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(54) Benævnelse: **NÆRINGSMIDDELSAMMENSÆTNINGER TIL MODULATION AF INFLAMMATION OMFATTENDE EXOGENT VITAMIN K2**

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DESCRIPTION

BACKGROUND

[0001] The present disclosure generally relates to health and nutrition. More specifically, the present disclosure relates to nutritional compositions including exogenous vitamin K_2 and methods of making and using the nutritional compositions.

[0002] There are many types of nutritional compositions currently on the market. Nutritional compositions can be targeted toward certain consumer types, for example, young, elderly, athletic, etc., based on the specific ingredients of the nutritional composition. Nutritional compositions can also be formulated based on the certain physiological conditions that the nutritional compositions are intended to treat or improve.

SUMMARY

[0003] Nutritional compositions having exogenous vitamin K2 and methods of making and using the nutritional compositions are provided. In a general embodiment, the present disclosure provides a nutritional composition including exogenous K2for use in modulating the effects of inflammation, wherein said modulation of the effects of inflammation is preserving the fractional rate of mixed muscle protein synthesis under conditions of inflammation. The nutritional composition can be a complete nutritional or as an oral nutritional supplement (incomplete nutritional). The nutritional composition can be in a formulation designed for any mammal such as a human or an animal. The active ingredients in the nutritional composition can also be provided as a modular product. A modular product can be defined as a method of delivering one or more specific nutrients as a supplement and not intended to be used for sole source nutrition.

[0004] In an embodiment, the nutritional composition further includes one or more prebiotics. The prebiotic can be fructooligosaccharides, inulin, lactulose, galactooligosaccharides, acacia gum, soyoligosaccharides, xylooligosaccharides, isomaltooligosaccharides, gentiooligosaccharides, lactosucrose, glucooligosaccharides, pecticoligosaccharides, guar gum, partially hydrolyzed guar gum, sugar alcohols, alpha glucan, beta glucan, or a combination thereof.

[0005] In an embodiment, the nutritional composition further includes one or more probiotics. The probiotic can be Saccharomyces, Debaromyces, Candida, Pichia, Torulopsis, Aspergillus, Rhizopus, Mucor, Penicillium, Bifidobacterium, Bacteroides, Clostridium, Fusobacterium, Melissococcus, Propionibacterium, Streptococcus, Enterococcus, Lactococcus, Staphylococcus, Peptostrepococcus, Bacillus, Pediococcus, Micrococcus, Leuconostoc, Weissella, Aerococcus, Oenococcus, Lactobacillus or a combination thereof.

[0006] In another embodiment, the nutritional composition further includes one or more amino acids. The amino acid can be Alanine, Arginine, Asparagine, Aspartate, Citrulline, Cysteine, Glutamate, Glutamine, Glycine, Histidine, Hydroxyproline, Hydroxyserine, Hydroxytyrosine, Hydroxylysine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Taurine, Threonine, Tryptophan, Tyrosine, Valine, HICA (Alpha-Hydroxyisocaproic Acid), HIVA (Alpha-Hydroxyisovaleric Acid), HIMVA (alpha-hydroxymethylvaleric acid) or a combination thereof.

[0007] In an embodiment, the nutritional composition further includes one or more proteins.

[0008] In an embodiment, the nutritional composition further includes one or more nucleotides.

[0009] In an embodiment, the nutritional composition further includes one or more synbiotics, fish oils, nonmarine omega-3 fatty acid containing dietary fat sources, phytonutrients and/or antioxidants. The antioxidants can be, for example, vitamin A , vitamin B_1, vitamin B_6, vitamin B_{12}, vitamin C, vitamin D, vitamin E, carotenoids, selenium, flavonoids, Lactowolfberry, Goji (wolfberry), polyphenols, lycopene, lutein, lignan, coenzyme Q10 ("CoQ10"), hesperidine and glutathione.

[0010] In an embodiment, the nutritional composition is in an administerable form such as pharmaceutical formulations, nutritional formulations, tube-feed formulations, dietary supplements, functional foods, beverage products or a combination thereof.

[0011] Additional features and advantages are described herein, and will be apparent from the following Detailed Description.

DETAILED DESCRIPTION

[0012] Vitamin K denotes a group of lipophilic, hydrophobic and essential vitamins having a common chemical ring structure (napthoquinone). The two most important forms of vitamin K are vitamin K_1, a single compound known as phylloquinone or phytomenadione, and vitamin K_2, a series of vitamers known as menaquinones or menatetrenones. There are also several synthetic forms of vitamin K including, for example, vitamins K_3, K_4 and K_5.

