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(54) **COMPOSITIONS COMPRISING SULFORAPHANE OR A SULFORAPHANE PRECURSOR AND MAGNESIUM**

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A61K 36/07	(2006.01)
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(57) **ABSTRACT**

The invention relates to the combination of a sulforaphane precursor, an enzyme capable of converting the sulforaphane precursor to sulforaphane, an enzyme potentiator, and magnesium or a salt or complex thereof. The invention also relates to the combination of a sulforaphane or a derivative thereof and magnesium or a salt or complex thereof. The invention also relates to the combination of a broccoli extract or powder and magnesium or a salt or complex thereof. The invention provides compositions and methods relating to these combinations.

FIG. 1

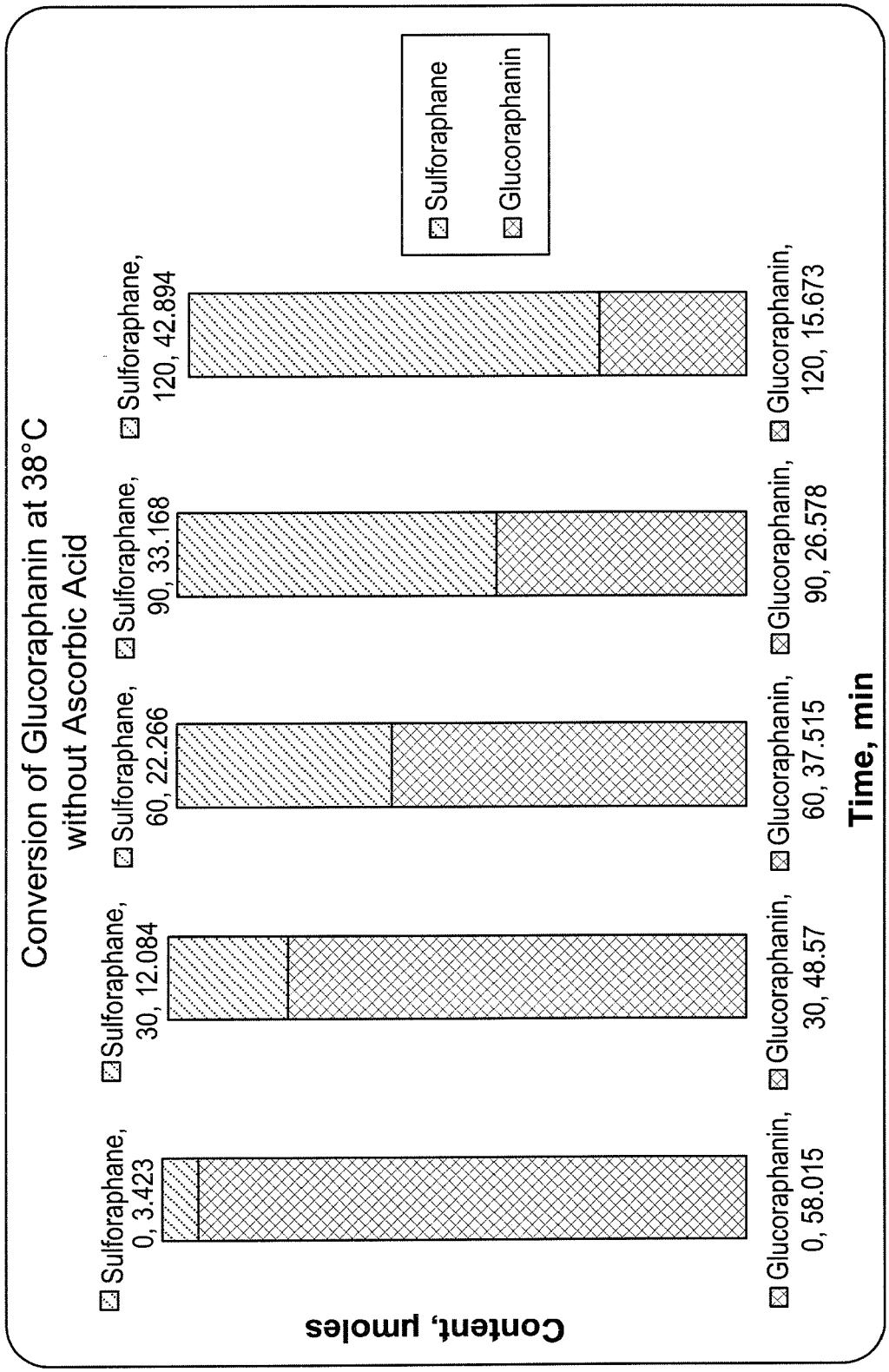

Conversion of Glucoraphanin at 38°C
without Ascorbic Acid
Sulforaphane
Glucoraphanin
Content, μmoles
Time, min
Sulforaphane, 0, 3.423
Glucoraphanin, 0, 58.015
Sulforaphane, 30, 12.084
Glucoraphanin, 30, 48.57
Sulforaphane, 60, 22.266
Glucoraphanin, 60, 37.515
Sulforaphane, 90, 33.168
Glucoraphanin, 90, 26.578
Sulforaphane, 120, 42.894
Glucoraphanin, 120, 15.673

FIG. 2

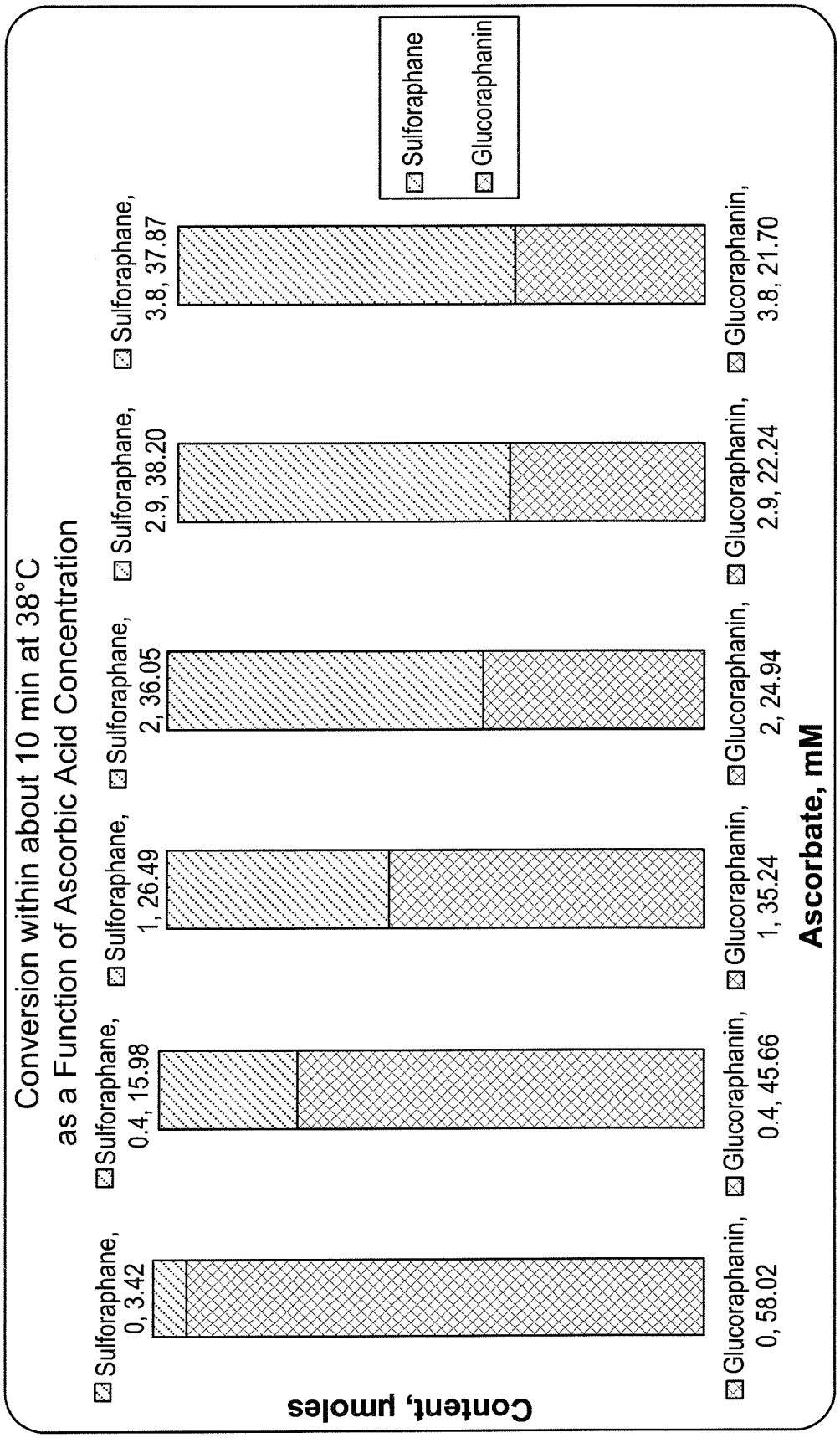

Conversion within about 10 min at 38°C
as a Function of Ascorbic Acid Concentration
Sulforaphane
Glucoraphanin
Content, µmoles
Ascorbate, mM
Sulforaphane, 0, 3.42
Glucoraphanin, 0, 58.02
Sulforaphane, 0.4, 15.98
Glucoraphanin, 0.4, 45.66
Sulforaphane, 1, 26.49
Glucoraphanin, 1, 35.24
Sulforaphane, 2, 36.05
Glucoraphanin, 2, 24.94
Sulforaphane, 2.9, 38.20
Glucoraphanin, 2.9, 22.24
Sulforaphane, 3.8, 37.87
Glucoraphanin, 3.8, 21.70

