mL was replaced with fresh medium on day 4. 1,25(OH)₂D₃ (10⁻⁸M) was added on days 0, 2 and 4, while test substances were added on days 2 and 4. Osteoprotegerin was used as a positive control in some experiments. After culture for 7 days, multi nucleated cells (≥3 nuclei) staining with tartrate-resistant acid phosphatase (TRAP) were counted. There were at least 8 wells for each group and each experiment was repeated 2 or 3 times.

Bone organ culture for assessing bone resorption

[0103] Mice were injected subcutaneously with 3 μCi ⁴⁵Ca at 1-3 days of age, and hemi calvariae were dissected out 3 days later. Hemi-calvariae were pre incubated for 24 hours in medium 199 with 0.1% BSA, then changed to fresh medium containing test substances or vehicle. Incubation was continued for a further 48 hours. There were 6-8 hemi-calvariae in each group and each experiment was repeated twice.

[0104] Culture media for all studies described above contained penicillin (100 units/mL) and streptomycin (100 µg/mL). All animal procedures were approved by the Animal Ethics Committee of our institution.

Lipid Preparations

- 5 [0105] Fatty acids were purchased from Sigma (St Louis MO) and milk fat fractions were provided by Fonterra Co-operative Group Limited (New Zealand). AMF and hard and soft fractions provided by Fonterra Co-operative Group Limited are described above. Monopalmitin, dipalmitin and tripalmitin were purchased from Sigma (USA). Soybean oil, coconut oil, safflower oil and palm oil were purchased locally and the oils were food grade oils.
- 10 [0106] The Ganglioside G600™ ganglioside fraction (available from Fonterra Co-operative Group Limited, New Zealand) is a spray dried milk ganglioside concentrate with a typical composition of lipids 30.0%, moisture 3.5%, ash 8.3%, and lactose 58.0%; a typical lipid composition of ganglioside GD3 1.4%, ganglioside GM3 0.3%, phosphatidyl serine 4.5%, phosphatidyl choline 5.1%, phosphatidyl ethanolamine 2.0%, and sphingomyelin 1.7%; and a typical
- 15 fatty acid composition of myristic acid (14:0) 4.7%, palmitic acid (16:0) 16.4%, palmitoleic acid (16:1) 1.2%, margaric acid (17:0) 0.5%, stearic acid (18:0) 17.0%, oleic acid (18:1) 33.4%, linoleic acid (18:2) 4.2%, linolenic acid (18:3) 1.4%, and arachidonic acid (20:4) 0.6%.

- [0107] The Phospholipid Concentrate PC600™ phospholipid fraction (available from Fonterra Co-operative Group Limited, New Zealand) is a spray dried milk phospholipid concentrate with a
- 20 typical composition of 75% polar lipids, 8.0% neutral lipids, and 4% moisture; a typical lipid composition of phosphatidyl serine 3%, phosphatidyl choline 32%, phosphatidyl ethanolamine 10%, and sphingomyelin 18%; and a typical fatty acid composition of myristic acid (14:0) 6.6%, palmitic acid (16:0) 27.1%, palmitoleic acid (16:1) 1.3%, margaric acid (17:0) 2.3%, stearic acid (18:0) 14%, oleic acid (18:1) 38.2%, linoleic acid (18:2) 6.5%, linolenic acid (18:3) 2%, cholesterol 0.1%, and
- 25 others 2%.

Statistics

[0108] Data were analyzed using analysis of variance with ANOVA, post-hoc Dunnett's or t-tests. A 5% significance level is used throughout. Data are presented as means ± SE, unless indicated otherwise. Analyses were performed using Prism v3 (Graphpad.com).

EXAMPLE 1 – Certain fatty acids inhibit osteoclastogenesis

[0109] The effects of butyric (C4:0), caprylic (C8:0), lauric (C12:0), myristic (C14:0), pentadecanoic (C15:0), palmitic (C16:0), heptadecanoic (C17:0) and stearic (C18:0) acids on osteoclastogenesis were assessed. Each fatty acid was assessed at three concentrations (0.1, 1.0 and 10 µg/mL) and results are shown in Figure 1.

EXAMPLE 2 – Certain C18 fatty acids inhibit osteoclastogenesis

[0110] C18 fatty acids having different numbers and positions of double bonds were assessed for their ability to inhibit osteoclastogenesis. The effects of stearic (C18:0), oleic (C18:1c9), linoleic (C18:2), alpha-linolenic (C18:3), elaidic (C18:1t9), vaccenic (C18:1t11) and cis-9, trans-11 conjugated linoleic (C18:2c) acids were assessed. Results are shown in Figure 2.

EXAMPLE 3 – ω3 and ω6 fatty acids are not particularly anti-osteoclastogenic

[0111] The anti-osteoclastogenic effects of a number of ω3 and ω6 fatty acids were assessed. The effects of stearic (C18:0), linoleic (C18:2) [ω6], arachidonic (C20:4) [ω6], alpha-linolenic (C18:3) [ω3], eicosapentaenoic (20:5) [EPA; ω3] and docosahexanoic (C22:6) [DHA; ω3] acids were assessed. The results are shown in Figure 3.

EXAMPLE 4 – Certain fatty acids stimulate osteoblast proliferation

[0112] Fatty acids with anti-osteoclastogenic effects consistently caused moderate stimulation of thymidine incorporation into osteoblasts at concentrations of 0.1 µg/mL and 1.0 µg/mL (Figure 4). The highest concentration (10 µg/mL) was less consistent in its effect, but did not decrease thymidine incorporation in any experiment.

EXAMPLE 5 – Certain fatty acids do not appear to be anti-resorptive

[0113] The effects of palmitic (C16:0) stearic (C18:0) oleic (C18:1) and linoleic (C18:2) acids were assessed on bone resorption in cultures of neonatal mouse calvariae. Ca⁴⁵ release from the calvariae was unaffected by any of these fatty acids when studied at concentrations of 1 and 10 µg/mL (Figure 5).

EXAMPLE 6 – Activity of milk fat fractions and hydrolysed milk fractions

[0114] Milk fractions and hydrolysed milk fractions were assayed for activity as described above and were found to inhibit osteoclastogenesis (Figures 6 and 7) and stimulate osteoblast proliferation (Figure 8). Hydrolysed typical milk fat (AMF) was found to inhibit osteoclastogenesis at a

concentration of 0.1 ug/ml ($P < 0.05$ compared to control; data not shown) but was not significantly active at concentrations of 1 or 10 ug/ml. The hydrolysed typical milkfat should have given a response that had a similar shape to Figure 7(D) but the magnitude of the response was less.