[0013] Vitamin K_1 is the major form of vitamin K in a normal diet and is synthesized by plants including, for example, certain plant oils such as canola and soybean and in green leafy vegetables such as spinach, swiss chard, broccoli, cabbage, cauliflower, kale, and brussels sprouts.

[0014] Vitamin K_2 is a group of compounds called menaquinones ("MK") having side chains

composed of a variable number of unsaturated isoprenoid residues generally designated as MK-n, where n specifies the number of isoprenoids. The most common MKs are MK-4 and MK-7. MK-4 is typically synthesized by animal organs and muscle, while MK-7 is typically synthesized by bacteria during fermentation. Accordingly, MK-7 is particularly abundant in fermented products including cheese, curd cheese and natto (fermented soybeans) and has a particularly long half-life when compared to vitamin K_1.

[0015] The estimated average requirement for vitamin K in children ages 1 to 18 years in the United States is based upon median intakes of vitamin K for adults. These levels are designed to meet the vitamin K levels required for normal blood coagulation and not other vitamin K-dependent proteins such as osteocalcin. The ratio of undercarboxylated (i.e., inactive) to carboxylated osteocalcin can be a surrogate marker for vitamin K status. Recent evidence suggests that children between the ages of 6 and 18 years of age have elevated levels of undercarboxylated osteocalcin relative to adults. Rather than attempting to increase the intake levels via higher vitamin K_1 intake, vitamin K_2 allows for administration of a more potent form of vitamin K without negatively impacting parameters of anticoagulation.

[0016] As compared to vitamin K_1, vitamin K_2 provides better absorption and more stable serum levels through a longer half-life. The improved bioavailability of vitamin K_2 to extrahepatic tissue may also allow for a greater impact on bone health (i.e. mineralization, micro architecture and strength) during normal growth and development. Therefore, vitamin K_2 provides for a more potent form of the vitamin in which its enhanced bioavailability can impact bone health during normal growth and development.

[0017] Rather than attempting to increase the intake levels via higher vitamin K1 intake, Vitamin K2 allows for a more potent form of vitamin K without negatively impacting parameters of anticoagulation. Specifically, vitamin K2 provides better absorption and more stable serum levels through a longer half-life when compared to phylloquinones (vitamin Kl). Improved bioavailability of vitamin K2 to extraheptic tissue may allow for a greater impact for improving musculoskeletal health in patients with inflammatory bowel disease (IBD) (Crohn's Disease and Colitis), especially pediatric patients. The incidence of low bone mass ranges from 30-50% in children with IBD. Vitamin K is a cited nutrition deficiency in this population and its limited availability may reduce osteocalcin carboxylation as well as reduce bone strength, bone mineralization and bone micro-architecture. In addition, a low vitamin K status may be a causative factor in Crohn's Disease-associated osteopenia. The osteopenia and elevated rate of bone resorption noted in some Crohn's Disease patients is a multifactorial process and vitamin K deficiency is certainly only one factor in this process. Low vitamin K levels can lead to an increase in the rate of bone resorption, without a compensatory increase in the rate of bone formation. An increased rate of bone turnover is associated with an increased risk of bone loss in Crohn's Disease patients. In terms of nutrition-related etiological factors for osteopenia, there are indications that in patients with longstanding Crohn's Disease, vitamin K deficiency has a greater influence on bone turnover than serum 25(OH) vitamin D concentrations. Vitamin K2 may serve as a critical micronutrient for optimizing bone regulation in this target population.

[0018] WO2008/006607 describes the use of vitamin K2 in combination with a PUFA in the treatment of cardiovascular, cartilage and bone disorders. WO2005/107731 reports menatetrenone as having activity in inhibition of NF-KappaB activation, IkappaB phosphorylation and cyclin D1 expression.

[0019] In a general embodiment, the present disclosure provides a nutritional composition including exogenous vitamin K_2 for use in modulating the effects of inflammation, wherein said modulation of the effects of inflammation is preserving the fractional rate of mixed muscle protein synthesis under conditions of inflammation The nutritional composition may further include a component selected from the group consisting of phosphorus, magnesium, zinc, iron, copper, manganese, calcium, vitamin D, osteopontin and combinations thereof.

