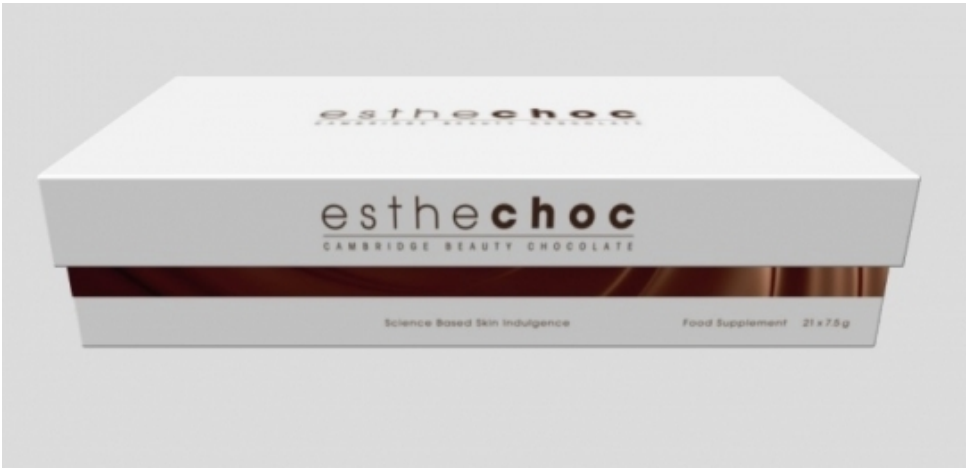
Ivan PETYAEV
Anti-Aging Chocolate

http://www.techtimes.com/articles/34476/20150222/forget-miracle-creams-anti-aging-chocolate-can-make-you-look-younger-by-30-years.htm
February 22, 2015

Forget Miracle Creams: Anti-Aging Chocolate Can Make You Look Younger... By 30 Years!

by Sumit Passary



For ages, people have been trying to find ways to suppress or hide their wrinkles, which is a natural phenomenon in humans due to old age. An anti-aging chocolate may soon come to the rescue of many who want to look 30 years younger.

Spas and retail stores in the UK are expected to get the first wrinkle removing chocolate in March. A Cambridge-based firm Lycotec has developed the anti-aging chocolate called Esthechoc, also known as Cambridge Beauty Chocolate.

The creators of the chocolate reveal that Esthechoc contains about 70 percent cocoa dark chocolate and is rich in antioxidants: astaxanthin and cocoa flavanols. The chocolate maker suggests that a small portion of the chocolate weighing just 7.5 grams can deliver the equivalent amount of flavanols to 100 grams of dark chocolate and of astaxanthin to 300 grams of wild Alaskan salmon.

Lycotec also reveals that the potency of the chocolate has also been demonstrated in large clinical trials.

"After three to four weeks of daily intake by 50 to 60-year-old volunteers, the Beauty Chocolate was able to not only suppress markers of sub-clinical inflammatory damage in their blood, but also reverse their age-related depression of microcirculation and blood supply to such peripheral tissues as subcutaneous fat and skin," stated Lycotec.

Dr. Ivan Petyaev, director of Lycotec and the inventor of the technology behind Esthechoc, revealed that clinical trials were conducted on over 3,000 participants between 50 and 60 years old. The findings of the research revealed that the biomarkers of the participant's skin were bought back to those of a 20 or 30-year-old person.

Dr. Petyaev said that it took several years to research the anti-aging product and it will soon be and it will soon be available on the market. In 2014, Lycotec entered a licensing agreement with a company aiming to

commercialize the product.

The company also claims that consuming one bar of Esthechoc each day is safe even for diabetic and calorie-conscious people. Esthechoc is said to come in packs of 21 bars, which a person should eat over three weeks. The retail price of the chocolate remains unknown.

Some market observers, however, believe that the company should conduct further studies before making strong claims about Esthechoc.

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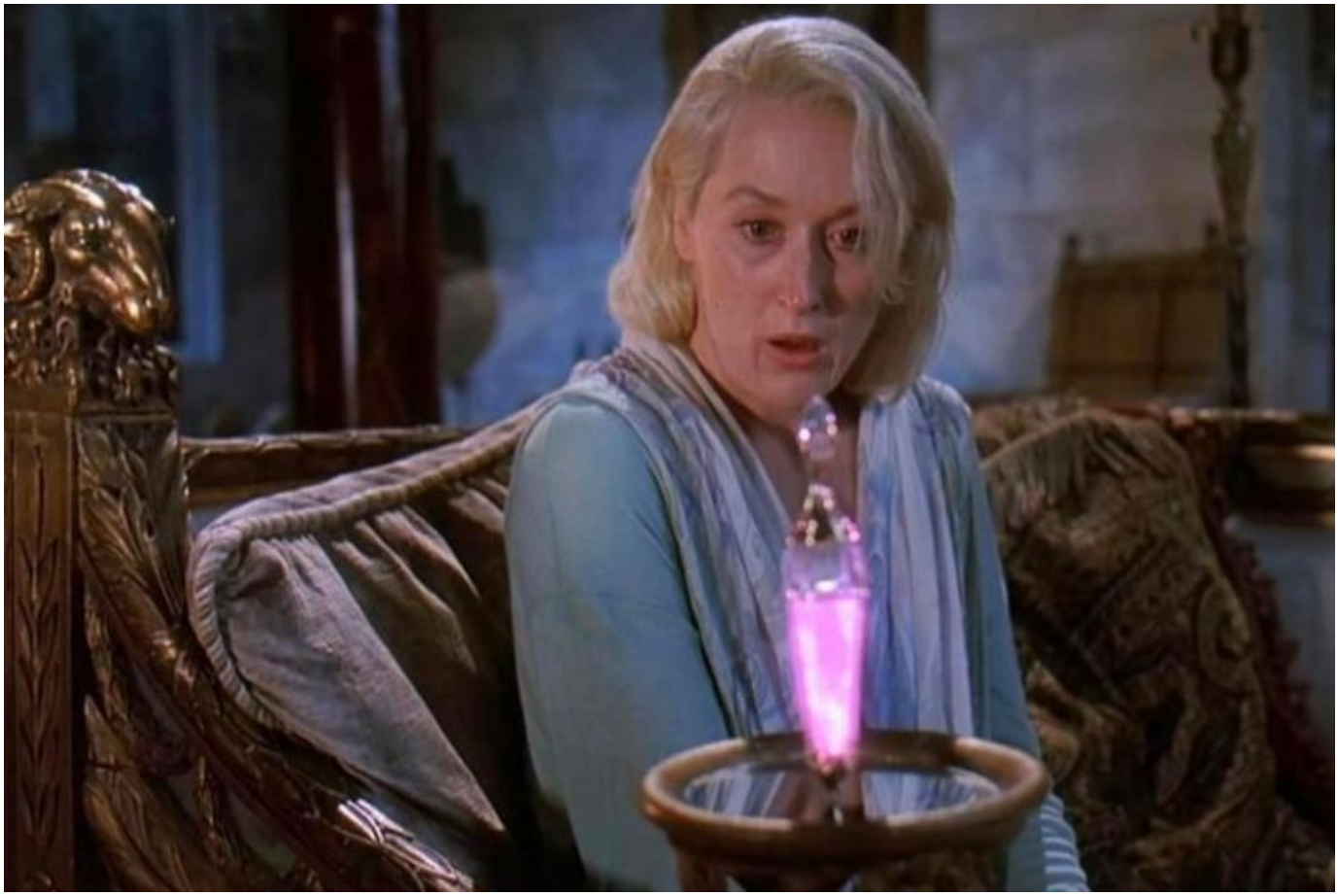
http://www.lycotec.com/Our_Team___Ivan_M_Petyaev.html

Lycotec Limited is registered in England, company number: 7397545

Dr. Ivan Mikhailovich Petyaev



Patents

COCOA-BASED FOOD PRODUCTS
US2014288187

The invention is concerned with food products comprising one or more cocoa bean products and a carotenoid compound, particularly with food products which are, or comprise, chocolate. The products of the invention may be used in reducing elevated total cholesterol, triglycerides and inflammatory damage, as well as improving tissue microcirculation and tissue oxygenation.

FIELD OF THE INVENTION

[0001] This invention relates to cocoa-based food products, such as chocolate, which have beneficial effects on parameters of metabolism in individuals including levels of triglycerides, cholesterol and other lipids, molecular oxygen transport and its metabolism, oxygen tissue saturation and microcirculation, control of hypoxia/ischaemia, as well as markers of inflammation and inflammatory oxidative damage.

BACKGROUND TO THE INVENTION

[0002] Food products based on cocoa beans, such as chocolate and other products containing cocoa solids, cocoa butter, cocoa liquor, and/or their derivatives, are widespread in the Western society. Although these cocoa-based products often contain flavonols and flavonoids, which have been associated with certain health benefits, they also contain high levels of cocoa butter or other high-fat ingredients. This high fat content means that cocoa-based food products are one of the dietary factors responsible for the growth of Metabolic Syndrome, Diabetes II and Obesity in the modern society.

[0003] Cocoa-based food products, such as chocolate, have been associated with health benefits, such as improvements in endothelial vascular function, including positive effects on blood pressure, and with antioxidant and anti-inflammatory properties [Keen et al Am J Clin. Nutr. 2005, 81(suppl): 298S-303S].

[0004] However, the effects of chocolate on blood lipid concentrations are either inconclusive or negative. Consumption of 105 g of dark chocolate has been reported to result in a mild reduction of total blood lipid concentrations of 11% [Cesar et al Clinical & Developmental Immunology, 2005; 12(1) 11-17], but this trial was done on an exceptional group of young elite sportsmen and positive blood lipid changes may be attributed to the overall improvement of their physical performance.

[0005] Meta-analysis of eight clinical trials on dark chocolate or cocoa powder showed an observed reduction in the cholesterol levels of up to 5.82 mg/dL, which was statistically insignificant [Lei et al Am J Nutr 2010; 92; 218-25]. Furthermore, the amount of the daily administered doses of cocoa phenols in these trials was equivalent of consumption of 100 g or more of the dark chocolate [Cheng et al Molecules 2009, 14: 200-209]. In other studies, when dark chocolate was consumed from 30 to 100 g daily, no changes were observed in the blood lipids [Taubert et al JAMA. 2007; 4, 298(1):49-60, Grassi et al Hypertension. 2005; 46(2):398-405].

[0006] The level of triglycerides in all the above studies above either had insignificant trends or did not change at all.

[0007] There are no reports of any health benefit, including on blood lipids, arising from consumption of the most common forms of chocolate, including milk chocolate and white chocolate.

[0008] Lycopene is known to be as a potent antioxidant. Its mild cholesterol-lowering effect has been reported from 13 clinical studies and was 7.55+6.15 mg/dL [Ried et: al Maturitas. 2011, 68(4):299-310]. However this effect was only observed for daily doses 25 mg of lycopene or above. There were no reports on the effect of lycopene on elevated triglycerides in human.

[0009] Although 25 mg of lycopene and above is considered to be safe for certain periods of administration, it is far above the daily level which could be consumed with a diet rich with tomato or tomato processed products (about 6-10 mg). Daily consumption of 6-10 mg lycopene has been reported to have no effect on cholesterol or other blood lipids [Bose et al Singapore Med J 2007; 48 (5); 415-420; Upritchard et al Diabetes Care, 2000, 236: 733-735]

SUMMARY OF THE INVENTION

[0010] The present invention is based on the unexpected finding that the combination of a carotenoid and a cocoa bean product can be used to reduced elevated cholesterol, reduce elevated triglyceride levels, reduce inflammatory oxidative damage and improve tissue microcirculation and oxygen transport. Given the high fat and sugar content of chocolate, such a finding was highly unexpected and indeed counterintuitive.

[0011] Accordingly, the invention provides a food product comprising one or more cocoa bean products and a carotenoid compound.

[0012] The invention also provides such a food and/or beverage and/or nutraceutical product for use in:

(a) reducing levels of elevated cholesterol, LDL and/or triglyceride in an individual, preferably where the individual has elevated levels of cholesterol, LDL and/or triglyceride;

(b) reducing subclinical or clinical inflammation; reducing anti-inflammatory oxidative damage; increasing plasma molecular oxygen transport, microcirculation and tissue oxygen saturation, reducing already developed liver (micro-) damage and liver steatosis, liver and other organs, including peripheral, tissue hypoxia or ischaemia, increasing antioxidant activity and/or reducing or delaying symptoms of ageing in an individual;

(c) reducing postprandial cholesterol- and triglyceride-aemias, reducing size of chylomicrons and increasing rate of their clearance, reducing postprandial inflammatory and oxidative stress, reducing postprandial or other liver (micro-) damage and liver steatosis, liver and other organs, including peripheral tissue hypoxia or ischaemia, or delaying of above mentioned symptoms of fat, or excessive, or imbalance food intake in an individual;

(d) increasing oxygen transport in a subject, preferably where the subject has a respiratory disorder and/or lung damage; and/or strenuous physical or mental performance; and/or muscle wasting conditions; and/or

(e) slimming, weight reduction or dieting.

[0018] The invention additionally provides a method of:

(a) improving the appearance and performance of an individual comprising administering a nutracosmetic formulation or food or beverage product of the invention to the individual; and/or

(b) reducing or delaying signs of aging in an individual, preferably visible signs of aging, comprising administering a food product of the invention to the individual.

[0021] The invention also provides a method of:

[0022] The invention also provides a method of:

(a) reducing levels of elevated cholesterol, LDL and/or triglyceride in the blood of an individual comprising administering a food product of the invention to an individual in need thereof;

(b) reducing subclinical or clinical inflammation; reducing anti-inflammatory oxidative damage; increasing plasma molecular oxygen transport, microcirculation and tissue oxygen saturation, reducing already developed liver (micro-) damage and liver steatosis, liver and other organs, including peripheral, tissue hypoxia or ischaemia; increasing antioxidant activity and/or reducing or delaying symptoms of ageing in an individual; comprising administering a food product of the invention to an individual in need thereof;

(c) reducing postprandial cholesterol- and triglyceride-aemias, reducing size of chylomicrons and increasing rate of their clearance, reducing postprandial inflammatory and oxidative stress, reducing postprandial or other liver (micro-) damage and liver steatosis, liver and other organs, including peripheral tissue hypoxia or ischaemia, or delaying of above mentioned symptoms of fat, or excessive, or imbalance food intake in an individual; comprising administering a food product of the invention to an individual in need thereof;

(d) providing nutrition to an individual comprising administering a food product of the invention to an individual in need thereof; and/or strenuous physical or mental performance; and/or muscle wasting conditions; and/or

(e) slimming, weight reduction or dieting comprising administering a food product of the invention.

[0028] The invention also provides a chocolate bar and/or chocolate beverage comprising a carotenoid, preferably where the carotenoid is a lycopene compound.

DETAILED DESCRIPTION THE INVENTION

[0029] This invention relates to the unexpected finding that incorporating carotenoid compounds into food products which contain cocoa-bean based products, such as cocoa solids, cocoa butter, cocoa liquor, and/or their derivatives, causes these food products to exert a positive effect on levels of triglycerides, cholesterol, LDL, and other metabolic parameters in individuals, despite being rich in saturated and unsaturated fats. Given the high fat content of food products such as chocolate, the finding that the combination of a carotenoid and chocolate is able to reduce those parameters was unexpected and counter-intuitive.

[0030] An aspect of the invention provides a food product which comprises one or more cocoa-bean products and a carotenoid compound. Typically, the carotenoid compound is an isolated carotenoid compound.

[0031] The food product may comprise a homogenous matrix which contains the cocoa-bean products and the carotenoid compound. For example, the cocoa-bean products and carotenoid compound may be blended together in a chocolate or cocoa-butter matrix.

[0032] A cocoa bean product is an extract, fraction or isolate from cocoa beans (i.e. beans of the cacao tree (Theobroma cacao)). Suitable cocoa bean products are well-known in the art and include cocoa solid, cocoa liquor and/or cocoa butter. For example, a food product may comprise one or more of cocoa solid, cocoa liquor and/or cocoa butter.

[0033] In some instances cocoa nibs or fragments thereof, chocolate liquor, partially and fully-defatted cocoa solids (e.g. cocoa powder), cocoa extract or a fraction thereof may be employed.

[0034] Cocoa solid (also known as cocoa powder) is a low-fat extract of cocoa beans, which contains flavanols, flavanoids, caffeine and theobromine. Cocoa solid may be produced by removing the fat component (cocoa butter) from the cocoa bean and grinding the remaining material, excluding the shell, to a powder using techniques which are well-known in the art, such as Broma processing. In some embodiments, cocoa powder may be treated with an alkaline substance such as potassium carbonate to reduce acidity and darken the colour (Dutch processing).

[0035] Cocoa butter is a high-fat extract of cocoa beans which is high in stearic acid, palmitic acid and other saturated fats. Cocoa butter may be produced from whole or ground cocoa beans using techniques which are well-known in the art.

[0036] Cocoa liquor is a cocoa bean extract which contains both cocoa solid and cocoa butter. Cocoa liquor may be produced by grinding and melting the cocoa bean nib (centre) to a smooth liquid state in accordance with techniques which are well-known in the art. Chocolate liquor does not contain non-cocoa vegetable fat and may also be referred to as “chocolate”, “unsweetened chocolate”, “baking chocolate”, or “bitter chocolate”.

[0037] In other embodiments, cocoa bean products may include derivatives or fermentation products of cocoa bean extracts, isolates or fractions.

[0038] Preferably, the food product comprises cocoa butter; cocoa solid; or both cocoa butter and cocoa solid.

[0039] For example the food product may contain at least 1% by weight, at least 10% by weight, at least 15% by weight, at least 20% by weight, at least 25% by weight or at least 30%, or at least 40% by weight cocoa butter. The food product may contain an amount of cocoa butter in a range comprising any of the above two values as endpoints.

[0040] In some embodiments, a food product may further comprise non-cocoa fats, such as vegetable or animal fats in addition to cocoa butter.

[0041] In some embodiments, a food product may be devoid of cocoa butter. For example, a food product may contain animal or non-cocoa vegetable fat instead of cocoa butter. Non-cocoa vegetable fats may include vegetable oils. Suitable vegetable oils, such as palm oil, soybean oil rapeseed oil and olive oil, are well known in the art.

[0042] The total fat content of a food product described herein may be at least 10% by dry weight, at least 15% by dry weight, at least 20% by dry weight, at least 25% by dry weight, at least 30% by dry weight or at least 35% by dry weight or at least 40% by dry weight. The fat content may be, for instance, in a range comprising any two such values as endpoints.

[0043] Additionally or alternatively, the food product may contain at least 5% by weight, at least 15% by weight, at least 20% by weight, at least 25% by weight, at least 30% by dry weight or at least 35% by weight, or at least 40% by weight dry cocoa solid. In some instances, the amount of cocoa solid may be at least 50% by weight, at least 60% by weight, at least 75% by weight, at least 80% by weight, at least 85% by weight, at least 90% by weight or even at least 95% by weight dry cocoa solid, particularly when the food stuff is a dark chocolate. The amount of weight of dry cocoa solid may be, for instance, in the range comprising any two of those values as endpoints.

[0044] In some embodiments, a food product may be devoid of cocoa solid.

[0045] For the avoidance of doubt, aspects of the invention provide food products which comprise all combinations of the above parameters of cocoa solid, cocoa butter and total fat.