[0115] Phospholipid fractions were separated from anhydrous milk fat (Fonterra Co-operative Group Limited, New Zealand) by thin-layer chromatography, and/or liquid chromatography. The fractions were PL1-1 (contained all three sphingomyelin peaks), PL1-2 (contained phosphatidylcholine and phosphatidylinositol in similar amounts, a small amount of phosphatidylserine, and the first of the three sphingomyelin peaks), and PL1-3 (contained a large amount of phosphatidylethanolamine and ceramides and lysophospholipid). Hydrolysed forms of these fractions were prepared (fractions PL2-1, PL2-2, and PL2-3, respectively). The samples were hydrolysed by adding 1% KOH (in ethanol), and stirring and heating for 10 minutes at pH 9.5-10.0. Hydrolysed samples were neutralised with acetic acid or hydrochloric acid to pH 7.0 and flushed dry in heated mantle under nitrogen. Samples neutralised with either acid gave the same result.

[0116] Commercial fractions were also tested. The Phospholipid Concentrate PC600™ phospholipid fraction and the Ganglioside G600™ ganglioside fraction (both available from Fonterra Co-operative Group Limited, New Zealand) were tested in non-hydrolysed form (fractions PL3-1 and PL3-3 respectively) and hydrolysed form (fractions PL3-2 and PL3-4 respectively). Fractions were hydrolysed as above.

[0117] Milk fat fractions were also prepared. MF1-1 was CLA-rich milk fat prepared by feeding cows with fish oil and sunflower oil according to known methods. MF1-2 was a milk fat fraction rich in saturated fats. MF2-1 was a hydrolysed form of MF1-1 and MF2-2 a hydrolysed form of MF1-2, both hydrolysed as described above. MF3-1 was a CLA-rich milk fat with a higher CLA concentration than MF1-1. MF3-2 was partially hydrolysed MF3-1, neutralised with acetic acid. MF3-3 was partially hydrolysed MF3-1, neutralised with hydrochloric acid.

EXAMPLE 7 – Activity of palmitic acid esters

[0118] Monopalmitin, dipalmitin and hydrolysed tripalmitin were assayed for activity as described above and were found to inhibit osteoclastogenesis (Figure 9). The tripalmitin was hydrolysed as described in Example 6.

EXAMPLE 6 – Activity of plant oils

[0119] Soybean oil, coconut oil, safflower oil and palm oil were hydrolysed as described in Example 6 and assayed for activity as described above and were found to inhibit osteoclastogenesis

(Figure 10). Safflower oil, soybean oil and coconut oil (Figure 10) were inactive. Palm oil (Figure 10(B)) was active at 0.1 ug/ml.

EXAMPLE 8 – Effects on bone density *in vivo* after injection

[0120] The mouse model described by Cornish et al (1993) may be used to assess effects on bone growth *in vivo*. Injections of active agents are given daily for 5 days, and the animals sacrificed a week later. Bone formation is determined by fluorescent labelling of newly formed bone. Indices of bone resorption and of bone mass are determined by conventional light microscopy, assisted by image analysis software.

EXAMPLE 9 – Effects on bone density *in vivo* after oral administration

[0121] The ability of active agents to reduce bone loss may be studied in the ovariectomised rat model (Kruger et al, 2005; Kruger et al, 2006).

[0122] 6-month-old female Sprague-Dawley rats are divided into sham-operated animals and OVX animals. Sham-operated animals undergo anaesthesia and an incision is made but the ovaries are left intact. The ovaries of OVX animals are removed.

[0123] Rats are fed appropriate treatment and control diets and are scanned regularly under anaesthesia to obtain bone mineral density (BMD) measurements. Bone mineral measurements can be taken using a Hologic QDR4000 bone densitometer using a pencil beam unit (Bedford, USA). Measurements are typically taken of the spine and left and right femurs while rats are positioned supine with right angles between the spine and femur, and femur and tibia

INDUSTRIAL APPLICATION

[0124] The present invention has utility in treating or preventing conditions likely to benefit from a reduction in bone resorption or an increase in osteoblast proliferation.

[0125] The described compositions may be employed as foods, drinks, food additives, drink additives, dietary supplements, nutritional products, medical foods, nutraceuticals, medicaments or pharmaceuticals. The described compositions and methods may be employed to treat or prevent one or more of the conditions discussed above.

[0126] 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.

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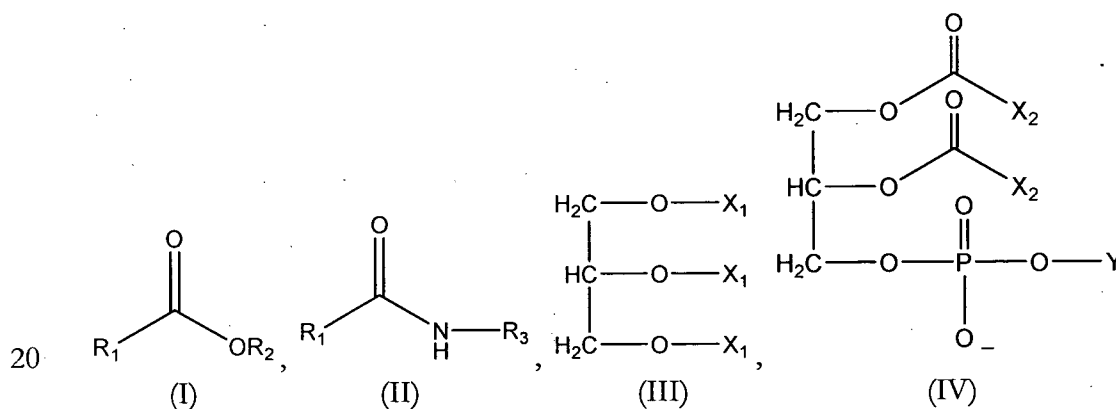
WHAT WE CLAIM IS

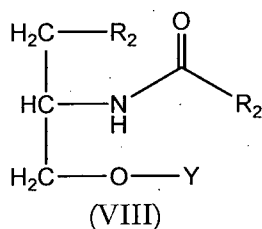
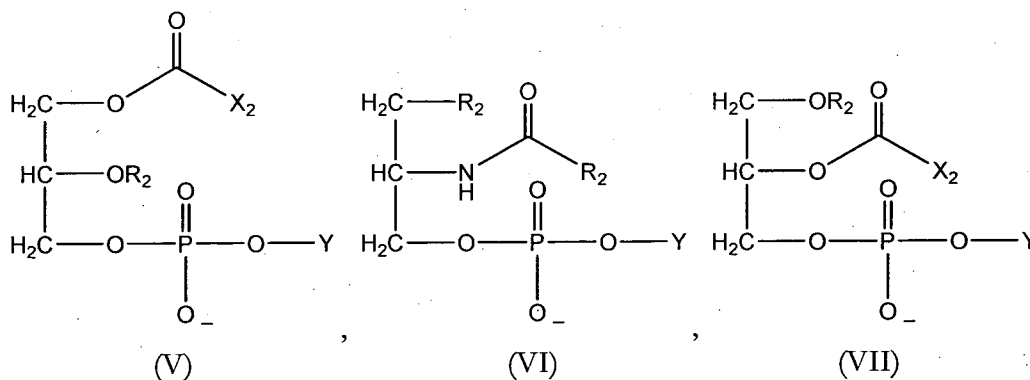
1. Use of one or more agents selected from:

- (a) one or more straight chain saturated carboxylic acids or esters or salts thereof comprising 3 to 9 or 11 to 24 carbon atoms, optionally substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups,
- (b) one or more derivatives of a straight chain carboxylic acid, wherein the acid moiety is replaced with an ester, ether, amide, amine, ketone or aldehyde moiety, or a salt thereof, wherein the carboxylic acid comprises 3 to 24 carbon atoms, optionally substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups, and wherein the carboxylic acid is saturated or monounsaturated, provided that when the derivative is an amide it is not an N-acylated lysophospholipid comprising a methoxycarbonyl group or a hydroxymethyl group at the sn-2 position,
- (c) milk fat, one or more milk fat fractions, or a combination thereof,
- (d) high CLA milk fat, one or more fractions thereof, or a combination thereof, and
- (e) a combination of any two or more agents selected from any of (a) to (d),

to treat or prevent a condition likely to benefit from a reduction in bone resorption or an increase in osteoblast proliferation.