[0020] As used herein, the term "nutritional composition" includes, but is not limited to, complete nutritional compositions, partial or incomplete nutritional compositions, and disease or condition specific nutritional compositions. A complete nutritional composition (i.e., those which contain all the essential macro and micro nutrients) can be used as a sole source of nutrition for the patient. Patients can receive 100% of their nutritional requirements from such complete nutritional composition. A partial or incomplete nutritional composition does not contain all the essential macro and micro nutrients and cannot be used as a sole source of nutrition for the patient. Partial or incomplete nutritional compositions can be used as a nutritional supplement. A disease or condition specific nutritional composition is a composition that delivers nutrients or pharmaceuticals and can be a complete or partial nutritional composition.

[0021] The exogenous vitamin K_2 can be combined with other ingredients for promotion of bone growth and bone quality. For example, exogenous vitamin K_2 could work more effectively to support bone health in pediatric patients when used in combination with a component selected from the group consisting of phosphorus, magnesium, zinc, iron, copper, manganese, calcium, vitamin D, osteopontin and combinations thereof. Exogenous vitamin K_2 may also work more effectively to support bone health when used in combination with amino acids (e.g., leucine), protein with low sulfur-containing amino acid content, lipids (n3:n6), bioactive peptides, protease inhibitors, creatine, etc.

[0022] In an embodiment, the nutritional composition further includes one or more prebiotics. As used herein, a prebiotic is a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora, that confers benefits upon host well-being and health. Non-limiting examples of prebiotics include fructooligosaccharides, inulin, lactulose, galactooligosaccharides, acacia gum, soyoligosaccharides, xylooligosaccharides, isomaltooligosaccharides, gentiooligosaccharides, lactosucrose, glucooligosaccharides, pecticoligosaccharides, guar gum, partially hydrolyzed guar gum, sugar alcohols, alpha glucan, beta glucan, or a combination thereof.

[0023] In an embodiment, the nutritional composition further includes one or more probiotics.

As used herein, probiotic micro-organisms (hereinafter "probiotics") are preferably microorganisms (alive, including semi-viable or weakened, and/or non-replicating), metabolites, microbial cell preparations or components of microbial cells that could confer health benefits on the host when administered in adequate amounts., more specifically that beneficially affect a host by improving its intestinal microbial balance, leading to effects on the health or well-being of the host. In general, it is believed that these micro-organisms inhibit or influence the growth and/or metabolism of pathogenic bacteria in the intestinal tract. The probiotics may also activate the immune function of the host. For this reason, there have been many different approaches to include probiotics into food products. Non-limiting examples of probiotics include Saccharomyces, Debaromyces, Candida, Pichia, Torulopsis, Aspergillus, Rhizopus, Mucor, Penicillium, Bifidobacterium, Bacteroides, Clostridium, Fusobacterium, Melissococcus, Propionibacterium, Streptococcus, Enterococcus, Lactococcus, Staphylococcus, Peptostrepococcus, Bacillus, Pediococcus, Micrococcus, Leuconostoc, Weissella, Aerococcus, Oenococcus, Lactobacillus or a combination thereof.

[0024] In another embodiment, the nutritional composition further includes one or more amino acids. Non-limiting examples of amino acids include Alanine, Arginine, Asparagine, Aspartate, Citrulline, Cysteine, Glutamate, Glutamine, Glycine, Histidine, Hydroxyproline, Hydroxyserine, Hydroxytyrosine, Hydroxylysine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Taurine, Threonine, Tryptophan, Tyrosine, Valine, HICA (Alpha-Hydroxyisocaproic Acid), HIVA (Alpha-Hydroxyisovaleric Acid), HIMVA (alpha-hydroxymethylvaleric acid) or a combination thereof. In a preferred embodiment, non-limiting examples of amino acids include proline, hydroxyproline, hydroxytyrosine, hydroxylysine and hydroxyserine and combinations thereof.

[0025] In an embodiment, the nutritional composition further includes one or more proteins.

[0026] In an embodiment, the nutritional composition further includes one or more nucleotides.