[0046] In some embodiments, the cocoa bean products may form a chocolate matrix. The carotenoid compound may be incorporated into the chocolate matrix by blending or admixing.

[0047] Any cocoa-based food product may be supplemented with a carotenoid compound as described herein. For example, the food product may be a foodstuff, a beverage or a dietary supplement or nutraceutical product.

[0048] Foodstuff products include bread, flour, cereal, biscuit, pastry, dairy products, such as cheese spread, cheese, cream and yoghurt, fillings, pastes, sauces and mousses. Other suitable foodstuffs are well known in the art.

[0049] In some preferred embodiments, foodstuff products may include confectionery products, such as chocolate. Especially preferred embodiments of the invention provide chocolate comprising a carotenoid compound, as described herein.

[0050] Chocolate may include dark chocolate, milk chocolate, or white chocolate.

[0051] In one preferred instance, the foodstuff of the invention may be a chocolate bar, for instance a dark, plain or milk chocolate bar comprising a carotenoid, such as any of those discussed herein. The amount of carotenoid in the bar may be, for instance, any of the amounts of carotenoid specified herein.

[0052] Dark chocolate, milk chocolate and white chocolate are subject to defined identity standards (for example, by the Food and Drug Administration (USA), EU and Food Standards Agency (UK); see for example EU directive 2000/36/EC; FDA 21 CFR Part 163 Federal Register: 2002 67 193 62171-62178). In one instance, a composition of the invention may be a standard of identity (SOI) chocolate, in others it is a non-SOI chocolate.

[0053] The ingredients of dark chocolate, milk chocolate, white chocolate or other forms of chocolate are well-known in the art. For example, dark chocolate typically comprises sugar, cocoa butter (e.g. at least 12% by weight), cocoa solids (e.g. at least 35% by weight), and optionally vanilla. Fat content may vary but averages between 30%-35%. Dark chocolate is sometimes referred to as sweet or semisweet chocolate. Milk chocolate may comprise sugar, cocoa butter, cocoa solids, vanilla or other flavourings, and milk, milk powder or cream. Milk chocolate typically contains at least 20% cocoa solid and at least 12% milk solids by weight. White chocolate may comprise sugar, cocoa butter, milk or milk powder, and vanilla and lacks cocoa solids. White chocolate typically contains at least 20% cocoa butter, 14% total milk solids, and less than 55% sugar.

[0054] In one instance, the food product of the invention may be about 100 g, 150 g, 200 g, 250 g, 300 g, 400 g or 500 g in weight or may have a weight in a range with any two of those values as endpoints. In a preferred instance, the foodstuff may be a chocolate bar of such weight.

[0055] The foodstuff may be a candy bar, for instance a chocolate coated candy bar. The foodstuff may take the form of individual chocolates, bagged chocolates or a box of chocolates. The chocolate may be in a formed shape. In one instance the foodstuff is an Easter egg. The invention may be provided in the form of chocolate icing or a cake comprising a carotenoid and chocolate. The invention also provides fruit or nuts coated with a chocolate of the invention. The invention also provides sweets or candy coated with a chocolate of the invention.

[0056] The invention also provides a chocolate of the invention provided in the form of a single serving dose, for instance in 10 to 30 g amounts, as well as a packet of such single serving doses. The invention also provides a chocolate bar of the invention segmented, for instance segmented so that it can be broken into single serving dosages.

[0057] The foodstuff of the invention may be, in other instances, a cake, cheesecake, baked snack, brownie,

cookie or biscuit, a meal replacement bar, a rice cake, ice cream or other pudding or dessert. In some instances, the invention provides such products coated in, or comprising, a chocolate of the invention. The products may for instance comprise the chocolate in the form of chips or in a central region.

[0058] Beverages may include any drink which comprises cocoa-bean products and may include cocoa, drinking chocolate, milk shakes, and other dairy and non-dairy drinks. Beverages may be non-alcoholic or alcoholic. The formulation of suitable beverages is well-known in the art. In one preferred instance, the beverage is a chocolate milkshake. In another instance, a powder, gel or cube for making up as a beverage is also provided. The invention also provides a hot chocolate, chocolate or cocoa drink, as well as a chocolate/cocoa shot drink comprising a product of the invention.

[0059] Dietary supplements or nutraceutical products may be in any form suitable for oral administration (e.g., by ingestion) and may be presented as discrete units such as capsules, cachets or tablets; as a powder or granules; as a solution or suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion; as a bolus; as an electuary; or as a paste.

[0060] The invention also provides a food-stuff intended for dieters which is, or comprises, a foodstuff of the invention. The invention provides for the use of the products described herein for slimming, dieting or weight reduction. The invention also provides for products for diabetics comprising, or consisting of, a foodstuff of the invention. In one instance, the invention provides a diabetic chocolate, where the chocolate is a chocolate of the invention.

[0061] In one preferred instance, a foodstuff of the invention may be provided with packaging and/or wrapping. Such packaging/wrapping may indicate the benefits of the invention and/or suggest consumption at, or near, mealtimes for maximal benefit. The packaging/wrapping may indicate the benefits of the product in slimming, decreasing cholesterol, and/or triglyceride levels. In another instance, the packaging may refer to the ability of the product to improve oxygen transport. The packaging may refer to treating or ameliorating any of the conditions mentioned herein. The packaging may be a sachet, for instance where the product is to be made up as a beverage.

[0062] The invention also provides food products targeted at sports people. For instance, the products may be used to reduce weight in such subjects or bring about any of the other benefits highlighted herein for the products of the invention. The products may be packaged or wrapped and include an indication of their ability to increase oxygen transport. The products of the invention may be used to reduce recovery time. The products may be used by climbers, particularly those climbing at altitude.

[0063] In addition to cocoa-bean products, food products described herein comprise a carotenoid compound.

[0064] The carotenoid compound may be isolated in the products of the invention. An isolated carotenoid compound is outside the physical milieu or environment in which it occurs in nature. For example, an isolated carotenoid compound may be free or substantially free from its natural environment e.g. it is not contained in the natural plant material with which it is naturally associated. Isolated carotenoid compounds include compounds which have been isolated, concentrated, purified or partially purified from natural sources, such as plants, and compounds which have been produced synthetically.

[0065] The food product will typically provide an effective amount of carotenoid, such as lycopene. The food product may comprise 0.0001% to 1%; 0.001% to 1%; or 0.01% to 0.1% by weight of carotenoid compound. For example, the food product may comprise 0.001 to 10 mg of carotenoid compound per one gram of food product, for example, 0.01 to 10 mg per gram or 0.1 to 1 mg per one gram of food product. The product of the invention will typically provide an effective amount of the carotenoid, for instance an amount effective to alter one of the parameters referred to herein.

[0066] The food product may be in a unit dose form which allows a controlled daily dose of carotenoid, preferably lycopene, to be consumed. For example, the food product may be formulated to provide a daily dose of 0.1 mg to 100 mg of lycopene, preferably 0.5 to 50 mg of lycopene. In some instances, a product of the invention may provide about 0.1 mg, 0.2 mg, 0.5 mg, 1 mg, 1.5 mg, 2 mg, 2.5 mg or more of carotenoid, such as about 3, 4, 5, 6, 7, 8, 9, or 10 mg of carotenoid. In some instances, the amount of carotenoid may be about 10, 15, 20 or 25 mg, or up to those levels. Preferably the carotenoid is lycopene. The product may comprise an amount of carotenoid which is in a range with any two of the values mentioned herein as endpoints.

[0067] In one instance, the foodstuff or product provides from 0.1 to 1.0 mg of carotenoid per gram of food product for example at least 0.2, 0.3, 0.4 or 0.5 mg carotenoid per gram of food product, with in some instances, up to 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1.0 mg of carotenoid per gram of product. In one preferred instance, those

values are employed where the product is chocolate and/or carotenoid is lycopene, preferably both. In another instance, the level of foodstuff administered is enough to reduce any of the makers discussed herein, preferably to near, or at, or below baseline levels for a healthy control or below baseline prior to administration of the product.

[0068] In some instances, the ratio of carotenoid to triglyceride or other fat molecules in the products of the invention may be from 1:1000 to 1:100,000, for instance from 1:2000 to 1:50,000, or from 1:5000 to 1:25,000.

[0069] The carotenoid and cocoa bean product may be present in a synergistic amount. For instance, they may be present where the combination produces a greater effect on any of the parameters mentioned herein than either individually when provided in the same amount. The invention therefore also provides a synergistic combination of a carotenoid and cocoa bean product. The invention also provides for the use of a carotenoid, such as any of those referred to herein, to treat any of the conditions mentioned herein, where the subject is also being administered chocolate and also the use of chocolate to treat any of the conditions mentioned herein where the subject is also being administered a carotenoid. Typically the carotenoid and chocolate will be administered together, for instance eaten together, or within 5, 10, 15, 30, 45 or 60 minutes of each other. The two may be given simultaneously.

[0070] Carotenoid compounds are tetraterpenoids which contain long polyene chains. Carotenoid compounds include xanthophylls such as lutein, capsanthin and zeaxanthin, and carotenes, such as beta-carotene, alpha-carotene, zeto-carotene, and lycopene compounds.

[0071] Lycopene compounds may include lycopene, 1-HO-3',4'-didehydrolycopene, 3,1'-(HO)2-gamma-carotene, 1,1'-(HO)2-3,4,3',4'-tetradehydrolycopene, 1,1'-(HO)2-3, 4-didehydrolycopene.

[0072] In preferred embodiments, the carotenoid compound is lycopene.

[0073] Lycopene is an open-chain unsaturated C40 carotenoid of structure I (Chemical Abstracts Service Registry Number 502-65-8),

[0000]

[0074] Lycopene occurs naturally in plants such as tomatoes, guava, rosehip, watermelon and pink grapefruit and any such sources of lycopene may be, for instance, employed.

[0075] Lycopene for use as described herein may comprise one or more different isomers. For example, lycopene may include cis-lycopene isomers, trans-lycopene isomers and mixtures of the cis- and trans-isomers. Lycopene may comprise at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% (Z)-isomers, (all-E)-isomers, or cis-isomers, such as 5-cis- or 9-cis- or 13-cis-isomers, which have improved bioavailability relative to trans isomers. Trans isomers may isomerise into cis forms in vivo, or during storage and processing.

[0076] Carotenoid compounds, such as lycopene, for use as described herein may be natural i.e. obtained from a natural source, for example, extracted from a plant, such as a tomato or melon. In one instance, oleoresin, particularly tomato oleoresin, may be employed in the invention. A range of methods for extracting, concentrating and/or purifying carotenoids from plants are known in the art. For example, solvent extraction using ethanol, DMSO, ethyl acetate, hexane, acetone, soya or other vegetable oil, or non-vegetable oils may be employed.

[0077] Carotenoid compounds, such as lycopene, for use as described herein may be synthetic i.e. produced by artificial means, for example, by chemical synthesis. A range of methods for chemical synthesis of lycopene and other carotenoids are known in the art.

[0078] For example, a three-stage chemical synthesis based on the standard Wittig olefination reaction scheme for carotenoid synthesis may be employed, in which an organic solution of C15 phosphonium methanesulfonate in dichloromethane (DCM) and an organic solution of C10 dialdehyde in toluene are produced, and the two organic solutions are gradually combined with sodium methoxide solution and undergo a condensation reaction to form crude lycopene. The crude lycopene may then be purified using routine techniques, for example by adding glacial acetic acid and deionized water to the mixture, stirring vigorously, allowing the aqueous and organic phases to separate, and extracting the organic phase containing DCM and crude lycopene with water. Methanol is added to the organic phase and the DCM removed via distillation under reduced pressure. The crude methanolic lycopene solution is then be heated and cooled to crystalline slurry that is filtered and washed with methanol. The lycopene crystals may then be recrystallized and dried under heated nitrogen. Synthetic

carotenoids, such as lycopene, are also available from commercial suppliers (e.g. BASF Corp, NJ USA).

[0079] Synthetic carotenoid compounds, such as lycopene, may comprise an increased proportion of cis isomers relative to natural carotenoid compounds. For example, synthetic lycopene may be up to 25% 5-cis, 1% 9-cis, 1% 13-cis, and 3% other cis isomers, whilst lycopene produced by tomatoes may be 3-5% 5-cis, 0-1% 9-cis, 1% 13-cis, and <1% other cis isomers. Since cis-lycopene has increased bioavailability relative to trans-lycopene, synthetic lycopene is preferred in some embodiments.

[0080] Derivatives of carotenoids as described above may be produced by chemical synthesis analogous to the synthesis described above or by chemical modification of natural carotenoids extracted from plant material.

[0081] A food product as described herein may contain a single carotenoid compound. (e.g. lycopene) or more than one carotenoid compound (e.g. lycopene and beta-carotene). Typically, each carotenoid compound will be present in a range of different isomeric forms.

[0082] The food product may be produced by admixing or blending the cocoa-bean products, such as cocoa butter and cocoa solids, and optionally one or more other ingredients, and the carotenoid compound under conditions which allow the carotenoid compound to incorporate into the matrix of the food product.

[0083] Other ingredients may include sugar, vanilla, milk, milk powder, emulsifying agents, such as soy lecithin or polyglycerol polyricinoleate (PGPR; E476), whey or potato peptides and/or proteins, soy products, such as soy proteins, soy extracts and/or soy isoflavones, vegetable oils or animal fats, nut-based products, such as nut powders and nut extract, starch and polysaccharides.

[0084] The cocoa-bean products may be in a dry, liquid, aerosol, frozen or melted form for admixing or blending with the carotenoid compound. For example, chocolate for blending may be in liquid form (i.e. melted chocolate).

[0085] In some preferred embodiments, the cocoa-bean products and the carotenoid compound in mixable forms and have the same or similar viscosities.

[0086] Suitable methods of mixing and blending, including mechanical blending, are well-known in the art.

[0087] In one instance, a carotenoid is added whilst the chocolate is being made or chocolate is melted and the carotenoid added. The chocolate may be added to a mould to give products of a particular shape and/or size.

[0088] The invention also provides for a method of producing a food product, such as a food product of the invention, which comprises adding a carotenoid during production of the food product. For instance, the carotenoid may be added during the preparation of chocolate.

[0089] Products of the invention may also contain other ingredients such as flavourings, emulsifiers, colourings and/or preservatives. In some cases the products may comprise nuts, particularly where the product is a chocolate, such as walnuts, hazelnuts, almonds or brazil nuts.

[0090] Food products as described herein are shown to have an unexpected effect on levels of blood cholesterol, low density lipoprotein, triglycerides and/or other lipids or lipid particles, such as LDL particles, in an individual. Given chocolate is perceived as a high fat food, that was unexpected.

[0091] Aspects of the invention provide a food product as described above for use in reducing blood levels of cholesterol, low density lipoprotein, triglycerides and/or other lipids or lipid particles, such as LDL particles, in an individual and a method of reducing blood levels of cholesterol, low density lipoprotein, triglycerides and/or other lipids or lipid particles, such as LDL particles, in an individual comprising administering a food product described above to the individual.

[0092] Another aspect of the invention provides the use of a carotenoid compound and one or more cocoa bean products, as described above, in the manufacture of a food product for use in reducing blood levels of cholesterol, low density lipoprotein, triglycerides and/or other lipids or lipid particles, such as LDL particles, in an individual.

[0093] This may be useful in the treatment or prevention of cardio- and cerebro-vascular disorders, or Metabolic Syndrome, high blood pressure, pre-diabetes and type II diabetes, being overweight (e.g. BMI>25), obesity (e.g. BMI>30) and hypercholesterolaemia. The invention may be employed, for instance, with any of those subjects. The products of the invention may be used in dieting.

[0094] The invention also provides a method of dieting comprising consuming a product of the invention as part of the diet.

[0095] An individual is preferably a human, though use in animals is also possible. The individual may have normal blood levels of cholesterol, LDL and/or triglycerides or elevated blood levels of cholesterol, LDL and/or triglycerides. In some instances, the subject may have a total serum cholesterol of more than 200 mg/dL, for instance more than 210 mg/dL. In some cases a subject may additionally, or alternatively have, triglyceride levels above 150 mg/dL. In some cases, the subject may be apparently healthy, but be identified as having such elevated levels of cholesterol and/or triglycerides, in other instances the subject may have a history of heart disease and/or atherosclerosis. The subject may be overweight and may be obese. The subject may be one taking statins, aspirin and/or blood pressure reducing medication. The subject may be one on a diet.

[0096] Methods of measuring levels of cholesterol, LDL, triglycerides and other lipids in an individual are well-known in the art.

[0097] In some embodiments, the individual may be at suffering from, or at risk of suffering from, a cardio- or cerebro-vascular disorders, such as coronary heart disease, metabolic syndrome, high blood pressure, pre-diabetes and type II diabetes, being overweight (e.g. BMI>25) or obesity (e.g. BMI>30). The subject may have had a heart attack. The subject may have had a stoke.

[0098] Food products as described herein are also shown to reduce levels of markers of inflammatory oxidative damage in an individual. In some cases the subject may have elevated levels of inflammatory oxidative damage. For instance, they may have ?20-39 μM MDA and/or at least 0.25 to 0.30 u/ml of Px-IgG Such levels may be in addition to, or alternative to, the above specified levels of total cholesterol and/or triglycerides.

[0099] The food products may therefore also be useful in reducing inflammation; reducing anti-inflammatory oxidative damage; increasing antioxidant activity and/or reducing or delaying symptoms of aging in an individual. The invention may be used to reduce the visible signs of aging.

[0100] Examples of possible daily doses of 0.1 mg to 100 mg of carotenoid compound, such as lycopene, preferably 0.5 to 50 mg, may be administered to the individual. Any of the amounts referred to herein may be administered.

[0101] In some embodiments, a suitable individual may be a mature or elderly individual, for example at least 50, 60, 65, 70, 75 or more years old or be of an age in the range defined by any of those two values.

[0102] Food products as described herein may also be useful in providing nutrition to an individual.

[0103] For example, food products may be useful as sports nutrition products or in providing nutrition to mature or elderly individuals (e.g. >50 years old) or individuals undergoing body mass control or reduction, i.e. for “slimming” purposes.

[0104] In other examples, food products may be useful in providing nutrition to individual having or recovering from a clinical condition. For example, food products described herein may be useful in the nutrition of an individual recovering from injury, operation, or trauma; an individual having or recovering from chemo- or radio-therapy; or an individual having or at risk of Metabolic Syndrome, obesity, diabetes II, atherosclerosis and their clinical complications.

[0105] The invention may be used to help treat ischemia or hypoxia. The invention may be, in some instances, administered after blood flow has been cut off to a particular tissue or organ. In one instance, the invention may be administered to subjects who have had a stroke.

[0106] Food products as described herein may also be useful in the treatment or prevention of cardio- and cerebro-vascular disorders, hypertension, metabolic syndrome, high blood pressure, pre-diabetes and type II diabetes, being overweight (e.g. BMI>25), obesity (e.g. BMI>30) or other medical conditions such as anaemia, rheumatism, rheumatoid arthritis, non-rheumatoid arthritis, prostate or testes malfunctions, erectile dysfunctions, loss of libido, cellulite, sarcopenia and cachexia.

