

**SYNERGIC COMPOSITIONS AND METHODS FOR INHIBITING COLLAGEN
LOSS**

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of U.S. Provisional Application No. 63/539,129, titled "SYNERGIC COMPOSITIONS AND METHODS FOR INHIBITING COLLAGEN LOSS", filed 19 September 2023, the contents of which are incorporated herein by reference in their entirety.

FIELD OF THE INVENTION

[0002] The present invention relates generally to the field of carotenoids and ceramides, and methods of using the same, such as for treating skin-related conditions, including collagen loss.

BACKGROUND

[0003] Dermal collagen represents the most abundant extracellular matrix (ECM) protein in human skin. The major components of the dermis are fibroblasts that produce, organize, and maintain collagen and elastic fibers. Collagen-1 is the main constituent of the dermal ECM composing approximately 85-90% of the total collagen in the skin and playing a major role in skin elasticity. Its synthesis is reduced in aged and photodamaged skin, thus, contributing to the wrinkled appearance of skin.

[0004] Inflammation and accumulation of reactive oxygen species (ROS) are believed to be the causative factors in aged and photodamaged skin. Several studies have linked the immune system and inflammation genes with photoaging. UV induces an array of events that can lead to inflammation: release of inflammatory cytokines such as Interleukin-1, ROS generation, production of inflammatory mediators, peroxidation of the membrane lipids, and skin cell death.

[0005] Neutrophils have been reported to infiltrate the skin within the epidermis and dermis following exposure to natural sunlight of UVB and solar simulating radiation (SSR),

infrared radiation, and heat. These neutrophils are packed with potent proteolytic enzymes for the clearance of UV-induced apoptotic cells and for killing skin cells with oxidized surface lipids, but they are also capable of degrading collagen and elastic fibers. Furthermore, it was reported that infiltrating neutrophils, rather than keratinocytes and fibroblasts, were the major source of proteolytic enzymes and free radicals following exposure to erythemogenic doses of SSR, thus neutrophils may be the key players in photoaging. Collagen is the only protein susceptible to fragmentation by superoxide anion as demonstrated by the liberation of small 4-hydroxyproline-containing-peptides. Hydroxyl radicals in the presence of oxygen or hypochlorous acid cleave collagen into small peptides, and the cleavage seems to be specific to proline or 4-hydroxyproline residues.

[0006] There is still a great need for compositions and methods for preventing collagen damage induced by activated neutrophils.

SUMMARY

[0007] The present invention, in some embodiments, is based, at least in part, on the surprising findings that a composition comprising a carotenoid mixture comprising phytoene, phytofluene, and zeta carotene, combined with ceramide provided a synergic effect in the prevention of collagen loss, and inhibition of myeloperoxidase (MPO) activity, compared to any one of the carotenoid mixture and ceramide alone.

[0008] The present invention, in some embodiments, is further based, at least in part, on the surprising finding that subjecting cells to the claimed composition prevented more than 80% collagen loss induced by neutrophils ex-vivo.

[0009] According to a first aspect, there is provided a composition comprising: (a) a carotenoid mixture comprising phytoene, phytofluene, and zeta carotene; and (b) a ceramide; wherein a weight per weight ratio (w/w) of the (a) and the (b) in the composition is between 25:1 (w/w) and 500:1 (w/w).

[0010] According to another aspect, there is provided a pharmaceutical composition comprising an effective amount of the composition of the invention, and a pharmaceutically acceptable carrier.

[0011] According to another aspect, there is provided a method for preventing or treating a collagen-loss related disease or disorder in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of the invention, thereby preventing or treating a collagen-loss related disease or disorder in the subject.

[0012] According to another aspect, there is provided a method for reducing the amount, activity, or both, of any one of: MPO, NO, superoxide, and any combination thereof, in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the composition of the invention.

[0013] According to another aspect, there is provided a kit comprising: (a) at least one carotenoid selected from the group consisting of: phytoene, phytofluene, zeta carotene, and any combination thereof; and (b) a ceramide.

[0014] In some embodiments, the carotenoid mixture comprises the phytoene in the amount of 55-65% (w/w) of the total carotenoids in the carotenoid mixture; the phytofluene in the amount of 10-20% (w/w) of the total carotenoids in the carotenoid mixture; and the zeta carotene in the amount of 15-25% (w/w) of the total carotenoids in the carotenoid mixture.

[0015] In some embodiments, a w/w ratio of the phytoene and the phytofluene combined to the zeta carotene ranges from 15:1 (w/w) to 2:1 (w/w).

[0016] In some embodiments, the carotenoid mixture further comprises an additional carotenoid selected from the group consisting of: lycopene, beta carotene, gamma carotene, and any combination thereof.

[0017] In some embodiments, the composition comprises the lycopene in the amount of less than 5% (w/w) of the total carotenoids in the carotenoids mixture, the beta carotene in the amount of less than 5% (w/w) of the total carotenoids in the carotenoids mixture, the gamma carotene in the amount of 0.2-1.5% (w/w) of the total carotenoids in the carotenoids mixture, or a combination thereof.

[0018] In some embodiments, the carotenoid mixture comprises a total carotenoid amount of 10-15% (w/w) of the carotenoid mixture.

[0019] In some embodiments, the carotenoid mixture further comprises a tocopherol in the amount of 10-30% (w/w) of the total carotenoids in the carotenoid mixture.

[0020] In some embodiments, the carotenoid mixture further comprises a phytosterol in the amount of 5-15% (w/w) of the total carotenoids in the carotenoid mixture.

[0021] In some embodiments, the ceramide comprises a plurality of types of ceramides.

[0022] In some embodiments, any one of the ceramide and plurality of types thereof, is selected from the group consisting of: C2-ceramide, C1 ceramide, C3 ceramide, C6-ceramide, Ceramide 9.

[0023] In some embodiments, the composition has a synergistic effect in inhibition of: (i) collagen-loss; (ii) production, secretion, activity, or any combination thereof of any one of: nitric oxide (NO), myeloperoxidase (MPO), superoxide (SO), and any combination thereof; and (iii) both (i) and (ii).

[0024] In some embodiments, the collagen is collagen-1.

[0025] In some embodiments, the pharmaceutical composition is for use in prevention or treatment of a collagen-loss related disease or disorder in a subject in need thereof.

[0026] In some embodiments, the collagen-loss related disease or disorder comprises collagen loss in skin of the subject.

[0027] In some embodiments, the subject is afflicted with a collagen loss-related disease or disorder.

[0028] In some embodiments, the collagen loss-related disease is selected from the group consisting of: an age-related disease, a skin disease, and an inflammatory disease.

[0029] In some embodiments, the skin disease comprises skin damage induced by any one of: radiation, oxidative stress, DNA damage, telomere shortening, inflammation, tobacco usage, and any combination thereof.

[0030] In some embodiments, the radiation comprises UV radiation, visible light radiation, infrared radiation, or any combination thereof.

[0031] In some embodiments, the UV radiation comprises UVA, UVB, UVC, or any combination thereof.

[0032] In some embodiments, the subject has increased amount, increased activity, or both, of any one of: MPO, NO, SO, and any combination thereof, in the skin, systemically, or both, compared to control.

[0033] In some embodiments, the method further comprises determining an amount, activity, or both, of any one of: MPO, NO, SO, and any combination thereof, in a sample obtained or derived from the subject.

[0034] In some embodiments, the administering comprises orally administering, topically administering, or both.

[0035] In some embodiments, the at least one carotenoid comprises phytoene, phytofluene, and zeta carotene.

[0036] In some embodiments, the kit further comprises instructions for mixing the (a) and the (b) in a w/w of between 25:1 (w/w) and 500:1 (w/w).

[0037] In some embodiments, the kit is for use in the preparation of a composition suitable for prevention or treatment of collagen-loss related disease in a subject in need thereof.

[0038] The following embodiments and aspects thereof are described and illustrated in conjunction with systems, tools and methods which are meant to be exemplary and illustrative, not limiting in scope.

[0039] Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

[0040] Further embodiments and the full scope of applicability of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes

and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

[0041] In addition to the exemplary aspects and embodiments described above, further aspects and embodiments will become apparent by reference to the figures and by study of the following detailed description.

BRIEF DESCRIPTION OF THE FIGURES

[0042] **Figures 1A-1D** include bar graphs showing a dose-dependent inhibition of nitric oxide (NO) production and cell viability induced by: a carotenoid mixture including phytoene, phytofluene, and zeta carotene (hereinafter “Lumenato”; **1A** and **1B**, respectively) or ceramide (**1C** and **1D**, respectively) in macrophages. In (**1A** and **1C**) the values represent % inhibition are the means $\pm$ standard error of the mean (SEM) of 3 independent experiments each done in triplicates; significance: * - $p<0.05$, ** - $p<0.01$, *** - $p<0.001$, significant inhibition from the stimulated macrophages.

[0043] **Figures 2A-2D** include bar graphs showing a dose-dependent inhibition of myeloperoxidase (MPO) secretion and superoxide production induced by: Lumenato (**2A** and **2B**, respectively) and ceramide (**2C** and **2D**, respectively) in neutrophils. In (**2A-2D**) the values represent % inhibition in and are the means $\pm$ SEM of 3 independent experiments each in triplicates; significance: * - $p<0.05$, ** - $p<0.01$, *** - $p<0.001$, significant inhibition from the stimulated neutrophils.

[0044] **Figures 3A-3D** include bar graphs showing synergistic inhibition of NO. Inhibition of nitric oxide (NO) production induced by: (**3A**) Lumenato; (**3B**) ceramide, (**3C**) a combination of 6.5 $\mu\text{g/ml}$ of Lumenato and 0.034 $\mu\text{g/ml}$, 0.17 $\mu\text{g/ml}$, or 0.34 $\mu\text{g/ml}$ of ceramide; and (**3D**) a combination of 13 $\mu\text{g/ml}$ of Lumenato and 0.034 $\mu\text{g/ml}$, 0.17 $\mu\text{g/ml}$, or 0.34 $\mu\text{g/ml}$ of ceramide. In (**3A-3D**) the values represent % inhibition and are the means $\pm$ SEM of 3 independent experiments each in triplicates. In (**3C-3D**) calculated additive effect (‘Additive’), and synergistic effect (‘Combined’) are provided; significance: * - $p<0.05$, ** - $p<0.01$, *** - $p<0.001$, significant increase from the additive effect.

