US 20090186803A1

(19) **United States**

(12) **Patent Application Publication**

Zwijsen et al.

(10) **Pub. No.: US 2009/0186803 A1**

(43) **Pub. Date: Jul. 23, 2009**

(54) **USE OF NUTRITIONAL COMPOSITIONS WITH PHOSPHOLIPID, SPHINGOLIPID AND CHOLESTEROL.**

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(21) Appl. No.: **12/158,931**

(22) PCT Filed: **Dec. 22, 2006**

(86) PCT No.: **PCT/NL2006/050331**

§ 371 (c)(1),
(2), (4) Date: **Oct. 22, 2008**

(30) **Foreign Application Priority Data**

Dec. 23, 2005 (EP) 05077972.7
Nov. 2, 2006 (NL) PCT/NL2006/050274

Publication Classification

(51) **Int. Cl.**
A61K 38/00 (2006.01)

(52) **U.S. Cl.** **514/2**

(57) **ABSTRACT**

A nutritional composition comprising phospholipids, sphingolipids and cholesterol for the prevention of obesity and/or diabetes is provided.

USE OF NUTRITIONAL COMPOSITIONS WITH PHOSPHOLIPID, SPHINGOLIPID AND CHOLESTEROL.

FIELD OF THE INVENTION

[0001] The present invention relates to preventing obesity by administering a nutritional composition to non-obese infants with the age below 3 years. In a further embodiment the present invention relates to the treatment and/or prevention of type 2 diabetes.

BACKGROUND OF THE INVENTION

[0002] Breast-feeding is the preferred method of feeding infants. However, there are circumstances that make breast-feeding impossible or less desirable. In those cases infant formulae are a good alternative. The composition of modem infant formulae is adapted in such a way that it meets many of the special nutritional requirements of the fast growing and developing infant.

[0003] Still it seems that improvements can be made towards the constitution of infant milk formulae. For example little is known about the effects of ingredients in the infant formulae on obesity later in life. The present invention relates to such future health.

[0004] EP 1566439 pertains to a method for preventing and/or treating obesity. WO 2005/051091 relates to a substantially homogenous lipid preparation comprising a combination of glycerophospholipids being phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI), wherein the quantitative ratio between said glycerophospholipids essentially mimics their corresponding ratio in naturally occurring human milk fat (HMF), optionally further comprising sphingomyelin (SM) and/or cholesterol, wherein the quantitative ratio between the glycerophospholipids in said combination and the sphingomyelin and/or cholesterol essentially mimics their corresponding ratio in said naturally occurring HMF. JP05292880 relates to buttermilk fat for inhibiting lipase activity.

SUMMARY OF THE INVENTION

[0005] The present invention provides a composition wherein the lipid fraction is enriched in polar lipids, more particularly in glycerophospholipids, sphingolipids (especially sphingomyelin) and cholesterol. It was surprisingly found by the inventors that administration of a composition comprising glycerophospholipids, sphingolipids (especially sphingomyelin) and cholesterol decreases insulin secretion when added to a nutritional composition, compared to the insulin secretion induced by the same nutritional formula without the present polar lipids.

[0006] In infants high blood insulin levels stimulate glucose uptake in adipose tissue, resulting in an increased adipose tissue mass. Furthermore, high blood insulin levels stimulate the formation of excess visceral fat. Excess visceral fat results in an increased chance to develop obesity later in life.

[0007] The present invention can therefore advantageously be used to prevent obesity later-in-life, but also to prevent the development of diabetes later in life. Inclusion of the present combination in infant nutrition reduces blood insulin levels and thereby the prevents the development of obesity and/or diabetes later in life. Since in infants high blood insulin levels particularly stimulate visceral adipocyte proliferation, the composition is particularly suitable to prevent visceral obesity.

[0008] Due the blood insulin reducing effects when the combination of glycerophospholipids, sphingolipids (especially sphingomyelin) and cholesterol is included in a nutritional composition, the present invention can also suitably be used for the manufacture of a nutritional composition for the prevention of type 2 diabetes, and/or for the dietary management of type 2 diabetes.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0009] The present invention provides the use of a composition comprising a lipid, protein and digestible carbohydrate component and (a) 0.5 to 20 wt. % phospholipids based on total lipid; (b) 0.1 to 20 wt. % sphingolipids based on total lipid; and (c) 0.005 to 10 wt. % cholesterol based on total lipid, for the manufacture of a nutritional composition to be administered to a (non-obese) infant with the age below 36 months for the prevention of obesity and/or type 2 diabetes.