FIG.3

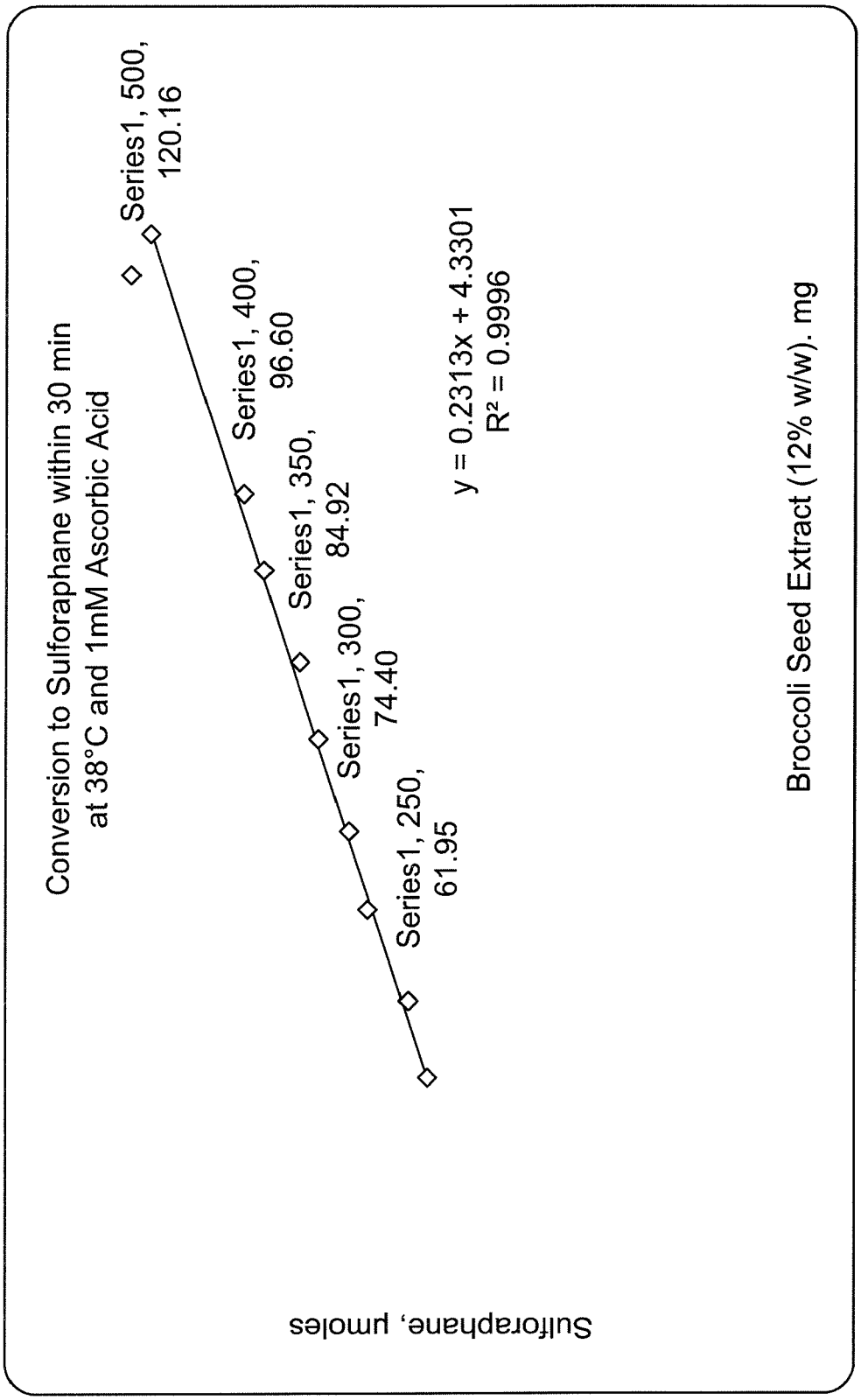

Conversion to Sulforaphane within 30 min
at 38°C and 1mM Ascorbic Acid
Series1, 500, 120.16
Series1, 400, 96.60
Series1, 350, 84.92
Series1, 300, 74.40
Series1, 250, 61.95
y = 0.2313x + 4.3301
R² = 0.9996
Sulforaphane, µmoles
Broccoli Seed Extract (12% w/w). mg

FIG.4

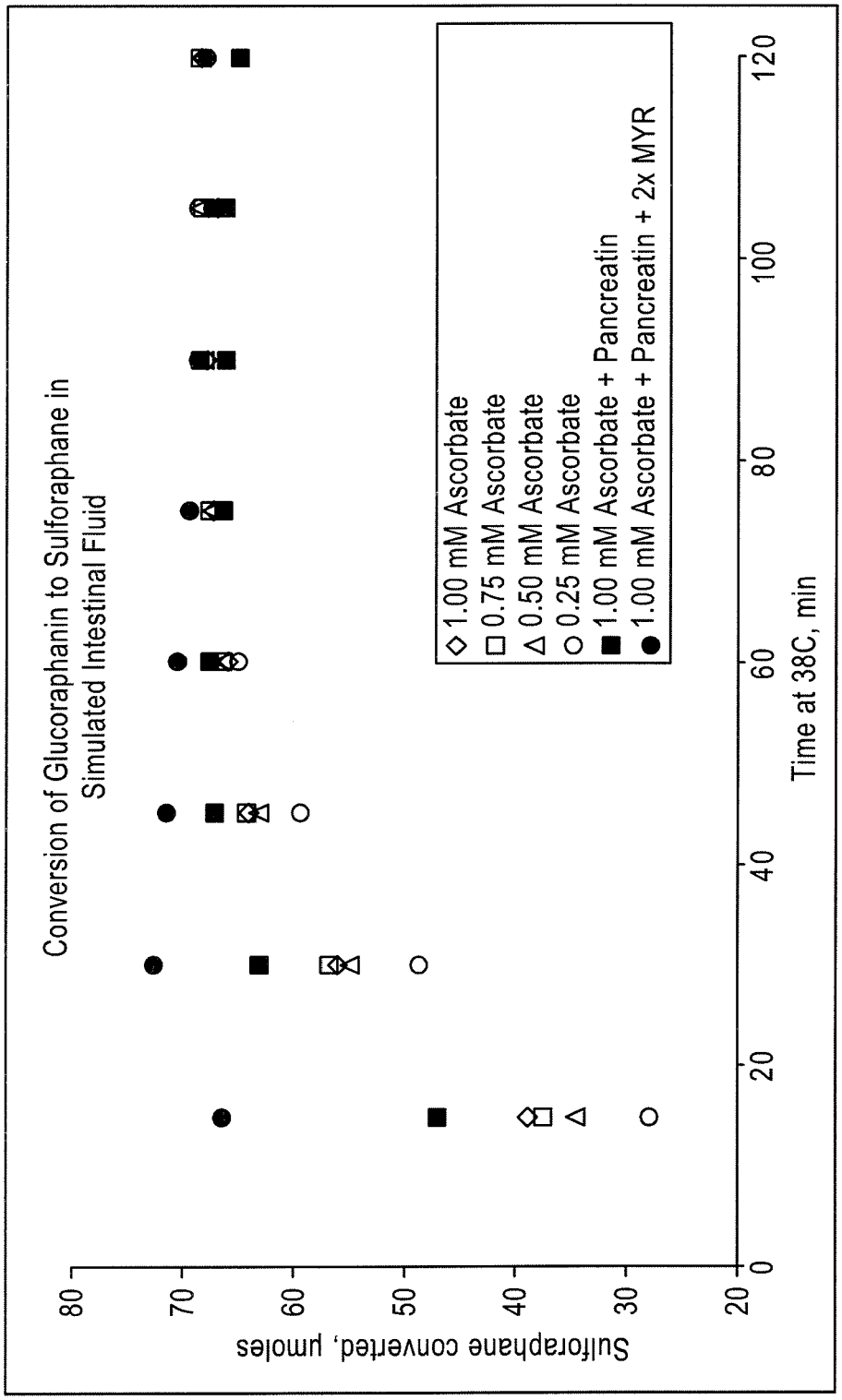
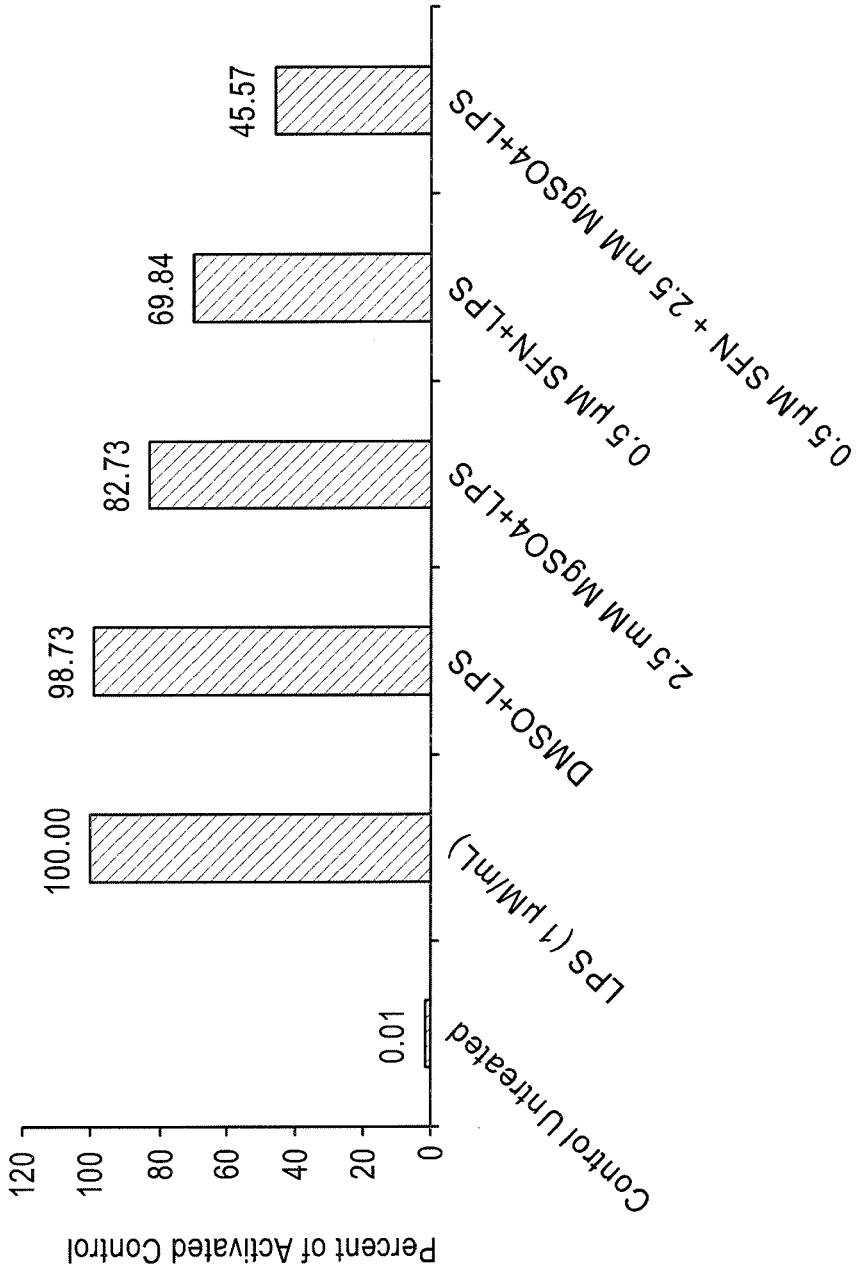