2. A use of claim 1, wherein the agent is a compound of Formula (I), (II), (III), (IV) (V), (VI), (VII) or (VIII)





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or a salt thereof, wherein

R_1 is independently selected from straight or branched chain C2 to C23 alkyl, straight or branched chain C2 to C17 alkenyl, straight or branched chain C2 to C17 alkynyl, C3 to C6 cycloalkyl and C4 to C6 cycloalkenyl; wherein R_1 may be optionally substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups,

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R_2 is selected from H, $-\text{CH}_3$ and R_1 ,

R_3 is selected from H, $-\text{CH}_3$, R_1 and the residue of an amino acid or protein such that $-\text{NHR}_3$ is an N-acyl amino acid or protein,

X_1 is independently selected from H, $-\text{CH}_3$, R_1 , $-\text{CH}_2-\text{R}_1$ and $-\text{C}(\text{O})-\text{R}_1$, provided that at least one X_1 is $-\text{CH}_2-\text{R}_1$ or $-\text{C}(\text{O})-\text{R}_1$,

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X_2 is independently selected from H, $-\text{CH}_3$ and R_1 , provided that at least one X_2 is R_1 , and

Y is selected from H, $-\text{CH}_2-\text{CH}(\text{OH})-\text{CH}_2\text{OH}$, $-\text{CH}_2-\text{CH}_2-\text{NH}_3^+$, $-\text{CH}-\text{CH}(\text{NH}_3^+)\text{COO}^-$, $-\text{CH}_2-\text{CH}_2\text{N}^+(\text{CH}_3)_3$, or a saccharide, wherein the saccharide is selected from a monosaccharide, a disaccharide, a trisaccharide, and a polysaccharide, including galactosyl-3-sulfate ester saccharides and saccharides comprising sialic acids (including N- or O-substituted derivatives of neuraminic acid),

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provided that when the agent is a compound of Formula (I), R₁ is selected from straight or branched chain C11 to C17 alkyl optionally substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups.

3. A use of claim 1, wherein the agent is an ester of the carboxylic acid and glycerol, a mono-glyceride, a di-glyceride, phosphatidic acid, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, sphingomyelin, lysophospholipid, ceramide, glucoceramide, lactoceramide, a glycolipid, a cerebroside, a sulfatide, a ganglioside, or a proteolipid.
4. A use of claim 1, wherein the agent is an ether derivative of an ester of the carboxylic acid and glycerol, a mono-glyceride, a di-glyceride, phosphatidic acid, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, phosphatidylcholine, phosphatidylglycerol, diphosphatidylglycerol, sphingomyelin, lysophospholipid, ceramide, glucoceramide, lactoceramide, a glycolipid, a cerebroside, a sulfatide, a ganglioside, or a proteolipid.
5. A use of claim 1, wherein the carboxylic acid or salt thereof is selected from propanoic acid (C3:0), butanoic acid (C4:0), pentanoic acid (C5:0), hexanoic acid (C6:0), heptanoic acid (C7:0), octanoic acid (C8:0), nonanoic acid (C9:0), decanoic acid (C10:0), undecanoic acid (C11:0), dodecanoic acid (C12:0), tridecanoic acid (C13:0), tetradecanoic acid (C14:0), pentadecanoic acid (C15:0), hexadecanoic acid (C16:0), heptadecanoic acid (C17:0), octadecanoic acid (C18:0), nonadecanoic acid (C19:0), eicosanoic acid (C20:0), heneicosanoic acid (C21:0), docosanoic acid (C22:0), tricosanoic acid (C23:0), and tetracosanoic acid (C24:0), optionally substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups.
6. A use of claim 1, wherein the carboxylic acid or salt thereof is a monounsaturated carboxylic acid selected from propenoic acid (C3:1), butenoic acid (C4:1), pentenoic acid (C5:1), hexenoic acid (C6:1), heptenoic acid (C7:1), octenoic acid (C8:1), nonenoic acid (C9:1), decenoic acid (C10:1), undecenoic acid (C11:1), dodecenoic acid (C12:1), tridecenoic acid (C13:1), tetradecenoic acid (C14:1), pentadecenoic acid (C15:1), hexadecenoic acid (C16:1), heptadecenoic acid (C17:1), and octadecenoic acid (C18:1), optionally substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups.
7. A use of claim 1, wherein the amide or salt thereof is selected from dodecanamide (C12:0), tridecanamide (C13:0), tetradecanamide (C14:0), pentadecanamide (C15:0), hexadecanamide (C16:0), heptadecanamide (C17:0), octadecanamide (C18:0), nonadecanamide (C19:0), eicosanamide (C20:0), heneicosanamide (C21:0), docosanamide (C22:0), and tetracosanamide (C24:0), optionally substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups.

(C22:0), tricosanamide acid (C23:0), and tetracosanamide acid (C24:0), including C1 to C6 alkyl N-substituted forms thereof and monounsaturated forms thereof, all optionally substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups.

8. A use of claim 1, wherein the amide or salt thereof is a monounsaturated amide selected from dodecenamide (C12:1), tridecenamide (C13:1), tetradecenamide (C14:1), pentadecenamide (C15:1), hexadecenamide (C16:1), heptadecenamide (C17:1), and octadecenamide (C18:1), including C1 to C6 alkyl N-substituted forms thereof and monounsaturated forms thereof, all optionally substituted with one or more groups selected from hydroxyl, methyl, ethyl and propyl groups.

9. A use of claim 2, wherein the amino acid is selected from glycine, alanine, beta-alanine, α -methylalanine, valine, leucine, isoleucine, alloisoleucine, phenylalanine, proline, serine, homoserine, threonine, allothreonine, glutamic acid, methionine, asparagine, aspartic acid, glutamine, histidine, lysine, arginine, tyrosine, and tryptophan, and including the D or L isomers thereof, and including α , β and γ -amino acids.

10. A use of any one of claims 1 to 9, wherein the salt is an ammonium (NH_4^+), boron, calcium, copper, iron (ferrous, Fe^{2+} and ferric, Fe^{3+}), magnesium, manganese, phosphorous, potassium, pyridinium ($\text{C}_5\text{H}_5\text{NH}^+$), quaternary ammonium (NR_4^+), silicon, sodium, strontium, or zinc salt, or a combination thereof.