[0045] **Figures 4A-4D** include immunofluorescence staining and quantitation by bar graphs showing synergistic prevention of collagen-1 damage induced by the combinations

of 6.5 µg/ml Lumenato and ceramide, as detected by immunofluorescence analysis. Immunofluorescence staining of collagen-1, densitometry of the staining (bar graph on the left) and cell count by DAPI staining in the cultures (bar graph on the right) are provided. Cells were co-cultured with neutrophils pre-incubated with: **(4A)** Lumenato; **(4B)** different concentrations of ceramide; and **(4C)** combinations of Lumenato and ceramide. **(4D)** A vertical bar graph showing the calculated % of prevention of collagen I damage after scanning the densitometry of the immunofluorescence staining of collagen-1. Also in **(4A)** a control wherein cells were co-cultured with non-preincubated neutrophils is provided. In **(4A-4C)** the values shown are the means $\pm$ SEM of three different experiments. Significance * - $p < 0.05$, *** - $p < 0.001$. In **(4D)** the horizontal lines represent the calculated additive effect, the effect above this line represents synergistic inhibition; significance: ** - $p < 0.01$, a significant increase from the additive effect. I didn't use tracked changes. Use this or make it similar in the subsequent figs.

[0046] **Figures 5A-5B** include a photograph and bar graphs showing synergistic prevention of collagen-1 damage induced by combinations of 6.5 µg/ml Lumenato and ceramide, as detected by western blot analysis. **(5A)** A representative western blot analysis of collagen-I and actin in cell lysates under the different treatments at 24 h of incubation. The intensity of each band was divided by the intensity of each actin band after quantitation by densitometry and expressed as arbitrary units in the bar graph; and **(5B)** the calculated % of prevention of collagen-I damages. The values shown in **(5A)** are the mean $\pm$ SEM of 3 different experiments. In **(5B)** the horizontal lines represent the calculated additive effect, the effect above this line represents synergistic inhibition; significance: *** - $p < 0.001$, significant increase from the additive effect.

[0047] **Figures 6A-6D** include fluorescent micrographs and bar graphs showing synergistic prevention of collagen-1 damage induced by combinations of 13 µg/ml Lumenato and ceramide, as detected by immunofluorescence analysis. Immunofluorescence staining of collagen-1, densitometry of the staining (bar graph on the left) and cell count by DAPI staining in the cultures (bar graph on the right) are provided. Cells were co-cultured with neutrophils pre-incubated with: **(6A)** Lumenato; **(6B)** different concentrations of ceramide; and **(6C)** combinations of Lumenato and ceramide. **(6D)** A vertical bar graphs showing the calculated % of prevention of collagen I damage after scanning the densitometry

of the immunofluorescence staining of collagen-1. Also in (6A) a control wherein cells were co-cultured with non-preincubated neutrophils is provided. In (6A-6C) the values shown are the means $\pm$ SEM of three different experiments. Significance * - $p < 0.05$, *** - $p < 0.001$. In (6D) the horizontal lines represent the calculated additive effect, the effect above this line represents synergistic inhibition; significance: ** - $p < 0.01$, significant increase from the additive effect.

[0048] **Figures 7A-7B** include bar graphs showing a synergistic prevention of collagen I damage induced by combinations of 13 $\mu\text{g/ml}$ Lumenato and ceramide as detected by western blot analysis. (7A) A representative western blot analysis of collagen I and actin in cell lysates under the different treatments at 24 h of incubation; and (7B) the calculated % of protection of collagen I damage. The intensity of each band in (7A) was divided by the intensity of each actin band after quantitation by densitometry and expressed as arbitrary units in the bar graph. In (7B) the values shown are the mean $\pm$ SEM of 3 different experiments and the horizontal lines represent the calculated additive effect, the effect above this line represents synergistic inhibition; significance: ** - $p < 0.01$, significant increase from the additive effect.

[0049] **Figures 8A-8D** include bar graphs showing a synergistic inhibition of MPO secretion. MPO inhibition induced by: (8A) Lumenato; (8B) ceramide; (8C) combinations of 6.5 $\mu\text{g/ml}$ of Lumenato and 0.034 $\mu\text{g/ml}$, 0.17 $\mu\text{g/ml}$, or 0.34 $\mu\text{g/ml}$ of ceramide; and (8D) combinations of 13 $\mu\text{g/ml}$ of Lumenato and 0.034 $\mu\text{g/ml}$, 0.17 $\mu\text{g/ml}$, or 0.34 $\mu\text{g/ml}$ of ceramide. In (8C and 8D) the calculated additive effect ('Additive'), and synergistic effect ('Combined') are presented; significance: * $p < 0.05$, significant increase from the additive effect.

[0050] **Figures 9A-9D** include bar graphs and fluorescent micrographs showing the effect of Lumenato or ceramide and their combinations on fibroblasts. Immunofluorescence staining of collagen-1, densitometry of the staining (bar graph on the left) and cell count by DAPI staining in the cultures (bar graph on the right), are provided. Fibroblasts were incubated with: (9A) Lumenato; (9B) different concentrations of ceramide; (9C) combinations of 6.5 $\mu\text{g/ml}$ of Lumenato and 0.034 $\mu\text{g/ml}$, 0.17 $\mu\text{g/ml}$, or 0.34 $\mu\text{g/ml}$ of

ceramide; and (9D) combinations of 13 µg/ml of Lumenato and 0.034 µg/ml, 0.17 µg/ml, or 0.34 µg/ml of ceramide.

DETAILED DESCRIPTION

[0051] According to one aspect of the invention, there is provided a composition comprising: (a) at least one carotenoid selected from: phytoene, phytofluene, zeta carotene, or any combination thereof; and (b) a ceramide, wherein a weight per weight (w/w) ratio of (a) and (b) is between 25:1 (w/w) and 500:1 (w/w).

[0052] In some embodiments, the composition of the invention comprises: (a) a carotenoid mixture; and (b) a ceramide. In some embodiments, the composition of the invention comprises: (a) a carotenoid mixture; and (b) a ceramide, in a w/w ratio of between 25:1 (w/w) and 500:1 (w/w). In some embodiments, the carotenoid mixture comprises phytoene, phytofluene, zeta carotene, or any combination thereof. In some embodiments, the carotenoid mixture comprises: phytoene, phytofluene, and zeta carotene.

[0053] In some embodiments, the w/w ratio of the carotenoid mixture and ceramide within the composition is between 30:1 (w/w) and 400:1 (w/w), between 40:1 (w/w) and 450:1 (w/w), between 50:1(w/w) and 200:1 (w/w), between 80:1 (w/w) and 240:1(w/w), between 4:1 (w/w) and 350:1 (w/w), or between 200:1 (w/w) and 500:1 (w/w). Each possibility represents a separate embodiment of the invention.

[0054] In some embodiments, the composition of the invention comprises an active agent and an acceptable carrier.

[0055] In some embodiments, the carotenoid mixture as disclosed herein is or consists of Lumenato. In some embodiments, the carotenoid mixture as disclosed herein is derived from Lumenato. In some embodiments, Lumenato is a source for the carotenoid mixture disclosed herein. In some embodiments, the carotenoid mixture as disclosed herein is extracted or obtained from Lumenato. In some embodiments, Lumenato is processed, extracted, fractionated, or any combination thereof, so as to obtain the carotenoid mixture as disclosed herein.

[0056] In some embodiments, the active agent consists essentially of: (a) at least one carotenoid selected from: phytoene, phytofluene, or zeta carotene; and (b) a ceramide.

[0057] In some embodiments, the active agent consists essentially of: (i) phytoene and ceramide; (ii) zeta carotene and ceramide; (iii) phytofluene and ceramide; (iv) phytoene, zeta carotene, and ceramide; (v) phytoene, phytofluene, and ceramide; (vi) zeta carotene, phytofluene, and ceramide; or (vii) phytoene, phytofluene, zeta carotene, and ceramide.

[0058] In some embodiments, the composition further comprises an acceptable carrier.

[0059] As used herein, the term “consists essentially of” denotes that a given compound or substance constitutes the vast majority of the active ingredient’s portion or fraction of the composition.

[0060] In some embodiments, consists essentially of means that: (a) a carotenoid mixture comprising phytoene, phytofluene, zeta carotene, or any combination thereof; and (b) a ceramide, constitutes at least 95%, at least 98%, at least 99%, or at least 99.9% by weight, of the active ingredient(s) of the composition, or any value and range therebetween. Each possibility represents a separate embodiment of the invention.

[0061] In some embodiments, ceramide concentration within the composition is of at least 0.025 µg/ml, at least 0.03 µg/ml, at least 0.05 µg/ml, at least 0.075 µg/ml, at least 0.1 µg/ml, at least 0.15 µg/ml, at least 0.20 µg/ml, at least 0.25 µg/ml, at least 0.30 µg/ml, at least 0.35 µg/ml, at least 0.40 µg/ml, at least 0.45 µg/ml, at least 0.5 µg/ml, at least 1 µg/ml, at least 5 µg/ml, at least 8 µg/ml, at least 10 µg/ml, at least 15 µg/ml, or any value and range therebetween. In some embodiments, ceramide concentration within the composition is between 0.025 µg/ml and 0.5 µg/ml, between 0.03 µg/ml and 0.4 µg/ml, between 0.034 µg/ml and 0.34 µg/ml, or between 0.03 µg/ml and 0.45 µg/ml. Each possibility represents a separate embodiment of the invention.

[0062] In some embodiments, the composition comprises the carotenoid mixture in a concentration of at least 1.5 µg/ml, at least 3.5 µg/ml, at least 4 µg/ml, at least 5 µg/ml, at least 6 µg/ml, at least 6.5 µg/ml, at least 8.5 µg/ml, at least 10 µg/ml, at least 15 µg/ml, at least 50 µg/ml, at least 75 µg/ml, at least 100 µg/ml, at least 150 µg/ml, at least 250 µg/ml, or any value and range therebetween. Each possibility represents a separate embodiment of the invention. In some embodiments, a carotenoid mixture amount within the composition is of between 1.5 µg/ml and 75 µg/ml, between 1.5 µg/ml and 50 µg/ml, between 1.5 µg/ml and 30 µg/ml, between 3.5 µg/ml and 30 µg/ml, between 3.5 µg/ml and 25 µg/ml, between

5 µg/ml and 20 µg/ml, between 6 µg/ml and 20 µg/ml, between 5 µg/ml and 15 µg/ml, between 6.5 µg/ml and 21.5 µg/ml, between 50 µg/ml and 250 µg/ml, between 75 µg/ml and 150 µg/ml, between 75 µg/ml and 150 µg/ml. Each possibility represents a separate embodiment of the invention.