[0010] In a further embodiment the present invention provides the use of a composition comprising a lipid, protein and digestible carbohydrate component and (a) 0.5 to 20 wt. % phospholipids based on total lipid; (b) 0.1 to 20 wt. % sphingolipids based on total lipid; and (c) 0.005 to 10 wt. % cholesterol based on total lipid for the manufacture of a nutritional composition to be administered to an infant with the age below 36 months for preventing the development of a disorder when said human has an age above 36 months, wherein said disorder is selected from the group consisting of type 2 diabetes, fasting hyperglycaemia, insulin resistance, visceral adiposity, hyperinsulinemia, hypertension, cardiovascular disease, cerebrovascular disease, arthrosclerosis, dyslipidaemia, hyperuricaemia, fatty liver, osteoarthritis and sleep apnoea.

[0011] In another embodiment the present invention provides the use of a composition comprising a lipid, protein and digestible carbohydrate component and: (a) 0.5 to 20 wt. % phospholipids based on total lipid; (b) 0.1 to 20 wt. % sphingolipids based on total lipid; and (c) 0.005 to 10 wt. % cholesterol based on total lipid, for the manufacture of a nutritional composition for the prevention of type 2 diabetes and/or for the dietary management of type 2 diabetes.

Obesity

[0012] The present composition is preferably administered to a non-obese human infant with the age below 36 months, preferably below 18 months, more preferably below 12 months, even more preferably below 6 months. Preferably the present composition is administered to a non-overweight human with the age below 36 months, preferably below 18 months, more preferably below 12 months, even more preferably below 6 months of age. The absence or presence of obesity and/or overweight in an infant can suitably be determined by a physician. Typically, a non-obese infant below 36 months of age has gender specific weight-for-length below the 95^{th} percentile, more preferably below the 85^{th} percentile. Gender specific weight-for-length percentiles have been published by Center for Disease Control and Prevention (CDC) in 2000.

[0013] Likewise the presence or absence of obesity and/or overweight in a human subject above 36 months of age can be

easily determined by a physician and/or with the gender specific weight-for-length percentiles published by CDC.

[0014] Health related problems are especially associated with a special form of obesity, namely central obesity. Preferably the composition is used to prevent central obesity later-in-life. The term 'central obesity' refers to a condition with increased visceral fat mass. A waist circumference above 102 cm in adult man or above 88 cm in adult women indicates central obesity. For children of 3-19 years old appropriate cut-offs for age- and sex-dependent waist circumferences can be found in Taylor et al, 2000 Am J Clin Nutr 72:490-495.

Lipid Component

[0015] The present composition comprises a lipid component. The present composition comprises phospholipids, sphingolipids and cholesterol. Additionally the present composition preferably comprises triglycerides. The lipid component provides preferably 35 to 55% of the total calories. More preferably the present composition comprises a lipid component providing 40 to 50% of the total calories. When in liquid form, e.g. as a ready-to-feed liquid, the composition preferably comprises 2.1 to 6.5 g lipid per 100 ml, more preferably 3.0 to 4.0 g per 100 ml. Based on dry weight the present composition preferably comprises 12.5 to 40 wt. % lipid, more preferably 19 to 30 wt. %.

Phospholipids, Sphingolipids and Cholesterol

[0016] As found by the inventors, oral administration of a composition comprising phospholipids, sphingolipids and cholesterol has the advantage that it decreases the post-prandial insulin response (see example 1), particularly when co-administered with a nutritional composition.

[0017] The present composition comprises phospholipids. The term phospholipids as used in the present invention particularly refers to glycerophospholipids. Glycerophospholipids are normally a class of lipids formed from fatty acids esterified at the hydroxyl groups on carbon-1 and carbon-2 of the backbone glycerol moiety and a negatively-charged phosphate group attached to carbon-3 of the glycerol via an ester bond, and optionally a choline group (in case of phosphatidylcholine), a serine group (in case of phosphatidylserine), an ethanolamine group (in case of phosphatidylethanolamine), an inositol group (in case of phosphatidylinositol) or a glycerol group (in case of phosphatidylglycerol) attached to the phosphate group. Preferably the present composition contains phosphatidylcholine (PC), phosphatidylserine, phosphatidylinositol and/or phosphatidylethanolamine, more preferably at least phosphatidylcholine.

[0018] The present composition comprises sphingolipids. The term sphingolipids as in the present invention particularly refers to glycolipids with an amino alcohol sphingosine. The sphingosine backbone is O-linked to a (usually) charged headgroup such as ethanolamine, serine or choline backbone. The backbone is also amide linked to a fatty acyl group. Sphingolipids include sphingomyelin, ceramides, and glycosphingolipids. Preferably the present composition contains sphingomyelin and/or glycosphingolipids. Glycosphingolipids are ceramides with one or more sugar residues joined in a β-glycosidic linkage at the 1-hydroxyl position. Glycosphingolipids may be further subdivided into cerebrosides, globosides and gangliosides. Cerebrosides have a single glucose or galactose at the 1-hydroxy position, while gangliosides have at least three sugars, one of which must be sialic acid. Sphingomyelins have a phosphorylcholine or phosphoroethanolamine molecule esterified to the 1-hydroxy group of a ceramide. Preferably the present composition contains gangliosides. Preferably the composition comprises sphingolipids, more preferably sphingomyelin and/or gangliosides, more preferably at least one ganglioside selected from the group consisting of GM3 and GD3.