[0027] In an embodiment, the nutritional composition further includes one or more synbiotics, fish oils, nonmarine omega-3 fatty acid containing dietary fat sources, Bowman Birk Inhibitor, phytonutrients and/or antioxidants. As used herein, a synbiotic is a supplement that contains both a prebiotic and a probiotic that work together to improve the microflora of the intestine. Non-limiting examples of fish oils include docosahexaenoic acid ("DHA") and eicosapentaenoic acid ("EPA"). Non-limiting examples of phytonutrients include quercetin, curcumin and limonin. Antioxidants are molecules capable of slowing or preventing the oxidation of other molecules. Non-limiting examples of antioxidants include vitamin A, carotenoids, vitamin C, vitamin E, selenium, flavonoids, Lactowolfberry, Goji (wolfberry), polyphenols, lycopene, lutein, lignan, coenzyme Q10 ("CoQ10"), hesperidine and glutathione.

[0028] In another embodiment, the present disclosure provides a method of making a nutritional composition. The method comprises adding an effective amount of exogenous K_2 and a component selected from the group consisting of phosphorus, magnesium, zinc, iron,

copper, manganese, calcium, vitamin D, osteopontin or combinations thereof to a nutritional composition, for example, to improve bone health of pediatric patients. The nutritional composition can be in an administerable form such as pharmaceutical formulations, nutritional formulations, tube-feed formulations, dietary supplements, functional foods, beverage products or a combination thereof.

[0029] As used herein, a "tube feed" formulation is preferably a complete or incomplete nutritional product that is administered to an animal's gastrointestinal system, other than through oral administration, including but not limited to a nasogastric tube, orogastric tube, gastric tube, jejunostomy tube (J-tube), percutaneous endoscopic gastrostomy (PEG), port, such as a chest wall port that provides access to the stomach, jejunum and other suitable access ports.

[0030] As used herein, "effective amount" is preferably an amount that prevents a deficiency, treats a disease or medical condition in an individual or, more generally, reduces symptoms, manages progression of the diseases or provides a nutritional, physiological, or medical benefit to the individual. A treatment can be patient- or doctor-related. In addition, while the terms "individual" and "patient" are often used herein to refer to a human, the invention is not so limited. Accordingly, the terms "individual" and "patient" refer to any animal, mammal or human having or at risk for a medical condition that can benefit from the treatment.

[0031] As used herein, animals include, but is not limited to mammals, which include, but is not limited to rodents, aquatic mammals, domestic animals such as dogs and cats, farm animals such as sheep, pigs, cows and horses, and humans. Wherein the terms animal or mammal or their plurals are used, it is contemplated that it also applies to any animals that are capable of the effect exhibited or intended to be exhibited by the context of the passage.

[0032] As used herein, "complete nutrition" are preferably nutritional products that contain sufficient types and levels of macronutrients (protein, fats and carbohydrates) and micronutrients to be sufficient to be a sole source of nutrition for the animal to which it is being administered to.

[0033] As used herein, "incomplete nutrition" are preferably nutritional products that do not contain sufficient levels of macronutrients (protein, fats and carbohydrates) or micronutrients to be sufficient to be a sole source of nutrition for the animal to which it is being administered to.

[0034] As used herein, "Long term administrations" are preferably continuous administrations for more than 6 weeks.

[0035] As used herein, mammal preferably includes but is not limited to rodents, aquatic mammals, domestic animals such as dogs and cats, farm animals such as sheep, pigs, cows and horses, and humans. Wherein the term mammal is used, it is contemplated that it also applies to other animals that are capable of the effect exhibited or intended to be exhibited by the mammal.

[0036] The term "microorganism" is meant to include the bacterium, yeast and/or fungi, a cell growth medium with the microorganism or a cell growth medium in which microorganism was cultivated.

[0037] As used herein, a "Prebiotic" is preferably a food substances that selectively promote the growth of beneficial bacteria or inhibit the growth of pathogenic bacteria in the intestines. They are not inactivated in the stomach and/or upper intestine or absorbed in the GI tract of the person ingesting them, but they are fermented by the gastrointestinal microflora and/or by probiotics. Prebiotics are for example defined by Glenn R. Gibson and Marcel B. Roberfroid, Dietary Modulation of the Human Colonic Microbiota: Introducing the Concept of Prebiotics, J. Nutr. 1995 125: 1401-1412.

[0038] As used herein, "Short term administrations" are preferably continuous administrations for less than 6 weeks.

[0039] As used herein, the terms "treatment", "treat" and "to alleviate" is preferably to both prophylactic or preventive treatment (that prevent and/or slow the development of a targeted pathologic condition or disorder) and curative, therapeutic or disease-modifying treatment, including therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition or disorder; and treatment of patients at risk of contracting a disease or suspected to have contracted a disease, as well as patients who are ill or have been diagnosed as suffering from a disease or medical condition. The terms "treatment" and "treat" also refer to the maintenance and/or promotion of health in an individual not suffering from a disease but who may be susceptible to the development of an unhealthy condition, such as nitrogen imbalance or muscle loss. The terms "treatment", "treat" and "to alleviate" are also intended to include the potentiation or otherwise enhancement of one or more primary prophylactic or therapeutic measure.