[0063] In some embodiments, a carotenoid is a natural carotenoid extracted, isolated, or purified from a fruit, a vegetable, or a plant (including a plant part), including any combination thereof. In another embodiment, a carotenoid is carotenoid extracted from a tomato plant. In another embodiment, a carotenoid is a carotenoid extracted from a tomato fruit. In another embodiment, a tomato carotenoid is a tomato extract enriched for a carotenoid. In another embodiment, tomato carotenoid is a carotenoid-rich tomato extract which is all-natural. In another embodiment, tomato carotenoid is a tomato carotenoid complex. In another embodiment, tomato carotenoid complex comprises a complex of phytonutrients including a plurality of carotenoids (such as phytoene, phytofluene, zeta carotene, beta-carotene, etc.), tocopherols and phytosterols. In some embodiments, a carotenoid is a synthetic carotenoid.

[0064] In some embodiments, the carotenoid mixture comprises at least one natural carotenoid or a combination thereof, a synthetic carotenoid or a combination thereof, or both.

[0065] In some embodiments, the carotenoid mixture comprising at least one carotenoid selected from: phytoene, phytofluene, zeta carotene, or any combination thereof (in amounts as specified hereinbelow), is a tomato extract. In some embodiments, the carotenoid mixture has reduced cellular toxicity. In some embodiments, reduced cellular toxicity is compared to other tomato extracts. In some embodiments, reduced toxicity enables to provide the composition of the invention to a subject in need, at a higher dose without reducing the survival, wellbeing, or both, of the subject. In some embodiments, administering the composition of the invention to a subject in need enables to increase the efficacy of the treatment by providing the active ingredients, such as phytoene, phytofluene, and zeta carotene, at higher amounts which increase the therapeutic effect, but without reducing the survival, wellbeing, or both, of the subject due to high cellular toxicity.

[0066] In some embodiments, reduced cellular toxicity is reduced toxicity to fibroblasts.

[0067] In some embodiments, the carotenoid mixture comprises phytoene in the amount of 10-40% (w/w), 15-35% (w/w), 20-45% (w/w), 25-35% (w/w), 20-30% (w/w), 35-65% (w/w), 45-65% (w/w), or 30-50 (w/w) of the total carotenoids of the carotenoid mixture. Each possibility represents a separate embodiment of the invention.

[0068] In some embodiments, the carotenoid mixture comprises phytofluene in the amount of 10-20% (w/w), 5-15% (w/w), 1-10% (w/w), 3-12% (w/w), 4-14% (w/w), 5-10% (w/w), 8-15% (w/w), or 2-9% (w/w) of the total carotenoids of the carotenoid mixture. Each possibility represents a separate embodiment of the invention.

[0069] In some embodiments, the carotenoid mixture comprises zeta carotene in the amount of 15-25% (w/w), 4-20% (w/w), 6-18% (w/w), 5-15% (w/w), 6-12% (w/w), 9-17% (w/w), or 10-17% (w/w) of the total carotenoids of the carotenoid mixture. Each possibility represents a separate embodiment of the invention.

[0070] In some embodiments, the weight ratio of phytoene and phytofluene combined to zeta carotene ranges from 20:1 (w/w) to 3:1 (w/w), 15:1 (w/w) to 3:1 (w/w), 20:1 (w/w) to 6:1 (w/w), 15:1 (w/w) to 2:1 (w/w), 17:1 (w/w) to 4:1 (w/w), 16:1 (w/w) to 7:1 (w/w), 13:1 (w/w) to 8:1 (w/w), 10:1 (w/w) to 3:1 (w/w), or 15:1 (w/w) to 10:1 (w/w). Each possibility represents a separate embodiment of the invention.

[0071] In some embodiments, the carotenoid mixture further comprises an additional carotenoid. As used herein, the term “an additional carotenoid” refers to any carotenoid or a metabolite thereof, other than or being different from phytoene, phytofluene, zeta carotene, or any combination thereof.

[0072] In some embodiments, the carotenoid mixture comprises an additional carotenoid in the amount of 0.1-3% (w/w), 0.2-3.5% (w/w), 0.5-2.5% (w/w), 0.15-1.75% (w/w), 0.35-2.75% (w/w), 0.8-4% (w/w), 1-5% (w/w), or 1.5-4.75% (w/w), of the total carotenoids in the carotenoid mixture. Each possibility represents a separate embodiment of the invention.

[0073] In some embodiments, the additional carotenoid is selected from: lycopene, beta carotene, gamma carotene, or any combination thereof.

[0074] In some embodiments, the carotenoid mixture comprises lycopene in an amount of less than 10% (w/w), less than 7% (w/w), less than 5% (w/w), less than 3% (w/w), less than

2% (w/w), or less than 1% (w/w), of the total carotenoids in the carotenoid mixture, or any value and range therebetween. In some embodiments, lycopene in an amount of less than 5% (w/w) of total carotenoids in the carotenoid mixture. Each possibility represents a separate embodiment of the invention.

[0075] In some embodiments, the carotenoid mixture comprises lycopene in an amount of 1-3% (w/w), 1-5% (w/w), 2-6% (w/w), 0.5-4.5% (w/w), 0.1-3% (w/w), 0.6-4.8% (w/w), or 2.5-4% (w/w) of the total carotenoids in the carotenoid mixture. Each possibility represents a separate embodiment of the invention.

[0076] In some embodiments, the carotenoid mixture comprises beta carotene in an amount of less than 10% (w/w), less than 7% (w/w), less than 5% (w/w), less than 3% (w/w), less than 2% (w/w), or less than 1% (w/w), of the total carotenoids in the carotenoid mixture, or any value and range therebetween. Each possibility represents a separate embodiment of the invention.

[0077] In some embodiments, the carotenoid mixture comprises beta carotene in an amount of 1-3% (w/w), 1-5% (w/w), 2-6% (w/w), 0.5-4.5% (w/w), 0.1-3% (w/w), 0.6-4.8% (w/w), or 2.5-4% (w/w) of the total carotenoids in the carotenoid mixture. Each possibility represents a separate embodiment of the invention.

[0078] In some embodiments, the carotenoid mixture comprises gamma carotene in an amount of at least 0.15% (w/w), at least 0.18% (w/w), at least 0.2% (w/w), at least 0.25% (w/w), at least 0.35% (w/w), at least 0.5% (w/w), at least 0.75% (w/w), at least 0.9% (w/w), at least 1% (w/w), at least 1.2% (w/w), at least 1.35% (w/w), or at least 1.7% (w/w) of the total carotenoids in the carotenoid mixture, or any value and range therebetween. Each possibility represents a separate embodiment of the invention.

[0079] In some embodiments, the carotenoid mixture comprises gamma carotene in an amount of 0.15-3% (w/w), 0.2-2% (w/w), 0.2-1.5% (w/w), 0.5-3% (w/w), 0.7-1.6% (w/w), 0.4-2.8% (w/w), or 1.2-3.2% (w/w) of the total carotenoids in the carotenoid mixture. Each possibility represents a separate embodiment of the invention.

[0080] In some embodiments, the carotenoid mixture comprises lycopene in an amount of less than 5% (w/w) of the total carotenoids in the carotenoid mixture, beta carotene in the

amount of less than 5% (w/w) of the total carotenoids in the carotenoid mixture, gamma carotene in the amount of 0.2-1.5% (w/w) of the total carotenoids in the carotenoid mixture, and any combination thereof.

[0081] In some embodiments, the carotenoid mixture comprises a total carotenoids amount of 5-25% (w/w), 10-15% (w/w), 12-35% (w/w), 3-17% (w/w), 2-20% (w/w), or 1-30% (w/w) of the carotenoid mixture. Each possibility represents a separate embodiment of the invention.

[0082] In some embodiments, the composition comprising the carotenoid mixture further comprises a tocopherol (e.g., vitamin E). In some embodiments, the composition comprises tocopherol and the carotenoid mixture and in an amount of 1-30% (w/w), 3-35% (w/w), 10-30% (w/w), 5-25% (w/w), 2-20% (w/w), 4-41% (w/w), 8-32% (w/w), or 13-39% (w/w). Each possibility represents a separate embodiment of the invention. In some embodiments, the carotenoid mixture further comprises a tocopherol in an amount of 1-30% (w/w), 3-35% (w/w), 10-30% (w/w), 5-25% (w/w), 2-20% (w/w), 4-41% (w/w), 8-32% (w/w), or 13-39% (w/w) by weight. Each possibility represents a separate embodiment of the invention.

[0083] In some embodiments, a weight ratio of phytoene and phytofluene combined to tocopherol ranges from 20:1 (w/w) to 3:1 (w/w), 15:1 (w/w) to 3:1 (w/w), 20:1 (w/w) to 6:1 (w/w), 17:1 (w/w) to 4:1 (w/w), 16:1 (w/w) to 7:1 (w/w), 13:1 (w/w) to 8:1 (w/w), 10:1 (w/w) to 3:1 (w/w), or 15:1 (w/w) to 10:1 (w/w). Each possibility represents a separate embodiment of the invention.

[0084] In some embodiments, a weight ratio of zeta carotene to tocopherol ranges from 3:1 (w/w) to 1:3 (w/w), 3:1 (w/w) to 1:2 (w/w), 3:1 (w/w) to 1:1 (w/w), 2:1 (w/w) to 1:1 (w/w), 2:1 (w/w) to 1:2 (w/w), 2:1 (w/w) to 1:3 (w/w), 1:1 (w/w) to 1:2 (w/w), or 1:1 (w/w) to 1:3 (w/w). Each possibility represents a separate embodiment of the invention.

[0085] In some embodiments, the composition comprising the carotenoid mixture further comprises phytosterol.