Conversion of Glucoraphanin to Sulforaphane in Simulated Intestinal Fluid
Sulforaphane converted, µmoles
Time at 38C, min
1.00 mM Ascorbate
0.75 mM Ascorbate
0.50 mM Ascorbate
0.25 mM Ascorbate
1.00 mM Ascorbate + Pancreatin
1.00 mM Ascorbate + Pancreatin + 2x MYR
80
70
60
50
40
30
20
0
20
40
60
80
100
120

FIG.5


Percent of Activated Control
120
100
80
60
40
20
0
0.01
100.00
98.73
82.73
69.84
45.57
Control Untreated
LPS (1 μM/mL)
DMSO+LPS
2.5 mM MgSO4+LPS
0.5 μM SFN+LPS
0.5 μM SFN + 2.5 mM MgSO4+LPS

COMPOSITIONS COMPRISING SULFORAPHANE OR A SULFORAPHANE PRECURSOR AND MAGNESIUM

RELATED APPLICATIONS

[0001] This application is a continuation of U.S. patent application Ser. No. 14/412,189 filed on Dec. 30, 2014, which claims priority to International Application No. PCT/US2013/049224 filed on Jul. 3, 2013, which claims priority to U.S. Provisional Patent Application No. 61/668,328, filed on Jul. 5, 2012; U.S. Provisional Patent Application No. 61/668,342, filed on Jul. 5, 2012; U.S. Provisional Patent Application No. 61/668,386, filed on Jul. 5, 2012; U.S. Provisional Patent Application No. 61/668,396, filed on Jul. 5, 2012; U.S. Provisional Patent Application No. 61/668,364, filed on Jul. 5, 2012; U.S. Provisional Patent Application No. 61,668,374, filed on Jul. 5, 2012; and U.S. Provisional Patent Application No. 61/794,417, filed on Mar. 15, 2013, the disclosure of each of which is hereby incorporated in its entirety by reference.

FIELD OF THE INVENTION

[0002] The present invention relates to the combination of a sulforaphane precursor, an enzyme capable of converting the sulforaphane precursor to sulforaphane, an enzyme potentiator, and magnesium or a salt or complex thereof. The present invention also relates to the combination of a sulforaphane or a derivative thereof and magnesium or a salt or complex thereof. The present invention also relates to the combination of a broccoli extract or powder and magnesium or a salt or complex thereof. The present invention provides compositions and methods relating to these combinations.

BACKGROUND OF THE INVENTION

[0003] The use of natural products is becoming increasingly popular with humans and companion animals. Some of these natural products are being incorporated into dietary supplements and medical foods. There is a need in the art for supplements which are useful as chemoprotective and/or antioxidant agents. In addition, there is a need in the art for pharmaceutical compositions and dietary supplements which are useful for conditions and disorders associated with cardiovascular health.

[0004] More than 1 in 3 (83 million) U.S. adults currently live with one or more types of cardiovascular disease. Respectively, heart disease and stroke are the first and third leading causes of death in the US with an estimated 935,000 heart attacks and 795,000 strokes occurring each year. Nearly 68 million adults have high blood pressure, and about half have uncontrolled hypertension. Additionally, an estimated 71 million adults have high cholesterol (i.e., high levels of low-density lipoprotein cholesterol), with an estimated ⅔ of this population having uncontrolled high cholesterol. These health problems are among the most widespread and costly of chronic illnesses in the United States today, accounting for approximately one out of every six dollars spent on healthcare. At the "heart "of cardiovascular disease is endothelial dysfunction. The endothelium, a thin membrane of flattened cells that line all blood vessels, is one of the largest organs in the body. The vascular endothelia play vital roles in blood flow, blood pressure regulation, coagulant and anticoagulant activity as well as control the selective adhesion and tissue migration of white blood cells. Endothelial dysfunction is the greatest underlying cause of atherosclerosis, hypertension, and inflammatory vascular diseases that lead to many of the catastrophic heart, circulatory, renal, and neurological conditions affecting the population. Endothelial dysfunction which invariably leads to major cardiovascular events, is most often due to a chronic inflammatory state brought on by conditions such as diabetes (both type 2 and insulin dependent diabetes), immune disorders (eg. lupus and rheumatoid arthritis), as well as a host of other chronic diseases. These conditions are positively associated with increased levels of specific inflammatory markers which include C-Reactive Protein (CRP), Interleukin-8 (IL-8), and monocyte chemoattractant protein (MCP-1). Recommendations to control endothelial dysfunction include lifestyle modifications such as eating a healthy diet, exercise, weight loss, avoidance of tobacco and second hand smoke, lowering cholesterol and controlling diabetes.

[0005] Chemoprotection through the use of natural products is evolving as a safe, effective, inexpensive, easily accessible, and practical means to prevent or reduce the occurrence of many conditions affecting humans and domesticated animals. It is known that carcinogens which can damage cells at the molecular level are often ingested and inhaled as non-toxic precursors. These non-toxic precursors may then convert into carcinogenic substances in the body. Chemoprotective agents, such as natural substances which can activate detoxifying enzymes or their co-factors, can counteract and allow for the elimination of carcinogens. These same natural substances can potentiate other naturally existing defenses such as the immune system.

[0006] Atherosclerosis results from a number of different inflammatory cascades. Evidence points to monocyte-derived macrophages as being one of the key cell types involved in endothelial inflammation. Interleukin-8 (IL-8) is responsible for recruiting monocytes and macrophages and monocyte chemoattractant protein-1 (MCP-1) plays a role in migration and infiltration of these cells. These proteins have been found to be significantly increased in the arterial atherosclerotic wall and worse, recruit additional inflammatory cells forming a vicious cycle. Mechanisms that can suppress both biomarkers (MCP-1 and IL-8) would diminish inflammation and subsequent atherosclerosis. The role of IL-8 in cardiovascular disease is discussed in *Apostolakis* et al. *Cardiovasc Res,* 2009, 84(3): 353-360; and Aukrust et al. *Arterioscler Thromb Vasc Biol,* 2008, 28:1909-1919. The role of MCP-1 in cardiovascular disease is discussed in Niu et al., *Clin Sci* (*Lond*), 2009, 117(3):95-109, and Hoogeveen et al, *Atherosclerosis,* 2005, 183(2): 301-307. MCP-1 and IL-8 is associated with a number of other inflammatory and vascular diseases and conditions. Examples of diseases and conditions associated with MCP-1 and IL-8 include, but are not limited to atherosclerosis, inflammatory bowel disease, inflammatory lung disease, chronic liver disease, inflammatory rheumatic disease, gingivitis, asthma, psoriasis, Alzheimer's disease, ischemic heart disease, acute coronary syndrome, arterial injury, and arteriogenesis.

[0007] Some natural products have antioxidant activity. Oxidative stress plays a major role in aging, the progression of neurodegenerative diseases as well as physiological trauma, such as ischemia. Antioxidant agents can reduce or inhibit the oxidation of vital biomolecules and may play a role in treating, preventing, or reducing the occurrence of conditions affected by oxidative stress.

[0008] An example of a natural product thought to have chemoprotective and antioxidant properties is sulforaphane. Sulforaphane is an organosulfur compound which is also known as 1-isothiocyanato-4-methylsulfinylbutane. The sulforaphane precursor, glucoraphanin, can be obtained from vegetables of the Brassicaceae family, such as broccoli, brussel sprouts, and cabbage. However, copious amounts of vegetables must be consumed in order to obtain levels adequate for chemoprevention. Glucoraphanin is converted into sulforaphane by a thioglucosidase enzyme called myrosinase, which occurs in a variety of exogenous sources such as Brassicaceae vegetables and endogenously in the gut microflora. However, upon ingestion of glucoraphanin, not all animals are capable of achieving its conversion to sulforaphane, most likely due to variations in microflora populations and overall health. In addition, in acidic environments such as the stomach, glucoraphanin can be converted to inert metabolites. The active metabolite, sulforaphane induces nuclear erythroid-2-related factor (Nrf2) which, in turn, upregulates the production of Phase II detoxification enzymes and cytoprotective enzymes such as glutathione S-transferases, NAD(P)H:quinine oxidoreductase (NQO1), and heme-oxygenase-1 (HO-1). Sulforaphane has been thought to induce the production of these enzymes without significantly changing the synthesis of P-450 cytochrome enzymes. The upregulation of Phase II enzymes is thought to play a role in a variety of biological activities, including the protection of the brain from cytotoxicity, the protection of the liver from the toxic effects of fat accumulation, and the detoxification of a variety of other tissues.

[0009] Sulforaphane and its precursor glucoraphanin have been studied extensively. Shapiro et al. (*Nutrition and Cancer,* (2006), Vol. 55(1), pp. 53-62) discuss a clinical Phase I study determining the safety, tolerability, and metabolism of broccoli sprout glucosinolates and isothiocyanates. Shapiro et al. discuss a placebo-controlled, double-blind, randomized clinical study of sprout extracts containing either glucosinolates such as glucoraphanin or isothiocyanates such as sulforaphane in healthy human subjects. The study found that administration of these substances did not result in systematic, clinically significant, adverse effects. Ye et al., (*Clinica Chimica Acta,* 200, 316:43-53) discuss the pharmacokinetics of broccoli sprout isiothiocyanates in humans.

[0010] Magnesium is a mineral that is important for many systems in the body, including muscles and nerves. Magnesium is vital for the metabolism of adenosine triphosphate (ATP) and is necessary for protein, fat, and nucleic acid synthesis. Adequate magnesium levels are important for the prevention of hypertension, which is a major cause of endothelial dysfunction. Magnesium is also important for calcium absorption, calcium regulation, and bone mineralization. While there are many magnesium salt forms that can augment dietary magnesium intake, the chelated form of magnesium (magnesium chelate) has been found to provide improved bioavailability of magnesium while providing a decreased side effect profile. For example, compared to salts of magnesium such as magnesium sulfate, magnesium chelates have been found to exert a decreased laxative effect.

[0011] Other supplements are also known to be beneficial, especially in the area of cardiovascular health. Vitamin K2, also known as menaquinone, is a fat-soluble vitamin that has been shown to be essential for calcium regulation in the body. Menaquinone occurs naturally in various molecular forms, characterized by the number of isoprene side chains (n), and designated as menaquinone-n (MK-n). MK-7 is most readily obtained through the fermentation of soybeans to make natto. Low levels of vitamin K2 are associated with vascular calcification, including calcification of atherosclerotic plaques, and osteopenic and osteoporotic conditions. Supplementation with vitamin K2 may decrease cholesterol plaque calcification, decrease arterial stiffness, and improve bone mineralization. Zhang et al. (Proc. *Natl. Acad. Sci.,* (1994), Vol. 91, pp. 3147-3150) discuss a study in Sprague-Dawley rats to determine the anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isiothiocyanates. The study determined that administration of sulforaphane was effective in blocking the formation of mammary tumors.