[0040] As used herein, a synbiotic is a supplement that contains both a prebiotic and a probiotic that work together to improve the microflora of the intestine.

[0041] As used herein, "normal bone growth" preferably includes: during childhood and adolescence bones are sculpted by modeling, which allows for the formation of new bone at one site and the removal of old bone from another site within the

[0042] As used herein, a "nucleotide" is preferably understood to be a subunit of deoxyribonucleic acid ("DNA") or ribonucleic acid ("RNA"). It is an organic compound made up of a nitrogenous base, a phosphate molecule, and a sugar molecule (deoxyribose in DNA and ribose in RNA). Individual nucleotide monomers (single units) are linked together to form polymers, or long chains. Exogenous nucleotides are specifically provided by dietary supplementation. The exogenous nucleotide can be in a monomeric form such as, for example, 5' Adenosine Monophosphate ("5'-AMP"), 5'-Guanosine Monophosphate ("5'-GMP"), 5'-Cytosine Monophosphate ("5'-CMP"), 5'-Uracil Monophosphate ("5'-UMP"), 5'-Inosine

Monophosphate ("5'-IMP"), 5'-Thymine Monophosphate ("5'-TMP") or a combination thereof. The exogenous nucleotide can also be in a polymeric form such as, for example, an intact RNA. There can be multiple sources of the polymeric form such as, for example, yeast RNA.

[0043] Nutritional products are preferably understood to further include any number of additional ingredients, including, for example one or more, vitamin, mineral, sugar, a pharmaceutically acceptable carrier, excipient, flavor agent, or colorants.

[0044] The term "protein", "peptide", "oligopeptides" or "polypeptide" as used herein is preferably understood to refer to any composition that includes, a single amino acids (monomers), two or more amino acids joined together by a peptide bond (dipeptide, tripeptide, or polypeptide), collagen, precursor, homolog, analog, mimetic, salt, prodrug, metabolite, or fragment thereof or combination. For the sake of clarity, the use of any of the above terms is interchangeable unless otherwise specified. It will be appreciated that polypeptides (or peptides or proteins or oligopeptides) often contain amino acids other than the 20 amino acids commonly referred to as the 20 naturally occurring amino acids, and that many amino acids, including the terminal amino acids, may be modified in a given polypeptide, either by natural processes such as glycosylation and other post-translational modifications, or by chemical modification techniques which are well known in the art. Among the known modifications which may be present in polypeptides of the present invention include, but are not limited to, acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of a flavanoid or a heme moiety, covalent attachment of a polynucleotide or polynucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycation, glycosylation, glycosylphosphatidyl inositol (GPI) membrane anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to polypeptides such as arginylation, and ubiquitination. The term "protein" also includes "artificial proteins" which refers to linear or non-linear polypeptides, consisting of alternating repeats of a peptide

[0045] As used herein, "phytochemicals" or "phytonutrients" are non-nutritive compounds that are found in many foods. Phytochemicals are functional foods that have health benefits beyond basic nutrition, and are health promoting compounds that come from plant sources. As used herein, "Phytochemicals" and "Phytonutrients" refers to any chemical produced by a plant that imparts one or more health benefit on the user. Phytochemicals can be administered by any means, including topically, enterally, and/or parenterally. As used herein, non-limiting examples of phytochemicals and phytonutrients include those that are:

1. 1. Phenolic compounds which include Monophenols (such as: Apiole, Carnosol, Carvacrol, Dillapiole, Rosemarinol); Flavonoids (polyphenols) including Flavonols (such as: Quercetin, Gingerol, Kaempferol, Myricetin, Rutin, Isorhamnetin), Flavanones (such as: Hesperidin, Naringenin, Silybin, Eriodictyol), Flavones (such as: Apigenin, Tangeritin,