[0107] In some instances, the subject the invention is applied to may have an auto-immune disease; an allergic condition; hypertension; atherosclerosis; cardio pathologies, such as Coronary Heart Disease; vascular pathologies, such as endocarditis, myocarditis, heart failure, heart valve disease, arrhythmias, atherosclerosis, hypertension, vasculitis, endarteritis, varicose veins, endophlebitis, endothelial damage; cerebral pathologies;

obesity; diabetes type 2; cancer, sarcopenia; metabolic dysfunction; Metabolic Syndrome; cellulite and aging tissue degradation; gastritis; stomach or duodenum ulcers; or arthritis; or dermatitis, psoriasis, acne, chronic skin ulcerations, or other age-related or not skin conditions, including skin and other tissues burns and wounds; sport, trauma, operation and other injuries; cachexia, side-effects of chemotherapies and radiation treatment, or radiation exposure; the subject may be at risk of such a condition.

[0108] Due to the ability of the invention to increase oxygen transport, the invention may also be used to treat conditions where such increased oxygen transport may be beneficial. For instance, a subject with a respiratory disorder such as emphysema, COPD, cystic fibrosis, asthma, or ARDS. The subject may have reduced lung function, for instance due to lung damage or lung cancer. In one instance, the subject may be a smoker.

[0109] The invention may also be used to treat impairment of tissue oxygenation, for instance due to reduction of blood supply due to circulatory dysfunction or circulatory disease. The subject may have had an injury, disease or disorder causing reduced blood flow, for instance one that results from blood flow to an organ and/or tissue being reduced or cut-off.

[0110] The invention may be used to increase tissue oxygenation and treat circulatory disease. In one instance the circulatory disorder may be due to traumatic, compressive, occlusive, tumors/malformations and/or vasospastic reduction in oxygenation. The subject may have atherosclerosis resulting in reduced tissue oxygenation or DVT. The subject may be one with angina, such as angina pectoris, acute coronary syndrome, or had a myocardial infraction. The invention may also be used to treat individuals with reduced tissue inflammation due to ongoing inflammatory conditions or processes in the tissue, such as any of those referred to herein.

[0111] Given the ability of the invention to reduce inflammatory markers, the invention may also be employed to help treat inflammatory or autoimmune disorders, for instance arthritis, inflammatory bowel disease and atherosclerosis.

[0112] Another aspect of the invention provides a nutracosmetic formulation comprising one or more cocoa bean products and a carotenoid compound.

[0113] Suitable cocoa bean products and carotenoid compounds are described in more detail above.

[0114] A nutracosmetic formulation which comprises one or more cocoa bean products and a carotenoid compound as defined above, may further comprise one or more cosmetically or nutritionally acceptable carriers, adjuvants, excipients, sweeteners, diluents, fillers, buffers, stabilisers, preservatives, colourings, lubricants, or other materials well known to those skilled in the art.

[0115] The term “nutraceutically acceptable” as used herein pertains to compounds, materials, compositions, and/or dosage forms which are in common or widespread usage in food and dietary products and are generally considered non-toxic, for example, compounds may have the US FDA designation “GRAS” (Generally Recognised as Safe), or equivalent food additive status in other jurisdictions.

[0116] Nutracosmetic formulations are generally intended for oral administration and may be formulated accordingly.

[0117] Nutracosmetic formulations may be useful in improving the appearance of an individual or in reducing, delaying or masking Visual signs of aging in an individual.

[0118] The invention may be administered to treat, ameliorate, prevent, or reduce the severity of symptoms in any of the conditions referred to herein. In one instance, the invention is administered prophylatically to help prevent the onset of any of the conditions mentioned herein. The invention may result in reduction of any of the parameters discussed herein, it may, for instance, reduce cholesterol, triglyceride, inflammatory damage, weight or body fat.

[0119] Various further aspects and embodiments of the present invention will be apparent to those skilled in the art in view of the present disclosure.

[0120] All documents mentioned in this specification are incorporated herein by reference in their entirety.

[0121] The term “and/or” where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example “A and/or B” is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein.

[0122] In instances herein where the terms “comprises” or “comprising” are used, the invention may also provide what is described when it “consists essentially of” or “consisting of” the specified constituents.

[0123] Unless context dictates otherwise, the descriptions and definitions of the features set out above are not limited to any particular aspect or embodiment of the invention and apply equally to all aspects and embodiments which are described.

[0124] The following is a list of some further numbered embodiments of the invention:

[0000] (1) A food product comprising one or more cocoa bean products and an isolated carotenoid compound.

(2) A food product according to (1) which comprises a homogenous matrix containing the cocoa-bean products and the carotenoid compound.

(3) A food product according to (1) or (2) wherein the cocoa bean products comprise one or more of cocoa solids, cocoa powder, cocoa liquor and/or cocoa butter.

(4) A food product according to any of (1) to (3) where the one or more cocoa bean products are in the form of a chocolate or cocoa butter matrix, said matrix incorporating the carotenoid compound.

(5) A food product according to any one (1) to (4) which comprises 0.001 to 10 mg of carotenoid compound per gram of food product.

(6) A food product according to any one of (1) to (5) the wherein the carotenoid compound is a lycopene compound.

(7) A food product according to any one of (1) to (6) wherein the carotenoid compound is comprised in a carotenoid-rich product, such as tomato or other fruit, vegetable or plant paste, sauce, concentrate, oleoresin, fraction or extract.

(8) A food product according to any one of (1) to (6) wherein the carotenoid compound is comprised in a carotenoid rich fruit, vegetable or other plant, or fungus, algae or bacterium.

(9) A food product according to any one of (1) to (8) wherein the lycopene compound is lycopene.

(10) A food product according to any one of (1) to (8) wherein the food product is produced by admixing or blending together the cocoa bean products, the carotenoid compound and optionally one or more additional ingredients.

(11) A food product according to (10) wherein the cocoa bean products are admixed or blended together with the carotenoid compound in a dry, liquid, aerosol, frozen or melted form.

(12) A food product according to any one of (1) to (11) wherein the food product is a foodstuff.

(13) A food product according to (12) wherein the foodstuff is bread, flour, cereal, biscuit, pastry, spread, filling, paste, sauce, mousse, cream, or yogurt.

(14) A food product according to (12) wherein the foodstuff is a confectionery product.

(15) A food product according to (14) wherein the foodstuff is chocolate.

(16) A food product according to (15) wherein the chocolate is dark, milk, or white chocolate.

(17) A food product according to any one of (1) to (11) wherein the food product is a beverage.

(18) A food product according to any one of (1) to (11) wherein the food product is a dietary supplement, nutracosmetic or nutraceutical product.

(19) A food product according to any one of (1) to (18) for use in reducing levels of cholesterol, LDL and/or triglyceride in an individual.

(20) A food product according to any one of (1) to (19) for use in reducing inflammation; reducing anti-inflammatory oxidative damage; increasing antioxidant activity and/or reducing or delaying symptoms of aging

in an individual.

(21) A food product according to any one of (1) to (20) for use in the nutrition of an individual.

(22) A food product for use according to (21) wherein the individual is mature or elderly.

(23) A food product: for use according to any one of (19) to (22) wherein the individual is undergoing body mass control or body mass reduction.

(24) A food product for use according to any one of (19) to (22) wherein the individual is suffering from; at risk of suffering from; or recovering from a clinical condition.

(25) A food product for use according to (24) wherein the individual is recovering from injury, operation, or trauma or undergoing or recovering from chemo- or radio-therapy; or having or being at risk of having Metabolic Syndrome, obesity, diabetes II, atherosclerosis and clinical complications thereof.

(26) A food product for use according to any one of (1) to (20) for the treatment of a clinical condition.

(27) A food product for use according to (26) wherein the clinical condition is a cerebro-vascular disorder, cardio-vascular disorder, hypertension, metabolic syndrome, high blood pressure, pre-diabetes, type II diabetes, being overweight (e.g. BMI>25), obesity (e.g. BMI>30), anaemia, rheumatism, rheumatoid arthritis, non-rheumatoid arthritis, prostate or testes malfunction, erectile dysfunction, loss of libido, cellulite, sarcopenia and cachexia.

(28) A method of improving the appearance of an individual comprising administering a nutracosmetic formulation according to any one of (1) to (18) to the individual.

(29) A method of reducing or delaying visible signs of aging in an individual comprising administering a nutracosmetic formulation according to any one of (1) to (18) to the individual.

(30) A method of reducing levels of cholesterol, LDL and/or triglyceride in the blood of an individual comprising administering a food product according to any one of (1) to (18) to an individual in need thereof.

(31) A method of reducing inflammation; reducing anti-inflammatory oxidative damage; increasing antioxidant activity and/or reducing or delaying symptoms of aging in an individual; comprising administering a food product according to any one of (1) to (18) to an individual in need thereof.

(32) A method of providing nutrition to an individual comprising administering a food product according to any one of (1) to (18) to an individual in need thereof.

(33) A method according to (32) wherein the individual is mature or elderly.

(34) A method according to any one of (30) to (33) wherein the individual is undergoing body mass control or body mass reduction.

(35) A method according to any one of (30) to (33) wherein the individual is suffering from; at risk of suffering from; or recovering from a clinical condition.

(36) A method according to (35) wherein the individual is recovering from injury, operation, or trauma or undergoing or recovering from chemo- or radio-therapy; or having or being at risk of having Metabolic Syndrome, obesity, type II diabetes, atherosclerosis and clinical complications thereof.

(37) A method of treatment: of a clinical condition comprising administering a food product according to any one of (1) to (18) to an individual in need thereof.

(38) A method of treatment according to (37) wherein the clinical condition is cerebra-vascular disorder, cardio-vascular disorder, hypertension, metabolic syndrome, high blood pressure, pre-diabetes, type II diabetes, being overweight (e.g. BMI>25), obesity (e.g. BMI>30), anaemia, rheumatism, rheumatoid arthritis, non-rheumatoid arthritis, prostate or testes malfunction, erectile dysfunction, loss of libido, cellulite, sarcopenia or cachexia.

[0125] Certain aspects and embodiments of the invention will now be illustrated by way of example and with reference to the tables described below.

[0126] Table 1 shows the effect of lycopene on lipid parameters, and markers of IOD and inflammation in CHD patients.

[0127] Table 1 shows the effect of 30 g dark chocolate on lipid parameters, and markers of IOD and inflammation in CHD patients.

[0128] Table 3 shows the effect of 30 g of L-chocolate on lipid parameters, and markers of IOD and inflammation in CHD patients.

[0129] The results presented in the other Tables are discussed in the individual Examples below.

EXAMPLES

Experiments

Lipid-Lowering Chocolate (L-Chocolate)

[0130] Commercially available dark chocolate (Green & Black's Dark Chocolate; 85% cocoa) was melted at 70° C. The melted chocolate was mixed with tomato oleoresin, containing 15% of lycopene (Lyc-O-Mato), in the ratio of 1.57 mg of oleoresin to 1 g of the chocolate. The mixture was blended for 10 minutes and then divided into daily 10 g or 30 g portions and cooled down to the room temperature.

[0131] Each 10 g or 30 g chocolate portion contained 47.1 mg of tomato oleoresin or about 7 mg of lycopene.

Control Samples of Chocolate

[0132] The melting and mixing procedures were performed as described above using the same commercially available dark chocolate, but instead of tomato oleoresin, sunflower oil (Floral™) was used.

Lycopene

[0133] 47.1 mg of tomato oleoresin was pre-dissolved in ethanol and mixed with Whey Protein as described in Richelle et al (2002) J Nutr 132 404-408, WO01/091588 and US2002/01072992. Then the mixture was placed into gelatine capsules.

[0134] All products were kept in cool dry, protected from light conditions.

Validation in Clinical Trials

CHD Patients

[0135] 18 male CHD patients, age 47-69, were recruited for this study.

[0136] Main inclusion criteria were:
elevated total serum cholesterol above 200 mg/dL and/or triglycerides above 150 mg/dL,
all patients were naive for any lipid-lowering medications,
stable clinical conditions and regimen of medications was t the last 3 months.

[0140] Secondary inclusion criteria were:
positive blood markers on inflammatory oxidative damage, IOD, >20-30 μM MDA
positive blood on an antibody inflammatory marker, Px-IgG>0.250-0.300 U/ml

[0143] All patients were randomised and divided into three equal groups of 6 patients each. Two groups receiving chocolate were blinded; the group receiving lycopene preparation along was open labelled. The period of the trial was 4 weeks.

Results

[0144] The results of the ongoing trial are presented in the tables 1 to 3 below.

[0145] It was observed that after two weeks of administration of 7 mg of lycopene, there were no changes in any patients on their levels of elevated cholesterol, triglycerides and markers of oxidative damage or inflammation (table 1).

[0146] Similar results were observed in the group where patients were taking 30 g of the control bar of dark chocolate (table 2).

[0147] However, in the group of patients taking 30 g of L-chocolate, a reduction in concentration of both total cholesterol and its LDL fraction was observed in every patient in the group even after the first seven days of the trial (table 3). The positive trend in triglyceride levels was also detected in 5 out of 6 patients.

[0148] Furthermore, the elevated level of transferases in two patients in this group also started to decline, indicating a positive effect of L-chocolate on their liver damage status. In addition, for the majority of the patients administration of the L-chocolate was accompanied by reduction of inflammatory oxidative damage markers, indicating that this product has not only lipid-lowering properties but anti-inflammatory as well.

[0000]
TABLE 1

Lycopene

IOD	Px-IgG	TC	TG	HDL	LDL	GL	AST	ALT
ID	Age	µM MDA	U/ml	mg/dL	mg/dL	mg/dL	mg/dL	mmol/L	U/L	U/L
Baseline										
13	48	101	0	765	225	161	39	153	6.5	44	25
14	69	162	0.698	231	150	42	159	5.6	45	36
15	54	79	0.811	204	134	41	135	3.8	34	24
16	49	95	0.803	219	126	44	161	4.4	27	35
17	66	83	0.751	243	165	37	186	5.9	49	29
18	53	49	0.743	210	157	40	147	6.1	25	26
		56.5	95	0.762	222	149	40.5	157	5.4	37.3	29.2
Week 1										
13	48	99	0.823	224	160	39	153	6.4	47	31
14	69	158	0.746	231	152	42	160	5.7	46	33
15	54	85	0.809	205	137	40	134	4.9	36	29
16	49	94	0.867	217	130	43	160	3.6	31	34
17	66	81	0.851	241	164	38	185	5.1	44	33
18	53	57	0.839	209	159	40	149	6.1	34	29
		96	0.823	221	150	40.3	157	5.3	39.7	31.5
Week 2										
13	48	95	0.812	223	159	39	152	6.6	43	29
14	69	139	0.809	230	151	41	158	5.2	42	31
15	54	84	0.815	208	138	40	133	5.4	31	28
16	49	91	0.844	216	132	42	159	4.1	38	32
17	66	75	0.830	239	162	39	183	3.9	41	31
18	53	68	0.799	208	158	41	150	6	30	34
		92	0.818	221	150	40.3	156	5.2	37.5	30.8
Week 3										
13	48	94	0.834	221	155	39	151	6.2	42	32
14	69	136	0.781	227	149	42	157	4.8	39	29
15	54	85	0.84	210	135	40	132	5.1	35	25
16	49	93	0.795	214	142	41	155	5	36	31
17	66	76	0.809	232	161	38	179	4.4	42	28
18	53	81	0.774	211	154	41	152	5.8	33	31
		94	0.806	219	149	40.2	154	5.22	37.8	29.3

[0000]
TABLE 2

Chocolate

IOD	Px-IgG	TC	TG	HDL	LDL	GL	AST	ALT
ID	Age	µM MDA	U/ml	mg/dL	mg/dL	mg/dL	mg/dL	mmol/L	U/L	U/L
Baseline										
7	49	132	0.902	209	165	41	157	5.6	35	25
8	55	96	0.933	232	183	40	153	4.2	42	36
9	51	145	0.998	198	182	45	124	6.6	28	24

10 62 53 0.756 227 144 39 179 5.9 41 35
11 50 61 0.854 217 136 41 166 3.8 33 29
12 53 110 0.941 221 179 38 148 5.8 37 26
53.3 99.5 0.897 217 165 40.7 155 5.3 36 29.2
Week 1
7 49 123 0.912 208 168 41 156 5.8 37 27
8 55 105 0.875 229 181 40 154 4.9 41 43
9 51 132 0.914 201 178 44 128 6.4 35 28
10 62 96 0.665 225 149 40 177 5.9 40 38
11 50 83 0.806 216 147 42 165 4.7 36 31
12 53 105 0.915 219 180 38 149 5.6 39 30
107 0.848 216 167 40.8 155 5.5 38 32.3
Week 2
7 49 119 0.945 207 169 40 157 5.9 32 29
8 55 99 0.927 230 179 40 154 5.6 39 44
9 51 141 0.983 197 185 45 126 6.4 29 32
10 62 67 0.844 223 155 40 176 5.8 42 37
11 50 78 0.915 218 146 41 165 3.7 35 33
12 53 114 0.926 215 182 39 147 5.9 35 35
103 0.923 215 169 40.8 154 5.55 35.3 35
Week 3
7 49 118 0.999 201 165 40 156 5.2 32 28
8 55 99 0.876 233 182 41 157 4.9 39 43
9 51 133 0.858 195 164 44 134 6.7 29 36
10 62 69 0.761 219 153 41 175 5.1 42 47
11 50 83 0.944 226 132 42 161 4.2 35 31
12 53 105 0.832 209 167 38 149 5 35 37
101 0.878 214 160 41 155 5.2 35.3 37

[0000]
TABLE 3

Chocolate + Lycopene

ID	Age	Px-IOD µM MDA	IgG U/ml	TC mg/dL	TG mg/dL	HDL mg/dL	LDL mg/dL	GL mmol/L	AST U/L	ALT U/L
Baseline										
1	52	73	0 904	217	121	40	132	4.5	22	40
2	55	46	0.842	211	200	37	169	4.7	30	33
3	63	88	0.871	249	199	42	174	5.3	30	27
4	59	150	0.901	136	170	37	167	6.2	48	110
5	47	112	0.660	228	168	40	150	4.6	40	45
6	49	123	0.789	227	113	42	130	5.5	120	154
	54	98.7	0.827	211	162	39.7	154	5.1	48.7	68.2
Week 1										
1	52	76	0.943	195	120	40	130	4.2	22	40
2	55	38	0.912	183	200	37	167	4.8	30	32
3	63	69	0.838	233	179	42	170	5	30	26
4	59	143	0.522	132	162	37	162	6.1	49	73
5	47	97	0.720	208	161	40	149	4.4	38	42
6	49	101	0.324	193	99	42	127	5	74	137
		87.3	.710	191	153	39.7	151	4.9	40.5	58.3
Week 2										
1	52	44	0.452	193	119	40	129	4.3	22	40
2	55	19	0.81	183	202	37	167	4.7	27	36
3	63	58	0.448	223	178	42	170	5.1	30	28
4	59	63	0 522	130	161	37	160	6.2	45	70
5	47	88	0.23	200	159	40	147	4.5	38	41
6	49	89	0.214	191	89	42	125	5.8	70	132
		60.2	0.446	187	151	39.7	150	5.1	38.7	57.8
Week 3										
1	52	53	0.129	193	119	40	129	4.3	22	40
2	55	21	0.742	182	188	38	160	4.5	27	35

3 63 41 0.081 225 171 42 170 5-2 30 27
4 59 50 0.096 130 158 38 159 6.1 44 70
5 47 61 0 189 154 40 137 5.0 38 39
6 49 54 0.120 190 84 43 122 5.5 72 130
46.7 0.195 185 146 40.1 146 5.1 38.1 56.8

Clinically Healthy Volunteers with Hypercholesterolaemia

Dose Effect

[0149] 52 clinically healthy volunteers 26 males and 26 females, age 35-61 years old, were recruited for this study.

