NUTRITIONAL COMPOSITIONS CONTAINING PUNI CALAGINS

DESCRIPTION

TECHNICAL FIELD

[0001] The present invention relates generally to nutritional compositions comprising punicalagins and methods of using nutritional compositions comprising punicalagins.

DISCLOSURE OF THE INVENTION

[0002] In an embodiment, the present disclosure is directed to nutritional compositions comprising a protein source, a fat source, a carbohydrate source, and punicalagins.

[0003] In another embodiment, the disclosure is directed to a method for enhancing the immune system in a pediatric subject via the administration of punicalagins.

[0004] Further, the disclosure, in an embodiment, is directed to a method for reducing allergic inflammatory responses in a pediatric subject via administration of punicalagins.

[0004A] In one aspect, there is provided a nutritional composition for aiding in immune system development in a pediatric subject comprising (a) a protein source; (b) a fat source; (c) a carbohydrate source; (d) punicalagins; (e) punicic acid; and (f) pomegranate leaf extract.

[0004B] In another aspect, there is provided the use of punicalagins, punicic acid and pomegranate leaf extract in the manufacture of a nutritional composition for enhancing the immune system of a pediatric subject.

[0004C] In a further aspect, there is provided the use of punicalagins, punicic acid and pomegranate leaf extract in the manufacture of a nutritional composition for reducing allergic inflammatory responses in a pediatric subject.

[0004D] In yet another aspect, there is provided an infant formula for aiding in immune system development comprising a protein source; a fat source; a carbohydrate source; punicalagins; pomegranate leaf extract and punicic acid.

[0004E] In a further aspect, there is provided a nutritionally complete composition for a pediatric subject comprising: a protein source; a fat source; a carbohydrate source; punicalagins; punicic acid; and pomegranate leaf extract.

BEST MODE FOR CARRYING OUT THE INVENTION

[0005] Reference now will be made in detail to the embodiments of the invention, one or more examples of which are set forth below. Each example is provided by way of explanation of the invention, not a limitation of the invention. In fact, it will be apparent to those skilled in the art that various modifications and variations can be made in the present

invention without departing from the scope or spirit of the invention. For instance, features illustrated or described as part of one embodiment, can be used on another embodiment to yield a still further embodiment.

[0006] Thus, it is intended that the present invention covers such modifications and variations as come within the scope of the appended claims and their equivalents. Other objects, features and aspects of the present invention are disclosed in or are obvious from the following detailed description. It is to be understood by one of ordinary skill in the art that the present discussion is a description of exemplary embodiments only, and is not intended as limiting the broader aspects of the present invention.

[0007] The present invention is directed, in some embodiments, to nutritional compositions comprising a protein source, a fat source, a carbohydrate source, and

punicalagins. The nutritional composition may be provided in any form known in the art, such as a powder, a gel, a suspension, a paste, a solid, a liquid, a liquid concentrate, or a ready-to-use product. The nutritional composition may, in certain embodiments, comprise a nutritional supplement, children's nutritional product, infant formula, human milk fortifier, or any other nutritional composition designed for children or infants. As used herein, the terms "children" or "child" refer to human subjects between the ages of one and 13 years old. In some embodiments, the terms "children" or "child" refer to human subjects that are two, three, four, five, or six years old. The term "children's nutritional product" means a composition that satisfies at least a portion of the nutrient requirements of a child. As used herein, the term "infant" means a postnatal human that is less than about one year of age. The term "infant formula" means a composition that satisfies the nutrient requirements of an infant by being a substitute for human milk.

[0008] Punicalagins are tannins, which are large polyphenol compounds that are isomers of 2,3-(S)-hexahydroxydiphenoyl-4,6-(S,S)-gallagyl-D-glucose, hydrolysable tannins with a molecular weight of 1084. Punicalagins are the predominant pomegranate tannin.

[0009] In some embodiments of the invention, punicalagins are present in a nutritional composition in an amount ranging from about 0.004 to about 0.20 mg/g of the composition. In other embodiments of the invention, punicalagins are present in an amount ranging from about 0.008 to about 0.12 mg/g of the composition.