[0019] The present composition comprises cholesterol. The cholesterol is believed to further contribute to a reduced occurrence of obesity and/or type 2 diabetes. A further beneficial effect of dietary cholesterol is that it inhibits the endogenous cholesterol synthesis during infancy and programmes the endogenous cholesterol synthesis to lower levels. Consequently, reduced blood cholesterol levels later in life will be achieved. This results in a drop of LDL-cholesterol value in blood and a raise of HDL cholesterol value in blood during adolescence and adulthood.

[0020] Preferably the present composition comprises 0.5 to 20 wt. % phospholipids based on total lipid, more preferably 1 to 10 wt. %, even more preferably 4 to 8 wt. %. Preferably the present composition comprises 0.1 to 20 wt. % sphingolipids based on total lipid, more preferably 0.5 to 10 wt. %, even more preferably 2 to 8 wt%. Preferably the present composition comprises 0.5 to 20 wt. % (sphingolipids plus phospholipids) based on total lipid, more preferably 1 to 10 wt. %, even more preferably 4 to 8 wt%. The present composition preferably comprises at least 0.005 wt. % cholesterol based on total fat, more preferably at least 0.01 wt. %, more preferably at least 0.05 wt. %., even more preferably at least 0.1 wt. %. Preferably the amount of cholesterol does not exceed 10 wt. % based on total lipid, more preferably does not exceed 5 wt. %, even more preferably does not exceed 1 wt. % of total lipid. Most preferably the amount of cholesterol is 0.5 to 0.7 wt. % based on total lipid.

[0021] Preferred sources for providing the phospholipids, sphingolipids and cholesterol are egg lipids, milk fat, buttermilk fat and butter serum fat. A preferred source for phospholipids, particularly PC, is soy lecithin. Hence the present composition preferably comprises soy lecithin, egg lipid, milk fat, butter serum fat and/or buttermilk fat, more preferably buttermilk fat.

[0022] The present composition preferably comprises 0.05 to 10 grams buttermilk fat per 100 gram dry weight of the composition, preferably 0.1 to 3 gram. These amounts of buttermilk fat provide the blood insulin reducing effects.

[0023] The buttermilk fat and butter serum fat is typically obtained from the manufacture of buttermilk and in particular from the whey that remains after manufacturing cheese from buttermilk. This milk whey comprises a relatively high amount of small fat globules, especially when macromolecules such as β-lactoglobulin have been removed. The concentration of small fat globules can be increased by applying filtration techniques on skimmed products, which concentrate the lipid layer on one side of the membrane and remove molecules like salts and lactose. Fractions that are enriched with acidic sphingoglycolipids can also be isolated by applying chromatographic methods known in the art such as ion exchange. Milks from many mammals are suitable for isolation of the active components, however, milk from mares, sheep, goat and camels are particularly suited. It is most preferred to use a lipid extract isolated from sheep milk. Preferably the buttermilk or butter serum fat contains at least

4 wt. % phospholipids per 100 gram lipid, more preferably 7 to 75 wt. %, most preferably 30 to 70 wt. % phospholipids per 100 gram lipid.

Digestible Carbohydrate Component

[0024] The composition comprises digestible carbohydrate. The digestible carbohydrate component preferably provides 30 to 60% of the total calories. Preferably the present composition comprises a digestible carbohydrate component provides 40 to 50% of the total calories.

[0025] Preferred digestible carbohydrate sources are lactose, glucose, sucrose, fructose, galactose, maltose, starch and maltodextrin. The maintenance of insulin sensitivity, while reducing blood insulin levels can improved by inclusion of a low glycaemic carbohydrate in the present composition, preferably lactose. Hence, the present composition preferably comprises lactose. The present composition preferably comprises a digestible carbohydrate component, wherein at least 35 wt. %, more preferably at least 50 wt. %, more preferably at least 75 wt. %, even more preferably at least 90 wt. %, most preferably at least 95 wt. % is lactose. The present composition preferably comprises at least 25 grams lactose per 100 gram dry weight of the present composition, preferably at least 40 grams lactose/100 gram.