11. A use of any one of claims 1 to 10, wherein the composition further comprises a lipid composition selected from animal oils, animal fats, plant oils, plant fats, marine oils, marine fats, and lipids produced by microorganism fermentation.

12. A use of any one of claims 1 to 10, wherein the agent or composition comprises palm oil.

13. A use of any one of claims 1 to 12, wherein the milk fat fraction is selected from the group consisting of cream, butter, anhydrous milk fat, butter milk, butter serum, a hard milk fat fraction, a combination of hard milk fat fractions, a soft milk fat fraction, a combination of soft milk fat fractions, a combination of hard milk fat fractions and soft milk fat fractions, a sphingolipid fraction, a milk fat globule membrane fraction, a phospholipid fraction, a complex lipid fraction, a ceramide fraction, a ganglioside fraction, any combination of any two or more thereof, a hydrolysate of any one or more thereof, a fraction of the hydrolysate, any combination of any two or more of the hydrolysates, and any combination of one or more hydrolysed milk fat fractions and one or more non-hydrolysed milk fat fractions.

14. A use of any one of claims 1 to 13, wherein the composition further comprises a pharmaceutically acceptable carrier.
15. A use of any one of claims 1 to 13, wherein the composition is or is formulated as a food, drink, food additive, drink additive, dietary supplement, nutritional product, medical food, nutraceutical, medicament or pharmaceutical.
16. A use of any one of claims 1 to 15, wherein the composition further comprises a carotenoid, calcium, fluoride, magnesium, zinc, a calcium salt, a fluoride salt, a magnesium salt, a zinc salt, vitamin A, vitamin B6, vitamin C, vitamin D, a vitamin D derivative, vitamin E, vitamin K, a vitamin K derivative, a vitamin K analog, vitamin K2, whey protein, a whey protein fraction, glycomacropeptide, lactoferrin, a functional lactoferrin variant, a functional lactoferrin fragment, or any combination thereof.
17. A use of any one of claims 1 to 16, wherein the composition further comprises one or more omega-3 or omega-6 fatty acids, or a combination thereof.
18. A use of any one of claims 1 to 17, wherein the condition is likely to benefit from decreased bone resorption or decreased osteoclastogenesis or both.
19. A use of any one of claims 1 to 17, wherein the condition is likely to benefit from increased bone formation or increased osteoblast proliferation or both.
20. A use of any one of claims 1 to 19, wherein the condition is a skeletal disorder.
21. A use of claim 20, wherein the disorder is selected from bone fracture, bone damage following surgery, osteoporosis, rheumatoid arthritis, osteoarthritis, 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, osteogenesis imperfecta and oral bone erosion.
22. A method of treating or preventing a condition likely to benefit from a reduction in bone resorption or an increase in osteoblast proliferation, the method comprising administering an effective amount one or more agents as defined in any one of the preceding claims to a subject in need thereof.
23. A method of claim 22, wherein the condition is selected from bone fracture, bone damage following surgery, osteoporosis, rheumatoid arthritis, osteoarthritis, 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, osteogenesis imperfecta and oral
- 5 bone erosion.

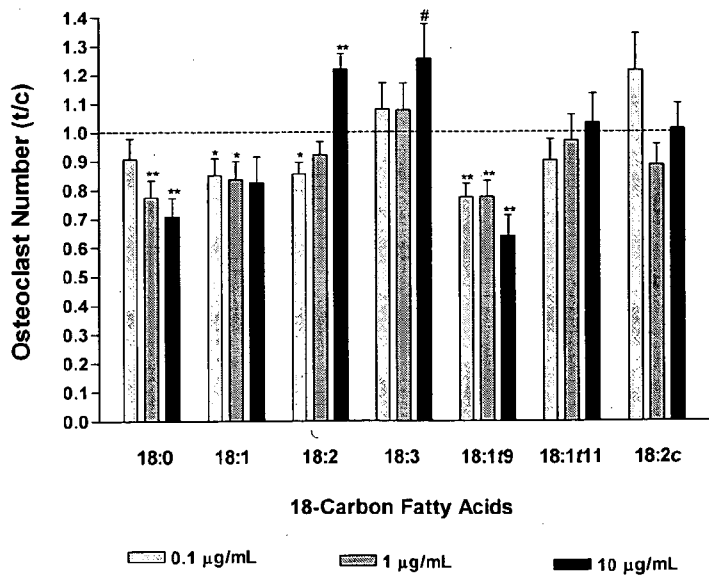
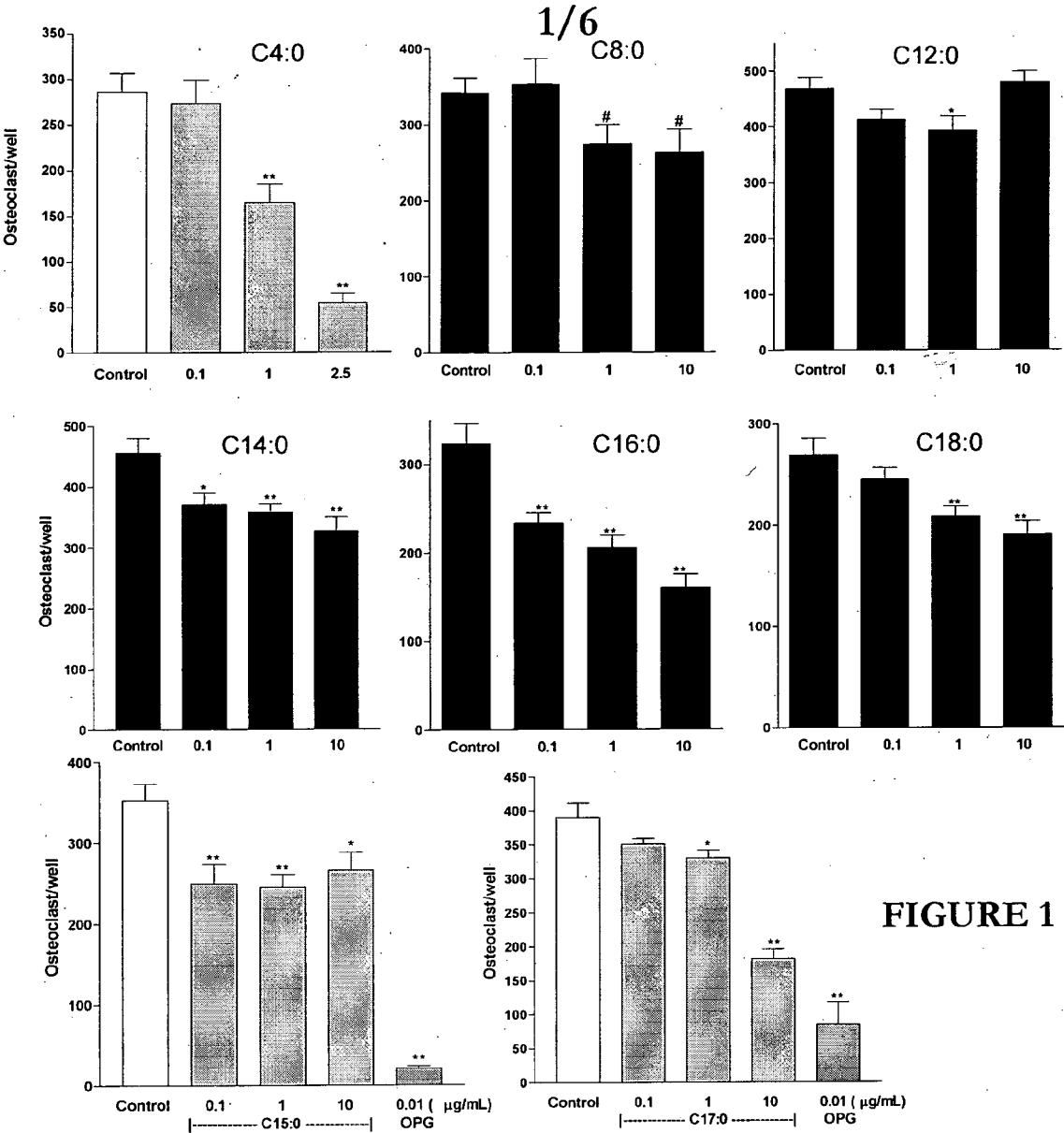