[0150] Main inclusion criteria were:

[0000] elevated total serum cholesterol above 200 mg/dL and/or triglycerides above 150 mg/dL,
all patients were naive, for at least 3 months prior to the study, for any lipid-lowering medications, dietary supplements or special lipid or weight management diets,
willing participate in the study.

[0151] All volunteers were randomised and divided into six groups. 10 participants were included in the control group and group which received chocolate containing 0.36 mg of lycopene per 1 gram of the product. Other four groups were formed from 8 volunteers each. Every participant received the one week supply of the same size of 10 g of the chocolate bar with different concentration of lycopene, or without it at all.

[0152] All chocolate samples were blinded so participants did not know what exactly composition of chocolate they were ingesting. All collected blood samples were also blinded so the analytical laboratory was not aware from which volunteers and from which group the samples were analysed. Every week participants were invited to the clinic when compliance of the ingesting chocolate was verified, blood from these persons was collected, and new batch of one week supply of chocolate was given.

[0153] The duration of the trial was 4 weeks. Effects of the following concentrations of lycopene, “L-tug”, in the chocolate was studied: 0.0, 0.1, 0.2, 0.3, 0.35, 0.7 mg of lycopene per 1 g of chocolate.

Results

Cholesterol

[0154] The effects of chocolate with different concentration of lycopene on the elevated level of the total cholesterol, and other biochemical parameters of the participants are presented in table 4a and table 5.

[0155] These results showed that the chocolate with 0.1 mg of lycopene per 1 g of the product was already able to reduce elevated total cholesterol although the changes were not statistically significant. However, chocolate with 0.2 mg of lycopene and above, per 1 g of the product, were consistently producing cholesterol lowering effect in the serum of the participants. The significant effect was already registered from the second week of the trial and reached it maximum by the fourth week.

Biochemistry

[0156] The effect of this lycopene/L-tug chocolate on other biochemical parameters was insignificant for the doses studied (table 5). Presumably this was because in most groups these parameters were within their physiological norms and there were no much room for their normalisation/“improvement”.

Inflammation and Oxidation

[0157] However, the majority of the participants were positive on markers of Inflammatory Oxidative Damage, IOD, or in some cases on presence of such inflammatory markers as LDL-Px and Chl.-IgG. This was probably due that the majority of the participants were between 50 and 60 years old, and these markers can frequently be detected on a subclinical level even in apparently healthy people of this age and above.

[0158] It all groups taking L-tug chocolate the reduction of the IOD was significant. However, reduction of two other inflammatory markers was observed only in some groups and was not apparently dose dependent. This inconsistency could be a result of a small number of participants in the tested groups.

Plasma Oxygen Transport

[0159] The other interest observation was that ingestion of the L-tug chocolate resulted in the dose-dependent increase of the plasma oxygen transport. This useful property could be used to increase and/or restore supply of the molecular O2 depression or reduction of which may occur not only in many clinical conditions but also during strenuous exercises, or with ageing.

Timing of the Ingestion of Chocolate in Relation to Food Intake.

[0160] To evaluate a possible hypothesis that incorporation of lycopene into chylomicrons and lipoproteins, during their re-assembly at the time of digesting food fat, we undertake the following study. We recruited a group of clinically healthy volunteers of similar age and similar level of hypercholesterolaemia.

[0161] The design of the study was the same as the study above but instead of taking L-tug chocolate with main food we asked participants to ingest the chocolate between meals—at least two hours after their breakfast, or lunch, and at least two hours before their next meal—lunch or dinner.

[0162] The product used was the same format of 10 g containing 0.7 mg of lycopene per 1 g of chocolate.

Results

[0163] The results of this study are presented in the table 4b.

[0164] It was observed that this regiment was also able to reduce elevated total cholesterol but the significant reduction was only observed on the week 3 of the trial. The maximum of the reduction was on the last 4th week.

[0165] It was interesting to note, that although the cholesterol-lowering effect was prominent it was still significantly lower that then when the same chocolate was taken during the main meal. If the week 4 is taken as a reference point, the 0.7 mg dose of the L-tug chocolate ingested on the “empty stomach” was more effective that the dose 0.1 but lees effective than dose 0.2 mg when they were taken with food.

[0000]
TABLE 4a

Dose dependency—chocolate ingestion with main food

L-tug, per 1 g chocolate	Total serum cholesterol, mg/dL 0 w	1 w	2 w	3 w	4 w
0.0 mg	217 + 4.2	215 + 5.6	215 + 5.1	214 + 4.9	213 + 5.3
	? = -2, p*	? = -2, p*	? = -3, p*	? = -4, p*	
0.1 mg	229 + 3.0	228 + 6.5	224 + 3.8	224 + 2.8	218 + 1.5
	? = -1, p*	? = -5, p*	? = -5, p*	? = -11,	
		p = 0.01			
0.2 mg	228 + 4.1	216 + 8.3	199 + 2.0	191 + 5.0	191 + 5.3
	? = -12, p*	? = -29,	? = -37,	? = -37,	
	p < 0.001	p < 0.001	p < 0.001		
0.3 mg	226 + 6.0	202 + 9.3	198 + 6.8	196 + 7.3	192 + 4.3
	? = -24, p*	? = -28,	? = -30,	? = -34,	
	p < 0.05	p < 0.05	p = 0.002		
0.35 mg	221 + 2.8	215 + 3.0	209 + 2.6	208 + 2.5	195 + 8.5
	? = -6, p*	? = -12,	? = -13,	? = -26,	
	p < 0.05	p < 0.05	p = 0.02		
0.7 mg	241 + 14.0	231 + 14.8	190 + 7.0	188 + 6.3	186 + 5.3
	? = -10, p*	? = -51,	? = -53,	? = -55,	
	< 0.05	p = 0.01	p < 0.01		

[0000]
TABLE 4b

The chocolate ingestion between meals two hours after and two hours before any food intake

0.7 mg	243 +	230 + 10.1	218 + 4.3	210 + 5.1	208 + 5.8
	13.7	? = -13, p*	? = -25,	? = -33,	? = -35,

? F[+/-] = -1 p < 0.05 p < 0.05 p < 0.05
pF[+/-] < ? F[+/-] = +28 ? F[+/-] = +22 ? F[+/-] = +22
0.05 pF[+/-] > 0.01 pF[+/-] > 0.05 pF[+/-] > 0.01
pF[+/-]—difference between the same time points in groups which ingested L-tug chocolate with or without food

[0000]
TABLE 5

Effect of different doses of L-tug Chocolate on biochemical parameters and markers of inflammation and oxidation

L-tug, per 1 g chocolate	TG mg/dL	HDL mg/dL	LDL mg/dL	IOD glucose	ELISA × AST	ELISA × ALT	LDL-Px CRP in μM	Chl.p-IgG 10<2>	IgG 10<2>	Plasma-O2
0	165 ± 12.8	40 ± 0.9	155 ± 9.9	5.9 ± 0.7	32 ± 4.1	41 ± 5.6	5.5 ± 1.4	138 ± 11.4	354 ± 41	675 ± 55	0.812 ± 75
	162 ± 11.5	40 ± 0.8	154 ± 8.7	5.8 ± 0.6	34 ± 3.9	40 ± 5.1	6.1 ± 1.2	124 ± 12.7	401 ± 38	722 ± 64	0.823 ± 66
	? = 3,	p > 0.05	p > 0.05	p > 0.05	p > 0.05	p > 0.05	p > 0.05	p > 0.05	p > 0.05	? = 11,	p > 0.05 p > 0.05
0.1 mg	99 ± 3.8	43 ± 1.8	131 ± 3.8	5.4 ± 0.6	40 ± 3.8	34 ± 3.0	6.0 ± 0.9	115 ± 10.8	175 ± 33	577 ± 98	1.153 ± 83
	88 ± 3.3	44 ± 1.3	127 ± 4.0	5.4 ± 0.3	36 ± 2.8	30 ± 2.7	5.4 ± 0.5	9 ± 4.6	212 ± 59	521 ± 67	1.263 ± 19
	? = 11,	p > 0.05	? = 4,	p > 0.05	p > 0.05	p > 0.05	p > 0.05	p < 0.001	p > 0.05	p > 0.05	? = 0.110,	p > 0.05 p > 0.05 p > 0.05
0.2 mg	146 ± 10.3	44 ± 0.6	145 ± 9.0	5.1 ± 0.5	29 ± 2.1	25 ± 3.0	5.1 ± 1.3	85 ± 7.8	142 ± 19	444 ± 22	0.811 ± 31
	120 ± 8.5	45 ± 0.5	135 ± 8.5	6.1 ± 0.4	28 ± 1.6	25 ± 2.3	4.9 ± 1.2	19 ± 6.5	? 0 ± 1.8	260 ± 18	1.363 ± 57
	? = 26,	p > 0.05	? = 10,	p > 0.05	p > 0.05	p > 0.05	p > 0.05	p < 0.001	p < 0.001	p < 0.001	? = 0.552,	p < 0.05 p > 0.05 p < 0.001
0.3 mg	162 ± 10.7	40 ± 1.0	154 ± 3.6	4.9 ± 0.4	49 ± 8.6	60 ± 9.1	6.7 ± 1.5	155 ± 10.7	511 ± 97	828 ± 59	0.731 ± 37
	142 ± 9.1	41 ± 0.8	142 ± 1.8	4.7 ± 0.2	36 ± 4.7	53 ± 10?	5.5 ± 1.1	36 ± 4.1	0 ± 38	286 ± 49	0.963 ± 24
	? = 20,	p > 0.05	? = 10,	p > 0.05	p > 0.05	p > 0.05	p > 0.05	p < 0.05	p < 0.05	p < 0.05	? = 0.223,	p > 0.05 p < 0.05 p < 0.01
0.35 mg	136 ± 28.1	43 ± 1.0	140 ± 10.0	6.3 ± 0.4	41 ± 2.4	49 ± 4.0	6.4 ± 1.7	130 ± 20.8	160 ± 36	552 ± 41	0.988 ± 24
	106 ± 16.5	44 ± 1.5	132 ± 9.3	5.5 ± 0.3	39 ± 2.3	45 ± 4.1	5.8 ± 1.6	55 ± 8.8	206 ± 47	506 ± 66	1.263 ± 19
	? = 30,	p > 0.05	? = 8,	p > 0.05	p > 0.05	p > 0.05	p > 0.05	p < 0.05	p > 0.05	p > 0.05	? = 0.275,	p > 0.05 p > 0.05 p < 0.001
0.7 mg	128 ± 10.8	42 ± 0.6	153 ± 3.8	5.4 ± 0.2	29 ± 2.5	29 ± 3.5	6.8 ± 0.8	86 ± 9.9	66 ± 12	negative	1.099 ± 87
	105 ± 6.6	43 ± 0.7	141 ± 3.0	6.0 ± 0.4	29 ± 2.0	27 ± 1.8	6.6 ± 0.5	25 ± 7.8	?3 ± 8.5	negative	1.430 ± 85
	? = 20,	p > 0.05	? = 8,	p > 0.05	p > 0.05	p > 0.05	p > 0.05	p < 0.05	p < 0.05	? = 0.331,	p > 0.05 p > 0.05 p < 0.01

Postprandial Study

[0166] 8 clinically healthy volunteers 4 males and 4 females, age 35-60 years old, were recruited for this study.

[0167] Main inclusion criteria were:
all patients were naive, for at least 3 months prior to the study, for any lipid-lowering medications, dietary supplements or special lipid or weight management diets,
willing participate in the study.

[0170] All volunteers received standardise fat rich meal comprising of 50 g of butter as a part of a sandwich with 2 slices of white bread. Then without any break volunteers were asked to ingestion 10 g chocolate bar without lycopene. During intake of this test meal volunteers was given 200 ml of warm decaffeinated tea with skimmed milk containing no more than 1% of dairy fat.

[0171] The blood was collected just before the intake of the meal and every hour for 4 hours after that.

[0172] After one week of break the same volunteers were ask to take exactly the same meal of 50 g of the same butter with two slices of the same white bread. Then each of them was asked to take 10 g of the chocolate bar

with 7 mg of lycopene blended in at the protocol described above. The same type and the volume of tea was allowed, and the blood was collected at the same protocol as in the previous week.

[0173] The results of this cross-over study are presented in the table 6. They show that ingestion of lycopene-contained chocolate was able to reduce elevation of postprandial triglycerides about two fold and cholesterol between 2 and 3 times. I was also interesting to note that the postprandial glycaemia was also reduced by this L-tug chocolate.

[0174] The most remarkable changes were observed when this chocolate with the embedded lycopene was not only able to prevent increase of the inflammatory oxidative markers in the postprandial blood but even cause their reduction below the baseline level (table 6).

[0000]
TABLE 6

Statistically significant between control and L-tug trials
Effect of L-tug chocolate on Postprandial Biochemistry Profile and Markers of Inflammatory Oxidation in Serum of Healthy Volunteers—Cross-over Study

	Trial A (control)					Trial B				
	50 g butter - 10 g chocolate (n = 0)					50 g butter + 10 g L-tug chocolate (n = 6)				
Postprandial time	TC in mg/dL	TG in mg/dL	LDL in mg/dL	Glucose in mmol/L	IOD, µM MDA	TC in mg/dL	TG in mg/dL	LDL in mg/dL	Glucose in mmol/L	IOD, µM MDA
baseline	184	108	153	5.0	87	180	109	135	5.0	86
1 h	208	119	136	6.0	94	206	174	136	5.9	87
	? = 24 ± 5.7	? = 11 ± 3.9	? = 3 ± 0.2	? = 1.0 ± 0.5	? = 7 ± 0.7	p(A-B) > 0.05	? = 18 ± 4.6 p(A-B) > 0.05	? = 15 ± 2.9 p(A-B) > 0.05	? = 3 ± 0.2 p(A-B) > 0.05	? = 0.9 ± 0.2 p(A-B) > 0.05	? = 1 ± 0.5 p(A-B) > 0.05
2 h	215	129	136	6.1	97	202	120	135	5.7	49
	? = 31 ± 5.2	? = 21 ± 5.3	? = 3 ± 0.2	? = 1.1 ± 0.1	? = 10 ± 0.6	p(A-B) < 0.01	? = 14 ± 4.7 p(A-B) < 0.05	? = 11 ± 2.6 p(A-B) > 0.05	? = 2 ± 0.1 p(A-B) < 0.05	? = 0.7 ± 0.1 p(A-B) > 0.05	? = 3 ± 0.6
3 h	204	120	135	5.6	97	194	114	135	5.4	72
	? = 20 ± 5.3	? = 12 ± 3.1	? = 2 ± 0.1	? = 0.6 ± 0.2	? = 10 ± 0.9	p(A-B) < 0.05	? = 6 ± 4.5 p(A-B) < 0.05	? = 5 ± 2.4 p(A-B) < 0.05	? = 0 ± 0.1 p(A-B) > 0.05	? = 0.4 ± 0.1 p(A-B) < 0.01	? = 14 ± 1.3
4 h	193	110	131	5.3	99	195	110	133	5.1	70
	? = 9 ± 4.4	? = 2 ± 3.4	? = 1 ± 0.2	? = 0.3 ± 0.2	? = 12 ± 1.3	p(A-B) > 0.05	? = 7 ± 4.2 p(A-B) > 0.05	? = 1 ± 2.7 p(A-B) > 0.05	? = 0 ± 0.2 p(A-B) > 0.05	? = 0.1 ± 0.2 p(A-B) > 0.05	? = 16 ± 1.1 p(A-B) < 0.01

TC—total cholesterol, TG—triglycerides, ?—changes in mean concentrations with the baseline, p(A-B)—statistical differences in same parameters at the same time points between trial A and trial B.

8 clinically healthy volunteers, 4 men and 4 women, average age—35-60 years old

New Opportunities to Control Lipid Metabolism Inflammation and Tissue Oxygenation

[0175] The results presented here open a possible new mechanisms and new ways not only to control already developed changes in the lipid metabolism but also to prevent these changes.

[0176] These results also provide for the development of new ways to control subclinical and other forms of inflammation and/or boosting transport of the plasma molecular oxygen, which could be useful to restore tissue oxygen saturation which could be important in many clinical conditions and to delay ageing.

[0177] The results described herein are unexpected because the benefit of adding any ingredient with additional health value to a chocolate product would be expected to be outweighed by the potential harmful consequences of consuming increased amounts of this high-fat food product.

[0178] Reducing fat content in food products is the standard way to minimise their fat load to the body. However this approach is not generally useful for cocoa-based products, such as chocolate, because fat reduction negatively affects the melting, feeling and taste properties.

[0179] The results described herein show the unusual and unexpected outcome of the blending of carotenoids such as lycopene with cocoa-based products. Not only is cocoa butter prevented from contributing to the rise of blood lipids, but the blend actively reduces lipids which are already at an elevated level.

[0180] In other words, the invention described herein not only makes cocoa products, such as chocolate, “safer” from the health impact point of view, but may also make it useful as a proactive interventional product for slimming, lipid-lowering purposes and anti-aging purposes, and for prevention and help in management of metabolic, pre-diabetes, cardio-vascular and other conditions.

CAROTENOID PARTICLES AND USES THEREOF
US2013337068

This invention relates to the incorporation of bioactive cargo molecules into particles with carotenoids, such as lycopene. The incorporation of a cargo molecule into a carotenoid particle may for example increase the bioavailability of the cargo molecule in the bloodstream compared to other delivery systems. Carotenoid particles as described herein may be useful in the formulation of therapeutic and nutritional compounds for oral administration to individuals.

[0001] This invention relates to vehicles for the delivery of molecules into the bloodstream of individuals.

[0002] Substances which are administered orally, such as pharmaceuticals and dietary supplements, are often modified or damaged, for example by enzymatic degradation, oxidation or stomach acidity, in the gastrointestinal tract. This modification or damage reduces the absorption and subsequent bioavailability of the substance in the blood stream.

[0003] Formulation with a carrier may increase the amount of a labile substance which is absorbed in an unmodified or undamaged form, thereby increasing its bioavailability in the bloodstream.

[0004] Whey protein has previously been used as a carrier to increase the activity of lycopene (Richelle et al J. Nutr. 132:404-408, 2002; PCT/EP01/06145). Lycopene formulated with a whey protein carrier has been reported to inhibit atherogenic serum abzymes and be useful in the treatment of atherosclerotic conditions (WO2007/010216).

[0005] This invention relates to the finding that carotenoids, such as lycopene, may be useful in delivering cargo molecules into the bloodstream. The incorporation of a cargo molecule into a carotenoid particle may lead to increased bioavailability of the cargo molecule in the bloodstream compared to other delivery systems, allowing the dose required to achieve efficacy to be reduced or increasing the efficacy of the same dose. Carotenoid particles may be useful in the formulation of therapeutic and nutritional compounds for oral administration to individuals.