[0012] Cornblatt et al. (*Carcinogenesis,* (2007), Vol. 38(7): pp. 1485-1490) discuss a study in Sprague-Dawley rats to determine the effect of sulforaphane in chemoprevention in the breast. The study determined that oral administration of sulforaphane resulted in a 3-fold increase in NAD(P)H:quinine oxidoreductase (NQO1) enzymatic activity and a 4-fold elevated immunostaining of the heme oxygenase-1 (HO-1) enzyme in the mammary epithelium.

[0013] European Patent Application No. 2 213 280 discloses formulations comprising glucosinolates such as glucoraphanin and myrosinase, wherein the formulation is encapsulated or coated.

[0014] All references cited herein are incorporated by reference in their entirety.

SUMMARY OF THE INVENTION

[0015] The present invention provides a composition comprising: (i) a sulforaphane precursor, preferably glucoraphanin; (ii) an enzyme capable of converting the sulforaphane precursor to sulforaphane, preferably a glucosidase enzyme, more preferably a thioglucosidase enzyme, and most preferably myrosinase; (iii) an enzyme potentiator, preferably ascorbic acid; and (iv) magnesium or a salt or complex thereof. The present invention also provides a method of treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of, conditions associated with the endothelium and cardiovascular system, comprising administering to the subject: (i) a sulforaphane precursor, (ii) an enzyme capable of converting the sulforaphane precursor to sulforaphane, (iii) an enzyme potentiator, and (iv) magnesium or a salt or complex thereof. The present invention also provides a method of decreasing levels or decreasing gene expression of interleukin-8 (IL-8) and/or monocyte chemoattractant protein-1 (MCP-1) in a subject, comprising administering to the subject: (i) a sulforaphane precursor, (ii) an enzyme capable of converting the sulforaphane precursor to sulforaphane, (iii) an enzyme potentiator, and (iv) magnesium or a salt or complex thereof. The present invention also provides a method of treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of a disease or condition associated with elevated levels of interleukin-8 (IL-8) and/or monocyte chemoattractant protein-1 (MCP-1), comprising administering to the subject: (i) a sulforaphane precursor, (ii) an enzyme capable of converting the sulforaphane precursor to sulforaphane, (iii) an enzyme potentiator, and (iv) magnesium or a salt or complex thereof.

[0016] The present invention provides a composition comprising: (i) sulforaphane or a derivative thereof, and (ii) magnesium or a salt or complex thereof. The present invention also provides a method of treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of, conditions associated with the endothelium and cardiovascular system in a subject, comprising administering to the subject: (i) sulforaphane or a derivative thereof, and (ii) magnesium or a salt or complex thereof. The present invention also provides a method of decreasing levels or decreasing gene expression of interleukin-8 (IL-8) and/or monocyte chemoattractant protein-1 (MCP-1) in a subject, comprising administering to the subject, comprising administering to the subject: (i) sulforaphane or a derivative thereof, and (ii) magnesium or a salt or complex thereof. The present invention also provides a method of treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of a disease or condition associated with elevated levels of interleukin-8 (IL-8) and/or monocyte chemoattractant protein-1 (MCP-1), comprising administering to the subject: (i) sulforaphane or a derivative thereof, and (ii) magnesium or a salt or complex thereof.

[0017] The present invention provides a composition comprising: (i) a broccoli extract or powder, and (ii) magnesium or a salt or complex thereof. The present invention also provides a method of treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of, conditions associated with the endothelium and cardiovascular system in a subject, comprising administering to the subject: (i) a broccoli extract or powder, and (ii) magnesium or a salt or complex thereof. The present invention also provides a method of decreasing levels or decreasing gene expression of interleukin-8 (IL-8) and/or monocyte chemoattractant protein-1 (MCP-1) in a subject, comprising administering to the subject, comprising administering to the subject: (i) a broccoli extract or powder, and (ii) magnesium or a salt or complex thereof. The present invention also provides a method of treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of a disease or condition associated with elevated levels of interleukin-8 (IL-8) and/or monocyte chemoattractant protein-1 (MCP-1) in a subject, comprising administering to the subject: (i) a broccoli extract or powder, and (ii) magnesium or a salt or complex thereof.

BRIEF DESCRIPTION OF THE FIGURES

[0018] FIG. **1** is a graph showing the conversion of glucoraphanin at 38° C. without ascorbic acid, as described in Example 4.

[0019] FIG. **2** is a graph showing the conversion within about 10 minutes at 38° C. as a function of ascorbic acid concentration, as described in Example 4.

[0020] FIG. **3** is a graph showing the conversion to sulforaphane within 30 minutes at 38° C. and 1 mM ascorbic acid, as described in Example 4.

[0021] FIG. **4** is a graph showing the conversion of glucoraphanin to sulforaphane in simulated intestinal fluid, as described in Example 5.

[0022] FIG. **5** is a graph showing the results of the experiment described in Example 6.

DETAILED DESCRIPTION OF THE INVENTION

[0023] The present invention relates to the combination of a sulforaphane precursor, an enzyme capable of converting the sulforaphane precursor to sulforaphane, an enzyme potentiator, and magnesium or a salt or complex thereof. The present invention also relates to the combination of sulforaphane or a derivative thereof and magnesium or a salt or complex thereof. The present invention also relates to the combination of a broccoli extract or powder and magnesium or a salt or complex thereof. The present invention also relates to the use of magnesium or a salt or complex thereof, with a mixture of one or more of the following: sulforaphane precursor, sulforaphane or a derivative thereof, and broccoli extract. The present invention provides compositions relating to these combinations.

[0024] The present invention also provides methods comprising administering these combinations. In some embodiments, the combination may be administered for treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of a disease or condition associated with the endothelium and cardiovascular system in a subject. In some embodiments, the combination may be administered for decreasing levels or decreasing gene expression of interleukin-8 (IL-8) and/or monocyte chemoattractant protein-1 (MCP-1) in a subject. In some embodiments, the combination may be administered for treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of a disease or condition associated with elevated levels of interleukin-8 (IL-8) and/or monocyte chemoattractant protein-1 (MCP-1) in a subject.

[0025] Sulforaphane is also known as 1-isothiocyanato-4-methylsulfinylbutane. Derivatives of sulforaphane include, but are not limited to sulfoxythiocarbamate analogues of sulforaphane, 6-methylsulfinylhexyl isothiocyanate (6-HITC), and compounds which comprise the structure of sulforaphane with different side chains and/or various lengths of spacers between the isothiocyanato and sulfoxide groups. Examples of derivatives of sulforaphane include those described in the following references, each of which is incorporated herein by reference: Hu et al., *Eur J Med Chem,* 2013, 64:529-539; Ahn et al., *Proc Natl Acad Sci USA,* 2010, 107(21):9590-9595; and Morimistu et al., *J. Biol. Chem.* 2002, 277:3456-3463, and Baird et al., *Arch Toxicol,* 2011, 85(4):241-272.

[0026] In some embodiments, the composition comprises sulforaphane or a derivative thereof, preferably sulforaphane, in an amount of about 1 μg to about 10 g, preferably about 3 μg to about 5 g, preferably about 5 μg to about 1000 mg, preferably about 7 μg to about 750 mg, more preferably about 10 μg to about 500 mg, and most preferably about 100 μg to about 100 mg. In some embodiments, compositions suitable for human use comprise about 1 mg to about 20 mg.

[0027] In some embodiments, the methods of the present invention comprise administration of sulforaphane or a derivative thereof to a subject, preferably sulforaphane, in an amount of about 1 μg to about 10 g, preferably about 3 μg to about 5 g, preferably about 5 μg to about 1000 mg, preferably about 7 μg to about 750 mg, more preferably about 10 μg to about 500 mg, and most preferably about 100 μg to about 100 mg. In some embodiments wherein the

subject is a human, the method comprises administration of about 1 mg to about 20 mg. In some embodiments, the methods of the present invention comprise administration of sulforaphane or a derivative thereof to a subject, preferably sulforaphane, in an amount of about 0.01 μg/kg to about 0.2 g/kg, preferably about 0.05 μg/kg to about 0.07 g/kg, more preferably about 0.07 μg/kg to about 15 mg/kg, more preferably about 0.1 μg/kg to about 11 mg/kg, and most preferably about 0.2 μg/kg to about 7 mg/kg. In some preferred embodiments wherein the subject is a human, the method comprises administration of about 2 μg/kg to about 2 mg/kg, and more preferably about 0.01 mg/kg to about 0.3 mg/kg. The above amounts may refer to each dosage administration or a total daily dosage. The total daily dosage refers to the total amount of a compound or ingredient which is administered to a subject in a twenty-four hour period.

[0028] In some embodiments, the method comprises administration of more than one of a sulforaphane or a derivative thereof. In some embodiments, the compositions comprise more than one of a sulforaphane or a derivative thereof. For example, the methods or composition may comprise both sulforaphane and one or more derivatives thereof, or two or more derivatives. In some embodiments wherein the method or composition comprise more than one of a sulforaphane or a derivative thereof, the above amounts may refer to the amount of each sulforaphane or a derivative thereof, or the total amount of the more than one sulforaphane or derivative thereof.

[0029] The term "sulforaphane precursor" refers to any compound, substance or material which can be used to produce sulforaphane. In preferred embodiments, the sulforaphane precursor comprises a compound which can be converted or metabolized to sulforaphane, preferably by an enzyme. In some preferred embodiments, the sulforaphane precursor comprises glucoraphanin. Glucoraphanin is a glucosinolate which is also known as 4-methylsulfinylbutyl glucosinolate and 1-S[(1 E)-5-(methylsulfinyl)-N-(sulfonatooxy) pentanimidoyl]-1-thio-β-D-glucopyranose.

[0030] In some embodiments, the composition comprises about 1 μg to about 10 g, preferably about 250 μg to about 5 g, more preferably about 500 μg to about 2000 mg, even more preferably about 1 mg to about 750 mg, even more preferably about 1.5 mg to about 250 mg, even more preferably about 2 mg to about 100 mg, and most preferably about 3 mg to about 75 mg of the sulforaphane precursor, preferably glucoraphanin. In some embodiments, compositions suitable for human use comprise about 3.5 mg to about 50 mg of the sulforaphane precursor, preferably glucoraphanin.