FIGURE 2

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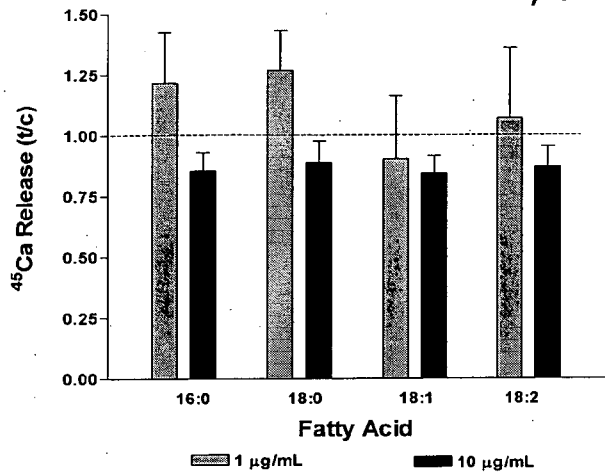


FIGURE 5

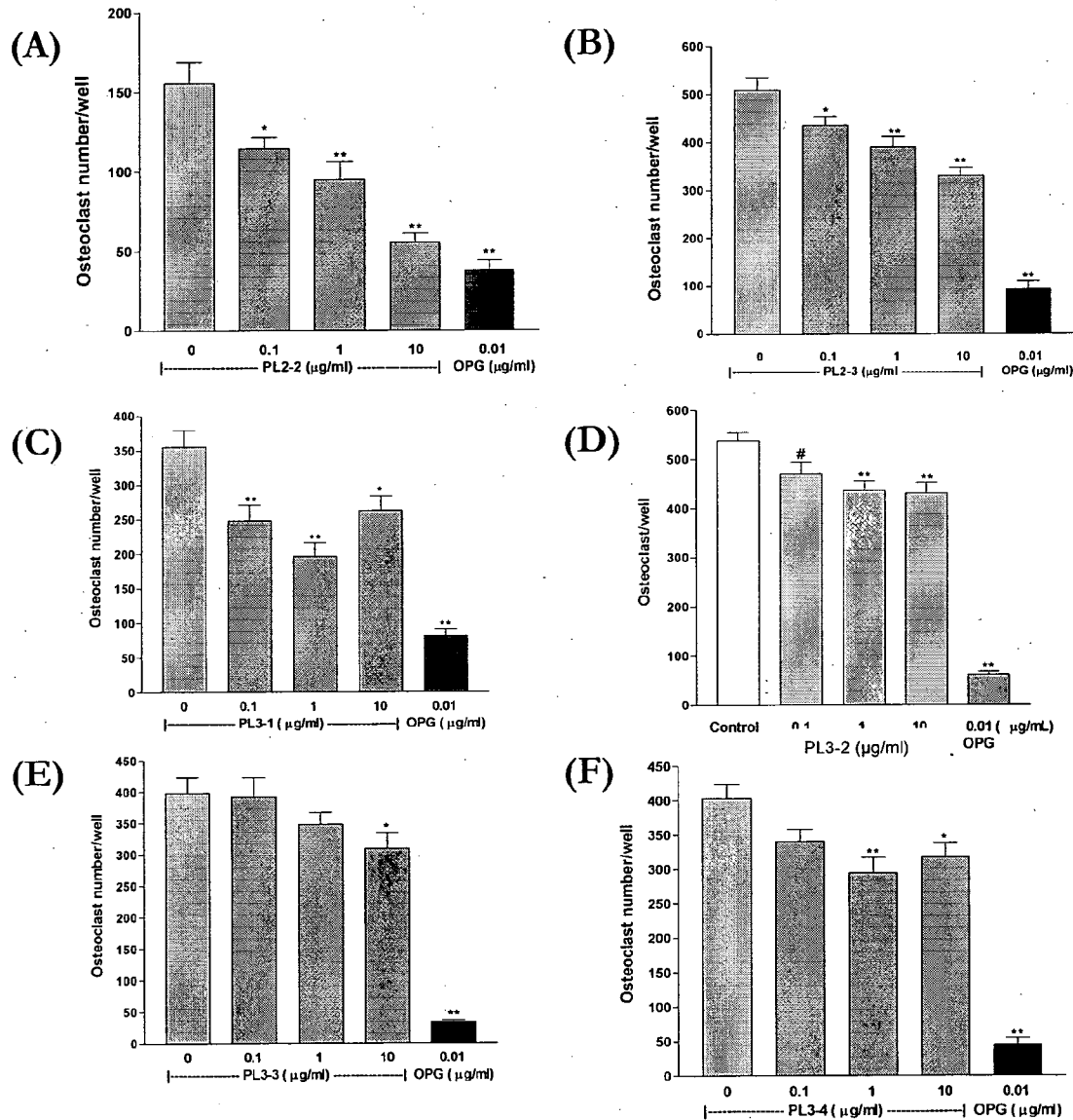