[0006] An aspect of the invention provides a population of particles, each particle comprising a carotenoid compound and one or more cargo molecules.

[0007] Carotenoids are resistant to enzymatic degradation in the gastrointestinal tract. The incorporation of cargo molecules into carotenoid particles as described herein provides protection from damage and/or modification in the gastrointestinal tract.

[0008] In some embodiments, in one or more of the carotenoid particles in the population, the carotenoid compound may form layer, for example an outer layer or interim layer which encapsulates an inner core comprising the one or more cargo molecules (i.e. micelles or reverse micelles). For example, 1% or more, 10% or more, 20% or more, 30% or more or 40% or more of the particles in the population may possess this micelle structure. Up to 100%, up to 95%, up to 90%, up to 80%, up to 70% or up to 60% of the particles in the population may possess this micelle structure. Carotenoid micelles may be soluble, and may for example exist in aqueous solution.

[0009] In some embodiments, in one or more of the carotenoid particles in the population, the carotenoid compound may form a matrix into which cargo molecules or their hydrophobic moieties are anchored or embedded (i.e. a non-micelle or composite particle). For example, 1% or more, 10% or more, 20% or more, 30% or more or 40% or more of the particles in the population may possess this composite structure. Up to 100%, up to 95%, up to 90%, up to 80%, up to 70% or up to 60% of the particles in the population may possess this composite structure. Non-micelle particles may exist in a dried form or as suspensions or colloids.

[0010] The proportion of particles in a population with micelle or non-micelle structure may be determined using routine techniques.

[0011] The carotenoid particles in a population may have uniform or substantially uniform structures (i.e. a homogenous population) or non-uniform or substantially non-uniform structures (i.e. a heterogeneous population).

[0012] The carotenoid particles may exist in aggregates or clusters within a population.

[0013] The structures adopted by carotenoid particles in a population depend on a number of factors, including the production method, the size, shape and hydrophobicity of the cargo molecule(s), the ratio of carotenoids to cargo molecules, the presence of surfactants, the ratio between the hydrophobic and hydrophilic parts of the cargo molecule(s) and the homogeneity and purity of the cargo molecule(s), especially if the particles contain more than one type of cargo molecule.

[0014] Carotenoid compounds are a class of tetraterpenoids which contain long polyene chains. Carotenoids include xanthophylls such as lutein and zeaxanthin, and carotenes, such as beta-carotene, alpha-carotene, zeto-carotene, and lycopene and related molecules, including 1-HO-3',4'-didehydrolycopene, 3,1'-(HO)2-gamma-carotene, 1,1'-(HO)2-3,4,3',4'-tetradehydrolycopene, 1,1'-(HO)2-3,4-didehydrolycopene.

[0015] Other suitable carotenoid compounds which may be used as described herein include hydrocarbons, such as lycopersene (7,8,11,12,15,7',8',11',12',15'-decahydro-?,?-carotene), phytofluene, hexahydrolycopene (15-cis-7,8,11,12,7',8'-hexahydro-?,?-carotene), torulene (3',4'-didehydro-ß,?-carotene) and a-zeacarotene (7',8'-dihydro-e,?-carotene); alcohols, such as alloxanthin, cynthiaxanthin, pectenoxanthin, cryptomonaxanthin, ((3r,3'r)-7,8,7',8'-tetradehydro-ß,ß-carotene-3,3'-diol), crustaxanthin (ß,-carotene-3,4,3',4'-tetrol), gazaniaxanthin ((3r)-5'-cis-ß,?-caroten-3-ol), oh-chlorobactene (1',2'-dihydro-f,?-caroten-1'-ol), loroxanthin (ß,e-carotene-3,19,3'-triol), lycoxanthin (?,?-caroten-16-ol), rhodopin (1,2-dihydro-?,?-caroten-1-ol), rhodopinol (aka warmingol; 13-cis-1,2-dihydro-?,?-carotene-1,20-diol), saproxanthin (3',4'-didehydro-1',2'-dihydro-ß,?-carotene-3,1'-diol) and zeaxanthin; glycosides, such as oscillaxanthin (2,2'-bis(ß-1-rhamnopyranosyloxy)-3,4,3',4'-tetradehydro-1,2,1',2'-tetrahydro-?,?-carotene-1,1'-diol), and phleixanthophyll (1'-(ß-d-glucopyranosyloxy)-3',4'-didehydro-1',2'-dihydro-ß,?-caroten-2'-ol); ethers, such as rhodovibrin (1'-methoxy-3',4'-didehydro-1,2,1',2'-tetrahydro-?,?-caroten-1-ol) and spheroidene (1-methoxy-3,4-didehydro-1,2,7',8'-tetrahydro-?,?-carotene), epoxides, such as diadinoxanthin (5,6-epoxy-7',8'-didehydro-5,6-dihydro-carotene-3,3-diol), luteoxanthin (5,6:5',8'-diepoxy-5,6,5',8'-tetrahydro-ß,ß-carotene-3,3'-diol), mutatoxanthin, citroxanthin, zeaxanthin (furanoxide 5,8-epoxy-5,8-dihydro-ß,ß-carotene-3,3'-diol), neochrome (5',8'-epoxy-6,7-didehydro-5,6,5',8'-tetrahydro-ß,ß-carotene-3,5,3'-triol), foliachrome, trollichrome, and vaucheriaxanthin (5',6'-epoxy-6,7-didehydro-5,6,5',6'-tetrahydro-ß,ß-carotene-3,5,19,3'-tetrol); aldehydes, such as rhodopinal, wamingone (13-cis-1-hydroxy-1,2-dihydro-?,?-caroten-20-al), torularhodinaldehyde (3',4'-didehydro-ß,?-caroten-16'-al); acids and acid esters, such as torularhodin (3',4'-didehydro-ß,?-caroten-16'-oic acid) and torularhodin methyl ester (methyl 3',4'-didehydro-ß,?-caroten-16'-oate); ketones, such as astaxanthin, canthaxanthin (aka aphanicin), chlorellaxanthin (ß,ß-carotene-4,4'-dione), capsanthin ((3r,3's,5'r)-3,3'-dihydroxy-ß,?-caroten-6'-one), capsorubin ((3s,5r,3's,5'r)-3,3'-dihydroxy-?,?-carotene-6,6'-dione), cryptocapsin ((3'r,5'r)-3'-hydroxy-ß,?-caroten-6'-one), 2,2'-diketospirilloxanthin (1,1'-dimethoxy-3,4,3',4'-tetradehydro-1,2,1',2'-tetrahydro-?,?-carotene-2,2'-dione), flexixanthin (3,1'-dihydroxy-3',4'-didehydro-1',2'-dihydro-ß,?-caroten-4-one), 3-oh-canthaxanthin (aka adonirubin; aka phoenicoxanthin; 3-hydroxy-ß,ß-carotene-4,4'-dione), hydroxyspheriodenone (1'-hydroxy-1-methoxy-3,4-didehydro-1,2,1',2',7',8'-hexahydro-?,?-caroten-2-one), okenone (1'-methoxy-1',2'-dihydro-c,?-caroten-4'-one), pectenolone (3,3'-dihydroxy-7',8'-didehydro-ß,ß-caroten-4-one), phoeniconone (aka dehydroadonirubin; 3-hydroxy-2,3-didehydro-ß,ß-carotene-4,4'-dione), phoenicopterone (ß,e-caroten-4-one), rubixanthone (3-hydroxy-ß,?-caroten-4'-one), siphonaxanthin (3,19,3'-trihydroxy-7,8-dihydro-ß,e-caroten-8-one); esters of alcohols, such as astacein (3,3'-bispalmitoyloxy-2,3,2',3'-tetradehydro-ß,ß-carotene-4,4'-dione or 3,3'-dihydroxy-2,3,2',3'-tetradehydro-ß,ß-carotene-4,4'-dione dipalmitate), fucoxanthin (3'-acetoxy-5,6-epoxy-3,5'-dihydroxy-6',7'-didehydro-5,6,7,8,5',6'-hexahydro-ß,ß-caroten-8-one), isofucoxanthin (3'-acetoxy-3,5,5'-trihydroxy-6',7'-didehydro-5,8,5',6'-tetrahydro-ß,ß-caroten-8-one), physalien, zeaxanthin dipalmitate ((3r,3'r)-3,3'-bispalmitoyloxy-ß,ß-carotene or (3r,3' r)-ß,ß-carotene-3,3'-diol dipalmitate) and siphonein (3,3'-dihydroxy-19-lauroyloxy-7,8-dihydro-ß,e-caroten-8-one or 3,19,3'-trihydroxy-7,8-dihydro-ß,e-caroten-8-one 19-laurate); apo carotenoids, such as ß-apo-2'-carotenal (3',4'-didehydro-2'-apo-b-caroten-2'-al), apo-2-lycopenal, apo-6'-lycopenal (6'-apo-y-caroten-6'-al), azafrinaldehyde (5,6-dihydroxy-5,6-dihydro-10'-apo-ß-caroten-10'-al), bixin (6'-methyl hydrogen 9'-cis-6,6'-diapocarotene-6,6'-dioate), citranaxanthin (5',6'-dihydro-5'-apo-ß-caroten-6'-one or 5',6'-dihydro-5'-apo-18'-nor-ß-caroten-6'-one or 6'-methyl-6'-apo-ß-caroten-6'-one), crocetin (8,8'-diapo-8,8'-carotenedioic acid), crocetinsemialdehyde (8'-oxo-8,8'-diapo-8-carotenoic acid), crocin (digentiobiosyl 8,8'-diapo-8,8'-carotenedioate), hopkinsiaxanthin (3-hydroxy-7,8-didehydro-7',8'-dihydro-7'-apo-b-carotene-4,8'-dione or 3-hydroxy-8'-methyl-7,8-didehydro-8'-apo-b-carotene-4,8'-dione), methyl apo-6'-lycopenoate (methyl 6'-apo-y-caroten-6'-oate), paracentrone (3,5-dihydroxy-6,7-didehydro-5,6,7',8'-tetrahydro-7'-apo-b-caroten-8'-one or 3,5-dihydroxy-8'-methyl-6,7-didehydro-5,6-dihydro-8'-apo-b-caroten-8'-one) and sintaxanthin (7',8'-dihydro-7'-apo-b-caroten-8'-one or 8'-methyl-8'-apo-b-caroten-8'-one); nor and seco carotenoids, such as actinioerythrin (3,3'-bisacyloxy-2,2'-dinor-b,b-carotene-4,4'-dione), ß-carotenone (5,6:5',6'-diseco-b,b-carotene-5,6,5',6'-tetrone), peridinin (3'-acetoxy-5,6-epoxy-3,5'-

dihydroxy-6',7'-didehydro-5,6,5',6'-tetrahydro-12',13',20'-trinor-b,b-caroten-19,11-olide), pyrrhoxanthininol (5,6-epoxy-3,3'-dihydroxy-7',8'-didehydro-5,6-dihydro-12',13',20'-trinor-b,b-caroten-19,11-olide), semi-a-carotenone (5,6-seco-b,e-carotene-5,6-dione), semi-ß-carotenone (5,6-seco-b,b-carotene-5,6-dione or 5',6'-seco-b,b-carotene-5',6'-dione) and triphasiaxanthin (3-hydroxysemi-b-carotenone 3'-hydroxy-5,6-seco-b,b-carotene-5,6-dione or 3-hydroxy-5',6'-seco-b,b-carotene-5',6'-dione); retro carotenoids and retro apo carotenoids, such as eschscholtzxanthin (4',5'-didehydro-4,5'-retro-b,b-carotene-3,3'-diol), eschscholtzxanthone (3'-hydroxy-4',5'-didehydro-4,5'-retro-b,b-caroten-3-one), rhodoxanthin (4',5'-didehydro-4,5'-retro-b,b-carotene-3,3'-dione) and tangeraxanthin (3-hydroxy-5'-methyl-4,5'-retro-5'-apo-b-caroten-5'-one or 3-hydroxy-4,5'-retro-5'-apo-b-caroten-5'-one); and higher carotenoids, such as nonaprenoxanthin (2-(4-hydroxy-3-methyl-2-butenyl)-7',8',11',12'-tetrahydro-e,y-carotene), decaprenoxanthin (2,2'-bis(4-hydroxy-3-methyl-2-butenyl)-e,e-carotene), c.p. 450 (2-[4-hydroxy-3-(hydroxymethyl)-2-butenyl]-2'-(3-methyl-2-butenyl)-b,b-carotene), c.p. 473 (2'-(4-hydroxy-3-methyl-2-butenyl)-2-(3-methyl-2-butenyl)-3',4'-didehydro-1',2'-dihydro-b,y-caroten-1'-ol) and bacterioruberin (2,2'-bis(3-hydroxy-3-methylbutyl)-3,4,3',4'-tetradehydro-1,2,1',2'-tetrahydro-y,y-carotene-1,1'-dio)

[0016] A carotenoid particle as described herein may contain a single carotenoid compound (e.g. lycopene) or more than one carotenoid compound (e.g. lycopene and beta-carotene). Typically, each carotenoid compound will be present in a range of different isomeric forms.

[0017] In some preferred embodiments, the carotenoid compound is lycopene. Lycopene is an open-chain unsaturated C40 carotenoid of structure I (Chemical Abstracts Service Registry Number 502-65-8).

[0000]

[0018] Lycopene occurs naturally in plants such as tomatoes, guava, rosehip, watermelon and pink grapefruit.

[0019] Lycopene for use as described herein may comprise one or more different isomers. For example, lycopene may comprise at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% (Z)-isomers, (all-E)-isomers, or cis-isomers, such as 5-cis- or 9-cis- or 13-cis-isomers, which have improved bioavailability relative to trans isomers. Trans isomers may isomerise into cis forms in vivo, or during storage and processing. Carotenoid particles comprising lycopene may be referred to herein as Lycosomes™.

[0020] Carotenoid compounds for use as described herein may be natural i.e. obtained from a natural source, for example, extracted from a plant, such as a tomato or melon. A range of methods for extracting, concentrating and/or purifying carotenoids from plants are known in the art. For example, solvent extraction using ethanol, DMSO, ethyl acetate, hexane, acetone, soya or other vegetable oil, or non-vegetable oils may be employed. A carotenoid compound may be isolated i.e. free or substantially free of other molecules found in its natural source or environment.

[0021] Carotenoid compounds for use as described herein may be synthetic i.e. produced by artificial means, for example, by chemical synthesis or fermentation. A range of methods for chemical synthesis of lycopene and other carotenoids are known in the art. For example, a three-stage chemical synthesis based on the standard Wittig olefination reaction scheme for carotenoid synthesis may be employed, in which an organic solution of C15 phosphonium methanesulfonate in dichloromethane (DCM) and an organic solution of C10 dialdehyde in toluene are produced, and the two organic solutions are gradually combined with sodium methoxide solution and undergo a condensation reaction to form crude lycopene. The crude lycopene may then be purified using routine techniques, for example by adding glacial acetic acid and deionized water to the mixture, stirring vigorously, allowing the aqueous and organic phases to separate, and extracting the organic phase containing DCM and crude lycopene with water. Methanol is added to the organic phase and the DCM removed via distillation under reduced pressure. The crude methanolic lycopene solution is then be heated and cooled to crystalline slurry that is filtered and washed with methanol. The lycopene crystals may then be recrystallized and dried under heated nitrogen. Synthetic carotenoids, such as lycopene, are also available from commercial suppliers (e.g. BASF Corp, NJ USA, DSM Nutritional Products, Basel, CH).

[0022] Synthetic carotenoids may comprise an increased proportion of cis isomers relative to natural carotenoids. For example, synthetic forms of carotenoids such as lycopene may be up to 25% 5-cis, 1% 9-cis, 1% 13-cis, and 3% other cis isomers, whilst natural forms of carotenoids, for example lycopene produced by tomatoes, may be 3-5% 5-cis, 0-1% 9-cis, 1% 13-cis, and <1% other cis isomers. Since cis-carotenoids, such as cis-lycopene, have increased bioavailability relative to trans-carotenoids, such as trans-lycopene, synthetic carotenoids may be preferred in some embodiments.

[0023] Derivatives of carotenoids as described above may be produced by chemical synthesis analogous to the

synthesis described above; by chemical modification of natural carotenoids extracted from plant material or by microbial, yeast, algal, or fungal fermentation. For example, lycopene may be produced by fermentation of the fungus Blakeslea trispora (e.g. Lyconat™, Vitatene SA).

[0024] The population of carotenoid particles may comprise 0.05 to 90% by weight of the carotenoid compound, preferably 0.1% to 10% by weight. For example, the population may be 0.01% or more, 0.05% or more, 0.1% or more, 0.2% or more, 0.5% or more, 1% or more, 10% or more, or 20% or more by weight of carotenoid compound. The population may be up to 90%, up to 80%, up to 70%, up to 60% up to 50%, up to 40%, up to 30%, up to 20% or up to 10% by weight of carotenoid compound.

[0025] The carotenoid particles in the population may contain the same or similar amounts of carotenoid compound or the amount of carotenoid compound may vary between particles in the population. Each carotenoid particle in the population may comprise 0.05 to 90% by weight of carotenoid compound. For example, each carotenoid particle in the population may be 0.05% or more, 0.1% or more, 1% or more, 10% or more, or 20% or more by weight of carotenoid molecules. Each carotenoid particle may be up to 90%, up to 80%, up to 70%, up to 60% up to 50%, up to 40% or up to 30%, up to 90% or more by weight of carotenoid compound.

[0026] The extent of particle variability within a population may vary depending on the production method. Preferably, at least 80%, at least 90%, at least 95%, at least 98% or at least 99%, for example 85% to 95%, of the carotenoid particles in the population contain the same or similar amounts of carotenoid compound.

[0027] Typically, a population of carotenoid particles may be comprised in a unit dosage formulation which contains 1 to 10 mg of carotenoid compound, such as lycopene, for example about 3.5 mg lycopene.

[0028] The cargo molecule which is incorporated into the carotenoid particle may be any compound, agent, drug or other product or combination thereof, which needs to be delivered to the blood stream. Typically, the cargo molecule will be a therapeutic or nutritional compound, such as a pharmaceutical, nutraceutical or a dietary or nutritional supplement.

[0029] Cargo molecules which are labile in the gastro-intestinal tract or poorly absorbed by the gastro-intestinal tract are especially suitable for incorporation into carotenoid particles.

[0030] Suitable cargo molecules include products of the fermentation, oxidation, processing or degradation of foods such as meat, fish, dairy, grain, bean, honey, tea or other foodstuffs or beverages. Products may include whey protein or peptides, carbohydrates, such as poly- or oligosaccharides, lipids, flavones, and other food derived bioactive molecules. Bioactive molecules may, for example, include antimicrobial peptides, defensins, cathelidins, whey acid proteins, bioactive fragments of food proteins; and peptides which display one or more of protease inhibiting, bactericidic, metabolic, anti-inflammatory, immune-stimulating, coagulation, angiogenesis and proliferation control activities, or exert a beneficial effect on neurotransmitters, angiotensin, hormones and/or other signalling pathways.