[0031] In some embodiments, the method comprises administering the sulforaphane precursor, preferably glucoraphanin to a subject, in an amount of about 1 μg to about 10 g, preferably about 250 μg to about 5 g, more preferably about 500 μg to about 2000 mg, even more preferably about 1 mg to about 750 mg, even more preferably about 1.5 mg to about 250 mg, even more preferably about 2 mg to about 100 mg, and most preferably about 3 mg to about 75 mg. In some embodiments wherein the subject is a human, the method comprises administration of about 3.5 mg to about 50 mg. In some embodiments, the method comprises administering an amount of sulforaphane precursor to a subject in an amount of about 1 μg/kg to about 1000 mg/kg, preferably about 5 pg/kg to about 500 mg/kg, more preferably about 7.5 μg/kg to about 100 mg/kg, even more preferably about 10 μg/kg to about 25 mg/kg, and most preferably about 25 μg/kg to about 10 mg/kg. In some embodiments wherein the subject is a human, the method comprises administration of about 50 μg/kg to about 800 μg/kg. The above amounts may refer to each dosage administration or a total daily dosage.

[0032] In some embodiments, the method comprises administration of more than one sulforaphane precursor. In some embodiments, the composition comprises more than sulforaphane precursor. In some embodiments wherein the method or composition comprises more than one sulforaphane precursor, the above amounts may refer to the amount of each sulforaphane precursor, or the total amount of the sulforaphane precursors.

[0033] The sulforaphane precursor may be converted or metabolized to sulforaphane. In some embodiments, the sulforphane precursor is converted to sulforaphane by an enzyme. In some embodiments, the enzyme capable of converting the sulforaphane precursor to sulforaphane comprises a glucosidase enzyme, preferably a thioglucosidase enzyme, and more preferably myrosinase. Myrosinase is also known as thioglucoside glucohydrolase.

[0034] In some embodiments, the composition comprises the enzyme in an amount of about 1 pg to about 1 μg, preferably about 50 pg to about 500 ng, and most preferably about 1 ng to about 150 ng. In some embodiments, compositions suitable for human use comprise about 5 ng to about 75 ng of the enzyme.

[0035] In some embodiments, the method comprises administering the enzyme, preferably myrosinase, in an amount of about 1 pg to about 1 μg, preferably about 50 pg to about 500 ng, and most preferably about 1 ng to about 150 ng. In some embodiments wherein the subject is a human, the method comprises administration of about 5 ng to about 75 ng of the enzyme. In some embodiments, the method comprises administering the enzyme to a subject in an amount of about 0.02 pg/kg to about 0.02 ug/kg, preferably about 0.7 pg/kg to about 7 ng/kg, and most preferably about 0.02 ng/kg to about 2 ng/kg. In some preferred embodiments wherein the subject is a human, the method comprises administration of about 0.1 ng/kg to about 1 ng/kg. The above amounts may refer to each dosage administration or a total daily dosage.

[0036] In some embodiments, the method comprises administration of more than one enzyme capable of converting the sulforaphane precursor to sulforaphane. In some embodiments, the composition comprises more than one enzyme capable of converting the sulforaphane precursor to sulforaphane. In some embodiments wherein the methods or compositions comprise more than one enzyme, the above amounts may refer to the amount of each enzyme, or the total amount of the enzymes.

[0037] The present invention also provides for the use of a broccoli extract and/or powder, including but not limited to broccoli seed and sprout extracts and powders. The present invention provides methods of administration of broccoli extract and/or powder, and compositions comprising broccoli extract and/or powder. In some embodiments, the broccoli extract or powder is standardized to contain about 1 to about 75% w/w, more preferably about 2.5% to about 50%, even more preferably about 5% to about 25%, and most preferably about 10% to about 20% of a sulforaphane precursor, preferably glucoraphanin. Examples of broccoli extracts and powders include but are not limited to those described in U.S. Patent Nos. 5,411,986; 5,725,895;

5,968,505; 5,968,567; 6,177,122; 6,242,018; 6,521,818; 7,303,770, and 8,124,135, each of which is incorporated by reference in its entirety. Powders of broccoli may be obtained, for example, by air drying, freeze drying, drum drying, spray drying, heat drying and/or partial vacuum drying broccoli, preferably broccoli sprouts. In some embodiments, the compositions and methods comprise use of about 1 μg to about 10 g, more preferably about 250 μg to about 5 g, even more preferably about 500 μg to about 1 g, preferably about 600 μg to about 500 mg, more preferably about 750 μg to about 400 mg, and most preferably about 1 mg to about 300 mg of the broccoli extract. In some embodiments, the broccoli extract or powder is present in a composition or administered to a subject in amounts sufficient to provide a sulforaphane precursor or sulforaphane in the amounts described above. In some embodiments, the composition may further comprise an enzyme potentiator, preferably ascorbic acid. In some embodiments, the method may further comprise administration of an enzyme potentiator, preferably ascorbic acid.

[0038] The sulforaphane or a derivative thereof, the sulforaphane precursor, and/or the enzyme capable of converting the sulforaphane precursor to sulforaphane may be obtained from any source, including but not limited to one or more plants from the Brassicaceae (also known as Cruciferae) family. Examples of plants from the Brassicaceae family include, but are not limited to, the following: broccoli, Brussels sprouts, cauliflower, cabbage, horseradish, parsnip, radish, wasabi, watercress, and white mustard. In some preferred embodiments, sulforaphane precursor, preferably glucoraphanin, and the enzyme, preferably myrosinase, are obtained from broccoli, broccoli sprouts, or broccoli seeds. The sulforaphane precursor and the enzyme may be obtained from the same source or from different sources. In some embodiments, both the sulforaphane precursor and the enzyme may be obtained from an extract or powder from these plants, preferably a broccoli seed or sprout extract or powder.

[0039] The present invention provides for the use of an enzyme potentiator. Enzyme potentiators may be used to enhance the activity of the enzyme that is capable of converting the sulforaphane precursor to sulforaphane. In some embodiments, the enzyme potentiator comprises an enzyme co-factor, preferably ascorbic acid. Ascorbic acid, also known as ascorbate or vitamin C, can potentiate the activity of myrosinase. In some embodiments, without an enzyme potentiator such as ascorbic acid, the conversion reaction to sulforaphane may be too slow to occur in the location needed for peak absorption. The enzyme potentiator may be obtained from a natural source, or it may be produced synthetically.

[0040] In some embodiments, the compositions may comprise about 1 mg to about 500 mg, preferably about 1 mg to about 250 mg, and most preferably about 1 mg to about 125 mg of the enzyme potentiator. In some preferred embodiments, compositions suitable for human use comprise about 1 mg to about 50 mg of the enzyme potentiator.

[0041] In some embodiments, the method of the present invention comprises administration of an enzyme potentiator, preferably ascorbic acid, in an amount of about 1 mg to about 500 mg, preferably about 1 mg to about 250 mg, and most preferably about 1 mg to about 125 mg. In some preferred embodiments wherein the subject is a human, the method comprises administration of about 1 mg to about 50 mg. In some embodiments, the method of the present invention comprises administration of the enzyme potentiator, preferably ascorbic acid, in an amount of about 0.01 mg/kg to about 3 mg/kg, and most about 0.02 mg/kg to about 2 mg/kg. In some preferred embodiments wherein the subject is a human, the method comprises administration of about 0.02 mg/kg to 0.7 mg/kg of the enzyme potentiator. The above amounts may refer to each dosage administration or a total daily dosage.

[0042] In some embodiments, the method comprises administration of more than one enzyme potentiator. In some embodiments, the composition comprises more than one an enzyme potentiator. In some embodiments wherein the method or composition comprises the use of more than one enzyme potentiator, the above amounts may refer to the amount of each enzyme potentiator, or the total amount of the enzyme potentiators.

[0043] The present invention further comprises magnesium or a salt or complex thereof. The present invention provides for the use of any compounds containing magnesium, such as magnesium oil. Salts of magnesium include, but are not limited to: magnesium sulfate, magnesium oxide, magnesium citrate, magnesium glutamate, magnesium gluconate, magnesium glycinate, magnesium bromide, magnesium carbonate, magnesium chloride, magnesium fluoride, magnesium iodide, magnesium nitrate, magnesium perchlorate , magnesium permanganate, magnesium phosphate, magnesium oratate, magnesium malate, magnesium aspartate, and dimagnesium malate. Complexes of magnesium include, but are not limited to magnesium chelates, such as magnesium bisglycinate chelate magnesium lysinate glycinate chelate, magnesium glycinate glutamine chelate. In some preferred embodiments, the magnesium or a salt or complex thereof comprises magnesium sulfate, magnesium oxide, magnesium citrate, magnesium malate, magnesium glycinate, dimagnesium malate, magnesium bisglycinate chelate, or dimagnesium malate. In some embodiments, the compositions the compositions and/or methods of the present invention comprise magnesium malate, or magnesium bisglycinate chelate, which is a form of bioavailable magnesium which presents a low adverse effect and drug interaction profile with a high level of absorption. In some embodiments, the compositions and/or methods comprise the use of more than one magnesium or salt or complex thereof.

[0044] In some embodiments, magnesium or a salt or complex thereof may be used. In some embodiments, the composition of the present invention comprises about 1 to about 1000 mg, preferably about 5 mg to about 750 mg, more preferably about 10 mg to about 500 mg, and most preferably about 15 mg to about 350 mg. In some preferred embodiments wherein the compositions are suitable for human use, the composition comprises about 20 mg to about 200 mg of elemental magnesium.

[0045] In some embodiments, the method of the present invention comprises administration of about 1 mg to about 1000 mg, preferably about 5 mg to about 750 mg, more preferably about 10 mg to about 500 mg, and most preferably about 15 mg to about 350 mg. In some preferred embodiments wherein the subject is a human, the method comprises administration of about 20 mg to about 200 mg of elemental magnesium. In some embodiments, the method of the present invention comprises administration of about 0.1 mg/kg to about 15 mg/kg, preferably about 0.15 mg/kg to

about 10 mg/kg, more preferably about 0.2 mg/kg to about 7.5 mg/kg, more preferably about 0.3 mg/kg to about 5 mg/kg, and most preferably about 0.3 mg/kg to about 4 mg/kg. In some preferred embodiments wherein the subject is a human, the method comprises administration of about 0.3 mg/kg to about 3 mg/kg of elemental magnesium. The above amounts may refer to each dosage administration or a total daily dosage.

[0046] In some embodiments, the method comprises administration of more than magnesium or a salt or complex thereof. In some embodiments, the composition comprises more than one type of magnesium or a salt or complex thereof. In some embodiments wherein the method or composition comprises more than one type of magnesium or a salt or complex thereof, the above amounts may refer to the amount of each magnesium or a salt or complex thereof, or the total amount of the magnesium or a salt or complex thereof.