FIGURE 6

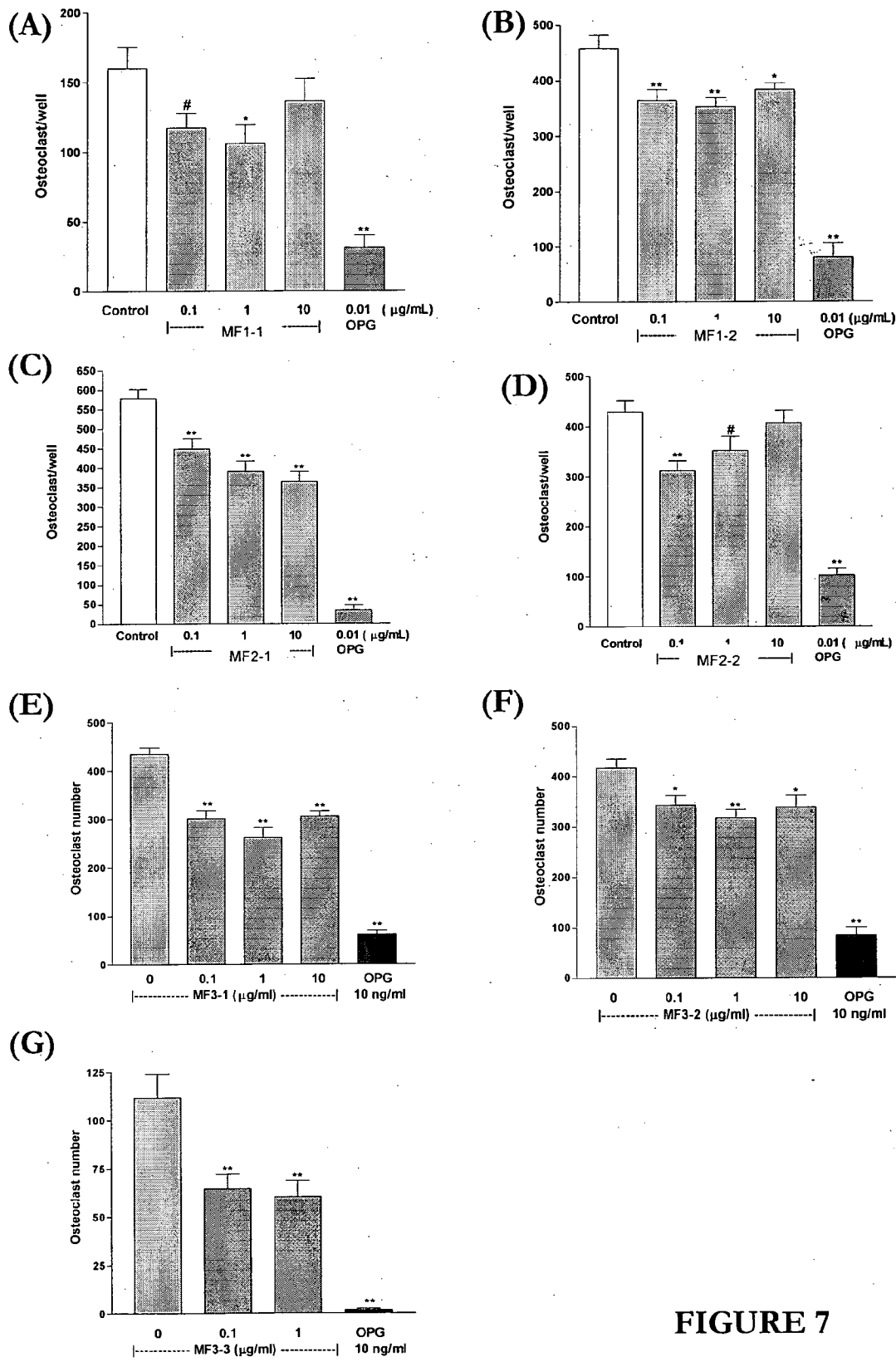


FIGURE 7

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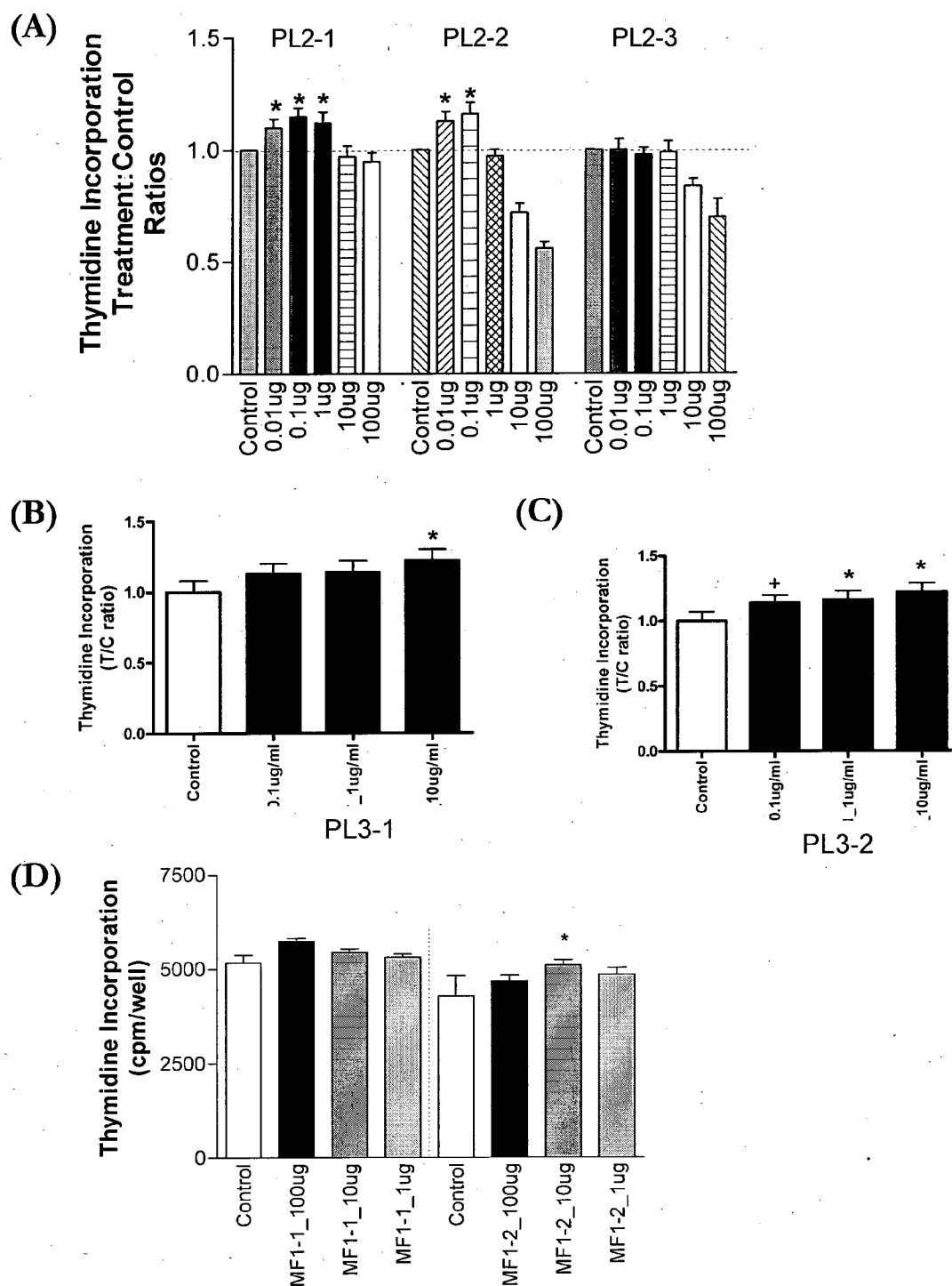