[0031] Suitable cargo molecules also include products of probiotic bacteria, yeast or other microbial metabolism, or the metabolism of fungi or moulds, in particular organisms which are used in food and beverage manufacturing or are associated therewith. Examples include bacteria such as Lactobacilli spp for example L. acidophilus, L. casei, L. lactis, L. plantarum, L. reuteri, L. rhamnosus, L. actococcus, L. garvieae and L. bulgaricus; Lactococci, such as L. raffinolactis; Bifidobacteria, such as B. animalis, B. breve and B. longu; E. coli such as E. coli M-17, E. coli Nissle 1917; Enterococci, such as Enterococcus faecium MG004 and Streptococci, such as Streptococcus thermophilus; yeasts, such as Dekkera intermedia, Candida, such as C. blankii and C. stellatam; Saccharomyces, such as S. cerevisiae, S. pastorianus, S. exiguus, S. boulardii and S. varum; Brettanomyces, such as B. bruxellensis and B. lambicus; Schizosaccharomyces pombe, Torulaspora delbrueckii and Zygosaccharomyces bailii; moulds, for example Aspergillus spp, such as A. oryzaeor, A. soyae, A. sojae, A. niger, A. terreus, A. tamari and A. flavus; Monascus spp, such as M. pupureus, M. ruber, and M. pilosus; Penicillium spp, such as P. chrysogenum, P. roqueforti, P. glaucum, P. candidum, P. camemberti, P. paneum, P. geotrichum, P. solitum, P. nalgiovense, P. commune, P. olsonii, P. verrucosum, P. oxalicum, and P. viridicatum; Tolypocladium inflatum; Rhizopus spp, such as R. artocarpi, R. nigricans, R. oligosporus, R. oryzae and R. stolonifer; Neurospora spp such as N. sitophilia and N. intermedia; and Fusarium venenatum.

[0032] Other suitable cargo molecules include lecithin, carbohydrates; amino acids; flavones, such as luteolin, apigenin, and tangeritin; flavonols, such as quercetin, rutin, kaempferol, myricetin, fisetin, isorhamnetin, pachypodol and rhamnazin; flavanones, such as hesperetin, naringenin, eriodictyol and homoeriodictyol; flavanonols, such as taxifolin (or dihydroquercetin), and dihydrokaempferol; isoflavones, such as genistein, daidzein and glycitein; catechins, gallocatechin, catechin 3-gallate, gallocatechin 3-gallate, epicatechins, epigallocatechin, epicatechin 3-gallate, flavon-3-ols such as epigallocatechin 3-gallate; proanthocyanidins, for

example as dimers, trimers, oligomers, or polymers with flavanols; anthocyanidins, such as cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin, aglycones of anthocyanins, such as betalain, amaranthine and isoamaranthine; silibinin or silymarin, curcuminoids, gingerols, ceramides; isoprene, prenol, isovaleric acid, geranyl pyrophosphate, eucalyptol, limonene, pinene, farnesyl pyrophosphate, artemisinin, bisabolol, geranylgeranyl pyrophosphate, retinol, retinal, phytol, taxol, forskolin, aphidicolin, squalene, lanosterol, and other terpenes and terpenoids; sterols and sterol esters, such as stanol ester; phytosterols; alpha-, beta-, gamma- and delta-tocotrienols; shark or other cartilaginous fish oils, vegetable oils, or oils from amaranth seed, rice, wheat germ or olives; squalenes; retinoids; garlic acid or salicylic acid or other hydrolysable tannins; cinnamic acid; lignins; polyphenols, such as catechol, hydroquinone, 2,6-dimethoxybenzoquinone, 3-acetyl-6-methoxybenzaldehyde, tyrosol, p-hydroxyphenylacetic acid, caffeic, ferulic acids, myristicin, eugenol, umbelliferone, aesculetin, bergenon, eugenin, juglone, plumbagin, mangiferin, resveratrol(3,5,4'-trihydroxy-trans-stilbene), emodin, cyanidin, pinoresinol, eusiderin, amentoflavone, ellagic acid, theaflavin, thearubigins, catechol melanins, condensed tannins, phlorotannins, and other polyphenols; vitamins, such as niacin (vitamin B3), folic acid (vitamin B9), ascorbic acid (vitamin C), riboflavin (vitamin B2), thiamine (vitamin B1), calciferol (vitamin D), cobalamins (vitamin 12), phylloquinone (vitamin K1), pantothenic acid (vitamin B5), biotin (vitamin B7) and pyridoxine (vitamin B6), minerals, such as calcium, selenium, chromium, magnesium, iron, zinc, copper and other metal ions; penicillins, cephalosporins, cardapenems, sulphonamides, quinolones, oxazodinones, macrolides and other antibiotics, anti-viral, anti-fungi, and anti-parasite drugs, in particular drugs targeting liver and other organs which express carotenoid receptors, such as liver, adrenal glands, lymphocytes, lymph nodes, prostate tissues, and testis; and statins, such as atorvastatin, cerivastatin, fluvastatin, lovastatin, mevastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin, either alone or in complexes or combinations.

[0033] A particle may contain a single type of cargo molecule or more than one type of cargo molecule, for example two, three, four or more different types of cargo molecule.

[0034] The carotenoid particle may comprise 0.05 to 90% by weight of cargo molecules. For example, the carotenoid particle may be 0.1% or more, 1% or more, 10% or more, or 20% or more by weight of cargo molecules. The carotenoid particle may be up to 90%, up to 80%, up to 70%, up to 60% up to 50%, up to 40% or up to 30%, up to 90% or more by weight of cargo molecules.

[0035] The ratio of carotenoid compound to cargo molecule in the carotenoid particle by weight may be 0.001 or more, 0.01 or more, 0.1 or more, 0.2 or more or 0.5 or more. The ratio of carotenoid compound to cargo molecule in the carotenoid particle by weight may be up to 1000, up to 100, up to 10, up to 5, or up to 2.

[0036] In some embodiments, a carotenoid particle may comprise lycopene and whey protein in the ratio (w/w) of 0.05 to 1, preferably 0.1. For example, a population of carotenoid particles in a unit dosage format may contain 2 to 5 mg, for example 3.5 mg, of lycopene and 20 to 50 mg, for example 35 mg, of whey protein.

[0037] In other embodiments, a carotenoid particle may comprise lycopene and resveratrol in a ratio (w/w) of from 0.02 to 0.2, preferably from 0.06 to 0.08. For example, a population of carotenoid particles in a unit dosage format may contain 2 to 5 mg, for example 3.5 mg, of lycopene and 30 to 70 mg, for example 50 mg, of resveratrol.

[0038] In other embodiments, a carotenoid particle may comprise lycopene and a statin, such as simvastatin, in a ratio (w/w) of from 0.1 to 0.5, preferably 0.3 to 0.4, for example 0.35. For example, a population of carotenoid particles in a unit dosage format may contain 2 to 10 mg, for example 7 mg, of lycopene and 20 mg of statin.

[0039] The bioavailability of the cargo molecule in circulation following oral administration of carotenoid particles incorporating the cargo molecule may be increased relative to bioavailability following oral administration of the cargo molecule alone.

[0040] Increased bioavailability may allow the dosage of the cargo molecule to be reduced when it is incorporated into carotenoid particles as described herein compared to administration without the particles, whilst achieving the same efficacy. This may be useful in reducing side-effects associated with the cargo molecule. For example, the dosage of the cargo molecule incorporated into a carotenoid particle may be 1% or less, 5% or less, 10% or less, 20% or less, 30% or less, 40% or less or 50% or less of the dosage of the cargo molecule on its own which is required for the same efficacy.

[0041] The bioavailability of the cargo molecule may be increased by two fold or more, three fold or more or four fold or more by incorporation into carotenoid particles as described herein. For example, the data herein shows that the bioavailability of resveratrol is increased by two fold and the bioavailability of simvastatin is increased by four fold by incorporation into a carotenoid particle. In some embodiments, the cargo molecule may display no bioavailability or substantially no bioavailability when it is administered without incorporation into a

carotenoid particle. For example, whey protein is shown to display little or no bioavailability when administered orally without incorporation into a carotenoid particle.

[0042] At the same dosage, the efficacy of the cargo molecule may be increased when it is incorporated into carotenoid particles as described herein compared to its efficacy without such incorporation. For example, the efficacy of the cargo molecule incorporated into a carotenoid particle may be increased by 2 fold or more, 3 fold or more, 5 fold or more, 10 fold or more or 100 fold or more compared to the efficacy of the same dosage of cargo molecule on its own.

[0043] Carotenoid particles as described herein may be useful in targeting cargo molecules to tissues which express carotenoid receptors.

[0044] A method of improving the delivery of a cargo molecule to a target tissue which expresses carotenoid receptors may comprise:

incorporating the cargo molecule into a carotenoid particle as described herein and,
administering the carotenoid particle to an individual.

[0047] Tissues which express carotenoid receptors include hepatocytes, liver, adrenal glands, lymphocytes, lymph nodes, prostate tissues and testis. In some preferred embodiments, the target tissue which expresses carotenoid receptors is liver.

[0048] Suitable cargo molecules may include compounds which are beneficially targeted to tissues which express carotenoid receptors, such as the liver.

[0049] Suitable cargo molecules for delivery to the liver include prodrugs which are activated in the liver, for example by the action of liver enzymes. Prodrugs which are activated by liver enzymes include aldehyde oxidase activated prodrugs, such as 5-ethynalyl-2(1H)-pyrimidinone, 5-iodo-2-pyrimidinone-2'-deoxyribose (IPdR), and 5-fluoro-2-pyrimidinone (5-FP); cytochrome P450 reductase activated prodrugs, such as menadione, mitomycin C, tirapazamine and E09 (3-hydroxymethyl-5-aziridinyl-1-methyl-2[1H-indole-4,7-dione]prop-2-en-1-ol); cytochrome p450 activated prodrugs, such as 4-ipomeanol, ftorafir, dacarbazine, trofosamide, ifosamide, cyclophosphamide, and 1,4-bis-{[2-(dimethylamino-N-oxide)ethyl]amino}-5,8-dihydroxyanthracene-9,10-dione (AQ4N); thymidine phosphorylase activated prodrugs such as 5'-deoxy-5-fluorouridine, and glutathione transferase activated prodrugs, such as ?-Glutamyl-a-amino-ß(2-ethyl-N,N,N',N'-tetrakis(2-chloroethyl)phosphoro-diamidate)sulfonyl-propionyl)-(R)-(-)phenylglycine (Ter286), S—CPHC-ethylsulfoxide (S—(N-p-chlorophenyl-N-hydroxycarbamoyl)ethylsulfoxide) and cis-3-(9H-Purin-6-ylthio) acrylic acid (PTA).

[0050] Other suitable prodrugs are well-known in the art and include lisdexamphetamine, codeine and tramadol.

[0051] Administration of carotenoid particles incorporating the cargo molecule may increase the concentration of the cargo molecule in the target tissue following relative to the concentration after administration of the same dose of cargo molecule on its own.

[0052] Administration of the carotenoid particle incorporating the cargo molecule may provide a decreased concentration of the cargo molecule in non-target tissue following relative to the concentration after administration of the same dose of cargo molecule on its own.

[0053] Methods as described herein are generally useful in increasing the availability of cargo molecules. A method of increasing the bioavailability of a cargo molecule may comprise:

incorporating the cargo molecule into a carotenoid particle as described herein.

[0055] Following incorporation of the cargo molecule into the carotenoid particles, and optional formulation into a composition, such as a pharmaceutical composition, food additive or dietary supplement, the carotenoid particles may be administered to an individual.

[0056] In some embodiments, the cargo molecule may be whey protein. Whey protein is shown herein to possess anti-Chlamydia and cholesterol lowering activity. Whey protein is a collection of globular proteins which are naturally found in milk. It is isolated from whey, which is a by-product of cheese manufacture. It is a mixture of beta-lactoglobulin (~65%), alpha-lactalbumin (~25%), and serum albumin (~8%), which are soluble in their native forms, independent of pH. Whey protein is commercially available from a number of suppliers (e.g. Eurosérum, France).

[0057] In some embodiments, the cargo molecule is not a lactoprotein, such as casein, beta-lactoglobulin, alpha-lactalbumin, and serum albumin. In such embodiments, carotenoid particles as described herein may be devoid of lactoproteins.

[0058] In some embodiments, the cargo molecules are not whey proteins and/or whey peptides. In such embodiments, carotenoid particles as described herein may be devoid of whey proteins and/or whey peptides.

[0059] In some preferred embodiments, the carotenoid particle may further comprise lecithin. Lecithin (E222) is commonly used as an emulsifier in food products and may be isolated from egg yolk or animal or soy or other plant tissue. Lecithin comprises a number of fatty acids, phospholipids, triglycerides, and glycolipids, as well as glycerol, choline and phosphoric acid. Lecithin is widely available commercially. Lecithin may include soy-lecithin.

[0060] A carotenoid particle as described herein may comprise from 1.5% to 98.5% (w/w) lecithin. For example, a particle may comprise at least 1.5%, at least 5%, or at least 10% (w/w) lecithin. A particle may comprise up to 98.5%, up to 90%, or up to 80% (w/w) lecithin.

[0061] The ratio of lecithin to carotenoid molecule in the carotenoid particle by weight may be 0.1 or more, 1 or more, 10 or more, or 20 or more. The ratio of lecithin to carotenoid molecule in the carotenoid particle by weight may be up to 1000, up to 500, up to 200, or up to 100.

[0062] The ratio of lecithin to cargo molecule in the carotenoid particle by weight may be 0.01 or more, 0.1 or more, 1 or more, or 2 or more. The ratio of lecithin to cargo molecule in the carotenoid particle by weight may be up to 100, up to 50, up to 20, or up to 10.

[0063] In some embodiments, a carotenoid particle may comprise lycopene, whey protein and lecithin in the ratio (w/w) of about 1:10:50. For example, a population of carotenoid particles in a unit dosage format may contain 3.5 mg of lycopene, 35 mg of whey protein and 175 mg lecithin.

[0064] Carotenoid particles as described herein may be packaged into chylomicrons upon absorption from the gastrointestinal tract for transport through the blood stream. The size of the particles is preferably suitable for chylomicron packaging. Carotenoid particles may be fine (100 nm to 2.5 μm), or ultrafine (1 to 100 nm). For example, the carotenoid particles may be from 0.1 nm to 1 μm in size, preferably 1 to 900 nm, more preferably 10 to 800 nm.

[0065] A suitable particle may be from 0.1 nm to 1 μm in its longest dimension (e.g. length, width, height and/or diameter). Preferably, all of the dimensions of the particle are from 0.1 nm to 1 μm.

[0066] Particle size may be determined by any convenient technique. For example, sieve analysis, laser diffraction, or photoanalysis.

[0067] A population of carotenoid particles may be uniform size (i.e. have a low size distribution) or non-uniform (i.e. have a high size distribution).

[0068] Preferably, at least 85%, at least 90%, at least 95% or at least 99% of the particles in the population display uniform size or substantially uniform size (e.g. within 5% or within 10% of the mean particle size).

[0069] A population of carotenoid particles may contain particles having a range of different shapes and sizes.

[0070] In some embodiments, a population of carotenoid particles may comprise inverse micelles in which carotenoids molecules are encapsulated by an outer later of cargo molecules, the hydrophobic structures of the cargo molecules facing the interior.

[0071] In some embodiments, a population of carotenoid particles may comprise aggregates comprising a carotenoid matrix which is embedded with hydrophobic parts of the cargo molecules. A range of different amphiphilic particles may be produced, depending on the nature and amount of the embedded and exposed regions of the cargo molecules.

[0072] When parts of the cargo molecule remain on the exterior of the carotenoid particle, the particles in the population may form clusters or aggregates. The size and shape of these clusters depends on the architecture of the cargo molecules and may also be influenced by the presence of other molecules, which may interact or complex with the cargo molecules.

[0073] Carotenoid particles as described herein may be produced by any convenient method.

[0074] In some embodiments, a population of carotenoid particles may be produced by a method comprising:

dissolving a carotenoid compound in a first solvent to produce a first solution and a cargo molecule in a second solvent to produce a second solution, and
admixing the first and second solutions under conditions which allow the cargo molecule to incorporate into the matrix of the carotenoid compound.

[0077] The carotenoid compound may be dissolved in any suitable pharmaceutically compatible solvent, for example oil, acetone, ethanol or isopropanol, most preferably ethanol or vegetable oil.

[0078] The cargo molecule may be dissolved in any suitable pharmaceutically compatible solvent. Suitable solvents include water, oil, acetone, ethanol or isopropanol. The choice of solvent will depend on the cargo molecule. For example, whey protein may be dissolved in water and resvertrol and statins, such as simvastatin, may be dissolved in ethanol. The skilled person is readily able to identify a suitable solvent for any given cargo molecule using readily available information or standard analytical techniques.

[0079] The first solvent and the second solvent may be the same or different depending on the carotenoid and cargo molecule used.

[0080] The carotenoid and the cargo molecule may be completely soluble in the first and second solvents or sufficiently soluble to facilitate incorporation of the cargo molecule, or its hydrophobic moieties, into the carotenoid matrix.

[0081] The first and second solutions may be mixed under conditions which allow the formation of matrix of carotenoid compound which incorporates the cargo molecule. For example, when an aqueous solution of cargo molecules is mixed with a solution of carotenoid compound in ethanol, a solvent/water ratio by volume of the order of 60/40 may be chosen.

[0082] Without being bound by any theory, carotenoid particles are driven to form spontaneously in solution through thermodynamics and the balance between entropy and enthalpy.

[0083] In aqueous solution, the hydrophobicity of the carotenoid compound drives the formation of particles, even though assembling molecules together into particles leads to a reduction in entropy. At very low concentrations of carotenoid, only monomers are present in true solution. As the concentration of the carotenoid increases, a point is reached at which unfavourable entropy considerations derived from the hydrophobic hydrocarbon chain of the carotenoid become dominant.

[0084] At this point, the hydrophobic end of the carotenoid is sequestered away from the water and carotenoid particles start to form. Above the critical carotenoid concentration, the entropic penalty of assembling the carotenoid monomers into particles is less than the entropic penalty of caging the carotenoid monomers with water molecules.

[0085] After mixing, the mixture of the first and second solvents may be left to stand for 30 to 60 min at a temperature slightly higher than ambient temperature. The solvents may then be evaporated or the mixture spray dried to produce a composition in emulsion or dispersion form. Evaporation may be conveniently achieved using reduced pressure (e.g. 200 to 300 mbar). The composition may then be further treated, for example by drying to produce a powder or by heat-treating to produce a gel.

[0086] In other embodiments, carotenoid particles as described herein may be produced by a method comprising:

dissolving a carotenoid compound in a first solvent to produce a first solution,
admixing the first solution with dried particles of the cargo molecule under conditions which allow the dried particles to be incorporated in liquid carotenoid droplets.

[0089] For example, lycopene dissolved in ethanol or acetone solution may be sprayed over a powder of dry particles of cargo molecule. When liquid droplets of lycopene crystallise on the surface of the powder, some of the dry particles are mechanically captured by the lycopene crystals.

[0090] The first solvent may then be dried out or evaporated to produce carotenoid particles in concentrated and/or dried form which incorporate the cargo molecule. Alternatively, the mixture of the pre-solubilised lycopene and the initially dried product may remain in a form of a suspension or emulsion in the first solvent.

[0091] In some embodiments, carotenoid particles may be produced by sonication of the mixture of first and second solutions. Sonication may be especially useful in admixing carotenoid compounds and cargo molecules which are dissolved in immiscible solvents. Ultrasound energy allows molecules to transiently cross the thermodynamic barrier imposed by the solvent environments, allowing blending and the formation of carotenoid particles, such as lycosome micelles.