[0047] In some embodiments, a vitamin K2 may be further used. The addition of vitamin K2, which is optional, may provide a synergistic effect. Vitamin K2, which is also known as menaquinone, can be provided in the form of menaquinone-4 (MK-4), menaquinone-5 (MK-5), menaquinone-6 (MK-6), menaquinone-7 (MK-7), menaquinone-8 (MK-8), menaquinone-9 (MK-9), menaquinone-10 (MK-10), menaquinone-11 (MK-11), and phylloquinone. Phylloquinone can be obtained from plant sources such as green leafy vegetables and has a short half-life in the plasma, but it can be converted to menaquinone-4 (MK-4) by the endothelium, testes and pancreas. It can be synthesized by intestinal bacteria and is also found in cheeses. In some preferred embodiments, vitamin K2 is provided through menaquinone-7 (MK-7).

[0048] In some embodiments, the composition comprises about 10 μg to about 500 μg, preferably about 20 μg to about 400 μg, more preferably about 40 μg to about 300 μg, and most preferably about 50 μg to about 250 μg of menaquinone (MK-7). In some preferred embodiments wherein the composition is suitable for human use, the composition comprises about 75 μg to about 250 μg of menaquinone-7 (MK-7).

[0049] In some embodiments, the method comprises administration of about 10 μg to about 500 μg, preferably about 20 μg to about 400 μg, more preferably about 40 μg to about 300 μg, and most preferably about 50 μg to about 250 μg of menaquinone-7 (MK-7). In some preferred embodiments wherein the subject is a human, the method comprises administration of about 75 μg to about 250 μg of menaquinone-7 (MK-7) to a subject. In some embodiments, the method comprises administration of about 0.1 μg/kg to about 8 μg/kg, preferably about 0.3 μg/kg to about 5 μg/kg, more preferably about 0.5 μg/kg to about 4.5 μg/kg, and most preferably about 0.75 μg/kg to about 3.5 μg/kg of menaquinone-7 (MK-7) to a subject. In some preferred embodiments wherein the subject is a human, the method comprises administration of about 1 μg/kg to about 3 μg/kg of menaquinone-7 (MK-7) to a subject. The above amounts may refer to each dosage administration or a total daily dosage.

[0050] Each of the components of the compositions and methods of the present invention, for example, the sulforaphane precursor, the enzyme capable of converting the sulforaphane precursor to sulforaphane, the enzyme potentiator, and/or magnesium or a salt or complex thereof may be obtained from a natural source or produced synthetically.

[0051] The methods of the present invention may further comprise administration of one or more additional components. The compositions of the present invention may further comprise one or more additional components. The additional components may include active pharmaceutical ingredients, nutritional supplements, and nutritional extracts. Examples of additional components include, but are not limited, glucan, ursolic acid, quercetin or a derivative thereof, an aminosugar such as glucosamine, a glycosaminoglycan such as chondroitin, avocado/soybean unsaponifiables, vitamins such as vitamin K2, coffee fruit, magnesium, ursolic acid, proanthocyanidins, alpha- and beta-glucans, curcumin, phytosterols, phytostanols, and S-adenosylmethionine (SAMe). These additional components may be present in milk thistle (Silybum marianum) extract (silymarin), cranberry (Vaccinium macrocarpon) extract (proanthocyanidins, quercetin, and ursolic acid), turmeric (Curcuma conga), medicinal mushrooms (such as maitake shiitake, or reishi mushrooms).

[0052] In some embodiments, the ratio of magnesium or a salt or complex thereof to sulforaphane or a derivative thereof is about 1:1 to about 50:1, preferably about 1.5:1 to about 20:1, more preferably about 1.75:1 to about 15:1, more preferably about 2:1 to about 11:1, and most preferably about 2:1 to about 8:1. In some embodiments, the ratio of magnesium or a salt or complex thereof to sulforaphane precursor is about 1:1 to about 25:1, preferably about 2:1 to about 10:1, more preferably about 3:1 to about 8:1, more preferably about 4:1 to about 7:1, and most preferably about 4:1 to about 6:1.

[0053] In some embodiments, the composition comprises a unit dosage form, including but not limited to pharmaceutical dosage forms suitable for oral, rectal, intravenous, subcutaneous, intramuscular, transdermal, transmucosal, and topical. In some preferred embodiments, the composition comprises an orally administrable dosage form or a rectally administrable dosage form. Examples of orally administrable dosage forms include, but are not limited to a tablet, capsule, powder that can be dispersed in a beverage, a liquid such as a solution, suspension, or emulsion, a soft gel/chew capsule, a chewable bar, or other convenient dosage form known in the art. In preferred embodiments, the composition comprises a tablet, capsule, or soft chewable treat. The orally administrable dosage forms may be formulated for immediate release, extended release or delayed release.

[0054] In some embodiments, at least the sulforaphane precursor, the enzyme, and the enzyme potentiator are provided in a dosage form which allows for the release in an area of the gastrointestinal tract having a pH of at least 4 and preferably at least 5, such as the small intestine, preferably the duodenum. In some embodiments, at least the sulforaphane or derivative thereof and/or the broccoli extract or powder are provided in a dosage form which allows for the release in an area of the gastrointestinal tract having a pH of at least 4 and preferably at least 5, such as the small intestine, preferably the duodenum. In some embodiments, the magnesium or a salt or complex thereof and/or any optional additional components are also released in an area of the gastrointestinal tract having a pH of at least 4 and preferably

at least 5, such as the small intestine, preferably the duodenum. The small intestine includes the duodenum, jejunum, and ileum.

[0055] In some embodiments, each of these components (i.e, sulforaphane precursor, enzyme, enzyme potentiator, sulforaphane or a derivative thereof, broccoli extract or powder, magnesium or a salt or complex thereof, and/or additional components) are released simultaneously or concomitantly (i.e., within a short period of time of each other). This provides benefits over glucoraphanin-containing compositions formulated to release the glucoraphanin in an area of the gastrointestinal tract having a pH below 4, such as the stomach. In low pH environments such as this, the acidic environment may divert conversion of sulforaphane precursor to other, physiologically inactive end products, such as sulforaphane nitrile and epithionitrile.

[0056] In some embodiments, the compositions may comprise orally administrable compositions which comprise gastroprotective formulations, including enteric coated dosage forms or any dosage form which is resistant to degradation in an area of the gastrointestinal tract having pH below 4, such as the stomach. For example, the orally administrable composition may comprise a tablet or capsule comprising an enteric coating. The enteric coating may comprise materials including, but not limited to cellulose acetate phthalate, hydroxypropyl methylcellulose phthalate, polyvinyl acetate phthalate, methacrylic acid copolymer, methacrylic acid:acrylic ester copolymer, hydroxypropyl methylcellulose acetate succinate, hydroxypropyl methylcellulose trimellitate, shellac, cellulose acetate trimellitate, carboxymethylethylcellulose, and mixtures thereof. The enteric coating may comprise any suitable enteric polymers known in the art. In some embodiments, one or more of the components in the composition may be embedded in a matrix of enteric polymers. In some embodiments, the orally administrable compositions comprise a capsule that dissolves slowly in gastric acid and travels to the small intestine, such as DRCAPS™ acid resistant capsules, which are marketed by CAPSUGEL® or any other acid resistant capsules.

[0057] In the most preferred form, the orally administrable composition is surrounded by a coating that does not dissolve unless the surrounding medium is at a pH of at least 4, and more preferably at least 5. Alternatively, a coating may be employed which controls the release by time, as opposed to pH, with the rate adjusted so that the components are not released until after the pH of the gastrointestinal tract has risen to at least 4, and more preferably at least 5. Thus, a time-release formulation may be used to prevent gastric presence of the sulforaphane precursor, the enzyme capable of converting the sulforaphane precursor to sulforaphane, and the enzyme potentiator, or of the sulforaphane. The coating layer(s) may be applied onto orally administrable composition using standard coating techniques. The enteric coating materials may be dissolved or dispersed in organic or aqueous solvents. The pH at which the enteric coat will dissolve can be controlled by a polymer, or combination of polymers, selected and/or ratio of pendant groups. For example, dissolution characteristics of the polymer film can be altered by the ratio of free carboxyl groups to ester groups. Enteric coating layers also contain pharmaceutically acceptable plasticizers such as triethyl citrate, dibutyl phthalate, triacetin, polyethylene glycols, polysorbates or other plasticizers. Additives such as dispersants, colorants, anti-adhering and anti-foaming agents may also be included.

[0058] The compositions may contain one or more non-active pharmaceutical ingredients (also known generally as "excipients"). Non-active ingredients, for example, serve to solubilize, suspend, thicken, dilute, emulsify, stabilize, preserve, protect, color, flavor, and fashion the active ingredients into an applicable and efficacious preparation that is safe, convenient, and otherwise acceptable for use. The excipients are preferably pharmaceutically acceptable excipients. Examples of classes of pharmaceutically acceptable excipients include lubricants, buffering agents, stabilizers, blowing agents, pigments, coloring agents, flavoring agents, fillers, bulking agents, fragrances, release modifiers, adjuvants, plasticizers, flow accelerators, mold release agents, polyols, granulating agents, diluents, binders, buffers, absorbents, glidants, adhesives, anti-adherents, acidulants, softeners, resins, demulcents, solvents, surfactants, emulsifiers, elastomers and mixtures thereof.

[0059] In some embodiments, the combination of (i) a sulforaphane precursor, preferably glucoraphanin, (ii) an enzyme capable of converting the sulforaphane precursor to sulforaphane, preferably a glucosidase enzyme, more preferably a thioglucosidase enzyme, and most preferably myrosinase, (iii) an enzyme potentiator, preferably an enzyme co-factor, more preferably ascorbic acid, and (iv) magnesium or a salt or complex thereof demonstrates a synergistic effect. In some embodiments, the combination of sulforaphane (or a derivative thereof) and magnesium or a salt or complex thereof demonstrates a synergistic effect. Synergy refers to the effect wherein a combination of two or more components provides a result which is greater than the sum of the effects produced by the agents when used alone. In preferred embodiments, the synergistic effect is greater than an additive effect. In some embodiments, the combination of a sulforaphane precursor, an enzyme capable of converting the sulforaphane precursor to sulforaphane, an enzyme potentiator, and magnesium or a salt or complex thereof has a statistically significant, greater effect compared to: (i) each component alone, (ii) the combination of sulforaphane precursor and the enzyme alone; and/or (iii) the combination of sulforaphane precursor, the enzyme, and the enzyme potentiator alone.

[0060] In preferred embodiments, the combination of the sulforaphane precursor, the enzyme, the enzyme potentiator, and magnesium or a salt or complex thereof demonstrates synergy by having a statistically significant and/or greater than additive effect compared to the sulforaphane precursor alone and magnesium or a salt or complex thereof alone. In some embodiments, the combination of glucoraphanin, myrosinase, ascorbic acid, and magnesium or a salt or complex thereof has a synergistic effect compared to the combination of glucoraphanin, myrosinase, ascorbic acid alone; and compared to magnesium.