FIGURE 8

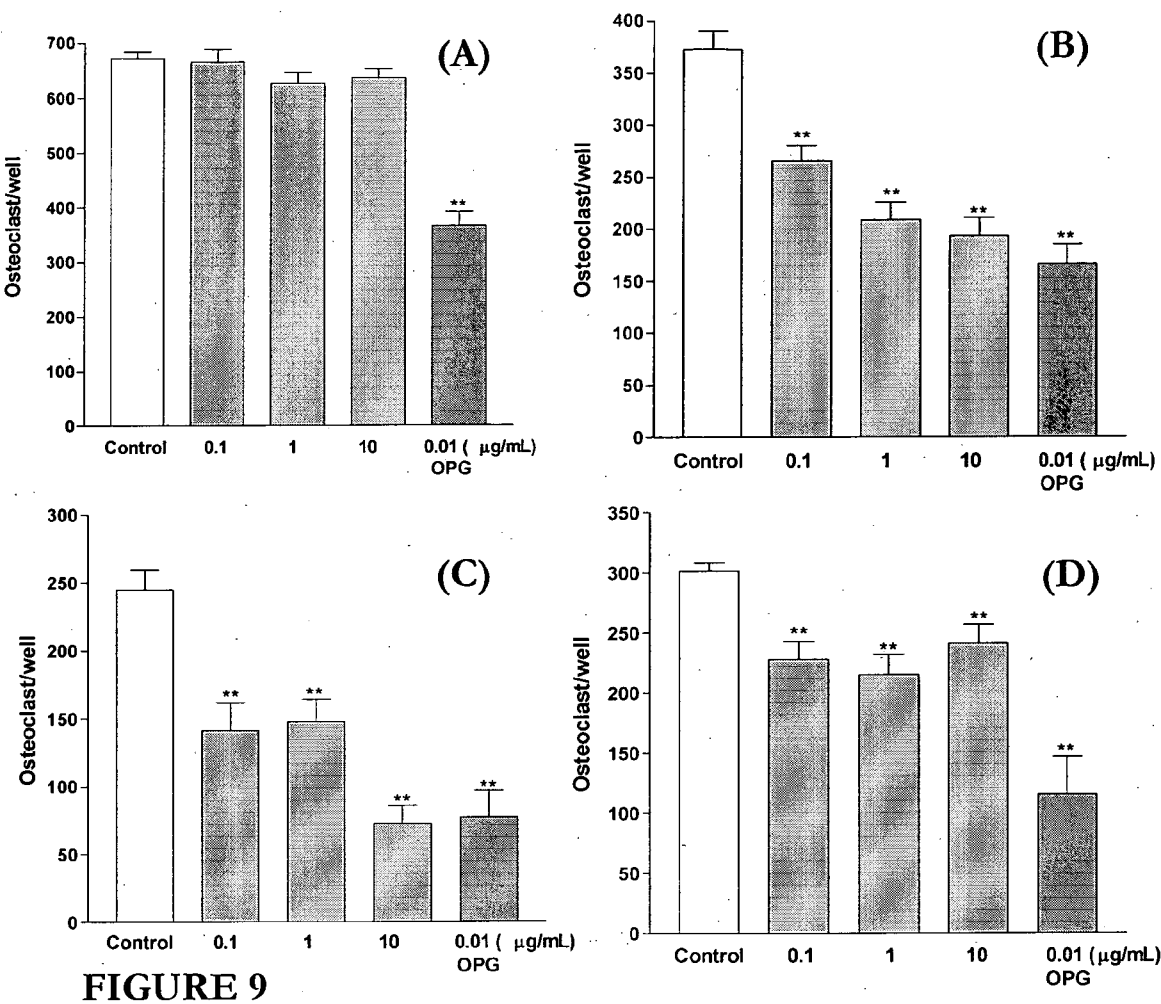


FIGURE 9

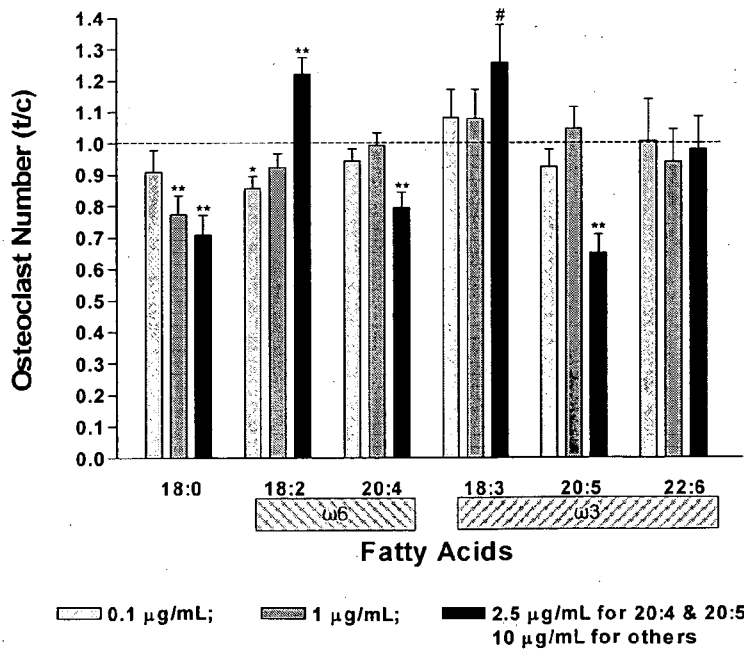


FIGURE 3

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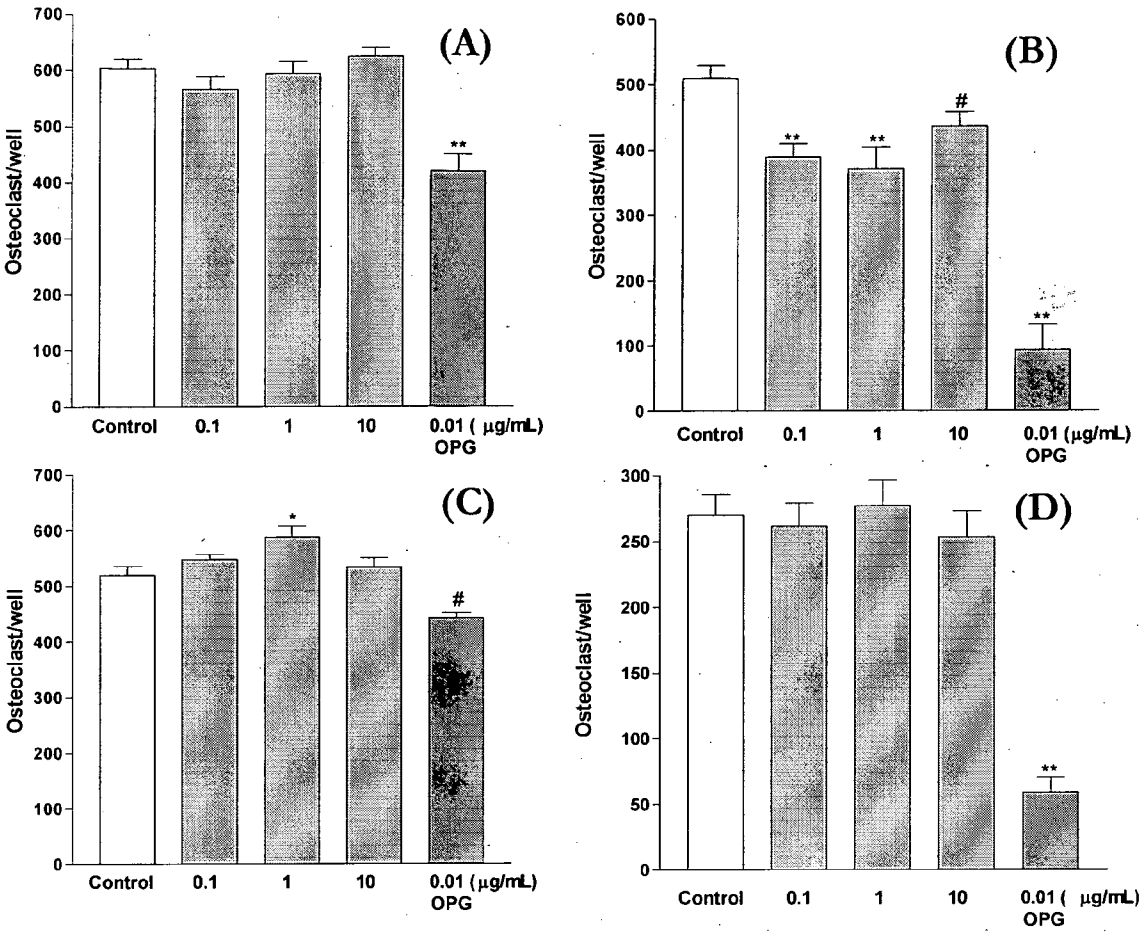


FIGURE 10

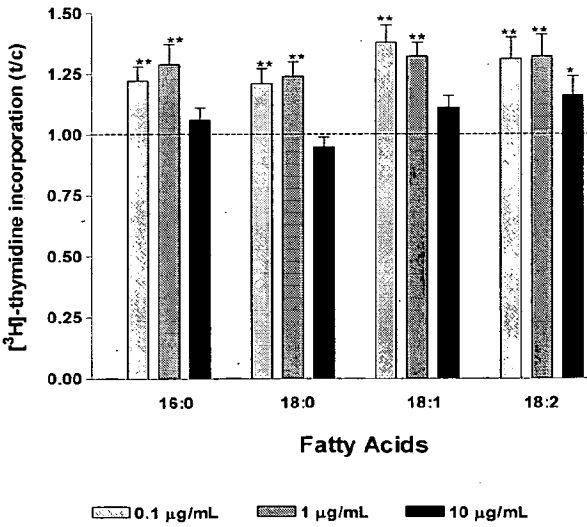


FIGURE 4

INTERNATIONAL SEARCH REPORT

International application No.

PCT/NZ2008/000127

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A61K 31/19 (2006.01) A61K 31/201 (2006.01) A61K 31/215 (2006.01) A61K 31/20 (2006.01) A61K 31/202 (2006.01)
A61K 35/20 (2006.01) A61P 19/00 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, EPODOC butyric acid, caprylic acid, lauric acid, myristic acid, pentadecanoic w acid, palmitic acid, heptadecanoic acid, stearic acid, oleic acid, linoleic acid, elaidic acid, arachidonic acid, eicosapentaenoic acid, docosahexanoic acid, propanoic acid, butanoic acid, pentanoic acid, hexanoic acid, heptanoic acid, octanoic acid, nonanoic acid, milk, dairy, fat, lipid, bone disease, bone resorption, bone formation, bone fracture, osteoclastogen, osteoblast

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2000/049885 A1 (SOCIETE DES PRODUITS NESTLE S. A.) 31 August 2000, See whole document.	1, 13-15, 18-23

☒ Further documents are listed in the continuation of Box C
 ☒ See patent family annex

* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "E" earlier application or patent but published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed		
"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	
"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	
"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	
"&"	document member of the same patent family	

 Date of the actual completion of the international search
 28 August 2008

 Date of mailing of the international search report
 - 8 SEP 2008

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/NZ2008/000127