[00010] If the composition of the invention is administered to an infant or child, an amount of punicalagins ranging from about 1 mg to about 100 mg per day may be administered. In other embodiments, the amount of punicalagins administered to an infant or child via the composition of the invention may range from about 5 mg to about 50 mg per day. In some embodiments the amount of punicalagins administered to an infant or child via the composition of the invention may range from about 10 mg to about 35 mg per day.

[00011] In some embodiments, the nutritional composition of the present invention further comprises punicic acid. Punicic acid is a polyunsaturated fatty acid, 18:3 (n-5). It is obtained from pomegranate seed oil. Its chemical formula is $C_{18}H_{30}O_2$. Punicic acid is a conjugated linolenic acid. It has three conjugated double bonds, and it is chemically similar to the conjugated linoleic acids, which have two.

[00012] The term "punicic acid," as used herein, refers to a conjugated linolenic acid isomer containing cis-9, trans-11, cis-13 double bonds in the C_{18} carbon chain, its non-toxic salts, active esters, active isomers, active metabolites, structural lipids containing punicic acid, and mixtures thereof.

[00013] In some embodiments of the invention, punicic acid is present in an amount ranging from about 0.01 to about 40 mg/g of the composition. In further embodiments of the invention, punicic acid is present in an amount ranging from about 0.02 to about 20 mg/g of the composition.

[00014] If the composition of the invention is administered to an infant or child, an amount of punicic acid ranging from about 25 mg to about 10 g per day may be administered. In further embodiments, the amount of punicic acid administered to an infant or child via the composition of the invention may range from about 50 mg to about 5 g per day.

[00015] If punicic acid is added to the nutritional composition of the present invention, the weight ratio of punicalagins:punicic acid may be from about 1:10,000 to about 4:1. In some embodiments of the present invention, the weight ratio of punicalagins:punicic acid may be from about 1:1,000 to about 1:1. In further embodiments, the ratio may be from about 1:500 to about 2:3.

[00016] In additional embodiments of the invention, the nutritional composition may further comprise pomegranate leaf extract. Although previous research has focused on the pomegranate fruit, the extract from pomegranate leaves may also provide health benefits. The use of pomegranate leaf extract in combination with punicalagins in accordance with the present invention may provide increased levels of health benefits in infants, children and adult mammal subjects.

[00017] In some embodiments of the invention, pomegranate leaf extract is present in an amount ranging from about 4 mg/g to about 60 mg/g of the composition. In further embodiments of the invention, pomegranate leaf extract is present in an amount ranging from about 12 mg/g to about 48 mg/g of the composition.

[00018] If the composition of the invention is administered to an infant or child, an amount of pomegranate leaf extract ranging from about 1 g to about 15 g per day may be administered. In further embodiments, the amount of pomegranate leaf extract administered to an infant or child via the composition of the invention may range from about 3 g to about 12 g per day. In some embodiments, the amount of

pomegranate leaf extract administered to an infant or child via the composition of the invention may range from about 5 g to about 10 g per day.

[00019] If the nutritional composition contains pomegranate leaf extract, the weight ratio of punicalagins:pomegranate leaf extract may be from about 1:15,000 to about 1:100. In some embodiments of the present invention, the weight ratio of punicalagins:pomegranate leaf extract may be from about 1:2,400 to about 1:60. In further embodiments, the ratio may be from about 1:500 to about 1:10.

[00020] In further embodiments, the nutritional composition of the present invention contemplates the use of punicalagins with punicic acid and pomegranate leaf extract. The combination of punicalagins, punicic acid, and pomegranate leaf extract may provide increased levels of health benefits in infants, children, and adult mammal subjects.

[00021] If punicalagins are provided in an infant formula or children's nutritional product, the formula or product may be nutritionally complete and may contain suitable types and amounts of lipid, carbohydrate, protein, vitamins, and minerals. As used herein, the term "nutritionally complete" refers to a nutritional composition that may be used as the sole source of nutrition, which would supply to a subject essentially all the required daily amounts of vitamins, minerals, and/or trace elements in combination with the proteins, carbohydrates, and lipids.