Non-digestible Oligosaccharides

[0026] As already described above a high blood insulin levels stimulate glucose uptake in adipose tissue, resulting in an increased adipose tissue mass. In infants the high insulin levels contribute to increased proliferation of adipocytes, at least partly due to the increased glucose uptake, and thereby in an increased chance of obesity later in life.

[0027] The present composition therefore preferably maintains low insulin levels. It was found that non-digestible oligosaccharides (NDO) that can be fermented (particularly galacto-oligosaccharides) have a blood insulin tempering effect, and consequently contribute to a reduced change on obesity later-in-life (see example 2). Furthermore, the combination of phospholipids, sphingolipids, cholesterol and non-digestible oligosaccharide is particularly effective in reducing blood insulin levels. It was surprisingly found that the combination of phospholipids, sphingolipids, cholesterol reduced the postprandial insulin secretion of non-digestible oligosaccharide containing nutritional compositions. This effect can be even further improved by including lactose as the (main) digestible carbohydrate source. The combination of phospholipids, sphingolipids, cholesterol and the non-digestible oligosaccharides synergistically reduces the obesity later in life

[0028] Preferably the present composition comprises non-digestible oligosaccharides with a DP between 2 and 60. The composition preferably prevents the onset of insulin resistance. The non-digestible oligosaccharide is preferably selected from the group consisting of fructo-oligosaccharides (including inulin), galacto-oligosaccharides (including transgalacto-oligosaccharides), gluco-oligosaccharides (including gentio-, nigero- and cyclodextrin-oligosaccharides), arabino-oligosaccharides, mannan-oligosaccharides, xylo-oligosaccharides, fuco-oligosaccharides, arabinogalacto-oligosaccharides, glucomanno-oligosaccharides, galactomanno-oligosaccharides, sialic acid comprising oligosaccharides and uronic acid oligosaccharides. Preferably the present composition comprises fructo-oligosaccharides, galacto-oligosaccharides and/or galacturonic acid oligosaccharides, more preferably galacto-oligosaccharides, most preferably transgalacto-oligosaccharides. In a preferred embodiment the composition comprises a mixture of transgalacto-oligosaccharides and fructo-oligosaccharides. Preferably the present composition comprises galacto-oligosaccharides with a DP of 2-10 and/or fructo-oligosaccharides with a DP of 2-60. The galacto-oligosaccharide is preferably selected from the group consisting of transgalacto-oligosaccharides, lacto-N-tetraose (LNT), lacto-N-neotetraose (neo-LNT), fucosyl-lactose, fucosylated LNT and fucosylated neo-LNT. In a particularly preferred embodiment the present method comprises the administration of transgalacto-oligosaccharides ([galactose]$_n$-glucose; wherein n is an integer between 1 and 60, i.e. 2, 3, 4, 5, 6, . . . , 59, 60; preferably n is selected from 2, 3, 4, 5, 6, 7, 8, 9, or 10). Transgalacto-oligosaccharides (TOS) are for example sold under the trademark Vivinal™ (Borculo Domo Ingredients, Netherlands). Preferably the saccharides of the transgalacto-oligosaccharides are β-linked. Fructo-oligosaccharide is a NDO comprising a chain of β linked fructose units with a DP or average DP of 2 to 250, more preferably 10 to 100. Fructo-oligosaccharide includes inulin, levan and/or a mixed type of polyfructan. An especially preferred fructo-oligosaccharide is inulin. Fructo-oligosaccharide suitable for use in the compositions is also already commercially available, e.g. Raftiline®HP (Orafti). Uronic acid oligosaccharides are preferably obtained from pectin degradation. Hence the present composition preferably comprises a pectin degradation product with a DP between 2 and 100. Preferably the pectin degradation product is prepared from apple pectin, beet pectin and/or citrus pectin. Preferably the composition comprises transgalacto-oligosaccharide, fructo-oligosaccharide and a pectin degradation product. The weight ratio transgalacto-oligosaccharide:fructo-oligosaccharide:pectin degradation product is preferably 20 to2:1:1 to 3, more preferably 12 to 7:1:1 to 2.

Fatty Acyl Chains

[0029] Herein LA refers to linoleic acid (18:2 n6); ALA refers to α-linolenic acid (18:3 n3); LC-PUFA refers to long chain polyunsaturated fatty acids and/or acyl chains comprising at least 20 carbon atoms in the fatty acyl chain and with 2 or more unsaturated bonds; DHA refers to docosahexaenoic acid (22:6, n3); EPA refers to eicosapentaenoic acid (20:5 n3); ARA refers to arachidonic acid (20:4 n6); DPA refers to docosapentaenoic acid (22:5 n3), and DHGLA refers to dihomogammalinolenic acid (20:3 n6). Medium chain fatty acids (MCFA) refer to fatty acids and/or acyl chains with a chain length of 6, 8 or 10 carbon atoms. MCFA may also be referred to as medium chain triglycerides (MCT).