[0092] In some embodiments, carotenoid particles may be produced by spray-drying the mixture of first and second solutions.

[0093] In some embodiments, lecithin may be incorporated into the carotenoid particles. In some embodiments, lecithin may be admixed with the first and second solutions. Alternatively, lecithin dissolved in oil may be admixed with the concentrated or dried admixture comprising the carotenoid particles after evaporation and/or spray drying.

[0094] While it is possible for carotenoid particles to be administered alone, it is preferable to present them as a composition (e.g., formulation), such as a food product, food additive, fortified food, dietary supplement, nutraceutical or pharmaceutical composition which comprises carotenoid particles, as defined above, together with one or more pharmaceutically or nutritionally acceptable carriers, adjuvants, excipients, diluents, fillers, buffers, stabilisers, flavourings, preservatives, sweeteners, colourings, lubricants, or other materials well known to those skilled in the art and optionally other food products, dietary supplements or nutraceutical, therapeutic or prophylactic agents.

[0095] Compositions or formulations comprising carotenoid particles as defined above, for example carotenoid particles admixed together with one or more pharmaceutically or nutraceutically acceptable carriers, excipients, buffers, adjuvants, stabilisers, or other materials, as described herein, may be used in the methods described herein.

[0096] The term “pharmaceutically acceptable” as used herein pertains to compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of a subject (e.g., human) without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Each carrier, excipient, etc. must also be “acceptable” in the sense of being compatible with the other ingredients of the formulation.

[0097] Suitable carriers, excipients, etc. can be found in standard pharmaceutical texts, for example, Remington's Pharmaceutical Sciences, 18th edition, Mack Publishing Company, Easton, Pa., 1990.

[0098] The term “nutraceutically acceptable” as used herein pertains to compounds, materials, compositions, and/or dosage forms which are in common or widespread usage in food and dietary products and are generally considered non-toxic, for example, compounds may have the US FDA designation “GRAS” (Generally Recognised as Safe), or equivalent food additive status in other jurisdictions.

[0099] The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy, food science or nutrition. Such methods include the step of bringing the carotenoid particles into association with a carrier which may constitute one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association the carotenoid particles with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

[0100] Formulations may be in the form of food products, beverages, liquids, solutions, suspensions, emulsions, elixirs, syrups, tablets, lozenges, granules, powders, capsules, cachets, pills, ampoules, ointments, gels, pastes, creams, sprays, mists, foams, lotions, oils, boluses, electuaries, or aerosols.

[0101] The carotenoid particles or compositions comprising the carotenoid particles are preferably in a form which is suitable for administration orally for delivery via the gastro-intestinal tract. Formulations suitable for oral administration (e.g., by ingestion) may be presented as discrete units such as capsules, cachets or tablets, each containing a predetermined amount of the active compound; as a powder or granules; as a solution or suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion; as a bolus; as an electuary; or as a paste.

[0102] A tablet may be made by conventional means, e.g., compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active compound in a free-flowing form such as a powder or granules, optionally mixed with one or more binders (e.g., povidone, gelatin, acacia, sorbitol, tragacanth, hydroxypropylmethyl cellulose); fillers or diluents (e.g., lactose,

microcrystalline cellulose, calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc, silica); disintegrants (e.g., sodium starch glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose); surface-active or dispersing or wetting agents (e.g., sodium lauryl sulfate); and preservatives (e.g., methyl p-hydroxybenzoate, propyl p-hydroxybenzoate, sorbic acid). Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active compound therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile.

[0103] Compositions for oral administration may further comprise sweeteners, texture modifiers, colourings and flavourings.

[0104] Aspects of the invention provide a method of producing a formulation, such as a nutraceutical or pharmaceutical composition, which has increased bioavailability of a cargo molecule comprising incorporating the cargo molecule into a carotenoid particle.

[0105] The cargo molecule may display increased bioavailability following oral administration when incorporated into a carotenoid particle relative to cargo molecule alone.

[0106] Methods of incorporating a cargo molecule into a carotenoid particle are described elsewhere herein.

[0107] Cargo molecules incorporated into carotenoid particles may be delivered via the blood stream to a tissue which expresses carotenoid receptors. Tissues which express carotenoid receptors may include hepatocytes, liver, adrenal glands, lymphocytes, lymph nodes, prostate tissues and testis. This may be useful in providing targeted delivery of an cargo molecule to a specific tissue.

[0108] An aspect of the invention provides the use of a carotenoid particle as described herein to deliver the cargo molecule to the blood stream via the gastrointestinal tract e.g. via oral administration.

[0109] Carotenoid particles as described herein may be used in methods of treatment of the human or animal body, including prophylactic treatment (e.g. treatment before the onset of a condition in an individual to reduce the risk of the condition occurring in the individual; delay its onset; or reduce its severity after onset). The method of treatment may comprise administering the carotenoid particles to an individual in need thereof.

[0110] Administration is normally in a “therapeutically effective amount” or “nutritionally effective amount”, this being sufficient to show benefit to the individual. Such benefit may be at least amelioration of at least one symptom or physiological parameter.

[0111] Determining the optimal dosage for an individual will generally involve the balancing of the level of dietary or therapeutic benefit or efficacy associated with a particular dosage of cargo molecule against any risk or deleterious side effects associated with the dosage.

[0112] The selected dosage level will depend on a variety of factors including, but not limited to, the nature and activity of the cargo molecule, the purpose of the treatment, the time of administration, the rate of excretion of the cargo molecule, the duration of the treatment, other drugs, compounds, and/or materials used in combination, and the age, sex, weight, condition, general health, and prior medical history of the individual. The amount of carotenoid particles will ultimately be at the discretion of the physician, dietician or other healthcare or wellness professional.

[0113] Administration in vivo can be effected in one dose, continuously or intermittently (e.g., in divided doses at appropriate intervals) throughout the course of treatment. Single or multiple administrations can be carried out with the dose level and pattern being selected by the supervising professional.

[0114] In general, a suitable dose of the cargo molecule is in the range of about 0.01 mg to about 1000 mg per kilogram body weight of the subject per day.

[0115] For example, when cargo molecule is whey protein, the composition may be for administration at a dose of 0.1 mg/Kg/day to 1000 mg/Kg/day of whey protein. When cargo molecule is resveratrol, the composition may be for administration at a dose of 0.1 mg/Kg/day to 100 mg/Kg/day of resveratrol. When cargo molecule is a statin, the composition may be for administration at a dose of 0.01 mg/Kg/day to 2 mg/Kg/day of statin. When cargo molecule is isoflavone, the composition may be for administration at a dose of 0.1 mg/Kg/day to 10 mg/Kg/day of isoflavone.

[0116] Where the cargo molecule is a salt, an ester, prodrug, or the like, the amount administered is calculated on the basis of the parent compound and so the actual weight to be used is increased proportionately.

[0117] Individuals suitable for treatment as described herein include individuals with a condition which is completely or partially (e.g. at least one symptom of the condition) ameliorated or alleviated by the cargo molecule, individuals at an increased risk of suffering from such a condition or patients who are predisposed to or at increased risk of suffering from such a condition, relative to the general population.

[0118] The condition which is ameliorated or alleviated by the cargo molecule will depend on the nature of the cargo molecule.

[0119] For example, a carotenoid particle comprising whey protein as described herein may be useful in treating Chlamydia infection, liver infections and/or in lowering cholesterol, for example in individual with elevated cholesterol levels or hypercholesterolemia.

[0120] A carotenoid particle comprising a statin, may be useful in the treatment and/or prevention of cardiovascular disease, dementia, hypertension, cancer, including lung cancer, cataracts, and elevated cholesterol or hypercholesterolemia. Carotenoid particles comprising statins may also be useful in the treatment and/or prevention of other diseases and conditions which may be ameliorated by the pleotropic effects of statins, but for which statin treatment has not previously been use because of possible side effects, such as diabetes, in particular type II diabetes, and Alzheimer's disease.

[0121] Carotenoid particles comprising resveratrol may be useful in the treatment and/or prevention of metabolic syndrome or one or more symptoms thereof, such as elevated cholesterol and/or triglycerides, diabetes, cardio- and cerebro-vascular disease, cancer, acute and chronic bacterial, fungal and viral infections, Alzheimer's and other neurodegenerative diseases, gastointestinal tract diseases, connective tissue disease, arthritis, and inflammatory conditions, as well as in anti-aging and beauty products and increasing wellness and longevity.

[0122] Carotenoid particles comprising isoflavones may be useful in the treatment and prevention of metabolic syndrome or one or more symptoms thereof, such as elevated cholesterol and/or triglycerides, diabetes, cardio- and cerebro-vascular disease, cancer, Alzheimer's and other neurodegenerative diseases, connective tissue diseases, and inflammatory conditions, as well as in anti-aging and beauty products and increasing wellness and longevity.

[0123] Various further aspects and embodiments of the present invention will be apparent to those skilled in the art in view of the present disclosure.

[0124] All documents mentioned in this specification are incorporated herein by reference in their entirety.

[0125] “and/or” where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example “A and/or B” is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein.

[0126] Unless context dictates otherwise, the descriptions and definitions of the features set out above are not limited to any particular aspect or embodiment of the invention and apply equally to all aspects and embodiments which are described.

[0127] Certain aspects and embodiments of the invention will now be illustrated by way of example and with reference to the figures and tables described below.

[0128] FIG. 1 shows the effect of whey protein on C. trachomatis in McCoy cells.

[0129] FIG. 2 shows the effect of incorporation of 100 mg resveratrol into lycosome particles on bioavailability. Data shows the combined serum concentrations of resveratrol and its metabolites in serum in ng/ml.

[0130] FIG. 3 shows comparative pharmacokinetics of two trans-Resveratrol products, free form and embedded into lycopene clusters.

[0131] FIG. 4 shows the effect on plasma cholesterol (FIG. 4A), plasma LDL (FIG. 48), and plasma HDL (FIG. 4C) of simvastatin alone at 20 mg, 40 mg and 80 mg daily dose and simvastatin (20 mg) incorporated into a lycopene particle (“Lycostatin™”).

[0132] FIG. 5 shows the combined isoflavone concentrations in ng/ml in the serum of SI only patients (green), SI+lycopene patients (red) and SI in lycopene particles patients (blue).

[0133] FIG. 6 shows the average serum concentrations of genistein in SI+lycopene patients (red) and SI in lycopene particles patients (blue).

[0134] FIG. 7 shows the average serum concentrations of daidzein in SI+lycopene patients (red) and SI in lycopene particles patients (blue).

[0135] FIG. 8 shows the average serum concentrations of lycopene in ng/ml in SI+lycopene patients (red) and SI in lycopene particles patients (blue).

[0136] FIG. 9 shows the serum concentrations of soy isoflavones in the serum of SI only patients.

[0137] FIG. 10 shows the serum concentrations of soy isoflavones in the serum of SI in lycopene particles patients (blue).

[0138] Table 1 shows the effect of WP and lycopene products on anti-Chlamydia IgG in CHD patients

[0139] Table 2 shows the effect of WP and lycopene products on serum cholesterol in CHD patients.

[0140] Table 3 shows the serum concentrations of trans-resveratrol 3-sulfate in ng/ml in patients administered 120 mg resveratrol alone or in a lycopene particle.

[0141] Table 4 shows the serum concentrations of trans-resveratrol 4'-o-ß-D-glucuronide in ng/ml in patients administered 120 mg resveratrol alone or in a lycopene particle.

[0142] Table 5 shows the area under the curve (AUC) for the pharmacokinetics of two trans-Resveratrol products, free form and embedded into lycopene clusters, as shown in FIG. 3.

[0143] Table 6 shows a comparison of the metabolic effect of soy-isoflavone (SI) administered with free lycopene (SI+lycopene) or incorporated into a lycopene particle (SI-lycosome).

EXPERIMENTS

1. Effect of Whey Protein on Chlamydia

[0144] The link between persistent Chlamydia infection and development of atherosclerosis has been has been established more than 25 years ago [1, 2]. Until recently a potential causative role of this infection has been occasionally questioned but remains unanswered. However, in the last year a number of publications started to shed some light on processes which may lay behind changes in lipid/cholesterol metabolism triggered by Chlamydia infection [3-5].

[0145] In this study, we investigated whether whey protein has anti-bacterial and in particular anti-Chlamydia properties.

1.1 Methods

Whey Protein

[0146] 10 mg of 100% whey protein (Multipower) were dissolved in 1 ml of PBS. Two fold dilutions were prepared in RPMI and used for cell culture.

Cell Culture and Organisms

[0147] McCoy cells were cultured in 5% CO2 in RPMI supplemented with 10% Fetal Bovine Serum (FBS) and 2 mM glutamine. Cells were grown in 24 well plates with round glass coverslips. Strain L2/Bu434 of C. trachomatis was kindly provided by Dr. P. Saikku (University of Oulu, Finland). Chlamydial strain was initially propagated in McCoy cells and purified by Renografin gradient centrifugation as described [6]. Chlamydial titers were determined by infecting McCoy cells with 10-fold dilutions of thawed stock suspension. Purified elementary bodies (EB) with known titer were suspended in sucrose-phosphate-glutamic acid buffer and used as inoculums for McCoy cells.

Cells Infection

[0148] McCoy plates were infected with C. trachomatis at multiplicity rate 2:1 in RPMI with 5% FBS and without cycloheximide and centrifuged for 1 hour at 1500 g at 25° C. Whey protein at concentration of 0.007-0.5 mg/ml was added to infected cells and plates were inoculated for 48 hours at 5% CO2 at 37° C.

Immunofluoresence Staining

[0149] Infected McCoy monolayers grown on coverslips in 24 well plates in the presence of different concentrations of Whey protein were fixed with methanol. Permeabilized cells were stained by direct immunofluoresence using FITC-conjugated monoclonal antibody against chlamydial lipopolysaccharide (NearMedic Plus, RF). Inclusion-containing cells were visualized using Nikon Eclipse 50i microscope fluorescence microscope at ×1350 magnification.

1.2 Results

[0150] Whey protein was observed to have a dose dependent effect on Chlamydia inclusions in McCoy cells (FIG. 1).

2. Production of Lycopene Particles

[0151] The main principle for the production of lycopene particles (Lycosomes™) is to facilitate the incorporation of the selected cargo molecules into the lycopene matrix.

[0152] This may be achieved by pre-solubilisation of lycopene and the cargo molecules in the same solvent, for example ethanol. Alternatively, the cargo molecules and lycopene may be dissolved in different solvents, for example, two different organic solvents, or an organic solvent and an oil. Preferably, the cargo molecules and lycopene are soluble either in full or in part in both solvents.

[0153] On the second step lycopene solution should be blended/mixed with the solution(s) of the product(s), under conditions which allow for incorporation the molecules of the products, or their parts, into the lycopene matrix.

[0154] Then the solvent(s) can be fully or partially evaporated which provides a residual substance in a dried, or a concentrated liquid form.

[0155] Production of lycopene particles may also be achieved by completely or partially dissolving lycopene in a solvent and spraying it over, or mixing it with cargo molecules in a dry, powdered form. The dried particles of the cargo molecules are then captured by the lycopene droplets. The lycopene solvent may then be dried out or evaporated to produce solid particles.

[0156] Alternatively, the mixture of solubilised lycopene and dried cargo molecules can stay in a form of suspension.

[0157] Techniques applied for preparation of the lycosome products in the examples described in this application are described below. For example, WP-Lycosomes were prepared using organic solvent(s) on the initial stage then vegetable oil; Resveratrol-Lycosomes and LycoStatin were prepared by separately dissolving lycopene and Simvastatin in ethanol, mixing them in the chosen ration and subsequently evaporating the solvent from the mixture, or spray-drying the mixture; IS-Lycosomes were prepared by mixing an ethanol (full or partial) solution of lycopene with granulated soy-isoflavones.

3. Preparation of Whey Protein Lycosomes

[0158] Lycopene particles incorporating whey proteins (WP-lycosomes) were produced as described in US20020107292. Briefly, 13.3 kg of whey protein isolate were dissolved in 330 l of demineralized water and the mixture stirred for 6 hours at 25-30° C. Separately, 550 g of Lycored™ oleoresin (LycoRed Corp NJ USA) comprising 6% of lycopene, was mixed in 438 l of acetone and the mixture was stirred.

[0159] The two solutions were subsequently mixed for 60 min at 30° C. The final mixture was moderately heated and the acetone was driven off at a moderate pressure. Finally, water was partially driven off at a pressure of 40-50 mbar. An aqueous solution of 200 kg of whey protein isolate and of oleoresin was obtained, which was subsequently spray dried.

[0160] Following spray drying, 186 g of soy lecithin in vegetable oil was added and mixed at 30° C. with the mass of the product.

[0161] Lycopene particles incorporating reveratrol were prepared using a spray dry method. The formulation components were prepared in absolute ethanol.

[0162] The material was prepared as follows:

[0000] 5 g 99% trans-resveratrol
3.333 g Lyc-O-Mato 15% OS (i.e. 500 mg lycopene)
0.110 g lecithin
Ratio: 100 mg resveratrol:10 mg lycopene:2.2 mg lecithin

[0163] 5 g resveratrol was dissolved in 100 ml absolute ethanol. 3.333 g Lyc-O-Mato 15% and 110 mg lecithin were dissolved in a separate volume of 100 ml absolute ethanol. The two solutions were mixed together and spray dried at 56° C. under nitrogen. The resulting powder was encapsulated as 203 mg per size 0 capsule to give a dose of 120 mg resveratrol/12 mg lycopene.

[0164] In other experiments, 20 g of 99% trans-Resveratrol was dissolved in 100 ml of 95% of ethanol, and 35 mg of tomato oleoresin (10% lycopene) was dissolved separately in 95% ethanol. Then the both solvents were mixed for 60 mins at 30° C. at then spray-dried.

[0165] Lycopene particles incorporating simvastatin were prepared using an evaporation method.

[0166] 180 size 0 capsules were prepared, each contained 10 mg lycopene and 20 mg simvastatin along with 106 mg lecithin.

[0167] An evaporation method was used and the resulting solid material was collected and ground in a mortar. The powder obtained was used for encapsulation. This method is comparable to rotogranulation of larger quantities of material in a commercial environment.

[0168] The production method for this 180 dose batch was as follows:

[0000] 19 g lecithin
48.5 g cornflour
3.6 g simvastatin
18 g Lyconattm 10% CWD (Vitatene Ltd) (i.e. 1.8 g lycopene) Total solids: 89.1 g

[0169] The 18 g Lyconat 10% CWD was dispersed in 180 ml RO H2O and made up to 1,800 ml with absolute ethanol to give a dispersion in 90% ethanol. 3.6 g simvastatin was dissolved in 90 ml ethanol, added to the lycopene dispersion in 90% ethanol and allowed to blend. 19 g lecithin was then added to the mixture and allowed to blend with the lycopene/simvastatin. The solvents were then evaporated.

[0170] The resulting solid was collected, ground to a powder and encapsulated. 1 dose contained 495 mg in a size 0 capsule. Each dose was composed of 10 mg lycopene, 106 mg lecithin, 20 mg simvastatin 269 mg cornflour, 90 mg starch from Lyconat 10% CWD

[0171] In other experiments, 20 g of Simvastatin was dissolved in 100 ml of 95% of ethanol, and 7 g of lycopene from Vitatene was dissolved separately in 95% ethanol. Both solvents are mixed for 60 mins at 30° C. and then spray-dried.