[0086] In some embodiments, the composition comprises phytosterol and the carotenoid mixture in the amount of 1-20% (w/w), 2-19% (w/w), 10-25% (w/w), 5-25% (w/w), 8-16% (w/w), 6-18% (w/w), 3-20% (w/w), 4-17% (w/w), or 5-15% (w/w). Each possibility

represents a separate embodiment of the invention. In some embodiments, the carotenoid mixture further comprises a phytosterol in the amount of 1-20% (w/w), 2-19% (w/w), 10-25% (w/w), 5-25% (w/w), 8-16% (w/w), 6-18% (w/w), 3-20% (w/w), 4-17% (w/w), or 5-15% (w/w) by weight. Each possibility represents a separate embodiment of the invention.

[0087] In some embodiments, a weight ratio of phytoene and phytofluene combined to a phytosterol ranges from 20:1 (w/w) to 3:1 (w/w), 15:1 (w/w) to 3:1 (w/w), 20:1 (w/w) to 6:1 (w/w), 17:1 (w/w) to 4:1 (w/w), 16:1 (w/w) to 7:1 (w/w), 13:1 (w/w) to 8:1 (w/w), 10:1 (w/w) to 3:1 (w/w), or 15:1 (w/w) to 10:1 (w/w). Each possibility represents a separate embodiment of the invention.

[0088] In some embodiments, a weight ratio of zeta carotene to a phytosterol ranges from 6:1 (w/w) to 2:1 (w/w), 5:1 (w/w) to 2:1 (w/w), 4:1 (w/w) to 2:1 (w/w), 3:1 (w/w) to 2:1 (w/w), 6:1 (w/w) to 3:1 (w/w), 5:1 (w/w) to 3:1 (w/w), 4:1 (w/w) to 3:2 (w/w), or 6:1 (w/w) to 3:1 (w/w). Each possibility represents a separate embodiment of the invention.

[0089] Methods for determining the amounts of phytonutrients, such as carotenoids, are common and would be apparent to one of ordinary skill in the art. Non-limiting examples for such methods include, but are not limited to, gas chromatography, liquid chromatography, and mass spectrometry.

[0090] In some embodiments, ceramide is a natural product. In some embodiments, ceramide is derived from a natural product. In some embodiments, the term “derived from” encompasses any industrial processing such as purification, isolation, fractionation, fermentation, chemical modification, etc. In some embodiments, ceramide is a synthetic product.

[0091] Non-limiting examples of ceramides include but are not limited to: ceramide 1 (e.g., EOS), ceramide 2 (e.g., ceramide NS), ceramide 3 (e.g., ceramide NP), ceramide 6-11 (e.g., ceramide AP), ceramide 9 (e.g., ceramide EOP), phytosphingosine, sphingosine, or any combination thereof.

[0092] In some embodiments, ceramide comprises a ceramide selected from: ceramide 1, ceramide 2, ceramide 3, ceramide 6-11, ceramide 9, or any combination thereof.

[0093] In some embodiments, ceramide is or comprises ceramide 2.

[0094] In some embodiments, the acceptable carrier is or comprises an oil. In some embodiments, the oil is or comprises vegetable oil. In some embodiments, vegetable oil is or comprises seed oil. In some embodiments, seed oil is or comprises tomato seed oil.

[0095] In some embodiments, a w/w percentage of an acceptable carrier within the composition is between 0.1% and 99.9%, between 10% and 99.9%, between 20% and 99.9%, between 1% and 50%, between 20% and 70%, or between 50 and 99.9%, between 30% and 90%. Each possibility represents a separate embodiment.

[0096] In some embodiments, the active agent consists essentially of: zeta carotene, phytosterol, phytoene, phytofluene, lycopene, tocopherol, beta carotene and gamma carotene, and ceramide.

[0097] In some embodiments, the composition of the invention consists essentially of: zeta carotene, phytosterol, phytoene, phytofluene, lycopene, tocopherol, beta carotene and gamma carotene, ceramide, and an acceptable carrier.

[0098] In some embodiments, the composition of the invention is a synergistic composition. In some embodiments, the composition of the invention provides a synergistic effect of: inhibition, reduction of expression, activity, or both, or any combination thereof, of: Matrix metalloproteinase (MMP), Myeloperoxidase MPO, superoxide (SO), elastase, nitric oxide (NO), reactive oxygen species (ROS), or any combination thereof.

[0099] In some embodiments, the synergistic effect is in a cell, or a subject. In some embodiments, the cell is a cell of a subject.

[00100] In some embodiments, the synergistic effect is compared to a control (or an effect exerted by same).

[00101] In some embodiments, the composition of the invention provides at least 5%, at least 10%, at least 15%, at least 20%, at least 30%, at least 50%, at least 75%, at least 100%, greater inhibition, prevention, or both, of compared to control. Each possibility represents a separate embodiment.

[00102] In some embodiments, control refers to the inhibition obtained by the individual compounds comprising the composition of the invention (e.g., (a) or (b)). In some embodiments, control refers to the inhibition obtained by any one of a carotenoid mixture and a ceramide individually. In some embodiments, the control refers to the inhibition obtained by a carotenoid

mixture or ceramide, individually, wherein the carotenoid mixture or ceramide are present in the control at the same concentration as within the composition of the invention.

[00103] In some embodiments, the composition of the invention provides a synergistic effect in inhibition, reduction, or prevention of collagen loss. In some embodiments, the composition of the invention provides greater inhibition reduction, or prevention of collagen loss compared to a control.

[00104] In some embodiments, collagen-loss comprises collagen degradation, collagen breakdown, or both. In some embodiments, prevention of collagen loss comprises increased collagen production, increased collagen stability, reduced collagen degradation or breakdown, or any combination thereof.

[00105] In some embodiments, the composition of the invention provides a synergistic effect or is characterized by a synergistic activity, wherein the activity comprises inhibition of NO production, secretion, or both. In some embodiments, the composition of the invention provides greater inhibition of NO production, secretion, or both, compared to a control.

[00106] In some embodiments, the composition of the invention provides a synergistic effect or is characterized by a synergistic activity, wherein the activity comprises inhibition of SO production, secretion, or both. In some embodiments, the composition of the invention provides greater inhibition of SO production, secretion, or both, compared to a control.

[00107] In some embodiments, the composition of the invention provides a synergistic effect or is characterized by a synergistic activity, wherein the activity comprises inhibition of MPO production, secretion, activity, or any combination thereof. In some embodiments, the composition of the invention provides greater inhibition of MPO production, secretion, activity, or any combination thereof, compared to a control. In some embodiments, MPO activity comprises peroxidase enzymatic activity comprising the production of hypochlorous acid (HOCl) from hydrogen peroxide (H₂O₂) and chloride anion (Cl⁻). In some embodiments, MPO activity comprises peroxidase enzymatic activity comprising the production of hypobromous acid (HOBr) from H₂O₂ and Bromide anion. In some embodiments, MPO activity as disclosed herein, is during a respiratory burst of a neutrophil. In some embodiments, MPO activity requires a heme group (e.g., as a cofactor). In some embodiments, MPO activity comprises oxidation of tyrosine to a tyrosyl radical.

[00108] In some embodiments, the composition of the invention provides a synergistic effect or is characterized by a synergistic activity, wherein the activity comprises inhibition of ROS production, secretion, or both. In some embodiments, the composition of the invention provides greater inhibition of ROS production, secretion, or both, compared to a control.

[00109] In some embodiments, the composition comprises a synergistically effective amount of: (a) a carotenoid mixture comprising at least one of phytoene, phytofluene, or zeta carotene; and (b) a ceramide.

[00110] In some embodiments, synergistically effective amount comprises (a) and (b) of the composition of the invention, in a w/w of between 10:1 (w/w) and 1:10 (w/w), between 10:1 (w/w) and 1:5 (w/w), between 5:1 (w/w) and 1:10 (w/w), between 5:1 (w/w) and 1:5 (w/w), between 4:1 (w/w) and 1:5 (w/w), between 3:1 (w/w) and 1:5 (w/w), between 2:1 (w/w) and 1:5 (w/w), between 1:1 (w/w) and 1:5 (w/w), between 1:1 (w/w) and 1:4 (w/w), or between 2:1 (w/w) and 1:2 (w/w). Each possibility represents a separate embodiment of the invention.

[00111] As used herein, the term “synergistically effective amount” refers to an amount of the composition of the invention being characterized by inhibition of at least one of: collagen loss, NO, MPO, SO, ROS, MMP, or any combination thereof, or prevention of a collagen-loss related disease or disorder, or both, that is at least 5%, at least 10%, at least 25%, at least 50%, at least 75%, greater than the inhibition of at least one of: collagen loss, NO, MPO, SO, ROS, MMP, or any combination thereof, or prevention of a collagen-loss related disease or disorder, by any one of (a) and (b) of the composition of the invention individually. Each embodiment represents a separate embodiment.

[00112] In some embodiments, the composition of the invention is for use in a reduction of collagen loss, increasing collagen levels, or both in a subject in need thereof.

[00113] In some embodiments, the composition of the invention has an activity of increasing collagen levels. In some embodiments, an activity of increasing collagen levels comprises any activity selected from: increasing collagen synthesis, increasing collagen weight per tissue weight, increasing collagen fibrils number, length, or both, increasing the amount of natively or properly folded collagen, reducing the amount of structurally damaged collagen, increasing collagen stability, reducing collagen degradation or breakdown, and any combination thereof.

[00114] In some embodiments, collagen is selected from: collagen I, collagen II, collagen III, collagen IV, collagen XI, collagen X, collagen XI, collagen V, or any combination thereof. In some embodiments, the collagen is or comprises collagen I.

[00115] Methods for measuring collagen levels are common and would be apparent to one of ordinary skill in the art. Non-limiting examples of methods for determining or measuring collagen levels include immunofluorescent microscopy, electron microscopy, western-blot, and QrT-PCR, some of which are exemplified hereinbelow. For example, by comparing collagen levels measured in the presence and absence of the composition of the invention, collagen loss, increased collagen levels, or both, can be determined.

[00116] In one embodiment, the composition of the invention can be provided to the individual *per-se*. In some embodiments, the composition of the invention is a pharmaceutical or a nutraceutical composition. In some embodiments, the pharmaceutical composition or a nutraceutical composition comprise a pharmaceutical or a nutraceutical-acceptable carrier or excipient, respectively. In some embodiments, the composition of the invention is a cosmeceutical composition. In some embodiments, the cosmeceutical composition comprises a cosmeceutical acceptable excipient. In some embodiments, a composition as described herein in an ingestible skin composition.