[0030] LA preferably is present in a sufficient amount in order to promote a healthy growth and development, yet in an amount as low as possible to prevent occurrence of obesity later in life. The composition therefore preferably comprises less than 15 wt. % LA based on total fatty acids, preferably between 5 and 14.5 wt. %, more preferably between 6 and 12 wt. %. Preferably ALA should be present in a sufficient amount to promote a healthy growth and development of the infant. The present composition therefore preferably comprises at least 1.0 wt. % based on total fatty acids. Preferably the composition comprises at least 1.6 wt. % ALA based on total fatty acids, more preferably at least 2.0 wt. %. Preferably the composition comprises less than 10 wt. % ALA, more preferably 5.0 wt. % based on total fatty acids. The weight

ratio LA/ALA should be well balanced in order to prevent obesity, especially central obesity, while at the same time ensuring a normal growth and development. Therefore, the present composition preferably comprises a weight ratio of LA/ALA between 2 and 15, more preferably between 2 and 7, more preferably between 3 and 6, even more preferably between 4 and 5.5, even more preferably between 4 and 5.

[0031] Since MCFA contribute to a reduced fat mass later in life when administered to an infant, the present composition preferably comprises at least 3 wt. % MCFA based on total fatty acids, more preferably at least 10 wt. %, even more preferably 15 wt. %. Since MCFA reduces body fat deposition with no preference for central fat mass, the present composition advantageously comprises less than 50 wt. % MCFA based on total fatty acids, more preferably less than 40 wt. %, even more preferably less than 25 wt. %.

[0032] Preferably the present composition comprises LC-PUFA, since LC-PUFA reduce obesity later in life, more preferably central obesity. More preferably, the present composition comprises n-3 LC-PUFA, even more preferably EPA, DPA and/or DHA, even more preferably DHA. It was found that these n-3 LC-PUFA decrease obesity.

[0033] Since a low concentration of DHA, DPA and/or EPA is already effective and normal growth and development are important, the content of n-3 LC-PUFA in the present composition, preferably does not exceed 15 wt. % of the total fatty acid content, preferably does not exceed 10 wt. %, even more preferably does not exceed 5 wt. %. Preferably the present composition comprises at least 0.2 wt. %, preferably at least 0.5 wt. %, more preferably at least 0.75 wt. %, n-3 LC-PUFA of the total fatty acid content.

[0034] As the group of n-6 fatty acids, especially arachidonic acid (AA) and LA as its precursor, counteracts the group of n-3 fatty acids, especially DHA and EPA and ALA as their precursor, the present composition comprises relatively low amounts of AA. The n-6 LC-PUFA content preferably does not exceed 5 wt. %, more preferably does not exceed 0.8 wt. %, more preferably does not exceed 0.75 wt. %, even more preferably does not exceed 0.5 wt. %, based on total fatty acids. Since AA is important in infants for optimal functional membranes, especially membranes of neurological tissues, the amount of n-6 LC-PUFA is preferably at least 0.02 wt. % more preferably at least 0.05 wt. %, more preferably at least 0.1 wt. % based on total fatty acids, more preferably at least 0.25 wt. %. The presence of AA is advantageous in a composition low in LA since it remedies LA deficiency. The presence of, preferably low amounts, of AA is beneficial in nutrition to be administered to infants below the age of 6 months, since for these infants the infant formulae is generally the only source of nutrition.

[0035] The weight ratio n-6 LC-PUFA/n-3 LC-PUFA in the present infant nutrition is preferably low in order to prevent obesity later in life. Preferably the composition comprises a weight ratio of n-6 LC-PUFA/n-3 LC-PUFA below 1.5, more preferably below 1.0, even more preferably below 0.6.

[0036] The amount of saturated fatty acids is preferably below 58 wt. % based on total fatty acids, more preferably below 45 wt. %. The concentration of monounsaturated fatty acids preferably ranges from 17 to 60% based on weight of total fatty acids

[0037] LA, ALA, MCFA and/or LC-PUFA are preferably provided as free fatty acids, in triglyceride form, in diglyceride form, in monoglyceride form, in phospholipid form, or as a mixture of one of more of the above. Preferably the present composition contains LC-PUFA in triglyceride and/or phospholipid form, even more preferably phospholipid form since LC-PUFA in phospholipid form are better incorporated into membranes. Preferably, the present composition contains MCFA in triglyceride form.