[0172] Lycopene particles incorporating soy isoflavones were prepared using an evaporation method.

[0173] 550 g of Lycored™ oleoresin (LycoRed Corp NJ USA) comprising 6% of lycopene, was mixed in 438 l of acetone (95% ethanol might be used as an alternative) and the solution was stirred. The lycopene solution was then mixed with soy isoflavones in a powdered form and the mixture spray dried.

[0174] In other experiments, lycopene particles incorporating soy isoflavones were produced by dissolving 550 g of Lycored™ oleoresin (LycoRed Corp NJ USA) comprising 6% of lycopene, was mixed in 438 l of ethanal and separately dissolving soy isoflavones in water.

[0175] The two solutions were subsequently mixed in the ratio of 50 g SI to 7 g or 14 g lycopene for 60 min at 30° C. The final mixture was moderately heated and the ethanol was driven off at a moderate pressure. Finally,

water was partially driven off at a pressure of 40-50 mbar and the resultant solution spray dried.

4. Effects of Whey Protein Incorporated into Lycopene Particles

[0176] To verify the potential effect of WP-Lycosomes™, a clinical trial was undertaken.

[0177] 20 Coronary Heart Disease (CHD) patients who were positive for anti-Chlamydia IgG and hypercholesterolaemia were identified. These patients were randomised in 4 groups of 5 patients, and each of them received daily either in:

1<st >group—7 mg of lycopene supplement (in 70 mg of tomato oleoresin), or
2<nd >group—700 mg of WP, or
3<rd >group—mechanical mixture of 7 mg of lycopene (in 70 mg of tomato oleoresin), and 70 mg of WP, or
4thgroup—WP-Lycosome comprising of 7 mg of lycopene (in 70 mg of tomato oleoresin) and 70 mg of WP.

[0182] Serum anti-Chlamydia IgG and total serum cholesterol were measured after 4 weeks.

[0183] The results show that WP itself has no ability to affect the level of Chlamydia infection in these patients, in terms of the specific IgG, or cholesterol concentration (Tables 1 and 2).

[0184] Lycopene on its own has some ability to reduce Chlamydia infection, but its effect is only observed from the 2<nd >week of its administration onwards and the total sero-negativity for all patients was only achieved in the last week of the trial.

[0185] Mechanical mixing of lycopene with whey protein substantially diminished the ability of lycopene to reduce Chlamydia infection and 4 out of 5 patients (80%) remained sero-positive by the end of the trial (4 weeks).

[0186] Lycopene on its own was observed to have a measurable effect on serum cholesterol. After 4 weeks it reduced cholesterol by 0.7 mmol/L. Mechanical mixing of lycopene with whey protein also substantially diminished this cholesterol-lowering effect.

[0187] However, whey protein incorporated into lycopene particles, as described herein (WP-Lycosome™) displayed a profound and very rapid effect on both Chlamydia infection and cholesterol levels. Anti-Chlamydia IgG were cleared from all patients serum by the end of the 1<st >week of the trial. Cholesterol levels in patients treated with WP-Lycosomes displayed a significantly deeper reduction than that produced by lycopene on its own (by 2 mmol/L).

[0188] These results show that, on top of “mild” anti-infective and cholesterol lowering properties of lycopene itself, there is a significant synergetic effect of the whey protein when it is incorporated into lycosomes.

[0189] By contrast, mechanical mixing of whey protein and lycopene was found to inactivate the latter without increasing the activity of the former.

[0190] These results show that incorporating whey protein into lycopene particles allows the anti-bacterial potential of the whey protein to be delivered to the liver.

[0191] These cell culture tests show that whey protein has a direct anti-Chlamydia effect. This effect has not been shown for lycopene. The effects of the whey protein are concentration dependent. There is no increase in lycopene concentration in carotenoid particles relative to lycopene itself. This indicates that the effect is due to Whey protein.

[0192] Although lycopene has been shown to reduce the symptoms of infection in vivo, this effect may be linked to its anti-oxidative and/or anti-inflammatory properties, and is generally evident after about 4 weeks. By contrast, whey protein acts much faster, and symptoms of Chlamydia infection, such as specific IgGs, disappear from the blood within days.

5. Effects of Resveratrol Incorporated into Lycopene Particles

[0193] To verify the potential effect of lycosome technology on Resveratrol bioavailability a pharmacokinetic study on volunteers was undertaken.

[0194] Resveratrol was incorporated into lycopene particles as described herein.

Clinical Protocol

[0195] The group of 5 volunteers comprised of 2 female and 3 male clinically healthy Caucasian persons, age between 23 and 35 years. They were asked, before commencing this experiment, to go for 3-4 days “wash-out”, when consumption of any grape, wine, peanut, chocolate and other products which might contain them.

[0196] In the morning of the experiment, one hour after light breakfast, volunteers were given 1 gelatine capsule containing 100 mg of a trans-Resveratrol product, tRSV. Blood samples were taken from their median cubital or cephalic veins at the baseline point. Then, after administration of tRSV, their blood was taken again at the following time points: 30 minutes, 1 hour, 2, 3, 4, 6 and 8 hours. After the 4 hours time point, volunteers had a light lunch which did not involve consumption of any grape, wine, peanut, chocolate and other products which might contain them.

[0197] After taking blood its serum was separated, aliquoted and stored at -80° C. for further testing. The study was blinded, cross-over, and each participant was involved in testing of all three resveratrol products.

[0000] tRSV Products

[0198] Two separately manufactured batches of tRSV-Lycosome, and one batch of tRSV itself. Resveratrol for all these products was from the same batch of the same manufacturer.

[0000] 5a. Study on Bioavailability of the Total Resveratrol

[0199] The results of this study are presented in the table 3 and table 4. These results demonstrate that, when resveratrol was administered in a form of lycosome, the level of two its major metabolites of, 3-sulfate and 4'-o-ß-D-glucuronide, was about 2-3 fold higher than when Resveratrol was administered on its own.

[0200] A comparison of the pharmacokinetics of all main resveratrol metabolites is presented in the FIG. 2. These data show that administration of resveratrol within a lycopene particle (i.e. a lycosome) increases bioavailability relative to the same 100 mg dose of resveratrol on its own.

[0000] 5b. Study on Bioavailability of Unmodified Trans-Resveratrol

[0201] A comparative pharmacokinetics of two trans-Resveratrol products, free form and embedded into lycopene clusters, is presented in the FIG. 3. A comparison of area under the curve, AUC, for two these products is presented in the Table 5. These results showed that incorporation of tRSV into lycopene was able to deliver this molecule in unmodified form, into the human blood, about 10 times more than when it was administered in free crystals.

6. Effects of Statin Incorporated into Lycopene Particles

[0202] 18 CHD patients with hypercholesterolaemia were randomised in 5 equal groups:

in 1<st >group—patients received daily 1 capsule of 7 mg of lycopene supplement,
in 2<nd >group—patients received daily 1 capsule 20 mg of Simvastatin,
in 3<rd >group—patients received daily 1 capsule 40 mg of Simvastatin,
in 4thgroup—patients received daily 1 capsule 80 mg of Simvastatin,
in 5thgroup—patients received daily 1 capsule of Lycosome-Simvastatin (Lycostatin™), which was comprised of 7 mg of lycopene and 20 mg of Simvastatin.

[0208] Capsules were of the same colour and size and all ingredients for Lycostatin™ were from the same batches of the same manufacturers as for the separated products.

[0209] The results of this trial are presented in FIGS. 4A to 4C.

[0210] Since in the lycopene control group there were significant changes in serum concentrations of tested lipid these results were not presented in the FIGS. 4A to 4C.

[0211] At the same time all Simvastatin-contained products demonstrated significant ability to reduce total and LDL-cholesterol. There was clear dose-dependency in three groups which received free Simvastatin.

[0212] However, in the group which received 20 mg, the smallest dose of the drug, but embedded into the

lycopene clusters, there were most powerful reduction in concentrations of total and LDL-cholesterol. Both the rate and the level of this reduction were more profound even than in the group which received 80 mg of free Simvastatin.

[0213] This provides indication that lycosome technology focuses drug delivery to the liver, which may potentially result in the reduction of the dose of the statin used, and consequently minimise its side effects.

7. Effects of Incorporation into Lycopene Particles of Soy Isoflavone

[0214] Soy, particularly soy isoflavones, are one of the key components of the Oriental Diet responsible for the prevention of the development of Metabolic Syndrome and Diabetes.

[0215] However, bioavailability and efficacy problems have been encountered in the extraction of isoflavones from their natural matrix and the development of dietary isoflavone supplements. Isolated isoflavones do not match the beneficial metabolic effects of the isoflavones within in a food matrix, even at the same dosages as the usual soy contained in the Oriental Diet.

[0216] One option to address low bioavailability is to increase the dose of isolated isoflavone which is administered. Increased dosages may lead to significantly increased concentrations of isoflavones in the blood and subsequently in the tissues, which may in turn, activate estrogenic hormone receptors. Although estrogenic hormone receptor activation is already used as a part of hormone-replacement therapy in post-menopausal women, activation of these receptors would not be desirable in women of other age groups or in men.

[0217] Another option is to focus the delivery of the isoflavones to the main metabolic organ (the liver) without increasing overall levels in the blood stream. Lycopene or other carotenoid compounds may be used as carriers to target the liver, which is rich with carotenoid receptors.

[0218] Soy Isoflavones were incorporated into lycopene particles (SI-Lycosomes™) and their metabolic activity and pharmacokinetics were compared with those of two other products: SI on its own and SI mechanically mixed with lycopene.

[0219] 42 patients with Metabolic Syndrome, elevated total cholesterol and/or triglycerides, were randomised in 3 equal groups:

in 1<st >group—patients received daily 50 mg of SI,
in 2<nd >group—patients received daily of the mechanical mixture of 50 mg of SI and 7 mg of Lycopene, [SI+Lycopene],
in 3<rd >group—patients received SI-Lycosome™, 50 mg:7 mg of SI and Lycopene respectively as a daily dose.

[0223] 3 patients in the second group and 4 in the third left the trial for low compliance reasons. Therefore only 34 patients managed to complete the trial.

[0224] Capsules used in all three groups were of the same colour and size and all ingredients for SI-Lycosomes™ were from the same batches of the same manufacturers as for the separated products.

[0225] Lipid parameters were measured and the results shown in Table 6. These results show that neither SI alone or mechanically mixed with lycopene had any significant effect on any analysed parameters of lipid metabolism in patient serum after 1 month of administration.

[0226] Mechanical mixing of SI with lycopene resulted in the significnat reduction of the absorption of the isoflavones, which was registered both in this (FIG. 5), and in additional 24 h pharmacokinetic trials (FIG. 6, 7).

[0227] However, the same dose of SI delivered in a lycopene particle has a significant lowering effect on elevated triglycerides, total cholesterol, LDL and Apo-proteins.

[0228] The observed metabolic effect of SI-Lycosome™ is unlikely to be due to the lycopene component itself because the increase in the lycopene concentration in serum of patients after 1 month of the administration of SI-Lycosome™ was about 3 times lower than in the group of patients who received the same dose of lycopene but in the mechanical mixture with SI. The increment in the former group was 150 ng/ml and in the latter 50 ng/ml (FIG. 8).

[0229] FIGS. 9 and 10 show that incorporation of SI into lycopene particles does not create a new serum profile of isoflavones, compared with the free SI.

[0230] Therefore, results herein show that the metabolic efficacy of SI is significantly boosted by incorporation into lycopene particles without increase of SI level sin blood. This liver response is possible due to the targetting of the liver by isoflavones incorporated into the carotenoid particle.

[0231] Since carotenoids get absorbed to a significant degree via mechanical pathways, as independent physical particles and/or as a part of chylomicron, without their chemical modification, they can serve not only as a protective parcel but also as a protective currier or vehicle for the incorporated molecules or substances which could deliver them into the circulation in unmodified form.

[0232] Therefore, if some molecules or compounds are captured, in full or in part, by lycopene molecules this can provide some protection, from such GIT factors as enzymatic degradation, oxidation, stomach acidity, gut flora, etc. The outcome of this could be an increase in absorption of these vulnerable substances and their delivery to the liver in their unmodified forms, i.e. increase in their bioavailability.

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[0000]
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[0000]
TABLE 1

Effect of different Lycopene products on Chlamydia pneumoniae load in IgG-ELISA*

Product	0 w	1 w	2 w	3 w	4 w
Controls:					
GA Lycopene 7 mg	0.997 ± 0.098 (10)**	(10)	(4)	(2)	0.288 ± 0.043 (0)
	$p < 0.001$				
Whey protein	0.976 ± 0.102 (10)	(8)	(10)	(8)	0.842 ± 0.095 (5)
700 mg	$p > 0.05$				
Mechanical	0.755 ± 0.091 (10)	(10)	(4)	(8)	0.510 ± 0.069 (4)
mixture of	$p < 0.01$				
Lycopene 7 mg +					
Whey protein					
70 mg					
WP-lycosome	1.047 ± 0.136 (10)	(0)	(0)	(0)	0.211 ± 0.054 (0)
"Delox"	$p < 0.001$				
Lycopene 7 mg +					
Whey protein					
70 mg					

*ELISA reading, below 0.300-0.400 is considered to be negative
**number of sero-positive patients

[0000]
TABLE 2

Cholesterol lowering effect of WP-lycosome

Total serum Cholesterol, in mmol/L	Total serum Lycopene, in ng/ml

Product 0 w 4 w ?* 0 w 4 w ?*
Controls:
GA Lycopene 7 mg 5.4 ± 0.23 4.7 ± 0.21 -0.7 179 ± 21 295 ± 23 +116
p < 0.05 p < 0.001
Whey protein 5.6 ± 0.35 5.5 ± 0.19 -0.1 192 ± 18 168 ± 15 -24
700 mg P > 0.05 P > 0.05
Mechanical mixture 5.2 ± 0.32 4.6 ± 0.35 -0.6 209 ± 22 173 ± 18 -36
of Lycopene 7 mg + p > 0.05 p > 0.05
Whey protein 70 mg
WP-lycosome 6.2 ± 0.36 4.2 ± 0.18 -2.0 124 ± 14 232 ± 17 +108
“Delox” p < 0.01 p < 0.001
Lycopene 7 mg +
Whey protein 70 mg

*difference in the parameter after 4 weeks of administration

[0000]
TABLE 3

Trans-resveratrol 3-sulfate in ng/ml
max concentration
Resveratrol-Lycosome
batch 1 Resveratrol-Lycosome batch 2 Resveratrol
ID 120 mg 120 mg 120 mg
1 1960 3230 1710
2 1900 1450 729
3 662 1810 415
4 2690 464 1056
5 824 2030 648
8036 8984 4558

[0000]
TABLE 4

Trans-resveratrol 4'-o-ß-D-glucuronide in ng/ml
max concentration
Resveratrol-Lycosome
batch 1 Resveratrol-Lycosome batch 2 Resveratrol
ID 120 mg 120 mg 120 mg
1 589 1000 289
2 1170 1130 73
3 173 306 140
4 891 266 361
5 781 732 205
3604 3434 1068
AUC for 24 Hours

[0239]

[0000]
TABLE 5

Volunteer ID
100 mg trans-Resveratol 1 2
Resveratrol-Lycosome?™ 02 1600 1900
Resveratrol-Lycosome?™ 03 1140 2890
Crystal Resveratrol 432 275

[0000]
TABLE 6

SI
gender age TC TG HDL LDL ApoA ApoB AST ALT

before

m 57 214 131 40 150 120 117 41 56
m 50 257 89 39 182 180 90 42 78
m 73 223 182 49 160 175 140 30 37
m 71 225 126 40 120 139 92 19 30
m 70 203 101 45 100 150 88 20 22
f 55 230 118 49 110 155 100 19 20
m 51 254 128 37 159 200 119 33 28
f 70 237 96 54 101 170 91 20 15
m 70 218 109 48 118 160 98 27 17
f 56 240 119 36 140 170 105 23 24
f 54 210 110 46 118 140 100 30 14
m 54 238 162 37 135 180 117 23 31
f 72 229 105 40 130 149 118 9 18
m 62 230 138 49 144 155 120 15 21
61.8 229 122 43.5 133 160 107 25.1 29.4
1 month after
200 130 40 150 121 117 32 52
240 89 39 178 172 90 40 75
220 176 49 160 172 140 25 36
222 120 40 120 137 90 19 27
200 100 45 100 149 86 20 22
230 113 49 120 150 100 18 20
247 129 37 157 200 119 23 26
240 95 53 104 170 92 17 17
210 100 48 112 145 92 26 19
236 119 37 140 172 105 22 24
205 113 46 118 139 100 25 14
233 159 37 133 181 117 23 32
228 111 38 126 144 110 10 16
235 140 48 143 155 122 14 19
225 121 43.3 133 158 106 22.4 28.5

SI + Lycopene
gender age TC TG HDL LDL ApoA ApoB AST ALT
before
m 70 220 161 32 150 173 140 27 32
f 81 222 200 40 180 169 173 22 37
f 43 164 140 37 120 144 130 18 25
m 48 218 96 51 123 140 93 24 49
m 57 227 93 40 127 130 78 32 42
f 58 250 200 35 180 177 149 11 29
f 70 213 74 45 130 150 80 30 17
m 70 232 137 41 127 139 119 21 19
f 48 237 163 39 170 160 130 20 28
f 72 242 146 40 152 180 121 19 38
m 47 240 140 37 155 170 119 13 43
60.36 224 141 39.7 147 157 121 21.5 32.6
1 month after
193 150 33 140 170 137 20 30
227 200 40 178 170 170 19 35
175 137 37 121 144 130 17 24
220 108 50 125 141 94 23 46
229 100 40 125 133 79 27 40
241 200 36 177 172 140 10 25
212 76 45 130 150 80 23 18
230 138 41 126 136 120 20 17
224 160 39 170 155 130 20 27
247 149 40 152 180 120 17 35
246 146 37 155 172 120 12 39
222 142 39.8 145 157 120 18.9 30.5

SI-Lycosome

gender	age	TC	TG	HDL	LDL	ApoA	ApoB	AST	ALT
before									
m	68	219	187	47	195	193	170	16	33
m	55	200	175	38	181	155	201	37	47
f	70	200	163	58	195	180	159	22	37
f	51	224	173	64	186	170	190	18	19
m	63	209	208	48	205	199	185	19	17
f	66	210	196	51	148	173	167	34	31
f	73	231	184	58	197	190	150	27	40
m	55	221	167	47	300	220	170	100	110
f	46	201	163	40	215	152	193	30	29
		60.78	213	180	50.1	202	181	176	33.7	40.3
1 month after									
		170	148	50	167	175	160	16	29
		167	139	42	150	140	191	30	40
		190	138	58	180	175	150	19	32
		188	153	62	178	168	162	18	19
		180	120	50	177	189	172	17	17
		170	130	53	132	160	160	30	30
		220	178	58	190	190	143	26	45
		180	149	48	230	220	170	80	100
		183	156	42	186	150	190	27	24
		183	146	51.4	177	174	166	29.2	37.3