[00117] In some embodiments, an ingestible skin composition comprises a composition administered or consumed orally so as to exert activity of the active agent in or on the skin of a subject administered with or consuming the composition.

[00118] In one embodiment, a “pharmaceutical composition”, a “cosmeceutical composition” or a “nutraceutical composition” refers to a preparation of a composition as described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition, a cosmeceutical composition, or a nutraceutical composition is to facilitate administration of the composition to an organism. In one embodiment, the phrases “physiologically acceptable carrier” refer to a carrier or a diluent that does not cause significant irritation to a mammal and does not abrogate the biological activity and properties of the administered composition. An adjuvant is included under these phrases.

[00119] According to another aspect of the present invention, there is provided a cosmeceutical composition comprising an effective amount of the composition of the invention, an acceptable salt thereof or both, and a cosmeceutical acceptable carrier.

[00120] In some embodiments, the cosmeceutical composition of the invention is a topical composition.

[00121] According to another aspect of the present invention, there is provided a pharmaceutical composition comprising an effective amount of the composition of the invention, an acceptable salt thereof or both, and a pharmaceutically acceptable carrier.

[00122] In some embodiments, a therapeutically effective amount is sufficient for a substantial reduction in collagen loss.

[00123] In some embodiments, the pharmaceutical composition of the invention is for use in prevention or treatment of collagen loss-related diseases or disorders in a subject in need thereof.

[00124] In some embodiments, the term “pharmaceutically acceptable” and “cosmeceutical acceptable” refer to a carrier approved by a regulatory agency such as the Federal or a state government for use in animals, and more particularly in humans.

[00125] In some embodiments, the pharmaceutical composition and the cosmeceutical composition include incorporation of the composition of the invention into or onto particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, hydrogels, etc., or onto liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spheroplasts. One skilled in the art will appreciate that such incorporation may influence the physical state, solubility, stability, rate of in vivo release, and rate of in vivo clearance.

[00126] In some embodiments, the pharmaceutical composition is an oral composition or a topical composition. In some embodiments, the pharmaceutical composition as described herein is a topical composition. In some embodiments, the pharmaceutical composition as described herein is an oral composition.

[00127] In some embodiments, an oral composition is in the form of a soft gel capsule. In some embodiments, an oral composition is in the form of a beverage, a shot, a gummy, or

a powder. In some embodiments, an oral composition is mixed or assimilated into a food stuff, such as chocolate, ice cream, or others.

[00128] In some embodiments, the pharmaceutical composition is an injectable composition. In some embodiments, the pharmaceutical composition is for systemic use.

[00129] In some embodiments, the composition of the invention is referred to herein as an active ingredient of a pharmaceutical composition. In some embodiments, the composition of the invention is the only active ingredient within the pharmaceutical composition.

[00130] In some embodiments, the composition of the invention present in the pharmaceutical composition is of pharmaceutical grade purity.

[00131] In some embodiments, pharmaceutical grade purity is characterized by a chemical purity of at least about 90%, at least about 95%, greater than 95%, greater than 99%, or any value and range therebetween. In some embodiments, pharmaceutical grade purity is characterized by a chemical purity of between 90 and 99.999%, between 90 and 95%, between 90 and 97%, or between 95 and 99%. Each possibility represents a separate embodiment of the invention.

[00132] In one embodiment, “excipient” refers to an inert substance added to a composition to further facilitate administration of an active ingredient. In one embodiment, excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols. In some embodiments, the excipient and/or carrier improve the stability of the active ingredient in a living organism. In some embodiments, the carrier improves the stability of the active ingredient within the pharmaceutical composition. In some embodiments, the carrier enhances the bioavailability of the active ingredient.

[00133] Techniques for formulation and administration of drugs are found in “Remington’s Pharmaceutical Sciences” Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference in its entirety. In one embodiment, suitable routes of administration, for example, include oral, rectal, transmucosal, transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections.

[00134] According to another aspect, there is provided a kit comprising: (a) a carotenoid mixture comprising at least one carotenoid selected from: phytoene, phytofluene, zeta carotene, or any combination thereof; and (b) a ceramide.

[00135] In some embodiments, the carotenoid mixture comprises at least one natural carotenoid or a combination thereof, a synthetic carotenoid or a combination thereof, or both.

[00136] In some embodiments, the carotenoid mixture, and the ceramide are stored in different compartments. In some embodiments, the carotenoid mixture and ceramide are stored in the same compartment. In some embodiments, the carotenoid mixture and ceramide are in a form of a powder or a liquid (e.g., solution).

[00137] In some embodiments, the kit further comprises instructions for mixing the carotenoid mixture and the ceramide at appropriate conditions, to obtain the composition of the invention.

[00138] In some embodiments, appropriate conditions refer to a final w/w ratio of the carotenoid mixture and the ceramide of between 1:5 and 5:1.

[00139] In some embodiments, appropriate conditions refer to a mixing temperature of between 20 °C and 40 °C, between 22 °C and 35 °C, between 20 °C and 35 °C, between 20 °C and 30 °C, between 22 °C and 27 °C, or between 20 °C and 27 °C. Each possibility represent a separate embodiment.

[00140] In some embodiments, the kit further comprises an acceptable carrier. In some embodiments, the acceptable carrier comprises or is a pharmaceutical, cosmetical, or nutraceutical acceptable carrier. In some embodiments, the acceptable carrier is or comprises a liquid carrier.

[00141] In some embodiments, any one of: carotenoid mixture, ceramide, and the acceptable carrier are stored in different compartments. In some embodiments, any one of: carotenoid mixture, ceramide, and the acceptable carrier are stored in same compartments.

[00142] In some embodiments, the kit further comprises instructions for mixing an acceptable carrier with a carotenoid mixture and ceramide, under suitable conditions.

[00143] In some embodiments, the mixing comprises obtaining a composition wherein a final w/w percentage of the acceptable carrier is of between 0.1% and 99.9%, between 10% and 99.9%, between 20% and 99.9%, between 1% and 50%, between 20% and 70%,

between 50 and 99.9%, between 30% and 90%. Each possibility represents a separate embodiment.

[00144] In some embodiments, the instructions for mixing can vary, e.g., in order to cope with the needs of a patient subpopulation to be treated or the needs of a single patient whose different needs can be due to a particular disease, severity of a disease, age, sex, or body weight as can be readily made by a person skilled in the art.

[00145] According to another aspect of the present invention, there is provided a method for preventing or treating collagen loss in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the composition of the invention, thereby preventing or treating collagen loss-related disease or disorder in the subject.

[00146] According to another aspect of the present invention, there is provided a method for preventing or treating collagen loss in a subject in need thereof, comprising administering to the subject a synergistically effective amount of a composition comprising: (a) a carotenoid mixture comprising at least one carotenoid selected from: phytoene, phytofluene, or zeta carotene; and (b) a ceramide, thereby preventing or treating collagen loss-related disease or disorder in the subject.

[00147] In some embodiments, the subject is a mammal. In some embodiments, the mammal is a human.

[00148] In some embodiments, the subject is afflicted with a collagen-loss related disease.

[00149] In some embodiments, the subject is characterized by increased expression, activity, or both, of any one of: MMP, MPO, superoxide (SO), elastase, nitric oxide (NO), or any combination thereof.

[00150] In some embodiments, the method further comprises determining expression level, amount, abundance, activity, or any combination thereof, in a sample obtained or derived from the subject.

[00151] In some embodiments, the determining is in vitro or ex vivo.

[00152] In some embodiments, the subject is a healthy subject. In some embodiments, the subject is afflicted with collagen loss induced: aging, exposure to an environmental factor, diet, lack of sleep, stress, or any combination thereof.

[00153] In some embodiments, an environmental factor is selected from: radiation, pollution, smoke, or any combination thereof.

[00154] In some embodiments, a subject afflicted with a collagen-loss related disease is characterized by having: reduced skin elasticity, reduced epidermal thickness, increased skin vulnerability to damage (such as a mechanical damage), increased skin wrinkles, sagging, or both, or any combination thereof.

[00155] Methods for determining any one of skin elasticity or epidermal thickness are common and would be apparent to one of ordinary skill in the art, and non-limiting examples of such methods include, but are not limited to skin suction or indentation and subsequent skin displacement detection by optical measurement, and histological analysis of a skin biopsy.

[00156] According to some embodiments, there is provided a method for inhibiting or reducing any one of: the amount, the activity, or both, of any one of: a MMP, MPO, superoxide (SO), elastase, nitric oxide (NO), and any combination thereof, in a subject in need thereof, comprising administering to the subject a synergistically (as above)/therapeutically effective amount of the composition of the invention.

[00157] In one embodiment, MMP is MMP-9, MMP-8, MMP-1, or any combination thereof.

[00158] In some embodiments, the method further comprises reducing ROS production, secretion, or both, in a cell or a subject.

[00159] Methods for determining the amount, the activity, or both, of any one of: MMP, MPO, SO, elastase, NO, ROS are common and would be apparent to one of skill in the art. Non-limiting example of these methods include, but are not limited to, ELISA, immunohistochemistry, oxidation assays, and enzymatic assays, such as exemplified hereinbelow, and/or others.

[00160] In some embodiments, inhibiting or reducing is by 20-90%, 30-90%, 40-95%, 50-99%, 50-95%, 70-90%, or 75-99%, compared to control. Each possibility represents a separate embodiment of the invention.

[00161] As used herein, a control comprises the skin of a healthy subject. In some embodiments, a control comprises a healthy skin sample derived, isolated, or obtained from the same subject (e.g., the subject afflicted with the collagen-loss related disease). In some embodiments, a control comprises a skin sample derived, isolated, or obtained from the same subject afflicted with the collagen-loss treated solely with a carotenoid mixture or a ceramide (but not with both).

[00162] In some embodiments, a control comprises an untreated cell-culture. In some embodiments, a control comprises a cell culture treated solely with a carotenoid mixture or ceramide (but not with both).

[00163] In some embodiments, administering comprises orally administering. In some embodiments, administering comprises topically administering. In some embodiments, administering comprises combined orally administering and topically administering.