[00022] If the composition of the present invention is provided as an infant formula, the amount of lipid or fat in an infant formula can vary from about 3 to about 7 g/100 kcal. Lipid sources can be any used in the art, *e.g.*, vegetable oils such as palm oil, canola oil, corn oil, soybean oil, palmolein, coconut oil, medium chain triglyceride oil, high oleic sunflower oil, high oleic safflower oil, fish oil, and the like. The amount of carbohydrate can vary from about 8 to about 12 g/100 kcal. Carbohydrate sources can be any used in the art, *e.g.*, lactose, glucose, corn syrup solids, maltodextrins, sucrose, starch, rice syrup solids, and the like.

[00023] The amount of protein, if the present composition is provided as an infant formula, can vary from about 1 to about 5 g/100 kcal. Protein sources can be any used in the art, *e.g.*, nonfat milk, whey protein, casein, soy protein, hydrolyzed protein, amino acids, and the like. In a particular embodiment, the protein source contains both bovine whey and bovine casein. In an embodiment, the whey may be a denatured whey protein concentrate. In another embodiment, the whey may be an

undenatured whey protein concentrate. In a specific embodiment, the protein source may contain both a denatured and an undenatured whey protein concentrate.

[00024] If the composition of the present invention is provided as an infant formula, suitable examples of infant formulas may include soy based, partially or extensively hydrolyzed, lactose-free, low-lactose, elemental, protein-free, anti-regurgitation, premature, or standard infant formulas. The type of infant formula used may be based on the needs of the infant for proper digestion and health.

[00025] The composition of the present invention may be provided to both term and preterm infants. As a result, if the composition of the present invention is provided as an infant formula, the infant formula may be a term infant formula or a preterm infant formula. Similarly, if the composition of the present invention is provided as a human milk fortifier, the human milk fortifier may be a term human milk fortifier or a preterm human milk fortifier.

[00026] Long chain polyunsaturated fatty acids (LCPUFA) have been shown to be important in infant development. Arachidonic acid (ARA; C20:4, n-6) and docosahexaenoic acid (DHA; C22:6 n-3) are of particular interest due to the high concentrations of each found in the infant brain and retina. ARA and DHA are synthesized from their respective 18 carbon precursors, linoleic acid (18:2, n-6) and α-linolenic acid (18:3, n-3) through alternate desaturation and elongation. DHA and ARA are typically obtained through breast milk in infants that are breast-fed. In infants that are formula-fed, however, DHA and ARA must be supplemented into the diet.

[00027] Evidence indicates that infants with altered LCPUFA levels, resulting from inadequate intake of dietary LCPUFA, may be at risk for neurological problems, may score lower on cognitive tests, and have lower retinal development than infants fed human milk. Thus, the provision of LCPUFA, in particular ARA and DHA, in amounts closely approximating those found in human milk may support adequate growth as well as neurological development in formula-fed infants. Thus, in some embodiments of the invention, the nutritional composition contains at least one LCPUFA. In a particular embodiment, the nutritional composition contains DHA and/or ARA.

[00028] If added to a nutritional composition, the weight ratio of ARA:DHA may be from about 1:3 to about 9:1. In one embodiment of the present invention, this ratio is from about 1:2 to about 4:1. In yet another embodiment, the ratio is from about 2:3

to about 2:1. In one particular embodiment the ratio is about 2:1. In another particular embodiment of the invention, the ratio is about 1:1.5. In other embodiments, the ratio is about 1:1.3. In other embodiments, the ratio is about 1:1.9. In a particular embodiment, the ratio is about 1.5:1. In a further embodiment, the ratio is about 1.47:1.

[00029] If DHA is included in the invention, the level of DHA may be between about 0.0% and 1.00% of fatty acids, by weight. In other embodiments, the level of DHA may be about 0.32% by weight. In some embodiments, the level of DHA may be about 0.33% by weight. In another embodiment, the level of DHA may be about 0.64% by weight. In another embodiment, the level of DHA may be about 0.67% by weight. In yet another embodiment, the level of DHA may be about 0.96% by weight. In a further embodiment, the level of DHA may be about 1.00% by weight.