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Derwent Abstract Accession No. 97-419377/39, Class B04 D13, JP 09187250-A (SNOW BRAND MILK PROD CO LTD) 22 July 1997.	1, 12-15, 18-23
X	Derwent Abstract Accession No. 2006-599862/62, Class B04 D13, KR 2005087089-A (LCS KOREA CO LTD) 31 August 2005.	1, 13-15, 18-23
X	US 2004/0058995 A1 (Shinohara et al) 25 March 2004, See whole document.	1- 8, 10-12, 14-23
X	Dongxu Sun et al, "Dietary n-3 Fatty Acids Decrease Osteoclastogenesis and Loss of Bone Mass in Ovariectomized Mice", Journal of Bone and Mineral Research, 2003, Vol 18, no 7, pages 1206-1216, See whole document.	1, 6, 8, 10, 15, 17-23
X	Sun Jin Hur and Yeonhwa Park, "Effect of Conjugated Linoleic Acid on Bone Formation and Rheumatoid Arthritis", European Journal of Pharmacology, 2007, Vol 568, pages 16-24, See whole document.	1, 6, 8, 10, 17-23
X	Derwent Abstract Accession No. 2008-B93278/14, Class B05, WO 2007080884 A1 (SEIKAGAKU CORP) 19 July 2007.	1- 2, 8-10, 17-23
X	US 2006/0035867 A1 (Janus et al) 16 February 2006, See whole document.	1-2, 8, 10, 16-23
X	WO 2004/098582 A2 (THE UNIVERSITY COURT OF THE UNIVERSITY OF ABERDEEN) 18 November 2004, See whole document.	1-2, 8, 10, 17-23
X	WO 2002/070517 A2 (MERCK FROSST CANADA & CO) 12 September 2002, See whole document.	1-2, 5, 8, 10, 17-23
X	Derwent Abstract Accession No. 2006-517776/53, Class B05, KR 2005051054 A (KOH J M), 1 June 2005.	1-2, 5, 8, 10, 17-23

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/NZ2008/000127

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report				Patent Family Member			
WO	0049885	AU	32824/00	BR	0008427	CA	2360490
		CN	1352528	EP	1062876	EP	1154701
		ZA	200107831				
JP	9187250		NONE				
KR	2005087089		NONE				
US	2004058995	CA	2442692	CN	1499965	EP	1380293
		WO	02078689				
WO	2007080884		NONE				
US	2006035867	US	2002055457	US	2005113306	US	2005113307
WO	2004098582	EP	1638544	US	2007027112		
WO	02070517	AU	2002242500				
KR	2005051054		NONE				
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.							
END OF ANNEX							