[0061] In some embodiments, the combination of a sulforaphane (or a derivative thereof) and magnesium or a salt or complex thereof has a statistically significant and/or greater than additive effect than: (i) sulforaphane (or a derivative thereof) alone, and/or (ii) magnesium or a salt or complex thereof alone. In some embodiments, the combination of sulforaphane and glucan has a synergistic effect compared to sulforaphane alone, and magnesium alone.

[0062] In some embodiments, the combination of broccoli extract or powder and magnesium or a salt or complex

thereof has a statistically significant and/or greater than additive effect than: (i) broccoli extract or powder alone, and/or (ii) magnesium or a salt or complex thereof alone. In some embodiments, the combination of broccoli extract or powder and MK-7 has a synergistic effect compared to broccoli extract or powder alone, and magnesium alone.

[0063] The present invention provides methods of use, including methods of administration to a subject in need thereof. In some embodiments, the method comprises administration of the combination of a sulforaphane precursor, an enzyme capable of converting the sulforaphane precursor to sulforaphane, an enzyme potentiator, and magnesium or a salt or complex thereof. In some embodiments, the method comprises administration of the combination of a sulforaphane or a derivative thereof and magnesium or a salt or complex thereof. In some embodiments, the method comprises administration of the combination of a broccoli extract or powder and magnesium or a salt or complex thereof.

[0064] In some embodiments, the methods relate to treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of diseases or conditions such as those associated with the endothelium and cardiovascular system in a subject. In some preferred embodiments, the diseases and conditions comprise atherosclerosis, ischemic heart disease, acute coronary syndrome, or arterial injury. The methods may also relate to decreasing endothelial C-Reactive Protein to reduce vascular inflammation, increasing endothelial nitric oxide to improve vasodilitation for improved blood flow, decreasing atherosclerotic plaque calcification, reducing vascular damage or stiffness, and/or increasing bone mineral density.

[0065] In some embodiments, the combination may be administered for decreasing levels or decreasing gene expression of interleukin-8 (IL-8) and/or monocyte chemoattractant protein-1 (MCP-1) in a subject. In some embodiments, the combination may be administered for treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of a disease or condition associated with elevated levels of interleukin-8 (IL-8) and/or monocyte chemoattractant protein-1 (MCP-1) in a subject.

[0066] Examples of diseases and conditions include, but are not limited to: atherosclerosis, inflammatory bowel disease, inflammatory lung disease, chronic liver disease such as cirrhosis, inflammatory rheumatic disease, osteoarthritis, gingivitis, asthma, psoriasis, Alzheimer's disease, ischemic heart disease, acute coronary syndrome, arterial injury, arteriogenesis, depression, type II diabetes, metabolic syndrome, colorectal cancer, migraines, asthma, renal disease, osteoporosis, lyme disease, ischemic disorders, neuropathy, gastrointestinal disease, and conditions occurring specifically in animals such as laminitis (e.g., in equines) and after heart worm treatments (e.g., in dogs and cats). The compositions may also be administered after surgery.

[0067] In some embodiments, the methods relate to providing a beneficial effect on biomarkers, and treating, preventing, reducing the occurrence of, decreasing the symptoms associated with abnormal levels of these biomarkers. Examples of such biomarkers include, but are not limited to NADPH-dependent enzymes, thioredoxin (TXN), thioredoxin reductase-1 (Txnrd-1), glutamate-cysteine ligase subunit (GCLC), sulfotransferase 1A1 (SULT1A1), heme oxygenase-1 (HMOX1), glutathione peroxidase-3 (GPx-3), glutathione S-transferse theta 2 (GSTT2), microsomal glutathione S-transferase 1 (MGST1), aldehyde oxidase (AOX1), aldo-keto reductase 1B8 (Akr1 b8), flavin-containing monooxygenase 2 (FMO2), Fc receptor region receptor III (Fcgr3), tryptase beta 1 (TPSB1), mast cell protease-6 (Mcpt6), neurexin-1-alpha (NRXN-1), microphthalmia-associated transcription factor (MITF), type II iodothyronine deiodinase (DIO2), angiopoietin-14 (Angpt14), cluster of differentiation (CD36), and Ntel. Diseases or conditions associated with elevated or abnormal levels of these biomarkers include, but are not limited to cancer, pulmonary and central nervous system tuberculosis, multiple sclerosis, Crohn's disease, atherosclerosis, osteoarthritis, asthma, stroke, emphysema, diabetic nephropathy, chronic histiocytic intervillositis of the placenta, hypertension, abdominal aortic aneurysm, inflammatory bowel disease, chronic rhinosinusitis, coronary artery disease, and kidney disease.

[0068] In some embodiments, the method comprises administering to a subject in need thereof a combination of sulforaphane and magnesium or a salt or complex thereof. In some embodiments the method comprises administering to a subject in need thereof a combination of broccoli extract or powder and magnesium or a salt or complex thereof. In some preferred embodiments, the method comprises administering to the subject a combination of glucoraphanin, myrosinase, ascorbic acid, and magnesium or a salt or complex thereof. In preferred embodiments, the combinations demonstrate a synergistic effect in the methods of the present invention.

[0069] In preferred embodiments, one or more components of the combinations (for example, the sulforaphane precursor, the enzyme capable of converting the sulforaphane precursor to sulforaphane, the enzyme potentiator, the magnesium or a salt or complex thereof; or the sulforaphane or derivative thereof and the magnesium or a salt or complex thereof; or the broccoli extract or powder and the magnesium or a salt or complex thereof) are administered together in one composition or dosage form, or separately, preferably within a period in which their therapeutic properties overlap. In some embodiments, the components of the combinations may be administered in two or more orally administrable compositions or dosage forms. For example, in some embodiments, the sulforaphane precursor, the enzyme capable of converting the sulforaphane precursor to sulforaphane, and the enzyme potentiator are administered in one orally administrable dosage form, while the magnesium or a salt or complex thereof are administered in one or more separate or additional orally administrable dosage form(s). In preferred embodiments, the components of the combination are administered in one dosage form.

[0070] In some embodiments, the combination may be administered at a frequency of 1 to 10 times daily, preferably 1 to 5 times daily, more preferably 1 to 3 times daily, and most preferably 1 time daily.

[0071] The dosages disclosed in this application refer generally to dosages suitable for humans. Dosage calculations can be determined by those of skilled in the art by evaluating body weight, surface area, metabolic rate, and species differences.

[0072] The term "subject" refers to any animal, including mammals and birds. Mammals include, but are not limited to, humans, dogs, cats, horses, cows, camels, elephants, lions, tigers, bears, seals, and rabbits. In preferred embodi-

ments, the subjects comprise mammals that are not consumed as food, such as humans, cats, and dogs.

EXAMPLES

Example 1

[0073] The following is an exemplary formulation:

[0074] Glucoraphanin-containing broccoli seed extract (about 12% w/w), 50 mg to 5 grams

[0075] Myrosinase-containing freeze-dried broccoli sprout powder, 25 mg to 500 mg

[0076] Ascorbic acid, 5 mg to 500 mg

[0077] Magnesium malate, providing 20 to 200mg elemental magnesium.

Example 2

[0078] A Hydrophobic Interaction Chromatographic (HILIC) method was developed, comprising the following conditions:

[0079] Column: Waters BEH Amide, 1.7-μm particle size; 2.1 mm×100 mm

[0080] Mobile Phase: 20% 10 mM Ammonium Acetate, pH 5.0; 80% Acetonitrile;

[0081] Separation mode: isocratic

[0082] Column Temperature: 70° C.

[0083] Flow Rate: 0.7 mL/min

[0084] The above conditions allow separation of five typical Brassicaceae glucosinolates, including the sulforaphane precursor, glucoraphanin.

Example 3

[0085] Consumption of Glucoraphanin as a Function of the Ascorbic Acid Concentration.

[0086] About 250 mg of broccoli seed extract containing about 12% (w/w) glucoraphanin were subjected to hydrolysis by a fixed concentration of broccoli sprout-derived myrosinase in the presence of variable concentration of ascorbic acid, ranging from 0 to 600 μmoles/Liter. The reaction mixtures were thermostated at 38° C.; aliquots were withdrawn every 15 minutes for 60 minutes, and concentration of glucoraphanin determined chromatographically. The rate of glucoraphanin consumption was interpreted as the rate its conversion to sulforaphane. Graphical representation of glucoraphanin content reduction as a function of increasing ascorbic acid concentration results in a series of linear plots; the slopes of the linear regression lines reflect the rate of glucoraphanin consumption, in μmoles/minute. It is apparent that in the presence of 600 μmoles/Liter concentration of ascorbic acid, the reaction rate increased 13-fold relative to that which proceeded in the absence of modulatory effects of ascorbic acid.

	Content of Ascorbic Acid							
Time, min	0 μM	50 μM	125 μM	250 μM	250 μM Filtered	400 μM	600 μM	
0	93.36	93.36	93.36	93.36	93.36	93.36	93.36	μmoles
15	92.24	89.20	84.52	80.95	86.31	78.32	75.02	GR
30	90.71	84.24	75.92	69.06	79.44	62.78	55.66	
45	89.44	80.30	68.09	57.63	71.94	47.67	37.50	
60	87.79	76.36	59.41	45.76	65.18	33.15	22.09	
Slope	−0.09293	−0.28599	−0.56217	-0.79012	−0.47140	−1.00714	−1.20029	μmol/min
Intercept	93.496	93.271	93.123	93.053	93.386	93.270	92.734	μmol

Example 4

[0087] Equimolar Conversion of Glucoraphanin to Sulforaphane.

[0088] A two-part experiment was conducted to further elucidate the role of ascorbic acid in modulating myrosinase activity. All solutions were prepared in 20 mM Tris-buffered saline, at pH 7.5, previously identified as an optimal for myrosinase activity; each sample tube had 100 mg of freeze-dried broccoli powder accurately weighed in as a source of myrosinase. Experiment was conducted at 38 QC for 2 hours, with sample aliquots removed in 30-minute increments, and both glucoraphanin and sulforaphane content assessed by HPLC. A strongly acidic "stop" solution was utilized to instantaneously inhibit further myrosinase activity in the removed aliquots. A control sample contained no ascorbic acid, and the enzymatic conversion proceeded unassisted by a co-factor.

[0089] PART 1. In the presence of the fixed concentration of ascorbic acid, 1 mmol/Liter, an increasing amount of broccoli seed extract (about 12% glucoraphanin, w/w) was added, ranging from 250 mg to 500 mg.