[00030] If ARA is included in the invention, the level of ARA may be between 0.0% and 0.67% of fatty acids, by weight. In another embodiment, the level of ARA may be about 0.67% by weight. In another embodiment, the level of ARA may be about 0.5% by weight. In yet another embodiment, the level of DHA may be between about 0.47% and 0.48% by weight.

[00031] If used, the amount of DHA in the present invention may be from about 2 mg/100 kilocalories (kcal) to about 100 mg/100 kcal. In another embodiment, the amount of DHA may be from about 5 mg/100 kcal to about 75 mg/100 kcal. In yet another embodiment, the amount of DHA may be from about 15 mg/100 kcal to about 60 mg/100 kcal.

[00032] If used, the amount of ARA in the present invention may be from about 4 mg/100 kilocalories (kcal) to about 100 mg/100 kcal. In another embodiment, the amount of ARA may be from about 10 mg/100 kcal to about 67 mg/100 kcal. In yet another embodiment, the amount of ARA may be from about 20 mg/100 kcal to about 50 mg/100 kcal. In a particular embodiment, the amount of ARA may be from about 25 mg/100 kcal to about 40 mg/100 kcal. In one embodiment, the amount of ARA is about 30 mg/100 kcal.

[00033] If the composition of the invention is supplemented with oils containing DHA and/or ARA, it may be accomplished using standard techniques known in the art. For example, an equivalent amount of an oil which is normally present in the composition may be replaced with DHA and/or ARA.

[00034] If utilized, the source of one or more of the LCPUFA can be any source known in the art such as marine oil, fish oil, single cell oil, egg yolk lipid, brain lipid, and the like. Any LCPUFA can be in natural form, provided that the remainder of the LCPUFA source does not result in any substantial deleterious effect on an infant or child. Alternatively, a LCPUFA can be used in refined form.

[00035] If used, the LCPUFA source may or may not contain eicosapentaenoic acid (EPA). In some embodiments, especially for feeding to infants the LCPUFA used in the invention contains little or no EPA. For example, in certain embodiments that the nutritional compositions used herein contain less than about 20 mg/100 kcal EPA; in some embodiments less than about 10 mg/100 kcal EPA; in other embodiments less than about 5 mg/100 kcal EPA; and in still other embodiments substantially no EPA.

[00036] In some embodiments of the invention, the nutritional composition contains additional components which may include probiotics or prebiotics. The term "probiotic" means a microorganism that exerts beneficial effects on the health of the host. Any probiotic known in the art may be added, provided it is suitable for combination with the other components of the composition. For example, the probiotic may be chosen from the group consisting of *Lactobacillus* and *Bifidobacterium*. Alternatively, the probiotic can be *Lactobacillus rhamnosus* GG.

[00037] In certain embodiments, the nutritional composition of the present invention additionally comprises at least one prebiotic. The term "prebiotic", as used herein, means a non-digestible food ingredient that stimulates the growth and/or activity of probiotics. In this embodiment, any prebiotic known in the art may be added, provided it is suitable for combination with the other components of the composition. In a particular embodiment, the prebiotic can be selected from the group consisting of fructo-oligosaccharide, inulin, gluco-oligosaccharide, galacto-oligosaccharide, isomalto-oligosaccharide, xylo-oligosaccharide, soybean oligosaccharides, chito-oligosaccharide, gentio-oligosaccharide, manno-oligosaccharide, lactulose, lactosucrose, raffinose, aribino-oligosaccharide, glucans, siallyl-oligosaccharide, polydextrose, inulin, fuco-oligosaccharide, and mixtures thereof.