[00164] In some embodiments, a collagen-loss related disease is selected from: osteogenesis imperfecta, osteoporosis, Ehlers-Danlos syndrome, infantile cortical hyperostosis (also known as Caffey's disease), collagenopathy (types II and XI), Dupuytren's contracture, Alport syndrome, Goodpasture's syndrome, Ulrich myopathy, Bethlem myopathy, Atopic dermatitis, Epidermolysis bullosa dystrophica, Posterior polymorphous corneal dystrophy 2, EDM2, EDM3, Schmid metaphyseal dysplasia, Bullous pemphigoid, psoriasis, eczema, solar-radiation induced hyperplasia.

[00165] In some embodiments, a collagen-loss related disease comprises loss of collagen-1.

[00166] As used herein, the phrase "collagen-loss related disease" refers to any disease, disorder, or a symptom associated therewith, being induced by, involving, promoted by, propagated by, enhanced by, any equivalent thereof, or any combination thereof, collagen loss.

[00167] In some embodiments, the method comprises treating or preventing a disease selected from: osteogenesis imperfecta, osteoporosis, Ehlers-Danlos syndrome, infantile cortical hyperostosis (also known as Caffey's disease), collagenopathy (types II and XI), Dupuytren's contracture, Alport syndrome, Goodpasture's syndrome, Ulrich myopathy, Bethlem myopathy, Atopic dermatitis, Epidermolysis bullosa dystrophica, Posterior polymorphous corneal dystrophy 2, EDM2, EDM3, Schmid metaphyseal dysplasia, Bullous pemphigoid, psoriasis, eczema, solar-radiation induced hyperplasia, or any combination thereof, in a subject in need thereof, comprising administering to the subject a therapeutically effective amount of the composition of the invention.

[00168] In some embodiments, collagen loss comprises reduced: expression (transcription and/or translation), assembly, stability, or any combination thereof, increased degradation and/or breakdown, or any combination thereof, of collagen.

[00169] In some embodiments, a collagen-loss related disease is selected from: an age-related disease, a skin disease, and an inflammatory disease.

[00170] As used herein, "age-related disease" refers to a disease or a condition associated therewith having an incidence which increases rapidly with age. As used herein, the term "rapidly" is exponentially.

[00171] Non-limiting examples of conditions associated with age, which can be collectively termed 'lesser ailments of aging' (LAA) include, but are not limited to general muscle weakness, low temperature intolerance, skin wrinkles, and slow healing of bruises in the skin, wasting (total weight loss) muscle volume loss and bone density decrease.

[00172] As used herein, the phrase "skin disease" refers to any skin pathological condition or a symptom thereof. In one embodiment, collagen loss is a pathogenic factor leading to the development of a skin disease. In one embodiment, collagen loss is a pathophysiological factor resulting from the onset, initiation, progression, or any combination thereof, of a skin disease. In one embodiment, collagen loss is a condition characteristic of a developing or an ongoing skin disease.

[00173] In some embodiments, a skin disease is an immune-related skin disease. In one embodiment, an immune-related skin disease is an inflammatory skin disease, an autoinflammatory skin disease, or an autoimmune skin disease.

[00174] As used herein, the phrase “inflammatory disease” refers to any disease involving a multicomponent response of an organism (e.g., cells of the immune system, molecular signal mediators such as cytokines, etc.) to a harmful exogenous entity, for example, bacteria, fungi, viruses, protozoa, allergens, etc.

[00175] In some embodiments, skin disease is characterized or determined based on skin damage. In some embodiments, skin damage is induced by any one of: radiation, oxidative stress, DNA damage, telomere shortening, inflammation, tobacco usage, and any combination thereof.

[00176] In some embodiments, radiation comprises any radiation wavelength within the light spectrum. As used herein, the term “light spectrum” encompasses wave lengths ranging from 10^{-9} m to 10^{-3} m. In some embodiments, radiation wavelength within the light spectrum comprises UV radiation, visible light radiation, infrared radiation, or a combination thereof. In some embodiments, exposure to radiation comprises exposure to sunlight.

[00177] As used herein, the term “ultraviolet (UV)” encompasses any wavelength of the UV range. In some embodiments, UV is UV radiation. In some embodiments, radiation is UV radiation. In some embodiments, UV radiation is UVA radiation, UVB radiation, UVC, or any combination thereof.

[00178] As used herein, the term “infrared” encompasses wavelength spanning from 700 nm to 1,000 nm (having frequency of 430 THz to 300 GHz).

[00179] In some embodiments, the subject has increased amount, increased activity, or both, of any one of: MMP, MPO, SO, elastase, NO, ROS, and any combination thereof, in the skin, systemically, or both, compared to control.

[00180] In some embodiments, increased is by 1-10%, 5-30%, 15-50%, 25-75%, 70-150%, 100-350%, 250-550%, 500-750%, or 700-1,000% compared to control. Each possibility represents a separate embodiment of the invention.

[00181] As used herein, the terms “treatment” or “treating” of a disease, disorder, or condition encompasses alleviation of at least one symptom thereof, a reduction in the severity thereof, or inhibition of the progression thereof. Treatment need not mean that the disease, disorder, or condition is totally cured. To be an effective treatment, a useful composition herein needs only to reduce the severity of a disease, disorder, or condition, reduce the severity of symptoms associated therewith, or provide improvement to a patient or subject’s quality of life.

[00182] As used herein, the term “prevention” of a disease, disorder, or condition encompasses the delay, prevention, suppression, or inhibition of the onset of a disease, disorder, or condition. As used in accordance with the presently described subject matter, the term “prevention” relates to a process of prophylaxis in which a subject is exposed to the presently described compositions or formulations prior to the induction or onset of the disease/disorder process. This could be done where an individual has a genetic pedigree indicating a predisposition toward occurrence of the disease/disorder to be prevented. For example, this might be true of an individual whose ancestors show a predisposition toward certain types of, for example, inflammatory disorders. The term “suppression” is used to describe a condition wherein the disease/disorder process has already begun but obvious symptoms of the condition have yet to be realized. Thus, the cells of an individual may have the disease/disorder, but no outside signs of the disease/disorder have yet been clinically recognized. In either case, the term prophylaxis can be applied to encompass both prevention and suppression. Conversely, the term “treatment” refers to the clinical application of active agents to combat an already existing condition whose clinical presentation has already been realized in a patient.

[00183] In some embodiments, preventing comprises reducing the disease severity, delaying the disease onset, reducing the disease cumulative incidence, or any combination thereof.

[00184] As used herein, the terms “subject” or “individual” or “animal” or “patient” or “mammal,” refers to any subject, particularly a mammalian subject, for whom therapy is desired, for example, a human.

[00185] In the discussion unless otherwise stated, adjectives such as “substantially” and “about” modifying a condition or relationship characteristic of a feature or features of an embodiment of the invention, are understood to mean that the condition or characteristic is defined to within tolerances that are acceptable for operation of the embodiment for an application for which it is intended. Unless otherwise indicated, the word “or” in the specification and claims is considered to be the inclusive “or” rather than the exclusive or, and indicates at least one of, or any combination of items it conjoins.

[00186] It should be understood that the terms “a” and “an” as used above and elsewhere herein refer to “one or more” of the enumerated components. It will be clear to one of ordinary skill in the art that the use of the singular includes the plural unless specifically stated otherwise. Therefore, the terms “a”, “an” and “at least one” are used interchangeably in this application.

[00187] For purposes of better understanding the present teachings and in no way limiting the scope of the teachings, unless otherwise indicated, all numbers expressing quantities, percentages or proportions, and other numerical values used in the specification and claims, are to be understood as being modified in all instances by the term “about.” Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained. At the very least, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

[00188] In the description and claims of the present application, each of the verbs, “comprise”, “include” and “have” and conjugates thereof, are used to indicate that the object or objects of the verb are not necessarily a complete listing of components, elements or parts of the subject or subjects of the verb.

[00189] Other terms as used herein are meant to be defined by their well-known meanings in the art.

[00190] Unless specifically stated or obvious from context, as used herein, the term “or” is understood to be inclusive.

[00191] Throughout this specification and claims, the word “comprise” or variations such as “comprises” or “comprising,” indicate the inclusion of any recited integer or group of integers but not the exclusion of any other integer or group of integers.

[00192] As used herein, the terms “comprises”, “comprising”, “containing”, “having” and the like can mean “includes”, “including”, and the like; “consisting essentially of” or “consists essentially” likewise has the meaning ascribed in U.S. patent law and the term is open-ended, allowing for the presence of more than that which is recited so long as basic or novel characteristics of that which is recited is not changed by the presence of more than that which is recited, but excludes prior art embodiments. In one embodiment, the terms “comprises”, “comprising”, “having” are/is interchangeable with “consisting”.

[00193] Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

[00194] Generally, the nomenclature used herein, and the laboratory procedures utilized in the present invention include chemical, molecular, biochemical, and cell biology techniques. Such techniques are thoroughly explained in the literature. See, for example, “Molecular Cloning: A laboratory Manual” Sambrook et al., (1989); “Current Protocols in Molecular Biology” Volumes I-III Ausubel, R. M., ed. (1994); “Cell Biology: A Laboratory Handbook”, Volumes I-III Cellis, J. E., ed. (1994); The Organic Chemistry of Biological Pathways by John McMurry and Tadhg Begley (Roberts and Company, 2005); Organic Chemistry of Enzyme-Catalyzed Reactions by Richard Silverman (Academic Press, 2002); Organic Chemistry (6th Edition) by Leroy “Skip” G Wade; Organic Chemistry by T. W. Graham Solomons and, Craig Fryhle.

Material and Methods

Macrophage isolation and culture—Peritoneal

[00195] Macrophages were collected from the peritoneal cavity of 6-8 weeks old male ICR mice (Harlan, Israel) following an intraperitoneal injection of 1.5 ml of thioglycollate broth (4%) 4 days before harvest as previously described by Hadad et al., 2012. Peritoneal macrophages were washed three times with PBS and, when appropriate, a hypotonic lysis of erythrocytes was performed, yielding a highly enriched (90-95%) macrophage cell population. Peritoneal macrophages (1×10^6 cells/well) were cultured in 96-well plates at 37 °C in 5% CO₂ atmosphere in RPMI 1640 medium containing 10% FCS, 2 MM L-glutamine; 100 U/ml penicillin; 100 µg/ml streptomycin (Beit-Haemek, Israel). Cells were stimulated with 1 µg/ml LPS from *Salmonella enterica serotype typhimurium*. The study was performed following approval by the Ben-Gurion University of the Negev committee for ethical care and use of animals in experiments, Authorization No. IL-33-06-2020 and was conducted according to the Israeli Animal Welfare Act following the Guide for Care and Use of Laboratory Animal (National Research Council, 1996).