[0090] PART 2. While keeping the amount of broccoli seed extract fixed at 250 mg, the concentration of ascorbic acid was varied from 0.4 mmol/Liter to 3.8 mmol/Liter.

[0091] The table below presents glucoraphanin and sulforaphane expressed in μmoles. It is apparent that within the first 30 minutes in almost all the reaction mixtures, conversion of glucoraphanin to sulforaphane was complete. However, careful examination of the enzymatic conversion occurring in the control sample, without the stimulating effects of ascorbic acid, reveals an equimolar conversion of glucoraphanin to sulforaphane, i.e., the amount of glucoraphanin consumed results in the equivalent amount of sulforaphane produced.

	Glucoraphanin, μmoles					Sulforaphane, μmoles				
Time, min	0	30	60	90	120	0	30	60	90	120
GR 250 mg AA 0.0 mM	58.02	48.57	37.52	26.58	15.67	3.42	12.08	22.27	33.17	42.89
GR 250 mg AA 1.0 mM	40.07					21.51	61.95	60.20	60.04	58.25

-continued

	Glucoraphanin, μmoles					Sulforaphane, μmoles				
Time, min	0	30	60	90	120	0	30	60	90	120
GR 300 mg AA 1.0 mM	49.31					24.18	74.40	73.04	72.19	70.56
GR 350 mg AA 1.0 mM	61.41					25.00	84.92	84.02	83.19	80.02
GR 400 mg AA 1.0 mM	71.35	1.56				26.71	96.60	95.38	93.39	91.16
GR 500 mg AA 1.0 mM	89.41	1.01				33.52	120.16	118.45	116.45	112.34
GR 250 mg AA 0.4 mM	45.66					15.98	62.06	61.01	60.88	58.90
GR 250 mg AA 1.0 mM	35.24					26.49	62.19	60.62	60.41	59.10
GR 250 mg AA 2.0 mM	24.94					36.05	60.85	59.78	59.65	58.08
GR 250 mg AA 2.9 mM	22.24					38.20	59.95	59.34	58.77	56.99
GR 250 mg AA 3.8 mM	21.70					37.87	58.77	57.79	58.41	56.17

[0092] In the Part 2 of the experiment, the modulatory effect of the increasing concentration of ascorbic acid on the activity of myrosinase was assessed. An initial, apparently linear, increase in myrosinase-promoted conversion of glucoraphanin to sulforaphane is observed to about 2 mmol/L of ascorbic acid concentration, followed subsequently by a considerable leveling off.

[0093] Finally, examination of sulforaphane yield of after 30 minutes within the PART 1 of the experiment, reveals that in the presence of 1 mmol/Liter of ascorbic acid, the fixed amount of myrosinase contained in 100 mg of freeze-dried broccoli sprout powder is capable of generating at least 200 μmoles of sulforaphane, in a predictably linear fashion. FIGS. **1**, **2**, **3**, and **4** demonstrate the results of this study.

Example 5

[0094] Conversion of Glucoraphanin to Sulforaphane in the Presence of Simulated Intestinal Fluid.

[0095] Simulated Intestinal Fluid (SIF) powder, a commercially supplied concentrate closely approximating the human intestinal content in terms of composition, pH and ionic strength, was used. The experiment utilized a USP Dissolution Apparatus 2 (paddles), where into six dissolution vessels 500 mL of Simulated Intestinal Fluid was dispensed, along with 150 mg of freeze-dried broccoli sprout powder as a source of myrosinase. In vessels 1-4, the concentration of ascorbic acid was varied from 0.25 to 1.00 mmol/Liter; in vessel 5, in addition to 1 mmol/Liter ascorbic acid, 3.125 g of pancreatin (8× USP) was suspended; in vessel 6, in addition to 1 mmol/Liter ascorbic acid, and 3.125 g of pancreatin (8× USP), a doubled amount of freeze-dried broccoli sprout powder (300 mg) was added. After vessels were brought to 38° C, 250 mg of glucoraphanin-rich (8%, w/w) broccoli seed extract was added to each, and the resulting suspensions were stirred at 75 RPM for 2 hours. Aliquots were withdrawn every 15 minutes, and assayed for sulforaphane. FIG. **4** shows direct correlation between larger yield of sulforaphane and higher concentrations of ascorbic acid, especially at the earlier stages of the experiment.

Example 6

[0096] The following study was conducted to determine the effect of the combination of sulforaphane and magnesium sulfate ($MgSO_4$) on expression of monocyte chemoattractant protein (MCP-1), which is a mediator of inflammation. MCP-1 is chemokine which attracts monocytes, macrophages, and lymphocytes to sites of inflammation. A reduction in MCP-1 is beneficial for the health of endothelial cells which line the cardiovascular system.

[0097] In the study, RAW cells were activated with 1μg/mL lipopolysaccharide (LPS) for 30 minutes to stimulate the induction of MCP-1 gene expression. Following LPS treatment, the cells were treated with one of the following: (i) DMSO (vehicle control), (ii) 0.5 μM SFN, (iii) 2.5 mM $MgSO_4$, or (iv) the combination of 0.5 μM SFN and 2.5 mM $MgSO_4$. After treatment for 24 hours, the MCP-1 levels were assessed via quantitative RT-PCR.

[0098] The results, which are shown in FIG. **5**, demonstrate that the combination of sulforaphane and magnesium sulfate had a synergistic (greater than additive effect) compared to sulforaphane and magnesium sulfate alone. The data shows that the following reduction in MCP-1 levels: magnesium sulfate alone resulted in an approximately 16% decrease, sulforaphane alone resulted in an approximately 29% decrease, and the combination of magnesium sulfate and sulforaphane resulted in an approximately 53% decrease. This shows that the combination had a greater than additive effect in reducing MCP-1 levels.

Example 7

[0099] A subject presents with atherosclerosis and is suffering from symptoms including damage to the arteries and chest pain. She is administered a tablet containing glucoraphanin, myrosinase, ascorbic acid, and magnesium malate. The tablet is an enteric coated formulation which releases the contents in the small intestine. After one month of daily administration of the tablet, the subject experiences modulation of surrogate biomarkers including interleukin-8 (IL-8) and/or monocyte chemoattractant protein-1 (MCP-1) which correlate with improvement in symptoms.

What is claimed:

1. An orally administrable composition comprising:

a sulforaphane precursor;

a glucosidase enzyme capable of converting the sulforaphane precursor to sulforaphane;

a cofactor of the enzyme; and

magnesium or a salt or complex thereof;

the orally administrable composition providing a magnesium:sulforaphane precursor ratio of from about 1:1 to about 25:1.

2. The orally administrable composition of claim **1**, wherein the sulforaphane precursor comprises glucoraphanin.

3. The orally administrable composition of claim 1, wherein the enzyme capable of converting the sulforaphane precursor to sulforaphane comprises myrosinase.

4. The orally administrable composition of claim 1, wherein the enzyme cofactor comprises ascorbic acid.

5. The orally administrable composition of claim 1, wherein the composition further comprises one or more additional components selected from the group consisting of:

vitamin K2, quercetin, an aminosugar, a glycosaminoglycan, avocado/soybean unsaponifiables unsaponifiable, a vitamin, coffee fruit, silymarin, proanthocyanidins, ursolic acid, curcumin, phytosterols, and phytostanols.

6. The orally administrable composition of claim 1, comprising glucoraphanin, myrosinase, ascorbic acid, and a magnesium salt.

7. The orally administrable composition of claim 1, wherein the composition comprises a broccoli extract or powder comprising the sulforaphane precursor.

8. The orally administrable composition of claim 1, wherein the composition comprises an enteric-coated dosage form.

9. A method of treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and reducing secondary recurrences of a cardiovascular condition or disorder, comprising administering to a subject in need thereof the orally administrable composition of claim 1.

10. The method of claim 8, comprising administration of glucoraphanin, myrosinase, ascorbic acid, and a magnesium salt.

11. An orally administrable composition comprising:

a broccoli seed extract or powder standardized to contain from about 500 μg to about 2000 mg of a sulforaphane precursor;

a broccoli sprout extract or powder comprising a glucosidase enzyme capable of converting the sulforaphane precursor to sulforaphane;

a cofactor of the enzyme; and

magnesium or a salt or complex thereof;

the orally administrable composition providing a magnesium:sulforaphane precursor ratio of from about 1:1 to about 25:1.

12. The orally administrable composition of claim 11, wherein the sulforaphane precursor comprises glucoraphanin.

13. The orally administrable composition of claim 11, wherein the enzyme capable of converting the sulforaphane precursor to sulforaphane is myrosinase.

14. The orally administrable composition of claim 11, wherein the enzyme cofactor is ascorbic acid.

15. The orally administrable composition of claim 11, wherein the composition comprises an enteric-coated dosage form.

16. The orally administrable composition of claim 11, wherein the composition further comprises one or more additional components selected from the group consisting of:

vitamin K2, quercetin, an aminosugar, a glycosaminoglycan, avocado/soybean unsaponifiables, a vitamin, coffee fruit, silymarin, proanthocyanidins, ursolic acid, curcumin, phytosterols, and phytostanols.

17. The orally administrable composition of claim 11, comprising glucoraphanin, myrosinase, ascorbic acid, and a magnesium salt.

18. An orally administrable composition comprising:

sulforaphane; and

magnesium or a salt or complex thereof;

the orally administrable composition providing a magnesium:sulforaphane ratio of from about 1:1 to about 50:1.

19. The orally administrable composition of claim 18, comprising the sulforaphane in an amount of from about 74 μg to about 750 mg.

20. The orally administrable composition of claim 18, wherein the composition further comprises one or more additional components selected from the group consisting of: vitamin K2, quercetin, an aminosugar, a glycosaminoglycan, avocado/soybean unsaponifiables unsaponifiable, a vitamin, coffee fruit, silymarin, proanthocyanidins, ursolic acid, curcumin, phytosterols, and phytostanols.

21. The orally administrable composition of claim 18, comprising sulforaphane and a magnesium salt.

22. The orally administrable composition of claim 18, wherein the composition comprises an enteric-coated dosage form.

23. The orally administrable composition of claim 18, wherein the composition comprises a broccoli extract or powder comprising sulforaphane.

24. A method of decreasing levels or decreasing gene expression of interleukin-8 (IL-8) and/or monocyte chemoattractant protein-1 (MCP-1) in a subject, comprising administering to the subject in need thereof the orally administrable composition of claim 18.

25. A method of treating, preventing, reducing the occurrence of, decreasing the symptoms associated with, and/or reducing secondary recurrences of a disease or condition associated with elevated levels of interleukin-8 (IL-8) and/or monocyte chemoattractant protein-1 (MCP-1) in a subject in need thereof, comprising administering to the subject in need thereof the orally administrable composition of claim 18.

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