Luteolin), Flavan-3-ols (such as: Catechins, (+)-Catechin, (+)-Gallocatechin, (-)-Epicatechin, (-)-Epigallocatechin, (-)-Epigallocatechin gallate (EGCG), (-)-Epicatechin 3-gallate, Theaflavin, Theaflavin-3-gallate, Theaflavin-3'-gallate, Theaflavin-3,3'-digallate, Thearubigins), Anthocyanins (flavonals) and Anthocyanidins (such as: Pelargonidin, Peonidin, Cyanidin, Delphinidin, Malvidin, Petunidin), Isoflavones (phytoestrogens) (such as: Daidzein (formononetin), Genistein (biochanin A), Glycitein), Dihydroflavonols, Chalcones, Coumestans (phytoestrogens), and Coumestrol; Phenolic acids (such as: Ellagic acid, Gallic acid, Tannic acid, Vanillin, Curcumin); Hydroxycinnamic acids (such as: Caffeic acid, Chlorogenic acid, Cinnamic acid, Ferulic acid, Coumarin); Lignans (phytoestrogens), Silymarin, Secoisolariciresinol, Pinoresinol and lariciresinol); Tyrosol esters (such as: Tyrosol, Hydroxytyrosol, Oleocanthal, Oleuropein); Stilbenoids (such as: Resveratrol, Pterostilbene, Piceatannol) and Punicalagins;

2. 2. Terpenes (isoprenoids) which include Carotenoids (tetraterpenoids) including Carotenes (such as: α-Carotene, β-Carotene, γ-Carotene, δ-Carotene, Lycopene, Neurosporene, Phytofluene, Phytoene), and Xanthophylls (such as: Canthaxanthin, Cryptoxanthin, Zeaxanthin, Astaxanthin, Lutein, Rubixanthin); Monoterpenes (such as: Limonene, Perillyl alcohol); Saponins; Lipids including : Phytosterols (such as: Campesterol, beta Sitosterol, gamma sitosterol, Stigmasterol), Tocopherols (vitamin E), and omega-3, 6, and 9 fatty acids (such as: gamma-linolenic acid); Triterpenoid (such as: Oleanolic acid, Ursolic acid, Betulinic acid, Moronic acid);
3. 3. Betalains which include Betacyanins (such as: betanin, isobetanin, probetanin, neobetanin); and Betaxanthins (non glycosidic versions) (such as: Indicaxanthin, and Vulgaxanthin);
4. 4. Organosulfides which include Dithiolthiones (isothiocyanates) (such as: Sulphoraphane); and Thiosulphonates (allium compounds) (such as: Allyl methyl trisulfide, and Diallyl sulfide), Indoles, glucosinolates which include Indole-3-carbinol; sulforaphane; 3,3'-Diindolylmethane; Sinigrin; Allicin; Alliin; Allyl isothiocyanate; Piperine; Syn-propanethial-S-oxide;
5. 5. Protein inhibitors which include protease inhibitors;
6. 6. Other organic acids which include Oxalic acid, Phytic acid (inositol hexaphosphate); Tartaric acid; and Anacardic acid; and
7. 7. combinations thereof.

[0046] As used herein the term "antioxidant" is preferably understood to include any one or more of various substances (as beta-carotene (a vitamin A precursor), vitamin C, vitamin E, and selenium) that inhibit oxidation or reactions promoted by Reactive Oxygen Species (ROS) and other radical and non-radical species.

[0047] As used herein the term "vitamin" is preferably understood to include any of various fat-soluble or water-soluble organic substances (non-limiting examples include vitamin A, vitamin B_1, vitamin B_6, vitamin B_{12}, vitamin C, vitamin D, vitamin E) essential in minute amounts for normal growth and activity of the body and obtained naturally from plant and animal foods

or synthetically made, and include their pro-vitamins, derivatives, and analogs.

[0048] As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a polypeptide" includes a mixture of two or more polypeptides, and the like.

[0049] As used herein, "about," is preferably understood to refer to numbers in a range of numerals. Moreover, all numerical ranges herein should be understood to include all integer, whole or fractions, within the range.

[0050] The present disclosure provides a method of making a nutritional composition. The method comprises adding exogenous vitamin K_2 and a component selected from the group consisting of phosphorous, magnesium, zinc, iron, copper, manganese, calcium, vitamin D, vitamin analogs, osteopontin or combinations thereof to a nutritional composition.