Neutrophil purification

[00196] Forty (40) ml of blood with a neutrophil count of between $3-7 \times 10^6$ neutrophils/ml were drawn from healthy volunteers with their written consent. Neutrophils at 95% purity were obtained by Ficoll/Histopaque centrifugation, dextran sedimentation, and hypotonic lysis of erythrocytes. Cells were counted and their viability was determined by trypan blue exclusion. The study was approved by the institutional Human Research Committee of the Soroka University Medical Center (No. 0370-16-SOR). Neutrophils were stimulated with TNFα (Peprotech, Rocky Hill, NJ, USA).

Ingredients

[00197] Lumenato, composed of 0.55% lycopene, 24.06% phytoene, 6.75% phytofluene, 11.27% tocopherols, 0.61% b-carotene, 3.15% phytosterols, 7.48% zeta-carotene, gamma carotene 0.16% and tomato seed oil was supplied by LycoRed Natural Products Industries Ltd. (Beer-Sheva, Israel). C2-Ceramide (d18:1/2:0), was purchased from Avanti Polar lipids Inc. (Alabaster, Alabama, USA). Working concentrations of phytonutrients were prepared from the stock solution dissolved in DMSO (in a final concentration of 5 MM for ceramide and 200 mg/ml for Lumenato) by addition of appropriate volumes to warmed culture medium.

Fibroblasts cell culture

[00198] Normal human dermal fibroblasts (NHDF) of adult donors (Promocell, Heidelberg, Germany), were cultured in and supplemented with Fibroblast Growth Medium-2 (Promocell) to final concentration in the medium: fetal calf serum 0.02 ml/ml, basic fibroblast recombinant human growth factor 1mg/ml, and 5 µg/ml recombinant human insulin, 2 MM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin (Beit-Haemek, Israel). The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO₂. When fibroblasts reached more than 80% confluence, the cells were seeded in 24-well plates.

Cell survival

[00199] Cell viability was assessed by cell count using trypan blue exclusion or the colorimetric MTT ([3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium) metabolic activity assay, as done before by Solomonov et al., 2021. For MTT measurement, cells were cultured in 96-well plates. The MTT was dissolved in medium (5 mg/ml) and added to each sample in an amount equal to 10% of the culture medium volume. After incubation for 30 min, the formazan crystals were dissolved in 100 MM HCl and 10% Triton X-100, all in isopropanol in an equal volume to the culture medium. The medium only served as a background. Absorbance intensity was measured by a Versamax Microplate Reader (Molecular Devices, Menlo Park, CA, USA) at 570 nm with a reference wavelength of 690 nm.

Nitric oxide (NO) production assay

[00200] NO levels in cell culture supernatants were determined by assaying nitrite levels using Griess reagent and sodium nitrite as a standard.

Immunofluorescence analysis

[00201] For immunofluorescence detection, fibroblasts or co-cultures of fibroblasts and neutrophils were fixed with methanol at -20 °C for 3 min followed by a wash in PBS. Fixed co-cultures were incubated with anti-collagen I antibodies (Southern Biotech Birmingham, Alabama, USA) 1:500 in 5% BSA/PBS for 90 min at room temperature as described before by Solomonov et al., 2018. The cells were washed three times in PBS and incubated with

Cy3 anti-mouse, (1:100 in 5% BSA/PBS; Jackson Immuno Research Laboratories, Inc., PA, USA) for 60 min at room temperature. The cells were washed three times in PBS, and the nuclei were stained with DAPI. Then a final wash was performed, and the cells were analyzed by fluorescence microscopy (Olympus, BX60, Hamburg, Germany). Fluorescence intensity for collagen III was determined using a CellProfiler Program.

Total cell lysates

[00202] Total cell lysates were prepared using 1% Triton X-100, 50 mM HEPES (PH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 10 % glycerol, 25 mM NaF, 10 μ M ZnCl₂, 1 mM PMSF, and 100 μ M leupeptin as described earlier by Hadad et al., 2012.

Immunoblot analysis

[00203] Lysate protein (10 μ g) were separated by electrophoresis on 7.5% polyacrylamide SDS gels. The resolved proteins were electrophoretically transferred to nitrocellulose, which was stained with Ponceau red to detect protein banding, and then blocked in 5% milk in TBS (10 mM Tris, 135 mM NaCl, PH 7.4). Immunoblot determination was done as described before by Hadad et al., 2012 using primary antibodies against Collagen I (Southern Biotech Birmingham, Alabama, USA) and against actin (MP Biomedicals, 9 Goddard Irvine, CA, USA), for overnight incubation at 4 °C and a second antibody, peroxidase conjugated goat anti-goat or anti-mouse (Amersham Biosciences, Buckinghamshire, United Kingdom) for 1 hour at room temperature, and developed using the enhanced chemiluminescence (ECL) detection system (Amersham Biosciences).

Superoxide generation

[00204] Horseradish peroxidase (HRP)-dependent oxidation of the highly sensitive fluorescent biosensor Amplex Red was employed. The oxidation of Amplex Red occurs outside the cells by HRP, which traps H₂O₂ as soon as it is generated by spontaneous dismutation of O₂⁻, the first product of NADPH oxidase. Resting and activated neutrophils (2×10⁵/well) were suspended in KRPG buffer (phosphate buffer, 145 mM NaCl, 4.86 mM KCl, 1.22 mM MgSO₄, 5.5 mM D-glucose, and 0.54 mM CaCl₂, PH 7.35) containing HRP (0.1 unit/ml) and Amplex Red (50 μ M). Fluorescence was recorded using a microplate

reader with 535 nm-excitation and 595 nm-emission wavelengths. Background fluorescence was measured in the absence of neutrophil cells.

Myeloperoxidase (MPO) activity

[00205] Hundred (100) μ l of 37 °C O-dianisidine hydrochloride solution (1 mg O-dianisidine, 10 ml phosphate buffer PH 6.0 + 0.0015% H_2O_2) were added to 100 μ l supernatant in a 96-well plate immediately before the optical density was followed by the change of absorbance at 450 nm at 2-min intervals on a Thermomax Microplate Reader (Molecular Devices, Menlo Park, CA).

Statistical analysis

[00206] Data are presented as the mean $\pm$ SEM. Significant differences from control conditions were determined using either one- or two-way analysis of variance (ANOVA) followed by a posteriori Bonferroni's test for multiple comparisons provided by GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA).

Results

[00207] The dose-response effect of Lumenato or ceramide on NO production assessed as NO secretion levels in culture supernatants of peritoneal macrophages was studied. Lumenato or ceramide was added 1 h before the addition of 1 mg/ml LPS to peritoneal macrophages cultured for 24 h at 37 °C. The secreted NO was determined in the cell culture supernatants. Either Lumenato (in the range of 6.5-208 μ g/ml) or ceramide (in the range of 0.034-17 μ g/ml) caused a dose-dependent inhibition of NO production (**Figures 1A and 1C**), without affecting cell survival detected by MTT (**Figures 1B and 1D**).

[00208] Similarly, the effect of Lumenato or ceramide in those ranges was studied on the activation of neutrophils. Lumenato or ceramide was added to neutrophils for 15 min at 37 °C before activation by $TNF\alpha$ overnight and MPO was determined in the supernatant (**Figures 2A and 2C**). The effect of Lumenato or ceramide on the production of short-lived superoxides was determined immediately after activation by cytochrome C reduction (**Figures 2B and 2D**). As shown in **Figures 2A-2D**, the addition of Lumenato or ceramide caused a dose-dependent inhibition of MPO release and superoxide production.

[00209] To study whether their combinations result in synergistic inhibition, Lumenato (6.5 or 13 $\mu\text{g/ml}$) or ceramide (0.034 $\mu\text{g/ml}$, 0.17 $\mu\text{g/ml}$, or 0.34 $\mu\text{g/ml}$) were added singly or in combinations to macrophages 1 h before the addition of LPS overnight. The secreted NO was determined in the cell culture supernatants. These combinations caused a synergistic inhibition of NO production of about twice the additive effect (**Figures 3A-3D**).

[00210] To study whether these combinations may cause a synergistic protection of the damage of collagen-1 induced by $\text{TNF}\alpha$ activated neutrophils, the inventors used the optimal conditions of co-cultures of fibroblasts and neutrophils that were determined by Solomonov et al., 2021. Neutrophils were stimulated with $\text{TNF}\alpha$ since upregulation of this cytokine is a key early response to sunlight and UVB by keratinocytes in the skin and represents an important component of the inflammatory cascade in the skin. During overnight the short-life neutrophils do not resist and thus do not interfere with collagen staining of the co-culture. For co-cultures, $1 \times 10^5/\text{ml}$ fibroblasts were plated overnight to obtain confluent cultures. Either Lumenato, ceramide, or their combinations were added for 15 min to 2×10^5 neutrophils/ml before activation with 100 ng/ml $\text{TNF}\alpha$. Activated neutrophils with and without the ingredients were added to the fibroblasts overnight. The addition of 2×10^5 activated neutrophils/ml with $\text{TNF}\alpha$ caused significant ($p < 0.001$) collagen-1 damage as shown by immunofluorescence staining and densitometry, reducing collagen-1 from $53 \pm 3.2\%$ to $38.3 \pm 1.9\%$ without affecting fibroblast cell number. (**Figure 4A**). The addition of 6.5 $\mu\text{g/ml}$ Lumenato had no effect in protecting from collagen-1 damage as shown by immunofluorescence staining (**Figure 4A**). The presence ceramide (0.034 $\mu\text{g/ml}$, 0.17 $\mu\text{g/ml}$, or 0.34 $\mu\text{g/ml}$) caused a low and similar protection of collagen-1 damage ($p < 0.05$) as shown by immunofluorescence staining and densitometry (**Figure 4B**). Combinations of Lumenato with each of ceramide concentrations caused a dose dependent protection of collagen-1 damage as shown by immunofluorescence staining and densitometry (**Figure 4C**). In all cultures the cell count was not affected (**Figures 4A-4C**). The calculated percentage of collagen-1 prevention loss by each ingredient and their combinations are presented in **Figure 4D**. The best and significant ($p < 0.01^{**}$) prevention of collagen-1 damage compared with the additive effect was achieved when 6.5 $\mu\text{g/ml}$ Lumenato was combined with 0.34 $\mu\text{g/ml}$ ceramide ($79.3 \pm 13.2\%$ compared with 43%). Similar results were obtained by western blot analysis as shown in **Figures 5A-5B**. The use of immunoblot

analysis technique showed a marked reduction in collagen-1 (of about 50%) by the addition of TNF α -activated neutrophils (**Figure 5A**). The significant ($p<0.001$) synergistic calculated prevention of collagen-1 loss compared with the additive effect was obtained with the combination of 6.5 $\mu\text{g/ml}$ Lumenato and 0.34 $\mu\text{g/ml}$ ceramide standing at $96\pm10.8\%$ compared with 46.4% of Lumenato alone (**Figure 5B**).