Lipid Sources

[0038] Preferably the present composition comprises at least one, preferably at least two lipid sources selected from the group consisting of linseed oil (flaxseed oil), rape seed oil (including colza oil, low erucic acid rape seed oil and canola oil), salvia oil, perilla oil, purslane oil, lingonberry oil, sea buckthorn oil, hemp oil, high oleic sunflower oil, high oleic safflower oil, olive oil, fish oil (especially tuna oil), single cell oil (including algal, microbial oil and fungal oil), black currant seed oil, echium oil, butter fat, coconut oil and palm kernel oil. Preferably the present composition comprises at least one, preferably at least two lipid sources selected from the group consisting of linseed oil, rapeseed oil, coconut oil, high oleic sunflower oil, butter oil, single cell oil and fish oil.

[0039] Preferably as a source of n-3 LC-PUFA single cell oil, including algal oil and microbial oil, is used, since these oil sources have a low EPA/DHA ratio, which results in an increased anti-obesity effect. More preferably fish oil (even more preferably tuna oil) is used as a source of n-3 LC-PUFA since they have a higher EPA concentration which is advantageous since EPA is precursor of eicosanoids which have an additional anti-obesity effect.

Uridine and Choline

[0040] The present composition preferably comprises a source of uridine and/or choline. Preferably the composition comprises a source of uridine and choline. Choline is preferably added as choline chloride. The present composition preferably comprises choline chloride. The present composition preferably comprises at least 0.035 wt. % choline based on dry weight of the composition, more preferably at least 0.045 wt. %. Preferably the present composition comprises no more than 1 wt. % choline based on total dry weight of the present composition, more preferably below 0.5 wt. %, even more preferably below 0.1 wt. %. The presence of choline has the advantage that it is an excellent methyldonor. In stages of quick growth such as in infancy, a sufficient amount of methyldonor is important to sustain differentiation and regulation and thereby result in a proper metabolic imprinting via DNA methylation. A proper metabolic imprinting is important for preventing obesity later in life. Therefore the composition of the present invention preferably comprises choline. The choline resulting from phospholipids is not calculated to contribute to this amount of methyl donor. In a preferred embodiment the present composition comprises uridine in the form of a nucleotide, nucleoside and/or base. Preferably, the present composition comprises uridine 5'-monophospate and/or salts thereof (collectively abbreviated to UMP), in particular monosodium salts thereof. Preferably the composition comprises 0.001 to 0.1 wt. % UMP based on dry weight of the present composition, more preferably 0.002 to 0.05 wt. %, most preferably 0.002 to 0.025 wt. %. UMP is preferably be added to the composition in a mixture of nucleotides.

Protein Component

[0041] The present composition comprises proteins. The protein component preferably provides 5 to 15% of the total

calories. Preferably the present composition comprises a protein component provides 6 to 12% of the total calories.

[0042] Preferably the composition comprises vegetable protein and/or animal (non-human) milk protein. Preferably the composition comprises hydrolyzed casein and/or hydrolyzed whey protein. It was found that administration of a composition wherein the protein comprises hydrolyzed casein and/or hydrolyzed whey results in reduced post-prandial levels of both insulin and glucose compared to the administration of a composition comprising intact casein and intact whey protein. The present composition more preferably comprises more preferably casein hydrolysate and whey protein hydrolysate because the amino acid composition of bovine casein is more similar to the amino acid composition found in human milk protein and whey protein is easier to digest and found in greater ratios in human milk.

Nutritional Composition

[0043] The present composition is particularly suitable for providing the daily nutritional requirements to an infant with the age below 36 months, particularly an infant with the age below 24 months, even more preferably an infant with the age below 18 months, most preferably below 12 months of age. The present composition comprises a lipid, protein and digestible carbohydrate component wherein the lipid component preferably provides 35 to 55% of the total calories, the protein component preferably provides 5 to 15% of the total calories and the digestible carbohydrate component preferably provides 30 to 60% of the total calories. Preferably the present composition comprises a lipid component providing 40 to 50% of the total calories, a protein component provides 6 to 12% of the total calories and a digestible carbohydrate component provides 40 to 50% of the total calories.

[0044] The present composition is not human breast milk. The present composition preferably comprises (i) vegetable lipid and/or animal (non-human) fat; and/or (ii) vegetable protein and/or animal (non-human) milk protein. The present composition preferably does not comprise a proteinase inhibitor, preferably not a trypsin inhibitor, chymotrypsin inhibitor or elastase inhibitor. The present composition is not human milk. The compositions of the inventions preferably comprise other fractions, such as vitamins, minerals according to international directives for infant formulae.