[0051] The nutritional composition can include the exogenous vitamin K_2 in an amount to be administered ranging from about 1 µg/day to about 100 µg/day. The exogenous vitamin K_2 can also be administered in an amount ranging from about 10 µg/day to about 95 µg/day, or from about 20 µg/day to about 90 µg/day, or from about 30 µg/day to about 85 µg/day, or from about 50 µg/day to about 80 µg/day, or 1 µg/day, 5 µg/day, or 10 µg/day, or 15 µg/day, or 20 µg/day, or 25 µg/day, or 30 µg/day, or 35 µg/day, or 40 µg/day, or 45 µg/day, or 50 µg/day, or 55 µg/day, or 60 µg/day, or 65 µg/day, or 70 µg/day, or 75 µg/day, or 80 µg/day, or 85 µg/day, or 90 µg/day, 95 µg/day, or 100 µg/day.

K2 AND INFLAMMATION

[0052] The acute control of global rates of protein synthesis is predominantly executed at the level of translational initiation with the modulation of various eukaryotic initiation factors (eIFs). The protein kinase referred to as the mammalian target of rapamycin (mTOR), which serves as a convergence point for signaling by growth factors and amino acids to the mRNA binding step of translation initiation is involved in modulation of the phosphorylation of the binding protein for the eukaryotic initiation factor 4E, *i.e.* 4E-BP1. It also acts to control the phosphorylation status of the 70-kDa ribosomal protein S6 kinase (S6K1). Modulation of these translation initiation events allows for more immediate control of protein synthesis and is responsive to changes associated with acute metabolic or nutritional alterations.

[0053] The canonical NF-κB pathway involves nuclear transport of a p65-p50 heterodimer. Activation of NF-κB occurs when IκBs are phosphorylated by the IκB kinase complex, leading to ubiquitination and degradation of IκB and nuclear translocation of the NF-κB dimer. Cytokines such as TNF-α are potent activators of the canonical NF-κB heterodimer, and this activation is associated with muscle protein loss.

METHODS

[0054] Male Sprague-Dawley rats (175 g) are kept on a 12-h light:dark cycle with food (Harlan-Teklad Rodent Chow, Madison, WI) and water provided freely. Animals are administered daily doses of vitamin K2 (MK-7) or saline (control) via oral gavage over 7 days. Stock solutions of vitamin K2 are prepared containing 3.5 g/L HCO-60 and 1 g/L of M&-7 in buffer A (0.15 M NaCl, 0.05 M Tris-HCl, pH 7.5). The K2 is dissolved by sonication during five pulses of 5 set with an amplitude of 6 pm. Solutions thus obtained are clear, homogeneous, and stable. Shortly before vitamin K administration the stock solutions are diluted five times with buffer A, leading to a final HCO-60 concentration of 0.7 g/L. Further dilutions (as required) are made with 0.7 g/L HCO-60 in buffer A. Each dilution step is followed by sonication as described above. In all cases vitamin K2 is administered to the rats in 0.5 mL samples, with either 25 or 50 microgram oral doses.

[0055] On the final day (Day 7), rodents are administered vitamin K2 and 2 hours later were given an IP dose of LPS (Escherichia coli serotype O111:B4, L2630, Sigma) intraperitoneally (0.5 mg/kg of body weight). Four hours later animals are sacrificed.

[0056] *Measurement of Protein Synthesis*-The fractional rate of synthesis *(Ks)* is estimated from the rate of incorporation of radioactive phenylalanine into total mixed muscle protein using the specific radioactivity of serum phenylalanine as representative of the precursor pool. The actual time for incorporation of the radiolabeled phenylalanine into protein is taken as the time elapsed from injection until freezing of muscle in liquid nitrogen.

[0057] *Analysis of mTOR Signaling to* eIFs-Gastrocnemius muscles are weighed and homogenized in 7 volumes of buffer containing 20 mM HEPES (pH 7.4), 100 mM potassium chloride, 0.2 mM EDTA, 2 mM EGTA, 50 mM sodium fluoride, 50 mM glycerophosphate, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine, 1 mM dithiothreitol (DTT), and 0.5 mM sodium vanadate. The remaining homogenate is centrifuged at 10,000 x g for 10 min at 4 °C. The resulting supernatant is combined with an equal volume of SDS sample buffer and then subjected to protein immunoblot analysis. Samples are analyzed for the phosphorylation status of 4E-BP1 (Thr37) and ribosomal protein S6 (Ser 235/236), the anti-phosphospecific antibodies were obtained from Cell Signaling Technology, Beverly, MA. Additionally, samples are analyzed for phosphorylated IKKα/β (Serl76/180; Cell Signaling Technology) and phosphorylated p65 (Ser536; Cell Signaling Technology).