[00211] The effect of 13 $\mu\text{g/ml}$ Lumenato was also studied in the same experiments described in **Figures 4-5**. Conditioning neutrophils in 13 $\mu\text{g/ml}$ Lumenato before their supplementation to fibroblast had provided low and insignificant protection of collagen damage (**Figure 6A**). The presence of the different doses of ceramide alone provided a low protection of collagen loss (**Figure 6B**, same as presented in **Figure 4B**). In sharp contrast, combinations of 13 $\mu\text{g/ml}$ Lumenato with different ceramide concentrations provided a significant ($p<0.001$) protection of collagen loss (**Figure 6C**). Cell number was not affected in all cultures. The calculated % prevention of collagen-1 loss by the combinations is presented in **Figure 6D**. The results show a significant ($p<0.01$) synergistic protection attributed to the combinations, at a magnitude of about twice the additive effect. Combinations of 13 $\mu\text{g/ml}$ Lumenato with: (i) 0.034 $\mu\text{g/ml}$ ceramide provided a synergistic effect of $83.7\pm6.4\%$ prevention of collagen-1 loss compared to the additive effect (39%); (ii) 0.17 $\mu\text{g/ml}$ ceramide provided $89.4\pm7.9\%$ protection compared to the additive effect (47.7%); and (iii) 0.34 $\mu\text{g/ml}$ ceramide provided $92.2\pm13.2\%$ protection compared to the additive effect (52%). Similar results were obtained using western blot analysis demonstrating significant synergistic ($p<0.01$) protection of collagen-1 loss induced by activated neutrophils by the different combinations of Lumenato with ceramide (**Figure 7**). MPO activity measured in the supernatant of the co-cultures showed that in the combinations of Lumenato and ceramide there was a synergistic activity in accordance with the synergistic protection of collagen damage induced by activated neutrophils (**Fig. 8**). Addition of Lumenato and ceramide to fibroblast cultures, either singly or in combinations, did not affect collagen-1 expression or fibroblast cell number (**Figures 9A-9D**).

Discussion

[00212] In the present study, the inventors showed that collagen-1 damage in fibroblasts cultures induced by activated neutrophils could be prevented by Lumenato or ceramide in a

dose-dependent manner. Previously reported by the inventors, an 80% prevention of collagen loss induced by neutrophils was reported in the presence of 208 µg/ml of Lumenato. Whereas in the current study the inventors used much lower concentrations of Lumenato (6.5 µg/ml or 13 µg/ml) and of ceramide (0.034-0.34 µg/ml), each showing no or low protection of collagen-1 damage, but in combinations reached a synergistic protection against collagen-1 damage that was higher than 80% protection. By using exemplary compositions of the invention (combinations of low concentrations of Lumenato and ceramide) the inventors showed that a significant synergistic prevention of collagen-1 loss is surprisingly achievable.

[00213] The effect of Lumenato and ceramide is probably by inhibiting neutrophil activation and not by increasing collagen-1 synthesis, as their addition singly or in combinations to fibroblast cultures did not affect collagen-1 expression or cell count. This suggestion is supported by the dose dependent inhibition of superoxide production and MPO release by each of these ingredients in stimulated neutrophils and the inhibition of MPO activity detected in the supernatant of the co-cultures.

[00214] The inventors also showed that ceramide or Lumenato inhibited NO production by LPS stimulated- macrophages in a dose dependent manner. In addition, the inventors clearly showed herein that the combinations of Lumenato with ceramide in low concentrations, caused a synergistic inhibition of NO production by stimulated macrophages.

[00215] The inventors showed that ceramide blocks ROS release from neutrophils and prevents collagen-1 damage induced by activated neutrophils, thus it may have valuable potential in protecting against collagen damage such as following exposure to natural sunlight, erythemogenic dose of UVB, or solar stimulating radiation that induce ROS release by infiltrating neutrophils into the skin.

[00216] Further, the inventors showed that the combination of low concentrations of Lumenato and ceramide caused significant synergistic protection of collagen-1 damage induced by activated neutrophils, thus indicating that this combination has potential for protecting the skin from photoaging.

[00217] While the present invention has been particularly described, persons skilled in the art will appreciate that many variations and modifications can be made. Therefore, the

invention is not to be construed as restricted to the particularly described embodiments, and the scope and concept of the invention will be more readily understood by reference to the claims, which follow.

CLAIMS

What is claimed is:

1. A composition comprising: (a) a carotenoid mixture comprising phytoene, phytofluene, and zeta carotene; and (b) a ceramide; wherein a weight per weight ratio (w/w) of said (a) and said (b) in said composition is between 25:1 (w/w) and 500:1 (w/w).
2. The composition of claim 1, wherein said carotenoid mixture comprises said phytoene in the amount of 55-65% (w/w) of the total carotenoids in said carotenoid mixture; said phytofluene in the amount of 10-20% (w/w) of the total carotenoids in said carotenoid mixture; and said zeta carotene in the amount of 15-25% (w/w) of the total carotenoids in said carotenoid mixture.
3. The composition of claim 2, wherein a w/w ratio of said phytoene and said phytofluene combined to said zeta carotene ranges from 15:1 (w/w) to 2:1 (w/w).
4. The composition of any one of claims 1 to 3, wherein said carotenoid mixture further comprises an additional carotenoid selected from the group consisting of: lycopene, beta carotene, gamma carotene, and any combination thereof.
5. The composition of claim 4, comprising said lycopene in the amount of less than 5% (w/w) of the total carotenoids in said carotenoids mixture, said beta carotene in the amount of less than 5% (w/w) of the total carotenoids in said carotenoids mixture, said gamma carotene in the amount of 0.2-1.5% (w/w) of the total carotenoids in said carotenoids mixture, or a combination thereof.
6. The composition of any one of claims 1 to 5, wherein said carotenoid mixture comprises a total carotenoid amount of 10-15% (w/w) of said carotenoid mixture.
7. The composition of any one of claims 1 to 6, wherein said carotenoid mixture further comprises a tocopherol in the amount of 10-30% (w/w) of the total carotenoids in said carotenoid mixture.

8. The composition of any one of claims 1 to 7, wherein said carotenoid mixture further comprises a phytosterol in the amount of 5-15% (w/w) of the total carotenoids in said carotenoid mixture.

9. The composition of any one of claims 1 to 8, wherein said ceramide comprises a plurality of types of ceramides.

10. The composition of any one of claims 1 to 9, any one of said ceramide and plurality of types thereof, is selected from the group consisting of: C2-ceramide, C1 ceramide, C3 ceramide, C6-ceramide, Ceramide 9.

11. The composition of any one of claims 1 to 10, wherein said composition has a synergistic effect in inhibition of: (i) collagen-loss; (ii) production, secretion, activity, or any combination thereof of any one of: nitric oxide (NO), myeloperoxidase (MPO), superoxide (SO), and any combination thereof; and (iii) both (i) and (ii).

12. The composition of claim 11, wherein said collagen is collagen-1.

13. A pharmaceutical composition comprising an effective amount of the composition of any of claims 1 to 12, and a pharmaceutically acceptable carrier.

14. The pharmaceutical composition of claim 13, for use in prevention or treatment of a collagen-loss related disease or disorder in a subject in need thereof.

15. The pharmaceutical composition for use according to claim 14, wherein said collagen is collagen-1.

16. The pharmaceutical composition for use according to claim 14 or 15, wherein said collagen-loss related disease or disorder comprises collagen loss in skin of said subject.

17. A method for preventing or treating a collagen-loss related disease or disorder in a subject in need thereof, the method comprising administering to said subject a therapeutically effective amount of the pharmaceutical composition of claim 13, thereby preventing or treating a collagen-loss related disease or disorder in the subject.

18. A method for reducing the amount, activity, or both, of any one of: MPO, NO, superoxide, and any combination thereof, in a subject in need thereof, the method comprising administering to said subject a therapeutically effective amount of the composition of any one of claims 1 to 12.

19. The method of claim 18, wherein said subject is afflicted with a collagen loss-related disease or disorder.

20. The method of claim 19, wherein said collagen loss-related disease is selected from the group consisting of: an age-related disease, a skin disease, and an inflammatory disease.

21. The method of claim 20, wherein said skin disease comprises skin damage induced by any one of: radiation, oxidative stress, DNA damage, telomere shortening, inflammation, tobacco usage, and any combination thereof.

22. The method of claim 21, wherein said radiation comprises UV radiation, visible light radiation, infrared radiation, or any combination thereof.

23. The method of claim 22, wherein said UV radiation comprises UVA, UVB, UVC, or any combination thereof.

24. The method of any one of claims 18 to 23, wherein said subject has increased amount, increased activity, or both, of any one of: MPO, NO, SO, and any combination thereof, in the skin, systemically, or both, compared to control.

25. The method of any one of claims 18 to 24, further comprising determining an amount, activity, or both, of any one of: MPO, NO, SO, and any combination thereof, in a sample obtained or derived from said subject.

26. The method of any one of claims 18 to 25, wherein said administering comprises orally administering, topically administering, or both.

27. A kit comprising: (a) at least one carotenoid selected from the group consisting of: phytoene, phytofluene, zeta carotene, and any combination thereof; and (b) a ceramide.

28. The kit of claim 27, wherein said at least one carotenoid comprises phytoene, phytofluene, and zeta carotene.

29. The kit of claims 27 or 28, further comprising instructions for mixing said (a) and said (b) in a w/w of between 25:1 (w/w) and 500:1 (w/w).

30. The kit of any of claims 27 to 29, for use in the preparation of a composition suitable for prevention or treatment of collagen-loss related disease in a subject in need thereof.