[00038] In some embodiments, the use of punicalagins alone, or in combination with punicic acid or pomegranate leaf extract, or the combination of all three aid in immune system development and function in pediatric subjects. For example, the

use of punicalagins alone, or in combination with punicic acid or pomegranate leaf extract, or the combination of all three may enhance resistance to infection and/or reduce allergic inflammatory responses including, but not limited to asthma, wheezing, atopic cough, bronchiolitis, bronchitis, and eczema. Thus, in some embodiments, the invention is directed to a method for enhancing the immune response in a pediatric subject comprising administering the nutritional composition of the present invention to the pediatric subject. In further embodiments, the invention is directed to a method for enhancing resistance to infection in a pediatric subject comprising administering the nutritional composition of the present invention to the pediatric subject. In other embodiments, the invention is directed to a method for reducing allergic inflammatory responses in a pediatric subject comprising administering the nutritional composition of the present invention to the pediatric subject. As used herein, the term "pediatric subject" refers to human subjects that are less than 13 years old. In some embodiments, the term "pediatric subject" refers to human subjects that are less than 8 years old.

[00039] Further, in some embodiments, the use of punicalagins alone or in combination with punicic acid, or pomegranate leaf extract, or the combination of all three may aid in enhancing cardiovascular health, eye health, brain development and function, gastrointestinal health and function. Further, the use of punicalagins alone, or in combination with punicic acid or pomegranate leaf extract, or the combination of all three may aid in reducing the risk of inflammation, cancer, or the metabolic syndrome, including obesity and diabetes mellitus. Thus, in some embodiments, the invention is directed to a method for enhancing cardiovascular health, and eye health in a pediatric subject by administering the nutritional composition of the present invention to the pediatric subject. In further embodiments the invention is directed to a method for enhancing brain development and function in a pediatric subject by administering the nutritional composition of the present invention to the pediatric subject. In other embodiments the invention is directed to a method of improving gastrointestinal health and function in a pediatric subject by administering the nutritional composition of the present invention to the pediatric subject. Such an embodiment of the invention could include providing a healthy intestinal micro flora balance in the pediatric subject. In some embodiments, the invention is directed to a method for reducing the risk of inflammation and cancer in a pediatric subject by administering the nutritional

composition of the present invention to the pediatric subject. In further embodiments, the invention is directed to a method for reducing the risk of metabolic syndrome in a pediatric subject by administering the nutritional composition of the present invention to the pediatric subject. In such an embodiment, the reduction of the risk of metabolic syndrome in pediatric subjects could include reduction in the risk of obesity and diabetes mellitus.

[00040] In certain embodiments, the combination of punicalagins with punicic acid or with pomegranate leaf extract, or the combination of all three provides an enhanced effect with respect to the health benefits listed above. It is believed that the activity of the combination of punicalagins with punicic acid or with pomegranate leaf extract, or the combination of all three is greater than the added activity expected when each of the compounds are administered separately.

[00041] The following example describes an embodiment of the present invention. Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from the consideration of the specification or practice of the invention disclosed herein. It is intended that the specification, together with the example, be considered to be exemplary only, with the scope of the invention being indicated by the claims which follow the example.

Example 1

[00042] Table 1 illustrates the nutrient components of one infant formula embodiment of a nutritional composition of the present invention.

Table 1

(Normal Dilution)	Per 100 Calories (5 fl oz)	Per 100 grams Powder
Protein, g	2.1	10.8
Fat, g	5.3	27
Linoleic acid, mg	860	4400
Linolenic acid, mg	85	440
DHA, mg	17	88
ARA, mg	34	175
Carbohydrate, g	10.9	56

Water, g	134	3.3
Punicalagins, mg	1.9	10
Vitamins/Other Nutrients		
Vitamin A, IU	300	1550
Vitamin D, IU	60	310
Vitamin E, IU	2	10.3
Vitamin K, mcg	8	41
Thiamin (Vitamin B1), mcg	80	410
Riboflavin (Vitamin B2), mcg	140	720
Vitamin B6, mcg	60	310
Vitamin B12, mcg	0.3	1.55
Niacin, mcg	1000	5200
Folic acid (Folacin), mcg	16	83
Pantothenic acid, mcg	500	2600
Biotin, mcg	3	15.5
Vitamin C (Ascorbic acid), mg	12	62
Choline, mg	24	124
Inositol, mg	6	31
Carnitine, mg	2	10.3
Taurine, mg	6	31
Minerals		
Calcium, mg	78	400
Phosphorus, mg	43	220
Magnesium, mg	8	41
Iron, mg	1.8	9.3
Zinc, mg	1	5.2