Infant

[0045] Adipocytes, including visceral adipocytes, proliferate during the first 36 months of life as well as (more limited) in puberty. The amount of adipocytes is an important determinant in the degree of obesity later-in-life. Hence the present composition is preferably administered to the infant during the first 3 years of life. It was found that there is a predominance of proliferation of (visceral) adipocytes in the first 12 months of life (with an optimum in perinatal adipocyte proliferation). Hence, it is particularly preferred that the present composition is administered to the infant in this period of life. The present composition is therefore advantageously administered to a human of 0-24 months, more preferably to a human of 0-18 months, most preferably to a human of 0-12 months. The present invention particularly aims to prevent obesity later-in-life and is preferably not an obesity treatment. Hence, the present composition is preferably administered to an infant not suffering from obesity or childhood obesity, particularly a non-obese infant more preferably an infant that does not suffer from overweight. The present composition is preferably administered orally to the infant.

Application

[0046] The present invention also aims to prevent the occurrence of obesity and or diabetes at the age above 36 months, particularly to prevent obesity at the age above 8 years, particularly above 15 years, more particularly above 18 years.

[0047] Preferably the composition is used to prevent obesity, more preferably central obesity, since especially central obesity is related to health disorders such as type 2 diabetes, fasting hyperglycaemia, insulin resistance, visceral adiposity, hyperinsulinemia, hypertension, cardiovascular disease, cerebrovascular disease, arthrosclerosis, dyslipidaemia, hyperuricaemia, fatty liver, osteoarthritis and sleep apnoea, more preferably type 2 diabetes.

[0048] In this document and in its claims, the verb "to comprise" and its conjugations is used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded. In addition, reference to an element by the indefinite article "a" or "an" does not exclude the possibility that more than one of the element is present, unless the context clearly requires that there be one and only one of the elements. The indefinite article "a" or "an" thus usually means "at least one".

EXAMPLES

Example 1: Phospholipids Beneficially Affect Insulin Sensitivity

[0049] Nutritional compositions: A complete infant formula comprising lactose, galacto-oligosacharides with a DP 2-6 and fructo-oligosaccharides with DP 7-60 with extra added phospholipids (0.2 g/100 ml) was manufactured using a commercially available buttermilk/butter serum concentrate of Lactalis as source. An infant formula with a comparable composition, but without added phospholipids was used as control. The concentration of phospholipids was about 6.3 wt. % based on total lipid in the experimental formula and about 0.75 wt. % based on total lipid in the control formula. The experimental composition comprised about 1.4 wt. % sphingomyelin based on total lipid and about 4 wt. % cholesterol based on total lipid. The amount of sphingomyelin and cholesterol was negligible in the control formula.

[0050] Methods: 20 adult male Wistar rats (aged 10 weeks at the start of the experiment) were housed individually. After a 4 h fasting period, 10 animals were fed 2 ml of a composition. Three different compositions were tested in a cross-over design (experiments separated by one week) i) Standard infant formula, ii) Phospholipid comprising formula. Subsequently, blood samples (200 μl) were collected in heparinised chilled tubes at t=0, 5, 10, 15, 30, 60 after feeding. Subsequently, plasma was separated after centrifugation (10 min, 5000 rpm) and stored at −20° C. until analysis. Plasma insulin was measured by radioimmunoassay (RIA, of Linco Research) according to the manufacturer's instructions with the following adjustment: all assay volumes were reduced four times.

[0051] Results: The area under the curve (AUC) of insulin was lower in rats fed phospholipd containing formula than in rats fed with standard formula. (Table 1). Administration of a phospholipid comprising formula resulted in post-prandial insulin levels and kinetics more similar to those previously

observed with human milk. Decreased levels of insulin indicate increased insulin sensitivity, which is believed contribute to the prevention of obesity, especially central obesity, later-in-life.

TABLE 1

Effects of phospholipids on post-prandial area under the curve of insulin.

Effect	Standard	Phospholipids	Human milk
AUC 10 (±SE)			
Insulin (pM*10 min) AUC 15 (±SE)	9.8 ± 1.4	9.5 ± 1.0	
Insulin (pM*15 min) AUC 30 (±SE)	14.8 ± 2.1	13.8 ± 1.6	
Insulin (pM*30 min) AUC 60 (±SE)	21.4 ± 2.9	18.7 ± 2.0	11.7 ± 4.7
Insulin (pM*60 min)	25.8 ± 3.3	23.6 ± 2.2	

Example 2: Blood Glucose/Insulin and Non-digestible Oligosaccharides

[0052] Animals and treatment: Adult male Wistar rats (n=7) were given a GOS fiber load, cellulose load or water via a gastric canula on day 1. A 6 ml bolus load was administered equal to 50% of their daily fiber intake; GOS fiber used was transgalacto-oligosaccharides obtained from Elix'or (Borculo Domo). Fiber was dissolved in water. About 24 h later (on day 2) an oral glucose tolerance test was carried out and the postprandial glucose and insulin course was monitored for 120 min upon the intragastric injection of a carbohydrate load (2 g/kg body weight). To this end blood samples were drawn repeatedly via a jugular vein canula. Intragastric injection of water or a cellulose solution in water on day 1 served as control. As the GOS fiber preparation consisted of 50% of digestible carbohydrates (mainly lactose), the two control injections were co-administered with carbohydrates to correct for this.