RESULTS

[0058] Treatment of rodents with K2 results in a significant decrease in the rise of plasma TNF-α compared to LPS treated animals. Additionally, vitamin K2 results in a significant blunting of the drop in phosphorlyation for IKKα/β and NFκB p65 induction compared to LPS treatment. Finally, K2 abrogates the decrease in 4E-BP1(Thr-37) and ribosomal protein S6

phosphorylation compared to LPS treatment along with a greater preservation of the fractional rate of mixed muscle protein synthesis under conditions of inflammatory sepsis.

[0059] In another embodiment, this invention provides a nutritional composition comprising exogenous vitamin K2 for use in modulating the effects of inflammation, wherein said modulating the effects of inflammation is preserving the fractional rate of mixed muscle synthesis. In another embodiment, this nutritional composition further comprises a component selected from the group consisting of phosphorus, magnesium, zinc, iron, copper, manganese, calcium, vitamin D, osteopontin and combinations thereof. In another embodiment, this nutritional composition further comprises at least one antioxidant. In another embodiment, this nutritional composition further comprises at least one phytonutrient. In another embodiment, the patient is a child.

[0060] In another embodiment, this invention provides for a method for preserving the fractional rate of mixed muscle protein synthesis under conditions of inflammation, the method comprising: administering to a patient in need of same a nutritional composition comprising exogenous vitamin K2. In another embodiment, this nutritional composition further comprises a component selected from the group consisting of phosphorus, magnesium, zinc, iron, copper, manganese, calcium, vitamin D, osteopontin and combinations thereof. In another embodiment, this nutritional composition further comprises at least one antioxidant. In another embodiment, this nutritional composition further comprises at least one phytonutrient. In another embodiment, the patient is a child.

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- WO2008006607A [0018]
- WO2005107731A [0018]

Non-patent literature cited in the description

- **GLENN R. GIBSONMARCEL B. ROBERFROID**Dietary Modulation of the Human Colonic Microbiota: Introducing the Concept of PrebioticsJ. Nutr., 1995, vol. 125, 1401-1412 [0037]

Patentkrav

1. Næringsmiddelsammensætning omfattende en effektiv mængde exogent vitamin K2 til anvendelse i modulation af virkningerne af inflammation, hvor modulationen af virkningerne af inflammation er bevarelse af fraktionsraten af blandet muskelproteinsyntese under tilstande af inflammation.

2. Næringsmiddelsammensætning ifølge krav 1, hvor det exogene vitamin K2 er valgt fra gruppen bestående af MK-4, MK-7 og kombinationer deraf.

3. Næringsmiddelsammensætning ifølge krav 1, hvor det exogene vitamin K2 er MK-7.

4. Næringsmiddelsammensætning ifølge krav 1, hvor den effektive mængde af exogent vitamin K2 er fra ca. 1 µg til ca. 100 µg fortrinsvis fra ca.
fra ca. 20 µg til ca. 90 µg.
fra ca. 50 µg til ca. 80 µg.

5. Næringsmiddelsammensætning ifølge krav 1, hvor næringsmiddelsammensætningen er i en administrerbar form valgt fra gruppen bestående af farmaceutiske formuleringer, næringsmiddelformuleringer, sondemadsformuleringer, kostsupplementer, funktionelle fødevarer og drikkeprodukter.

6. Næringsmiddelsammensætning ifølge krav 1, næringsmiddelsammensætningen yderligere omfatter mindst én af præbiotika, probiotika, symbiotika, aminosyre, protein, nukleotider, en fiskeolie, ikke-marine omega-3 fedtsyreindeholdende kostfedtkilde, phytonæringsmidler, antioxidant, og kombinationer deraf.

7. Næringsmiddelsammensætning ifølge krav 6, hvor aminosyren er valgt fra gruppen bestående af prolin, hydroxyprolin, hydroxytyrosin, hydroxylysin og hydroxyserin og kombinationer deraf.

8. Næringsmiddelsammensætning ifølge krav 1, hvor patienten har mindst én af udviklingsforsinkelse, fejl-trivsel, inflammatorisk tarmsygdom, Crohn's sygdom,

Crohn's Sygdom-associeret osteopeni, Colitis, ulcerativ colitis, cøliakisygdom, glutenintolerans, neuromuskulær dysfunktion, cystisk fibrose, nyredysfunktion, androgen mangel, alvorlig fødevareallergi, kort tarmsyndrom, eller kombinationer deraf.