Manganese, mcg	15	77
Copper, mcg	75	390
Iodine, mcg	10	52
Selenium, mcg	2.8	14.5
Sodium, mg	27	139
Potassium, mg	108	560
Chloride, mg	63	330
Molybdenum, mcg	NA	NA
Chromium, mcg	NA	NA

[00043] All references cited in this specification, including without limitation, all papers, publications, patents, patent applications, presentations, texts, reports, manuscripts, brochures, books, internet postings, journal articles, periodicals, and the like, are hereby incorporated by reference into this specification in their entireties. The discussion of the references herein is intended merely to summarize the assertions made by their authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinence of the cited references.

[00044] These and other modifications and variations to the present invention may be practiced by those of ordinary skill in the art, without departing from the scope of the present invention, which is more particularly set forth in the appended claims. In addition, it should be understood that aspects of the various embodiments may be interchanged in whole or in part. Furthermore, those of ordinary skill in the art will appreciate that the foregoing description is by way of example only, and is not intended to limit the invention so further described in such appended claims. Therefore, the scope of the appended claims should not be limited to the description of the preferred versions contained therein.

CLAIMS

1. A nutritional composition for aiding in immune system development in a pediatric subject comprising:
    a. a protein source;
    b. a fat source;
    c. a carbohydrate source;
    d. punicalagins;
    e. punicic acid; and
    f. pomegranate leaf extract.

2. The nutritional composition according to claim 1, wherein the amount of punicalagins in the composition is between about 0.004 to about 0.20 mg/g of the composition.

3. The nutritional composition according to claim 1, wherein the amount of punicic acid in the composition is between about 0.01 to about 40 mg/g of the composition.

4. The nutritional composition according to claim 1, wherein the amount of pomegranate leaf extract in the composition is between about 4mg/g to about 60 mg/g of the composition.

5. The nutritional composition according to claim 1, wherein the nutritional composition is an infant formula.

6. Use of punicalagins, punicic acid and pomegranate leaf extract in the manufacture of a nutritional composition for enhancing the immune system of a pediatric subject.

7. The use of claim 6, wherein the enhanced immune system function comprises an improved resistance to infection.

8. The use of claim 6, wherein the amount of punicalagins is between about 0.004 to about 0.20 mg/g of the composition.

9. The use according to claim 6, wherein the amount of punicic acid is between about 0.01 to about 40 mg/g of the composition.

10. The use according to claim 9, wherein the amount of pomegranate leaf extract is between about 4mg/g to about 60 mg/g of the composition.

11. Use of punicalagins, punicic acid and pomegranate leaf extract in the manufacture of a nutritional composition for reducing allergic inflammatory responses in a pediatric subject.

12. The use according to claim 11, wherein the amount of punicalagins is between about 0.004 to about 0.20 mg/g of the composition.

13. The use according to claim 11, wherein the amount of punicic acid is between about 0.01 to about 40 mg/g of the composition.

14. The use according to claim 11, wherein the amount of pomegranate leaf extract is between about 4mg/g to about 60 mg/g of the composition.

15. An infant formula for aiding in immune system development comprising:

a protein source;

a fat source;

a carbohydrate source;

punicalagins;

pomegranate leaf extract and punicic acid.

16. The infant formula of claim 15 further comprising long chain polyunsaturated fatty acids.

17. The infant formula of claim 16, further comprising docosahexaenoic acid.

18. The infant formula of claim 15 further comprising a prebiotic.

19. The infant formula of claim 15 further comprising a probiotic.

20. A nutritionally complete composition for a pediatric subject comprising:

a protein source;

a fat source;

a carbohydrate source;

punicalagins;

punicic acid;

and pomegranate leaf extract.

21. The composition of claim 20, further comprising long chain polyunsaturated fatty acids.