[0053] Results: pre-treatment with GOS fibers clearly decreased the amount of insulin secreted, resulting in significant ($p<0.05$) lower incremental AUC values. Blood glucose levels were not affected significantly. Pre-treatment with cellulose or water did not modulate the insulin secretion, see Table 2.

TABLE 2

Insulin and glucose levels in rats.

Pre-treatment with:	AUC insulin (pM*30 min)	AUC glucose (mM*30 min)
Water	41 ± 7	69 ± 10
Cellulose	46 ± 8	75 ± 9
GOS	22 ± 4	74 ± 15

Example 3: Infant Nutrition

[0054] Infant nutrition comprising a lipid component providing 48% of the total calories, a protein component providing 8% of the total calories and a digestible carbohydrate component providing 44% of the total calories;

[0055] (i) the lipid component comprising based on total fatty acids: 14 wt. % LA; 2.6 wt. % ALA, 3.7 wt. % MCFA; 0.2 wt. % DHA, 0.05 wt. % EPA; 0.02 wt. % DPA, 0.35 wt. % AA, 0.03 wt. % DHGLA. The composition comprises about 5.6 wt. % phospholipids, 1.4 wt. % sphingomyelin and about 4 wt. % cholesterol based on total lipid.

[0056] (ii) the carbohydrate component comprising 50.9 gram lactose/100 gram powder, 5.22 g galacto-oligosaccharides with DP 2-6 and 0.58 g fructo-oligosaccharides with DP 7-60 per 100 g powder; (ii) the protein component comprising cow milk protein, including casein. Furthermore the composition comprises 73 mg choline and 5.6 mg UMP per 100 g powder. The composition comprises vitamins and minerals according to EU guidelines. The label of the package of this infant nutrition indicates that the nutrition prevents the development of obesity.

1. A method to prevent obesity and/or type 2 diabetes in a non-obese human infant, comprising feeding a human infant younger than 36 months of age with nutritional composition that comprises a lipid component, a protein component and a digestible carbohydrate component, and:

(a) 0.5 to 20 weight (wt.) % phospholipids based on weigh of total lipid in the composition;

(b) 0.1 to 20 wt. % sphingolipids based on weight of total lipid in the composition; and

(c) 0.005 to 10 wt. % cholesterol based on weight of total lipid in the composition.

2. The method of claim **1** wherein the composition further comprises at least 0.5 wt. % of at least one soluble, non-digestible oligosaccharide based on dry weight of the composition.

3. The method of claim **1**, wherein the lipid component comprises

(a) linoleic acid (LA) and alpha-linolenic acid (ALA) in a weight ratio of LA to ALA of between 2 and 7;

(b) less than 15 wt. % LA based on weight of total fatty acids in the composition; and

(c) at least 1 wt. % ALA based on weight of total fatty acids in the composition.

4. The method of claim **1**, wherein

(a) the lipid component provides 35 to 55% of the total calories of the composition,

(b) the protein component provides 5 to 15% of the total calories of the composition, and

(c) the digestible carbohydrate component provides 30 to 60% of the total calories of the composition.

5. The method of claim **1** wherein the feeding of said infant prevents development of obesity at an age above 36 months.

6. The method of claim **1**, wherein the feeding of an infant aged below 12 months.

7. The method of claim **1** wherein the composition comprises at least one soluble, non-digestible oligosaccharides selected from the group consisting of fructo-oligosaccharides, galacto-oligosaccharides, gluco-oligosaccharides, arabino-oligosaccharides, mannan-oligosaccharides, xylo-oligosaccharides, fuco-oligosaccharides, arabinogalacto-oligosaccharides, glucomanno-oligosaccharides, galactomanno-oligosaccharides, sialic acid comprising oligosaccharides and uronic acid oligosaccharides.

8. The method of claim **1** wherein the composition comprises galacto-oligosaccharides.

9. The method of claim **1** wherein the feeding of said infant prevents the development of a disease or disorder that is present after the age of 36 months, which disease or disorder is selected from the group consisting of type 2 diabetes, fasting hyperglycemia, insulin resistance, visceral adiposity, hyperinsulinemia, hypertension, cardiovascular disease, cerebrovascular disease, arthrosclerosis, dyslipidemia, hyperuricemia, fatty liver, osteoarthritis and sleep apnea.

10. The method of claim **1** wherein the feeding results in

(a) prevention, and/or

(b) treatment, and/or

(c) dietary management of type 2 diabetes.

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